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EVALUATION OF 17 MEDICINAL PLANTS FROM NORTHERN CÔTE D'IVOIRE FOR THEIR *IN VITRO* ACTIVITY AGAINST *STREPTOCOCCUS PNEUMONIAE*

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Abstract

Twenty crude extracts from 17 species out of 11 families were assessed for their antibacterial activity against *Streptococcus pneumoniae* (Pneumococcus*)*. The selected plants are used in Northern Côte d'Ivoire to treat various infections including respiratory track diseases. From all the tested extracts, only 7 from 6 plants showed a promising *in vitro* bactericidal activity against Peumococcus, including strains resistant to penicillin. The most active extracts were from *Erythrina senegalensis* (Fabaceae), *Piliostigma thonningii* (Caesalpiniaceae), *Waltheria indica* (Sterculiaceae), *Andira inermis* (Fabaceae), *Uapaca togoensis* (Euphorbiaceae), *Keetia hispida* (Rubiaceae) and *Combretum molle* (Combretaceae). This is the first time that the antipneumococcal activity of the tested plants is reported. The results of this preliminary investigation support the traditional use of these plants in the treatment of pneumococcal infections. The most active of them could be candidates for isolation of compounds which could serve as lead structures for the development of new drugs against *Streptococcus pneumoniae*.

Keywords : Antibacterial activity ; Côte d'Ivoire ; Medicinal plants ; *Pneumococcus* ; *Streptococcus pneumoniae.*

Introduction

Infections caused by *Streptococcus pneumoniae* continue to be a growing public health concern. In many African countries including Côte d'Ivoire, Pneumococcus was found to be responsible for extensive morbidity and mortality (Benbachir et al., 1998) in vulnerable persons icluding infants (Kacou-N'Douba et al., 2001 ; Campbell et al., 2004) and severe immunodeficient subjects such as AIDS patients (Attia et al., 2001 ; Anglaret et al., 2002 ; Zar, 2004).

In addition, the occurrence of resistant strains of *Pneumococcus* or reduced sensitivity to many antibiotics (Benbachir et al., 1998 ; Kacou-N'Douba et al., 2001) curbs effective control of diseases caused by *S. pneumoniae*. Also, antibiotics are relatively expensive for a large portion of the rural population, many of whom, even if they could afford them, live far from the health centres. Herbal therapy remains the only health care alternative for those populations.

Given the clinical impact of *Streptococcus pneumoniae*, it is important to find alternative treatments which are affordable to the local population. Traditional medicine in Africa is an important field for ethnopharmacological investigation.

The aim of this study was to determine between 17 traditional medicinal plants used in Northern Côte d'Ivoire, those with strong activity against the *Pneumococcus* for further investigation and possible production of standardized traditional medicine for the Ivorian health care system.

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Material and Methods

Plant materials

The 17 plant species were selected after ethnomedical surveys carried out in 2001 in the Ferkessedougou region, about 585 km from Abidjan, the economic capital. Most of these plants were reported by healers as used in the treatment of diseases such as pneumonia and other respiratory track infections, (unpublished data). The list of the plants is given in table 1. Plant species were identified by the ivorian botanist Henri Téré and were deposited in the herbarium of the Centre Suisse de Recherches Scientifiques (CSRS) in Adiopodoumé (Côte d'Ivoire).

Plant crude extracts

Plant samples were harvested at the start of the dry season (October 2003) around the town of Ferkessesougou (Northern Côte d'Ivoire). After collection, plant parts were left to dry in the shade. For laboratory analysis, they were dried in an air-conditioned room (18°C) and then crushed. Plant powders were extracted in a 10-fold excess of 90 % ethanol, by mechanical stirring, during 14 hours at room temperature (25°C). After complete elimination of the ethanol in a rotary evaporator at 40°C, the extracts in water solution were frozen, lyophilised and stored at 4°C until the *in vitro* screening.

Antibacterial test

Antibacterial assays were carried out on one reference culture ATCC 49619 and 11 hospital isolates of *Streptococcus pneumoniae*, Gram positive bacteria provided by the Department of Bacteriology-Virology of Institut Pasteur in Abidjan, Côte d'Ivoire. Some of the strains were sensitive to Penicillin while others were resistant to it.

Two different techniques were used to test the extracts: (1) a diffusion method on agar plate (Courvalin et al., 1985; Dosso et Faye Kette, 1995), (2) a liquid medium microdilution method (Dosso et Faye Kette, 1995). The sensitivity of the bacteria was determined with the diffusion method on plates of agar mixed with fresh blood (5%). Two agar plates were seeded, one with the culture of a reference collection strain and other with the culture of a hospital isolate one. Paper disks were soaked in one of four dilutions (1500, 750, 375 and 188 µg/ml) of each crude extract and placed on the prepared plates. These were incubated in a CO_2 atmosphere during 18 hours. The inhibitory (IC_{100}) and bactericidal concentrations (B.C.) were assessed by microdilution in liquid medium only for extracts that showed an inhibitory diameter of at least 10 mm. For the quantitative assessment, all the 12 strains were included. Crude extracts (1500 to 3 µg/ml) were serially diluted into Dynatech microplates and an *inoculum* (5.10^6 bacteria/ml) was added to wells. Microplates were incubated in a CO_2 atmosphere (5%), at 37°C during 18 hours.

The IC_{100} was defined as the lowest concentration of crude plant extract at which visible growth of a strain was completely inhibited (no turbidity in the wells) (Koné et al., 2004). The value BC/IC_{100} determined whether an extract was bactericidal ($BC/IC_{100} < 4$) or bacteriostatic ($BC/IC_{100} > 4$). Tests were repeated 3 times for each extract that showed some antipneumococcal activity.Tetracycline (Sigma) and Gentamicin (Sigma) were used as controls.

Results

Of the 17 plant species tested, except *Acacia polyacantha*, the other species were found to display activity against at least one tested strain of *Streptococcus pneumoniae*, some of which were resistant to penicillin. However, while considering an IC_{50} value of 94 µg/ml or lower as a reasonable cut-off point for crude extracts, 7 extracts from 6 plants had a promising level of bactericidal activity. These were from *Erythrina senegalensis* followed by *Piliostigma thonningii*, *Waltheria lanceolata*, *Andira inermis*, *Uapaca togoensis* and *Keetia hispida* (table 2).

Discussion

In this study, the activity of 20 crude ethanol extracts from 17 plants of Northern Côte d'Ivoire was evaluated *in vitro* against *Streptococcus pneumoniae*. Of these extracts, 19 exhibited activity against tested bacteria. This is the first report of the activity of these plants against Pneumococcus. The results demonstrated a link between the usage of some of those plants in traditional healing and their effective antipneumococcal activity. For example, active plants such as *Andira inermis*, *Combretum molle*, *Keetia hispida* and *Garcinia afzelii* were reported by healers to have a curative effect on pneumonia, an infection often caused by

Table 1: Plants from the Ferkessedougou region screened for their *in vitro* activity against *Streptococcus pneumoniae*.

Plant species	**Local names in Niarafolo**	**Plant parts used**	**Indications**	**Usage in folk medicine : preparation and administration**
Acacia polyacantha Willd. *subsp. campylacantha* (Hochst. ex A. Rich) Brenan (Mimosaceae)	Guhonrugô	Stem bark	Cough	Chew raw stem bark and swallow the juice, once a day
Alternanthera pungens Kunth (Amaranthaceae)	Kabélisaharga	Whole plant	Cough, fever, malaria, mouth ulcer	Decoction, for drink and bath, twice a day
Andira inermis (Wright) DC. (Fabaceae)	Luhôdjimin	Leaves	Pneumonia, fever	Decoction, drink and bath, twice a day
Asparagus africanus Lam. (Asparagaceae)	Tutcholo	Leaves	Pneumonia, cough,	Decoction in association with *Maytenus senegalensis* for drink and bath, twice a day
Combretum molle R. Br. ex G. Don (Combretaceae)	Kahadjaba	Leaves	Cough, fever, wounds	Decoction for drink and hot inhalation, twice a day
Cussonia arborea Hochst. ex A. Rich (Araliaceae)	Nadoli	Leaves	Cough in children, fever	Decoction in association with *Imperata cylindrica* and *Borreria verticillata* for drink, twice a day
Daniellia oliveri Hutch.et Dalz. (Caesalpiniaceae)	Siltigué	Leaves (young)	Cough in children, fever	Decoction in association with young leaves of *Piliostigma thonningii* for drink, twice a day
Entada abyssinica Steud. ex A. Rich. (Mimosaceae)	Yiriwaha	Stem bark/roots	Cough, fever, malaria, cold	Decoction, for drink, twice a day or chewed
Erythrina senegalensis DC. (Fabaceae)	Katchignien	Roots/ stem bark	Dry cough, fever, urinary infection, tuberculosis	Macerate of stem bark or decoction of roots for drink and bath, twice a day

Table 1 (Continued)

Plant species	**Local name in Niarafolo**	**Plant part used**	**Indications**	**Usage in folk medicine: preparation and administration**
Garcinia afzelii Engl. (Clusiaceae)	Nessole	Roots/leaves	Pneumonia, cough, fever	Decoction, for drink and bath, twice a day
Keetia hispida (Benth.) Bridson (Rubiaceae)	Yulnumonnin	Leaves	Pneumonia, fever, cough in children	Decoction, for drink, hot inhalation and bath, twice a day
Phyllanthus muellerianus (O. Ktze) Exell (Euphorbiaceae)	Pkukadala	Leaves	Pneumonia, fever, cough, malaria	Decoction, for drink, hot inhalation and bath, twice a day
Piliostigma thonningii (Schum.) Milne-redhead (Caesalpiniaceae)	Tchaman	Leaves (young)	Cough in children, wounds, jaundice, abdominal pain	Decoction, for drink and bath, twice a day Application of the paste on wounds
Pseudarthria hookeri Wight et Arn. (Fabaceae)	Tchéguenimin	Leaves	Cough	Burn to a cinder and suck three times a day
Terminalia schimperiana Hochst. (Combretaceae)	Sangopènin	Leaves (young)	Cough in children, fever, rheumatism	Decoction, for drink, hot inhalation and bath, twice a day
Uapaca togoensis Pax. (Euphorbiaceae)	Kassérigué	Leaves/ stem bark	Pneumonia, cough, fever, rheumatism, vomiting, epilepsy	Decoction, for drink, hot inhalation and bath, twice a day
Waltheria lanceolata R. Br. Ex Mast. (Sterculiaceae)	Wôfigué	Roots	Cough, diarrhoea, vomiting,	Decoction, for drink and bath, twice a day

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

Plant species	**Tested part**	**ATCC and hospital strains of *Streptococcus pneumoniae* (n = 12)**														
		ATCC	Sensitive strains							Penicillin-resistant strains						
		49619	24	615	624	1948	2474	6771	554	52	59	525	563			
		IC_{100}												**IC_{50}**	**IC_{100}m**	**Range of IC_{100}**
Erythrina senegalensis	Roots	**6 (2)**	**12(2)**	**12(2)**	**12(2)**	**12(2)**	**6(2)**	**12(2)**	**6(4)**	**6(2)**	>1500	**3(2)**	**23(4)**	**12(2)**	10	23-3
Piliostigma thonningii	Leaves (young)	**47(2)**	**23(2)**	**47(4)**	**47(2)**	**47(4)**	**94(2)**	**94(4)**	188	**47(4)**	**47(2)**	>1500	>1500	**47(2)**	68,1	47-23
Waltheria lanceolata	Roots	188	**47(2)**	**94(4)**	188	**47(2)**	**94(4)**	**47(4)**	**47(4)**	**47(4)**	>1500	**94(4)**	**23(2)**	**47(4)**	83,27	188-23
Uapaca togoensis	Stem bark	**94(4)**	**6(2)**	**94(4)**	188	**94(4)**	**94(4)**	188	375	**6(2)**	**94(4)**	>1500	>1500	**94(4)**	100	94-23
	Leaves	188	**94(4)**	**94(4)**	188	**94(4)**	188	188	188	**94(4)**	>1500	188	**23(2)**	**94(4)**	> 100	188-94
Andira inermis	Leaves	**47(2)**	**23(2)**	**94(4)**	**94(4)**	**94(4)**	188	**94(4)**	**94(4)**	**23(4)**	>1500	**94(4)**	**94(4)**	**94(4)**	> 100	188-94
Keetia hispida	Leaves	**94(2)**	**94(2)**	188	188	**94(4)**	188	188	**94(4)**	**94(4)**	>1500	**94(4)**	**94(4)**	**94(4)**	> 100	188-6
Garcinia afzelii	Leaves	**94(4)**	**94(2)**	**94(4)**	**94(4)**	**94(4)**	188	188	**94(4)**	**94(4)**	**94(4)**	>1500	>1500	> 100	> 100	188-94
	Roots	**94(2)**	**23(2)**	188	188	188	188	188	375	**23(2)**	188	>1500	>1500	> 100	> 100	375-23
Combretum molle	Leaves	**94(4)**	**6(2)**	188	188	188	375	188	**94(4)**	**6(4)**	>1500	**47(2)**	**47(2)**	> 100	> 100	375-6
Antibiotics																
Gentamicin		25	6,25	6,25	12,5	25	1,6	>50	50	>50	>50	50	50	12,5	15,6	>50-1,6
Tetracycline		1,6	6,25	3,215	6,25	>50	6,25	1,6	50	>50	>50	50	50	6,25	9,2	>50-1,6

Bold type : value of IC_{100} = 94 µg/ml or lower ; in brackets : value of BC/ IC_{100} ; IC_{100}m = average
All experiments were run in triplicate.

Streptococcus pneumoniae. Some other plants such as *Erythrina senegalensis*, *Waltheria lanceolata*, *Uapaca togoensis* and *Piliostigma thonningii* were said to be used traditionally in the treatment of respiratory diseases such as coughing. These 4 plants showed a strong activity against *Streptococcus pneumoniae*. The result is interesting as the traditional use of these 4 plants against symptoms switched in a promising activity against *Streptococcus pneumoniae*. In a previous study, *Erythrina senegalensis*, *Waltheria lanceolata*, *Uapaca togoensis* (Koné et al., 2004) and *Piliostigma thonningii* (Akinpelu and Olorunmolo, 2000) were shown to be very active against *Staphylococcus aureus*, bacteria also implicated in respiratory track infections. Therefore, the potency of these four plants against *Streptococcus pneumoniae* and *Staphylococcus aureus*, largely supported their traditional use in the Ferkessedougou region in the treatment of respiratory infections.

Although the phytochemical constituents of some of those studied species such as *Erythrina senegalensis* (Taylor et al., 1986; Wandji et al., 1990) are known, the exact active principles should be identified in order to standardise traditional medicines. Further work on *Uapaca togoensis* and *Waltheria lanceolata* is underway in order to identify their phytochemical constituents. Toxicology assessment of all these active plants is needed for a more secure use of these remedies.

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