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Research Article

Assessment of antibacterial activity of some extracts of *Securinega virosa* (Roxb. ex Willd.) Baill on pathogens bacteria

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Abstract

Today, faced with the emergence of microbial resistance to antibiotics, the renewal of the arsenal of anti-infective drugs is acutely posed. In order to explore other sources of anti-infective drugs, this study therefore proposed to evaluate the antibacterial activity in vitro of several extracts of *Securinega virosa*, a well-known combretacea of populations in northern Côte d'Ivoire. To achieve this objective, the method of determining the diameters of the zones of inhibition on wells in an agar medium as well as that of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration were carried out. The results showed that the aqueous extract was not active on all the bacteria tested. The dichloromethane fraction at 500 mg/mL inhibited growth of *Shigella Typhi* (12.5 mm), followed by *Streptococcus* sp. (12.33 0.25 mm) and *Staphylococcus aureus* Meti-R (11.75 1.25 mm). The ethyl acetate fraction inhibited primarily Gram-positive bacteria with average diameters of 12 mm to 500 mg/mL. The ethanol fraction was most active on all bacteria with inhibition diameters ranging from 9 to 13.33 mm to 500 mg/mL. It showed the lowest MIC (3.12 mg/mL) on gram-positive and large Gram-negative levels ranging from 12.5 to 50 mg/mL. This study through its results provides data in favor of the traditional use of *Securinega virosa* in therapy.

Keywords : *Securinega virosa*, in vitro, antibacterial, activity

INTRODUCTION

Both in industrialized countries and in countries around the tropics, infectious diseases continue to rank among the causes, the most common causes of human mortality in the world[1]. Yet, the chemotherapy of bacterial infections that began in the early 1940s with Flemming's discovery of penicillin followed by the advent of new molecules in therapeutics had the important benefit of increasing human life expectancy. After less than half a century of existence, this brilliant picture is darkened by the progressive appearance of pathogen resistance to antimicrobials. This phenomenon, which is making the drugs used to treat infections less effective, has emerged as one of the major public health threats of the 21st century[2]. According to the British government, antimicrobial resistance could kill 10 million people a year by 2050[3]

Faced with this emerging public health problem, the renewal of the arsenal of anti-infective molecules has become a priority. This leads to the search for new antimicrobial agents mainly among plant extracts with the aim of discovering new chemical structures that are effective and free of toxicity[4]. Indeed, plants have been used for centuries to treat infectious diseases and are considered an important source of new antimicrobial agents[5]. In this perspective, more and more researchers are directing their work in the evaluation of antimicrobial effects of plant extracts namely root, stem, leaf or flower extracts[6]

Like these colleagues, the present study focused on the root barks of *Securinega virosa* (Roxb. ex Willd) Baill. In Côte d'Ivoire, ethnobotanical studies have shown that this plant is used in the traditional treatment of infectious and metabolic diseases[7]. Pharmacological studies attribute anti-diabetic[8], anti-diarrheal[9], anti-oxidant[10] and anti-malarial[11] properties to it.

This study plans to explore the antibacterial potential of different extracts of *Securinega virosa* (Roxb. ex Willd) Baill on pathogenic bacteria (Gram negative and Gram positive) to humans.

II-MATERIAL AND METHODS

II-1- Material

II-1-1 Biological material

❖ **Plant material**

Securinega virosa root barks were used. These organs were collected in January 2013 in Kouto (Bagoué region), a town located 725 km north of Abidjan (Côte d'Ivoire), following an ethnobotanical survey conducted by Koné et al.[7] among traditional healers in the locality. The authentication of the plant species collected was done by **Professor AKE ASSI Laurent**, thanks to the herbarium of the Centre National Floristique (CNF) of the Université Félix Houphouët Boigny.

❖ **Microorganisms**

Nine (9) bacterial strains involved in gastrointestinal disorders were used: *Escherichia coli* CIP 7624 (ATCC 25922) (reference strain), eight (08) clinical strains isolated from biological products: *E. coli* ESBL 13Y016 (isolated from urine), *Salmonella Typhi* 1586 (isolated from stool), *Salmonella Typhi* 43PI16 (isolated from stool), *Pseudomonas aeruginosa* 131813 (isolated from stool), *Shigella dysenteriae* 1079PI/15 (isolated from stool), *Klebsiella oxytoca* (isolated from urine) and *Staphylococcus aureus* Meti-R 1532C/10 (isolated from pus) and *Streptococcus sp*. These strains come from the bio-bank of the Institut Pasteur of Côte d'Ivoire.

II-1-2 Culture media and antimicrobial agents

Müller-Hinton agar (Liofilchem®, Italy) for the study of bacterial susceptibility to different plant extracts, ordinary agar (Liofilchem®, Italy) and Methylene Blue Eosin agar (Cultimed®, USA) for the isolation and maintenance of bacterial strains were used.

Cefotaxime (Himedia®, India) and Gentamycin (Himedia®, India) disks were used as reference antibiotics.

II-1-3 Technical material

The following equipment was used for this study: an oven (Med Center Venticell®), a refrigerator (XPer®), a rotary evaporator (Buchi®), a magnetic stirrer (IKAMAG-RCT®), a grinder (IKAMAG-RCT®), a centrifuge (Rotina 380, HETTICH®), an autoclave (Autotester E, DRY-PV®).

II-2- Methods

II-2-1 Preparation of the total aqueous extract and organic fractions of *A. leiocarpa*

Total aqueous extract was prepared according to **Guede-Guina**[12] and organics fractions according to Manga et al.[13].

II-2-2 Preparation of the bacterial inoculum

A volume of 0.01 mL or 0.1 mL or 1 mL of opalescent pre-culture broth was collected for *Pseudomonas*, enterobacteria and *Staphylococci*, respectively, and then diluted in a tube containing 10 mL of physiological saline to constitute dilution inoculum 10^0.

II-2-3 Preparation of concentration ranges

A concentration range of 500 to 7.81 mg/mL was prepared by the double dilution method in 7 test tubes. These tubes were then sterilized by autoclaving at 121 °C for 15 minutes and stored in a refrigerator at + 4 °C.

II-2-4 Preparation of culture media

Culture media were prepared according to manufacturers' instructions (Liofilchem® and Cultimed®).

II-2-5 Antibacterial sensitivity test

Agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts as displayed by **Daoud et *al.***[14] Cefotaxime (CTX 30 µg) for enterobacteria and gentamycin (GEN 10 µg) for anothers bacteria served as positive controls.

II-2-6 Determination of antibacterial parameters

II-2-6-1 Minimum Inhibitory Concentration (MIC)

Broth dilution technique in Mueller Hinton were used according to **Black and Black**[15]. Nine experimental tubes whose concentration varies to double dilution from 50 to 0.195 mg/ml and 2 control tubes, the growth control tube (TC) and the sterility control tube (TS) are prepared. The slope of the experimental tubes and that of the TC tube was seeded. The tubes were incubated at 37 °C for 24 hours. The MIC was the concentration of the first tube from which no microbial visible growth[16].

II-2-6-2 Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of substance that leaves at most 0.01 % of surviving germs.

Using a loop calibrated at 2 µL the contents of the tubes in which no haze was observed were seeded on MH (Box B) in parallel streaks 5 cm in length at the surface, starting with by the MIC tube. After 24 hours incubation in an oven at 37 °C, the numbers of colonies on the streaks of box B with those of box A were compared. In practice, the CMB corresponds to the concentration of the experimental tube whose number of colonies present on the streak is less than or equal to the number of colonies present on the streak of the dilution 10^{-4}.

III- RESULTS

III-1 Results of sensitivity testing of bacteria to aqueous extract and organic fractions *Securinega virosa*

III-1-1- Aqueous extract sensitivity tests

Table 1 shows the diameters of the bacterial growth inhibition zones with respect to the total aqueous extract of *Securinega virosa.* For all bacteria tested, the diameters of the growth inhibition zones are all less than 8 mm. However, a slight sensitivity is observed on the growth of Gram + bacteria (*S. aureus* Meti-R and *Streptococcus* sp) for which, the average diameter of the inhibition zone is 8 mm at the concentration of 250 mg/mL.

Table 1: Diamètre d'inhibition de l'extrait aqueux de *Securinega viros*a

Tested strains	Diameters of growth inhibition zones (mm)				
	Concentrations (mg/mL)			Antibiotics (µg)	
	C_1 = 500	C_2 = 250	C_3 = 125	CTX (30)	GEN (10)
E. coli ATCC 25922	00.00±00	00.00±00	00.00±00	30	ND
E. coli ESBL	00.00±00	00.00±00	00.00±00	10	ND
P. aeruginosa 131813	00.00±00	00.00±00	00.00±00	ND	21
S. Typhi 43PI16	00.00±00	00.00±00	00.00±00	25	23
S. Typhi 1586	00.00±00	00.00±00	00.00±00	28	30
S. dysenteriae 1079PI15	00.00±00	00.00±00	00.00±00	ND	ND
K. oxytoca	00.00±00	00.00±00	00.00±00	ND	ND
S. aureus Meti-R	00.00±00	08.09±1.33	00.00±00	ND	ND
Streptococcus sp	00.00±00	08.69±0.53	00.00±00	ND	15

00.00±00 : diameter of the inhibition zone < 8 mm. Values are means of three replicates affected by the standard error of the mean (m±esm). CTX: Cefotaxime; GEN: Gentamycin; ESBL: Extended spectrum beta-lactamase; Meti-R: Meticillin resistant; ND: not determined

III-1-2- Diameters of growth inhibition zones obtained with the dichloromethanic fraction of *Securinega virosa*

The diameters of the growth inhibition zones obtained with the dichloromethanic fraction of *Securinega virosa* are presented by **Table 2**. The results show that only the high concentrations (250 and 500 mg/mL) inhibit the *in vitro* growth of bacteria to varying degrees. The largest inhibition zone diameters of 12.58±0.23 and 12.58±0.23 are obtained at the concentration of 500 mg/mL on the growth of *E. coli* ESBL and *S. dysenteriae* 1079PI15 respectively.

Table 2 : Diameters of growth inhibition zones obtained with the dichloromethanic fraction of *Securinega virosa*

Tested strains	Diameters of growth inhibition zones (mm)				
	Concentrations (mg/mL)			Antibiotics (µg)	
	C_1 = 500	C_2 = 250	C_3 = 125	CTX (30)	GEN (10)
E. coli ATCC 25922	11.02±0.55	09±0.48	00.00±00	30	ND
E. coli ESBL	12.58±0.23	09.33±0.67	00.00±00	10	ND
P. aeruginosa 131813	09±0.68	00,00±00	00.00±00	ND	21
S. Typhi 43PI16	11.72±2.25	10,07±0.22	00.00±00	25	23
S. Typhi 1586	12.33±0.25	10.91±1.22	00.00±00	28	30
S. dysenteriae 1079PI15	12.33±0.25	10.91±1.22	00.00±00	ND	ND
K. oxytoca	10.33±0.81	08.11±0.32	00.00±00	ND	ND
S. aureus Meti-R	11.14±1.20	08.19±1.33	00,00±00	ND	ND
Streptococcus sp	10.66±0.69	08.69±0.53	00.00±00	ND	15

00.00±00 : diameter of the inhibition zone < 8 mm. Values are means of three replicates affected by the standard error of the mean (m±esm). CTX: Cefotaxime; GEN: Gentamycin; ESBL: Extended spectrum beta-lactamase; Meti-R: Meticillin resistant; ND: not determined

III-1-3- Diameters of growth inhibition zones obtained with the acetate fraction of *Securinega virosa*

The results of the diameters of growth inhibition zones are shown in **Table 3**. The acetate fraction did not inhibit the *in vitro* growth of *E. coli* ATCC 25922, *E. coli* ESBL and *P. aeruginosa* 131813. For these bacteria, the inhibition diameters were all less than 8 mm. The greatest sensitivity to this fraction was observed on the growth of *Streptococcus* sp. (12.33±0.25 mm) followed by *S. aureus* Meti-R (11.75±1.25 mm) at the concentration of 500 mg/mL.

Table 3 : Diameters of growth inhibition zones obtained with the acetate fraction of *Securinega virosa*

Tested strains	Diameters of growth inhibition zones (mm)				
	Concentrations (mg/mL)			Antibiotics (µg)	
	C_1 = 500	C_2 = 250	C_3 = 125	CTX (30)	GEN (10)
E. coli ATCC 25922	00.00±0.00	00.00±0.00	00.00±0.00	30	ND
E. coli ESBL	00.00±0.00	00.00±0.00	00.00±0.00	10	ND
P. aeruginosa 131813	00.00±0.00	00.00±0.00	00.00±0.00	ND	21
S. Typhi 43PI16	10.14±0.80	08.09±0.33	00.00±0.00	25	23
S. Typhi 1586	10.50±0.44	10.16±0.84	00.00±0.00	28	30
S. dysenteriae 1079PI15	09.21±0.81	00.00±0.00	00.00±0.00	ND	ND
K. oxytoca	10.50±0.40	08.61±0.10	00.00±0.00	ND	ND
S. aureus Meti-R	11.75±1.25	09.80±0.58	00.00±0.00	ND	ND
Streptococcus sp	12.33±0.25	10.91±0.22	08.25±0.84	ND	15

00.00±00 : diameter of the inhibition zone < 8 mm. Values are means of three replicates affected by the standard error of the mean (m±esm). CTX: Cefotaxime; GEN: Gentamycin; ESBL: Extended spectrum beta-lactamase; Meti-R: Meticillin resistant; ND: not determined

III-1-4- Diameters of growth inhibition zones obtained with the ethanolic fraction of *Securinega virosa*

The results of the diameters of growth inhibition zones are reported in the Table 4.

With respect to the ethanolic fraction, the highest sensitivity was observed with *S. aureus* Meti-R 1532C/10 (13.66 mm) and *Streptococcus* sp. (13.33 mm) strains, followed by *S. dysenteriae* 1079PI15 (12.93 mm) at 500 mg/mL. The *E. coli* ESBL strain was the least sensitive with 9.46 mm at 500 mg/mL.

Table 4 : Diameters of growth inhibition zones obtained with the ethanolic fraction of *Securinega virosa*

Tested strains	Diameters of growth inhibition zones (mm)				
	Concentrations (mg/mL)			Antibiotics (µg)	
	C_1 = 500	C_2 = 250	C_3 = 125	CTX (30)	GEN (10)
E. coli ATCC 25922	10,25±0,33	08.08±0.69	00.00±0.00	30	ND
E. coli ESBL	09.46±0.55	00.00±0.00	00.00±0.00	10	ND
P. aeruginosa 131813	11.44±0.08	00.00±0.00	00.00±0.00	ND	21
S. Typhi 43PI16	10.18±0.55	10.30±0.00	00.00±0.00	25	23
S. Typhi 1586	10.01±0.22	08.66±0.23	00.00±0.00	28	30
S. dysenteriae 1079PI15	12.93±0.11	10.60±0.20	08.81±0.80	ND	ND
K. oxytoca	12.03±0.76	09.50±0.86	08.36±0.55	ND	ND
S. aureus Meti-R	13.66±0.30	11.06±0.69	09.08±0.74	ND	ND
Streptococcus sp	13.33±0.52	10.15±0.46	08.68±0.58	ND	15

00.00±00 : diameter of the inhibition zone < 8 mm. Values are means of three replicates affected by the standard error of the mean (m±esm). CTX: Cefotaxime; GEN: Gentamycin; ESBL: Extended spectrum beta-lactamase; Meti-R: Meticillin resistant; ND: not determined

III-2- Antibacterial parameters (Minimum Inhibitory Concentration and Minimum Bactericidal Concentration)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) The antibacterial parameters obtained with the organic fractions and the total aqueous extract are presented in **Table 5**. Only for acetatic and ethanolic fractions, MIC and MBC could be determined. For these two fractions, the MBC /MIC ratio is less than 2, implying that they are bactericidal.

Moreover, ethanolic fraction showed the lowest MIC (3.12 mg/mL) observed with *S. aureus* Meti-R 1532C/10 and *Streptococcus* sp strains, while the highest MIC was 50 mg/mL obtained with *S. Typhi* 43PI16 strain.

Table 6 : Antibacterial parameters (Minimum Inhibitory Concentration and Minimum Bactericidal Concentration) of aqueous extract and organic fractions of *Securinega virosa*

Extracts	**Antibacterial parameters** (mg/mL)	Gram-negative bacteria							Gram-positive bacteria	
		E. coli ATCC	*E. coli* BLSE	*S. Typhi* 43PI16	*S. Typhi* 1586	*P. aeruginosa* 131813	*S. dysenteriae* 1079PI15	*K. oxytoca*	*S. aureus* Meti-R	*Streptococcus sp*
EDMS	CMI	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	CMB	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	CMB/CMI	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Effet	ND	ND	ND	ND	ND	ND	ND	ND	ND
EAS	CMI	> 50	> 50	6,25	6,25	> 50	25	12,5	12,5	6,25
	CMB	> 50	> 50	6,25	6,25	> 50	25	50	12,5	12,5
	CMB/CMI	ND	ND	1	1	ND	1	2	1	2
	Effet	ND	ND	Bcid	Bcid	ND	Bcid	Bcid	Bcid	Bcid
ETHS	CMI	25	25	50	25	12,5	12,5	12,5	3,12	3,12
	CMB	25	50	50	50	25	25	25	6,25	6,25
	CMB/CMI	1	2	1	2	2	2	2	2	2
	Effet	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid
ETAS	CMI	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	CMB	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	CMB/CMI	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Effet	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: not determined; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; ETHS: Ethanolic fraction of S. virosa; ETAS: total aqueous extract of *S. virosa*; EDMS: dichloromethane fraction of *S. virosa*; EAS: acetate fraction of *S. virosa*; Bcid: Bactericidal

IV- DISCUSSION

This study was intended to evaluate *in vitro* the antibacterial potential of *Securinega virosa* on some pathogenic strains of enterobacteria commonly involved in diarrheal diseases and on strains of Gram positive bacteria including *Staphylococcus aureus* Meti-R and *Streptococcus* sp.

As presented by the results in **Table 1**, the total aqueous extract was inactive on all bacteria tested despite the high concentrations. Similar findings were made in Benin by Onzo et al.[17] with extracts from four leaves (*Thalia geniculota, Musa spp, Manihot esculenta, and Daniella oliveri*) used as food packaging, then in India by Patel et al.[18] with methanol and acetonic extract from some medicinal plants and in China by Sukesh et al.[19] with hexanic and chloroformic extracts from two plants (*Gymnema sylvestre and Andrographis paniculata*). These results can be explained by the concentration of active ingredients and their solubilization in water used for extraction[17]. Moreover, the explanation can also be found in the inefficiency of the active molecules in these plants in relation to the membrane structure and the origin of the strains.

Yala et al.[20] explain this lack of antibacterial activity by the fact that some strains have developed resistance mechanisms to the antibacterial molecules present in the aqueous extract.

In addition, for Yala et al.[20] it is also possible that the solvents used in the extraction are responsible for the lack of activity of the plant extracts. Undoubtedly, the solvent used may not have been able to retain the molecules sought because of its polarity.

Dichloromethane extract inhibited growth of bacterial strains tested at higher concentrations (250 and 500 mg/mL). This study confirms previous findings in the literature that antimicrobial activities have a direct relationship to increased extract concentrations[21]. Indeed, in their work on the antimicrobial activity of plant extracts, several authors have suggested in most cases the use of high concentrations of extracts to obtain proven effects[22].

However, the diameters of the growth inhibition zones were low. This could be explained by the presence in this extract of very few bioactive molecules. Indeed, the phytochemical screening performed by Kouangbé et al.[23] only revealed the presence of polyterpenes more precisely sterols. Although the antibacterial activity of these substances is demonstrated by several authors[24], their low activity here, would be related either to their low concentration in the extract or they did not cross bacterial barriers.

The ethyl acetate fraction of *Securinega virosa* was more active on the *Shigella Typhi* strain (10 mm), and gram-positive bacteria such as *Streptococcus* sp. (12.33±0.25 mm) and *S. aureus* Meti-R (11.75±1.25 mm) at 500 mg/mL with MICs ranging from 6.25 to 12.5 mg/mL. No susceptibility to this fraction was observed with *Escherichia coli* strains. These results could be explained by the absence of anti-*Escherichia* compounds. A plant extract may contain several phytomolecules. However, they may have targeted

antibacterial activities that take into account their polarity, their concentration in the extract and the phenotype of the target bacteria.

The ethanolic fraction was active on all bacteria tested in this study. The smallest MICs (3.12 mg/mL) were recorded with Gram-positive bacteria in contrast to Gram-negative bacteria, with which MICs are higher (12.5 to 25 mg/mL). In the same direction, this fraction induced inhibition diameters ranging from 9 to 13 mm. These results could be explained by the choice of solvent, the methods of preparation of extracts and the part of the plant used.

Ethanol would concentrate much better the bioactive compounds responsibles for antibacterial activity. Similar results were previously obtained by Dickson et al.[25] and later confirmed by Amenu et al.[26]. These authors showed that of all the root extracts of *Securinega virosa* tested, only the ethanol extract was active on all the bacteria used in their study. Yéo et al.[27] also reported that among the ethanolic fractions, acetatic, dichloromethanic and acetatic obtained by exhaustion of the total aqueous extract of the roots of *Cochlospermum planchonii* and tested on the *in vitro* growth of strains of *Salmonella. Typhi*, *Vibrio. cholerea*, *Staphylococcus. aureus* ATCC, *Staphylococcus* aureus Méti-R, *Pseudomonas aeruginosa* Imip-I, *Pseudomonas aeruginosa* ATCC, *Salmonella Typhi* ESBL, *Escherichia coli* ATCC and *Escherichia coli* ESBL, only the ethanolic and acetatic fractions showed a proven antibacterial activity. The conclusion of this study thus corroborates that proposed by Yéo et al.[27].

The active ingredients would therefore be intermediate polarity compounds, better concentrated in ethanol and making ethanol as the best extraction solvent.

In short, the inefficiency of aqueous extracts and the low activity of dichloromethane fraction could be explained by the extraction method used to concentrate the active ingredients in the solvents with intermediate polarity (ethyl acetate and ethanol). However, other work has shown better antimicrobial activity with chloroformic fractions[28] and petroleum ether[29].

The highest inhibition diameters were obtained with *Staphylococcus aureus* Meti-R (13.66 0.30 mm) and *Streptococcus* sp (13.33 0.52 mm) showing the high sensitivity of Gram-positive bacteria to Gram-negative bacteria. The high sensitivity of *Staphylococcus aureus* Meti-R to the alcoholic fractions of *Securinega virosa* was confirmed by Enwa et al.[30]. The high sensitivity of Gram-positive bacteria to plant extracts compared to Gram-negative bacteria has been reported by several authors[31]. This difference in sensitivity between Gram-negative and Gram-positive bacteria is believed to be due to the variation in parietal structure of both cell types. In fact, the cell wall of Gram-positive bacteria consists of 70 to 90 % peptidoglycan unlike Gram-negative bacteria whose wall has only 20 % and an external membrane with two lipid layers. These structural differences between Gram-positive and Gram-negative bacteria would result in variation in the penetration of antimicrobial substances[32]. The inhibitory effect of the extracts on the synthesis of the bacterial cell wall (reticulation of peptidoglycan), which is less concentrated in gram-negative bacteria, may also be responsible for their reduced sensitivity to aqueous extract and organic fractions compared to Gram-positive bacteria[33]

The results of this study would argue in favour of a real antibacterial profile of *Securinega virosa*. This property was also highlighted by **Anarado et al.**[34] then **Ezeabara et al.**[35] during their work.

CONCLUSION

Securinega virosa is a plant well known by rural populations for its antimicrobial properties. The results of this study provide scientific arguments supporting its properties. Antibacterial tests carried out *in vitro* should be supplemented by *in vivo* tests in order to consolidate the results obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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