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SHORT COMMUNICATION

FREE RADICAL SCAVENGING CAPACITY AND ANTIBACTERIAL ACTIVITY OF DITERPENOIDS FROM STEM BARK OF *CROTON MEGALOCARPOIDES* FRIIS & M. G. GILBERT

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ABSTRACT

Background and aim: Medicinal plants represent the bedrock of traditional medicine practice which most people in developing countries still rely on for treatment of various diseases. Root and stem bark of *Croton megalocarpoides* Friis & M. G. Gilbert (Euphorbiaceae) are used to treat infections, parasitic diseases, wounds and whooping cough. These traditional medicinal uses have not been validated scientifically, hence the need to provide scientific-based evidence involving antioxidant and antimicrobial activities of isolated diterpenoids to support these folkloric claims.

Methods: Clerodane diterpenoids previously isolated from *C. megalocarpoides* Friis & M. G. Gilbert stem bark (crotocorylifuran, megalocarpodolide H, 1,2-dehydrocrotocorylifuran-2-one, 7,8-dehydrocrotocorylifuran and 12-epi croton zambefuran) were screened for antioxidant activities for the first time using 2,2 -Diphenylpicrylhydrazine (DPPH) radical scavenging activity at concentrations of 1 to 0.0625 mg/ml. Antibacterial activities of these diterpenoids were also investigated using Gram positive bacteria at concentration range of 200 - 6.25 mg/ml.

Results: The antioxidant tests revealed the diterpenoids exhibited incomparable radical scavenging activity (average 30.2-33.2%) when compared with the standard drug, ascorbic acid (89.8-91.5%). The antibacterial tests revealed moderate activity against tested microorganisms viz: *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at the peak concentration (200 mg/ml).

Conclusion*:* The presence of diterpenoids with moderate antioxidant capacities in *C. megalocarpoides* stem bark may be responsible for its ethnomedicinal uses with added pharmacological value.

Keywords: *Croton megalocarpoides*, antioxidant activity, antibacterial activity, diterpenoids, stem bark

INTRODUCTION

Antioxidants are believed to play a very important role in the body defense against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration. Natural antioxidants occur in all parts of plant and plant derived antioxidants have been shown to function as free radical scavengers [1]. Also, microorganisms causing diseases are becoming more resistant to existing drugs, therefore there is urgent need to discover new drugs that will be effective against this disease resistant microorganisms.

Croton megalocarpoides Friis & M. G. Gilbert a monoecious shrub, belongs to Euphorbiaceae family, grows up to 8 m and is found in coastal bushland of East Africa and Somalia [2]. The root and stem bark are used to treat infections and parasitic diseases, wounds and whooping cough [2]. The root and stem are a rich source of diterpenoids [3,4]. Diterpenoids which are characteristic of *Croton* species, have been found to exhibit various bioactivities including cytotoxic, antifungal, antiinflammatory and acetycholinesterase inhibitory activities [5]. From previous studies, crotonolide G, isolated from *Croton laui*, and ursane triterpenoid from *C. bonplandianum* roots were found to exhibit potent antibacterial activities against gram positive bacteria [6] antifungal activities [7], respectively. Diterpenoids are known to exhibit cytotoxic, antifungal, anti-inflammatory and acetycholinesterase inhibitory activities [5]. In our previous study, five diterpenoids were isolated from the stem bark of *C. megalocarpoides* [3]. To date, there is no scientific data on the antioxidant capacities and antibacterial activities of these diterpenoids from *C. megalocarpoides*. As a part of our ongoing efforts to search for biologically-significant diterpenoids, we investigated the antioxidant and antibacterial activities of the diterpenoids from *C. megalocarpoides* for the first time.

MATERIALS AND METHODS

C. megalocarpoides stem bark strips had been collected in city of Mombasa in Kenya, from our previous study [4], and identified with voucher specimen number BN 2009/8 deposited. Diterpenoids were then isolated and characterised using various spectroscopic techniques: IR, UV, NMR (H NMR, CNMR, HMBC, HSQC, DEPT) and MS [4]. They were preserved in a refrigerator 4^{o}C.

Determination of DPPH radical scavenging capacity: The effect of previously isolated five diterpenoids on DPPH radical was estimated by adopting the method of Panda *et al.* [8]. A solution of 0.135 mL DPPH in methanol was prepared, and 1.0 mL mixed with 1.0 mL of different diterpenoid isolates in methanol to give concentration range of 0.02-0.1 mg/mL. The reaction mixture was vortexed thoroughly, and left in the dark at room temperature for 30 min. and absorbance measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard.
Percentage DPPH scavenging effect was calculated using the formula:

Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance of standard sample.

Antibacterial testing: Agar diffusion method used for antibacterial assay [10]. An overnight culture of different Gram positive bacterium: *Staphylococcus aureus*, and Gram negative bacteria: *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas*

aeruginosa was prepared. Each organism (0.1 ml) was taken into 9.9 ml of sterile distilled water to give 10 ml at 1:100 (10^{-2}) dilution from 10^{-2} dilution. Aliquot of 0. 2 ml was taken into sterile molten nutrient agar at 45^{o}C. This was aseptically poured into the sterile plates and allowed to set on the bench for about 45 minutes. Concentrations of 200 - 6.25 mg/ml of diterpenoids were prepared. A sterile cork-borer was used to create wells/holes inside the set plate. Different concentrations of diterpenoids, as well as positive (gentamicin, 10 mg/ml) and negative (methanol) controls, were introduced into separate wells. These were allowed to stay on the bench for two hours before incubation at 37^{o}C for 24 h [9].

RESULTS AND DISCUSSION

The antioxidant activities in Table 1 revealed that the diterpenoids exhibited moderate % radical scavenging activity as follows: 12-epi crotozambefuran A (42.89-34.46%), crotocorylifuran (32.9-26.9%), megalocarpodolide H 33.8-32.6%), 1,2-dehydrocrotocorylifuran-2-one and 7,8-dehydrocrotocorylifuran (33.3-30.2% each).

Table 1: Antioxidant Activities of *Croton megalocarpoides* diterpenoids

	Scavenging activity (%)					
Conc. (mg/ml)	Crotocorylifuran	Megalocarpodolide H	1,2-dehydrocrotocorylifuran-2-one	7,8 dehydro crotocorylifuran	12 – epicrotonzambefuran A	Ascorbic acid
1.0	32.9	32.9	33.8	33.2	33.2	91.5
0.5	32.6	32.6	33.3	32.8	32.8	90.0
0.25	31.5	31.5	32.8	32.3	32.3	90.3
0.125	29.3	29.3	32.8	31.4	31.4	89.8
0.0625	26.9	26.9	32.6	30.2	30.2	89.8

The antibacterial tests (Table 2) revealed weak activities against tested microorganisms: *Staphylococcus aureus*, *Staphylococcus typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at 200 mg/ml. Antibacterial activities were only observed at the peak concentration of 200 mg/ml for the five diterpenoids tested: crotocorylifuran, 12-epi-zambefuran A, megalocarpodolide H, 1,2-dehydrocrotocorylifuran-2-one and 7,8-dehydrocrotocorylifuran. These activities were incomparable to that of gentamicin.

Diterpenoids which are characteristic of *Croton* species have been found to exhibit various bioactivities such as cytotoxic, antifungal, anti-inflammatory and acetycholinesterase inhibitory activities [5]. From previous studies, crotonolide G from *C. laui*, was found to exhibit potent antibacterial activities against Gram positive bacteria [6] and also ursane triterpenoid from root of *C. bonplandianum* displayed antifungal activities [7]. Crotofolanes diterpenoids from *C kilwae* were reported to display antiplasmodial activities [10]. Other diterpenoids, crotofoligandrin, from *C. oligandrus* and floridolide A, a clerodane diterpenoid from the stem bark of *C. macrostachys* were known to possess antioxidant [11], and significant antibacterial and antifungal [12] activities. In this present study, the five diterpenoids from *C. megalocarpoides* exhibited moderate antioxidant and weak antibacterial activities, hich may be responsible for its ethnomedicinal uses.

Table 2: Antibacterial activities of *Croton megalocarpoides* diterpenoids at 200 mg/ml

Microorganism	Zone of inhibition (mm)					
	Crotocorylifuran	Megalocarpodolide H	1,2-dehydrocrotocorylifuran-2-one	7,8-Dehydrocrotocorylifuran	12-Epicrotonzambefuran A	Gentamicin (10 mg/ml)
Staphylococcus aureus	10	10	-	10	10	38
Salmonella typhi	10	-	10	10	10	38
Klebsiella pneumoniae	10	10	10	10	10	40
Pseudomonas aeruginosa	10	10	10	10	10	38

CONCLUSION

The presence of five known diterpenoids in *C. megalocarpoides* with moderate antioxidant and weak antibacterial potentials may be responsible for its ethnomedicinal uses with further pharmacological value.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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