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(RESEARCH ARTICLE)

Triphytochemistry and antibacterial activity of ethanolic 70% and aqueous extracts of *Lecaniodiscus cupanioides* Planch. Stem bark (Sapindaceae)

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Abstract

The aim of this work is to carry out the phytochemical study and to evaluate the antibacterial activity of the aqueous (EAq) and ethanolic 70% (EEth70%) extracts of *Lecaniodiscus cupanioides* Planch (Sapindaceae) bark, on four strains. Thus, after the extractions, the best yield was observed in EEth70%. The chemical screening revealed the presence of most of the desired compound in both extracts. However, alkaloids, saponosides, anthocyanins, gallic tannins, quinones, sterols and polyterpenes were absent in EAq. Also, alkaloids, anthocyanins and gall tannins are absent in both extracts. Regarding antibacterial activity, all tested microorganisms reacted in dose response to the extracts. However, Pseudomonas aeruginosa and *Klebsiella pneumoniae* strains were found to be resistant to both extracts. Nevertheless, the results obtained with EEth 70% showed the most significant antibacterial activity. *Escherichia coli* and *Staphylococcus aureus* strains had inhibition diameters of 20 and 18 mm respectively at the concentration of 200 mg/mL in the presence of the ethanolic extract. For the aqueous extract the inhibition diameters of the latter are 14 and 10 mm respectively at 200 mg/mL. On the basis of the Minimum Inhibitory Concentrations (MIC), *E. coli* and *S. aureus* strains were the most sensitive to both plant extracts with MICs ranging from 6.25 to 25 mg/mL. On the other hand, *P. aeruginosa*, and *K pneumoniae* strains were less sensitive on both extracts (EAq and EEth).

Keywords: *Lecaniodiscus cupanioides*; Triphytochemistry; Antibacterial; *Côte d'Ivoire*

1. Introduction

The phenomenon of antibiotic resistance is increasingly observed for certain drugs. This resistance has led researchers to look for natural molecules, effective and without any adverse effect, with antibacterial [1], antioxidant [2] and anti-inflammatory [3] properties in medicinal and culinary plants. The African flora is very rich in medicinal species used for the treatment of various infections. This is the case of *Lecaniodiscus cupanioides* Planch (Sapindaceae), a plant widely used in traditional medicine in Côte d'Ivoire against inflammations, pulmonary diseases, sexual impotence, liver hypertrophy and some bacterial infections [4]. The bark is made into a powder for inhalation or as tobacco to relieve headaches, sinusitis and nasal congestion, as well as otitis (ear infection) and ophthalmia (eye problem). Fresh bark is also used to treat earaches [5]. A decoction of the bark is applied externally as a general restorative or revulsive for

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chest pain, bronchitis, pleurisy, and kidney pain [4]. Several works have shown that *Lecaniodiscus cupanioides* is used for the treatment of cysts, myomas, fibroids and dental caries in Benin [6]. In Côte d'Ivoire, the Abbey and Krobou peoples use these stem barks in decoction to facilitate childbirth [7]**.** Scientific research has shown the existence of a number of medically active compounds that often verify traditional uses in this plant. The antimicrobial activity of the methanolic extract of the leaves of the plant in Nigeria was found to be effective on *B. cereus, S. aureus, M. kristine* and *S. pyrogens* [8]**.** Thus, the present study also aims at evaluating the antibacterial power of the extracts of stem bark of *Lecaniodiscus cupanioides* Planch (Sapindaceae), a plant used for traditional treatments of sexual impotence and urinary tract infections in the mountain district more precisely in the department of Man.

2. Material and methods

2.1. Plant material

The plant material consisted of *Lecaniodiscus cupanioides* stem bark collected at the University of Man site, located 7 km from the city of Man, on the Man-Danané axis (Côte d'Ivoire).

2.2. Microorganisms

The bacterial support used in this study consists of four (4) strains namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These bacterial organisms are from the strain bank Unit Antibiotics Natural Substances and Monitoring Microorganisms for Anti-Infective (ASSURMI) of the Department of Bacteriology and Virology of the Pasteur Institute in Ivory Coast (IPCI).

2.3. Preparation of plant extracts

2.3.1. Preparation of aqueous extract

100 g powder of the leave of *M. holstii* were macerated for 24 hours in 1L of distilled water [9]. The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one fold on filter paper (Whatman paper® 2 mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4 °C [10].

2.3.2. Preparation of ethanolic 70% extract

It was carried out using modified [9] method. A mass of 100 g of plant powder was added in 1L of ethanol 70% and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

2.4. Preparation of bacterial inoculum

Two isolated colonies from each bacterial culture for 18 hours were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37 °C for preculture. A levy of 0.1 mL of the preculture broth was diluted in a tube containing 10 mL of Mueller-Hinton (MH). This bacterial suspension was made consisting of 10^0 dilution of bacterial inoculum so as to obtain a bacterial load estimated to 10^6 Unit Format colonies per milliliter (CFU / mL).

2.4.1. Preparation of extracts concentration ranges

A range of concentration of each extract was prepared with a series of ten vice tubes through the method of double dilution an in medium liquid. This range of concentration is 200 mg / mL to 0.39 mg / mL numbered T1 to T10. For this, 10 mL of a mixture solution of DMSO / sterile distilled water (V / V) were placed in the tubes T1 and 5mL in all the other tubes. Two grams (2g) of each extract were dissolved in the tubes T1 to obtain a concentration of 200 mg / mL. A 5 mL volume of the tubes T1 was transferred into the tubes T2 and then homogenized. This operation was repeated until T10 tubes where 5 mL of T10 tubes are rejected. All tubes are kept refrigerated at 4 °C[11,1].

2.4.2. Determination of growth inhibition zones

The method of holes punch in the MH agar described by [12] has been accepted. Each pit or holes of 6 mm diameter was filled with 80 µL of extract concentrations of 200 and 100 mg / mL, taking care to separate two holes of at least 20 mm. A negative control wells was performed for each bacterial strain with 80 µL of the mixture of DMSO / sterile distilled water solution (V/V). After a pre-release of 45 minutes at laboratory temperature to 16 °C, all the Petri dishes were incubated in an incubator at 37 °C for 18-24h. Meanwhile, Ceftriaxone (CRO 30µg) for Enterobacteriaceae and oxacillin (OX 5µg) for staphylococci were used as positive controls. After incubation, the activities of the extracts were assessed

by measurement of a growth inhibition area around the wells using a caliper. According to [13], a strain is called insensitive or resistant, sensitive and very sensitive if the diameters of inhibition are respectively less than 8 mm, between 9 and 14 mm and between 15 and 19 mm.

2.4.3. Determination of Minimum Inhibitory Concentration (MIC)

The macro dilution method in liquid medium described by [14] was used to determine these antimicrobials parameters. Thus, in a series of 10 hemolysis tubes numbered C1 to C10 for each extract was introduced 1 mL of the bacterial inoculum. Then 1 mL of each extract concentration well known by the range of prepared concentration was added in the same tubes. This distribution of plant extract is made so that 1 ml of plant extract of 200 mg / mL was transferred in the tube C1, that of 100 mg / mL in the tube so C2 to C9 tube receive 1mL plant extract of 0.78 mg / mL. C10 has been tube, received instead of plant extract, 1 mL of DMSO / Sterile distilled water (V/V), was used as a control. This distribution of plant extract concentration is well known in each tube already containing 1 mL of inoculum reduced the concentration of plant extract in medium at its half. Tube and the concentration of C1 increased from 200 mg / mL to 100 mg / mL. 100 mg / mL to 50 mg / mL for C2 so on until a concentration of 0.39 mg / mL for T9. This experiment was performed identically for each sample tested. The first nine (9) tubes (C1 to C9) are called "experimental tubes" and the last tube (C10) is rated "growth control tube or TC." The loaded tubes were incubated at 37 °C for 24 h. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye.

2.4.4. Determination of Minimum Bactericidal Concentration (MBC)

From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours corresponds to the CMB. It is determined by plating by a streak on Mueller-Hinton agar by streaking 5 cm using a loop, beginning with the first and incubated undisturbed at 37 ° C for 24 h tube.

2.4.5. Antibacterial activity of the extracts tested

The antibacterial effect of different extracts tested was considered bactericidal or bacteriostatic depending on the MBC / MIC ratio. According [15] when this ratio is greater than 4, the extract has bacteriostatic and bactericidal, if the ratio is less than or equal to 4.

2.5. Phytochemical screening

2.5.1. Test for sterols and polyterpenes (reaction LIEBERMANN)

After evaporation to dryness 5mL of each solution in a capsule on a sand bath without charring, the residue was dissolved in hot acetic anhydride and 1 mL in a test tube, we poured cautiously with 0.5 mL of concentrated sulfuric acid along the tube wall to the solution. The applications to the interphase of a purple or purple ring, turning blue to green, indicate a positive reaction [16].

2.5.2. Test for alkaloids (reactions Dragendorff and Bouchardat)

Six milliliters of plant extract were evaporated. The residue was taken up in six milliliters of alcohol at 60 ° and the alcoholic solution thus obtained was divided into two test tubes.

In the first tube was added two drops of Dragendorff reagent. The appearance of a precipitate or an orange color indicated the presence of alkaloids.

In the second tube was added two drops of reagent Bouchardat. The appearance of a reddish brown color indicated a positive reaction to the presence of alkaloids [17].

2.5.3. Test for polyphenols

Two milliliter of extract was added a drop of alcoholic solution of ferric chloride at 2%. The appearance of a dark green or lighter or darker blue color indicated the presence of polyphenolic derivatives [18]

2.5.4. Test for flavonoids

For this research, two milliliter of the extract was evaporated to dryness in a porcelain dish on a sand bath. The residue was taken after cooling in five milliliter hydrochloric alcohol half. The successive addition of three magnesium shavings and three drops of isoamylic alcohol showed an intense pink or violet in the presence of flavonoids [19].

2.5.5. Test for saponosides

A volume of two milliliters of each extract was evaporated and taken up in five milliliters of water. After vigorous stirring, the foaming of more than one centimeter, stable and persistent high for 30 minutes indicated the presence of saponins [20].

2.5.6. Test for catechol or condensed tannins (reaction Stiasny)

A volume of five milliliter of each extract was evaporated and an amount of 10 ml of a reagent solution Stiasny was added to the residue. This mixture was placed in a water bath at 80 °C for 30 minutes and was cooled to room temperature. Positive feedback had resulted in the formation of large flakes brown clear or dirty precipitates [16].

2.5.7. Quinonic substances research

For this research, 2 mL of each extract solution is first evaporated to dryness in a sand-bath capsule without charring, then the residue is triturated in 5 mL of 1: 5 hydrochloric acid. Then the solution obtained is brought to the boiling water bath for half an hour. Finally, after cooling on a current of cold water, the hydrolyzate is extracted with 20 ml of chloroform and the chloroform phase is collected in another test tube supplemented with 0.5 ml of ammonia diluted by half. The appearance of a color ranging from red to purple indicates the presence of quinones [16].

2.5.8. Search anthocyanins

The presence of anthocyanins in an extract solution is indicated by a red color which increases with the addition of dilute HCl and turns purplish-blue-green by the addition of ammonia [16].

2.5.9. Test for Gallic tannins

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2% have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic [16].

3. Results

3.1. Yield

The yield values were calculated relative to the initial mass of *Lecaniodiscus cupanioides* powder for one trial. The extraction yields of the plant were 7.13% for the ethanolic extract **70%** (EEth 70%) and 6% for the aqueous extract (EAq) (Table 1).

Table 1 Yield of *Lecaniodiscus cupanioides* extracts

Extracts	Weight (g)	Yield (%)	Colors	Aspects
EAq	9	6	Brown	Glitter
EEth 70%	10.7	7. 13	Yellow-brown	Powder

EAq = Aqueous extract of bark; EEth 70% = Ethanolic extract 70% of bark

3.2. Triphytochemistry

Table 2 shows the major groups of chemical families contained in the two extracts of *L. cupanioides*. Phytochemical screening of the Aqueous Extract (EAq) and Ethanolic Extract (EEth 70%) of *L. cupanioides* stem barks shows the presence of several families of chemical compound. These are sterols+polyterpenes, alkaloids, gallic tannins and catechins, quinones, saponosides, anthocyanins, flavonoids and total polyphenols. On the other hand, sterols and polyterpenes and quinones are absent in EAq and saponosides are absent in EEth. However, alkaloids, anthocyanins and gallic tannins were completely absent in both extracts.

3.3. Antimicrobial activity

Table 3 presents the antibacterial activity of EAq and EEth 70% of *Lecaniodiscus cupanioides* stem barks. These results show that the best bacterial activity is obtained with EEth with *E. coli* (20 mm) and *S. aureus* (18 mm) 200 mg/mL.

For EAq, the highest sensitivity was observed with the E. coli strain (14 mm), followed by S. aureus with 10 mm inhibition diameter at 200 mg/mL. On the other hand, with both extracts, *P. aeruginosa* and *K. pneumoniae* strains showed the least sensitivity with 8 mm in diameter. Both *K. pneumoniae* and *P. aeruginosa* strains were resistant to the extracts.

Table 2 Phytochemical analysis of Aqueous and Ethanolic 70% Extracts of *Lecaniodiscus cupanioides* barks

Extracts		**EAq**	**EEth 70%**
alkaloids	B	-	-
	D	-	-
Saponosides		-	+++
Anthocyanins		-	-
Tannins	Cat	+++	+++
	Gal	-	-
Flavonoids		+++	+++
Polyphénols		++	++
Quinones		-	+++
Polyterpènes and Stérols		-	+++

- : Absent + : Présence, ++ : strong presence, +++ :very strong presence, EEth 70% : Ethanolic extract 70% ; EAq : Aqueous extracts ; Gal : Gallic ; Cat : Catéchiques ; B : Bouchardât ; D : Dragendorff

Table 3 Antibacterial parameters of ethanolic 70% and aqueous extracts of *Lecaniodiscus cupanioides* barks on the *in vitro* growth of tested germs

Extracts	**Tested strains**	**Concentrations of extracts (mg/mL)**				**Antibiotics**
		200	**100**	**50**	**25**	**CRO**
EAq	*E. coli*	14 ± 0.6	11 ± 0.3	08 ± 0.9	06 ± 00	12
	S. aureus	10 ± 0.3	08 ± 0.6	06 ± 00	06 ± 00	15
	P. aeruginosa	06 ± 00	06 ± 0.3	06 ± 00	06 ± 00	10
	K. pneumoniae	08 ± 0.9	06 ± 00	06 ± 00	06 ± 00	10
EEth 70%	*E. coli*	20 ± 0.6	16 ± 0.9	13 ± 0.9	11 ± 0.9	12
	S. aureus	18 ± 0.3	15 ± 0.9	10 ± 0.3	08 ± 0.6	15
	P. aeruginosa	08 ± 0.9	06 ± 00	06 ± 00	06 ± 00	10
	K. pneumoniae	06 ± 00	06 ± 00	06 ± 00	06 ± 00	10

with : **Ts = T = 0** : Sterility control including well diameter (6 mm) with DMSO/Water (0,5 : 0,5 ; V/V) ; **CRO** = Ceftriaxon , **EEth 70%**: éthanolic Extract 70% ; **EAq** : Aqueous Extract

3.4. Antibacterial parameters

After 24 h of incubation at 37 °C in liquid medium (BMH), the study of the action of *Lecaniodiscus cupanioides* extracts on the in vitro growth of strains presented the results recorded in Table 4. On the basis of the Minimum Inhibitory Concentrations (MIC), *E. coli* and *S. aureus* strains were the most sensitive to both extracts of the plant with MICs ranging from 6.25 to 25 mg/mL. On the other hand, *P. aeruginosa*, and *K pneumoniae* strains were less sensitive on both extracts (EAq and EEth 70%).

Regarding the ratio of Minimum Bactericidal Concentrations (MBC) to MIC, the antibacterial parameters of EAq and EEth 70% were bactericidal for most of the strains tested except for E. coli which was bacteriostatic with EAq

Table 4 Antibacterial parameters of the ethanolic 70% and aqueous extracts of *Lecaniodiscus cupanioides* barks on the in vitro growth of the tested germs

Extracts	Antibacterial parameters mg/mL	STRAINS			
		E. coli	***S. aureus***	***P. aeruginosa***	***K pneumoniae***
EAq	MIC	12.5	25	200	200
	MBC	50	25	200	200
	MBC / MIC	4	1	1	1
	effect	Bacteriostatic	Bactericide	Bactericide	Bactericide
EEth 70%	MIC	6.25	12.5	200	200
	MBC	12.5	12.5	200	200
	MBC / MIC	2	1	1	1
	effect	Bactericide	Bactericide	Bactericide	Bactericide

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration EEth 70%: Ethanolic extract 70%; EAq: Aqueous extract

4. Discussion

The extraction method used, is the maceration of *Lecaniodiscus cupanioides* barks (planch) with two solvents, one inorganic (distilled water) and the other organic (ethanol 70%). From the results obtained, it appears that the yields of the extractions varied considerably from one extract to another. Thus, ethanol has the highest yield value with 7.30% compared to water 6% taking into account the initial mass of *Lecaniodiscus cupanioides* bark powder. This could be explained by the fact that ethanol concentrates the compounds better than water in our plant. The variations in extraction yields can be attributed to the difference in solubility of the compounds in the extraction solvents. Besides the extraction method, other factors can influence the efficiency of the extraction of compounds. It depends on several parameters such as: nature and volume of the solvent used, pH, temperature, extraction time and sample composition, sample harvesting, etc [16].

Then, the extracts of *L. cupanioides* were subjected to a qualitative phytochemical analysis. It revealed the presence of polyphenols, tannins, flavonoids, saponosides, anthocyanins, alkaloids, quinones, sterols and polyterpenes in the bark of the plant. Anthocyanins and gallic tannins are absent in both extracts. Besides that, the presence of the majority of these sought metabolites was detected at the level of EEth 70%. According to [21], the absence of a compound could be explained by the nature of the solvent used, the study area or the quality of the soil.

Regarding the study of antibacterial activity, both extracts (EEth70%, EAq) of *Lecaniodiscus cupanioides* bark, gave diameters of inhibition on the growth of some tested germs namely *Escherichia coli, S. aureus, P. aeruginosa* and *K pneumoniae*. EEth 70% gave good inhibitory activity on the growth of the tested strains compared to EAq. The inhibition diameters were generally between 06 and 20 mm for the ethanolic extract and between 06 and 14 mm for the aqueous extract. The highest antibacterial activities are observed with the aqueous and ethanolic extracts on *Escherichia coli* and *Staphylococcus aureus*. They show respective inhibition diameters of 20 mm and 14 mm against the strain at concentrations of 200mg/mL. According to [22]**,** there is a good correlation between this positive activity and the polyphenol content. Moreover, these results consolidate those reported in the literature, phenolic compounds show the greatest antibacterial activity and that the *Staphylococcus aureus* strain is more particularly sensitive to phenolic compounds. This sensitivity could be explained by the presence of flavonoids, polyphenols, and polyterpenes [23]. This activity depends on several factors, namely the plant species, the method chosen for the preparation of the extract, the solvent used and the sensitivity of the bacterial species [24]**.** The observed difference can be explained by the variation of the concentration of active ingredients and the solubilization of these active ingredients in ethanol.

On the other hand, it should also be noted that *P. aeruginosa* and *K pneumoniae* strains showed resistance to both extracts at concentrations of 200, 100, 50 and 25 mg/mL. It should be noted that overall, and especially in the case of the present study, most Gram-positive as well as Gram-negative bacteria were sensitive, with the exception of *P. aeruginosa* and *K pneumoniae* strains, which did not show inhibition in the presence of the aqueous and ethanolic extracts. Several works, in particular those of [25], have confirmed the high resistance of certain Gram-negative bacteria

compared to Gram-positive bacteria. This resistance of Gram-negative bacteria would be linked to the presence of their external membrane which would function as an effective barrier against biomolecules [26].

Analysis of these results reveals that the MIC values are consistent with those of the diameters of the growth inhibition zones. This is the case for the aqueous extract and the ethanolic extract on the E. coli strain with MICs of 6.25 and 12.5 mg/mL for 14 and 20 mm of inhibition zone respectively. For the Staphylococcus aureus strain, when the MIC = 25 mg/mL, we obtain 10 mm with the aqueous extract. Similarly for the ethanolic extract for an MIC = 12.5 mg/mL, the inhibition diameter is 18 mm. However, it should be noted that, the antimicrobial activities of secondary plant metabolites depend on several factors including the origin of the plant, the extraction methods, the nature of the solvent, the concentration of active compounds, the nature of the tests applied as well as the strains tested [27].

Overall, the greatest sensitivity was found with the ethanolic extract for Staphylococcus aureus and E. coli strains. It is difficult to compare our results with those of the bibliography because no similar scientific study has been conducted on the organ of this plant. The antibacterial activity may depend on the composition of the culture medium [28]. On the other hand, the BMC/MIC ratio was used to determine the bactericidal and bacteriostatic powers of plant extracts. According to [15] when this ratio is greater than 4, the extract is said to be bacteriostatic and bactericidal when it is less than or equal to 4. These results allow us to affirm that each extract showed a bactericidal power against E. coli, Staphylococcus aureus, and P. aeruginosa because the values of the BMC/MIC ratios are lower or equal to 4. But bacteriostatic with the aqueous extract on *E coli*.

5. Conclusion

This work has allowed to valorize not only the therapeutic virtues of *Lecaniodiscus cupanioides* Planch. (Sapindaceae) but also to identify its chemical constituents with pronounced activity for a better therapy. This study showed the richness of some chemical compounds and its antibacterial activity. It allowed to highlight the presence of polyphenols, flavonoids, saponosides, sterols and polyterpenes in the bark of the plant. Saponosides, anthocyanins, quinones, sterols and polyterpenes are absent in EAq and the same is true for alkaloids, anthocyanins and gallic tannins in EEth. On the other hand, the antibacterial activity showed that the ethanolic extract of *Lecaniodiscus cupanioides* bark is more active on most of the tested strains, especially on *Staphylococcus aureus* and *E. coli*. However, these two extracts were less effective on *P. aeruginosa* and *K pneumoniae*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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