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Phytochemical composition, antimicrobial and cytotoxicity activities of *Parkia biglobosa* (Jacq) benth extracts from Benin

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Abstract
Parkia biglobosa is a plant used in traditional medicine for the treatment of certain diseases. In order to scientifically appraise some of the anecdotal, folkloric ethno medical uses of *P. biglobosa,* this study aimed at making phytochemical screening and evaluating some biological activities of *P. biglobosa* leave and bark extracts. These organs powdered were used for qualitative phytochemical screening and extractions with 4 solvents (dichloromethane, ethyl acetate, ethanol and water). Total Polyphenols content were determined by spectrophotometer method. The antibacterial activity was tested on 10 references strains and 9 meat isolated *Staphylococcus* by disc diffusion method. The DPPH method was used to evaluate extracts antioxidant activity. *Artemia salina* larvae were used to evaluate the extracts cytotoxic effects. The major phytochemical constituent of interests such as polyphenol, glycosides are found to be present in the both organ. Leave ethanolic extract have total polyphenolic content three times higher than those of bark ethanolic extract. Whereas, total flavonoïd content was approximativly the same for the two extracts. Susceptibility of *P. biglobosa* extracts with the reference strains varies ($p < 0.05$) according to the strains and extracts. The ratio of the two parameters (CMI and CMB) shows that the bark extracts are more active on the reference strains with the same bactericidal power (54.14%). The leave ethanolic extract shows a highest value (17.86 ± 0.60 mM) of ascorbic acid as an antioxidant, while the bark ethanolic extract shows the lowest (14.28 ± 0.95 mM). These results confirm some uses of *P. biglobosa* in traditional medicine and pave the way for further studies.

Keywords: Extracts, *Parkia biglobosa*, meat product, *Staphylococcus*, biological activity

Introduction
Since several years, plants have been used as valuable sources of natural products to maintain human and animal health. Despite the discovery of modern pharmaceuticals, plants remain recourse for traditional and natural medicine in several countries [1]. The interest of antimicrobial activity in plants has been established due to the resistance of microorganisms to antimicrobial agents. Indeed, there are microorganisms useful to humans and other pathogens that cause infectious diseases and collective foodborne illnesses [2].
Currently, foodborne illness is a public health problem and an important cause of mortality in developing countries [3] Several strains are responsible for this, such as those of the genus *Staphylococcus* and specifically the methicillin-resistant *aureus* species, which is one of the three most frequently, implicated bacteria in 90% of collective foodborne infections [3]. Unfortunately, the resistance of these bacteria to antibiotics is one of the most important problems of anti-infectious drugs in the world and in the pharmaceutical industry [4] because almost of the antibiotics are facing to this resistance phenomenon [5]. Even cases of resistance have been reported with the strains isolated to the consumption products [6]. Indeed, the control of bacterial infections becomes complex with conventional treatment [7].
To face this resistance problem, several tracks can be explored, including the formalization of endogenous knowledge through the traditional pharmacopoeia plants study. In this direction, several studies have shown that the pharmacopoeia plants contain secondary metabolites which confer their biological activities [8, 9]. With fewer side effects [10, 11]. *Parkia biglobosa* is used in traditional medicine as remedy for many diseases: dental caries, pneumonia, bronchitis, violent colic, severe cough, diarrhea, wounds, otitis, dermatoses, amoebiasis, hemorrhoids, bilharziosis, leprosy, hookworms, tracheitis, conjunctivitis [12, 13, 14]. Despite these medicinal virtues, very few scientific studies have focused on the chemical composition and biological activities of this plant. It is in this perspective that this study proposes to search the phytochemicals compounds and evaluate in vitro, the antimicrobial activity of the extracts of

P. biglobosa with the strains of the genus *Staphylococcus* isolated from the meat products and on some reference strains.

Material and methods

Tested microorganisms

The tested microorganisms include 10 references and 9 *Staphylococcus* meat isolated strains The 10 reference strains obtained from the National Laboratory for Quality Control of Medicines and Medical Consumables (Ministry of Heath, Benin) are *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilus* A24974, *Micrococcus luteus* ATCC 10240, *Proteus vulgaris* A25015, *Streptococcus oralis*, *Enterococcus faecalis* ATCC 29212, *Candida albicans* MHMR. The 9 *Staphylococcus* species (*S. sciuri, S. aureus, S. simulans, S. xylosus, S. cohnii, S. equorum, S. saprophyticus, S. haemolyticus* and *S. lentus*) use in this study were those isolated from three different meat products in Ivory Coast by [15]. and stored in the Laboratory of Biology and Molecular Typing in Microbiology (University of Abomey-Calavi, Benin).

Plant material collection

P. biglobosa bark and leaves were collected in the village of Pobe department of plateau, southern Benin. The plants materials were air dried at 25°C - 30°C for two weeks, grinded and sieved into a bark powder. The smooth powder was stored in airtight glassware and kept in darkness at -20°C until use.

Preparation of aqueous, ethanol ethyl acetate and dichloromethan extracts

The extract were obtained according to the method describe by [8] and [16] Briefly, the powder (50 g) of bark, leaf powder was macerated into 500 ml of each solvent (water, ethanol, ethyl acetate and dichloromethane**)** under agitator for 72 h at room temperature. The each homogenate was then filtered two times on absorbent cotton and once on Whatman N°1 paper (125 mm ø, Cat No 1001 125). For the aqueous extract the filtrate was dried in the oven at 40°C. While for the organic (ethanol, ethyl acetate and dichloromethan) extract the filtrate were concentrated in vacuum using a rotary evaporator (Heidolph Instruments GmbH & Co. KG No: 591-28000-00-1) to obtain the extract. All extracts were stored in labeled sterile bottles and kept at -20°C until further use.

Phytochemical screening

Preliminary qualitative phytochemical investigation

The bark and leaves powder were subjected to the qualitative phytochemical investigation to identify the major constituents (nitrogenous, polyphenolic and terpenic compound, and glycosides) was done according to Houghton et Raman (1998).

Quantitative phytochemical screening of the bark and leaves extracts

Total phenolics content

Total polyphenols were determined by using adapted Folin-Ciocalteu method as described by [17]. Briefly, the methanolic solution of each extract (10 mg/ml) was diluted to 1/100 with distilled water. 125µl of this solution was then mixed with 625 µl of Folin-Ciocalteu reagent (10%). After 5 min, 500 µl of aqueous sodium carbonate (Na_2CO_3 ; 75 g/l) were added. After 2h of incubation in dark at the room temperature, the absorbances were measured in triplicate at 760 nm against a blank (0.5 ml Folin-Ciocalteu and 1 ml of Na_2CO_3) with spectrophotometer (BIOMATES 3S). The total phenolics content was determined using the plotted standard calibration curve with gallic acid (0-10 mg/ml).

DPPH radical scavenging essay

DPPH scavenging effect of *P. biglobosa* extract was assessed by the method described by [19]. With slight modifications. A volume of 1.5 ml of the extract solution (10µg/ml) was added to 3 ml of the methanolic solution of DPPH (0.4 mg/ml). The mixture was lifted in a dark area at room temperature for 15 min, and then the absorbance was measured at 517 nm against the blank (1.5 ml of methanol and 3 ml of DPPH at 10 µg/ml). Radical scavenging activity was determined using calibration curve ($R^2 = 0.99$) with ascorbic acid (0-10 mg/ml).

Antimicrobial activity

Sensitivity test

The disc diffusion method [20]. was used to screen the antimicrobial activity. The *in vitro* antibacterial assay was done using Muller Hinton Agar (Oxoid, England). The dishes were prepared by pouring approximately 15 ml of molten media into sterile petri plates. The bacterial suspension turbidity adjusted to McFarland standard number 0.5, in Mueller Hinton Broth. With a sterile cotton swab bacterial culture was streaked on previously prepared Mueller Hinton agar dish. Dried and sterilized paper discs (6 mm of diameter) were treated with 25 µl of previously prepared *P. biglobosa* extract solution (20 mg/ml) using a micropipette under aseptic condition and placed at equidistance in a circle on the seeded dish. These dishes were kept for 15-30 min at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The dishes were then incubated at 37 °C for 24 and 48 hours.

After the incubation period, the dishes were examined for inhibitory zones (including the diameter of the disc) [21]. Each sample was used in triplicate for the determination of antibacterial and antifungal activity. Blank disc impregnated with solvent was used as negative control.

Determination of Minimum Inhibitory Concentrations (MIC)

The Minimum Inhibitory Concentrations (MIC) of crude extract of plants extract was performed by macrodilution method [22]. First, the extracts were diluted in sterilized distilled water to the highest concentration of 20 000 µg/ml and then nine dilution were performed to obtain the concentrations of 10 000 µg/ml, 5 000 µg/ml, 2 500 µg/ml, 1 250 µ/ml, 625 µ/ml, 312.5 µg/ml, 156.25 µg/ml, 78.12 µg/ml and 39.06 µg/ml in screw caped. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10^6 UFC/) to obtain 2 ml as a final volume. Culture medium without samples and others without microorganisms were used in the tests as control. Tubes were incubated at 37°C for 18-24 hours and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth (turbidity).

Minimum Bactericidal Concentration (MBC)

It was determined by sub culturing the test dilutions onto a fresh solid medium and incubated further for 18-24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC [23].

Cytotoxicity activity of the extracts

The cytotoxic effect of the extracts was evaluated according to an adaptation of the method described by [24]. The tests are carried out twice on 72 h larvae of *Artemia salina*. Briley, a test was constituted of 16 *A. salina* larvae in a 2 ml solution containing 1 ml of the extract tested concentration and 1 ml of sea. The number of surviving larvae is counted after incubation at room temperature (24 h) and the DL_{50} was calculated using the regression line obtained from the surviving larvae in function of the extracts concentration representation.

Statistical analysis

The results of the experience were expressed as mean ± standard deviation. Data were analyzed using Ducan test and ANOVA with the software SAS 9.2. p values less than 0.05 ($p < 0.05$) were considered significant.

Results

Phytochemical screening

The major phytochemical constituent of interests such as polyphenol, glycosides are found to be present (Table 2) in the both organ (bark and leaves) of *P. biglobosa*

Table 1: Phytochemical components of *P. biglobosa* bark and leave

Chemical compound	bark	leaf
Alkaloids	−	−
Tannins	+	+
Saponins (MI)	+ (167)	+ (167)
Anthocyanins	+	+
Flavonoids	+	+
Steroids	−	−
Triterpenes	−	−
Coumarin	−	−
Reducing compound	−	−
Glycosids	+	+
Cyanogenic derivate	−	−

MI : index moss

Polyphenolic content

The total polyphenolic of the extracts are showin in table 2. Leave ethanolic extract have total polyphenolic content three times higher than those of bark ethanolic extract. Whereas, content was approximativly the same for the two extracts.

Table 2: Total phenolic content of the *P. biglobosa* extracts

	Total phenolics (µg GAEq/mg)
Bark ethanolic extract	2.91 ± 0.5888
Leave ethanolic extract	6.54 ± 0.494

GAEq : Equivalent gallic acid

Susceptibility of *P. biglobosa* extracts with the reference strains

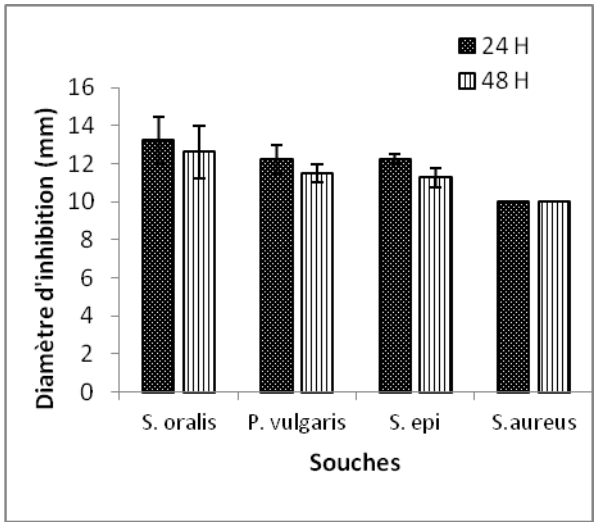
Susceptibility of *P. biglobosa* extracts with the reference strains

vary according to the strains and extracts (Table 3). Of the ten reference strains tested, only *S. epidermidis*, *S. aureus*, *S. oralis* and *P. vulgaris* were sensitive to the bark ethanolic and ethyl acetate extracts. On the other hand, at 20 mg / ml, the leaves ethanolic extract has no effect on the strains tested while the ethyl acetate extract of this organ inhibits the growth of 70% of the strains (Table 3). For this reason, only the bark ethanolic and ethyl acetate extracts and the leaves ethyl acetate extract were taken into account for the subsequent antimicrobial tests.

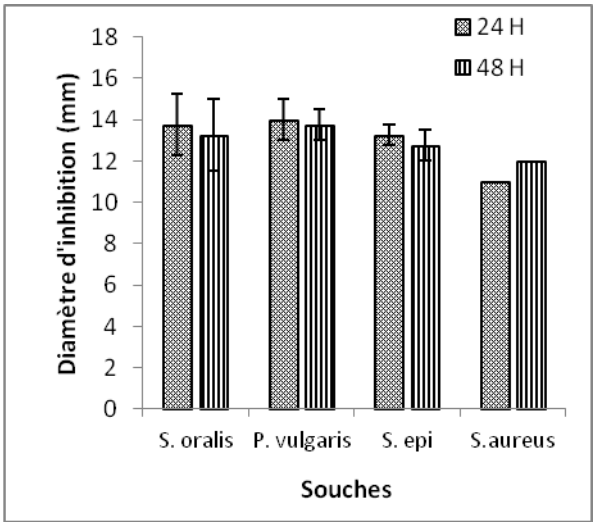
Table 3: Reference strains sensibility test to *P. biglobosa* extracts

Reference strains	ECET	ECAT	ECA	ECDCM	FET	FAT	FA	FDCM
S. epidermidis	+	+	-	-	-	+	-	-
P. vulgaris	+	+	-	-	-	+	-	-
S. oralis	+	+	-	-	-	+	-	-
S. aureus	+	+	-	-	-	+	-	-
C. albicans	-	-	-	-	-	-	-	-
E. foecalis	-	-	-	-	-	-	-	-
P. mirabilis	-	-	-	-	-	+	-	-
P. aeruginosa	-	-	-	-	-	+	-	-
M. luteus	-	-	-	-	-	+	-	-
E. coli	-	-	-	-	-	-	-	-

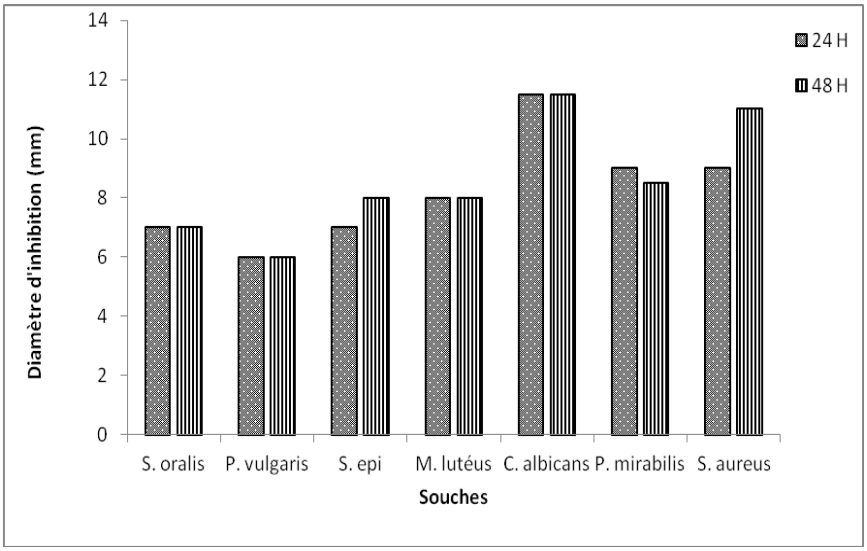
(+): inhibiton, (-) : no inhibition ; ECAT : bark ethyl acetate extract ; ECET : bark ethanolic extract ; ECDCM : bark dichloromethane extract; ECA : bark aqueous extract ;FET :leave ethanolic extract ;FAT :leave ethyl acetate extract ; FA :leave aqueous extract ; FDCM: leave dichloromethane extract. *S. aureus: Staphylococcus aureus, M. luteus: Miccrococcus luteus, S. epidermidis: Staphylococcus epidermidis, S. oralis: Streptococcus oralis, P. aeruginosa*: *Pseudomanas aeruginosa*, E. *foecalis*: *Enterococcus foecalis, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli, C. albicans: Candida albicans, P. mirabilis: Proteus mirabilis.*



bark Ethanolic extract



bark ethyl acetate extract



Leave ethyl acetate

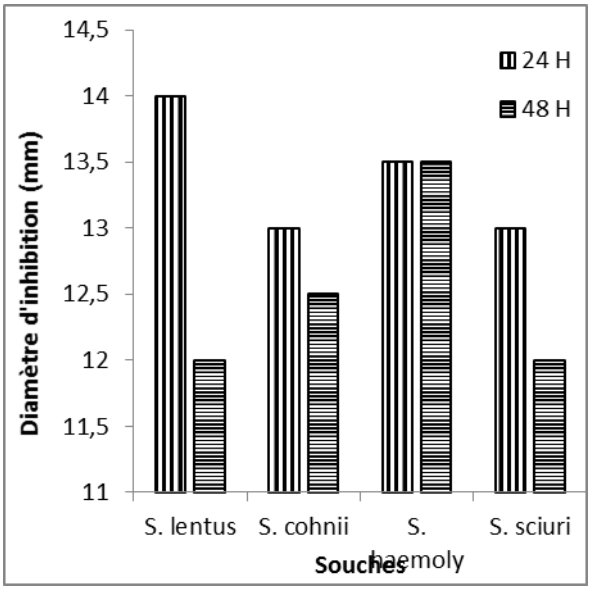
S. aureus: Staphylococcus aureus, M. luteus: Miccrococcus luteus, S. epidermidis: Staphylococcus epidermidis, S. oralis: Streptococcus oralis, P. aeruginosa: *Pseudomanas aeruginosa*, E. *foecalis*: *Enterococcus foecalis*, *P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli, C. albicans: Candida albicans, P. mirabilis: Proteus mirabilis*

Fig 1: Inhibitory diameter zone of bark ethanolic, ethyl acetate and leave ethyl acetate extracts of *Parkia biglobosa* with the reference strain.

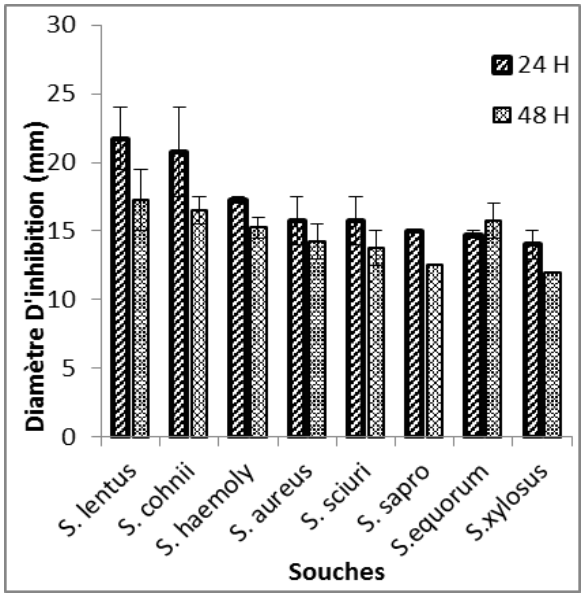
Susceptibility of meat isolated *Staphylococcus* strains with *P. biglobosa* extracts

The inhibitory diameters zone of the bark acetate and ethanol extracts vary ($p < 0.05$), mostly according to food strains (Figures 2a and 2b). On the other hand, for the leaves acetate extract (Figure. 2c), only *S. cohnii* diameters varying ($p > 0.05$) through the time (24h and 48h). It is apparent from the analysis that the bark ethyl acetate extract is less active (inhibiting 44.44% of the strains) than the ethanolic extract which inhibits the proliferation of 88.88% of the food strains. *S. lentus* was the most sensitive to both extract of the same organ. It should be noted that a remanence effect was observed after 48 hours with this same strain (*S. lentus*) for the two extracts of bark and with *S. sciuri* for the bark ethyl acetate extract.

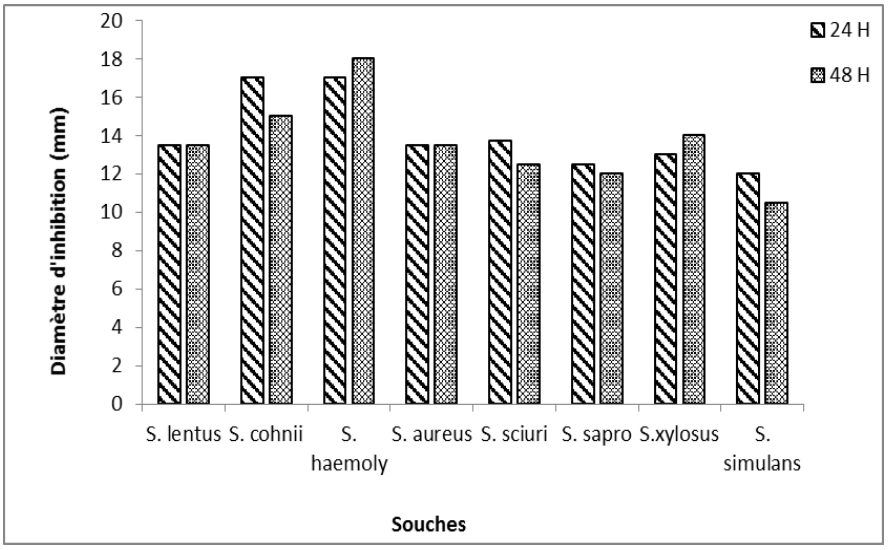
Moreover, the leaves ethyl acetate extract had the same spectrum activity as the bark ethanolic extract to inhibiting 88.88% of the meat isolated strains but with relatively less inhibitory powers (Figue. 2c). Contrary to the bark ethanolic extract *S. haemolyticcus* and *S. cohnii* showed the greatest inhibitory diameters to the leaves ethyl acetate extract. The remanence effect observed with the bark extracts with *S. lentus* and *S. sciuri* was also observed with the leaves ethyl acetate extract with *S. cohnii*.



a. Bark ethyl acetate extract



b. Bark ethanolic extract



c. Leave ethyl acetate extract

S. xylosus: Staphylococcus xylosus, S. lentus: Staphylococcus lentus, S. simulans: Staphylococcus simulans, S. sciuri: Staphylococcus. sciuri, S. cohnii: Staphylococcus cohnii, S. equorum: Staphylococcus. equorum, S.. saprophyticus: Staphylococcus. saprophyticus, S. haemolyticus: Staphylococcus. haemolyticus and *S. aureus* : *Staphylococcus. Aureus*

Fig 2: Inhibitory diameter zone of bark ethyl acetate (a), ethanolic (b) and leave ethyl acetate (c) extracts of *Parkia biglobosa* with meat *Staphylococcus* isolated strain.

Minimum Inhibitory and Bactericidal Concentrations of *P. biglobosa* extracts and their power action on the microorganisms studied

Like the inhibitory diameters, the Minimum Inhibitory and Bactericidal Concentrations of the active extracts vary according to the reference (Table 4) and meat *Staphylococcus* isolated strains (Table 5). From the analysis, it appear that for the reference strains, among the bark extracts, the ethyl acetate extract had the smallest MIC (0.156 mg / ml) with *S. aureus*, which remained lower than that (0.625 mg / ml) obtained with the leaves extract. The same trend was observed with meat *Staphylococcus* strains but with a concentration (0.078 mg / ml) twice small as that obtained with the reference strains. Overall, MICs obtained with bark extracts on meat *Staphylococcus* strains are lower than those of reference strains. On the other hand, the opposite effect was observed with leaf extract.

Table 4: Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of *P. biglobosa* extracts with reference strains

Strains		MIC (mg/ml)			BMC (mg/ml)			BMC /MIC		
	Extracts	ECAC	ECET	FAT	ECAC	ECET	FAT	ECAC	ECET	FAT
	P. vulgaris	0.625	0.625	1.875	0.625	1.25	5	1*	2*	2.667
	S. oralis	0.625	1.25	0.625	0.625	1.25	10	1*	1*	16
	S. aureus	0.156	0.625	2.5	0.312	1.25	5	2*	2*	2*
	C. albicans	-	-	0.625	-	-	1.25	-	–	2*
	P. mirabilis	-	-	0.625	-	-	5	-	–	8
	M. luteus	-	-	0.937	-	-	2.50	-	–	2.668
	S. epidermidis	0 .312	1.25	0.625	0.625	2.50	10	2*	2*	16

ECAT: Bark ethyl acetate extract; ECET : Bark ethanolic extract; FAT :Leave ethyl acetate extract; MIC= Minimum Inhibitory Concentration, BMC= Minimum Bactericidal Concentration, **With ***= Bactericidal effects and without *** = Bacteriostatical effects. *S. aureus: Staphylococcus aureus, M. luteus: Miccrococcus luteus, S. epidermidis: Staphylococcus epidermidis, S. oralis: Streptococcus oralis*, *P. aeruginosa*: *Pseudomanas aeruginosa*, E. *foecalis*: *Enterococcus foecalis, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli, C. albicans: Candida albicans, P. mirabilis: Proteus mirabilis*

The lowest CMB (0.312 mg / ml) was obtained with the bark ethyl acetate extract both with the reference strains (*S. aureus*) and the meat *Staphylococcus* strains (*S. saprophyticus*). With the exception of *S. equorum* strain, the strains with the lowest MIC also have the lowest MBC (Table 4). Contrary to the observation made with the MICs, the CMBs of the meat *Staphylococcus* strains are globally superior to those obtained with the reference strains. Indeed, *S. lentus, S. xylosus, S. sciuri* and *S. haemolyticus* strains had no CMB with the bark extracts at the dose (20 mg / ml) tested (Table 5).

The ratio of the two parameters (CMI and CMB) shows that the bark extracts are more active on the reference strains with the same bactericidal power (54.14%). With its MICs and CMBs more high than those of bark, the leaves ethyl acetate extract has nevertheless a bactericidal effect with two reference strains (*S. aureus* and *C. albicans*). Despite the fact that bark extracts have the lowest CMB with meat *Staphylococcus* strains, they show only bacteriostatic effects on these strains. On the other hand, the leaves ethyl acetate extract has a bactericidal effect with 33.33% of the strains (Table 5).

Table 5: Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of *P. biglobosa* extracts with meat *Staphylococcus* strains

Strains		MIC (mg/ml)			BMC (mg/ml)			BMC/MIC		
	Extracts	ECAC	ECET	FAT	ECAC	ECET	FAT	ECAC	ECET	FAT
	S. lentus	0 .312	0.625	2.50	20	>20	10	64	–	4
	S. xylosus	0.3125	-	2.50	>20	-	10	-	–	4
	S. aureus	0.3125	-	2.50	20	-	10	64	–	4
	S. sciuri	0.3125	1.25	2.50	20	>20	5	64	–	2*
	S. cohnii	0.625	0.156	2.50	10	0.625	2.5	8.01	4	1*
	S. equorum	0.078			0.625	-	-	16	–	-
	S. simulans	-	-	2.50	-	-	5	-	–	2*
	S. saprophyticus	0.078	-	0 .625	0.312	-	2.5	4	–	4
	S. haemolyticus	0.625	0.625	2.50	20	>20	10	32	–	4

ECAT: Bark ethyl acetate extract; ECET : Bark ethanolic extract; FAT :Leave ethyl acetate extract; MIC= Minimum Inhibitory Concentration, BMC= Minimum Bactericidal Concentration, **With ***= Bactericidal effects and without *** = Bacteriostatical effects. *S. xylosus: Staphylococcus xylosus, S. lentus: Staphylococcus lentus, S. simulans: Staphylococcus simulans, S. sciuri: Staphylococcus. sciuri, S. cohnii: Staphylococcus cohnii, S. equorum: Staphylococcus. equorum, S.. saprophyticus: Staphylococcus. saprophyticus, S. haemolyticus: Staphylococcus. haemolyticus* and *S. aureus* : *Staphylococcus. aureus*

DPPH radical scavenging activity

The result display in table 6 show the *P. biglobosa* extracts free radical scavenging activity, which was carried out by using the DPPH assay. The leave ethanolic extract shows a highest value of ascorbic acid as an antioxidant, while the bark ethanolic extract shows the lowest.

Table 6: Ascorbic acid content of *P. biglobosa* extract

Ascorbic acid content (mM)	
Bark ethanolic extract	14.28 ± 0.95
Leave ethanolic extract	17.86 ± 0.60

Cytotoxicity assay of *P. biglobosa* extracts

The regression line of each curve show that the survival of *A. salina* larvae respects a dose-response relation. Thus, with the different concentration of the extracts, the cytotoxicity assay shows the variation on the lethal dose (LD_{50}) according to the extract. Indeed, the lowest lethal dose (10.10 mg/ml; R^2 = 0.99) was obtained with the bark ethanolic extract while the highest (19.9 mg/ml, R^2 = 0.99) was obtained with the leave ethanolic extract.

Discussion

P. biglobosa which is a plant used in Benin traditional pharmacopoeia has shown variable antibacterial activities according to the extracts. Several studies relating to antimicrobial activity have been conducted with different plant species and findings have shown that medicinal plants can be used as alternative to modern medicine [25]

Among the tested extracts, only the ethanolic and ethyl acetate extracts inhibited the microorganisms growth while the aqueous and dichloromethane extracts had no effect on the tested strains growth. Similar observations on the inactivity of the dichloromethane extract have already been made by other authors such as [26] which showed that the dichloromethane extract of *P. biglobosa* bark at 20 mg / ml had no inhibitory action with *Staphylococcus aureus* isolated from various infections (Pus, vaginal specimens, sperm, uroculture, etc.). This observation can be explained by the affinity of phytomolecules for solvents. Indeed, the solvent dichloromethane would not concentrate in sufficient quantity the antimicrobial active ingredients contained in this plant. On

the other hand, [27] showed that *P. biglobosa* leaves aqueous extract at 100 mg / ml exerts an inhibitory effect against *Staphylococcus aureus* (NCTC 6571) and *Bacillus cereus*. The concentration 5 times higher than that used in our study, may be at the origin of this difference. In addition, it may be related to the origin of the strains used.

Considering the type of solvent used, the ethanolic extracts are less effective than the ethyl acetate extracts with the studied strains. The high efficiency observed with the acetate extracts is similar to the results observed by [28] with *Terminélia glauscesens* ethyl acetate extract against *Salmonella typhimurium*. From these observations the antibacterial substances contained in *P. biglobosa* are then more soluble in ethyl acetate than the water. With meat isolated *Staphylococcus* strains, the leaves ethyl acetate extract is more effective than the bark extract. This observation is contrary to that of [29], which, through a comparative study between the leaves and bark extracts of *P. biglobosa* with the enteropathogenic bacteria and staphylococci, showed that the bark extracts are much more effective than those of leaves on some enterobacteria and *Staphylococcus aureus*. This difference can be explained by difference of physiological characteristics of a plant according to its maturity, the soil nature and the environmental microclimate [30]. These factors are very important in the chemical principles biosynthesis and, therefore, influence the plant pharmacological activity [30].

In relation to the tested microorganisms, Gram + bacteria, composed of meat isolated strains and reference strains, are more sensitive to the different extracts than Gram-bacteria. The same observation was made by [31] in Nigeria. These results can be explained by the fact that, in their structure, Gram-bacteria have one membrane more than those with Gram +. The Gram- bacteria wall is more complex and constitute of succession of capsular proteins, lipid bilayer, a periplasmic space before the peptidoglican. However, antimicrobial substances act mainly on the synthesis of the wall micopeptides (peptidoglycans) by pertubation or inhibition of certain enzymes such as peptidoglycalle synthetase [32]. Thus, this additional external membrane observed on Gram- could be the factor responsible for the difference in susceptibility observed between Gram + and Gram- bacteria with *P. biglobosa* extracts.

Conclusion

The present work made enable to underline the antimicrobial properties of *P. biglobosa* leaves and bark extracts. *P. biglobosa* have low-dose antimicrobial activity with meat isolated Staphylococcus strains as well as on reference strains. These extracts (acetate and ethanol) have bactericidal and bacteriostatic effects. This study, beyond its long-term objectives of revealing new anti-infectious molecules, is above all a validation of the use of this plant in traditional medicine. Thus, *P. biglobosa* extracts may be considered after further complementary studies in ethnopharmacological use for the treatment of food poisoning.

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