Phytochemical study and antimicrobial activity of various extracts of *Merremia tridentata* (Convolvulaceae) leaves

ABSTRACT

Merremia tridentata (Convolvulaceae) is a plant belonging to the African pharmacopoeia. Its leaves are traditionally used in Senegal to treat a variety of illnesses, including cancer, diabetes, ulcers, hemorrhoids, and urinary tract infections. The present study aims to highlight the presence or absence of certain families of chemical compounds through phytochemical screening, and then evaluate the antimicrobial activities of the various extracts from the leaves of *Merremia tridentata*. The sensitivity of the various extracts to gram-positive *Staphylococcus aureus* ATC C29212 and gram-negative isolates of bacteria (*Escherichia coli* ATCC25922) has been studied using the disc diffusion method. This technique allows for the determination of the minimal bactericidal concentration (MBC), the minimal inhibitory concentration (MIC), and the inhibition diameter. The results showed that the strain of *Staphylococcus aureus* is more sensitive to the various extracts. The raw ethanol extract showed better inhibition at a concentration of 3.125 mg/mL followed by the raw methanol extracts at 12.5 mg/mL. The raw methanol, dichloromethane, and defattedethanol extracts evaluated on the *Staphylococcus aureus* strain ATCC29212 showed bactericidal action, however the raw ethanol extract was bacteriostatic. But, when the same extracts were tested on the *Escherichia coli* strain ATCC25922, only the defatted ethanol extract showed bactericidal action.

Keywords: Merremia tridentata, Antimicrobial Activities, Phytochemical Screening.

1. INTRODUCTION

Traditional medicine has always played and continues to play an important role in the live of millions of people all over the world, particularly in Africa. In fact, medicinal plants are an accessible source of health care for rural and poor populations, who often have limited access to modern health care. Medicinal plants are used to treat a wide range of diseases and conditions, including infections, pain, high blood pressure, digestive problems, skin diseases, and more. Some medicinal plants contain bioactive compounds such as alkaloids, flavonoids, essential oils and terpenoids that give them antimicrobial properties, which can kill or inhibit the growth of bacteria. However, bacteria and other microbes are responsible for many infectious diseases. Bacteria are ubiquitous, unicellular and nucleus-free microorganisms (prokaryote) whose genome is made up of DNA. It consists of a single chromosome and the presence of plasmids (a small piece of circular DNA) is noted. Some bacteria can be pathogenic. Food-borne diseases related to Gram-positive and Gram-negative bacteria, especially *Escherichia*, *Staphylococcus*

and constitute a major public health problem in the world [1]. Moreover, with the emergence of multi-resistant pathogens, the clinical effectiveness of many antibiotics is threatened [2]. So, infectious diseases have become the leading cause of death in the world. Bacteria also have the ability to adapt rapidly to the presence of antimicrobial molecules and thus create resistance to the abuse of antibiotics [3]. Today, there has been growing interest in developing more effective alternative medicines from natural sources with less toxicity to humans and the environment. These alternative medicines may come from certain plants such as *Merremia tridentata* (L.) Hallier F which belongs to the Convolvulaceae family and is a perennial, dicotyledonous herbaceous plant. It is native to tropical regions of Africa [4] where it has been a source of natural remedy for several diseases, for the majority of the population. This plant is used for the treatment of various diseases such as: cancer, diabetes, hemorrhoids, swelling, ulcers, rheumatisms, stiffness of the joints, hemiplegia, urinary infections [5]. The phytochemical screening already carried out in the laboratory revealed the presence of compounds of types: catechols, polyphenols, tannins, proanthocyanidins, flavonoids and alkaloids. In addition, the quantitative characterization also carried out in the laboratory resulted in high levels of polyphenols and flavonoids [6]. These results obtained in previous studies [7], motivated the choice of this species in this study whose objective is to evaluate, the antibacterial activities of different extracts of the leaves of *Merremia tridentata* with a view to its potential use in antimicrobial control.

2. MATERIALS AND METHODS

2.1 Vegetable material

The choice of the plant is largely justified according to the literature; few studies have been conducted on this plant, particularly in sub-Saharan Africa [8] and more specifically in Senegal.
The leaves of *Merremia tridentata* were collected in a field in the municipality of Ndoffane, a town located in the Kaolack region in the center-west of the country. It borders with Gambia, on the southern Sahel and northern Sudan geographical coordinates 14° 00′ 00″ north, 16° 00’ 00″ west. This plant was identified at the Institut Fundamental d’AfriqueNoire (IFAN) in the Lebrun and Stork Plant Database of Africa.

2.2 Extraction process

The samples were rinsed with distilled water and then dried in the dark. The leaves were then ground using an electric grinder and kept for later use. The powders obtained were macerated at room temperature with three solvents (Hexane, Methanol, Ethanol) in two different ways:
First, 30 g of leaf powder was inserted into a 1000 mL balloon containing 200 mL of hexane. The resulting mixture was left in maceration for 24 Hours. After filtration, the residues are macerated successively with ethanol and methanol. At the same time, raw macerations for 24H with ethanol, methanol, water, acetyl acetate and dichloromethane were also carried out. The resulting filters were subjected to the rotary evaporator to remove the extraction solvents.

2.3 Phytochemical Study

Phytochemical screening is a qualitative analysis based on precipitation and/or coloration reactions. It was performed according to the methods of Ronchetti and Russo [9-11]. The various extracts from the leaves of M. tridentata have been tested to determine the presence or absence of secondary metabolites that can be found in plant. The qualitative results are expressed by (+) for the presence and by (-) for the absence of a tested compound family. In this work, screening focuses on the search for alkaloids, polyphenols, tannins, flavonoids, sterols and polyterpenes, coumarin and catechols.

The solution was prepared by dissolving each extract in small amount of solvent(S). To the latter, various reagents were added according to the following experimental protocols:
- Polyphenols and tannins were identified by the $FeCl_3$ test and the Stiasny reagent.
- Flavonoids and catechols by reaction with cyanidine.
- Sterols and polyterpenes by the Liebermann-Burchard test.
- Coumarin by ammonium hydroxide test.
- Alkaloids by the Mayer test [12,13].

2.4 Antibacterial activity

2.4.1 Sterility test of extracts

The purpose of this test is to verify that the extracts contain no germs. For this purpose, 0.1 g of the extract was added in 10mL of thioglycolate brothand incubated at 37°C for 24 Hours. After this period, the broth was sown on one petri box containing nutrient gelose and on another containing Sabouraud gelose.The petri boxes were incubated at 37°C. The substance is declared sterile, if no colonies are visible on the frozen box after 24; 48 and 72 hours of incubation at 37°C.

2.4.2 Test of germ sensitivity to various extracts

The activity of the extracts against the bacterial strains used is based on the disc diffusion method.

2.4.3 Test strains

The bacterial strains used in this work, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive), were provided by the Microbiology Laboratory of the Cheikh Anta Diop Polytechnic School of the University of Dakar.

2.4.4 Preparation of McFarland Standard Solution

To avoid any bias that may be due to the variability of the turbidity of the inoculum, it is recommended to standardize it with a McFarland solution 0.5. The preparation of the standard solution was achieved by mixing 0.05 mL of 1% $BaCl_2$ and 9.95 mL of 1% H_2SO_4 to form a $BaSO_4$ suspension. This mixture was placed in a screw tube covered with an aluminum sheet, stored at room temperature and protected from light.

2.4.5 Preparation of the inoculum

Using a loop, a few fresh colonies of the strain to be tested were taken from a Petri dish incubated the day before and suspended in a tube containing sterile physiological saline (8.5 g NaCl/L).The resulting suspension is compared to the McFarland turbidity standard of 0.5 and adjusted, if necessary, by adding saltwater or cells.

2.4.6 Gelose diffusion test

The Mueller-Hinton (Condalab) gelose culture medium is prepared according to the manufacturer's instructions, left to cool to approximately 45-50°C, then poured into Petri boxes (25 mL per box).After solidification, the Petri boxes are inoculated with the 0.5 McFarland standard inoculum using a sterile shell. The seeding is done by stripping 3 times the surface of the crop medium while rotating the Petri box at an angle of 60° after each application. After 5 to 10 minutes of drying at room temperature, sterile diffusion discs of 0.6 cm in diameter were then placed on the upper layer of the frozen medium seeded with bacteria. The discs were then impregnated with 50 µL of plant extract solution at a concentration of 100 mg/mL and the boxes were incubatedat37°C for 24 hours.During the incubation period (24 hours at 37 °C), the strain to be studied competes

with the inhibitory effect of the plant extract.When the strain is sensitive to the extracts, a circular zone of inhibition forms, and if the strain is resistant, there is an absence of the inhibition zone. 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; and it is very effective if you have a diameter between 15 and 19 mm and, finally, it is extremely effective when it is greater than 20 mm. The concentration used is 100 mg/mL.

2.4.7 Determination of Minimum Inhibitory Concentrations (MIC)

The determination of antibacterial parameters is carried out by dilution in a liquid medium. MIC is the lowest concentration at which no growth is observed with the naked eye after a given incubation period. MIC was determined using the experimental test tube method [14] for extracts that presented areas of inhibition of the microorganism. The concentration range is prepared by the half-dilution method. It required preparing a mother solution of the extracts with a concentration of 100 mg/mL. Subsequently, a series of 1/2 dilutions from this mother solution was performed to obtain concentration ranges ranging from 50 to 0.78 mg/mL.In each tube, 1 mL of plant extract concentration is placed in contact with 1 mL of bacterial culture inoculum suspension. For each strain of bacteria, gentamicin was used as positive test, and as negative test, we used distilled water. The tubes were then incubated for 24 hours at 37 °C. A clear solution (no disturbance) of the tube indicates that there is no growth, and the turbidity of the tubes indicates bacterial growth. Each experiment was repeated three times.

2.4.8 Determination of Minimum Bactericidal Concentrations (MBC)

For the determination of minimum bactericidal concentrations (MBCs), a series of dilutions of the initial inoculum with a dilution factor of 10 was performed (4 concentrations were obtained: 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). The different concentrations were sown using a 2 µL calibrated dough in 5 cm long strips, on a Muller-Hinton gelose, incubated for 24 hours at 37°C. These Petri boxes were named A. The contents of tubes in which there was no visible growth after MIC reading were used to sow Muller-Hinton Gelose on 5 cm stripes. This series of Petri boxes was named B. MBC was determined by comparing bacterial growth in boxes A and B. The Minimum Bactericidal Concentration (MBC) is the lowest concentration that kills 99.99% of bacteria in culture compared to the initial inoculum.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screwing

Table 1 summarizes all phytochemical screening of leaf extracts from the *M. tridentata* plant. Phytochemical screening results show that M. tridentata extracts contain various secondary metabolites such as polyphenols, flavonoids, sterols, polyterpenes, catechols and tannins. The presence of all these compounds largely justifies the use of this plant in traditional medicine for the treatment of several pathologies.

Table 1. Phytochemical results of M. tridentata leaves

		polyphenols	Flavonoïds	Alcaloïds	Sterols& polyterpenes	Catechols	Coumarins	T.Catechiques	T. Galliques
tridentata	Hex*	-	-	++	-	-	-	-	-
	MeOH*	+++	+++	++	+	+	+++	-	+
	EtOH*	+++	+++	-	-	++	+	+++	+++

	Aqueous**	**+++**	**+++**	-	**++**	**+**	-	**+**	**+**
	EtOH**	**+++**	**++**	**-**	-	**++**	**-**	-	**+++**
	AcOEt**	**++**	-	-	++	-	-	-	-
	DCM**	**++**	-	+	+	-	+	-	-
	MeOH**	**+++**	**+++**	**-**	-	**++**	**-**	-	-

* Extraction of defatted residues after hexane maceration
** Direct extraction of raw material
DCM: Dichloromethane
+++ Strong presence; ++ Medium Presence; + Low presence: - Absence; **Rough extract.

The analysis of these results showed that methanol, ethanol and aqueous raw extracts, as well as the delipidated methanol and ethanol, contain polyphenols and flavonoids. In parallel, ethyl acetate extract showed an average concentration of polyphenols but no presence of flavonoids. The presence of alkaloids was observed in the raw hexane and defatted methanol extracts;however, these substances were absent from the other extract. Raw methanol, aqueous,dichloromethane and ethyl acetate extracts showed an average presence in terpenes while other extractions revealed the absence of these compounds. For catechols, all extracts contain these compounds except for the raw hexane, DCM and ethyl acetate extracts. It is noted that raw ethanol extracts and delipidated ethanol extractshad high tannins content while raw aqueous and defatted methanol extract reveal their low presence.Defatted methanol extract contains a high concentration of coumarin, while defatted ethanol extracts showed a low concentration. Nevertheless, there was a total absence of coumarin in the other extracts. Under the same conditions, saponins were also not detected in any extract.

3.2 Antibacterial activities

3.2.1 Determination of the sensitivity of different extracts at a concentration of 100 mg/mL to different strains of bacteria

The sensitivity test revealed variable inhibition diameters depending on the extraction solvent and the strain of bacteria tested (table 2).

Table 2. Sensitivity test: inhibition diameter in millimeters (mm)

Stems	**Inhibition diameters by extraction solvent**					**Reference Control**	
	MeOH*	EtOH*	Hex*	DCM*	EtOH**	Gent	H_2O
Escherichia coli ATCC25922	16.89 ± 0.91	16.14 ± 0.30	11.38 ± 0.67	14.12 ± 0.71	16.54 ± 0.65	34.31 ± 1.24	00

Staphylococcus aureus ATCC29212	15.46 ± 0.45	15.74 ± 0.69	12.64 ± 0.28	19.09 ± 0.51	08.85 ± 0.36	38.00 ± 00	00

MeOH*: Methanol; EtOH*= Ethanol; Hex*= Hexane; DCM**= dichloromethane; EtOH**= Ethanol; H_2O: Water; Gent: Gentamicine

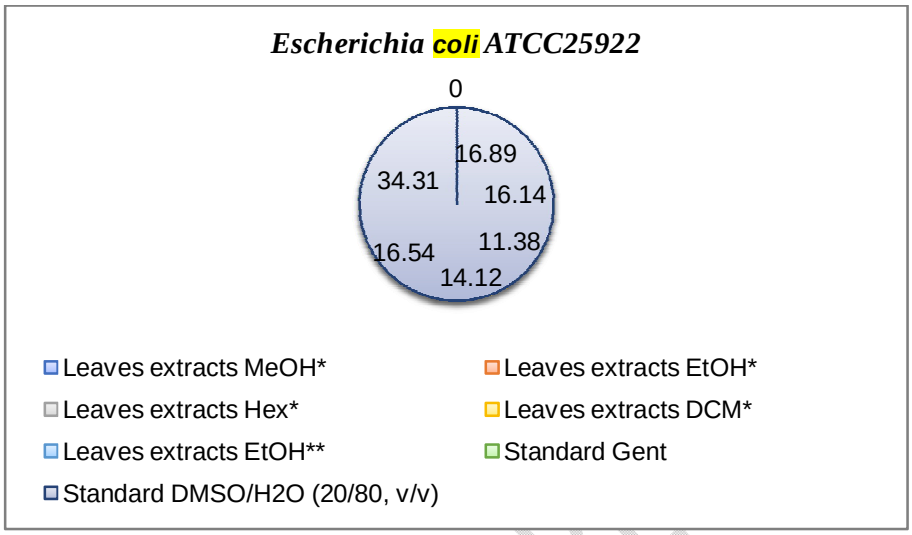

Figure 1 : Sensitivity of various extracts to *Escherichia coli* strain ATCC25922

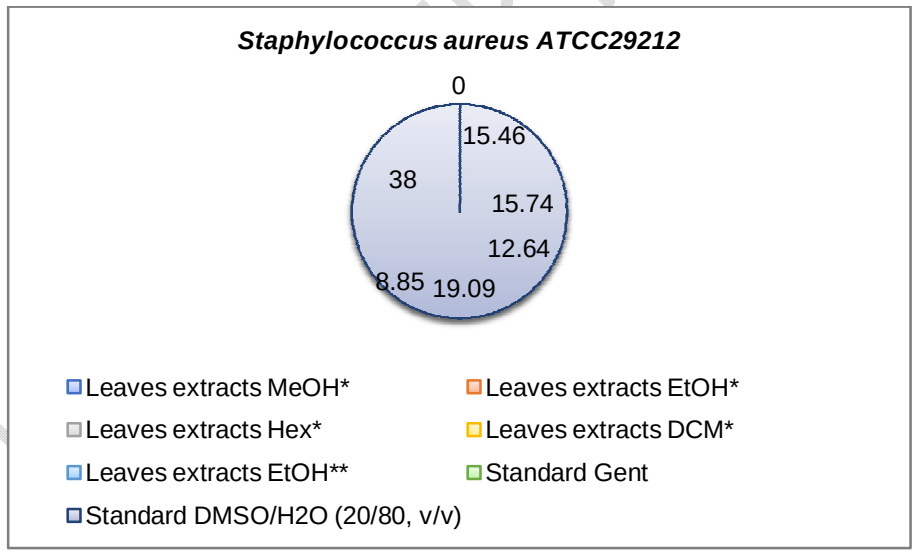

Figure 2: Sensitivity of various extracts to the *Staphylococcus aureus* strain ATCC29212

Our results are corroborated by several studies that have shown that M. tridentata has significant antibacterial and/or antimicrobial properties [15]. In this study, the different extracts from *M. tridentata* leaves show inhibitory action on the two bacterial strains studied. Indeed, the different extracts of *M. tridentata* leaves such as methanol, ethanol and dichloromethane, hexane and ethanol extracts, were evaluated against a Gram positive strain (*Staphylococcus aureus*) and a Gram negative bacterium. (*Escherichia coli*).The results from Table 2 showed that the strains *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATSC29212 are sensitive to the extracts. The strain *Escherichia*

coli ATCC25922, is sensitive to the extracts of methanol, ethanol, hexane, dichloromethane and ethanol crude defatted leaves with inhibition diameters in mm of 16.89 ± 0.91; 16.14±0.30; 11.38 ± 0.67; 14.12 ±0.71; 16.54 ± 0.065, respectively (Figure 1). Under the same conditions, the strain of *Staphylococcus aureus* ATCC29212 has inhibition diameters in mm of 15.46 ± 0.45; 15.74 ± 0.069; 12.64 ± 0.28; 19.09 ± 0.51 and 08.85 ± 0.36, respectively (Figure 2). These results are consistent with those of Kaladhar et al. [16]. Gentamicin used as positive test is tested on the strains of *Escherichia coli* ATCC25922 and *Staphylococcus aureus*. The latter are gentamicin-sensitive with inhibition diameters of 34.31 ± 1.24 and 38.00 ± 00 mm, respectively. The inhibition diameters for sensitive strains range from 9 to 14 mm for a concentration of 100 mg/mL. Raw methanol, ethanol, dichloromethane and ethanol defatted extracts have shown significant antibacterial effects comparable to those of standard drugs such as gentamicin.

3.2.2 Determination of minimum inhibitory concentrations (MIC) in mg/mL of M. tridentata leaf extracts

After sensitivity tests, the minimum inhibitory concentrations of the extracts on the strains were determined. For this purpose, a range of concentrations was prepared by dilution in test tubes and applied to the strains. The readings are performed after 24 hours of incubation at 37°C and the results are shown in the diagram in Figure 3. A clear solution shows that the extract inhibits the proliferation of the bacteria, while a troubled solution shows resistance or bacterial proliferation.

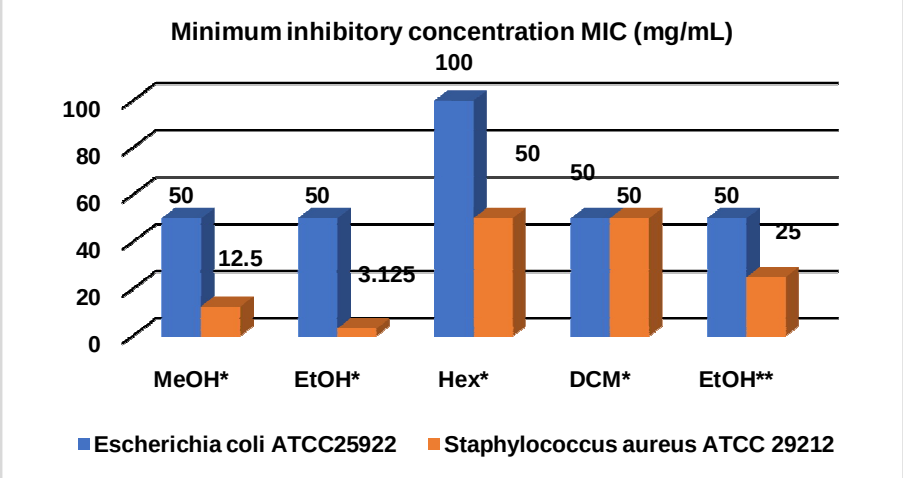

Figure 3: Average inhibitory concentrations (MIC in mg/mL)

For every extract, the minimum inhibitory concentration ranges from 3.125 to 100 mg/mL (Figure 3).The extracts were more effective against *staphylococcus aureus* with lower MICs (3.125 to 50 mg/mL) than those obtained with *Escherichia coli*. It was noted that the raw ethanol extract had the lowest MIC (3.125 mg/mL) against *staphylococcus aureus*. The hexane extract had the highest MIC with *Escherichia coli* therefore it was the least active against this strain. However, all other extracts had the same MIC (50 mg/mL) against *Escherichia coli*. These results showed that *M. tridentata* leaf extracts were more active against *Staphylococcus aureus* compared to *Escherichia coli*.

3.2.3 Determination of minimum bactericidal concentrations (MBCs) in mg/mL of M. tridentata leaf extracts

The minimum bactericidal concentration (MBC) was determined by comparing bacterial growth in petri boxes and the results were shown in the diagram in Figure 4.

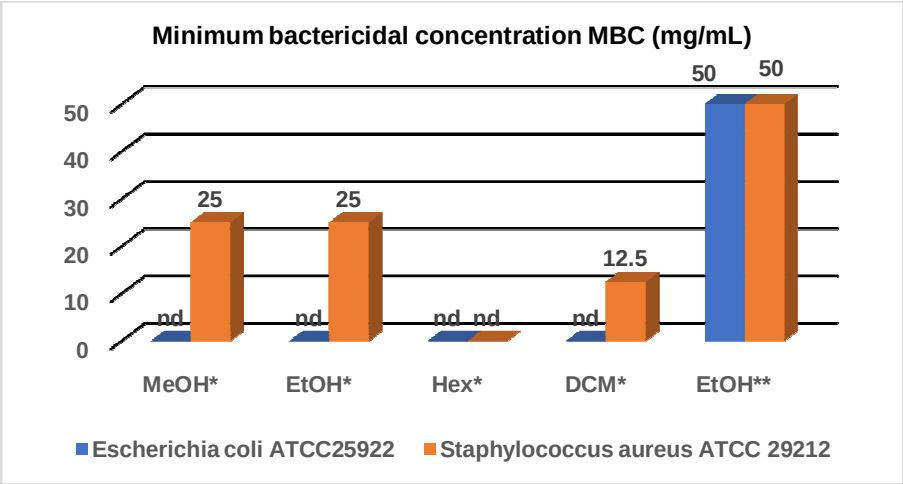

Figure 4: Average bactericidal concentrations (in mg/mL)

The MBC/MIC report is clarified the method of action of the substance. In fact, the extract was bactericidal when the MBCwas equal to the MIC or if the ratio was less than 4. However, it was bacteriostatic when MBCwas higher than MIC or if the MBC/MIC ratio was greater than 4. The determination of the minimum inhibitory concentration and the minimum bactericidal concentration of extracts on different micro-organisms was evaluated using the liquid medium method. The MIC and MBC values were shown in Table 3 below.All *M. tridentata* leaf extracts showed significant antibacterial activity against the Gram positive bacterium with MIC values ranging from 3.125 to 100 mg/mL. In contrast, antibacterial activity was more moderate against Gramnegative bacteria with an MIC of 50 mg/mL except for hexane extract, which had shown no inhibitory activity. The ratio of Minimum Bactericidal Concentration (MBC) to Minimum Inhibitory Concentrated (MIC) of extracts such as dichloromethane, methanol and ethanol defatted for *Staphylococcus aureus* strain ATCC29212 was less than 4. Thus, it can be concluded that these extracts were bactericidal. However, the one obtained with the raw ethanol extract was greater than 4 where it was bacteriostatic.

Table 3: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MCCs) of the various extracts.

Bacterial strains	Minimum Inhibitor Concentration MIC / Minimum Bactericidal Concentrations in (mg/mL)				
	Leaf Extracts				
	MeOH*	EtOH*	Hex*	DCM*	EtOH**
Escherichia coli ATCC25922	$50^{(a)}$ $und^{(b)}$ $und^{(c)}$	$50^{(a)}$ $und^{(b)}$ $und^{(c)}$	$100^{(a)}$ $und^{(b)}$ $und^{(c)}$	$50^{(a)}$ $und^{(b)}$ $und^{(c)}$	$50^{(a)}$ $50^{(b)}$ $1^{(c)}$
Staphylococcus aureus ATCC 29212	$12,5^{(a)}$ $25^{(b)}$ $2^{(c)}$	$3,125^{(a)}$ $25^{(b)}$ $8^{(c)}$	$50^{(a)}$ $und^{(b)}$ $und^{(c)}$	$50^{(a)}$ $12,5^{(b)}$ $0,25^{(c)}$	$25^{(a)}$ $50^{(b)}$ $2^{(c)}$

MIC(^{a}): Minimum inhibitory concentration (mg/mL). MBC(^{b}): Minimum bactericidal concentration (mg/mL). MBC/MIC(^{c}): Minimum bactericidal concentration/ Minimum inhibitory concentration.und: undefined.

For *Escherichia coli* strain ATCC25922, the MIC and MBC of raw methanol, dichloromethane, ethanol and hexane extracts have not been determined while the MBC/MIC ratio of the defatted ethanol extract was less than 4. So, we can conclude that only the defatted ethanol extract was bactericidal. It was noted that the best activity was recorded with the defatted ethanol extract and dichloromethane extract against the strains of *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATSC29212, respectively. The bactericidal effect of these different extracts could be attributed to phytochemical compounds such as alkaloids, polyterpenes, tannins, flavonoids and steroids. These antibacterial activities of M. tridentata largely justify the traditional use of the leaves of the plant for the treatment of bacterial infections such as diarrhea, dysentery, and cough [7].

4. CONCLUSION

The phytochemical screening carried out on the species M. tridentata revealed in leaves extracts, the presence of several secondary metabolites such as: polyphenols, alkaloids, tannins, polyterpenes, sterols, catechols, coumarins and flavonoids. Some compounds were present in several extracts. In this study, almost all extracts showed antibacterial activity against both strains, including the grampositive strain *Staphylococcus aureus* ATCC29212 with inhibition diameters greater than 9. The evaluation of antimicrobial activity, through the MBC/MIC report, showed that the methanol, dichloromethane and defatted ethanol extracts were bactericidal against the *Staphylococcus aureus* strain ATCC29212, whereas the crude ethanol extract was bacteriostatic. However, only the defatted ethanol extract was bactericidal against the gramnegative strain *Escherichia coli* ATCC25922 despite the presence of secondary metabolites in these different extracts. This study has made it possible to evaluate the activities described on this part of the plant *Merremiatridentata* because it has just confirmed the ethnopharmacological properties described by its use in traditional medicine.To better support the results of this work, the antimicrobial activity of *Merremiatridentata* leaves extracts should be studied in vivo models infected by clinically isolated strains and specific microorganisms in order to demonstrate their effectiveness and clarify their mechanism of action.

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