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ANTIBIOTIC ACTION OF CONSTITUENTS OF ROOT BARK OF *EUCLEA NATALENSIS A.DC.*

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Petroleum ether and then chloroform extracts of *Euclea natalensis* root bark showed marked antibiotic activity against *Staphylococcus aureus,* 7-Methyljuglone, mamegakinone and diospyrin were isolated from it and found active against a number of pathogenic organisms including *Neisseria gonorrhoeae* and *Shigella Spp.* A triterpenoid lupeol isolated from *E. natalensis* was found to be inactive against *Staphylococcus aureus.*

INTRODUCTION

Euclea natalensis, a shrub of family *Ebenaceae* is widely distributed throughout Africa and is believed to be a natural remedy for diarrhoea, dysentery, gonorrhoea, in various skin infections and as an antiseptic mouth wash [1]. A number of naphthoquinones had been isolated from *Euclea natalensis* [2] and other plants [3] of family *Ebenaceae.* The presence of biologically active naphthoquinones in plants, fungi, lichines and echinoderms mainly in sea urchins has been known for sometimes [4]. These compounds have been particularly associated with antibacterial activity. Plumbagin from a few species of *Plumbago,* family *Plumbaganaceae* showed marked antibiotic properties [5]. Recently the antifungal activity of some 1,4-naphthoquinones including lawsone, juglone, and 7-methyljuglone has been reported [6]. In addition to antibacterial and antifungal action, a number of naphthoquinones are haemostatic. Juglone, a taxonomic characteristic of family *fuglonadaceae,* is a well known haemostatic agent [7].

Since in Tanzania the root bark of *Euclea natalensis* is extensively used in various infectious diseases by traditional practitioners, the current investigation of the possible occurence of *in vitro* antimicrobial compounds in this plant was undertaken. So far there have been no reports on the *in vitro* and *in vivo* antibiotic activity of the plant and the naphthoquinones it contains.

MATERIALS AND METHODS

The powdered root bark of *Euclea natalensis* collected during the rainy season (April) from the University campus in Dar es Salaam, Tanzania, was extracted in a Soxhlet first with petroleum-ether (b.p. 80–100°) and then with chloroform. Both these extracts gave positive antibiotic activity against *Staphylococcus aureus* and no activity against *Escherichia coli.*

Petroleum-ether Extract. The petroleum-ether extract was evaporated and the residue was chromatographed on a column of silica gel using petroleum ether and then methylated spirit. The methylated spirit fraction on evaporation gave a black solid which was refractionated on a column of silica gel using chloroform as eluent. The chloroform was evaporated off and the residue was repeatedly recrystallized from chloroform – methanol (3:2) to give mamegakinone [8] (0.15% yield). The filtrate from mamegakinone after decolorization (activated charcoal) and several recrystallizations from methanol – water gave pure lupeol [9] (0.10% yield).

Chloroform Extract. The residue after evaporation of the chloroform was extracted several times with methanol. The combined methanol extracts on evaporation gave a dark brown mass which after several fractional crystallizations from chloroform – methanol gave mamegakinone (0.001% yield) and lupeol (0.004% yield). A small amount of 7-methyljuglone [10] crystals were mechanically separated from the crystals of mamegakinone. The methanol insoluble residue indicated the presence of diospyrin [2] (by comparison with authentic sample TLC), but it was not isolated at this stage. Diospyrin [2] was isolated by immersing twice the powdered root bark (wet season) of *E. natalensis* in petroleum-ether for three days at room temperature.

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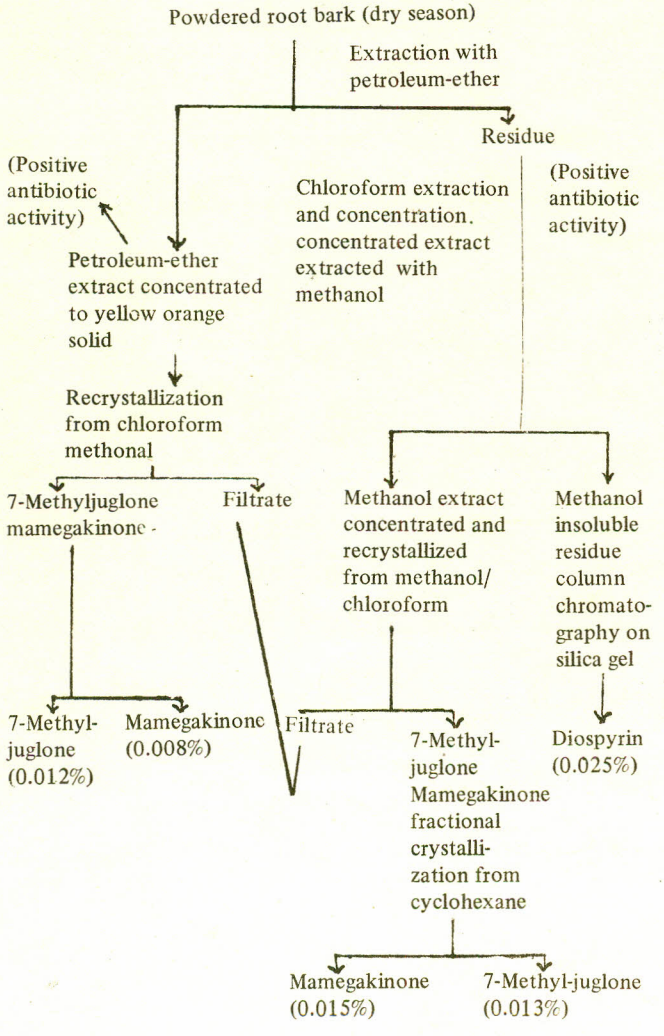
The solvent on evaporation gave a black residue which was dissolved in methanol and then absorbed on activated charcoal. The filtrate from this gave lupeol, while the chloroform extraction of the charcoal on evaporation deposited red crystals of crude diospyrin. Recrystallization from methanol – chloroform (1:1) gave pure diospyrin (0.024%).

The dried powdered root bark of *E. natalensis* collected during the dry season (October) from the same plant was subjected to the separation scheme outlined below. This gave a higher yield (0.025%) of 7-methyljuglone while the relative concentration of lupeol was much less (0.002% yield).

Separation Scheme



All the compounds isolated were identified by comparison with authentic sample or with literature data (by IR, NMR, MS, UV, TLC).

Assay of Antimicrobial Activity. Filter-paper disc method was used for the assay of antimicrobial activity.

Test Cultures: With the exception of *N. gonorrhoeae* and *H. influenzae* the test organisms employed for assaying antibiotic activity (Table 1) were obtained from stock cultures in the Department of Microbiology and Immunology, Faculty of Medicine, University of Dar es Salaam. The *Neisseria gonorrhoeae* and *Haemophilus influenzae* cultures were isolated from clinical cases.

Media: The culture media used for the study were drugsensitivity agar (DST Agar Oxoid) and chocolate agar. For *Neisseria gonorrhoeae* and *Haemophilus influenzae* chocolated agar plates were heavily streaked with the organism, while for other bacteria DST agar plates were used. A 24-hr broth culture of the organism under test was poured on to the surface of the medium. It was allowed to stay for 10 min and then pipetted off. The various compounds (about 0.3 0 mg/ml of chloroform) and crude extracts tested were applied on to 6-mm sterile absorbent filter paper discs which then were placed on agar surface. The plates were incubated at 37° (in a 5–10% CO_2 atmosphere for *N. gonorrhoeae* and *H. influenzae*)and zones of inhibition of bactaria growth after 24–48 hr were measured. The results are given in Table 1.

Table 1. Susceptibility of microorganisms to constituents of *E. natalensis*

Bacteria	Zone of inhibition (mm) 7-Methyl-juglone	Diospyrin	Mamega-kinone
Klebsiella aerogenes (from urine)	11	9	11
Shigella dysenteriae	14	14	9
Shigella flexnerii	12	11	0
Corynebacterium diph-theriae	13	14	–
Bacillus anthracis	17	13	0
Bacillus cereus	9	10	0
Salmonella hidelberg	8	8	8
Haemophilus influenzae	11	12	10
Pseudomonas areuginosa	0	0	0
Escherichia coli	0	0	0
Clostridium welchii	8	0	0
Staphylococcus aureus	11	0	22
Neisseria gonorrhoeae	24	0	14

0, No antibiotic activity

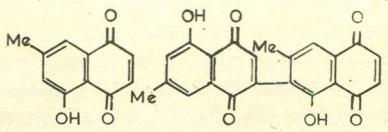
Petroleum-ether and chloroform extracts were active against *Staphylococcus aureus*, the zone of inhibition was about 15 mm in both the extracts. They were not active against *Escherichia coli.*

RESULTS AND DISCUSSION

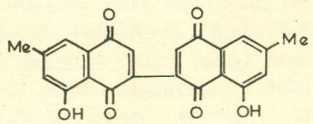
The preliminary screening for antibacterial action of the extracts from the root bark of *Euclea natalensis* showed

high level of activity against *Staphylococcus aureus* but the same did not inhibit the growth of *Escherichia coli.* The crude extracts were not further tested against other organisms.

The active principles isolated were 7-methyljuglone (I), diospyrin (II) and mamegakinone (III). These showed



(I) (II)



(III)

a varied degree of antibiotic activity against a number of selected microorganisms as shown in Table 1. Mamegakinone was most effective against *Staphylococcus aureus* (22 mm zone of inhibition) while 7-methyljuglone demonstrated activity against *Neisseria gonorrhoea* (24 mm zone of inhibiton). Although it is not known at this stage, without clinical evaluation, whether the rootbark is active or not, possible effectiveness against sores, purulent lesions and skin infections could be attributed to the activity of mamegakinone against *Staphylococcus aureus.* 7-Methyljuglone with its significant *in vitro* antigonococcal activity could possibly give curative properties to the rootbark against gonorrhoea. All the three naphthoquinones showed good inhibition of *Shigella spp.* – a well known etiological agent of bacillary dysentery. It would be interesting to report that the 7-methyljuglone was found to be active against an isolate of *Neisseria gonorrhoeae* which exhibited marked resistance to Penicillin G. (1 unit).

The above *in vitro* observations on the antibacterial activity of these naphthoquinones against the tested organisms justify further investigation on their curative properties.

As reported elsewhere 7-methyljuglone also has antifungal activity [6]. The believed but not established effectiveness of *E. natalensis* in curing some cutaneous fungal infections could be due to this compound.

Besides *E. natalensis,* 7-methyljuglone, mamegakinone, diospyrin and some other quinones have bean isolated from other species of *Euclea* [10–12] and *Diospyros,* [3,8] which are commonly used in traditional medicine for various pathological conditions. The plants used against gonorrhoea almost always contained 7-methyljuglone.

In the present study a triterpenoid, lupeol isolated from *E. natalensis* did not show any *in vitro* activity when tested against *Staphylococcus aureus,* and *Escherichia coli.* Lupeol is said to be effective in urinary infections and rheumatism [1b], but as far as we know there is no reported attempt to ascertain its antibiotic activity *in vitro* or *in vivo.*

It was interesting to note that the *E. natalensis* roots collected in rainy and dry seasons showed marked variation in chemical composition. In the wet season the plant was rich in lupeol and the relative concentration of 7-methyljuglone was negligible, while in the dry season the concentrations of the two compounds were reversed. Biogenetically the two compounds are not directly related, lupeol type of compounds are derived from squalene [13] while 7-methyljuglone and other naphthoquinones are biogenetically derived from acetate units [4].

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