EFFECTS OF DERIVITIZATION ON ANTIMICROBIAL ACTIVITY OF EMBELIN ISOLATED FROM *EMBELIA SCHIMPERI* BERRIES

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ABSTRACT

Chromatographic separation of ethyl acetate extract from *Embelia schimperi* berries led to isolation of embelin 2,5-dihydroxy-3-undecyl- 1,4-benzoquinone(**1**). Embelin was identified on the basis of physical and spectroscopic data. Three quantities of compound **1** weighing 0.02g each were used to synthesize three derivatives by addition of sulphonyl, acetyl and alkyl imino groups to the parent compound's structure. The synthesized compounds were identified as 2,5-dihydroxy-5-sulpho-3-undecyl-1,4-benzoquinone (**2**), 1,2,4,5 tetra acetoxy-3-undecyl benzene (**3**) and 2,5-dihydroxy-1-di-ethyl imino-3-undecyl-4- benzoquinone (**4**) on the basis of MS and FTIR spectroscopic data. Embelin and its synthetic derivatives were tested against clinical strains of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans.* Plate titration technique was used; and presence or absence of microbial growth after respective incubation periods used to determine antimicrobial activity. The incubation period for antibacterial test was 24 hours and 48 hours for antifungal test respectively. Compound **1** and **4** were found to be active against *S. aureus* (gram positive bacteria) at dilutions of 100 ppm and 200 ppm respectively while compounds **2** and **3** were inactive. Compounds **1, 2, 3** and **4** were

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inactive against *P. aeruginosa, E. coli* (gram negative bacteria) and *C. albicans* (fungi) at all dilutions. The inactivity of embelin and its synthetic derivatives could possibly be attributed to inability of compounds to penetrate through the cell walls of *P.aeruginosa, E.coli* (gram negative bacteria) and *C. albicans* (a fungus). Retention of activity against *S. aureus* in compound **4** is an indication that effect of functional groups on properties of parent compound should be considered prior to derivatization. Addition of certain functional groups may affect compounds properties such as solubility, polarity and stability which determine the uptake and metabolism of a drug in the target organism.

Key words: Embelin, *Embelia schimperi,* derivatives, benzoquinones, antimicrobial, spectroscopic.

Introduction

In traditional medicinal practice, plant preparations have always been used to treat infectious diseases such as malaria and skin infections with a varying degree of success [11]. Use of *Embelia schimperi* and *Embelia ribes* plant extracts as de-wormers and wound cleaners has been reported in many parts of Africa and Asia [8].

Embelin from *Embelia schimperi* was reported against a number of micro organisms [6]. Antifungal and antibacterial activities were reported in embelin from *Oxalis erythrorhiza* [2]. Research on bio active plants with ethno-pharmacological uses, has attracted a lot of interest all over the world. Today in USA and Canada, 25% of prescription drugs are modeled after plant based compounds. Many of these drugs were discovered following leads provided by indigenous knowledge systems [1].The aim of this research was to isolate embelin and synthesize new derivatives with enhanced antimicrobial activity.

Materials and methods

Collection of plant samples

Berries of *embelia schimperi* were collected from the western slopes of Mau ranges in Kericho district about 300km west of Nairobi. The plant was identified by the Herbarium staff in the Department of Botany, Moi University. 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 quantity weighing 500g of the material was soaked in ethyl acetate at room temperature for 48 hours. The mixture was filtered and the excess solvent evaporated using a rota vapor affording 25g of a dark brown solid. A quantity of 10g of the crude extract was subjected to column chromatography using a column packed under n-hexane with deactivated silica.

The column was first eluted with pure n-hexane followed by a mixture of n-hexane\ethyl acetate with increasing polarity [7]. Elution of the column with n-hexane\ethyl acetate mixture (1:10 v\v) led to the isolation of bright orange crystalline compound labeled **1** [6, 2]. Compound was characterized on the basis of physical and spectroscopic (MS and FTIR) data and identified as 2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone (embelin).

Synthesis of embelin derivatives

Sulphonation reaction

A quantity of 0.02g of compound **1** was dissolved in ethyl acetate and concentrated sulphuric acid added drop wise until the color of the solution changed from yellow to dark green [10]. The solution was warmed in a water bath for 5 minutes at 50°C. The solution was left to stand after which it was concentrated to remove excess solvent. The solution was labeled **2.**

Acetylation Reaction

A quantity of 0.02g of embelin was suspended in 5ml of acetic anhydride. Pyridine was added drop-wise until embelin dissolved to form a pale yellow solution. The solution was covered and allowed to stand overnight after which it was concentrated to remove excess solvents [10]. The solution was labeled **3.**

Alkylamination reaction

A quantity of 0.02g of embelin was dissolved in 5ml chloroform and 2ml of tri-ethyl amine is dissolved in ethanol. The two solutions are mixed and warmed in a water bath for one minute where a purple solution was formed [10]. The solution was left to stand for five days after which it was concentrated to remove excess solvents. The solution was labeled **4**. Compounds 2, 3 and 4 were purified by preparative TLC, recrystallization was done and the percentage yields calculated. The derivatives were characterized on basis of spectroscopic data [9].

Antimicrobial assay

Plate titration method was used in antimicrobial assay [4].The Muller Hinton agar was prepared as per the manufacturer's instructions. A range of (100ppm to 200ppm) dilutions of the test compounds **1, 2, 3** and **4** were made. Petri dishes for each dilution were labeled and set in replicates of three Petri dishes. Starting from the weakest dilution of the test compound (100ppm), 1ml of each dilution was transferred to each dish. Using a pipette, 19ml of media was transferred to each dish and mixed well. The controls were left untreated. The plates were dried in the incubator for 30 minutes with the lids tilted. Once dried, the plates were stored at 4°C for one week. Ditches were cut on the media creating four portions on each plate. Each portion was inoculated with test organisms from 24 hour old cultures and incubated overnight for bacteria and for 48 hours for fungus after which presence or absence of growth is observed.

Results and discussion

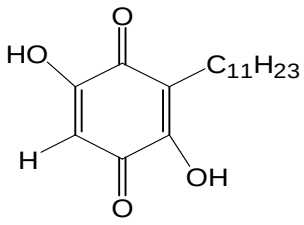
Table 1: Physical characteristics of embelin and its synthetic derivatives

Compound	Color of the crystals	Melting point	Yield
1	Yellow	142-143ºC	2%
2	Green	173-175ºC	67%
3	Dark orange	121-122ºC	15%
4	Purple	135-136ºC	37%

Table 2: Spectroscopic data on embelin and its synthetic derivatives

Test compounds	MS data molecular ion peaks	FTIR data on functional groups						
		OH	C=O	C=C	S=O	S-O	C-N	C=N
1	m\z 294 M^+	3307 (S)	1614 (S)					
2	m\z 392 M^+	3309 (S)	1720 (W)	1616 (S)	1190 (S)	1060 (M)		
3	m\z 445 M^+-18		1720 (W)	1616 (S)				
4	m\z 349 M^+	3315 (W)	1650 (M)	1548 (S)			1122 (M)	2750-2362(W)

Structure of embelin

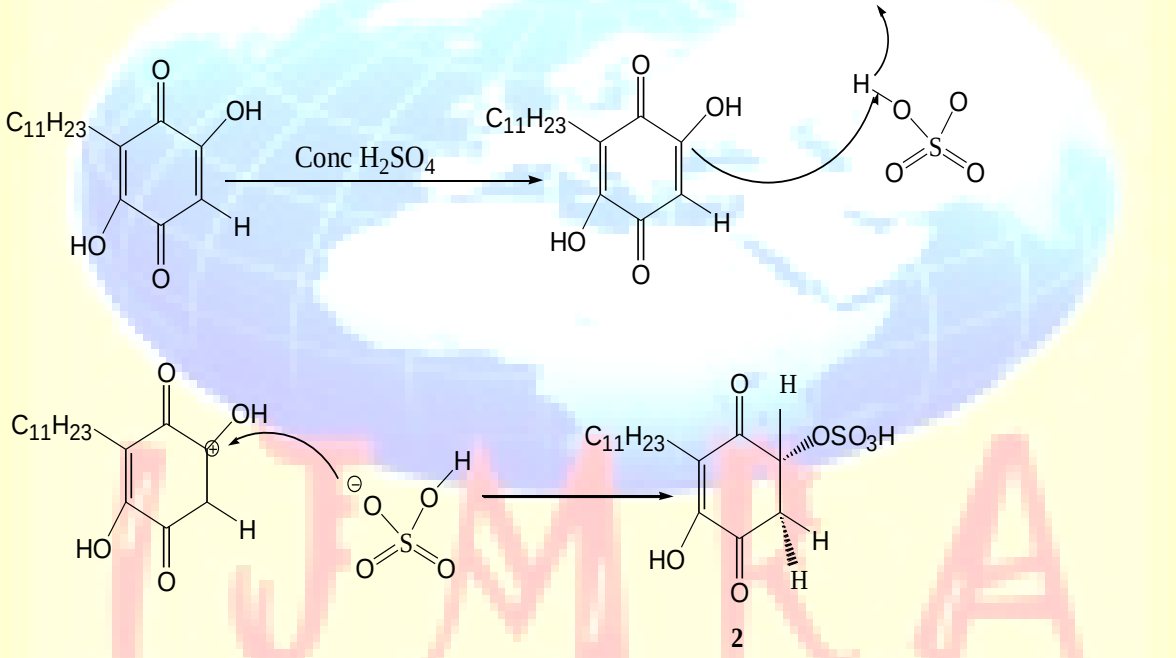


1

Reactivity of embelin

The reaction mechanisms for the formation of compounds **2, 3** and **4** were formulated based on the known reactivity treads of benzoquinone class of compounds to which embelin belongs and are shown in scheme i, ii and iii below [10].

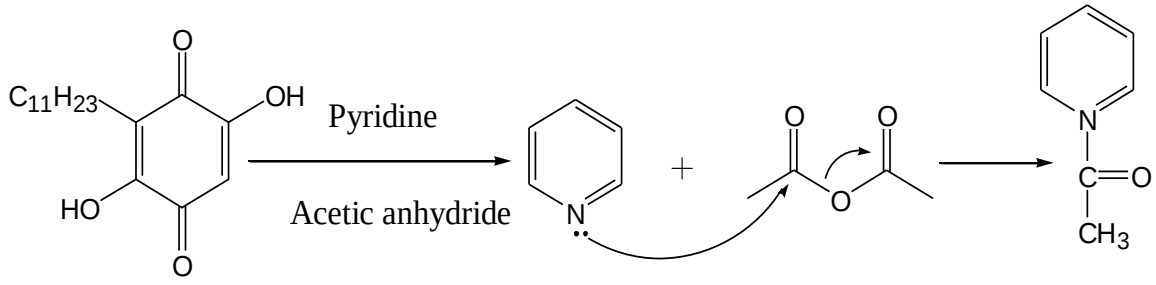
Scheme I: Synthesis of compound 2



2

2,5-dihydroxy-5-sulpho-3-undecyl-1,4 BQ

Scheme II: Synthesis of compound 3



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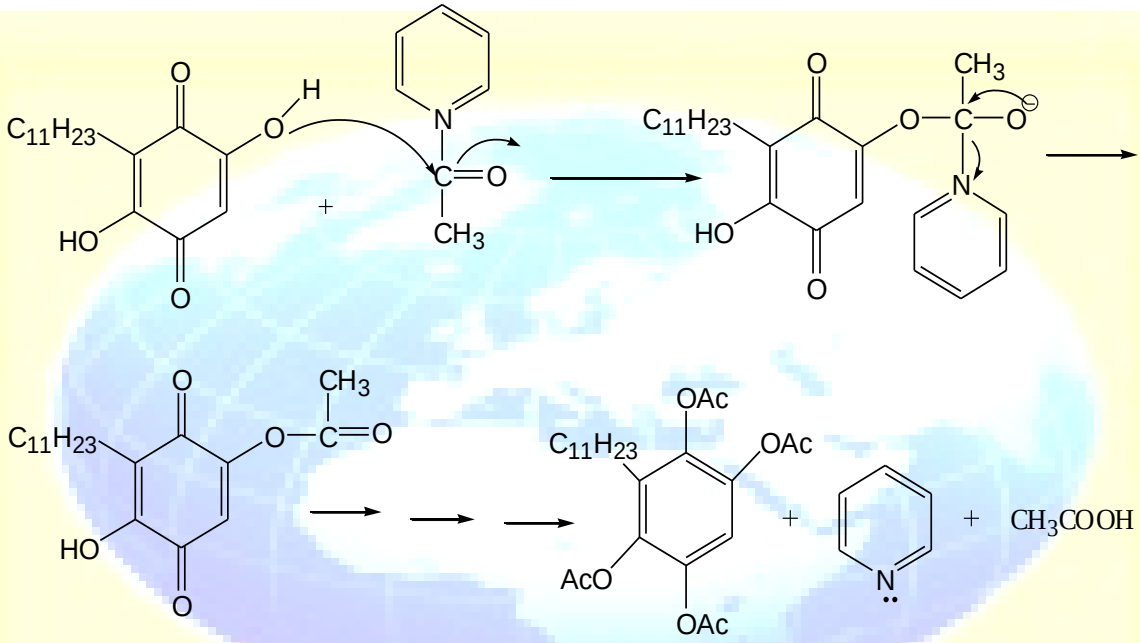
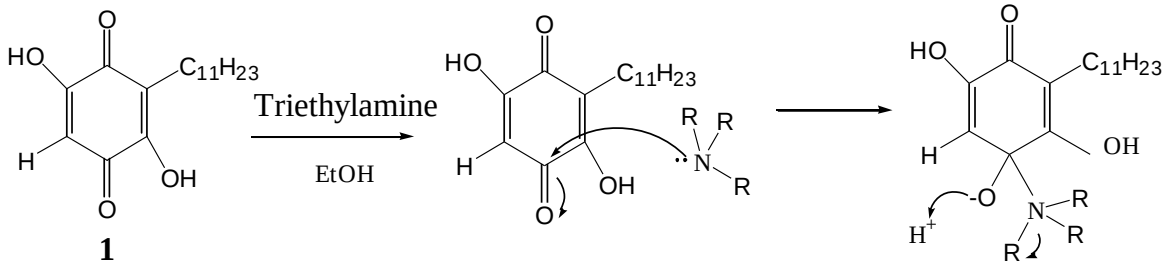


3

1,2,4,5-tetra acetoxy-3- undecyl benzene

Scheme III: Synthesis of compound 4



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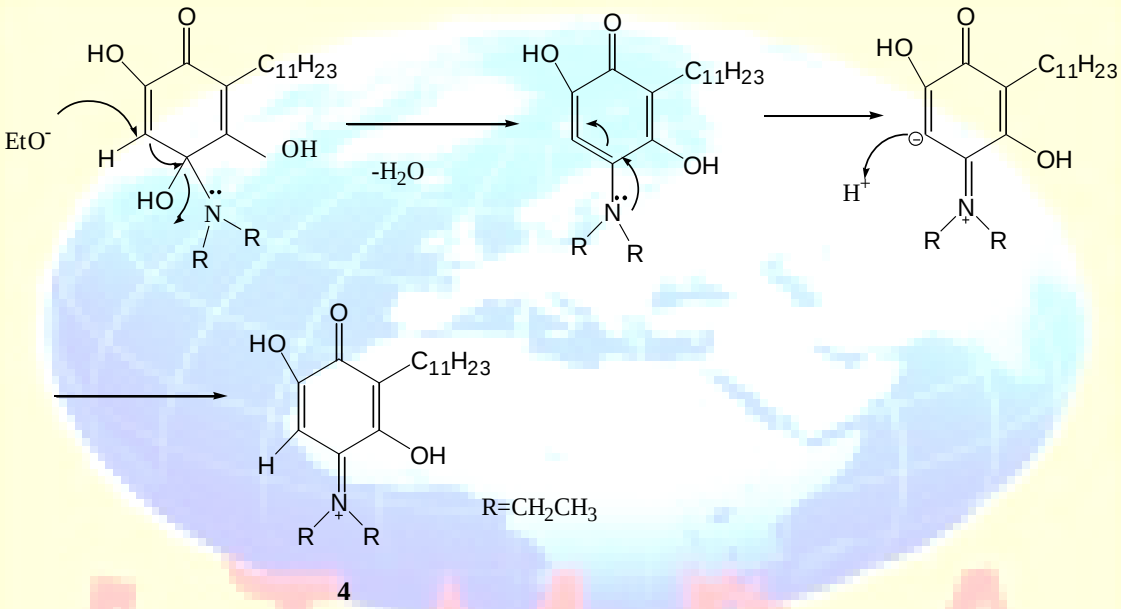

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2,5-dihydroxy-1-diethyl imino-3-undecyl-4-BQ

Table 3: Antimicrobial activity of embelin and its synthetic derivatives

Test micro organisms	**Test compounds (100-200 ppm)**			
	1	**2**	**3**	**4**
E. coli	Growth	Growth	Growth	Growth
S. aureus	No growth	Growth	Growth	No growth
P. aeruginosa	Growth	Growth	Growth	Growth
C. albicans	Growth	Growth	Growth	Growth

Compounds **1** and **4** were found to against *S. aureus* while compounds **1, 2, 3** and **4** were inactive against *E. coli*, *P. aeruginosa* and *C. albicans* within the dilution range of 100-200 ppm. The inactivity of embelin against *E. coli* had been reported earlier [6].

Embelin is cell permeable and lipid soluble hence the ability to act the cell membrane of the micro organism making tunnels to drain the cell contents and eventually kill the cell [14]. The double bonds in embelin interact with the receptor via van der Waal bonding. The inactivity of embelin and its synthetic derivatives could possibly be attributed to inability of compounds to penetrate through the cell walls of *P.aeruginosa, E.coli* (gram negative bacteria) and *C. albicans* (a fungus) [5].

The low molecular weights of compounds **1** (294) and **4** (350) could have enhanced the compounds' cell membrane penetration in gram positive bacteria, *S. aureus*, hence the observed activity [5, 11, 12]. Imines are weak bases which are easily ionized and have good binding interactions with drug receptors as proton donors. The branched alkyl groups could interact with the hydrophobic sites of a receptor hence the retention of activity in compound **4** [3].

Presence of sulphanato group in compound **2** increases the acidity of the compound. This makes it easy for the O-H bond to break releasing a proton. Water molecules easily solvate the ionized form of acid hence the increased polarity. Polar compounds bind easily on drug receptors but on the other hand they little chance of crossing the fatty cell membrane hence the elimination of activity in compound **2** [5].

Acetylation of embelin led to the replacement of all the OH functional groups in the benzoquinonic structure. The possibility of the molecule taking part in hydrogen bonding as a proton donor to its receptor is ruled out. Electronic properties of an ester are also affected in that the carbonyl group can pull away electrons from the neighbouring oxygen to give resonance structures. Since the lone pair is involved in such an interaction, it cannot take part effectively in hydrogen bonding. The extra bulk of the acyl group will also hinder close approach to the receptor hence the loss of activity in compound **3** [3].

Conclusion

The antimicrobial activity of embelin against *S. aureus* supports the fact that *Embelia schimperi* plant extracts have been traditionally used to clean wounds. This gram positive bacterium is responsible for wound infections, boils and urinary tract infections [6]. Retention of activity against *S. aureus* in compound **4** is an indication that effect of addition of functional groups on properties of parent compound should be considered prior to derivatization. Addition of

functional groups may affect properties such as solubility, polarity and stability which determine the uptake and metabolism of a drug in a target organism [3, 5].

Acknowledgement

The authors are grateful to the staff of Botany Department of University of Eldoret for plant identification, Mr. Ekeyya, Chief technician and the technical staff in the department of Chemistry of University of Eldoret for their support. We also acknowledge International Centre for Insect Physiology and Ecology (ICIPE) for the MS data, Jomo Kenyatta University of Agriculture and Technology (JKUAT) Chemistry department for the FTIR data and the faculty of Health Sciences, Moi University for performing the bioassay.

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