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Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity

W.M. Koné[a,b,*], K. Kamanzi Atindehou[a,b], C. Terreaux[c], K. Hostettmann[c], D. Traoré[a], M. Dosso[d]

[a] *Laboratoire de Botanique, U.F.R. Biosciences, Université de Cocody, 22 B.P. 582 Abidjan, Côte-d'Ivoire*
[b] *Centre Suisse de Recherches Scientifiques (CSRS), 01 B.P. 1303 Abidjan 01, Côte-d'Ivoire*
[c] *Institut de Pharmacognosie et de Phytochimie, Université de Lausanne, BEP, CH-1015, Lausanne, Suisse, Switzerland*
[d] *Laboratoire de Bactériologie-Virologie, Institut Pasteur d'Abidjan, 01 B.P. 490 Abidjan 01, Côte-d'Ivoire*

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Abstract

Sixty-seven crude ethanol extracts from 50 plants (31 families), which are used in North Côte-d'Ivoire as traditional remedies for bacterial diseases, were screened for in vitro activity against Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive (*Staphyloccocus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus subtilis*) bacteria. Thirty-one extracts showed antibacterial activity only on Gram positive bacteria. Of these, 10 extracts from 10 plant species had a promising level of activity against bacteria including strains resistant to antibiotics such as aminosides, penicillin M, macrolides, lincosamide and streptrogramin B. The most active was *Erythrina senegalensis* DC (Fabaceae) followed by *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr. & Wiersema (Caesalpinaceae), *Waltheria lanceolata* R. Br. ex Mast. (Sterculiaceae), *Uapaca togoensis* Pax. (Euphorbiaceae), *Ximenia americana* L. (Olacaceae), *Khaya senegalensis* (Ders.) A. Juss. (Meliaceae), *Lannea acida* A. Rich. (Anacardiaceae), *Cissus populnea* Guill. & Perr. (Vitaceae), *Keetia hispida* (Benth.) Bridson (Rubiaceae) and *Ficus thonningii* (Miq.) A. Rich. (Moraceae). This is the first report of the antibacterial potency of these 10 plant species on a range of bacteria.

The results provided evidence that some of the studied plants might indeed be potential sources of new antibacterial agents, also against some antibiotic-resistant strains.

Keywords: Antibacterial activity; Medicinal plants; Ferkessedougou; Côte-d'Ivoire

1. Introduction

Despite the existence of a wide variety of antibacterial agents, the search for new ones is of great importance. Bacterial infections are the cause of a large burden of diseases and bacteria are listed in the first position among the common microorganisms responsible for opportunistic diseases occurring associated with AIDS. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics (Keasah et al., 1998; Marimoto and Fujimoto, 1999).

Many people still use traditional herbs to treat a variety of diseases including bacterial infections. According to Pousset (1994), more than 70% of the people in West African countries still use traditional medicine. The use of herbal remedies is likely to be particularly important in places where modern medicines are too expensive for the local population. Thus, a large variety of traditional medicines is often found in remote areas. One such area is the Ferkessedougou region, in the north of Côte-d'Ivoire where people have low incomes, mostly for subsistence. At first, an ethnobotanical survey was carried out in this region in 1998 to collect information on cures used in traditional medicine (Koné et al., 2002).

Plants are among the most important common sources of potentially valuable new drugs. There is, therefore, an urgent need to investigate the biological properties of additional medicinal plants in order to develop new drugs. In Côte-d'Ivoire, a lot of work had been carried out on the herbal treatment of various diseases (Adjanohoun and Aké

* Corresponding author. Tel.: +1-225-23-45-52-58; fax: +1-225-23-45-12-11.
E-mail address: mamidou.kone@csrs.ci (W.M. Koné).

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Assi, 1979; Vangah-Manda, 1986; N'guéssan, 1995). However, only a few reports exist on the antibacterial activity of plants from the Ivorian flora (Agbassi, 1989; Bouboutou, 1991; Weiss et al., 1996).

The present paper concentrates on 50 of the 97 plants collected in a 1998 survey, most of which were reported by healers of the Ferkessedougou region to have a curative effect on bacterial diseases such as urinary infections, respiratory diseases, boils, etc.

2. Materials and methods

2.1. Selection of plants

Plants were selected after an ethnobotanical survey carried out in the Ferkessedougou region (Koné et al., 2002). This area is located in the North of Côte-d'Ivoire about 585 km from Abidjan. The uses of plants selected in traditional medicine are shown in Table 1. These plants were used alone by healers, rather than in mixtures, for the treatment of diseases including bacterial infections.

The identification of species was performed by the Ivorian botanist Téré Henri in the "Centre Suisse de Recherches Scientifiques" (CSRS) in Adiopodoumé (Côte-d'Ivoire). Voucher specimens are preserved in the herbarium of CSRS.

2.2. Plant material extraction

Leaves, stem barks and roots were dried at ambient temperature after collection. For laboratory analysis, they were dried in an air-conditioned room (18 °C) and then pounded by hand. Crude plant extracts were prepared for in vitro screening by extracting 25 g of powdered plant material with 250 ml of ethanol 90%, with mechanical stirring, during 12 h at room temperature (25 °C). The ethanol was completely evaporated at 40 °C. The extract was lyophilized and stored at 4 °C until use.

2.3. Antibacterial screening

The biological assays were carried out on 39 reference cultures and hospital isolates, provided by the Virological and Bacteriological Laboratory of the "Institut Pasteur de Côte-d'Ivoire". The bacteria included Gram positive strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Streptococcus pyogenes*) and Gram negative ones (*Escherichia coli* and *Pseudomonas aeruginosa*). Some strains were sensitive and some were resistant to antibiotics (penicillin M, tetracycline, aminoside, macrolide, lincosamide and streptrogramine B). The details are in Table 2.

The lyophilized plant extracts were solubilized in DMSO (30 mg/ml) and serially diluted in Mueller-Hinton medium, from 1500 to 3 μg/ml.

Two methods were used to assess the antibacterial activity: a diffusion method on agar plates (Sanofi-Diagnostics-Pasteur, 1993; Dosso and Faye Kette, 1995) and microdilution in liquid medium (NCCLS, 1990; Dosso and Faye Kette, 1995). First, susceptibility of all the bacteria to crude extracts was determined by using the diffusion method. Agar plates were seeded with bacterial cultures from the reference collection and one sensitive strain of each bacteria species (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*). Paper disks soaked in one of four dilutions of each extract were placed on the prepared plates. After 18 h, the inhibitory diameters were measured. Tests were repeated three times with all the extracts that showed some inhibitory effect. The active extracts at this level were tested on resistant strains.

The extracts showing an inhibitory diameter of at least 10 mm were selected for quantitative assessment of inhibitory (IC_{100}) and bactericidal (BC) concentrations in liquid medium. The IC_{100} was defined as the lowest concentration of crude plant extract at which the visible growth of a strain was completely inhibited (no turbidity in wells). We considered as IC_{50} the minimum IC_{100} at which 50% of all the tested strains were inhibited.

All the tested bacteria were used with an initial *inoculum* of 5×10^6 bacteria/ml. The BC was the lowest concentration of crude extract in which less than 0.01% of the initial *inoculum* survived after 18–24 h. Medium from wells with no visible growth and from the initial *inoculum* were plated on agar, and colonies counted.

The value BC/IC_{100} determined whether an extract was bactericidal ($BC/IC_{100} < 4$) or bacteriostatic ($BC/IC_{100} > 4$).

Tetracycline (Sigma) and gentamicin (Sigma) were used as controls.

3. Results

Of 67 extracts tested, 31 (46.27%) exhibited some activity against Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Streptococcus pyogenes*). However, only 10 extracts showed a considerable antibacterial activity, with IC_{50} values ranging between 12 and 94 μg/ml, against at least one of the bacteria species. These 10 extracts derived from 10 plants belonging to 10 families.

Only six extracts were bactericidal on *Staphylococcus aureus* strains, nine of which were Methicillin resistant *Staphylococcus aureus* (MRSA) and strain MLS_B/R. The most active extracts were *Erythrina senegalensis*, *Bobgunnia madagascariensis* and *Waltheria lanceolata* (Table 2).

Eight extracts were bactericidal and two bacteriostatic on *Enterococcus faecalis* strains, eight of which were resistant to aminoside; the most active extracts came from *Erythrina senegalensis* followed by *Bobgunnia*

Table 1
Traditional uses of medicinal plants from the Ferkessédougou region selected for antibacterial screening

Plant species (family)	Plant parts used	Indications
Acacia polyacantha Willd. (Forssk.) Willd. (Mimosaceae)	Stem bark	Cough
Alternanthera pungens H. B. & K. (Amaranthaceae)	Whole plant	Diarrhoea, mouth wounds, eyes infections
Ampelocissus africana (Lour.) Merrill. (Vitaceae)	Roots	Constipation
Andira inermis (Wright) DC. (Fabaceae)	Leaves	Cough, respiratory diseases
Annona senegalensis Pers. (Annonaceae)	Roots	Diarrhoea, boils
Apodostigma pallens (Planch. ex Oliv.) Wilczek (Hyppocrateaceae)	Leaves	Gonorrhoea
Asparagus africanus Lam. (Asparagaceae)	Whole plant	Respiratory diseases, wounds
Baissea multiflora A. DC. (Apocynaceae)	Roots	Dry cough, gonorrhoea
Bobgunnia madagascariensis (Desv.) J. H. Kirkbr. & Wiersema (Caesalpinaceae)	Roots	Malaria
Cissus populnea Guill. & Perr. (Vitaceae)	Roots	Infected wounds, boils
Cochlospermum planchoni Hook. f. ex Planch. (Cochlospermaceae)	Roots	Diarrhoea, malaria
Combretum molle R. Br. Ex G. Don. (Combretaceae)	Leaves	Respiratory diseases, wounds
Combretum racemosum P. Beauv. (Combretaceae)	Roots	Boils, wounds
Cussonia arborea Hochst. ex A. Rich. (Araliaceae)	Leaves	Cough, respiratory diseases
Cyperus articulatus L. (Cyperaceae)	Rhizome	Diarrhoea, vomiting
Dichrostachys cinerea (L.) Wight & Arn. (Mimosaceae)	Leaves	Toothache
Entada abyssinica Steud. ex A. Rich. (Mimosaceae)	Stem bark	Mouth wounds, malaria
Entada africana Guill. & Perr. (Mimosaceae)	Roots	Respiratory diseases
Erythrina senegalensis DC. (Fabaceae)	Stem bark, roots	Dry cough, gonorrhoea, stomach ache
Euphorbia hirta L. (Euphorbiaceae)	Whole plant	Dysentery, gonorrhoea, sore throat
Evolvulus alsinoides L. (Convolvulaceae)	Whole plant	Boils
Ficus vallis-choudae Del. (Moraceae)	Stem bark	Heart problems
Ficus thonnigii (Miq.) A. Rich. (Moraceae)	Leaves	Rheumatism
Hibiscus asper Hook. f. (Malvaceae)	Leaves	Toothache, eye infections
Hymenocardia acida Tul. (Hymenocardiaceae)	Roots	Diarrhoea, abdominal pain
Keetia hispida (Benth.) Bridson (Rubiaceae)	Leaves	Respiratory diseases
Khaya senegalensis (Ders.) A. Juss. (Meliaceae)	Stem bark	External and internal wounds, diarrhoea, dysentery
Kigelia africana (Lam.) Benth. (Bignoniaceae)	Roots	Wounds, abdominal pain
Landolphia owariensis P. Beauv. (Apocynaceae)	Leaves	Diarrhoea
Lannea acida A. Rich. (Anacardiaceae)	Stem bark, roots	Diarrhoea, stomach-ache, gonorrhoea, rheumatism
Maranthes floribunda (Bak.) White (Chrysobalanaceae)	Stem bark	Diarrhoea, dysentery
Maytenus senegalensis (Lam.) Exell (Celastraceae)	Leaves	Diarrhoea, malaria
Morinda lucida Benth. (Rubiaceae)	Roots	Stomach ache, conjunctivitis
Olax subscorpioidea Oliv. (Olacaceae)	Roots	Intestinal worms
Opilia amentacea Roxb. (Opiliaceae)	Leaves	Intestinal worms
Phyllanthus muellerianus (O. Ktze) Exell (Euphorbiaceae)	Leaves	Respiratory diseases, malaria
Premna lucens A. Chev. (Verbenaceae)	Roots	Stomach ache, wounds
Pseudarthria hookeri Wight & Arn. (Fabaceae)	Leaves	Cough
Pseudocedrela kotschyi (Schweinf.) Harms (Meliaceae)	Roots	Internal wounds, toothache
Saba senegalensis var. *glubriflora* (Hua) Pichon (Apocynaceae)	Roots	Diarrhoea
Sansevieria forskaliana (Schult. f.) Hepper & Wood (Agavaceae)	Rhizome	Gonorrhoea
Securidaca longepedunculata Fres. (Polygalaceae)	Roots	Wounds, infected eruption, intestinal worms
Spermacoce verticillata L. (Rubiaceae)	Leaves	Gonorrhoea, abdominal pain, fever
Strychnos spinosa Lam. (Loganiaceae)	Roots	Cough, gonorrhoea, malaria
Strychnos innocua Del. (Loganiaceae)	Leaves	Infected eruption
Stylosanthes erecta P. Beauv. (Fabaceae)	Whole plant	Dermatitis
Terminalia schimperiana Hochst. (Combretaceae)	Young leaves	Cough, respiratory diseases
Trichilia emetica Vahl. (Meliaceae)	Roots	Abdominal pain
Uapaca togoensis Pax. (Euphorbiaceae)	Leaves	Vomiting, rheumatism, fever
Waltheria lanceolata R. Br. ex Mast. (Sterculiaceae)	Roots	Diarrhoea, vomiting, cough
Ximenia americana L. (Olacaceae)	Roots	Mouth wounds, rheumatism, diarrhoea

madagascariensis, *Waltheria lanceolata*, *Ximenia americana*, *Uapaca togoensis* and *Khaya senegalensis* (Table 2).

Of 10 active extracts against *Streptococcus pyogenes*, 8 were bactericidal and 2 bacteriostatic. The most active were *Erythrina senegalensis*, *Bobgunnia madagascariensis*, *Waltheria lanceolata*, *Cissus populnea*, *Lannea acida*, *Ximenia americana*, *Uapaca togoensis* and *Khaya senegalensis* (Table 2).

One strain of *Bacillus subtilis* was sensitive to five extracts (three bactericidal and two bacteriostatic). The most active ones derived from *Erythrina senegalensis*, *Bobgunnia madagascariensis* and *Uapaca togoensis* (Table 2).

No extract was active against Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) on agar plates. Therefore, the microdilution method was not applied.

Table 2
Values of inhibitory concentrations (μg/ml) of active plant species and antibiotics

Plant species	Tested part	ATCC strain	Hospital strains of *Staphylococcus aureus* ($n = 12$)													
		ATCC	Methicillin resistant *Staphylococcus aureus* (MRSA)									Sensitive	MLSB/R			
		29213	1167	3073	4698	4699	4942	17232	542	18417	Trazié	802	421			
		IC$_{100}$												IC$_{50}$	IC$_{100}$m	Range
Erythrina senegalensis	Roots	**23**	**12**	**47**	**12**	**12**	**12**	**23**	**12**	**12**	**12**	**23**	**47**	**12**	20.6	47–12
Bobgunnia madagascariensis	Roots	**94**	**94**	**47**	**47**	**23**	**23**	**23**	**94**	**23**	**23**	**47**	**94**	**47**	52.7	94–47
Waltheria lanceolata	Roots	**94**	**94**	188	**94**	188	**47**	**94**	**94**	188	**94**	**94**	188	**94**	121.4	188–47
Cissus populnea	Roots	188	188	188	375	**94**	188	375	>1500	188	375	188	188	188	230.5	375–94
Lannea acida	Roots	188	1500	188	188	**94**	1500	188	>1500	188	1500	188	188	188	537.3	1500–94
Ximenia americana	Roots	188	1500	375	375	1500	1500	1500	>1500	188	1500	188	188	375	818.4	1500–188
Gentamicin		0.2	50	25	>50	>50	25	50	0.4	0.8	>50	1.6	>50	25	19.1	>50–0.2
Tetracycline		0.8	>50	50	>50	>50	50	>50	0.8	0.8	0.8	3.125	25	25	16.4	>50–0.8

Plant species	Tested part	ATCC strain	Hospital strains of *Enterococcus faecalis* ($n = 10$)										
		ATCC	Aminoside resistant strains							Sensitive			
		29212	16/9	166	1104	1194	2503	4673	10706	Madou	4922			
		IC$_{100}$										IC$_{50}$	IC$_{100}$m	Range
Erythrina senegalensis	Roots	**12**	**23**	**12**	**12**	**23**	**23**	**12**	>1500	**12**	**23**	**12**	16.9	23–12
Bobgunnia madagascariensis	Roots	**94**	188	**94**	**12**	**47**	**47**	**23**	>1500	**6**	**94**	**47**	67.2	188–6
Waltheria lanceolata	Roots	**94**	**94**	**94**	**47**	**47**	**47**	**47**	**94**	188	**94**	**94**	84.6	188–47
Cissus populne	Roots	188	188	**94**	**94**	**94**	188	188	188	**94**	188	188	150	188–94
Lannea acida	Roots	188	188	188	**94**	188	**94**	188	375	**23**	188	188	171	375–94
Ximenia americana	Roots	188	**94**	**94**	**94**	188	**94**	188	375	**47**	188	**94**	155	375–94
Uapaca togoensis	Leaves	**94**	**94**	**94**	**94**	**94**	188	**94**	375	**23**	**94**	**94**	124	375–23
Khaya senegalensis	Stem bark	188	**94**	**94**	**94**	**94**	188	**94**	375	375	188	**94**	178	750–94
Keetia hispida	Leaves	**94**	188	188	**94**	188	188	**94**	375	**47**	188	188	164	375–47
Ficus thonningii	Leaves	188	375	375	188	375	750	**94**	750	375	188	375	431	750–188
Gentamicin		12.5	25	25	25	12.5	>50	25	25	>50	50	25	25	>50–12.5
Tetracycline		25	0.4	>50	>50	50	>50	>50	>50	>50	3.125	>50	19.6	>50–0.4

Table 2 (*Continued*)

Plant species	Tested part	Hospital strains of *Streptococcus* (n = 4)							Hospital strains of *Bacillus subtilis*
		3476	15876	Group A	Beta-hemolytic				Sensitive
		IC$_{100}$				IC$_{50}$	IC$_{100}$m	Range	IC$_{100}$
Erythrina senegalensis	Roots	**12**	**23**	**6**	**6**	**12**	11.7	12–6	**12**
Bobgunnia madagascariensis	Roots	**94**	188	**47**	**23**	**94**	88	188–23	**23**
Waltheria lanceolata	Roots	**94**	**94**	**23**	**23**	**94**	58.5	94–23	188
Cissus populnea	Roots	188	**94**	**23**	**47**	**94**	88	188–23	>1500
Lannea acida	Roots	**94**	**94**	**23**	**47**	**94**	64.5	94–23	188
Ximenia americana	Roots	**94**	**94**	**6**	**47**	**94**	54.3	94–6	>1500
Uapaca togoensis	Leaves	**94**	**94**	**6**	**23**	**94**	54.3	94–6	**23**
Khaya senegalensis	Stem bark	**94**	**94**	**6**	**23**	**94**	54.3	94–6	>1500
Keetia hispida	Leaves	188	188	**23**	**47**	188	111.5	188–23	>1500
Ficus thonningii	Leaves	375	375	**23**	**47**	375	205	375–23	>1500
Gentamicin		50	>50	**6.25**	**0.8**	50	19	>50–0.8	**0.8**
Tetracycline		50	>50	50	**1.6**	50	33.9	>50–1.6	>50

Bold type: values of IC$_{100}$ or IC$_{50}$ = 94 or lower. R = resistant; MLSB = Macrolide, lincosamide, streptrogramine B; IC$_{100}$m = average.

4. Discussion

In the present investigation, 31 of 67 crude extracts, obtained from 50 plant species from the flora of Côte-d'Ivoire were identified as having some in vitro antibacterial activity. Many of these plants are used locally for the treatment of various bacterial diseases. These results are in agreement with the statements of healers on the traditional uses of the plant species tested. The most active extracts were those from *Erythrina senegalensis*, *Bobgunnia madagascariensis*, *Waltheria lanceolata*, *Cissus populnea*, *Lannea acida*, *Xemenia americana*, *Uapaca togoensis*, *Khaya senegalensis*, *Keetia hispida*, *Ficus thonnongii*. To our knowledge, this is the first report on the antibacterial potency of crude extracts of these plants on a range of bacteria.

An IC_{50} value of 94 μg/ml or lower was considered a reasonable cut-off point for crude extracts. Thus plants with an interesting activity are those showing an inhibitory concentration below this limit.

Of the plants tested, *Erythrina senegalensis* contained very active bactericides with IC_{50} values of 12 μg/ml against all the Gram positive bacteria studied, some of which were methicillin resistant (MRSA) and MLS_B resistant. *Erythrina senegalensis* has been chemically investigated (Taylor et al., 1986; Wandji et al., 1990) and was found to contain pterocarpans, isoflavonoids and terpenes which could contribute at least in part to the antibacterial activity. Antimicrobial pterocarpans have been isolated from many other species of *Erythrina* such as *Erythrina mildbraedii* (Lester et al., 1988).

Some other extracts also showed a promising activity. For example, *Bobgunnia madagascariensis* showed a low IC_{50} of 23–47 μg/ml against some MRSA strains, MLS_B *Staphylococcus aureus* and *Enterococcus faecalis* strains resistant to aminoside. This plant species has already been shown to have antifungal (Rahalison, 1994; Schaller et al., 2001), molluscicidal (Sarda et al., 1986; Borel and Hostettmann, 1987) and antimalarial (Minjas and Sarda, 1986) activities. But there are no previous reports of its antibacterial properties.

Waltheria lanceolata gave IC_{50} values of 94 μg/ml on seven MRSA strains and six *Enterococcus faecalis* strains resistant to aminoside. *Ximenia americana*, *Uapaca togoensis* and *Khaya senegalensis* were active with an IC_{50} of 94 μg/ml only on *Enterococcus faecalis* and *Streptococcus pyogenes*; some strains were resistant to aminoside. No literature reports of the antibacterial activity of these four plants were found.

This study demonstrates that many of the 50 plants used by traditional healers in the Ferkessedougou area against bacterial diseases showed antibacterial activity when tested in the laboratory. This was the first study for most of these plants. Our results are a contribution to a better knowledge of medicinal plants of Côte-d'Ivoire, and indicate that some may become a source of new antibacterial agents. Phytochemical investigations of the most active plants are planned, to identify and characterize the antibacterial active principles, and assess toxicity by laboratory assays.

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