Ethnobotanical Drug Discovery Based on Medicine Men's Trials in the African Savanna: Screening of East African Plants for Antimicrobial Activity II

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ETHNOBOTANICAL DRUG DISCOVERY BASED ON MEDICINE MEN'S TRIALS IN THE AFRICAN SAVANNA: SCREENING OF EAST AFRICAN PLANTS FOR ANTIMICROBIAL ACTIVITY II[1]

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ABSTRACT.—Antimicrobial activity of a number of East African plants was evaluated. The plants collected based on information provided by medicine men showed a much higher probability of finding active extracts than the plants collected randomly.

There is no doubt that plants are a good source of biologically active natural products. In addition, these phytochemicals are all biodegradable and, more importantly, they are renewable. The efficient use of such renewable natural resources is becoming increasingly important worldwide.

We have previously reported our preliminary antimicrobial screening data of East African medicinal plants (1). It should be emphasized that these medicinal plants were collected primarily based on information provided by native people, especially "Bwana Mganga" (Swahili or similar terminology meaning medicine man in other languages) (2). Naturally, a Bwana Mganga possesses a wealth of empirical knowledge on local plants. Besides the information gathered from Bwana Mganga, some plants were selected based on information from books (3,4) in which plants were described as sources of treatment for various infectious diseases.

Although it is essential to test against the specific target microorganisms themselves, in order to avoid handling numerous pathogenic microorganisms, four typical microorganisms (*Bacillus subtilis*, a Gram-positive bacterium; *Escherichia coli*, a Gram-negative bacterium; *Saccharomyces cerevisiae*, a yeast; and *Penicillium crustosum*, a mold) were utilized as taxonomical representatives for the initial screening. As a result, of 79 extracts from 72 species of plants, we found that 40 extracts initially gave positive results indicative of antimicrobial activity against one or more of the microorganisms tested (1). This number represents a much higher probability of finding the active extracts than previous reports (5,6). In addition, the concentration of 100 μg/ml of the plant crude extracts used for the assay was also much lower than earlier reports (5,6), in which the concentrations were reported in the range of 500–2000 μg/ml. About the same number of plants were then collected at random in the same area and assayed for comparison purposes.

RESULTS AND DISCUSSIONS

We evaluated 65 extracts representing 64 plant species distributed among 35 families. Although the plants were collected randomly in this experiment, the aforementioned previous results were kept in mind during the plant collections. Thus, collection of different parts from the active plants and of plants belonging to the same genus as the active plants was the primary focus. In contrast to the previous result, only 6 extracts

[1]For Part I see Taniguchi *et al.* (1).

TABLE 1. Antimicrobial Activity of Crude Extracts of East African Plants.

Plant			Microorganism tested[a]			
Family	Species	Part	Ec	Bs	Sc	Pc
Apocynaceae	*Hunteria zeylanica*	Bark	−	+	−	−
Guttiferae	*Harungana madagascariensis*	Root	−	+	−	−
Polygonaceae	*Emex spinosus*	Leaf	−	−	+	−
Rosaceae	*Hagenia abyssinica*	Leaf	−	+	−	−
Solanaceae	*Solanum nigrum*	Leaf	−	−	+	−
Umbelliferae	*Ammi majus*	Leaf	−	−	+	−

[a]Ec, *Escherichia coli;* Bs, *Bacillus subtilis*; Sc, *Saccharomyces cerevisiae*; Pc, *Penicillium crustosum.* −, No effect; ±, Partial growth inhibition; +, Complete growth inhibition.

exhibited activity against either *Bac. subtilis* or *Sacch. cerevisiae.* They are presented in Table 1. The concentration for the assay was even increased to 500 μg/ml, since only a few of the extracts showed some activity at 100 μg/ml. None of the extracts showed any activity against *Es. coli* and *Pen. crustosum* at 500 μg/ml. A summary of the comparison of the two different collections mentioned above is shown in Table 2.

The extract of the root bark of *Bersama abyssinica* (Melianthaceae) showed activity against *Bac. subtilis* at 100 μg/ml, but the extract from the leaves of the same plant did not exhibit any activity even at 500 μg/ml. Four antibacterial bufadienolide steroids, abyssinin [**1**] and abyssinols **2**–**4** (7) were found in the root bark but not in the leaves. By contrast, the extract of the leaves of *Trema guineensis* (Ulmaceae) showed activity against *Bac. subtilis* but the bark did not.

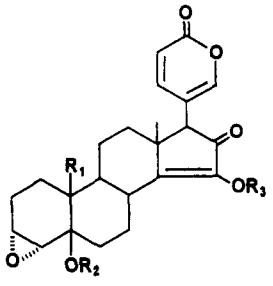
The extracts of the root-bark of *Erythrina abyssinica* (Leguminosae) exhibited a broad spectrum of antimicrobial activity at 100 μg/ml, but those of *Erythrina excelsa* did not show any activity at 500 μg/ml. Interestingly, Bwana Mganga uses *Er. abyssinica* as a "dawa ya miti" (Swahili or similiar terminology meaning medicinal plant) and it was also

TABLE 2. Antimicrobial Activity of Crude Extracts of East African Plants.

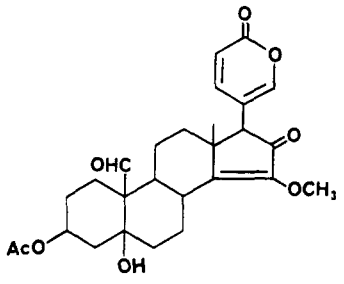
Screening[a]	Plant		Microorganism tested[b]			
	Species	Part	Ec	Bs	Sc	Pc
I.....	*Bersama abyssinica*	Root bark	−	+	−	−
II	*Bersama abyssinica*	Leaf	−	−	−	−
I.....	*Trema guineensis*	Leaf	−	+	−	−
II	*Trema guineensis*	Bark	−	−	−	−
I.....	*Erythrina abyssinica*	Root bark	−	+	+	±
II	*Erythrina excelsa*	Root bark	−	−	−	−
I.....	*Fagara chalybea*	Bark	−	±	+	+
I.....	*Fagara holtziana*	Bark	−	±	+	+
II	*Fagara macrophylla*	Bark	−	±	−	−
I.....	*Teclea trichocarpa*	Root bark	−	+	−	−
II	*Teclea nobilis*	Bark	−	−	−	−
I.....	*Croton macrostachyus*	Bark	−	+	−	−
II	*Croton sylvaticus*	Bark	−	−	−	−
I.....	*Indigofera paniculata*	Root bark	−	+	−	−
II	*Indigofera africeps*	Root bark	−	−	−	−
II	*Indigofera circinella*	Root bark	−	−	−	−

[a]I, Previous experiment; II, Current experiment.

[b]Ec, *Escherichia coli*; Bs, *Bacillus subtilis*; Sc, *Saccharomyces cerevisiae*; Pc, *Penicillium crustosum.* −, No effect; ±, Partial growth inhibition; +, Complete growth inhibition.



1 R_1=CHO, R_2=Ac, R_3=Me
2 R_1=CHO, R_2=H, R_3=Me
3 R_1=CH_2OH, R_2=H, R_3=OH



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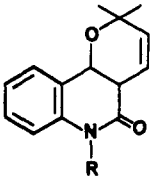
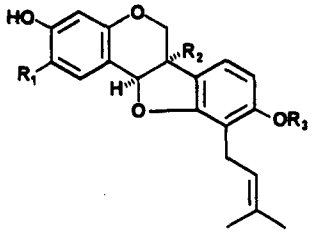
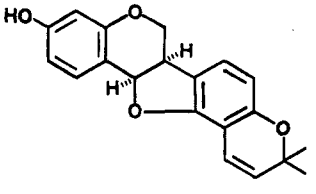


5 R=Me
6 R=H



7 R_1=H, R_2=OH, R_3=Me
8 R_1=CH_2CH=CMe_2, R_2=H, R_3=H
10 R_1=H, R_2=H, R_3=H



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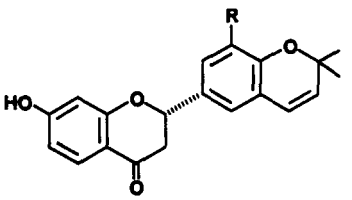
listed as a medicinal plant in the Kokwaro book (3), while *Er. excelsa* was not. The active principles in *Er. abyssinica* are described below in detail. Similarly, the extracts of the barks of *Fagara chalybea* and *Fagara holtziana* (Rutaceae) and *Croton macrostachyus* (Euphorbiaceae) showed activity, but those of *Fagara macrophylla, Croton sylvaticus,* and *Croton megalocarpus* did not. In the case of the *Fagara* trees, the antimicrobial alkaloids *N*-methylflindersine [**5**] and flindersine [**6**] were identified in *F. chalybea* and *F. holtziana*, but not in *F. macrophylla* (8). The extracts of the root bark of *Teclea trichocarpa* (Rutaceae) and *Indigofera paniculata* (Leguminosae) exhibited activity against *Bac. subtilis*, but *Teclea nobilis, Indigofera africeps,* and *Indigofera circinella* did not.

In conclusion, the information provided by Bwana Mganga through his practices with humans proved to be useful. Among the plants (1) collected based on his information, *Er. abyssinica, Warburgia ugandensis,* and *Warburgia stuhlmannii* (Canellaceae) were studied in greater detail, since these extracts exhibited a broad antimicrobial spectrum (1).

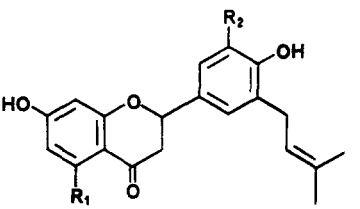
The root bark of *Er. abyssinica* is widely used in East Africa in various folk remedies for ailments such as malaria and syphilis (3). Bioassay-guided fractionation using *Bac.*

subtilis led to the isolation of two new pterocarpans, erythrabyssins I [**7**] and II [**8**]; five new flavonones, abyssinones I [**9**], II [**10**], III [**11**], IV [**12**], and V [**13**]; and a new chalcone, abyssinone VI [**14**], in addition to two known pterocarpans, phaseollin [**15**] and phaseollidin [**16**] (9). The antimicrobial activity of the purified compounds is shown in Table 3. Although the activity of each compound is moderate, the total quantity of the active compounds **7–10**, **12**, **13**, **15**, **16** in the root bark of *Er. abyssinica* is actually quite large (about 1% dry wt). They may, therefore, play an important role in the plant's defense, especially against soil microbial attack in the living plant.

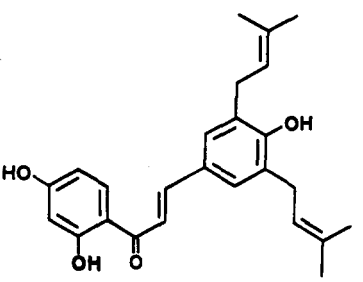
The genus *Warburgia* consists of two species, *W. ugandensis* and *W. stuhlmannii*, the barks of which are widely used in folk medicine and food spices in East Africa (3,4).



11 R=H
13 R=$CH_2CH=CMe_2$



12 $R_1=R_2=H$
14 $R_1=H$, $R_2=CH_2CH=CMe_2$
15 $R_1=OH$, $R_2=CH_2CH=CMe_2$



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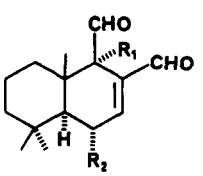
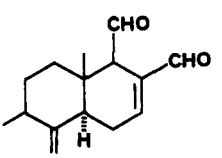


17 $R_1=OH$, $R_2=H$
19 $R_1=R_2=H$
20 $R_1=OH$, $R_2=OH$



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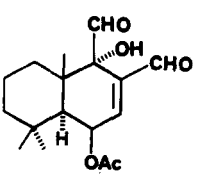

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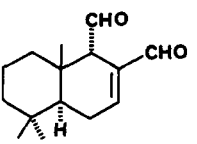

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TABLE 3. Antimicrobial Activity (MIC, μg/ml) of the *Erythrina abyssinica* Compounds.

Microorganism tested	Compound									
	7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Staphylococcus aureus	12.5	3.13	25	50	>100	25	50	>100	12.5	50
Bacillus subtilis	6.25	3.13	25	50	>100	12.5	25	>100	6.25	25
Escherichia coli	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Saccharomyces cerevisiae	50	>100	100	100	>100	>100	>100	>100	25	>100
Candida utilis	50	>100	100	100	>100	>100	>100	>100	50	>100
Mucor mucedo	25	>100	50	50	>100	>100	>100	>100	12.5	100
Penicillium crustosum	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

Preliminary tests indicated that the bark extract possessed a broad spectrum of antimicrobial activity. The barks were originally extracted with aqueous MeOH. However, MeOH inactivated, in part if not all, the active dialdehyde compounds through acetal (or hemiacetal) formation. Therefore, the use of alcohols was avoided throughout the isolation procedure. Bioassay-directed fractionation of the *n*-hexane extract of *W. ugandensis* led to the isolation of two new active sesquiterpene dialdehydes, warburganal [**17**] and muzigadial [**18**] (10) in addition to a known congener, polygodial [**19**] (11,12). Interestingly, these hot-tasting sesquiterpenoids were identical to potent African armyworm antifeedants from the same source (13–16). In addition, three additional sesquiterpene dialdehydes, mukaadial [**20**] (17), ugandensidial [**21**] (18,19), and epipolygodial [**22**] (14,15), were isolated from the active fraction. However, none of the latter sesquiterpenoids exhibited any antimicrobial activity up to 100 μg/ml. Nevertheless, all of the purified sesquiterpenes were tested against fifteen additional microorganisms. The results are shown in Table 4. Warburganal, muzigadial, and polygodial exhibited broad antimicrobial activity against all yeasts and molds tested. In particular, they were highly active against *Sacch. cerevisiae, Candida utilis,* and *Sclerotinia libertiana*. Among these three sesquiterpene dialdehydes, polygodial exhibited the most potent activity. Its activity against these microorganisms is comparable to that of amphotericin B and may be potent enough to be considered for practical application. In addition, the same antimicrobial sesquiterpene dialdehydes were isolated from the bark of *W. stuhlmannii*. Some of the results have already been reported (20–22).

A large number of phytochemicals have already been isolated as antimicrobial agents (23,24). However, their activity is usually not potent enough to be considered for practical application, even though each of the antimicrobial phytochemicals may play an important role in the defense against microbial attacks in living plants. This is always a dilemma when the antimicrobial activities of phytochemicals are considered. Hence, studies to enhance their biological activity are needed. This strategy seems to be a most promising approach for efficient utilization of renewable natural resources. Therefore, an attempt to enhance antimicrobial activity of some of the purified antimicrobial agents was made. As a model experiment, polygodial was combined with several antibiotics such as actinomycin D and rifampicin. This was done in order to enhance polygodial

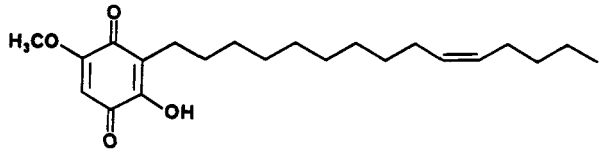
TABLE 4. Antimicrobial Activity (MIC, μg/ml) of the *Warburgia ugandensis* Compounds.

Microorganism tested	Compound					
	17	**18**	**19**	**20**	**21**	**22**
Staphylococcus aureus	>100	>100	>100	>100	>100	>100
Bacillus subtilis	>100	>100	>100	>100	>100	>100
Micrococcus luteus	>100	>100	>100	>100	>100	>100
Escherichia coli	>100	>100	>100	>100	>100	>100
Proteus vulgaris	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa	>100	>100	>100	>100	>100	>100
Saccharomyces cerevisiae	3.13	1.56	0.78	>100	>100	>100
Schizosaccharomyces pombe	12.5	25	6.25	>100	—[a]	>100
Hansenula anomala	12.5	25	1.56	>100	—[a]	>100
Candida utilis	3.13	3.13	1.56	>100	>100	>100
Sclerotinia libertiana	3.13	3.13	1.56	100	>100	>100
Mucor mucedo	25	25	6.25	>100	>100	>100
Rhizopus chinensis	100	100	12.5	>100	>100	>100
Aspergillus niger	50	50	25	>100	>100	>100
Penicillium crustosum	50	50	25	>100	>100	>100

[a]Not tested.

activity. As a result, polygodial significantly enhanced the antifungal activity of these antibiotics but not vice versa (25). Polygodial also synergized the antifungal activity of a benzoquinone, maesanin [**23**], against *Ca. utilis* (26). Maesanin was isolated from the fruit of *Maesa lanceolata* (Myrsinaceae) (27). The reason for these combination effects seems to be based on a polygodial-induced increase in the permeability of the plasma membrane to antibiotics (25,26). Furthermore, several additional combinations of polygodial and warburganal with other phytochemicals have been recently reported (28–30).

The use of antimicrobial compounds in combination may, in addition to enhancing and broadening the total biological activity, also hinder the development of resistant mechanisms in microorganisms (31).



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EXPERIMENTAL

PLANT MATERIALS.—The plant materials for screening were collected from different regions of East Africa, mainly in Kenya and Tanzania, and identified by Dr. J.B. Gillet and his assistants at the East African Herbarium, Nairobi, Kenya.

EXTRACTION PROCEDURE.—Botanically identified plant materials (100–500 g), for the most part air-dried, were extracted with 80% aqueous MeOH at ambient temperature. The solvent was removed in vacuo below 40° to give the crude extracts.

ANTIMICROBIAL ACTIVITY SCREENING.—The extracts were tested at a concentration of 500 μg/ml against *Bac subtilis* ATCC 6633, *Es. coli* IFO 3545, *Sacch. cerevisiae* IFO 0203, and *Pen. crustosum* Thom (32,33). *Bac. subtilis* and *Es. coli* were cultured in a peptone medium at 37°, and *Sacch. cerevisiae* and *Pen. crustosum* in wort at 25°. After 2 days, their growth was examined with the naked eye.

MINIMUM INHIBITORY CONCENTRATION (MIC).—The purified active principles from *W. ugandensis* and *Er. abyssinica* were also tested against eleven additional microorganisms, *Staphylococcus aureus* NCTC 8530, *Micrococcus luteus* IFO 3333, *Proteus vulgaris* IAM 12003, *Pseudomonas aeruginosa* IAM 1007, *Schizosaccharomyces pombe* IFO 0342, *Hansenula anomala* IFO 0136, *Ca. utilis* ATCC 42402, *S. libertiana* Ss, *Mucor mucedo* IFO 7684, *Rhizopus chinensis* IFO 4772, and *Aspergillus niger* ATCC 6275. The MIC was measured by the two-fold serial broth dilution method (34,35). The lowest concentration of the test compounds in which no growth occurred was defined as the MIC.

CHEMICALS.—The compounds for further study with the additional microorganisms were obtained as follows. Erythrabyssins I [**7**] and II [**8**], abyssinones I [**9**], II [**10**], III [**11**], IV [**12**], V [**13**], and VI [**14**], phaseollin [**15**] and phaseollidin [**16**] were from our previous work (9). Warburganal [**17**], muzigadial [**18**], mukaadial [**20**], ugandensidial [**21**], and epipolygodial [**22**] were also from our previous work (10,13). Polygodial [**19**] was isolated from the seeds and fresh sprouts of *Polygonum hydropiper* (Polygonaceae) by repeated cc over Si gel using a solvent gradient with *n*-hexane increasing the amount of EtOAc.

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