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ACTIVITY OF *MITRACARPUS SCABER* ON *ENTEROCOCCUS FAECALIS*

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ABSTRACT

In order to ascertain the antibacterial activity of *Mitracarpus scaber* (Rubiaceae), we tested five different extracts (aqueous extract, ethanolic extract 70%, insoluble residue in ethanol 70%, ethanolic extract 100% and butanolic extract) obtained from this plant on the *in vitro* growth of *Enterococcus faecalis* in Mueller-Hinton broth and Mueller-Hinton naehrboden agar. The results showed that all extracts were actives. Among those, the butanolic extract showed the highest bactericidal activity, with those values: MIC = 12,5 mg/ml, MBC = 1,56 mg/ml et IC_{50} = 0,06 mg/ml.

KEYWORDS: Bactericidal, extracts, *Enterococcus faecalis*, *in vitro*, *Mitracarpus scaber*.

INTRODUCTION

The emergence of resistant bacterial strains and treatment failures observed in modern therapy to usual molecules against these strains have led our research team, to scrutinize the Ivorian floristic heritage, in order to isolate molecules to overcome this state of fact. In the search for effective new drugs against diseases, some scientific groups show an interest in medicinal plants. The African floristic heritage considered in terms of the pharmacopoeia and traditional medicine is very rich in medicinal plants whose effectiveness is recognized. Indeed, our continent is full of over 5,000 medicinal plant species.[1] Research in our different laboratories have the desire to make rational and efficient, exploitation of this heritage by

providing a scientific basis for the use of these plants. *Mitracarpus scaber* (MISCA) is one of the plants tested extensively in the evaluation of its antifungal components.[2] Clinical trials on the effectiveness of MISCA have even been made to the development of pharmaceutical creams dealing successfully superficial and cutaneous mycoses.[3,4,] Studies conducted elsewhere have also confirmed its antimicrobial activity.[5,6,7,8] and even anti-parasitic.[9] Insofar, the antibacterial activity of *Mitracarpus scaber* is very little known, we initiated this study to test the action of plant extracts MISCA on the in vitro growth of *Enterococcus faecalis*. These strains are with *Staphylococci* and *Streptococci* β hemolytic, the most incriminated Gram + cocci in human skin pathology.

MATERIALS AND METHODS

Plant material

Mitracarpus scaber (Rubiaceae) were collected in Abidjan (Ivory Coast) and identified by the Botany Department of Université Félix Houphouet Boigny of Cocody. Voucher specimens are preserved in the herbarium for future reference. This plant is traditionally used against skin infections.

Preparation of extracts

As described by[10] whole *Mitracarpus scaber* plants were washed, cut and dried at room temperature. After drying, the plant organs were ground to a fine powder and 100 g were macerated for 24 h in 1 L of distilled water on a shaker-type IKA Labortechnik water. The aqueous homogenate was filtered through hydrophilic cotton and Whatman paper 3 mm. The filtrate was evaporated to dryness under reduced pressure at 70 °C with the Buchi Rotavapor to give a dark brown powder which formed the crude aqueous extract noted: MISCA-Aq. From MISCA-Aq, other fractions were prepared according to the method of.[11] 10 g of MISCA-Aq were dissolved in 200 ml ethanol : water (70/30, v/v). The supernatant and pellet were concentrated separately at 50 °C to obtain MISCA-70Et (70% ethanol extract from supernatant) and MISCA-70R (70% residual extract from pellet). Another part of MISCA-Aq was used to prepare the ethanol extract 100% (MISCA-100Et) simply by maceration 30 g MISCA-Aq in 600 ml of absolute ethanol under magnetic stirring until complete homogeneity. Then the two phases obtained after decantation were concentrated separately to obtain MISCA-100Et (supernatant) and MISCA-100R (pellet). MISCA-100Et has been used as basis for preparing further extracts. From this sample, 15 g were first defatted with hexane in soxhlet, and the aqueous fraction was subjected to partition into 600 ml ethyl

acetate : water (50/50, v/v) and then decanted. After separation, the two phases were concentrated separately at 50 °C to obtain MISCA-Ac^+ (from supernatant) and MISCA-Ac^- (from pellet). With MISCA-Ac^- 15 g were added in 600 ml butanol : water (50/50, v/v) and then decanted. Evaporation of these two fractions gave extracts MISCA-But^+ (supernatant) and MISCA-But^- (pellet). The MISCA-Aq, MISCA-70Et, MISCA-70R, MISCA-100Et and MISCA-But^+ extracts were tested on *in vitro* growth of *Enterococcus faecalis.*

Antibacterial activity

The minimum inhibitory concentration (MIC) values were evaluated by the Mueller-Hinton broth macrodilution test, as described by[12] and using the standard inoculum of 1.10^5–5.10^5 cfu ml^{-1}. The concentration ranges of each plant extract were prepared according to the method of double dilution with a geometric progression. The range from 200 to 0.39 mg/ml for MISCA-Aq, 25 to 0.39 mg/ml for MISCA-70Et and MISCA-100Et, 200 to 12.5 mg/ml for MISCA-70R and 12.5 to 0.05 mg/ml for MISCA-But^+. The MIC is defined as the lowest concentration of drug which inhibits the visible growth after 18 h. With fusidic acid (reference antibiotic), we used concentrations ranges from 500 µg/ml to 3.91 g/ml and we used the same procedure.

To determine the minimal bactericidal concentration (MBC), each tube of the experimental series was separately diluted according to a geometric progression of ratio 10^{-1}. Then each of the different dilutions were plated with Mueller-Hinton agar. All inoculated plates were then incubated at 37 °C for 24 h. After incubation, the enumeration of surviving bacteria per tube was done by establishing a relation between the diameter (2.1 mm) of the calibrated loop, the sampled volume (3 µl), the number of surviving bacteria on a streak data and subsequent dilutions made to obtain isolated colonies.

Number of bacteria = Number of colonies x 1 ml / volume sampled

The MBC was defined as the lowest concentration that did not permit any visible microorganism colony growth on the medium after the period of incubation fixed in the present study. The tests (MIC and MCB) were repeated three times consecutively over 3 successive different experiments. The bactericidal determination was based on the principle of [13]. According to this principle, if the MBC/MIC ratio is less than or equal to 4, the test substance is bactericidal.

RESULTS AND DISCUSSION

In liquid medium, no turbidity was observed for strains of *Enterococcus faecalis* from concentrations of.

-50 mg/ml for MISCA-Aq,

-25 mg/ml for MISCA-70 Et,

-50 mg/ml for MISCA-70R,

-25 mg/ml for MISCA-100Et,

-12.5 mg/ml for MISCA-But $^{+}$.

In solid medium, seeding inoculum growth control lamp tube inocula where turbidity was not visible and some previous inoculated the tube that was used to determine the MIC (high bacterial load) have allowed have the curves in Fig. 1 and Table I.

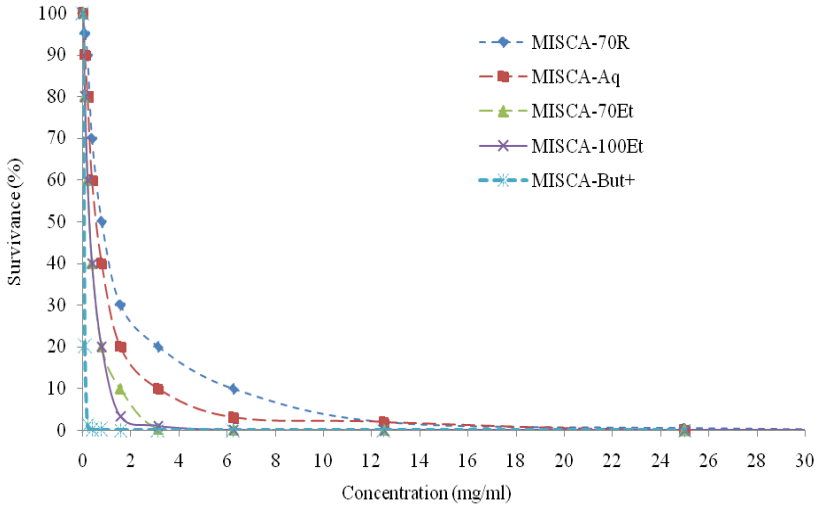

Figure 1: Curves of the sensitivity of Enterococcus faecalis of MISCA extracts

The results in Fig. 1 show decreasing curves until become zero on the x-axis. However, we note that the passage of the curve described by MISCA-70R to MISCA-But $^{+}$, the curves resemble more of the axes of the graph. These observations mean that the curve described by MISCA-Purpose + has antibacterial parameter values lower than those of MISCA-100Et. While MISCA-100Et has relatively lower values than those of MISCA-70˚ and. As described by MISCA-70˚ and has smaller values of parameters than MISCA-Aq. Finally, the curve described by MISCA-Aq has smaller values of parameters as described by MISCA-70R.

Table (1). Antibacterial parameters values of MISCA extracts.

	CMI (mg/ml)	CMB (mg/ml)	CI_{50} (mg/ml)
MISCA-Aq	50	50	1,72
MISCA-70Et	25	12,5	0,55
MISCA-100Et	25	6,25	0,49
MISCA-But$^+$	**12,5**	**1,56**	**0,06**
MISCA-70R	50	100	3,28

The comparison between these values of MBC (Minimum Bactericidal Concentration) of the different MISCA extracts tested on *Enterococcus faecalis* strains of table I shows that:

$MBC_{MISCA\text{-}But+} < MBC_{MISCA\text{-}100Et} < MBC_{MISCA\text{-}70\ Et} < MBC_{MISCA\text{-}Aq} < MBC_{MISCA\text{-}70R}$.

The results of this analysis confirms that the most bactericidal extract is MISCA-But$^+$ because its MBC (1.56 mg/ml) is the lowest. While MISCA-70R is the lowest of all bactericidal because of the high value of the MBC (100 mg/ml). However, in addition to MISCA-But$^+$ and MISCA-70R, it was obtained MISCA-100Et, MISCA-70 Et and MISCA-Aq extracts that are bactericidal in the following descending order : MISCA-100Et (6.25 mg/ml) , MISCA-70 Et (12.5 mg/ml) and MISCA-Aq (50 mg/ml). The phytochemical sort MISCA-But$^+$ showed the presence of polyphenols and abundant terpenes and sterols. However, the use of fusidic acid (steroid standard) revealed that the liquid medium was not turbid from the concentration 250 µg/ml. In solid medium, we noted that the isolated colonies appear at the concentration 7.81 ug/ml.

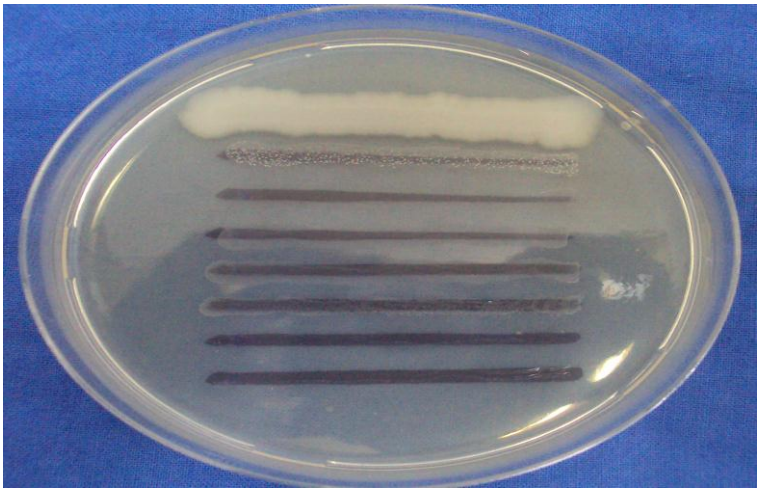

Figure 2: Action of fusidic acid in vitro on the growth of *Enterococcus faecalis*. From top to bottom, Pure inoculum without fusidic acid; inoculum with 7.81 µg/ml; inoculum with 15.63 µg/ml; inoculum with 31.25 µg/ml; inoculum with 62.5 µg/ml; inoculum with 125 µg/ml ; inoculum with 250 µg/ml; inoculum with 500 µg/ml.

DISCUSSION

Inasmuch no turbidity was observed for each sample tested from MISCA-Aq (50 mg/ml); MISCA-70Et (25 mg/ml); MISCA-70R (50 mg/ml); MISCA-100Et (25 mg/ml); MISCA-But$^+$ (12.5 mg/ml); fusidic acid (0.25 mg/ml).

These concentrations are therefore MICs of these substances.[13] If we consider only MISCA-Aq which is the basic extract, antibacterial parameter values (MIC) and (MBC) are identical (50 mg/ml). The ratio $MBC_{MISCA\text{-}Aq}/MIC_{MISCA\text{-}Aq}=50/50=1<4$. Therefore, we can deduce that the action of MISCA-Aq on *in vitro* growth of *Enterococcus faecalis* has bactericidal activity. It improves when we pass from MISCA-Aq to MISCA-But$^+$. This proves that the bactericidal action is related to the state of purity of the extract tested. The results in Fig. 1 indicate that *Enterococcus faecalis* are much more sensitive to MISCA-But$^+$ > MISCA-100Et > MISCA-70Et > MISCA-Aq > MISCA-70R. It appears from the analysis of these results that the most bactericidal extract is MISCA-But$^+$ because its MBC value (1,56 mg/ml) is the lowest. While the less bactericidal extract is MISCA-70R because of the high value of MBC (100 mg/ml). MISCA-70Et (12.5 mg/ml) and MISCA-100Et (6.25 mg/ml) have average bactericidal actions on the in vitro growth of *Enterococcus faecalis*.

We can compare the bactericidal potential of extracts by dividing the MBC value of each one by the most bactericidal (MISCA-But$^+$, 1.56 mg/ml). In addition, the ratio on the basis of MBC shows that MISCA-But$^+$ is 4 times more bactericidal than MISCA-100Et; 8 times more bactericidal than MISCA-70Et; 32 times more bactericidal than MISCA-Aq; 64 times more bactericidal than MISCA-70R. These results reveal that the extraction method which implements the total aqueous extract partition in successive combinations of solvents allows a better concentration of MISCA active compound. Because it helped lead to MISCA-But$^+$ which improves significantly the activity of the basic extract. On the other side, MISCA-70R is the least active extract. This lower performance of MISCA-70R is normal. It contains much more macromolecules (sugars, proteins and glycoproteins). These substrates generally have no inhibitory effect on the growth of strains of *Enterococcus faecalis*. The apparent activity MISCA-70R can be explained by the presence of trace amounts of active compound left in the settling of the phases during the preparation of the extracts. Phytochemical screening of MISCA-But$^+$ revealed that its active components are mainly sterols and polyphenols. In this extract, there is much more sterols than polyphenols. Studies performed by[6,7,14] revealed that MISCA also contained alkaloids possessing quinone structure and

tannins. All these results show that MISCA have abundant active substances which are expressed by the method and the extraction solvent used. Indeed, the work of[15] revealed that generally, the use of a specific solvent for the extraction of active compounds from a medicinal plant put more in evidence specific active substances.

Insofar different bacterial cultures show the same decrease colonies as the concentrations of fusidic acid gradually increase and the total disappearance of colonies is visible at a concentration of 250 µg/ml of standard steroid; it is possible that this compound has a dose-dependent anti-bacterial activity on these bacterial strains. The antibacterial activity of fusidic acid was demonstrated by.[16,17] They have even shown that this substance was also active on other bacteria Gram$^+$ like *Enterococcus faecalis.*

The MIC of the butanolic extract of MISCA (MISCA-But $^+$) is 12.5 mg/ml; while that of fusidic acid (standard molecule) is 250 µg/ml against strains of *Enterococcus faecalis*. Fusidic acid is about 50 times more inhibitory than MISCA-But$^+$ extract. Also the completely disappearance of *Enterococcus faecalis* colonies using the concentration of 12.5 mg/ml MISCA-But$^+$ and 250 µg/ml fusidic acid can be explained by the purity of fusidic acid (standard steroid) unlike the butanolic extract of MISCA is still a pool of active molecules (terpenes and polyphenols).

CONCLUSION

This study allowed us to show *Enterococcus faecalis* tested were sensitive to the different MISCA extracts used. All these samples have a bactericidal dose response on their *in vitro* growth. However, the most improved extract (butanol extract) has the most bactericidal activity (MBC = 1,56 mg/ml). MBC is 32 times smaller than crude aqueous (basic extract). The partition extraction of crude aqueous extract in various solvents allowed to better focus the substances responsible of the bactericidal activity. Phytochemical screening showed that bactericidal effect is related to the presence in the extract of polyphenols, and abundant terpenes and sterols. These compounds are among the many molecules with anti-bacterial action synthesized by medicinal plants. Testing a standard steroid, fusidic acid confirmed the anti-bacterial activity of terpenes and sterols on *Enterococcus faecalis aureus*. Indeed , it was demonstrated that compared with the pure molecule of MISCA butanolic extract is about 50 times more active against strains of *Enterococcus faecalis*.

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