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PHYTOCHEMISTRY AND STUDY OF ANTIMICROBIAL ACTIVITY OF FRACTIONS OF HYDRO ETHANOLIC EXTRACT OF *LECANIODISCUS CUPANIOIDES* (SAPINDACEAE)

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SUMMARY

Lecaniodiscus cupanioides is a plant of the family Sapindadaceae and the genus lecaniodiscus. It is a tree of 9 meters high with strong woody branches with flowers in fascicles in axillary cluster of purplish green color and perfumed with slender petioles. The species is present in tropical Africa from Sierra Leone to Sudan also in Angola to the South of the Democratic Republic of Congo and in Uganda. The species knows multiple uses in traditional medicine. In the form of inhalation, the bark is used to treat headaches, sinusitis, otitis, eye and ear problems. The leaves are reputed to be antibacterial and rubefacient. They are applied on boils, bruises, but are likely to cause burns if left on too long. It is to have scientific evidence of the use of these leaves that we wanted to study the phytochemistry and the anti microbial activity of the fractions of the hydro ethanolic extract of the dried leaves of the plant used in traditional medicine.

We prepared the hydro ethanolic extract from the dried powdered leaves of these plants and the yield of crude extract is 20%. We then fractionated the hydroethanolic extract obtained by the liquid-liquid extraction method using successively the following solvents of increasing polarity: cyclohexane, dichloromethane, ethyl acetate, and metanol. The flavonoids and polyphenols were determined by the colorimetric and Folin Ciocalteu methods in these different fractions and then the hydroethanolic extract and the fractions were tested on four reference microbial strains (*Esherichia coli* ATCC 25922; *Salmonella typhi* ATCC 14028; *Staphylococcus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 27853) The results showed

us that the polar fractions are very rich in polyphenols which confirms the results obtained during the phytochemical screnning carried out on the hydro ethanolic extract; and these fractions have a certain antimicrobial activity than the hydro ethanolic extract. The strongest bactericidal activity is observed in the ethyl acetate fraction of the hydro ethanolic extract. This plant could be an alternative in primary care systems for microbial infections.

KEYWORDS: *Lecaniodiscus cupanioides* Polyphenol Minimum Bactericidal Concentration Minimum Inhibitory Concentration.

INTRODUCTION

Infections have always been one of the major causes of mortality. Indeed, history is marked by epidemics or endemics whose social impact has been significant. The progress of medicine and hygiene, as well as the voluntarism of great scientists, have allowed a spectacular decline in infectious diseases. Antibiotic-resistant bacteria and their spread in human populations have become one of the most important infectious phenomena of the last twenty years. Antibiotic resistance is an aggravating factor in infections, which are also a major public health issue. In primary care, one third of pneumococci are now resistant to penicillin, whereas fifteen years ago they were largely susceptible. The prospects for the discovery of new classes of antibiotics are limited, and certain little-used antibiotics are no longer available or are in the process of disappearing, even though they will most certainly be useful in the near future.

Medicinal plants constitute an inexhaustible source of molecules with varied biological and pharmacological activity and an alternative to the use of synthetic antibiotics. For this reason we have studied *Lecaniodiscus cupanioides*, a plant with antimicrobial potentiality among the medicinal plants widely used in traditional medicine.

Lecaniodiscus cupanioides is a plant of the family Sapindadaceae and the genus lecaniodiscus. It is a tree of 9 meters high with strong woody branches with flowers in fascicles in axillary cluster of purplish green color and perfumed with slender petioles. The species is present in tropical Africa from Sierra Leone to Sudan also in Angola to the South of the Democratic Republic of Congo and in Uganda. The species knows multiple uses in traditional medicine. In the form of inhalation, the bark is used to treat headaches, sinusitis, otitis, eye and ear problems. The leaves are reputed to be antibacterial and rubefacient. They are applied on boils, bruises, but are likely to cause burns if left on too long.

MATERIAL AND METHOD

Plant material

The plant material consists of dried leaves of *Lecaniodiscus cupanioides* harvested in December 2021 in Abomey-Calavi. The plant was identified at the National Herbarium of the University of Abomey Calavi. The harvested leaves were washed and then dried at room temperature in a ventilated room of the Pharmacognosy laboratory for three weeks before being reduced to powder.

Extraction

The extraction was done for the hydro ethanolic extract by mixing 50g of powder in 500 ml of a hydro ethanolic mixture (40V/60V respectively) for 48 hours. After respective filtration on Whatman paper N°1 the filtrates obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in the oven for 48 hours at 40°C to obtain the dry extracts

Liquid-liquid extraction method

The liquid-liquid extraction is carried out by the intimate contact of the solvent with the solution in a separating funnel. The separation of the phases is obtained by gravimetric or centrifugal decantation after stirring of the whole. The solution consists of the hydroethanolic crude extract dissolved in 50 mL of distilled water. We used successively during the extraction 500 mL of cyclohexane, dichloromethane, ethyl acetate and methanol. The different fractions collected were evaporated with a rotavapor.

Phytochemical screening

The presence of the main chemical groups in the extracts was investigated using the tests described by Bassene (2012): flavonoids (Shibata test) tannins (Stiasny reaction followed by ferric chloride reaction), carotenoids (Carr-Price reaction), anthracenosides (Dragendorff Reagent), sterols (Libermann-Buchard reaction), cardiotonic heterosides 'Baljet, Kedde and Raymond-Marthoud reaction) and saponosides (**foam** index**)**

Polyphenol content

The polyphenol contents of the extracts are determined by the Folin - Ciocalteu method. In 1mL of the solution of each extract (10mg/mL) is added 1mL of Folin's reagent, then 3 minutes later 1 mL of sodium carbonate (Na_2C0_3) at 20% (2g in 10ml of distilled water). After 2 hours of incubation, the samples were centrifuged at 4000 rpm for 4 minutes. The

absorbances were then read with a spectrophotometer at nm730. Three trials were performed for each concentration of product tested.

A calibration curve from a dilution series of gallic acid (0.005-0.01-0.015-0.02-0.025-0.03-0.025-0.03-0.035-0.04 mg/ml) was treated in the same manner as the extracts. Results are expressed as milligram equivalent of gallic acid per gram of dry extract 'mg EAT/g).

Flavonoids assay

The flavonoid content of the extract was determined using the aluminum trichloride colorimetric method. A quantity of 100µL of the extract was mixed with 0.4 mL of distilled water and subsequently with 0.03 mL of 10% $ALCL_3$ solution was added. To the mixture 0.2 µL of $NaNO1M_2$ solution and 0.25 mL of distilled water were added after 5 min of rest. The mixture was vortexed and the absorbance was measured at 510 nm. The results are expressed as milligrams of catechin equivalent per g of dry plant material.

Bacterial material

Consisting of four reference strains provided by the Research Unit in Applied Microbiology and Pharmacology of Natural Substances.

Table 1: Bacterial material.

Strains	Reference
Pseudomonas aeroginosa	ATCC 27853
Salmonella Typhi	ATCC 14028
Staphylococcus aureus	ATCC 25923
Escherichia coli	ATCC 25922

Determination of the MIC in liquid medium

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of the substance for which there is no growth visible to the naked eye after an incubation time of 18 to 24 hours. Using a platinum loop, a quantity of bacterial strain previously preserved in Mueller Hinton agar was picked by simple scraping and then plated per quadrant on a regular agar plate and then incubated at 37°C for 18 to 24 hours in order to get isolated colonies. After this incubation time, 3 to 5 colonies were picked, plated in 10 mL of broth and incubated at 37°C for 3 to 5 hours. During this incubation time and in parallel, The concentration ranges of each plant extract were prepared using the liquid double dilution method with a geometric progression of extract concentrations. They generally varied from 0.781 mg/mL to 100 mg/mL. For each concentration range, 0.2 mL was taken and then deposited in a specific tube

of a series of experimental tubes. In this series called the test series, one tube served as a growth control (containing 0.2 mL of sterile distilled water). After 3 to 5 hours of incubation, 0.2 mL of the inoculated broth was removed and homogenized with a "VLEP Scientifica" vortex mixer in 20 mL of sterile Mueller Hinton broth. Then, 1.8 mL of the latter broth was taken to complete the volume (0.2 mL) of the test series tubes to 2 mL. Next to the test series, a reference series was prepared. In the latter, the experimental tubes each contained 0.2 mL of each concentration of plant extract previously prepared and the control tube 0.2 mL of sterile distilled water. To all the tubes of the reference series, 1.8 mL of sterile broth was added. The set of experimental tubes of the test series and the experimental tubes of the reference series were homogenized using a "VLEP Scientifica" type vortex shaker and then incubated at 37°C for 18 to 24 hours (Nassif et al., 1990; Okou et al., 2015). One day after incubation, the minimum inhibitory concentration (MIC) was determined by direct, naked eye, daylight reading. For the determination of this parameter, we compared concentration by concentration, the tubes of the test series with those of the reference series in search of absence of turbidity (Marmonier, 1990; Okou, 2012). This MIC determination was repeated during three successive experimental tests

Determination of the MBC in solid media

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of the substance that leaves no more than 0.01% surviving germs.

After MIC determination, the growth control tube of a given bacterial strain was diluted from 10 to 10^{-4}in a geometric progression of reason 10^{-1}. Then the various dilutions were plated on a Mueller Hinton agar plate, on 5 cm strips using a calibrated loop (Box A). To better appreciate the evolution of the sensitivity of the bacterial strains used in the presence or absence of plant extract, inocula obtained from a given bacterial strain were plated on a Mueller-Hinton agar plate on 5 cm streaks using a calibrated loop. The inocula plated were the inoculum from the growth control tube, inocula where turbidity was not visible, and some inocula preceding the tube that determined MIC (high bacterial load) (Box B). Finally, Boxes A and B were incubated at 37°C for 18 to 24 hours. After this incubation time, comparison of the number of colonies on the streak at dilution 10^{-4} of Box A with that of each streak of Box B allowed to determine the minimum bactericidal concentration. According to Marmonier in 1990:

- If the MBC/CMI ratio ≤ 4, the tested substance is bactericidal.

- If the MBC/MIC ratio > 4, the test substance is bacteriostatic.

RESULTS AND DISCUSSION

Extraction yields

Table 2: Fractionation efficiency of the crude extract of *lecaniodiscus cupanioides.*

Plant material	Extract	Mass	Yield
Crude extract (20g)	C_6H_6 Extract	0,45 g	2 ,25%
	C_2HCL_2 Extract	0,60 g	3 %
	AcOEt Extract	10,65 g	53,25 %
	MeOH Extract	15,22 g	76,1 %
	Aqueous Residu	9,25 g	46,25 %

Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins and saponosides in the hydroethanol extract of the plant Reducing compounds, quinonics anthracenosides, steroides, coumarins are also present in the plant extract; On the other hand, alkaloids, triterpenes, cardiotonic heterosides, anthocyanins, were not found in the plant extract which is the subject of the present study.

Total polyphenol content

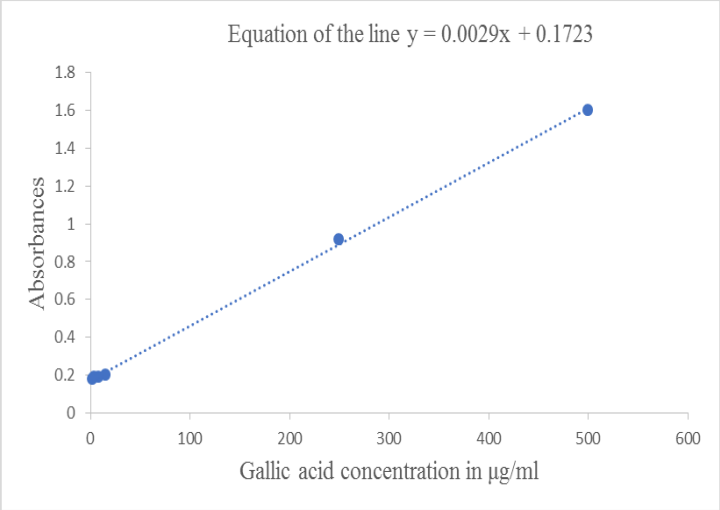
The determination of the total polyphenol content in the extract was done by the Folin-Ciocalteux method. The content was reported in mg gallic acid equivalent/g dry plant material. It is an extract rich in total polyphenols. This is confirmed by phytochemical screening which reveals the presence of flavonoids, tannins and saponosides in the extract.

Table 3: Total Flavonoid Concentration; Tannin; Total Polyphenols.

	Phenolic compounds dosage		
	PT (AGE)	FLA (EQ)	TAC (EC)
Lecaniodiscus cupanioides	203 ± 3,2	92,76 ± 3,42	143,61 ± 6,62

Table 4: Total polyphenol concentration (mg EAG/L) of the fractions of the hydroethanolic extract of *Lecaniodiscus cupanioides.*

Extracts	Total polyphenol concentration (mg EAG/L)
Raw Extract	203,62 ± 3,2
C_6H_6 Extract	25,9 ± 0,3
C_2HCl_2 Extract	82,9 ± 2,0
AcOEt Extract	703,7 ± 4,3
MeOH Extract	812,7 ± 1,8
Aqueous Extract	147,7 ± 2,8



Picture 1: Calibration line for gallic acid.

Table 5: Phytochemical screening of the hydro ethanolic extract.

Compounds		*Lecaniodiscus cupanioides*		Compounds	*Lecaniodiscus cupanioides*
Tannins	Gallics	+		Reducing compounds	+
	Catechics	+		Quinonics	+
Flavonoids		+		Mucilage	+
Anthocyanin		-		Free anthracenics	+
Leuco anthocyanin		+		O-heterosides	-
Saponosides		+		C- heterosides	+
Cyanogenic derivative		-		Cardiotonic derivatives	-
Triterpenes		+		Alkaloids	+
Steroids		+		Coumarins	+

Determination of the minimum inhibitory concentration (MIC) in liquid medium

In liquid medium the absence of turbidity was observed for the different strains studied from the concentrations of -1,25mg/L; 5mg/L; 1.25mg/mL; 0,625mg/L for the ethyl acetate fraction of the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively*;*

-2,5mg/L; 6,25 mg/L; 5 mg/L; 2,5mg/L for the methanolic fraction of the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively -3,25mg/L; 2,25mg/L; 3.125mg/L; 3.125mg/L for the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa* respectively;

We observed turbidity for the dichloromethane fraction of the hydroethanol extract for all concentrations.

Determination of the Minimum Bactericidal Concentration (MBC) in solid media

Comparison of the number of colonies on the streak at dilution 10^{-4} of box A with that of a streak of box B allowed to determine the concentrations of :

- 7 mg/L; 12 mg/L; 5mg/L; 2.5mg/L for the action of the ethyl acetate fraction of the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* strains respectively*;*

- 6,5 mg/L; 6.25mg/L; 12,5mg/L; 8,5mg/L for the action of the methanolic fraction of the hydro ethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa ; Staphylococcus aureus* respectively.

- 10 mg/L; 8 mg/L; 12.5mg/L; 12.5mg/L for the action of the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* respectively*;*

DISCUSSION

1. Phytochemical Screening and Determination of total polyphenols

We had polar fractions rich in polyphenols compared to the apolar fractions of the extract, which confirms their presence in the hydroethanolic extract

2. Determination of the antibacterial activity of different plant extracts

2.1 Determination of the minimum inhibitory concentration (MIC) in liquid medium

Insofar as the absence of turbidity was observed for the different strains studied from the concentrations of

-1,25 mg/L; 5mg/L; 1.25mg/mL; 0,2 65mg/L for the ethyl acetate fraction of the hydro ethanolic extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively*;*

- 2,5mg/L; 6,25 mg/L; 5 mg/L; 2,5mg/L for the methanolic fraction of the hydroethanol extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively

-3,25 mg/L; 2.25 mg/L; 3.125mg/L; 3.125mg/L for the hydro ethanolic extract of *Lecaniodiscus cupanioides* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* respectively*;*

It is possible to deduce that these concentrations constitute the minimum inhibitory concentrations (MIC) of these tested substances

2.2 Determination of the Minimum Bactericidal Concentration (MBC) in solid medium

The results in the table show that these different fractions tested have activity on these strains. Based on the MBC, the ethyl acetate fraction of the hydroethanol extract is more active on *Salmonella tyhi* (MBC equal to 12mg/L) and *Eschericha coli* strains than on *Pseudomonas aeruginosa* (MBC equal to 5mg/L) and *Staphylococcus aureus* strains.

The methanolic fraction of the hydro ethanolic extract of *Lecaniodiscus cupanioides* is more active on *Pseudomonas aeruginosa* (MBC equal to 12.5mg/L) and *Staphylococcus aureus* (MBC equal to 8,5mg/L) strains than *Salmonella tyhi* and *Eshericha coli* (MBC equal to 6.50 mg/L) strains.

The hydroethanol extract of *Lecaniodiscus cupanioides* is more active on *Pseudomonas aeruginosa* (MBC equal to 12.5mg/L) and *Staphylococcus aureus* strains than on *Escherichia coli* and *Salmonella typhi* strains (MBC equal to 8 mg/L).

On the basis of the comparisons of the MBC of the various extracts tested with those of the hydroethanolic extracts (MBC_{brut}/ $MBC_{ethyl\ acetate}$; MBC_{brut} / $MBC_{methanolic}$) and on the in vitro growth of the various strains studied it is possible to say that.

The ethyl acetate fraction is 5more bactericidal than the hydro ethanolic extract of the plant on the strain of *Staphylococcus aureus* and 2 times more bactericidal than the hydro ethanolic extract of the plant on the strain of *Pseudomonas aeruginosa.*

The methanolic fraction of *Lecaniodiscus cupanioides* has the same bactericidal power as the hydro ethanolic extract of the plant on the different strains studied.

The dichloromethane extract of the hydro ethanolic extract of the plant is neither bactericidal nor bacteriostatic on the different strains.

CONCLUSION

The phytochemical study of the crude extract of *Lecaniodiscus cupanioides* and its fractions from the liquid-liquid extraction showed a high content of total polyphenols; especially for the polar fractions. The results of the antibacterial activity study showed that the ethyl acetate

fraction of the hydro ethanolic extract of *Lecaniodiscus cupanioides* has a promising antibacterial activity on the studied strains.

This bactericidal action is dose-dependent because it is linked to the increase of the concentrations of the studied extract.

Table 6: Summary of the antibacterial parameters of the effects of the different extracts of *Lecaniodiscus cupanioides* on the in vitro growth of the strains studied.

		MICROBIAL STRAINS STUDIED			
		E. coli	***S. typhi***	***P. aeruginosa***	***S. aureus***
Ethyl acetate fraction *Lecaniodiscus cupanioides*	MIC (mg/mL)	1,25	5	1,25	0,625
	MBC (mg/mL)	7	12	5	2,5
	CMB/CMI	5,6	2,4	4	4
Methanolic fraction *Lecaniodiscus cupanioides*	MIC (mg/mL)	2,5	6,25	5	2,5
	BMC (mg/mL)	6,5	6,25	12,5	8,5
	CMB/CMI	2,6	1	2,5	3,4
Raw extract *Lecaniodiscus cupanioides*	MIC (mg/mL)	3,25	2,25	3,125	3,125
	BMC (mg/mL)	10	8	12,5	12,5
	CMB/CMI	3,07	3,05	4	4

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