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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF FOUR MEDICINAL PLANTS ON THE *IN VITRO* GROWTH OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

Philomène Aya KOKORA[1], ***Jacques Auguste Alfred Bognan ACKAH[1]**, Zinzindorf Yessé NANGA,[2] Mathieu Adou Koffi KRA[1], Guillaume Yao LOUKOU[2], Adama COULIBALY [1], Joseph DJAMAN[1,3]

[1]*Laboratoire de Pharmacodynamie – Biochimique, UFR Biosciences, Université de Cocody Abidjan,*

[2]*UFR des Sciences Pharmaceutiques & Biologiques, Université de Cocody, Laboratoire National de la Santé Publique (Côte d'Ivoire)*

[3]*Departement de biochimique clinique et fondamentale, Institut Pasteur de Côte d'Ivoire. BP490 Abidjan 01*

**Corresponding Author's Email: jacquackah@yahoo.fr*

ABSTRACT

Evaluation of antibacterial activity of four medicinal plants used in the treatment of diarrheal diseases. To determine the antibacterial activity of total aqueous and ethanolic extracts of different plants, agar diffusion (Muller-Hinton) and broth macrodilution (Muller-Hinton) methods(DMHB) were used tested on *Staphylococcus aureus* ATCC 25923 and *Escherichia* coli ATCC 25922 and two clinical strains *Escherichia coli* 18170 and *Staphylococcus aureus* 1315. The diameters of inhibition and minimum inhibitory concentrations were determined. The studies carried out by the method of agar diffusion method and the double dilution revealed that extracts of *Terminalia mantaly* H. Perrier (*Combretaceae*) has an inhibitory activity on all tested strains. The inhibitory diameters vary between 7.66 mm and 35mm. The minimum inhibitory concentrations (MIC) of the extracts ranged from 0.078 mg /mL and 2.5 mg /mL. The ethanolic extracts inhibit the growth of bacteria at lower concentrations than the aqueous extracts. *Terminalia mantaly* has a stronger antibacterial activity and thus can be useful in the search for new molecules to fight against bacterial resistance.

Keywords: antibacterial activity, inhibition diameters, minimum inhibitory concentration, *Terminalia mantaly*

INTRODUCTION

In Africa, as elsewhere in the world, plants are widely used in the treatment of various ailments. Adjanohoun and Aké[1] have identified more than 5000 medicinal plant species. The properties of some of them have long been exploited by people. In the olden days in Africa priests and medical doctors had the ancient manuscripts describing many herbal recipes[8]. The safety, dosage and usage description were passed down from generation to generation. Thus most of the diseases known by the populations had a corresponding treatment.

Today, traditional medicine rivaled with modern medicine despite the exploits of the latter. According to the World Health Organization (WHO), nearly 80% of people in developing countries depend on this traditional medicine for their primary health care needs due to the high cost of modern medicines [11], this is justified by the fact that these herbal recipes continue to show their efficacy in healing many people. In Côte d'Ivoire people using traditional medicines is increasingly high due to, poverty of the population, prolong political instability, war and access to modern medicine for health care is becoming increasingly difficult.

However, knowledge of the healing power of plants by the people is acquired empirically [13, 14]. They were unaware of the chemical composition of these plants and also various other properties they might possess. Thus, in order to provide a scientific justification for the utilization of these plants, verification of the efficacy and safety of medicinal plants through ethnopharmacological studies have been conducted by several scientific groups. Four plants were selected for the purpose of this study, in order to verify the validity of anti-infective qualities assigned to them. They are: *Erigeron floribundus, Acanthospermum spidum, Melanthera scadens* and *Terminalia mantaly*. The antibacterial activity of total aqueous and ethanolic extracts of these plants was determined *in vitro* on the growth of *Escherichia coli* (*E. Coli*) and *Staphylococcus aureus* (*S. Aureus*).

MATERIALS AND METHODS

Plant material

The leaves and bark of *Erigeron floribundus* (*Asteraceae*) (EF), *Acanthospermum hispidum* Schrank. (*Asteraceae*) (AH) *Melanthera scadens* Schum. & Thonn. (*Asteraceae*) (MS) and *Terminalia mantaly* H.Perrier (*Combretaceae*) (LYTER) were collected, dried and transformed into powder. All plants used in this study were provided by traditional healers in Côte d'Ivoire and identified by the National floristic Center University of Cocody Abidjan. The homogenates obtained were used in the preparation of different extracts.

Microorganisms used

The strains used for the purpose of these study are widely found in various human pathologies. They were

supplied by the National Laboratory of Public Health. Different strains are shown in Table 1.

Table 1: Bacterial Strains

Strains	Origin	Antibacterial profile
***E. coli* ATCC 25922**	Collection	AMX^S AMC^S TIC^S TCC^S PIP^S CF^S FOX^S CTX^S CAZ^S ATM^S GM^S TM^S K^S AN^S NET^S TE^S CS^S SXT^S NA^S PEF^S CIP^S
***E. coli* 28170**	ECBU	AMX^R PIP^R CF^R CXM^R CMX^R TM^R NET^R TE^R SXT^R NA^R PEF^R NOR^R GM^R
***S. aureus* ATCC 25923**	Collection	P^S AM^S AMX^S AMC^S OX^S TIC^S CTX^S GM^S K^S TM^S TE^S E^S SP^S L^S CIP^S
***S. aureus* 1326**	Cathéter central	PT^R P^R L^R E^R RA^R K^R TM^R GM^R

ECBU: Urine culture, ATCC Américan Type Culture Collection, R: resistant, AMC: Amoxicillin + Clavulanic acid, PIP: Piperacillin, CF: Cephalothin, CXM: Cefuroxime, CMX: cefmenoxime, TM: tobramycin, NET netilmicin, TE : Tetracycline, SXT: + Trimethoprim Sulfonamides, NA: Nalidixic acid, PEF: pefloxacin, NOR: Norfloxacin, PT: pristinamycin, P: Penicillin G, L: Lincomycin, E: Erytromycine, C: Chloremphenicol, RA: Rifampicin, K: Kanamycin , TM: Tobramycin, GM: gentamicin, CIP: ciprofloxacin, AMX Amoxicillin ICT Tecarcilline, CTX: cefotaxime, OX: Oxacillin, TCC: Tticarcilline + clavulanic acid, FOS: Fosfomycin, CAZ: ceftazidime, ATM: aztreonam, AN: Amikcine, CS: Colistin.

Preparation of extracts

The extracts were prepared according to the method described by Zihiri and Kra [17]. For the preparation of total aqueous and ethanolic extracts 70%, 100 g of plant powder were extracted in one liter of distilled water (or a mixture of ethanol - water (70/30)) by maceration using a magnetic agitator (the process is repeated 3 times). The homogenate obtained was first spun in a square of fabric, and then filtered twice in succession on cotton wool and once on Whatman 3mm paper. The filtrate was concentrated using a rotary evaporator at 70 ° C. The concentrate was evaporated at 50 ° C. in an oven for 48 hours. The extracts obtained from the total aqueous and ethanolic extracts 70%. The various extracts obtained are: Aqueous extracts: EFaq, ASaq MSaq and LYTERaq; Ethanolic Extracts 70%: EFeth, Aseth, Mseth and LYTEReth.

Determination of antibacterial activity

Agar diffusion method

The agar diffusion Muller-Hinton in agar plates was used to evaluate the activity of different extracts obtained. Culture medium has been in contact with inocula (prepared from pure culture of 18 to 24 hours) density of 0.5 McFarland approximately 10^6 CFU / ml for 5 minutes. The petri dishes were then dried for 15 minutes at 37 ° C. With sterilized object wells of 5 mm in diameter were punched in the agar, 6 wells maximum par each petri dish. 100 mg / ml extract solutions were prepared by diluting 100 mg of extracts in 1 ml of distilled water. 50 ml of each extract solution were distributed into the wells. The plates are then incubated in an oven at 37 ° C for 18 to 24 hours.

Method of double dilution in liquid medium

The method of broth macrodilution (Muller-Hinton) was used to determine the minimum inhibitory concentration (MIC) of plant extracts. In a series of seven hemolytic tubes, 1 ml of each extract concentration (80 mg to 0.078 mg / ml) was added to each tube containing 1 ml of Muller-Hinton broth and germ. The final concentration of extract varied between 40 mg / ml and 0.039 mg / ml. Other two tubes were used as control tubes. One for sterility control containing 2ml of sterilized broth and other containing 2 ml contaminated broth for growth control. The tubes were incubated at 37 ° C for 18 to 24 hours. The MIC determination was performed by finding the difference between the turbidity values obtained before incubation (di) and those obtained after incubation (df) of each tube. These values were measured using a turbidimeter densimat type. When df - di = 0 for a given tube then extract concentration of the tube corresponds to the MIC.

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RESULTS AND DISCUSSION

Table 2: Diameters of inhibition zones in mm extracts of *Erigeron floribundus, Heliotropium indicum, Acanthospermum spidum, Melanthera scadens* and *Terminalia mantaly*

	Extract	*E. coli* ATCC 25922	*E. coli* souche clinique	*S. aureus* ATCC 25923	*S. aureus souche clinique*
Erigeron floribundus	Aqueous Ethanolic	<10 <10	<10 <10	<10 28	<10 11±0,57
Heliotropium indicum	Aqueous Ethanolic	<10 <10	<10 <10	<10 <10	<10 <10
Acanthospermum hispidum	Aqueous Ethanolic	<10 <10	<10 <10	<10 12,33±1,52	<10 11,33±0,57
Melanthera scadens	Aqueous Ethanolic	<10 <10	<10 <10	<10 24,66±5,03	<10 12,33±1,52
Terminalia mantaly	Aqueous Ethanolic	12 14,33±0,57	10,66±0,57 12,33±0,57	29±1 35±1	23±1 30,66±1,15

Table 3: Minimum Inhibitory Concentration (MIC) in mg / ml extracts of *Erigeron floribundus, Heliotropium indicum, Acanthospermum spidum, Melanthera scadens* and *Terminalia mantaly*

	Extraits	*E. coli* ATCC 25922	*E. coli* clinical strain	*S. aureus* ATCC 25923	*S. aureus clinical strain.*
Erigeron floribundus	Aqueous Ethanolic	> 40 > 40	> 40 > 40	> 40 20	> 40 > 40
Heliotropium indicum	Aqueous Ethanolic	> 40 > 40	> 40 > 40	> 40 > 40	> 40 > 40
Acanthospermum hispidum	Aqueous Ethanolic	> 40 > 40	> 40 > 40	> 40 40	> 40 > 40
Melanthera Scadens	Aqueous Ethanolic	> 40 > 40	> 40 > 40	> 40 40	> 40 > 40
Terminalia Mantaly	Aqueous Ethanolic	0,625 0,3125	2,5 1,25	0,3125 0,078	1,25 0,3125

Agar diffusion method revealed that only *Terminalia mantaly* presented inhibitory activity on all strains tested. The diameter of the inhibition zones ranged from 7.66 mm to 29 mm for total aqueous extract and 11.33 mm to 35 mm for total ethanolic 70% extract (Table 1). The highest zones of inhibition are obtained on the strain of *S. aureus* ATCC 25923 and are 29 mm in diameter for LYTERaq and 35 mm diameter for LYTEReth. Total ethanolic extracts 70% AHeth Mseth and EFeth showed inhibition zones on the strain of *S. aureus* ATCC 25923 with diameters of 12.33 mm, 24.66 mm and 28 mm respectively.

The results obtained with the method of double dilution in liquid medium confirmed the results obtained above. *Terminalia mantaly* is active in all strains. And that is represented by minimum inhibitory concentrations (MIC) obtained (Table 2). MIC values varied from 0.3125 mg / ml to 2.5 mg / ml for aqueous extract and from 0.078 mg / ml to 1.25 mg / ml for total ethanolic extract.

The analysis result shows that *Terminalia mantaly* has the strongest inhibitory activity than the other three plants regardless of the method used. No cases of resistance have been noted with the different extracts of this plant. This is verified by the inhibition diameters from 7.66 mm to 36mm and MIC from 0.078mg/ml to 2.5 mg / ml. Thus MIC obtained with *Terminalia mantaly* are 0.078mg/ml, 0.3125 mg / ml, 0.3125mg/ml and 1.25 mg / ml respectively on *S. aureus* ATCC 25923, *S. aureus* clinical strain, *E. coli* ATCC 25922 and *E. coli* clinical strain. The highest MIC (2.5 mg / ml) was obtained on the clinical strain of *E. coli*. These results are comprehensive, because according to Baba - Moussa et al[6] and Mann et al[10], most of the families *Combretaceae* species are known to contain antimicrobial compounds. Also the antibacterial activity of *Terminalia mantaly* had already been mentioned by Andrianstsoa and Andriantsiferana [4]. He revealed that the extract from the decoction of the leaves and stems of *Terminalia mantaly* inhibited the growth of *Shiguella dysenteriae* at concentration between 600µg/ml and 800µg/ml . Yayé et al [16] also showed that the total aqueous extracts and ethanolic 70% of this plant showed antifungal activity against *Candida albicans* at concentrations of 97.5 micrograms / ml and 48.75 micrograms / ml.

The ethanolic extracts have a better antibacterial activity than the aqueous extracts and this regardless of the germ and the plant tested. The method of preparation of the ethanolic extract by the mixture of ethanol-water solvent (70/30) was initiated by Zihiri et al.[17] Several studies including the Ahon et al.[3], Ackah et al.[2] and Yayé et al [16]

showed that ethanol 70% extract prepared by this method is more active than the aqueous extract. Our results therefore confirm theirs. This method therefore produces a better concentration of active ingredients.

CONCLUSION

This study has allowed us to determine the antibacterial properties of Erigeron floribundus, Acanthospermum hispidum, Melanthera scadens and Terminalia mantaly. It shows from these investigations that Terminalia mantaly is the most active plant among the four plants studied. The most sensitive strains were S. aureus. The hydroalcoholic extract (70/30) concentrate most the active ingredient of plants studied. Further studies will produce a series of chromatography to optimize the active ingredients, and determine the chemical compounds responsible for the antibacterial activity of Terminalia mantaly.

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