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Research Article

ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF TETRAPLEURA TETRAPTERASCHUMACH. THONN. (FABACEAE)

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ABSTRACT
Facing the recrudescence of infectious diseases due to the resistance of bacteria because of the abusive use of conventional antibiotics, it appears urgent to propose other more effective molecules based on plants. It is in this perspective that the present study was initiated in order to verify the antibacterial activity of aqueous extracts of fruits and barks of the trunk of *Tetrapleura tetraptera Tchum* on the *in vitro* growth of *Escherichia coli* and *Staphylococcus aureus* strains. This work was devoted to the phytochemical study and to the demonstration of antibacterial activity by the methods of diffusion in solid medium and dilution in liquid medium. From the phytochemical study, it appears that the bark of the trunk and the fruits of *Tetrapleura tetraptera Tchum* contain polyphenols, flavonoids and saponins. Alkaloids and tannins are only present in the fruits. Microbiological tests showed that in solid and liquid media, the extract from the maceration of the bark is active on all the germs studied. The largest diameters obtained with this extract were 19,33 mm on *E. coli* and 18,66 mm on *S. aureus* at 400 mg/mL. Depending on the germs, this extract presents MICs ranging from 3,125 to 25,00 mg/mL and BMCs ranging from 6,25 to 25,00 mg/mL. The BMC/MIC ratios obtained indicate that the extract is bactericidal

Keywords*: Tetrapleura tetraptera, In Vitro, Growth, Antibacterial Activity, Aqueous Extracts*

INTRODUCTION
Infectious diseases are one of the leading causes of morbidity and mortality in the world, especially in developing countries (Yala *et al.*, 2001). The agents responsible for these infections are diverse and varied including fungi, bacteria, protozoa that viruses (Yala *et al*., 2016*).* This resurgence of infectious diseases is due to the numerous resistance mechanisms that microorganisms have developed, particularly bacteria, due to the massive and sometimes abusive use of antibiotics (Yala *et al*., 2001). The genetic instability of bacterial strains and the difficulties related to the limitations of antibiotic therapy diagnostic tools are the basis of therapeutic failures (Okou *et al., 2018*). For the fight against the many therapeutic failures, the traditional plant-based pharmacopoeia remains one of the most coveted avenues. Indeed, plants have long been used as an essential source of nutrients and medicines, without knowing what their beneficial actions were due to (Mosa'd, 2012). Given their important and primary roles in the case of health care, medicinal plants are an alternative or at least a complement to modern medicine. However, the problem related to the use of traditional herbal remedies is the lack of knowledge regarding the mode of action, active ingredients, doses to be used, indications and quality control (Golly, *et al*., 2012). It is to contribute to a

thorough knowledge and rational use of traditional plant medicine that this study was conducted. It consisted in verifying the antibacterial activity of extracts of *Tetrapleura* fruits and barks on the in vitro growth of *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Bacterial strains

The bacterial strains used in this study consisted of six strains: one Cefoxitin-sensitive *Staphylococcus aureus* strain (*S. aureus* 1174), one Cefoxitin-resistant *Staphylococcus aureus* strain (*S. aureus*1225C/19), two Cefoxitin-sensitive *Escherichia coli* strains (*E. coli* 1219 and 1178), and two reference strains *S. aureus* ATCC 29213 Cefoxitin-sensitive and *E. coli* ATCC 25922 Cefotoxin-sensitive. All these strains were provided by the bacteriology department of the Institut Pasteur de Côte d'Ivoire (IPCI).

Plant material

The plant material used consisted of the bark of the trunk and fruits of *Tetrapleura tetraptera*. These organs were harvested in August 2020 in the department of Abengourou in the east of Côte d'Ivoire. After harvesting, the plant was identified at the Centre National de Floristique of the University Félix Houphouët-Boigny in Abidjan-Cocody as corresponding to herbarium N° 8816. The collected plant parts were cut into fine pieces, washed and dried at room temperature (27± 2°C), protected from the sun for one month. After drying, with the help of an electric grinder, the different organs were pulverized to obtain plant powders packaged in polyethylene bags and stored at 4°C until the preparation of the different aqueous extracts.

Preparation of aqueous extracts of T. tetraptera

The different extracts were prepared by maceration, decoction and infusion. According to the method of preparation of extracts by maceration (Zihiri *et al.,* 2003, 100 g of plant powder of the trunk bark or fruit of *T. tetraptera* was dissolved in one liter of distilled water and cold macerated for 24 hours. For extracts by decoction, the mixture of plant powder and distilled water (100 g in 1L) was first heated for 15 min before being homogenized by maceration for 24 hours. Concerning the extracts by infusion, 100 g of plant powder were dissolved in one liter of distilled water, previously boiled and then homogenized by maceration for 24 hours. After homogenization of each mixture, the homogenates were successively filtered three times on absorbent cotton and once on Whatman 3 mm filter paper. Each filtrate was frozen and then freeze-dried to obtain the different total aqueous extracts which were kept in the refrigerator at 4° C for bacteriological tests.

Study of the antibacterial activity

The study of the antibacterial activity was made following two methods:

- the diffusion method in solid medium to study the efficiency of the extracts;
- the dilution method in liquid medium to determine the antibacterial parameters (MIC : Minimum Inhibitory Concentration and MBC : Minimum Bactericidal Concentration).

Efficacy tests of plant extracts in solid media

The efficacy test is used to detect the antimicrobial activity of a substance. For this test, Mueller Hinton agar was the main culture medium (Soro *et al*., 2010; Golly *et al*., 2012; Outtara *et al*., 2016). For extract preparation, three concentrations (400 mg/mL ; 200 mg/mL ; 100 mg/mL) of each extract were obtained with sterile distilled water. The tests were performed on a bacterial inoculum of 5.106 CFU/ mL. For the efficiency test, the well method was used. Indeed, using a Pasteur pipette, wells were made in agar plates previously inoculated with E. coli or S. aureus bacterial inocula. A 60 µL volume of each concentration of plant extract to be tested was introduced into each well. The inoculated dishes were incubated in an oven at 37 °C for 18 to 24 hours. These tests were repeated three times. The observation of a zone of bacterial growth inhibition indicates the existence of antimicrobial activity. The diameter of the zone of inhibition is used to judge the effectiveness of the extract. Control wells inoculated with 60 µL of the extract preparation solvent were used to judge the effect of the solvent on the germs. In parallel, comparative

tests with cefoxitin (FOX-30 µg) *for S. aureus* and *E. coli* were performed under the same conditions.inimum Inhibitory Concentration and MBC: Minimum Bactericidal Concentration).

Test to determine the antimicrobial parameters of the extracts

The antimicrobial parameters that are the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were determined using the liquid dilution method (Soro *et al*., 2010; Golly *et al*., 2012; Ouattara *et al*., 2016). A concentration range of plant substances was prepared by the double dilution method with concentrations ranging from 200.00 to 1.56 mg/mL. The tests were performed by introducing into a series of hemolysis tubes 1 mL of the plant substance solution and 1 mL of the bacterial inoculum described according to Moro *et al*, (2008). Instead of plant extract, the control tube received 1 mL of the extract preparation solvent and 1ml of the bacterial inoculum. The whole set was incubated at 37°C for 18 to 24 hours. The results were read by daylight and with the naked eye. The clearness of the medium implies the antimicrobial effect of the tested extract while the presence of a cloudiness indicates its inefficiency (sign of bacterial growth). The Minimum Inhibitory Concentration (MIC) will correspond to that of the first clear tube. The Minimum Bactericidal Concentration (MBC), is the lowest concentration of extract that kills at least 99.99% of bacteria in culture. For its determination, the contents of the control tube were diluted to 10^{-4}, which corresponds to 0.01% survival of bacteria in culture. The clear contents of the experimental tubes from the MIC were transferred by 5 cm streaks onto Mueller Hinton agar and incubated at 37 °C for 24 hours. The first experimental tube in which the number of germs determined is less than or equal to that of the 10^{-4} dilution will correspond to the BMC.

Phytochemical study

Phytochemical study of T. tetraptera trunk fruits and barks, based on staining and/or precipitation tests, was carried out on the aqueous extracts of the fruit and barks crush (Oyetayo *et al*., 2007; Reza *et al*., 2007; Tiwari *et al*., 2011; Ashafa and Umebese, 2012). The families of molecules targeted by this study are saponins, tannins, polyphenols, flavonoids, alkaloids, steroids, terpenoids and quinones.

Antibacterial activity of the extracts on the in vitro growth of germs

Activity in solid medium

Efficiency tests carried out before determination of the antibacterial parameters of the extracts at concentrations of 400, 200 and 100 mg/mL led to the results summarized in Table I. These results are expressed by the inhibition diameters of the extracts. The Bark Maceration (BM) extract is active on all S. aureus strains at the different concentrations (400 mg/mL, 200 mg/mL and 100 mg/mL) with respective diameters of:

- 19.00 mm, 16.33 mm and 13.00 mm for *S. aureus* strain ATCC29213 ;
- 18.66 mm, 16.33 mm and 13.00 mm for *S. aureus* strain 1225C/19 and
- 14.33 mm, 12.00 mm and 10.66 mm for *S. aureus* strain 1174C/19.

On the other hand, on the E. coli strains tested, the extract (ME) is active only on the in vitro growth of the *E. coli* 1178C/19 strain. The observed inhibition diameters vary from 19.33 mm to 13.00 mm for concentrations of 400 mg/mL, 200 mg/mL and 100 mg/mL respectively. For all the germs studied, the different fruit extracts (MF, DF, IF) were active only on the single strain of E. coli1178C/19 with respective diameters of:

- 9.66 mm, 7.33 mm and 6.33 mm at concentrations 400 mg/mL, 200 mg/mL and 100 mg/mL for the Fruit Maceration (MF) ;
- 10.00 mm, 6.66 mm, at concentrations 400 mg/mL and 200 mg/mL for Fruit Decoction (FD) and
- 10.66 mm and 9.00 mm at concentrations 400 mg/mL and 200 mg/mL for Fruit Infusion (IF). DF and IF extracts were not active at the 100 mg/mL concentration.

Cefoxitin 30 µg/mL inhibited the growth of all strains with inhibition diameters ranging from 15.00 to 30.00 mm.

Table 1: Inhibition diameters of extracts and antibiogram against microbial strains

Extracts	ME			MF			DF			IF			EDS	Céfoxitine
Concentrations	400 mg/mL	200 mg/mL	100 mg/mL	400 mg/mL	200 mg/mL	100 mg/mL	400 mg/mL	200 mg/mL	100 mg/mL	400 mg/mL	200 mg/mL	100 mg/mL	0 mg/mL	(30 µg)
E. coli ATCC25922	**0**	0	0	0	0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**
E. coli 1219	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**
E. coli 1178	**19.33**	**15**	**13**	**9.33**	**7,33**	**6.33**	**11.33**	**6.66**	**0**	**11.33**	**9.00**	**0**	**0**	**20**
S. aureus ATCC29123	**19**	**16,33**	**13**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**30**
S. aureus 1225C/19	**18.66**	**16.33**	**13**	**0**	**0**	**0**	**9,33**	**0**	**0**	**0**	**0**	**0**	**0**	**15**
S. aureus 1174	**14.3**	**12.66**	**10,66**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**29**

ME : Maceration of the Barks, MF : Maceration of the Fruits, DF : Decoction of fruits, IF : Infusion of Fruits, EDS : Sterile Distilled Water

Antibacterial parameters of the extract by maceration of barks (ME) in liquid medium

Considering the good activity of the ME extract in solid medium, it was retained for the determination of antimicrobial parameters in liquid medium. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), as well as the MBC/MIC ratio of the action of the extract (ME) of *T. tetraptera* on the *in vitro* growth of the different germs studied are recorded in Table 2. The analysis of the results shows that the extract (ME) inhibits the *in vitro* growth of all S. aureus strains with MICs of :

- 12.50 mg/mL for the reference strain S. aureus ATCC29213 ;
- 12.5 mg/mL for S. aureus strain 1225C/19;
- 25 mg/mL for S. aureus strain 1174C/19

For *E. coli* strains, the MIC was 3.125 mg/mL for E. coli 1178C/19 and greater than 100 mg/mL for the other two *E. coli* strains.

The minimum bactericidal concentrations (MBC) of the extract (ME) on the S. aureus strains are equal to the MICs.

On the other hand, on the *E. coli*1178C/19 strain its MBC is 6.25 mg/mL and higher than 100 mg/mL on the *E. coli* 1219C/19 and reference *E. coli* strains. The result of the BMC/MIC ratio is 1 for all *S. aureus* strains and 2 for the *E. coli* 1178C/19 strain. This ratio is indeterminate for the other two *E. coli* strains.

Table 2 : Antimicrobial parameters of the bark extract (ME) of *T. tetraptera* on the studied germs

	Bacterial strains					
	***E. coli* ATCC25922**	***E. coli* 1219**	***E. coli 1178***	***S. aureus* ATCC29213**	***S. aureus* 1225C/19**	***S. aureus* 1174**
CMI (mg/mL)	>100	>100	3,125	12,5	12.5	25
CMB (mg/mL)	>100	>100	6.25	12.5	12.5	25
BMC/MIC	**>1**	**>1**	**2**	**1**	**1**	**1**
Bactericidal effects	-	-	**Bactericide**	**Bactericide**	**Bactericide**	**Bactericide**

Secondary metabolite composition of T. tetraptera extracts

Table 3 below presents the results of the secondary metabolite composition of *T. tetraptera* extracts. From the analysis of the table, it appears that the different extracts of *Tetrapleura tetraptera* fruits (maceration (MF), infusion (IF) and decoction (DF)) contain alkaloids, tannins, polyphenols, flavonoids and saponins. The extract of the trunk bark of *T. tetraptera* (ME) is rich in polyphenols, flavonoids and saponins. Quinones and sterols are absent in all extracts of *T. tetraptera*.

Table 3 : Secondary metabolite composition of aqueous extracts of bark and fruit of *T. tetraptera*

Secondary metabolites	Aqueous extracts of T. tetraptera			
	DF	IE	MF	ME
Alkaloids	+	+	+	-
Polyphenols	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Saponins	+	+	+	+
Stéroids et Terpénoids	-	-	-	-
Quinones	-	-	-	-

ME : Maceration of the Barks, MF : Maceration of the Fruits, DF : Decoction of fruits, IF : Infusion of Fruits, EDS : Sterile Distilled Water,

DISCUSSION

The study of the antibacterial activity of the different extracts carried out in solid medium by the diffusion method led to the determination of the most active extract. The study in liquid medium by the method of double dilutions allowed to obtain the antimicrobial parameters of this extract. Indeed, the diffusion method in agar medium is a pre-test or an efficacy test that allows the detection of the existence of antibacterial activity of a substance (Oussou *et al*., 2008). Although it is recognized as reliable and reproducible, it is mainly preliminary to the double dilution method, as it allows access to essentially qualitative results (Hayes and Markovic, 2002). The first method thus allows to choose among the extracts those that should be used in the second method for the determination of their antimicrobial parameters.

The diffusion method revealed that on *S. aureus* strains, the *T. tetraptera* fruit infusion (IF) extract was active on the single *S. aureus* strain 1225C/19 at the concentrions of 400 mg/mL with a mean inhibition diameter of 9.33 mm. But the other extracts from the fruit (maceration and decoction) of *T. tetraptera* had no effect on the *in vitro* growth of the three strains of *S. aureus* at the concentrations of 100 mg/mL, 200 mg/mL and 400 mg/mL. In contrast, *T. tetraptera* trunk bark maceration (TMB) extract inhibited the growth of all *S. aureus* strains with mean inhibition diameters ranging from 10.66 mm to 19.00 mm. All *T. tetraptera* extracts inhibited the growth of *E. coli* strain 1178C/19 with higher inhibition diameters recorded with the ME extract (19.33 mm, 15.00 mm and 13.00 mm). The *in vitro* growth of *E. coli* 1119 and *E. coli* ATCC25922 strains was not inhibited by any *T. tetraptera* extract. These results indicate that the two *S. aureus* strains ATCC29123 and 1225C/19 tested and the E.coli 1178C/19 strain are very sensitive to the ME extract (200 mg/mL and 400 mg/mL) as they induced inhibition zone diameters greater than 15 mm. These results are similar to those of Ponce *et al*, (2003) and Golly *et al*, 2015. However, the comparative results between the extracts of the fruit (MF, DF, IF), the trunk bark (ME) of *T. tetraptera* and the reference substance (Cefoxitin 30 µg/mL) allow to affirm the existence of an antimicrobial power in the trunk bark of *T. tetraptera*. However, the high inhibition zone diameter values of the reference product at low concentrations compared to those of the ME extract (high concentration) could be explained according to Sourabie *et al*. (2010). These authors justify this by the fact that the reference substances are pure, isolated molecules of well known concentration. This is not the case with

the ME extract which is a concentrate of various unpurified chemical constituents. Also the lack of activity of the different *T. tetraptera* extracts on the two *E. coli* strains (one Cefoxitin 1219C/19 sensitive and one reference strain ATCC25922 Cefoxitin sensitive) could indicate the absence of active substance on these germs in these extracts or simply be related to a diffusion issue (Zakaria *et al*., 2006; Golly *et al.,* 2015). From the solid-state tests, it was known that the trunk bark macerate (TMB) has the best activity on bacterial strains.

The antibacterial parameters (MIC and BMC) obtained in liquid medium with the ME extract confirmed the existence of an antibacterial power of it on all the *S. aureus* strains studied, and on the *E. coli* 1178C/19 strain. Indeed, the BMC/MIC ratios recorded on each of these germs remain lower than 4. This result reveals that the ME extract has a bactericidal effect on these germs (Marmonier, 1990). In the present study, GRAM positive bacteria (*S. aureus*) were all sensitive to the ME extract compared to GRAM negative bacteria (*E. coli*). In the literature, different studies have been done on the resistance of GRAM positive and GRAM negative bacteria. Thus, several authors have confirmed the high resistance of 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 (Bagamboula *et al*., 2004). However, according to Rath *et al*. (2009), the resistance of GRAM-negative and GRAM-positive bacteria to plants would depend on the nature of the plant extract but also on the nature of the strain tested. Also, it is known that the same bacterial species do not have the same sensitivity towards an antimicrobial agent. Thus, in a given bacterial population, there may be individual differences in sensitivity. The research of the composition in secondary metabolites of the aqueous extracts of barks and fruits of *T. tetraptera* allowed to highlight the chemical groups likely to be at the origin of the antibacterial power of *T. tetraptera*. In particular, alkaloids, tannins, polyphenols, flavonoids and saponins. These results corroborate with those obtained by the West African Health Organization (OOAS, 2013). The antimicrobial activity of these compounds has already been confirmed by several authors (Jayasinghe *et al*., 2003; Konate *et al*., 2012). The antimicrobial activity of ME extract could be attributed to the high presence of saponin, phenolic compounds and flavonoids with proven antimicrobial properties (Bssaibis *et al*., 2009). The work of Vincken *et al.,* (2007) showed that saponins isolated from the medicinal plant, have antibacterial properties. Thus, according to Maillard *et al.,* (1989) the saponins of *T. tetraptera* are the most powerful natural molluscicides. Moreover, the literature reports that the alcoholic and aqueous extract inhibited the growth of *S. aureus in vitro*.

CONCLUSION

This study is part of the vast program of the valorization of the Ivorian pharmacopoeia by the research of new anti-infectious molecules. It appears from this work that of all the extracts of *Tetrapleura tetraptera* used, the extract of the bark of the trunk (maceration of the bark of the trunk ME) was the most active compared to the maceration (MF), the decoction (DF) and the infusion (IF) of the fruits of this plant. The phenolic compounds, saponins, flavonoids revealed in the extracts could be at the origin of the observed activities. These results therefore provide a scientific justification for the traditional use of this plant species in traditional medicine for its antibacterial activity.

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