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*Full Length Research Paper*

# Antimicrobial effect analysis of *Verbena officinalis*, *Malva sylvestris* and *Ageratum conyzoides* on *Gardnerella vaginalis* and *Candida* spp. isolated from vaginal secretionin Palmas, TO, Brazil

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**Vaginitis is a condition that involves vulva and vaginal inflammation or infection, caused mainly by imbalance of vaginal microbiota. Its treatment is performed by the use of antimicrobials which are related with the appearance of resistant microorganisms, adverse effects and high rates of recurrence. Thus, interest in plants with antimicrobial activity has grown exponentially in academic area. For the first time, the antimicrobial activity of gervao (*Verbena officinalis*), malva branca (*Malva sylvestris*) and mentrasto (*Ageratum conyzoides*) ethanolic extractsis evaluated against clinical specimens of *Candida* spp. and *Gardnerella vaginalis*– important microorganisms in the etiology of vaginitis. Although the extracts did not present antifungal activity against *Candida* spp. specimens, the combination of the three extracts at 50, 100 and 200 mg.mL$^{-1}$ exhibited antibacterial activity against all specimens of *G. vaginalis*. Gervao and mentrasto extracts combined at 50, 100 and 200 mg.mL$^{-1}$were also able to inhibit the bacterial growth. Besides, those extracts had the lowest values of MIC (6.25 mg.mL$^{-1}$), followed by the combination of mentrasto with malva branca– 6.25-25 mg.mL$^{-1}$– and the combination of the three extracts – 12.5-25 mg.mL$^{-1}$.Therefore, extracts of mentrasto and gervao are promising for the development of new strategies for the treatment of bacterial vaginosis.**

**Keywords:***Verbena officinalis*, *Malva sylvestris*, *Ageratum conyzoides*, *Gardnerella vaginalis*, *Candida* spp., antimicrobial activity.

## INTRODUCTION

Vagina and cervix are embedded in a complex ecosystem, containing numerous species of aerobic and anaerobic microorganisms, and environmental variation of that ecosystem (caused by vaginal acidity reduction, decreased immunity, diabetes, and iatrogenic factors) may lead to the appearance of vaginitis. Vaginitis is a condition involving inflammation or infection of the vulva and vaginal wall, with leukorrhea, erythema, pain and

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dyspareunia. Bacterial vaginosis and vulvovaginal candidiasis are the main types of vaginitis (CASSONE, 2015; MILLS, 2017).

In Brazil, bacterial vaginosis is very common, affecting most women with complaints of vaginal discharge. In addition, it affects about 10% to 30% of pregnant women and 10% of women attended in basic care and may also be present in women asymptomatically (MS, 2015). It is characterized by an imbalance of the vaginal microbiota, in which there is an overgrowth of anaerobic bacteria, such as *Gardnerella vaginalis*, *Peptostreptococcus*, *Mobiluncus*, *Prevotella*, *Bacteroides* and *Mycoplasma hominis*, and a concomitant decrease of *Lactobacillus* (ALMEIDA et. al., 2013; REID, 2018).

*Gardnerella vaginalis* is one of the bacterial agents that are most frequently associated with bacterial vaginosis, having as main characteristics: shortcocci-bacilli, negative-Gram or variable-Gram, pleomorphic, uncapsulated, immobile and facultative anaerobic; capable of modifying the vaginal pH (above 4.5); generation of abundant greyish-white and foul-smelling discharge from the production of volatile aminopeptidases (ALMEIDA et al., 2013; MILLS, 2017).

Vulvovaginal candidiasis, on the other hand, is caused by the intense growth of the opportunistic yeast *Candida* spp. in the genital mucosa. *Candida albicans* is the most prevalent etiology agent in vulvovaginal candidiasis, accounting for 80% to 90% of infections (MS, 2015). Despite this, other species, such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis* have been reported to be involved (FULE et al. 2015; ZAHEDI et al., 2016). This group has saprophytic behavior in the lower genital tract, which under certain conditions multiplies excessively, becoming pathogenic. About 75% of women develop vulvovaginal candidiasis at least once in their lifetime and the disease is clinically characterized by intense vulvar pruritus, burning, leucorrhoea, dyspareunia, dysuria, edema and vulvovaginal erythema (CASSONE, 2015; DENNING et al., 2018).

The treatment of vaginal infections is carried out by the use of topical or oral antimicrobials that are associated with the occurrence of side effects, high recurrence rates and appearance of resistant microorganisms (CÓRDOBA et al., 2018; DENNING et al., 2018; MAHBOUBI, 2018). In this way, the interest in alternative and complementary medicine has been growing exponentially since the beginning of the twenty-first century (FELIX et al., 2019). Indeed, a number of plants with antimicrobial activity have been studied and used (ESSIEN et al., 2001; JUTEAU et al., 2002; HOFFMAN et al., 2004; LEITE et al., 2011; CARDOSO et al., 2012; MACEDO, 2013; GIORDANI et al., 2015; AZUERO et al., 2016; AHMED et al., 2017; KUOK et al., 2017; FEIZI et al. 2018; MAHBOUBI, 2018).

Gervao (*Verbena officinalis*, Verbenaceae), malva branca (*Malva sylvestris* L., Malvaceae) and mentrasto (*Ageratum conyzoides* L., Asteraceae) are examples of plants whose medicinal use is widespread in Brazil and in other countries, with a range of pharmacological properties: antimicrobial, anti-inflammatory, analgesic, gastroprotective, antihelmintic, antiulcerogenic, expectorant, hypoglycemic and immunostimulating action (ALONSO, 1998; SILVA, 2001; CASTRO et al., 2004; SILVEIRA, et al. 2007; BOSCOLO & VALLE, 2008; MILLANI et al., 2010; FILTER et al., 2014). Although these plants are traditionally used against vulvovaginal candidiasis, there are few studies that verify the antimicrobial activity of them against the main etiological agents of vaginitis. The objective of the present study was to analyze whether the extract of gervao, malva branca and mentrasto leaves have antimicrobial action against specimens of the genus *Candida* and *Gardnerella vaginalis*– isolated from samples of vaginal secretion collected during the clinical examination of patients with discharge characteristic of vaginitis in health services from Palmas, TO, Brazil.

## MATERIAL AND METHODS

### Botanical Material Collection and Plant Extracts Obtaining

One kilogram of gervao, malva branca and mentrasto leaves were collected in the city of Porto Nacional and region (10.7006368 latitude and 48.397708 longitude), TO, Brazil. The material was transported to the Laboratory of General and Applied Microbiology (LGAM) of the Universidade Federal do Tocantins (UFT) for processing. One specimen of each plant was deposited in the UFT herbarium. The fresh leaves were dried in an oven (45-48 °C) for 6 h and then crushed. The dry material was submitted to extraction of chemical constituents according to OLIVEIRA and collaborators (2016), using the Soxhlet extractor (SOXHLET, 1879). Firstly, the dry leaves were weighed directly in cellulose cartridges (21.58 g of gervao, 24.05 g of malva branca and 17.53 g of mentrasto) and placed in the Soxhlet apparatus. Ethanol (VETEC) was used as extractor and the extraction process was carried out for 5 hours. The condenser water cooling system was used at 18 ºC and the extracts were concentrated in a rotary evaporator. The yield (Y%) of each extract was calculated based on the dry matter according to the following equation:

$$Y\% = [Bm - (Mo)].100$$

Where Bm = vegetal aerial biomass in grams (g); Mo = mass of the extract in grams (g); and 100 is the conversion factor for percentage.

### GC/MS Analysis

Plant extracts were submitted to derivatization (transesterification reaction) by acidcatalysis of boron trifluoride in methanol with heat conditions according to MEHER and collaborators (2006). The analyses were carried out in a Shimadzu type GC/MS QP, 2010Plus

Model, which has a capillary column of fused silica HP-5MS (30m × 0.25mm × 0.25 $\mu$m), using the method of OLIVEIRA and collaborators (2016). The compounds were identified comparing their peak mass data with the data in the NIST-08 (National Institute of Standards and Technology) library.

**Pathogens Obtaining**

Samples of vaginal secretion were collected with sterile Swabs during the clinical examination of patients who presented vaginal discharge characteristic of vaginitis. These samples were immediately immersed in tubes with saline solution 0.85% (w/v) and sent immediately to the LGAM for experimental processing.

In order to obtain specimens of *Candida* spp., clinical samples were seeded in Petri dishes containing Sabouraud dextrose agar culture medium, supplemented with chloramphenicol (10 $mg.mL^{-1}$), and incubated at 35 °C for 2–7 days. From the dishes where yeast colonies developed, four morphologically representative colonies per clinical specimen were chosen for subculture in the same culture medium without antibacterial drug. After 24–48 h of incubation at 35 °C, mineral oil was added to the pure cultures obtained for preservation, and they were stored under refrigeration (4°C).

In order to obtain *Gardnerella vaginalis* specimens, clinical samples were seeded in Petri dishes containing *Gardnerella* selective agar with 5% Human Blood BD culture medium and in Petri dishes containing BD Columbia Agar medium plus blood of sheep 5% (v/v). Samples were seeded in both culture media and incubated in microaerophilic atmosphere at 36 °C for 48–72 h. Then, the presence of small and medium colonies, surrounded by diffuse beta-hemolysis was verified in *Gardnerella* selective agar with 5% Human Blood BD. This result was compared with the growth obtained on the BD Columbia Agar medium plus blood of sheep 5% (v/v). The colonies that presented beta-hemolysis in the selective medium of *Gardnerella* were classified as *G. vaginalis*, according to the manufacturer's instructions (BD, 2011), and the beta-hemolytic colonies present in both media were submitted to Gram staining for the certification of that the microorganism in the selective medium of *Gardnerella* was a small gram-variable diphtheroid rod (BD, 2011). Four representative colonies morphologically distinct per clinical specimen were selected, sub-cultured on Mueller-Hinton agar and incubated at 36 °C for 24h in microaerophilic atmosphere. After obtaining pure cultures, mineral oil was added to them and they were stored under refrigeration (4 °C).

**Antimicrobial Sensitivity Test**

The antimicrobial sensitivity assays were carried out in triplicate through the agar well diffusion method (CLSI, 2009) using Petri dishes with 50 mL of Sabouraud dextrose agar, in the case of yeasts, and with 50 mL of Mueller-Hinton agar, in the case of bacteria. The number of yeast cells was standardized in Neubauer's chamber, diluting 3–4colonies of pure culture in 0.85% saline solution (w/v) until the concentration reached $1x10^6$ $UFC.mL^{-1}$. The number of bacterial cells was standardized by diluting the same number of colonies of pure culture in 0.85% saline solution (w/v) until reaching the turbidity corresponding to 0.5 of the MacFarland scale (CLSI, 2009), equivalent to $1,5 \times 10^8$ $UFC.mL^{-1}$. As negative control, 10% dimethyl sulfoxide (DMSO) solution (v/v) was used, and as positive control, Fluconazole (200 $mg.mL^{-1}$) was used for yeasts and Chloramphenicol (200 $mg.mL^{-1}$) was used for bacteria.

The inoculum solutions were seeded on Petri dishes using sterile swab. Then, wells of 5 mm of diameter were made in the media. In such wells, 50 µL of the three simultaneously combined extracts (gervao – G, malva branca – MB and mentrasto – M) diluted in 10% DMSO solution (v/v) at the concentrations of 200, 100 and 50 $mg.mL^{-1}$ were added. Individual and paired extracts were also diluted at the mentioned concentrations and 50 µL of each dilution were added to the wells. The same volume of positive and negative controls was introduced into the wells. Thus, dishes were incubated for 24h at 35 °C under aerobic condition, in the case of yeasts, and at 36 ° C under microaerobic condition, in the case of bacteria. Finally, the growth inhibition halo measurement was carried out using a digital caliper.

**Determination of Minimum Inhibitory Concentration (MIC)**

To determine the minimum inhibitory concentration (MIC) of the plant extracts against microorganisms with inhibited growth in the agar well diffusion method, the broth microdilution technique was used (CLSI, 2009). Then, 100 µL of Mueller-Hinton broth was added to 96-well microplates, followed by the addition of 100 µL of the plant extracts (individual or combined) at 400 $mg.mL^{-1}$. The following series of extracts concentrations were obtained at the end of the process: 200, 100, 50, 25, 12, 5, 6, 25, 3.125 and 1.5625 $mg.mL^{-1}$.

As positive control, 100 µL of chloramphenicol at 2000 $\mu g.mL^{-1}$ were added and serially diluted at 1000, 500, 250, 125, 62.5, 31.25 and 15.625 $\mu L.mL^{-1}$. As negative control, 100 µl of 100% DMSO was added and diluted at 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78%. As growth control, 100 µL of the bacteria solution at $10^7$ $CFU.mL^{-1}$ were added and serially diluted. Finally, as a sterility control, 100 µL of culture medium was added into one well of the microplate.

Then, 5 µL of the bacterial inoculum solution at 107 $CFU.mL^{-1}$ were added to the wells containing the extracts, both positive and negative controls. At the end of the procedure, microplates were incubated at 37 °C for 24 h. Thus, 30 µL of 1% resazurin (7-hydroxy-3H-pheno-

xazine-3-one-10-oxide) aqueous solution (v/v) was added to the wells and homogenized. The microplates were incubated at 37 °C for 1 h and the results were read. MIC was considered the lowest concentration of extract(s) capable of killing the bacterial cells.

**Statistical Analysis**

The results were submitted to analysis of variance and to the comparison of means by Student's T test, at a 5% probability level, through software R, version 3.3.1.

**RESULTS**

**Plant Extracts Obtaining**

About 1 kg of gervao, malva branca and mentrasto was collected in the city of Porto Nacional and region, TO, Brazil. The crude ethanolic extract of each of the three vegetal species using a Soxhlet extractor resulted in a yield of 24.80% for gervao,5,43% for malva branca and 13,39% for mentrasto.

**GC/MS Analysis**

The chemical composition of the three extracts is listed in Table 1. In general, palmitic acid is one of the main components of the three extracts. Besides, gervao extract is composed mainly by linoleoyl chloride and trans-13-octadecenoic acid. On the other hand, part of malva branca and mentrasto extracts consists of linoleic acid and 9,12-hexadecadienoic acid. Moreover, malva branca extract has a significative amount of stearic acid and 2-pentadec-12-ynoxyoxane.

**Pathogen Obtaining**

Among the patients that participated in this study, which presented vaginal discharge characteristic of vaginitis during clinical examination (n=75), vaginal secretion culture allowed the recovery of *Candida* spp. From 22 clinical samples and *Gardnerella vaginalis* from 6 ones.

**Antimicrobial Sensitivity Test**

The antimicrobial sensitivity test was performed through the agar well diffusion method with the combined and individual extracts at concentrations of 200, 100 and 50 $mg.ml^{-1}$. Regarding the clinical isolates of *Candida* spp., no growth inhibition halo was visualized from the growth conditions imposed using the plant ethanolic extracts. Therefore, neither the combined gervao, malva branca and mentrasto extracts nor the individual ones affected the growth of the yeast specimens.

However, the growth of *G. vaginalis* specimens was inhibited by all concentrations of the mixture of three extracts simultaneously (Table 2, Figure 1). When the extracts were tested in pairs, only the combination of mentrasto and gervao (M + G) was able to inhibit bacterial growth, even so, of only one of the samples (Table 2, Figure 2A). A similar result was observed when mentrasto (M) and gervao (G) were individually imposed, being the halos formed in the mentrasto extract presence larger than those formed in the gervao extract presence (Table 2, Figure 2B). Thus, the antibacterial activity of mentrasto is the most intense. The results of the other combinations of extracts, which did not inhibit the growth of *G. vaginalis* specimens, are not shown.

The diameter of inhibition halos varied from 19.45 to 21.75 mm when the three extracts were used at the same time (M + MB + G) at 200 $mg.mL^{-1}$; from 18.34 to 20.15 mm at 100 $mg.ml^{-1}$; and from 14.92 to 16.99 mm at 50 $mg.ml^{-1}$(Table 2). The diameter of inhibition halo was 28.87 mm when mentrasto (M) was imposed at 200 $mg.ml^{-1}$, 25.35 mm at 100 $mg.ml^{-1}$ and 15.91 at 50 $mg.ml^{-1}$(Table 2). The diameter of inhibition halo was 11.15 mm when gervao (G) was imposed at 200 $mg.ml^{-1}$, 9.96 mm at 100 $mg.ml^{-1}$ and 9.85 mm at 50 $mg.ml^{-1}$ (Table 2). Therefore, it is notable that mentrasto (M) was the major protagonist of this experiment, considering the highest values of bacterial growth inhibition obtained when it was used individually. Nevertheless, all values of the growth inhibitory halos were lower than those promoted by the reference antibiotic (chloramphenicol 200 $mg,mL^{-1}$,Table 2).

**Determination of Minimum Inhibitory Concentration (MIC)**

Broth microdilution technique (CLSI, 2009) was used to determine the minimum inhibitory concentration (MIC) of plant extracts able to prevent the growth of *G. Vaginalis* specimens. The results are presented in Table 3. The concentrations used in the test ranged from 200 $mg.mL^{-1}$ to 1.5625 $mg.mL^{-1}$ and it was observed that MICs varied among bacterial isolations. The lowest MIC values were those exhibited from simultaneous combination of the three extracts (M + MB + G) – between 12.5 and 25 $mg.mL^{-1}$; followed by mentrasto and malva branca (M + MB) – between 6.25 and 25 $mg.mL^{-1}$;mentrasto (M) – between 6.25 and 12.5 $mg.mL^{-1}$; and gervao (G) – between 6.25 and 25 $mg.mL^{-1}$.

**DISCUSSION**

For the first time, the antimicrobial activity of extracts of gervao (*V. officinalis*, Verbenaceae), malva branca (*M. sylvestris* L., Malvaceae) and mentrasto (*A. conyzoides* L., Asteraceae) against yeasts and bacteria important in the etiology of vaginitis was evaluated. It is known that vaginitis – such as bacterial vaginosis and vulvovaginal candidiasis –are the main complaint of women in gynecological clinics (MILLS, 2017). In Brazil, for example, bacterial vaginosis affects most women with

**Table 1.** Chemical composition of the plant ethanolic extracts according to GC/MS analysis.

| Compounds | Gervao | | Malva Branca | | Mentrasto | |
|---|---|---|---|---|---|---|
| | RT | Area (%) | RT | Area (%) | RT | Area (%) |
| *Succinic acid C4:0* | 5.762 | 0.95 | Nd | Nd | Nd | Nd |
| *Caproic acid, C6:0* | Nd | Nd | 21.170 | 1.16 | Nd | Nd |
| *Tridecanoic acid, C13:0* | 22.506 | 1.35 | 15.872 | 0.88 | 12.916 | 1.10 |
| ***Palmitic acid, C16:0*** | 19.946 | **24.36** | 19.955 | **25.78** | 19.964 | **26.16** |
| ***Stearic acid, C18:0*** | Nd | Nd | 25.417 | **7.26** | 25.412 | 3.86 |
| ***Linolenic acid, C18:3 (ω3)*** | Nd | Nd | 24.729 | **32.50** | 24.756 | **40.57** |
| *11-Tridecenol, $C_{13}H_{26}O$* | Nd | Nd | 18.036 | 0.89 | Nd | Nd |
| ***2-Pentadec-12-ynoxyoxane, $C_{20}H_{36}O_2$*** | Nd | Nd | 23.667 | **7.78** | 23.664 | 4.09 |
| ***9,12-Hexadecadienoic acid, $C_{16}H_{28}O_2$*** | Nd | Nd | 24.512 | **16.38** | 24.513 | **10.69** |
| *d-Ribose, 2-deoxy-bis(thiononyl)-dithioacetal, $C_{23}H_{48}O_3S_2$* | Nd | Nd | 27.102 | 1.02 | Nd | Nd |
| *Phytol, $C_{20}H_{40}O$* | Nd | Nd | Nd | Nd | 18.556 | 2.49 |
| *1-Methyl-2-acetyl-3-(1-methylethenyl)-cyclopentane, $C_{11}H_{18}O$* | Nd | Nd | Nd | Nd | 18.671 | 0.97 |
| *Hexadecyloxirane, $C_{18}H_{36}O$* | Nd | Nd | Nd | Nd | 19.833 | 1.03 |
| *Methylphoshonic acid, $C_8H_{18}FO_2P$* | Nd | Nd | Nd | Nd | 20.566 | 1.26 |
| *2-Pentadec-12-ynoxyoxane, $C_{20}H_{36}O_2$* | Nd | Nd | Nd | Nd | 22.858 | 1.50 |
| *2-Ethylbutyric acid, $C_6H_{12}O_2$* | Nd | Nd | Nd | Nd | 27.102 | 2.25 |
| *Laevulinic acid, $C_5H_8O$* | 5.127 | 2.18 | Nd | Nd | Nd | Nd |
| ***Linoleoyl chloride, $C_{18}H_{31}ClO$*** | 24.734 | **46.15** | Nd | Nd | Nd | Nd |
| ***Trans-13-octadecenoic acid, $C_{18}H_{34}O_2$*** | 24.834 | **9.73** | Nd | Nd | Nd | Nd |
| *8-Nonynoic Acid, $C_9H_{14}O_2$* | 25.423 | 3.41 | Nd | Nd | Nd | Nd |

RT: retention time in minutes; Area: proportional peak area; Nd: not detected. Expressive compounds and areas are marked in bold.

complaints of vaginal discharge (MS, 2015), and the main etiological agent is *G. vaginalis* (ALMEIDA et al., 2013). However, conventional treatment of vaginitis is often ineffective, considering the increased frequency of microorganism resistant; besides, it is related to the occurrence of side effects and high recurrence rates (CÓRDOBA et al., 2018; DENNING et al., 2018; MAHBOUBI, 2018). In this way, treatment using alternative and complementary medicine (as medicinal plant), becomes promising to combat infectious diseases, such as vaginitis (FELIX et al., 2019).Gervao, malva branca and mentrasto are examples of plants whose medicinal use is widely spread, being traditionally used against vulvovaginal candidiasis. Nevertheless, there are few studies that verify their antimicrobial activity against the main etiological agents of vaginitis.

Here, ethanolic extracts of gervao, malva branca and mentrasto leaves were obtained and GC–MS analysis of those extracts revealed the presence of fatty acids that were the main compounds in the three plants, but their percentages in each plant were greatly different (Table 1). Through the agar well diffusion method, it was verified that the three vegetal extracts, when simultaneously combined, have antibacterial activity against *G. vaginalis* specimens. Gervao and mentrasto extracts tested either individually or combined presented growth inhibitory effect against one specimen. Malva branca was not able to inhibit the growth of any bacterial specimen. Interestingly, mentrasto extract antimicrobial activity was the most significant when compared to the others. However, when compared to the positive control, the plant extract growth inhibition halos are still smaller than the control halos. This indicates that high concentrations of gervao and mentrasto could be more effective, being potential forms of treatment of bacterial vaginosis. Similar results were observed when MIC was determined. The lowest MIC values were those exhibited when mentrasto and gervao were individually used and when three extracts were simultaneously combined. Mentrasto and malva branca pair exhibited MIC values lower than gervao

**Table 2.** Diameters of the growth inhibition halo (mm) of *G. Vaginlais* specimens cultured in the presence of the combined extracts (M + MB + G, M + MB, M + G and G + MB) and individual ones (M, MB e G). Chloramphenicol was used as positive control. Hyphen means absence of halo.

| ***G. vaginalis*specimens** | **M + MB + G** | | | **Chloramphenicol** |
|---|---|---|---|---|
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | 19.78 ± 0.44 | 18.82 ± 0.25 | 14.92 ± 1.15 | 46.7 |
| *4* | 19.45 ± 1.42 | 18.34 ± 1.02 | 15.74 ± 0.36 | 48.97 |
| *II* | 21.75 ± 0.09 | 20.15 ± 0.33 | 16.99 ± 0.28 | 50.25 |
| | **M + MB** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | - | - | - | 47.3 |
| *4* | - | - | - | 48.09 |
| *II* | 20.27 ± 0.94 | - | - | 45.42 |
| | **M + G** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | 8.85 ± 0.58 | - | - | 47.3 |
| *4* | - | - | - | 48.09 |
| *II* | 19.55 ± 0.32 | 17.08 ± 1.40 | 15.32 ± 1.16 | 45.42 |
| | **G + MB** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | - | - | - | 47.3 |
| *4* | - | - | - | 48.09 |
| *II* | - | - | - | 45.42 |
| | **M** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | - | - | - | 52.67 |
| *4* | - | - | - | 50.96 |
| *II* | 28.87 ± 2.21 | 25.35 ± 3.00 | 15.91 ± 1.72 | 54.2 |
| | **G** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | - | - | - | 52.67 |
| *4* | - | - | - | 50.96 |
| *II* | 11.15 ± 0.39 | 9.96 ± 0.01 | 9.85 ± 0.67 | 54.2 |
| | **MB** | | | **Chloramphenicol** |
| | *200 mg.mL$^{-1}$* | *100 mg.mL$^{-1}$* | *50 mg.mL$^{-1}$* | *200 mg.mL$^{-1}$* |
| *2* | - | - | - | 52.67 |
| *4* | - | - | - | 50.96 |
| *II* | - | - | - | 54.2 |

and mentrasto pair. Once again, mentrasto has stood out the inhibition of *G. vaginalis*, indicating its high antimicrobial potential in this vaginitis type treatment.

In the literature, the evaluation of antibacterial activity of gervao, malva branca and mentrasto extracts against *G. vaginalis* specimens is unpublished. Most of the studies

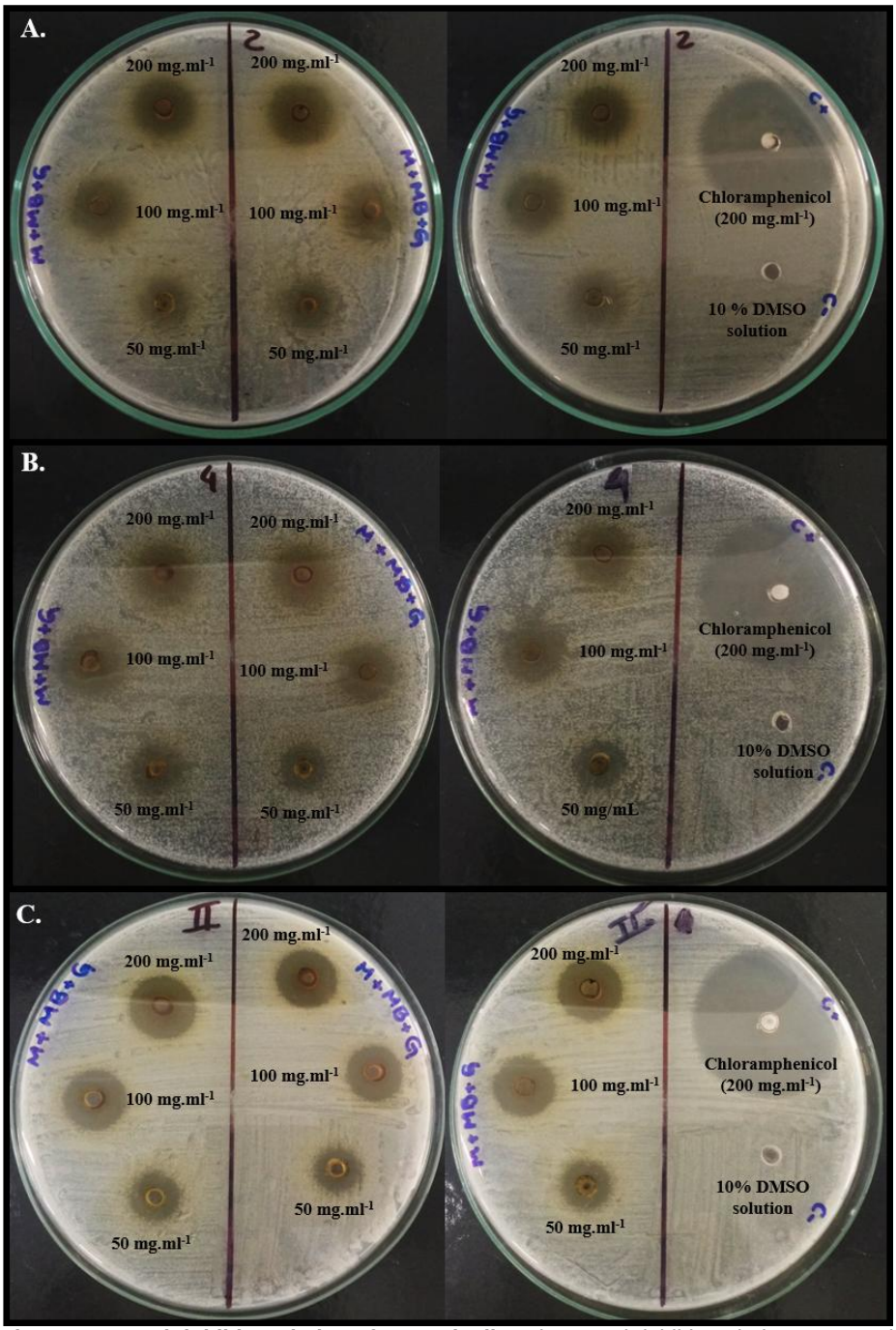


**Figure 1. Growth inhibitory halos of *G. vaginalis* –**The growth inhibitory halos presented by three clinical isolations of *G. vaginalis* (2, 4 and II), which grown in the presence of the mixture of M + MB + G extracts simultaneously at 200, 100 and 50 mg.ml$^{-1}$, are showed. As positive control, chloramphenicol at 200 mg·mL$^{-1}$ was used and, as negative control, 10% DMSO solution (v/v) was used.

report great antibacterial activity against other bacterial species, such as: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Citrobacter freundii* and *Bacillus subtilis* in the presence of gervao extracts (HRYTSYK et al., 2016; AHMED et al., 2017; KUOK et al., 2017; SISAY et al., 2019); *P. aeruginosa*, *B. subtilis* and *Aggregatibacter actinomycetemcomitans* in the presence of malva branca extracts (FEIZI et al., 2018; VAHABI et al., 2019); and *S. aureus*, *P. aeruginosa*, *E. coli* and *Klebsiella pneumonia* in the presence of mentrasto extracts (HOFFMAN et al., 2004; TRINH et al., 2020).

The only studies that evaluated *G. vaginalis* sensitivity explored potential of other plants. Two of them showed

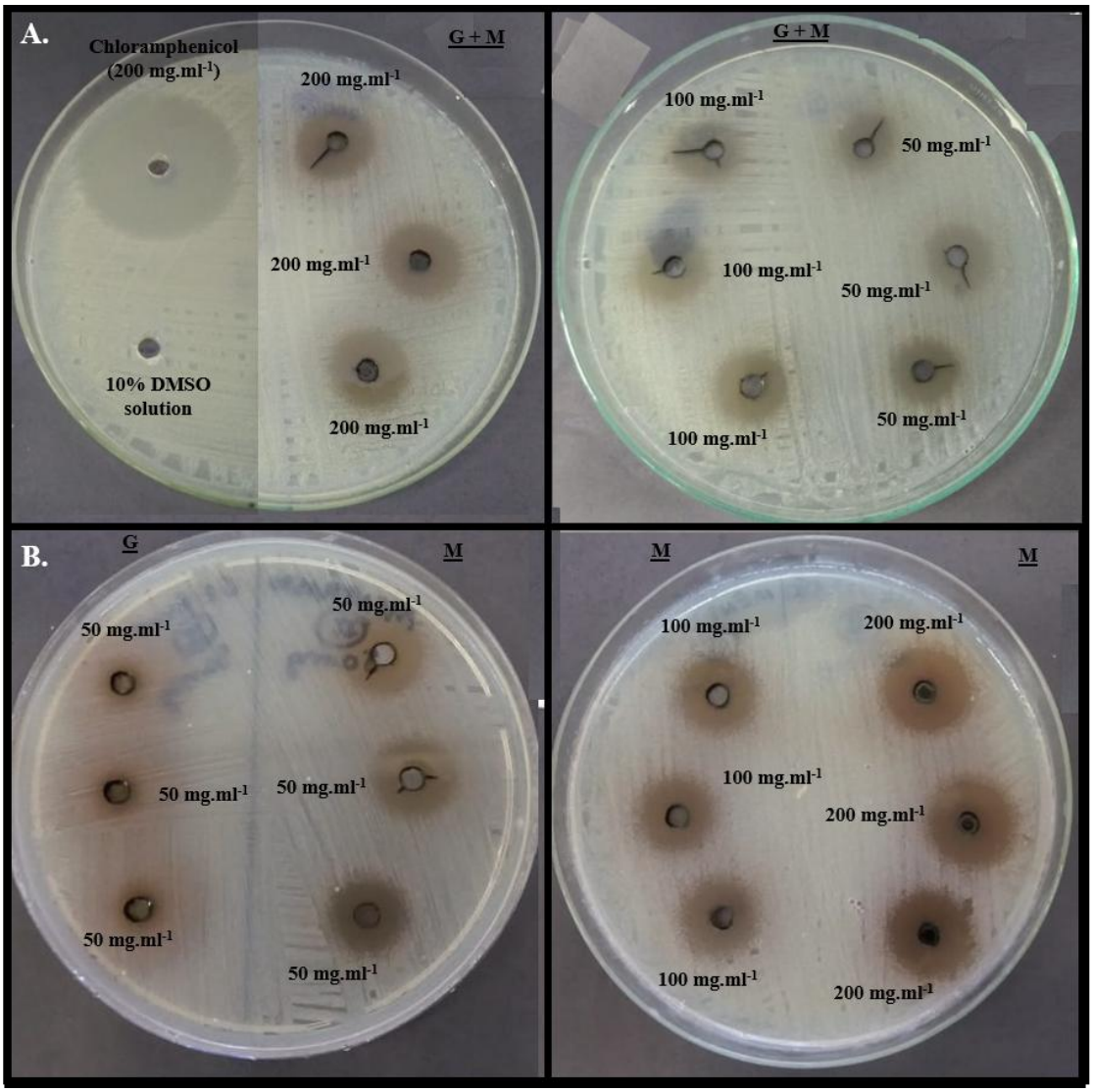


**Figure 2. Growth inhibitory halos of *G. vaginalis*–**The growth inhibitory halos presented by clinical isolation II of *G. vaginalis*, which was cultured in the presence of the mixture of extracts mentrasto and gervao (M + G), mentrasto (M) and gervao (G) at 200, 100 and 50 mg.ml$^{-1}$, are showed. As positive control, chloramphenicol at 200 mg·mL$^{-1}$was used and, as negative control, 10% DMSO solution (v /v) was used.

the effect of *Gossypium barbadense* L extracts–from both leaf and essential oil –on *G. vaginalis* growth (ESSIEN et al., 2001; MACEDO, 2013). In both cases, the antibacterial activity was proven. In addition, the antibacterial activity of *Schinus terebinthifolius* Raddi extract was compared to a reference drug against *G. vaginalis* growth (LEITE et al., 2011). Although it has some activity, the effect of this extract is still ineffective in curing bacterial vaginosis.

Regarding *Candida* spp. specimens, no effect on growth inhibition was observed when they were exposed to gervao, malva branca and mentrasto extracts, although they are traditionally used against vulvovaginal candidiasis. It is the first time that a work evaluates the antifungal activity of gervao extract. However, other studies have already addressed the antifungal effect of malva branca and mentrasto extracts or their essential oils on the yeast growth. The review of GIORDANI and collaborators (2015) affirms that malva branca hydroalcoholic extract has an antifungal effect on *C. albicans*, *C. tropicalis*, *C. krusei* and *C. stelatoidea* growth. Other studies report the same effect from the malva branca aqueous extract on *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis* and *C. orthopsilosis* growth (CARDOSO et al., 2012; FEIZI et al. al., 2018). Mentrasto ethanolic extract and its essential oil inhibited the growth of *C. Albicans* and *Candida* spp., respectively (AZUERO et al., 2016; TRINH et al., 2020). Then, none of those studies evaluated the action of the ethanolic extract, as presented here. This could explain

**Table 3.** Minimum Inhibitory Concentration (MIC) of the combined plant extracts (M + G + MB, MB + M, M + G, G + MB) and individual ones(M, MB e G). The values are in $mg.ml^{-1}$. Hyphen means absence of MIC.

| PlantExtracts | *G. vaginalis*specimens | | |
|---|---|---|---|
| | *2* | *4* | II |
| *M + MB + G* | 12.5 | 25 | 25 |
| *M + G* | 50 | 50 | 12.5 |
| *G + MB* | 50 | 25 | 25 |
| *M + MB* | 25 | 12.5 | 6.25 |
| *G* | 25 | 25 | 6.25 |
| *M* | 12.5 | 6.25 | 6.25 |
| *MB* | 25 | - | - |

the lack of antifungal effect from such extracts, although they are traditionally used in the vulvovaginal candidiasis treatment.

## CONCLUSIONS

For the first time, a study analysed the antimicrobial activity of gervao, malva branca and mentrasto extracts against yeasts and bacteria that cause the main vaginitis. It was verified that ethanolic extracts of those plants are composed mainly of fatty acids and they do not present antifungal activity against *Candida* spp. specimens obtained from clinical samples. However, it was observed that the combination at 50, 100 and 200 $mg.mL^{-1}$of three extracts exhibited antibacterial activity against all *G. vaginalis* specimens. In addition, extracts at all concentrations of gervao and mentrasto were able to inhibit the growth of one bacterial specimen, the highest inhibition values being those from mentrasto extract. Through broth microdilution technique, it was observed that mentrasto and gervao extracts presented the lowest MIC values, followed by the combination of mentrasto and malva branca, and the combination of the three extracts. Therefore, both mentrasto and gervao ethanolic extracts are promising for the bacterial vaginosis treatment. From this work, the identification of the active principle(s) present in these two extracts can be carried out and, thus, the establishment of partnerships with drug companies can be done to develop topical medications containing the active ingredient of the plant extracts.

### Conflicts of interest

Declarations of conflicts of interest: none

### Ethical approval

This study was approved by the Research Ethics Committee of the Universidade Federal do Tocantins, opinion no. 3,014,990.


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### Authors and contributors

Considering the conceptualization, we believe that it is appropriate to include the following authors in the manuscript: VFO, MCTA, JFMS and RSP. Conceived of methodology, validation, formal analysis and investigation: VFO, MCTA, AITO and EPLPF. Provided research resources and acquired funding: VFO, JFMS and RSP. Prepared original draft and visualized: MCTA. Reviewed and edited: VFO, MCTA, EPLPF and RSP. Supervised: RSP. Administrated the project: VFO.

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