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Phytochemical screening and antimicrobial activity of *Corymbia torelliana* (Myrtaceae) bark extracts

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Abstract

Infectious diseases represent a real public health problem. They are widespread in several regions of the world, particularly in developing countries. Opportunistic pathogens from fungal, bacterial and parasitic infections are the main causes. The aim of this study is to scientifically validate the therapeutic properties attributed to the bark of *Corymbia torelliana* (Myrtaceae), a plant used in the treatment of various infections in Côte d'Ivoire. It consisted of carrying out a phytochemical screening and assessing the antimicrobial activity of aqueous (EAq), hexanolic (Ehex) and ethanolic (Eeth) extracts of the plant bark on *Escherichia coli*, *Enterobacter cloacae, Enterobacter aerogenes, Trichophyton rubum* and *Aspergillus fumigatus*. After the extractions had been carried out, phytochemical analysis of the three extracts revealed the presence of gall tannins, sterols/terpenes, polyphenols, saponosides, flavonoids and quinones, and a total absence of catechic tannins and alkaloids. The assessment of the antimicrobial activity of *Corymbia torelliana* bark extracts showed that the plant has bacteriostatic activity for all three extracts on all the bacterial strains tested, namely *Escherichia coli* (MBC=32mg/mL), *Enterobacter cloacae* (MBC>32mg/mL) and *Enterobacter aerogenes* (MBC>32mg/mL). Similarly, the hexanic extract showed fungicidal activity on *Trichophyton rubum* (MBC=50mg/mL) and *Aspergillus fimugatus* (MBC=25mg/mL) compared to the other two extracts.

Key words: *Corymbia torelliana*; Phytochemical sorting; Antibacterial activity; Fungicidal activity

1. Introduction

In recent decades, microbial diseases, transmitted by bacteria, viruses, fungi and other parasites, have claimed around 17 million victims worldwide. Africa, which accounts for two-thirds of the burden of this mortality, is the continent that pays the highest price [1, 2]. Faced with this situation, mankind has found and developed knowledge and practices to treat itself using natural products of plant, mineral or animal origin [3]. Thus, from the chemical compounds present in these natural products, synthetic products have been developed to replace conventional medicines, in particular antibiotics, which often prove ineffective due to the resistance developed by microorganisms and the manifestation of severe, and in some cases even toxic, side [4,5,6]. In addition, the main difficulties associated with the treatment of these microbial diseases are the inaccessibility and high cost of these drugs [7]. Even today, medicinal plants have become a precious heritage for the survival of humanity. Indeed, the WHO recognises that traditional, complementary and alternative medicine has many benefits [8]. From the wide range of medicinal plants used in Côte d'Ivoire, we chose

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Corymbia torelliana, a plant in the Myrtaceae family. This plant has therapeutic virtues and is used in the treatment of several infectious diseases [9].

The aim of our study is to investigate the antimicrobial activity of this plant on a number of enterobacteria and two moulds, by assessing the antimicrobial potency of plant extracts derived from the bark and determining the composition of secondary metabolites in the bark of the plant.

2. Materials and methods

2.1. Material

The plant material used was a powder obtained from *Corymbia torelliana* bark collected on the morning of 22 September 2021 at the Université Félix HOUPHOUËT-BOIGNY de Cocody (Abidjan, Côte d'Ivoire). The plant has been identified at the CNF under herbarium number UCJ019051. The microbiological material consisted of three (03) species of bacteria from the Enterobacteriaceae family, namely *Enterobacter aerogenes, Enterobacter cloacae* and *Escherichia coli* taken from patients: *Enterobacter aerogenes, Enterobacter cloacae* and *Escherichia coli* taken from patients and two fungal species, *Trichophyton rubum* and *Aspergillus fumigatus* supplied by the laboratory of the Centre National Floristique of the Université Félix Houphouët Boigny. The culture media consisted of Muller Hinton agar, Muller Hinton broth and Sabouraud agar (Ref: 64494, Batch: 64406698).

2.2. Methods

2.2.1. Preparation of extracts

The crude hydroethanol extract was prepared from *C. torelliana* powder according to the modified method of Zirihi [10]. Thus, 50g of *C. torelliana* powder was extracted in 500mL of a solvent mixture comprising 70% ethanol and 30% distilled water, homogenized in a blender. This operation was repeated with 50 g of *C. torelliana* powder. The total homogenate obtained was wrung out in a square of clean white cloth, then filtered four (04) times on absorbent cotton and once (01) on filter paper. The filtrate obtained was concentrated in a rotary evaporator and evaporated to dryness in an oven at 50°C. After drying, the extract was collected in a jar. This operation was performed three times. The hydroethanol extract was used to prepare two (02) other extracts. A 10 g portion of the extract was weighed and subjected to liquid/liquid separation in 300 mL of 2 solvent mixtures of 150 mL distilled water and 150 mL hexane. Maceration was carried out for 24 h, with stirring using a magnetic stirrer. After 24 h settling, two (02) phases (aqueous phase and hexane phase) were collected separately and oven-dried at 500C. In total, we have three (03) extracts, namely the crude ethanolic extract, the aqueous and hexanolic extracts from the water-hexane partition, which will be used for the rest of the study.

2.3. Phytochemical screening

Phytochemical screening was carried out using colorimetric methods and the precipitation method [11, 12], highlighting polyphenols, catechic and gallic tannins, quinones, saponins, alkaloids, sterols/terpenes and flavonoids.

2.4. Microbiological methods

2.4.1. Preparation of culture media and inoculation of young colonies

Muëller-Hinton (MH) agar and broth and Sabouraud agar were prepared according to the manufacturer's instructions, using 1.9 g homogenized in 50 mL distilled water for the agar and 1.26 g homogenized in 30 mL distilled water for the twice-concentrated broth.

For Sabouraud agar, 4.62 g of agar powder were dissolved in 110 mL of distilled water. The prepared medium was transferred to a series of tubes for double dilution.

To inoculate colonies of *Enterobacter cloacae, Enterobacter aerogenes* and *Escherichia coli*, an oese of the colony was taken from the stock culture and homogenized in 10mL of sterile distilled water. Another oese was then taken and inoculated onto the previously prepared agar. After inoculation, the culture was incubated for 24 hours.

For *Trychophyton rubrum* and *Aaspergillus fumigatus*, young colonies were obtained after subculturing for 5 days and 48 hours respectively on new sterilized agar.

2.5. Evaluation of antimicrobial parameters (MIC, CMB) using the liquid dilution method

2.5.1. Inoculum preparation

A few young colonies were picked with a platinum loop and homogenized in 10mL EDS. The turbidity of this suspension was adjusted to 0.5 Macfarland using an opacity control (Bio-Rad). This bacterial suspension, estimated at 10^8 cfu/mL, was diluted 1:100 in sterile Müeller-Hinton broth prepared as a twofold concentrate (0.29 mL bacterial suspension in 29 mL broth) to give a bacterial inoculum of 10^6 cfu/mL [13].

2.5.2. Preparation of extract concentration ranges

Concentration ranges were prepared in sterile hemolysis tubes. To achieve double dilution, the first tubes of each extract corresponding to each species contained 2 mL of sterile stock extract solution at a concentration of 64 mg/mL: The other tubes contain 1mL of EDS. Thus, 1mL of the stock solution was taken from the first tube and diluted in the 2nd tube. After shaking, 1mL was also taken from the 2nd tube and diluted in the 3rd tube. This operation was successively repeated for the other tubes of each extract corresponding to each species, giving respective concentrations of 64; 32; 16; 8; 4; 2; 1 and 0.5 mg/mL, before adjusting the concentration present in the 6 tubes with 1mL of bacterial inoculum 10^6ufc/mL. Finally, the final concentrations become 32; 16; 8; 4; 2; 1 and 0.25 mg/mL. A sterility control containing EDS and M-H broth and a growth control containing bacterial suspension and EDS were prepared. In addition to the experimental tubes, we prepared a germ-free growth control tube and a germ-free extract-free broth sterility control tube.

All tubes and the bactericidal control were incubated in the oven for 24 h at $37^{o}C$.

2.5.3. Determination of MIC and BMC

The MIC is the lowest concentration that does not show visible bacterial growth.

After incubation, bacterial growth was observed in the tubes. Tubes with no visible bacterial growth were plated on MH agar by streaking from highest to lowest concentration and incubated for 24h. The BMC is the smallest concentration for which subculturing shows growth of less than or equal to 0.01% survivors. After inoculation of the concentration range, the inoculum was counted to determine the BMC. A bactericidal control for each germ was performed by streaking on Müeller-Hinton agar in petri dishes after dilutions of 10^0, 10^{-1},10^{-2} ,10^{-3} and 10^{-4} of the inoculum corresponding to 100% ,10%, 1% 0.1% and 0.01% survivors respectively. After the MIC reading, tubes with no visible growth were incubated for 24 hours at 37°C. The streaks were then compared with the bactericidal control

2.6. Evaluation of antifungal parameters (MIC, FMC) using the solid-state dilution method

2.6.1. Preparation of inoculum

The inoculum was prepared from a young colony 5 days old for *T. rubrum* and 48 hours old for *Aspergillus fumigatus*. For each species, the parent suspension, known as suspension 10^0 (with a load of 10^6 cells/mL), was first prepared by homogenizing a fungal colony in 10 mL of sterilized distilled water. From suspension 10^0, a second suspension (10^{-1}) was prepared by a 1/10th dilution of the parent suspension by transferring 1 mL of suspension 10^0 into 9 mL of sterilized distilled water to give a final volume of 10 mL. The latter has a load of 10^5 cells/mL [14,15].

2.6.2. Incorporation of extracts into the culture medium

Plant extracts were incorporated into Sabouraud agar using the double dilution method, in inclined tubes. We had 8 test tubes with concentrations ranging from 100 mg/mL to 1.56 mg/mL and 2 control tubes without plant extract; one serving as a germ growth control (TC) and the other germ-free serving as a sterility control (TS) for the culture medium [15]. After incorporation of the extract, all 10 tubes in each series were autoclaved at 121°C for 15 minutes and then tilted to allow cooling and solidification of the agar.

2.6.3. Antifungal tests in the presence of plant extract

For each test tube, germs were cultured on the previously prepared media, by streaking (until exhaustion) 10 µL of the 10^{-1} suspension, corresponding to 1000 seeded cells. The resulting cultures were incubated at 30°C for 5 days for *T. rubrum* and 48 h for *A. fumigatus* [10].

Tests were carried out under standard culture conditions for antifungal tests (normal loads of 10^5-10^7 cells/mL). Tests were repeated 3 times for each extract.

2.6.4. Colony count

After the various incubation times, fungal colonies were counted directly using a colony-counting pen. Growth in the 10 experimental tubes was assessed as percentage survival, calculated in relation to 100% survival in the growth control tube [10]. The percentage survival of fungal species in experimental tubes can be calculated using the following formula:

S = N/ n x 100; S = Germ survival (expressed as a percentage); N = Number of colonies in the control tube; n = Number of colonies in the experimental tube.

3. Results

3.1. Extraction yields

The hydroethanol extract gave a brown powder with a yield of 31.45% noted EEth. For the preparation of partitioned extracts, yields varied from one partitioned extract to the next, giving a yield of 17.69% for the hexanic phase (EHex) and 57.10% for the aqueous phase (EAq).

3.2. Phytochemical sorting

Phytochemical analysis revealed the presence of gall tannins, sterols/terpenes, polyphenols, saponosides and quinones, although flavonoids were absent in the Hexanic extract (Ehex) and a total absence of catechic tannins and alkaloids in all extracts (Table 1).

Table 1 Chemical compounds in each extract

Extract/ Tests	**Eeth**	**Ehex**	**EAq**
polyphenols	+++	++	+++
Catechic tannins	-	-	-
Gallic tannins	+++	++	+++
Quinones	+	++	+
Saponosides	+	+	+
Terpene compounds	++	++	++
Flavonoïdes	++	-	+
Alcaloïdes	-	-	-

- : Absence of compounds ; +: Presence in small quantities ; ++ : Presence in medium quantities; +++: abundantly present

3.3. Microbiological tests

After incubation of the tubes in the oven for 24h, 48h and 5days respectively for the bacterial species (*Enterobacter cloacae, Enterobacter aerogenes* and *Escherichia coli*), *A. fumigatus* and *T. rubrum*, the growth of germs in each tube at the level of each extract is different for each species.

Table 2 MIC and BMC values for the different extracts on *E. coli, E. aerogenes* and *E. cloacae*

Strains bacterial	**Extracts**	**Antibacterial parameters**		**Effects**
		MIC	MBC	
E. coli	E_{Aq}	2 mg/mL	32 mg/mL	Bacteriostatic
	E_{Eth}	2 mg/mL	32 mg/mL	Bacteriostatic
	E_{Hex}	2 mg/mL	32 mg/mL	Bacteriostatic
E. cloacae	E_{Aq}	8 mg/mL	> 32mg/mL	Bacteriostatic
	E_{Eth}	16 mg/mL	> 32mg/mL	Bacteriostatic

	E_{Hex}	16 mg/mL	> 32mg/mL	Bacteriostatic
E. aerogenes	E_{Aq}	16 mg/mL	> 32mg/mL	Bacteriostatic
	E_{Eth}	16 mg/mL	> 32mg/mL	Bacteriostatic
	E_{Hex}	16 mg/mL	> 32mg/mL	Bacteriostatic

Table 3 MIC and FMC values for the various extracts on *T. rubum* and *A. fumigatus*

Fungal strains	**Extracts**	**Antifungal parameters**			**Effects**
		MIC	MFC	IC50	
T rubrum	E_{Aq}	>100 mg/mL	ND	ND	Inactive
	E_{eth}	>100 mg/mL	ND	ND	Inactive
	E_{Hex}	25 mg/mL	50 mg/mL	14,538 mg/mL	Fungicide
A. fimugatus	E_{Aq}	>100 mg/mL	ND	ND	Inactive
	E_{eth}	25 mg/mL	>100mg/mL	8,58 mg/mL	Fungistatic
	E_{Hex}	12,5 mg/mL	25 mg/mL	12,6 mg/mL	Fungicide

4. Discussion

The yield obtained after hydroethanol extraction (EEth Extract) of *C. torelliana* powder shows that the bark of this plant is rich in metabolites extractable by this solvent, which is used for most tests and has the capacity to extract the majority of polar liposoluble and water-soluble compounds. This confirms that it is therefore a very good solvent for analyzing the totum of extracts [16]. In addition, the choice of these two solvents was based on the fact that water is the solvent most commonly used in the preparation of traditional remedies (infusion, decoction, maceration, etc.) and hexane, which is an apolar solvent, is used for oil extractions, so it was possible to extract the lipid compounds present in the ethanolic extract. The presence of the chemical compounds determined after phytochemical analysis can be explained by the type of solvents used for the extractions. Our results are in line with those of Agré (2015) [9], who highlighted the same compounds. In tests carried out to determine the antibacterial activity of three different *C. torelliana* extracts (EHex ,EAq and EEth), only *E. coli* was sensitive to the EEth and EAq extracts, with a MIC equal to 2 mg/mL and a BMC equal to 32 mg/mL. *E. aerogenes* and *E. cloacae* were less sensitive to all three extracts. In fact, the active extracts inhibited the growth of these two bacterial species with a MIC value of 8 mg/mL for the EAq extract and 16 mg/mL for the EEth and EHex extracts concerning *E. cloacae*; for *E. aerogenes*, we obtained a MIC value equal to 16 mg/mL for the three extracts. The extracts generated an activity with a BMC value greater than 32mg/mL (> 32 mg/mL) for both strains. Other previous studies point in the same direction as our results, namely those of Koffi *and collaborator* in (2013) [17] and Leelaprakash and collaborator in (2011) [18]. which, using liquid diffusion and dilution methods, show that the aqueous extract of *Momordica charantia* leaves induces remarkable inhibition of *E. coli* growth, while the methanolic and 100mg/mL aqueous extracts are active on the same bacterial species. Furthermore, Fokunang and collaborator in (2012) [19] report that hexanic extract also has no effect on *E. coli* and *Enterobacter aerogenes* strains.

As far as fungal germs are concerned, *A. fumigatus* and *T. rubrum* showed sensitivity to hexanic extract. The same inhibitory activity was observed in the work of Ouattara *and collaborator* in 2009[20]. They tested the antifungal activity of *Terminalia Ivorensis* extracts (tekam 2) on the in vitro growth of *A. fumigatus*. The results showed that organic phase X12 (hexane extract from hexane/water partition) with a MFC = 0.09 mg/mL was more active on *Aspergillus fumigatus*. These results may provide some insight into the sensitivity of *A. fumigatus* to hexanic extracts. As for the aqueous and ethanolic extracts, they showed no activity on these fungal germs given the range of concentrations used. On the other hand, with *A fumigatus*, a MIC= 25mg/mL could be determined with the ethanolic extract. However, the MFC was higher than 100mg/mL. In fact, after subculturing, we found that this extract was not fungicidal, because after 48 h, several *A. fumigatus* colonies grew on the culture medium.

5. Conclusion

Our study showed that the aqueous and hexanolic extracts derived from water-hexane partitioning (EAq, EHex) and the crude ethanolic extract (EEth) have bacteriostatic activity on *Escherichia coli, Enterobacter aerogenes* and *Enterobacter cloacae* strains, as the MBC/MIC activity ratio is greater than 4. However, EEth and EAq extracts are more active on E. coli. Hexanic extract (EHex) has good fungicidal activity on *T. rubrum* and *A. fumigatus*. Ethanol and water would therefore be the solvents with the best extraction power, and the presence of the chemical groups listed in these different extracts would be at the origin of the antimicrobial activity observed.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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