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Research Article

PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF *EXTRACTS OF PARKIA BIGLOBOSA* (*MIMOSACEAE*) AND CARISSA EDULIS (APOCYNACEAE)

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Abstract

Parkia biglobosa (Jacq) Benth (Mimosaceae)and *Carissa edulis* (Apocynaceae)are two herbs that have been used traditionally for various conditions including hypertension and microbial diseases. Herbal remedies are an alternative in primary care systems and thus, a promising way for the development of traditionally improved medicines. We had studied in the present work the phytochemistry and anti-microbial activity of these two plants used in traditional medicine. We prepared the two hydro ethanolic extracts from the powdered dried leaves of these plants and the crude extract yields are 20% and 17% respectively. We then fractionated the hydro ethanolic extracts obtained by the liquid-liquid extraction method and assayed the flavonoids and polyphenols by the colorimetric and FolinCiocalteu methods in these different fractions and then tested the hydro ethanolic extracts and fractions on four reference microbial strains (Escherichia coli ATCC 25922; Salmonella typhi ATCC 14028; Staphylococcus aureus 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 screening; and these fractions have a more interesting bactericidal activity than the crude extracts (hydro ethanolic). These plants could be an alternative in primary care systems for microbial infections.

Keywords: *Parkia biglobosa Carissa edulis* Polyphenol Minimum Bactericidal Concentration Minimum Inhibitory Concentration.

INTRODUCTION

Bacterial infections are caused by different microorganisms and are the cause of the most fatal diseases and widespread epidemics. Many antibiotics are developed to treat them among which beta-lactam antibiotics are currently the most widely used throughout the world and especially in developing countries like Benin. They are used to such an extent because of their broad spectrum of action, safety, efficacy and especially their low cost. However, their abusive use is at the basis of the appearance of bacterial multiresistance. The control of bacterial infections is becoming complex as many bacteria have developed resistance to most antibiotics, which has become a major health problem worldwide. However, there is concern about the adverse effects of synthetic molecules intended to combat oxidative stress and bacterial infections. It therefore seems important to find an alternative to the use of conventional antibiotics. Herbal remedies are an alternative in primary care systems and therefore a promising way for the development of traditionally improved medicines. It is in this context that we wanted to study the phytochemistry and the anti-microbial activity of two plants used in traditional medicine, *Parkia biglobosa* and *Carissa edulis Parkia biglobosa* (Jacq) Benth. (Mimosaceae) *is a* tree of 10 m to 15 m high with a parasol-like port, with alternate bipinnate leaves traditionally used in febrile states and bronchitis for its leaves in decoction. The decoction of the bark used in gargle and mouthwash also calms toothache. *Carissa edulis* (Apocynaceae) is a thorny shrub with a height of over 5 m and a trunk of 30 cm in diameter. Its bark is brown with fibrous streaks and long spines. Its leaves are simple with veins having a white latex. *Carissa edulis* is used in traditional medicine, in food for humans and also as fodder. This plant is used in nine different categories of uses, the most frequent being for sexual weakness.

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MATERIALS AND METHODS

Plant material

The plant material consists of dried leaves of *Parkia biglobosa* and *Carissa edulis* collected in December 2020 in Abomey-Calavi. These two plants were identified at the National Herbarium of the University of Abomey Calavi. The leaves of the two plants were washed and 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 extracts by mixing 50g of powder in 500 ml of hydro ethanolic mixture (40V/60V respectively) for 48 hours. After respective filtration on Whatman paper N°1 the filtrate 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 crude hydroethanolic extract dissolved in 50 mL of distilled water. We used successively during the extraction 500 mL of cyclohexane, dichloromethane, ethyl acetate and butanol. The different fractions (phase from the operation containing the extracted solutes) collected are 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), anthracenes (Dragendorff reagent), sterols (Libermann-Buchard reaction), cardiotonic heterosides 'Baljet, Kedde and Raymond-Marthoud reaction) and saponosides (Foam index**)**

Polyphenol content

The polyphenol content of the extracts is determined by the Folin - Ciocalteu method. 1 mL of Folin's reagent is added to 1 mL of the solution of each extract, then 3 minutes later 1 mL of 25% sodium carbonate. After 2 hours of incubation, the samples were centrifuged at 4000 rpm for 4 minutes. The absorbances were then read with a spectrophotometer at 670 nm. Three tests were performed for each concentration of product tested. A calibration curve based on a dilution series of tannic 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 way as the extracts. The results are expressed as milligram equivalent of tannic acid per gram of dry extract 'mg ETA/g).

Determination of Flavonoids

The flavonoid content of the extracts was determined using the aluminium 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 ml of 1M $NaNO_2$ solution and 0, 25 ml of distilled water were added after a 5min rest. The whole 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

Strain	Reference
Pseudomonas aeruginosa	ATCC 27853
Salmonella Typhi	ATCC 14028
Staphylococcus aureus	ATCC 25923
Escherichia coli	ATCC 25922

Determination of MIC in liquid environment

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 transferred by quadrant onto a plain agar plate and incubated at 37°C for 18 to 24 hours to obtain isolated colonies. After this incubation time, 3 to 5 colonies were picked, inoculated 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 range from 0.781 mg/mL to 100 mg/mL. For each concentration range, 0.2 mL was taken and placed 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 tubes of the 2 mL test series. 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 reading, by eye, in daylight. 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 MBC in solid environment

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of the substance that leaves no more than 0.01% surviving germs. After the MIC determination, the growth control tube of a given bacterial strain was diluted from 10 to 10^{-4}in a geometric progression rate of 10^{-1}. The various dilutions were then 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 seeded on a Mueller-Hinton agar plate on 5 cm strips using a calibrated loop. The inoculaseeded were the inoculum from the growth control tube, the inocula where turbidity was not visible, and some inocula preceding the tube that determined the 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 the determination of the minimum bactericidal concentration. According to Marmonier in 1990:

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

RESULTS AND DISCUSSION

Table 2. Fractionation yield of the crude extract of *Parkia biglobosa*

Plant material	Extract	Mass	Yield
Crude extract (20g)	Extract C_6H_{12}	0.25 g	1.25%
	Extract CH_2CL_2	0.29 g	1.45%
	AcOEt extract	3.58 g	17.9%
	BuOH extract	3.4 g	17%
	Aqueous Extract	9.25 g	46.2%

Table 3. Fractionation yield of crude extract of *Carissa edulis*

Plant material	Extract	Mass	Yield
Crude extract (17g)	Extract C_6H_6	O.19g	1.12%
	Extract CH_2CL_2	0.25g	1.47%
	AcOEt extract	3.58g	21.06%
	Buol Extract	3.4g	20%
	Aqueous Extract	7.5g	44.12%

Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins and saponosides in the extracts of both plant species. Reducing compounds, anthracenes, steroids, coumarins are also present in both leaf extracts; however, alkaloids, triterpenes, cardiotonic heterosides, quinones, anthocyanins, were not found in the two plant extracts which are the subject of the present study.

Total polyphenol content

The determination of the total polyphenol content in both extracts was done by the Folin-Ciocalteux method for each extract. The content was reported in mg gallic acid equivalent/g dry plant material. These extracts are rich in total polyphenols. This was confirmed by phytochemical screening which revealed the presence of flavonoids, tannins and saponosides in both extracts.

Table 4. Total Flavonoïde ; Tanin ; Polyphénols

	Phenolic compounds dosage			Antiradical activity
	PT (AGE)	FLA (EQ)	Tac (EC)	DPPH (IC_{50})
Carissa edulis	180.0 ± 1.01	81.76 ± 3.42	43.61 ± 6.62	0.03
Parkia biglobosa	196.5 ± 1.32	77.52 ± 0.28	48.13 ± 0.66	0.09
Standards				
AG				0.03
BHA				0.09
Q				0.1

Table 5. Total polyphenol concentration (mg EAG/L) of the fractions of the hydro ethanolic extract of *Parkia biglobosa*

Extracts	Total polyphenol concentration (mg GAE/L)
Crude extract	99.7 ± 3.2
Extract C_6H_{12}	15.9 ± 0.3
CH_2Cl_2 extract	68.9 ± 2.0
AcOEt extract	303.7 ± 4.3
BuOH extract	212.7 ± 1.8
Aqueous Extract	157.7 ± 2.8

Table 6. Total polyphenol concentration (mg EAG/L) of the fractions of the hydro ethanolic extract of *Carissa edulis*

Extracts	Total polyphenol concentration (mg GAE/L)
Crude extract	275.6 ± 2.2
Extract C_6H_{12}	83.4 ± 1.3
CH_2Cl extract	57.8 ± 2.0
AcOEt extract	304.8 ± 3.3
BuOH extract	247 ± 1.5
Aqueous Extract	210.4 ± 1.8

Determination of the Minimum Inhibitory Concentration (MIC) in liquid environment

In liquid environment, the absence of turbidity was observed for the different strains studied from the concentrations of

- 6.25mg/L; 4.25mg/L; 3.125mg/mL; 6.5mg/L for the ethyl acetate fraction of *Carissa edulis*;
- 6.25mg/L; 6.25 mg/L; 3.125mg/ L; 3.125mg/L for the hydroethanolic extract of *Carissa edulis* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa* respectively;
- 25mg/L for the butanol fraction of *Carissa edulis*;
- We observed a lack of turbidity from the
- -6.25mg/L; 6.25mg/L; 1.56mg/L; 3.12mg/L for the ethyl acetate fraction of *Parkia biglobosa* on all.
- 3.12 mg/L for the hydroethanolic extract of *Parkia biglobosa* on all the strains used (*E coli*; *S typhi*; *P aeruginosa*; *S aureus*)*;*

Table 7. Phytochemical Screnning for the hydrothanolic extract

Compounds		*Parkia biglobosa*	*Carissa edulis*
Tannins	Gallic	+	+
	Catechin	+	+
Flavonoids		+	+
Anthocyanins		-	-
Leuco-anthocyanidins		+	-
Saponosides		+	+
Cyanogenic derivative		-	-
Triterpenes		-	-
Steroids		+	+
Reducing compounds		+	+
Quinonic		-	-
Mucilage		+	-
Free anthracene		+	+
O-heterosides		-	-
C- heterosides		+	-
Cardiotonic derivatives		-	-
Alkaloids		-	-
Coumarins		+	+

Table 8. Summary of the antibacterial parameters of the effects of the different extracts of *Carissa edulis* and *P. biglobosaon* the in vitro growth of the strains studied

		MICROBIAL STRAINS STUDIED			
		E. coli	***S. typhi***	***P. aeruginosa***	***S. aureus***
Ethyl acetate fraction *C. edulis*	MIC (mg/mL)	6.25	4.25	3.125	6.5
	MBC (mg/mL)	6.5	6.25	12.5	12.5
	MBC/MIC	1.04	1.5	4	2
Butanol fraction *C. edulis*	MIC (mg/mL)	25	50	25	50
	MBC (mg/mL)	50	50	100	100
	MBC/MIC	2	1	4	2
Crude extract *C. edulis*	MIC (mg/mL)	6.25	6.25	3.125	3.125
	MBC (mg/mL)	25	25	12.5	12.5
	MBC/MIC	4	4	4	4
Ethyl acetate fraction *P. biglobosa*	MIC (mg/mL)	6.25	6.25	1.56	3.12
	MBC (mg/mL)	25	25	6.25	12.5
	MBC/MIC	4	4	4	4
Butanol fraction *P. biglobosa*	MIC (mg/mL)	0.39	3.12	0.78	1.56
	MBC (mg/mL)	1.56	6.25	1.56	3.12
	MBC/MIC	4	2	0.02	2.3
Crude extract *P. biglobosa*	MIC (mg/mL)	3.12	3.12	4.56	3.1
	MBC (mg/mL)	50	25	6.25	6.25
	MBC/MIC	16	8	2.7	2

- 0.39 mg/L; 3.12mg/L; 0.78mg/L; 156mg/L for the butanol fraction of *Parkia biglobosa*on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively
- We observed turbidity for the dichloromethane fractions of both extracts for all concentrations.

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

- 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:
- 6.5mg/L; 6.25mg/L; 12.5mg/L; 12.5mg/L for the action of the ethyl acetate fraction of *Carissa edulis* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively*;*
- 25mg/L; 25mg/L; 12.5mg/L; 12.5mg/L for the action of the hydro ethanolic extract of *Carissa edulis;*
- 50 mg/L for the butanol fraction *Carissa edulis* on all strains used (*E coli*; *S typhi*; *P aeruginosa*; *S aureus*);
- 25mg/L; 25mg/L; 6.25mg/L; 6.25mg/L for the action of the ethyl acetate fraction of *Parkia biglobosa;*
- 25mg/L; 25mg/L; 12.5g/L; 6.25mg/L for the action of the hydroethanolic extract of *Parkia biglobosa;* respectively on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus*
- -1.56mg/L; 6.25mg/L; 1.56mg/L; 3.52mg/L for the action of the butanol fraction of the hydroethanolic extract of *Parkia biglobosa* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus,* respectively

DISCUSSION

Phytochemical screening and determination of total polyphenols

We have polyphenols rich polar fractions compared to the polar fractions of the two extract confirming their presence in the hydroethanolic extracts

Determination of the antibacterial activity of different plant extracts

Determination of the minimum inhibitory concentration (MIC) in liquid environment: Insofar as the absence of turbidity was observed for the different strains studied from the

- 6.25mg/L; 4.25mg/L; 3.125mg/mL; 6.5mg/L for the ethyl acetate fraction of *Carissa edulis*;
- 6.25mg/L; 6.25 mg/L; 3.125mg/L; 3.125mg/L for the hydro ethanolic extract of *Carissa edulis* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* respectively*;*
- 25mg/L for the butanol fraction of hydro ethanolic;
- We observed a lack of turbidity from the
- -6.25mg/L; 6.25mg/L; 1.56mg/L; 3.12mg/L for the ethyl acetate fraction of *Parkia biglobosa*;
- 3.12 mg/L; 3.12mg/L; 4.56mg/L; 4.56mg/L for the hydro ethanolic extract of *Parkia biglobosa* on *Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa; Staphylococcus aureus* respectively*;*
- 0.39 mg/L; 3.12mg/L; 0.78mg/L; 156mg/L for the butanol fraction of *Parkia biglobosa*;

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

Determination of the minimum bactericidal concentration (MBC) in solid environment:

The results in the table show that these different extracts tested have activity on these strains. On the basis of MBC, the ethyl acetate fraction of *Carissa edulis* is more active on *Escherichia coli* (MBC equal to 6.25mg/L) and *Salmonella typhi* strains than on *Pseudomonas aeruginosa* (MBC equal to 12.5mg/L) and *Staphylococcus aureus* strains. The butanol fraction of hydro ethanolic extract of *Carissa edulis* is more active on *Escherichia coli* (MBC equal to 50mg/L) and *Salmonella typhi* strains and less active on *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains (MBC equal to 100mg/L). The hydroethanolic extract of *Carissa edulis* 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 25mg/L). The *Pseudomonas aeruginosa* strain is more sensitive to the ethyl acetate fraction of hydro ethanolic of *Parkia biglobosa* (MBC equal to 1.56mg/L) than *Staphylococcus aureus* (MBC equal to 3.12mg/L) and *Escherichia coli* and *Salmonella typhi* (MBC equal to 25mg/L). The butanol fraction of hydro ethanolic extract of *Parkia biglobosa* is more active on the *Escherichia coli* (MBC equal to 1.56mg/L) and *Pseudomonas aeruginosa.* It is less active on *Salmonella typhi* (MBC equal to 6.25mg/L) and *Staphylococcus aureus* (3.12mg/L) strains. The hydroethanolic extract of *Parkia biglobosa* is more active on *Pseudomonas aeruginosa and Staphylococcus aureus* strains *(*MBC equal to 6.25mg/L) than on *Escherichia coli* and *Salmonella typhi* strains (MBC equal to 25mg/L). On the basis of the comparison of the MBCs of the various extracts tested with those of the hydro ethanolic extracts (MBC_{crude}/ $MBC_{ethyl\ acetate;}$$MBC_{crude}$/ MBCbutanoic MBC) and on the in vitro growth of the various strains studied it is possible to say that: the ethyl acetate extract of the two plants is 4 times more bactericidal than the hydro ethanolic extract of the plants on the *Escherichia coli* and *Salmonella typhi* strains

The ethyl acetate extract of *Carissa edulis* is 4 times more bactericidal than the crude (hydroethanolic) extract of *Carissa edulis* on *Escherichia coli and salmonella typhi* strains *and as bactericidal as* the crude (hydroethanolic) extract on *Pseudomonas aeruginosa and Staphylococcus aureus.* The ethyl acetate extract of *Parkia biglobosa* is 2 times more bactericidal than the crude (hydro ethanolic) extract of *Parkia biglobosa* on Escherichia coli and *Pseudomonas aeruginosa* and equally bactericidal on *Salmonella typhi and Staphylococcus aureus* strains. The butanoic extract of *Carissa edulis* is less bactericidal than the crude extract on *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* The butanoic extract of *Parkia biglobosa* is 4 times more bactericidal on *Salmonella typhi* strains, 2 times more bactericidal on *Staphylococcus aureus,* and 8 times more *Pseudomonas aeruginosa* and 32 times more bactericidal on *Escherichia coli* than the crude extract of *Parkia biglobosa.* The dichloromethane extracts of both plants are neither bactericidal nor bacteriostatic on the different strains.

Conclusion

The phytochemical study of the crude extracts of *Parkia biglobosa* and *Carissa edulis* and their fraction from liquid-

liquid extraction showed high content of total polyphenols; especially the polar fractions. The results of the antibacterial activity study showed that the different extracts of *Parkia biglobosa* and *Carissa edulis* have antibacterial activity on the studied strains. It is mainly the polar fractions of the two plants. This bactericidal action is dose-dependent because it is linked to the increase in the concentrations of the extract studied.

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