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Phytochemical screening and determination of antimicrobial activity of different extracts from the roots and leaves of *Stylocheaton Hypogaeus, a* plant used for the treatment of prostatitis in Senegal

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Abstract

Stylocheaton hypogaeus is a medicinal plant belonging to the Araceae family of African origin. It is widely used traditionally in southern Senegal to treat various diseases, mainly prostatitis, lung cancer and hemorrhoids. The main objective of this study is to evaluate the antibacterial properties of different leaf and root extracts of the *Stylocheaton hypogaeus* plant. A phytochemical screening test was previously carried out to highlight the presence of certain families of secondary metabolites, generally responsible for the biological activity of plants. For antibacterial activity, the extracts are tested on Gram-positive bacteria *Staphylococcus aureus* ATCC29213 and Gram-negative *Escherichia coli* ATCC25922. Thus, standard agar diffusion and macrodilution techniques were used to determine the inhibition diameters, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The results revealed a very good antibacterial activity of the different extracts of these two organs, with in fact an inhibition diameter of 21.11 mm for the ethyl acetate extract of the roots on the *Escherichia coli* ATCC25922 strain. The delipidated methanolic extracts of the roots and leaves showed the best antibacterial activity with a MIC of 1.5625 mg/mL, respectively on the *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 strains. This remarkable antibacterial activity of the methanolic extract of the roots of *Stylocheaton hypogaeus* justifies its use in traditional medicine for the treatment of prostatitis, a disease whose infection is generally caused by the bacterium *Escherichia coli* ATCC25922.

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Introduction

Bacterial resistance to antibiotics is a major challenge in human and animal health, requiring the search for new alternatives. Plants play an essential role in human life, meeting daily needs. They serve as sources for cosmetics, food and medicines, while being widely used in the treatment of many infectious diseases worldwide (J. D. Barak *et al.*, 2012). Medicinal plants represent one of the best sources for the identification and development of new bioactive substances (P. Khan *et al.* , 2009) . Many studies have shown that they contain various compounds, including peptides, long-chain unsaturated fatty acids, alkaloids, essential oils, phenols and flavonoids (M. M. Cowan *et al.*, 1999). These substances have great potential for therapeutic applications targeting human and animal pathogens, such as bacteria, fungi and viruses (P. Khan *et al.*, 2009). In addition, these substances confer antimicrobial properties, thus eliminating or inhibiting the growth of bacteria. Indeed, many medicinal plants contain compounds with potent antimicrobial activity against bacteria, viruses, and fungi (M. M. Cowan *et al.*, 1999). Some plants may help reduce inflammation, which may be beneficial in fighting infections (O. Oguntibeju *et al.*, 2018). Other plants can help strengthen the immune system, making it better equipped to fight infections (O. O. Olarewaju *et al.*, 2022). In addition, traditional medicinal plants can be more affordable than conventional medicines (R. S. Chaughule *et al.*, 2023) and therefore more accessible to populations.

Some foodborne diseases are caused by Gram-positive bacteria, such as *Staphylococcus aureus*, and Gram-negative bacteria, such as Escherichia coli, representing a major public health problem worldwide (R. Chawech *et al.*, 2015). Infectious diseases are now the leading cause of death worldwide (B. R. Ama *et al.*, 2021). In addition, bacteria have the ability to adapt to antimicrobial molecules, thus promoting the development of resistance linked to the excessive use of antibiotics (U.-C. Renata *et al.*, 2022). Plants represent a privileged natural source for the development of new drugs. Among them, *Stylochaeton hypogaeus*, a species of the Araceae family, stands out in particular. This plant, widely recognized in traditional medicine in southern Senegal, particularly in Ziguinchor, is used to treat various diseases such as prostatitis, cancer and hemorrhoids. Indeed, prostatitis is an inflammation of the prostate gland, generally of infectious origin (bacteria or not) (B. T. NIARE, 2007), most often *Escherichia coli* ATCC2592. However, in the Senegalese context, the antibacterial properties of this plant are not yet documented.

It is with this in mind that we proposed to carry out an in-depth chemical study on this plant, in order to have verified scientific information, to justify the preferred choice reserved for this plant for the treatment of several diseases, particularly prostatitis. Indeed, in this study, we will determine the antibacterial activity of certain extracts of the leaves and roots of the plant on the bacterial strains Staphylococcus aureus, and Escherichia coli. The choice of these strains is justified by the nature of the bacteria, which is *Escherichia coli* ATCC2592, responsible for prostatitis. Phytochemical screening has highlighted the richness of this plant in secondary metabolites, generally responsible for the biological activity of plants. The general objective of this study is to test the antibacterial properties of the plant *Stylochaeton hypogaeus* in order to isolate and then identify the bioactive molecules responsible for the treatment of prostatitis.

Materials and methods

Plant material

The choice of *Stylocheaton hypogaeus* is based on an ethnobotanical survey associated with a bibliographic study of the plants listed with certain traditional practitioners in the department of Bignona (Ziguinchor). The plant material consists of leaves and roots harvested in August 2022 in a field located in the commune of Djinaky, department of Bignona, in the south of Senegal with geographical coordinates 12°15'48" North and 16°27'46" West. The identification of the plant was made at the Fundamental Institute of Black Africa (IFAN), in the Lebrun and Stork database. The leaves and roots of

the plant were rinsed with distilled water, then dried in the open air, away from sunlight. Once dried, the samples were crushed using an electric grinder. The fine powder obtained is used as raw material for various chemical experiments.

Extraction process

The secondary metabolites were extracted by maceration in order to avoid possible degradation of the thermosensitive molecules present in the plant.

The plant material was successively placed in solvents of increasing polarity. Indeed, 20 g of powder from each part of the plant were introduced into 100 mL of hexane. The maceration was carried out for 24 hours. The macerate is filtered on filter paper, the collected filtrate is called hexane extract and the marc obtained is subjected, after drying, to a new maceration in hexane. This process is repeated only once with the marc obtained previously. After the two successive extractions with hexane, the marc is treated in the same way with 100 mL respectively with the solvents of ethyl acetate, methanol and aqueous, leading respectively to the extracts of ethyl acetate, methanol and aqueous. In parallel, 24 hours crude macerations with methanol, dichloromethane and ethyl acetate were also carried out. The filtrates obtained were subjected to the rotary evaporator to remove the extraction solvents.

The extracts obtained are covered with aluminum foil and kept in the fridge during the whole process.

Phytochemical screening

Phytochemical screening is a qualitative analysis based on precipitation or coloring reactions. These allow to define the presence or absence of secondary metabolites that may be found in a plant sample. In this work, screening concerns the search for: alkaloids, polyphenols, tannins, flavonoids, saponins, sterols and polyterpenes, leucoanthocyanins, catechols, mucilages. We tested the presence of these different chemical groups by referring to the techniques described in the work of Ronchetti and Russo (B. Mbow *et al.*, 2022). Polyphenols and tannins were identified by the $FeCl_3$ test and the Stiasny reagent; flavonoids, leucoanthocyanins and catechols by reaction with cyanidin; saponins by the foam test; sterols and polyterpenes by the Liebermann-Burchard test; mucilages by the absolute ethanol test and alkaloids by the Mayer test (A. R. Rechner *et al.*, 2002).

Antibacterial activity

Sterility test of extracts

This test is intended to verify the contamination or not of the extracts by germs. To do this, 0.1 g of the extract is added to 10 mL of thioglycollate broth, then the broth is incubated at 37 °C for 24 hours. After this period, the broth is inoculated on a petri dish containing the nutrient agar and another containing the sabouraud agar, then this dish is incubated at 37 °C (Y. Vaghasiya, 2009). The substance is declared sterile if no colony is visible on the agar dish after 24, 48 and 72 hours of incubation at 37 °C.

Test of sensitivity of germs to different extracts in solid medium

Test on solid medium

In this test, the previously prepared inoculum (from the colonies of bacterial strains of interest) is inoculated by swabbing on the agar. Holes are dug on the surface of the agar to introduce the solutions to be tested (M. Gulluce *et al.*, 2007). The dishes are carefully closed and the whole is left for 30 minutes at room temperature for pre-diffusion of the substances then incubated at 37 °C for 24 hours. After this time, the dishes are removed from the incubator and the diameters of the inhibition zones around the cylinders are measured using a graduated ruler. These diameters in mm are then considered proportional to the sensitivity of the germ studied with respect to the extract considered (F. Bssaibis *et al.*, 2009). The bacterial strains used in this work, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive), were provided by the Microbiology Laboratory of the Polytechnic School of Cheikh Anta Diop University in Dakar.

Preparation of the McFarland standard solution

To avoid any bias that may be due to the variability of

the inoculum, it is recommended to standardize it with a McFarland 0.5 solution. The preparation of the standard solution was carried out by mixing 0.05 mL of 1% $BaCl_2$ and 9.95 mL of 1% H_2SO_4 to form a suspension of $BaSO_4$. This mixture is placed in a screw-top tube covered with aluminum foil, stored at room temperature and protected from light.

Preparation of the inoculum for the solid medium test

From a 24-hour culture, one (or more) fresh colony (s) of bacteria are collected using a loop (loop), suspended in a tube containing a sterile normal saline solution (physiological water), then mixed using a vortex. This suspension is compared with the microbial suspension with the 0.5 MacFarland turbidity standard and is adjusted if necessary by adding saline or cells. It is evaluated at approximately 106 bacteria/mL (Murray P R *et al.*, 1983).

Diffusion test on agar

The determination of the sensitivity of bacterial strains was carried out by the diffusion method in wells on agar medium. This is a variant of the Kirby-Bauer disk diffusion test method (A. W. Bauer *et al.*, 1966).

The previously prepared inoculum (from the colonies of bacterial strains of interest) is inoculated by swabbing on the agar. Holes are made on the surface of the agar to introduce 50 µL of plant extract solution at a concentration of 100 mg/mL (M. Gulluce *et al.*, 2007). The dishes are carefully closed and the whole is left for 30 minutes at room temperature for pre-diffusion of the substances and then incubated at 37 °C for 24 hours. During the incubation period, the strain to be studied competes with the inhibitory effect of the plant extract. When the strain is sensitive to the extracts, an inhibition zone around the cylinders is formed. And if the strain is resistant, there is an absence of inhibition zone. These diameters in mm are then considered proportional to the sensitivity of the germ studied with respect to the extract considered (F. Bssaibis *et al.*, 2009). The extract is not effective if the inhibition diameter is less than 8 mm; it is effective if it is between 9 and 14 mm; it is very effective if the diameter is between 15 and 19 mm and, finally, it is extremely effective if it is greater than 20 mm (A. G. Ponce *et al.*, 2003).

Liquid medium tests

Preparation of the inoculum for liquid medium tests

Using a loop, the bacterial inoculum will be prepared from two (2) bacterial colonies of less than 24 hours using a Pasteur pipette and then emulsified in a test tube containing 10 mL of Mueller-Hinton broth (MHB). The mixture is incubated at 37 °C for a period of 3 hours to obtain a preculture. After this incubation, a suspension of 0.3 mL of this preculture is taken and diluted in 10 mL of sterile MHB which is homogenized by vortexing.

Preparation of the concentration range of plant extracts

The concentration range was prepared by the double dilution method. From a stock solution with a concentration of 50 mg/mL of the extracts, a series of dilutions of a factor of 2 is carried out in order to obtain concentration ranges from 50 to 0.78 mg/mL. This range of plant extract was made so that we have a concentration of plant extract of 50 mg/mL in tube C_1, 25 mg/mL in tube C_2 and so on up to tube C_7 which will have a concentration of 0.78125 mg/mL. We thus obtain 7 dilutions with 7 concentrations from C_1 to C_7 which will be used for the following tests.

Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration is the lowest concentration of the substance for which there is no visible growth of the bacteria to the naked eye after an incubation time of 18 to 24 hours. It was determined according to the following method: In a series of seven (7) test tubes from C_1 to C_7, 1 mL of pure inoculum was introduced. Then, 1 mL of plant extract was added to the tubes according to the concentration range prepared. This distribution of plant extract was done so that 1 mL of plant extract with a concentration of 50 mg/mL was transferred to tube C_1, tube C_2 received 1 mL of 25 mg/mL and so on up

to tube C_7 which received 1 mL of the solution at 0.78125 mg/mL. In a growth control tube, 1 mL of sterile distilled water and 1 mL of inoculum were placed. In another sterility control tube, 2 mL of sterile BMH are introduced. All tubes are incubated for 24 hours at 37 °C. After incubation, the MIC is determined by observing the turbidity induced by the growth of the germs studied in each tube. It corresponds to the smallest concentration for which there is no turbidity observed with the naked eye (M.C. Ploy *et al.*, 2016).

Determination of MBC

The minimum bactericidal concentration (MBC) was determined by subculture of the tube with inhibition of an agar plate. The MBC is the lowest concentration of substance that will leave 0.01% of surviving germs. For its determination, the inoculum count and the plant extract count are carried out.

For the inoculum count, a series of 10-fold dilutions were carried out from the solution to obtain 4 dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). Each dilution was inoculated using a calibrated 10 µL loop in 5 cm long streaks, on Muller-Hinton agar that was incubated at 37° for 24 hours.

For the plant extract count, the contents of the tubes in which there was no visible growth after reading the MICs are used to inoculate Petri dishes containing Muller-Hinton agar on 5 cm streaks and incubated. After 24 hours of incubation in an incubator at 37 °C, the minimum bactericidal concentration (MBC) is determined by comparing the number of colonies on the streaks of the plant extract count and that of the inoculum numbering dish.

Thus, the first experimental tube whose number of germs present on the streak is less than or equal to that of the 10^{-4} dilution corresponds to the MBC.

The MBC/MIC ratio makes it possible to specify the modality of action of the substance. Indeed, the extract is judged:

Bactericidal (kills bacteria) when MBC = MIC or if the MBC/MIC ratio is less than or equal to 4.

Bacteriostatic (inhibits bacteria, but does not kill them) when MBC> MIC or if the MBC/MIC ratio is higher than 4.

Results and discussions

Phytochemical screenin

The results of the phytochemical screening recorded in Table 2 above reveal the presence of all the families of secondary metabolites tested in the different extracts of this plant.

Table 1. Profile of bacteria tested.

Strains	Profile	Origins
Escherichia coli ATCC 25922	Sensitive	References
Staphylococcus aureus ATCC 29213	Low production □-lactamase	References

However, these results show mainly a variability of the compounds depending on the parts of the plant and the solvents used for extraction. The methanolic and aqueous extracts are distinguished by their richness in polyphenols, flavonoids and alkaloids in the three organs of the plant. On the other hand, the ethyl acetate and hexane extracts are less concentrated in these compounds, with the exception of some, such as sterols. Methanol and water seem to be the most effective solvents for the extraction of secondary metabolites contained in the different organs of this plant.

Antibacterial activity

Sensitivity test

This test is used to determine the presence or absence of inhibition zones around the wells. A circular halo appears around the well when the strain is sensitive to the deposited extract fractions, while no halo is observed if the strain is resistant.

Table 2. Results of phytochemical screening of leaf and root extracts of *Stylocheaton hypogaeus*.

	Secondary metabolites / Extracts	Polyphenols	Flavonoids	Alkaloids	Sterols and Polyterpenes	Leucoanthocyanins	Catechols
Leaves	Hexane*	-	-	-	+++	++	-
	Ethyl acetate **	+++	+	-	+++	++	-
	Methanol**	+++	+++	+++	+++	-	+++
	Aqueous**	+++	+	+++	+++	-	+++
Roots	Hexane*	-	-	-	+	-	-
	Ethyl acetate **	+	-	-	+	+	-
	Methanol**	+++	-	-	+	-	+++
	Aqueous**	++	+	+	-	-	++

+++: Strong presence, ++: Moderate presence, +: Low presence, -: Absence

*Raw extract; **Sequential extract.

Evaluation of the sensitivity of different root powder extracts to various bacterial strains.

The results of the inhibition diameters of *Stylocheaton hypogaeus* root extracts are shown in Figure 1.

The sensitivity test showed inhibition diameters that vary depending on the extraction solvent and the bacterial strain studied. The different *Stylocheaton hypogaeus* root powder extracts show significant inhibitory action on both strains. Indeed, the strain of Escherichia Coli ATCC 25922 is sensitive to extracts by methanol (previously delipidated), by dichloromethane and by ethyl acetate with inhibition diameters of 12.79, 13.47 and 21.11 mm respectively. Under the same conditions, the strain of *Staphylococcus aureus* ATCC 29213 gives inhibition diameters to extracts by delipidated methanol and by ethyl acetate of 15.58 and 14.8 mm respectively. Gentamicin, used as a positive control, is applied to both strains. They react favorably to gentamicin with inhibition diameters of 34 mm for Escherichia coli ATCC 25922 and 38 mm for *Staphylococcus aureus* ATCC 2921. These results show the effectiveness of root extracts obtained with dichloromethane and delipidated methanol against *Escherichia coli*.

In addition, the ethyl acetate extract records the highest antibacterial activity with an inhibition diameter of 21.11 mm on *Escherichia Coli*, showing an extremely effective activity. Overall, the delipidated methanol extract has a very effective antibacterial activity while that of ethyl acetate is simply effective on *Staphylococcus aureus*.

Evaluation of the sensitivity of the different leaf powder extracts on bacterial strains.

The results of the inhibition diameters of *Stylocheaton hypogaeus* leaf extracts are shown in Figure 2.

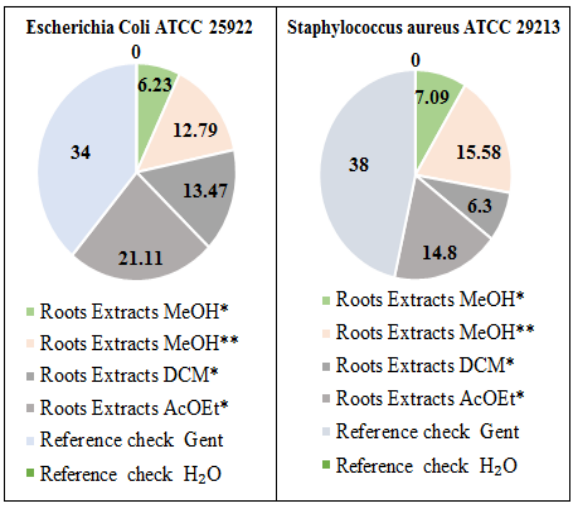

Fig. 1. Sensitivity diagram of various *Stylocheaton hypogaeus* root extracts on *Escherichia coli* ATCC 25922 and Staphylococcus aureus ATCC 29213 strains.

The sensitivity test revealed variations in inhibition diameters depending on the extraction solvent and the strains tested. The results indicate that both strains exhibit sensitivity on some extracts. *Escherichia Coli* strain ATCC 25922 is sensitive to crude methanol and delipidated methanol extracts with inhibition diameters of 20.02 and 15.87 mm, respectively.

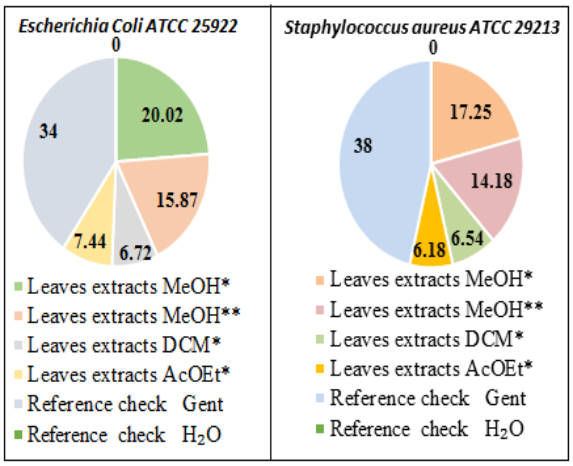

Fig. 2. Susceptibility diagram of different leaf extracts on *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 strains.

In addition, *Staphylococcus aureus* ATCC 29213 shows inhibition diameters to the extracts by crude methanol and by methanol (previously delipidated) of 17.25 and 14.18 mm respectively. Gentamicin, used as a positive control, is applied to both strains. They react favorably to gentamicin with inhibition diameters of 34 mm for Escherichia coli ATCC 25922 and 38 mm for *Staphylococcus aureus* ATCC 2921 Analyzing the results of the inhibition diameters, it can be said that, on the strain *Escherichia coli* 25922, the extract by crude methanol is extremely effective while that by delipidated methanol is very effective. In addition, on *Staphylococcus aureus* ATCC 29213, the extract by crude methanol is very effective while that by delipidated methanol is simply effective.

I.2.2 Determination of minimum inhibitory concentrations MIC in mg/mL

The diagrams in Figure 3 show the minimum inhibitory concentrations (MIC) of the different extracts of roots and leaves of *Stylocheaton hypogaeus* against the strains *Escherichia coli* ATCC 25922 and Staphylococcus aureus ATCC 29213.

The efficacy of the extract by delipidated methanol of the roots is more remarkable on *Escherichia coli* ATCC 25922 than on *Staphylococcus aureus* ATCC 29213 with respective MICs of 1.5625 and 6.25 mg/mL. On the other hand, the extracts of ethyl acetate and dichloromethane have a MIC of 12.5 mg/mL, showing their moderate efficacy on both strains. However, the delipidated methanol extract of the leaves has a better efficacy on *Staphylococcus aureus* ATCC 29213 than on *Escherichia coli* ATCC 25922 with respective MICs of 1.5625 and 3.125 mg/mL. On the other hand, the crude methanol extract displays MICs of moderate efficacy on *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 respectively of 25 and 50 mg/mL.

These results show the same efficacy of the delipidated methanol extract of the roots on Escherichia coli ATCC 25922 as that of the delipidated methanol extract of the leaves on *Staphylococcus aureus* ATCC 29213. II.2.3 Determination of minimum bactericidal concentrations MBC in mg/mL. The minimum bactericidal concentration (MBC) was determined by comparing bacterial growth in petri dishes from the inoculum count and the plant extract count. The results are shown in the diagrams in Figure 4.

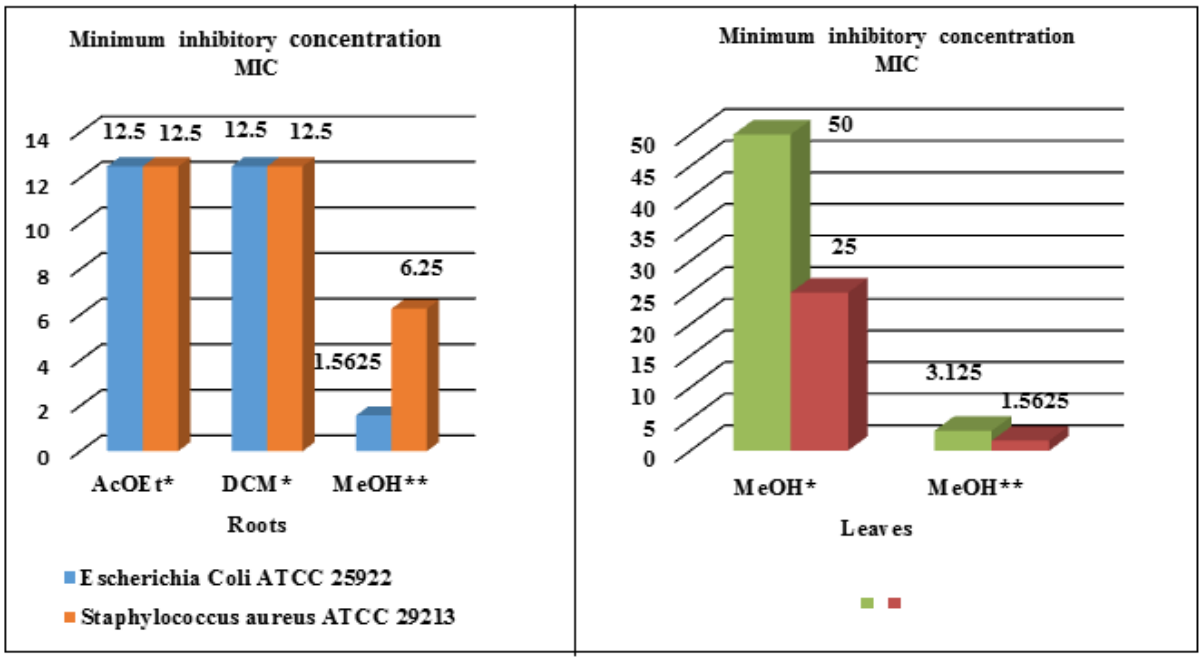

Fig. 3. Minimum inhibitory concentrations MIC in mg/mL.

The results show that the extracts by delipidated methanol and by dichloromethane of the roots are more active against *Escherichia coli* ATCC 25922. On the other hand, the extract by delipidated methanol of the leaves is more effective against *Staphylococcus aureus* ATCC 29213 with a concentration of 12.5 mg/mL.

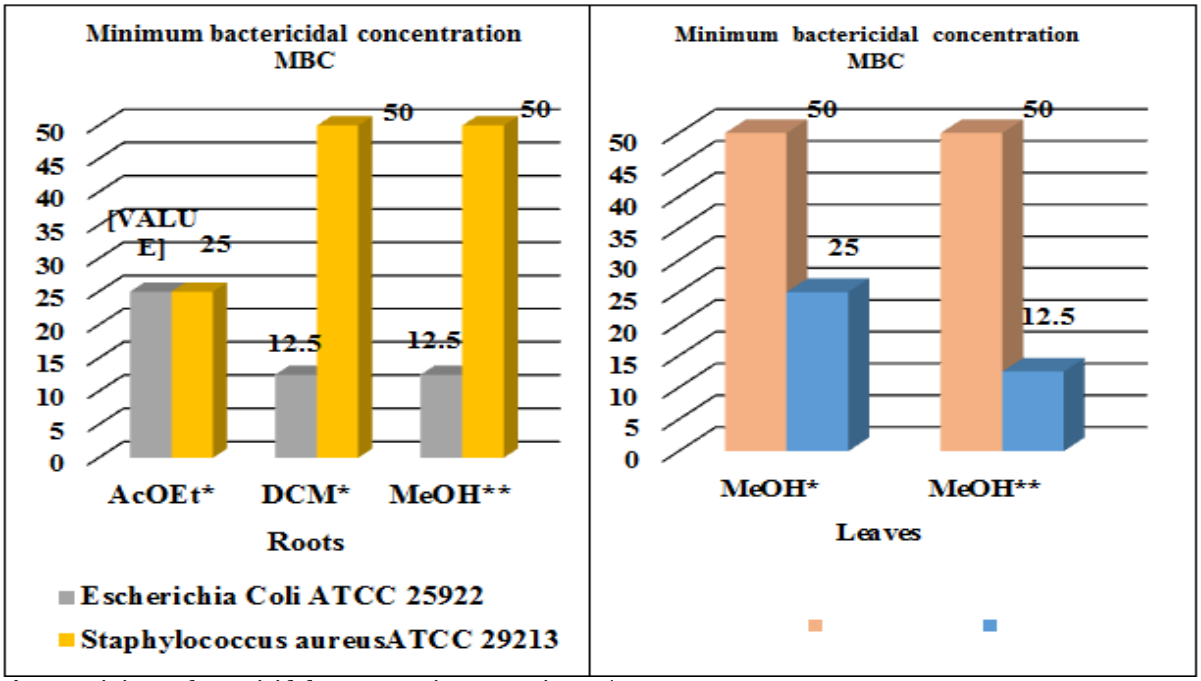

Fig. 4. Minimum bactericidal concentrations MBC in mg/mL.

Determination of the MBC/MIC ratios (mg/mL).

The determination of the ratio of the minimum bactericidal concentration (MBC) to the minimum inhibitory concentration (MIC) of each extract on the different microorganisms was done by the liquid medium method. The values of the MBC/MIC ratio are recorded in the diagram in the figure 5.

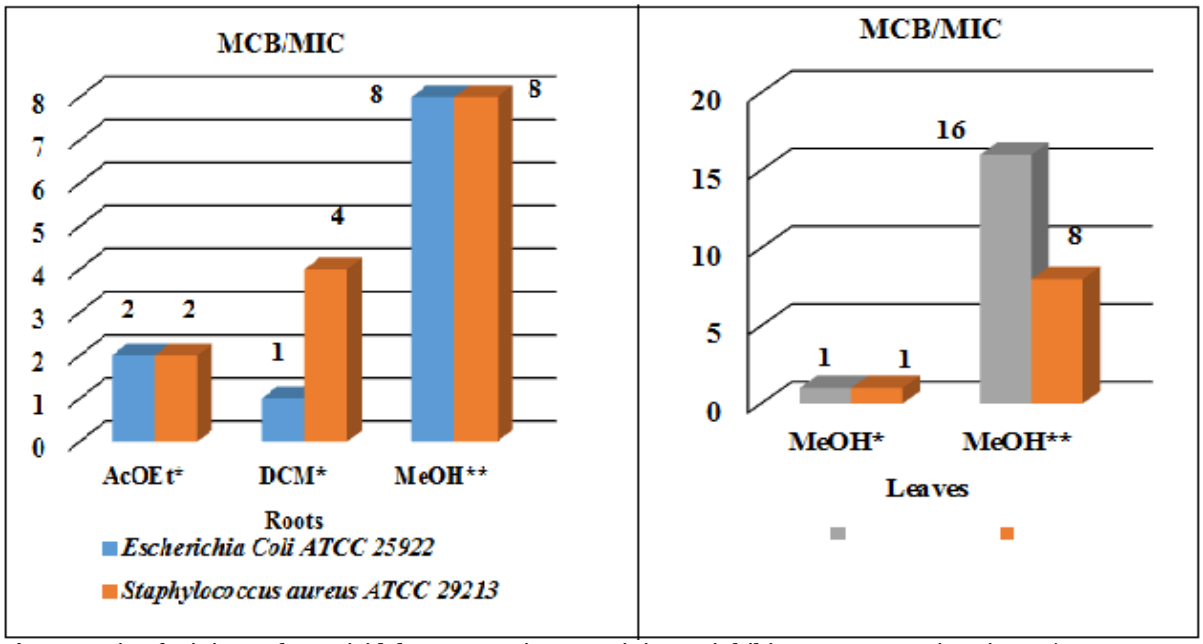

Fig. 5. Ratio of minimum bactericidal concentrations to minimum inhibitory concentrations in mg/mL.

For the leaves, the MBC/MIC ratio value of the extract by crude methanol is less than 4, showing its bactericidal efficacy on both strains. While that by delipidated methanol is greater than 4, also showing its bacteriostatic efficacy. In the case of the extracts by ethyl acetate and by dichloromethane of the roots, the values of the MBC/MIC ratios are less than or equal to 4. This explains why these extracts are bactericidal on both strains. However, for the extract by delipidated methanol, the MBC/MIC ratio is greater than 4, showing its bacteriostatic efficacy on both strains.

Conclusion

In this work, we evaluated the antibacterial activity of root and leaf extracts of *Stylocheaton hypogaeus*. The antimicrobial test is preceded by a phytochemical screening that showed the richness of this plant in secondary metabolites, generally responsible for the biological activity of plants.

The results of the antibacterial activity revealed that the majority of extracts have activity against Gram-positive strains *Staphylococcus aureus* ATCC 29213 and Gram-negative *Escherichia coli* ATCC 25922. The analysis of this antibacterial activity, based on the MBC/MIC ratio, showed that the extracts by ethyl acetate and by dichloromethane of roots have bactericidal properties against both strains, while that by delipidated methanol is bacteriostatic. On the other hand, for the leaves, the extract by crude methanol is bactericidal and that by delipidated methanol is bacteriostatic on both strains. These remarkable results justify the use of *Stylocheaton hypogaeus* in traditional therapy by local populations to treat various types of microbial infections, including prostatitis.

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