Antimicrobial effects of Sophora Flavescens Alkaloids on metronidazole-Resistant Gardnerella vaginalis in planktonic and biofilm formation

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Original article

Keywords: Bacterial vaginosis, Gardnerella vaginalis, Biofilm, Sophora Flavescens Alkaloids, Metronidazole

Posted Date: April 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-354686/v1

Abstract

Objectives: This study aims to explored the effects and mechanisms of Sophora Flavescens Alkaloids (SFAs) on metronidazole-resistant *Gardnerella vaginalis* under planktonic and biofilm conditions.

Methods: The main components of SFAs were analyzed using HPLC. Clinical strains were cultured from the vaginal secretions of BV patients, followed by PCR and 16s rRNA gene sequencing identification**.** According to the 2012 CLSI guidelines for anaerobic drug sensitivity testing, the sensitivity of *Gardnerella vaginalis* to metronidazole and SFAs were determined using the microdilution broth method. The formation of biofilms was evaluated, and the biofilm microstructure was observed using scanning electron microscopy.

Results: The main active contents of SFAs were matrine and oxymatrine. Nineteen metronidazole-resistant strains were isolated and identified (minimum inhibitory concentration, MIC≥32 μg/mL), of which four clinical strains were observed to be strong biofilm producer and the final minimum biofilm inhibitory concentration (MBIC) of metronidazole was increased to 512 μg/mL. SFAs could not only inhibited the growth of metronidazole-resistant *Gardnerella vaginalis* in planktonic (MIC: 0.3125-1.25 mg/mL), but also eliminated the biofilm formation (MBIC: 0.625-1.25 mg/mL). Also, there was less biomass biofilm formation which was sparse and relatively thin. In the high-magnification scanning electron, it was observed that the water channels were destroyed, and furturemore, the biofilm morphology changed from a thick to flaky shape and was nearly depleted.

Conclusions: Our results indicate that SFAs could not only inhibite the growth of metronidazole-resistant *Gardnerella vaginalis* in planktonic and biofilm levels, but also destroyed the biofilm morphology and microstructure, which may contribute to the prevention of BV recurrence.

Introduction

Bacterial vaginosis (BV) is an infectious disease of the lower female reproductive tract and frequently affects women of childbearing age. It causes an abnormal proliferation of anaerobic microorganisms, primarily *Gardnerella (G.)vaginalis*. This further leads to a decrease or disappearance of *Lactobacillus vaginalis* altering the composition of the vaginal flora [1-2].The reproductive health of millions of women in the world is seriously affected by BV every year. According to published data, the morbidity rate for BV is 5-15% in white women and 45-55% in black women [3].BV causes adverse pregnancy outcomes, such as spontaneous abortion, premature delivery, amniotic fluid infection, puerperal endometritis, cesarean incision infection, and perinatal complications[4].The recurrence rate of BV is high and gradually increases with time. Disease relapse has been observed in one-third of patients at three months after treatment. Moreover, persistent BV infection and recurrence also can increase the risk of trichomonas vaginitis, vulvovaginal candidiasis, cervical cancer, and human immune deficiency virus infection [5].

G.vaginalis is a facultative anaerobic Gram-negative hemophilic bacillus that is difficult to culture and can be transmitted by sexual contact. *G.vaginalis* adheres tightly to the surface of vaginal epithelial cells and can form densely clustered biofilms with potent cytotoxicity against vaginal epithelial cells. Other BV-related bacteria are less able to adhere to vaginal epithelial cells and form biofilms. Therefore, the *G.vaginalis* plays a

predominant role in BV occurrence [6].Also, *G.vaginalis* can form a specialized adhesive biofilm that competes with lactobacillus and coexists with normal dormant vaginal anaerobic bacteria to increase their numbers, which also contribute to BV. Current studies have demonstrated that biofilm formation plays a critical role in the pathogenesis of BV. The occurrence and repeated failure to successfully treat BV is related to *G.vaginalis* biofilm formation in the vagina [7].

Currently, BV is primarily treated with systemic or local administration of anti-anaerobic antibiotics. As the first-line of therapy for BV, metronidazole was recommended by Centers for Disease Control and Prevention (CDC). While the recurrence rate of BV is as high as 33% and 49-66% within three months and one year after treatment, respectively [8]. Metronidazole-resistant *G.vaginalis* might be one reason for BV recurrence and treatment failure. Sophora Flavescens Alkaloids (SFAs), a traditional chinese medicine, has multiple antibacterial and anti-inflammatory activities, as well as pharmacological antipruritic potential. Previous studies showed that SFAs also has the ability to restore vaginal microbiota and mucosal repair, resulting in decreased recurrence of vaginitis effects [9]. Furthermore, researches found that SFAs can also inhibit bacterial biofilm formation by regulating the activity of autoinducer [10]. Based on clinical research, SFAs effectively treats BV and significantly improves clinical symptoms. However, the antibacterial activity and mechanism of SFAs against *G.vaginalis*, especially the metronidazole-resistant clinical strains, has not been reported. This study evaluated the susceptibility of planktonic *G. vaginalis* and the biofilms formation of metronidazole-resistant strains to SFAs, which will provide a theoretical basis of SFAs in BV clinical treatment.

Materials And Methods

High performance liquid chromatography analysis

Matrine and oxymatrine were the main contents of SFAs and were analyzed by HPLC methods. The standard control sample of matrine and oxymatrine were both purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), the chromatographic analyses were implemented on the Agilent UPLC system (Shimadzu, Japan), and the Agilent XDB C18 5μm (4.6×150mm) was used for the chromatographic separation. Mobile phase consisted of solvent A (10% water) and B (90% methanol solution), and the flow rate was set to 1 ml/min with the injection volume was 4 μL. A sample of 1g SFAs was put into a 100ml volumetric flask with constant volume of methanol to obtain 10mg/ml test solution, the control sample matrine and oxymatrine were used as the the referenced sample. The operating parameters were as follows: both the reference and test samples were prepared into different gradient solutions with the same concentration. The reference substance and the test substance solution were injected respectively at the same time to obtain the peak area of the different component. Since the response value factor (*F*) of each component is unchanged, therefore, the concentration of test sample can be calculated according to *F* value. *F* is calculated as follows: *F*= reference concentration/reference peak area = test concentration/test solution peak area. So the oxymatrine content =[(concentration× dilution ratio)/test product weight sample volume] ×100%. Results are presented as mean ± SD of the mean of at least triplicates.

Isolation and culture of *G.vaginalis* strains

The strains used in this study were isolated from vaginal secretions of BV patients in the Gynecology Clinic as per the following procedure. The vaginal secretions were obtained from one-third of the vaginal wall using a sterile cotton swab and applied to a Casman agar plate (Beijing AOBOX Biotechnology Co., Ltd.).Then the agar plate was placed into an aerobic bag and incubated with 5% CO_2 at 37°C for 48 h. Round, needle-like, and translucent single colonies were selected and applied in line to a new Casman agar plate followed by anaerobic culture for 24-48 h. After three generations of pure cultures, single colonies were collected, mixed evenly with 30% glycerol, and stored at -80°C.

Identification of *G.vaginalis*strains

The clinically isolated strains were identified using colony polymerase chain reaction (PCR) and 16s rRNA gene sequencing. 16S rRNA gene hypervariable V1–V3 region was amplified using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTA GACTT-3') .The protocol used was as follows: total volume of the PCR mixture was 50 μL, and the reaction conditions included pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, then annealing and extension at 72°C for 80 s. The cycle was repeated 33 times. The PCR amplification products were sent to Beijing SinoGenoMax Co., Ltd. for sequencing, and the sequences of the splicing results were compared with the bacterial 16s rRNA gene sequences in GenBank data library for identification and confirmation of *G.vaginalis*. *Bacteroides fragilis* ATCC 25285 was used as a control for the tests carried out under anaerobic conditions.

Antimicrobial susceptibilitytesting

SFAs (NMPN Z20050058) was provided by Guiyang Xintian Pharmaceutical Co., Ltd. (Guizhou, China) and metronidazole (purity: 99.97%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). SFAs was dissolved in ethanol with concentration of 400 mg/mL and metronidazole dissolved by sterile distilled water with 2.56 mg/mL concentration.. The antimicrobial susceptibility activity was detected by the microdilution broth method as described by Sutyak Noll et al with minor modifications [11]. Briefly, the antimicrobials were diluted (a series of 2-fold dilutions) with an appropriate volume of fresh BHI broth (Beijing AOBOX Biotechnology Co., Ltd.) in 96-well culture plate. The final concentration of the SFA was 0.039-20 mg/mL and metronidazole was 0.125-128 μg/mL. An equal proportion of ethanol solution at the highest final concentration of 5% and sterile distilled water were served as the negative control, respectively. Sterile distilled water served as the negative control. *G.vaginalis* and control were cultured until they achieved the logarithmic phase and was diluted in BHI to the final 5×10^6 CFU/mL. From the diluted bacterial cells, 100 μL was transferred in the wells containing predetermined concentrations of antimicrobials. The inoculated plate was placed into an anaerobic chamber and incubated under 5% CO_2 at 37°C for 48 h. The lowest antibiotic concentration yielding marked reduction to no growth was read as the Minimum Inhibitory Concentration (MIC). According to the 2012 Clinical and Laboratory Standards Institute guidelines for anaerobic drug sensitivity testing, metronidazole MIC was evaluated as follows, MIC ≤8 μg/mL: sensitive to metronidazole, MIC =16 μg/m: moderate sensitivity, and MIC ≥32 μg/mL: resistant to metronidazole.

As previous described, after the plate was inoculated for 48h, 100 μL of the bacterial solution was separately taken from the non-turbidity and control wells, and then were evenly applied to a drug-free solid medium,

followed by anaerobic culture for 48 h. The drug concentration in the well where the total bacterial count declined by 99.9% or more was compared against the control well to reveal the minimum bactericidal concentration (MBC).

Bacterial biofilm formation assay

To develop the biofilm formation level of *G.vaginalis* clinical strains, a starting inoculum of 5×10^6 CFU/mL of prepared bacterial suspension in the BHI broth was planted in 96-well culture plate. The microplate was incubated anaerobically for 48 h. Crystal violet staining (Beijing AOBOX Biotechnology Co., Ltd.) was used to quantify the total amount of biofilm biomass [12]. After the incubation period, each well was gently washed twice with 200μL of PBS to remove the non-adhered bacteria, and dried for 15 min. The dried biofilm was then stained with 200 μl crystal violet (1%　w/v, Sigma) and incubated for 30min. Finally, the well was rinsed with PBS three times and decolorized with 95% alcohol 200μL for 5min. Then the liquid was moved to a new microtitre plate and absorbance at 595nm was measured by a microplate reader (Bio-Rad Laboratories, Hercules,CA, USA). The ability of biofilm formation was evaluated according to OD value, which which was defined as three standard deviations (SD) above the mean OD of the blank control (ODc), OD ≥4 × ODc indicates strong biofilm producer[13].

Determination of the minimal biofilm inhibitory concentration (MBIC)

The inhibitory concentration of SFAs against *G.vaginalis* biofilm formation was determined using the above described. The logarithmic-phase bacteria were cultured with antimicrobial-containing medium and anaerobically cultured for 48 h. The *G.vaginalis* biofilm was detected by violet solution as previously described, and the minimum biofilm inhibition concentration (MBIC) was defined as the lowest concentration of an antibiotic that completely inhibited the growth of microorganisms compared with control.

Observation of biofilm microstructure using scanning electron microscopy

The morphology and structure of biofilms changes were described by scanning electron microscopy examination. Sterile cover glass was placed at the bottom of each well of a 24-well plate, and bacteria were inoculated into each well glass, followed by anaerobic culture for 48 h to form biofilm. Then the cover glasses were removed, washed three times with PBS, fixed with 2% cold glutaraldehyde for 15 h, vacuum-dried for 72 h, and plated with gold. The morphological features of biofilm was observed using a scanning electron microscope (HITACHI S-3400, Japan).

Statistical analysis

GraphPad Prism 8 software was used for statistical analysis. Non-parametric Mann-Whimey U test was used for comparison because the data did not conform to normal distribution. $P < 0.05$ indicated a statistically significant difference.

Results

Quantitative analysis of the oxymatrine and matrine in SFAs

Previous research results indicated that matrine and oxymatrine were the main active components in the SFAs. The content ratio of oxymatrine and matrine were analysed by matching retention times and the HPLC chromatograms was shown in Figure 1. According to previous reports and corresponding standard compound, peaks 1 was identified as oxymatrine (retentiontime, 5.128 min) and peaks 2 was matrine (retentiontime, 7.007 min). Based on the standard curve of oxymatrine reference, the value of $F_{(oxymatrine)}$ is 7010090, thus y= $F_{(oxymatrine)}$x+5621990 (y is the peak area, and x is the concentration). In the same way, we calculated $F_{(matrine)}$ is 8198700 and y= $F_{(matrine)}$x+10199700. The final concentrations of oxymatrine is 4.840 ±0.3005 mg/ml and matrine is 2.673±0.2155 mg/ml, in other words, the ratio of oxymatrine and matrine in SFAs is 48.40 ±3.05% and 26.73±2.155% (Table1).

Table 1. The peak area of HPLC and final calculated concentration of oxymatrine and matrine.

Sample	Oxymatrine Area	Oxymatrine Concentration (mg/ml)	Matrine Area	Matrine Concentration (mg/ml)
Sample 1	41713562	5.15	33473267	2.84
Sample 2	37512681	4.55	32770519	2.75
Sample 3	39426564	4.82	30135852	2.43

Antimicrobial susceptibility of metronidazole against 30 clinical *G. vaginalis*

During the study period, a total of 30 isolates of *G. vaginalis* were isolated and detected, the identity of all presumptively isolated *G. vaginalis* was confirmed by PCR (Figure 2) and 16s rRNA gene sequencing. According to the 2012 CLSI guidelines for anaerobic drug sensitivity testing, MIC was determined using the broth microdilution method, with the standard strains of *Bacteroides fragilis* (ATCC 25285) as the quality control and *G.vaginalis* (ATCC 49145) served as the standard strains control. Antimicrobial susceptibility testing results indicated that only 30% (9/30) clinical strains were sensitive to metronidazole, whereas the resistance rate was as high as 63.33% (19/30) (Table 2).

Table 2. Antimicrobial susceptibility of metronidazole against 30 clinical *Gardnerella vaginalis.*

Metronidazole	S (MIC ≤8 μg/mL)	I (MIC =16 μg/mL)	R (MIC ≥32 μg/mL)
N, %	9, 30.00%	2, 6.67%	19, 63.33%

The antimicrobial effects of SFAs on 30 *G.vaginalis* clinical strains

The MIC and MBC values of SFAs were detected as previous described The results showed that the MIC range of total matrine against *G.vaginalis* strains was 0.1563-2.5mg/mL, which was only 1/128-1/8 of the clinical dose (about 20 mg/mL).The MBC value was in the range of 0.3125-5.0 mg/mL, which was also far lower than

the clinical dose, indicating that the SFAs had significant inhibitory and killing effects on clinical strains of *G.vaginalis* (Table 3). When in contrast with metronidazole-sensitive strains, the resistant group showed prominently increased MIC value (0.3551±0.1413 *vs* 0.9211±0.5460, $P<0.05$) and the MBC value (0.6534±0.2190 *vs* 1.8420±1.0920, $P<0.05$), which suggested that the metronidazole-resistant group may have some drug resistance characteristics.

Table3. Minimum inhibitory concentration and Minimal bactericidal concentration concentrations of SFAs against 30 *G.vaginalis* clinical strains

	SFAs MIC(mg/mL)		SFAs MBC(mg/mL)		
Range	**Mean±SD**		**Range**	**Mean±SD**	
metronidazole-sensitive group (n=11)	0.1563-0.6250	0.3551±0.1413	0.3125-1.2500	0.6534±0.2190	
metronidazole-resistant group(n=19)	0.3125-2.5000	0.9211±0.5460*	0.6250-5.0000		1.8420±1.0920*

- * $P<0.05$

The antimicrobial activity of SFAs against metronidazole-resistant *G. vaginalis* withstrong biofilm formation ability

Many studies have reported that biofilm formation may increase drug resistance, thus 4 metronidazole-resistant strains which had strong biofilm formation ability were chosen to the next study. *G.vaginalis* planktonic and biofilm-associated growth was inhibited only when high concentrations of metronidazole were used (MIC≥128μg/mL, MBIC 256~512 μg/mL). Furthermore, the MIC of standard strains of G.vaginalis was 2 μg/mL and its MBIC increased to 128 μg/mL. While the MIC of SFAs was 0.3125-1.25 mg/mL, which is only 1/32-1/16 of the clinical dosage of the vaginal administration. The MBIC of the SFAs against *G.vaginalis* biofilm formation was 0.625-1.25 mg/mL, which was increased only one to two times as compared with the MIC. Moreover, the MBIC of SFAs against standard strains of *G.vaginalis* was 1.25mg/mL, which was increased two times with MIC (0.0625 mg/mL) (Table 4). These results showed that, although the biofilm has formed, SFAs still exhibited a bactericidal effect to inhibite the continued growth and biofilm formation.

Table 4. The inhibitory concentrations of metronidazole and SFAs against 4 metronidazole-resistant *G.vaginalis* strains.

Strains	Metronidazole (mg /mL)		SFAs (mg/mL)			
MIC	MBIC	MBIC/MIC	MIC	MBIC	MBIC/MIC	
Clinical strain 1#	≥128	512	≥4	0.3125	0.625	2
Clinical strain 2#	≥128	512	≥4	0.3125	0.625	2
Clinical strain 3#	≥128	256	≥2	1.25	1.25	1
Clinical strain 4#	≥128	512	≥4	0.3125	0.625	2
ATCC 49145	2	128	64	0.625	1.25	2

Effect of SFAs on the ultrastructural morphology of GV biofilm

Furthermore, the effect of SFAs on *G.vaginalis* biofilm morphology was observed using a scanning electron microscope to illustrated the bactericidal mechanisam. The gradual formation of *G.vaginalis* biofilms was observed with increased time in culture. In the control group, *G.vaginalis* strains were primarily short bacillus with normal morphology at low magnification. As the incubation time increases, *G.vaginalis* single colonies were gathered in groups, and biofilm was preliminary form (Figure 3A).These colonies grew continuously and started to fuse, followed by an increase in the secretion of intercellular matrixes, which surrounded the *G.vaginalis* strains, thus causing the unclear-outline of strains (Figure 3B).Under high-magnification, thick biofilms generated by many extracellular matrixes were observed and the interconnected water channels among the bacteria also were present (Figure 3C). However, in the experimental group, The number of bacteria was significantly reduced after the addition of SFAs (0.3125 mg/mL) and bacterial count declined sharply (Figure 3D). and altered *G.vaginalis* morphology was observed (probably dead bacteria). Due to the reduced secretion of intercellular matrixes, there was less biofilm formation, and the biofilms became discontinuous and fragmented (Figure 3E).It was also observed that the biofilms were relatively thin and changed from thick to a flaky shape, the biofilms were nearly depleted with destroyed water channels (Figure 3F).These findings suggested that SFAs inhibited biofilm formation by destroying the *G.vaginalis* biofilm structure, thereby suppressing the growth of *G.vaginalis* and reducing the recurrence of BV.

Discussion

BV is characterized by a relative decrease in beneficial lactobacillus and the eaugmentation of anaerobic bacteria. G. vaginalis is frequently present in women and the detection rate of *G.vaginalis* was 87.5% in BV patients, 26.4% in healthy women, and 34.0% in women in an intermediate state [14]. In this study, a total of 76 clinical vaginal strains were isolated from 100 BV patients, and the number of *G.vaginalis* strains is 30 after sequence dection. As the predominant bacterial species, *G.vaginalis* can facilitate the growth of BV-related anaerobic bacteria by reducing H_2O_2, which can utilize metabolites of anaerobic bacteria and further raise the vaginal pH and reducing lactobacillus. *G.vaginalis* inimitably has three toxic characteristics: cytotoxicity, vaginal epithelial adhesion, and biofilm formation, thus possesses higher virulence than other BV-associated bacteria [15].

Metronidazole, a first-generation nitroimidazole, is effective against anaerobes by inhibiting nucleic acid synthesis and had became the current treatment of choice for BV. However, with the widespread use of metronidazole in BV clinical patients, the rate of BV recurrence within three months was highly reached 58% with drug-resistant strains emerged[16]. In our study, we found 63.33% (19/30) of *G.vaginalis* clinical strains of BV patients exhibited resistance to metronidazole. The mechanisms of bacterial resistance to metronidazole may be associated with decreased uptake, a decline in the reduction activation rate of metronidazole, inactivation of resistance determinants, and increased DNA repair efficiency [16]. Therefore, controlling resistance to metronidazole and finding more effective drugs for BV requires prompt solutions.

Matrine and oxymatrine was the active ingredient of SFAs, which not only exerted anti-cancer effects by regulating GADD45B, Bcl-2, and caspase-3 [17-18],but also regulated anti-inflammatory responses through AGE expression and Nrf translocation [19] and assist therapy for cardiovascular disease by affecting the JAK2/STAT3 and ATF6 signaling pathways [20-21]. Previous clinical studies demonstrated that SFA can effectively treat BV and significantly improve clinical symptoms but the actual mechanism is unclear. In this *in vitro* study, SFAs exhibited inhibitory and bactericidal effects on clinical metronidazole-resistant strains, and the MBC and MIC were much lower than the doses used clinically. This further substantiated use of SFAs as an option for clinical use.

It has been hypothesized that the biofilm establishment plays a key role in the pathogenesis of BV and increases the resistance to the host immune defense system and phagocytosis [22]. Swidsinsk et al [23] found that at 10-12 weeks after treatment of BV, *G.vaginalis*-dominated bacterial biofilms were detected in 40% of BV patients and concluded that the primary reason was the reactivation of biochemically inactivated biofilms, not the occurrence of a new infection. *G.vaginalis* biofilms cannot be effectively cleared by the human immune system or completely inactivated by antibacterial drugs, therefore, remains chronic and persistent infections[24]. Studies have shown that the antimicrobial response is significantly different which is associated with a planktonic or biofilm-associated style. The slow or no growth allows bacteria in biofilms to be safe from antibacterial drugs, thereby reducing their sensitivity to these drugs. Also,the biofilm matrix can serve as a barrier that reduces the penetration of antibacterial drugs[25].Therefore, *G.vaginalis* in a biofilm can tolerate high concentration of H_2O_2 and lactic acid against planktonic[26]. In this study, it was found that the inhibitory concentration of metronidazole against standard strains of *G.vaginalis* was increased by 63 times due to biofilm formation. The inhibitory concentration of SFAs against biofilms was increased by only 1 fold compared with MIC, which suggested that SFAs could effectively inhibited *G.vaginalis* in biofilm–associated form. Furthermore, the inhibition of biofilm and ultrastructure changes of *G.vaginalis* biofilm morphology was also observed by transmission electron microscope.

Even taking in consideration the limited survey samples the study, current data suggest SFAs could not only inhibited the growth of metronidazole-resistant *G. vaginalis* in planktonic and biofilm levels, but also destroyed the biofilm formation and microstructure. According to previous results, the antibiotic tolerance of *G. vaginalis* and recurrence of BV is associated with the ability of biofilm formation, thereby, identifying more novel therapeutics that target vaginal biofilms may contributed to the prevention of BV recurrence. In summary, SFAs seems a better choice to prevent the formation of new biofilms and to eliminated the presence of persistent biofilms in patients with BV, especially for metronidazole-resistant patients.

Declarations

Acknowledgements

The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for expert linguistic services.

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Contributions

LZH designed, supervised, and coordinated the study. FLY, ZZ and BHH carried out the experiments. LT and ZXN performed the sample collection. ZX contributed to scanning electron microscopy examination. FLY finished the data analyses and wrote the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Beijing, China (2018-KY-060-01). Sample collection was conducted after participants written informed consent and carried out in accordance with the declaration of Helsinki

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials statement

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Abbreviations

BV: Bacterial vaginosis

BHI: Brain heart infusion

CLSI: Clinical and Laboratory Standards Institute

CFU: Colony forming units

HPLC: High Performance Liquid Chromatography

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

MBIC: Minimum Biofilm Inhibitory Concentration

MRS: De Man, Rogosa, and Sharpe

ODc: Cut-off OD

PBS: phosphate buffered saline

SFAs: Sophora Flavescens Alkaloids

References

1. Janulaitiene M, Paliulyte V, Grinceviciene S, Zakareviciene J, Vladisauskiene A, Marcinkute A, Pleckaityte M (2017) Prevalence and distribution of Gardnerella vaginalis subgroups in women with and without bacterial vaginosis.BMC Infect Dis 17(1):394. doi:10.1186/ s12879-017-2501-y
2. Nelson DB, Macones G (2002) Bacterial vaginosis in pregnancy:current findings and future directions. Epidemiol Rev 24(2):102-108. doi: 10.1093/epirev/mxf008
3. Sherrard J, Wilson J, Donders G, Mendling W, Jensen JS (2018) 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organisation (WHO) guideline on the management of vaginal discharge. Int J STD AIDS 29(13):1258–1272. doi:10.1177/0956462 418785451
4. Schwebke J R, Muzny C A, Josey W E (2014) Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model. J Infect Dis 210(3):338-343. doi: 10.1093/infdis/jiu089
5. Onderdonk AB, Delaney ML, Fichorova RN (2016) The Human Microbiome during Bacterial Vaginosis. Clin Microbiol Rev 29(2):223–238. doi:10.1128/ CMR.00075-15
6. Patterson JL, Stull-Lane A, Girerd PH, Jefferson KK (2010) Analysis of adherence□biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial

vaginosis-associated anaerobes. Microbiology 156: 392-399. doi: 10.1099/mic.0.034280-0

7. Alves P, Castro J, Sousa C, Cereija TB, Cerca N (2014) Gardnerella vaginalis outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. J Infect Dis 210(4):593-596. doi: 10.1093/infdis/jiu131.
8. Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, Horvath LB, Kuzevska I, Fairley CK (2006) High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis 193(11):1478-1486. doi: 10.1086/503780.
9. He X, Fang J, Huang L, Wang J, Huang X (2015) Sophora flavescens Ait.: Traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine. J Ethnopharmacol. 172:10-29. doi: 10.1016/j.jep.2015.06.010.
10. Jia F, Zhou Q, Li X, Zhou X (2019) Total alkaloids of Sophora alopecuroides and matrine inhibit auto-inducer 2 in the biofilms of Staphylococcus epidermidis. Microb Pathog 136:103698. doi: 10.1016/j.micpath.2019.103698.
11. Noll KS, Prichard MN, Khaykin A, Sinko PJ, Chikindas ML (2012) The natural antimicrobial peptide subtilosin acts synergistically with glycerol monolaurate, lauric arginate, and ε-poly-L-lysine against bacterial vaginosis-associated pathogens but not human lactobacilli. Antimicrob Agents Chemother 56(4):1756-1761. doi: 10.1128/AAC.05861-11.
12. Hooven TA, Randis TM, Hymes SR, Rampersaud R, Ratner AJ (2012) Retrocyclin inhibits Gardnerella vaginalis biofilm formation and toxin activity. J Antimicrob Chemother 67(12):2870-2872. doi: 10.1093/jac/dks305.
13. Hymes SR, Randis TM, Sun TY, Ratner AJ (2013) DNase inhibits Gardnerella vaginalis biofilms in vitro and in vivo. J Infect Dis 207(10):1491-1497. doi: 10.1093/infdis/jit047.
14. Mendling W (2016) Vaginal Microbiota. Adv Exp Med Biol 902:83–93. doi:10.1007/978-3-319-31248-4_6
15. Aroutcheva AA, Simoes JA, Behbakht K, Faro S (2001) Gardnerella vaginalis isolated from patients with bacterial vaginosis and from patients with healthy vaginal ecosystems. Clin Infect Dis 33(7):1022–1027. doi:10.1086/323030
16. Dingsdag SA, Hunter N (2018) Metronidazole: an update on metabolism, structure-cytotoxicity and resistance mechanisms. J Antimicrob Chemother 73(2):265-279. doi: 10.1093/jac/dkx351.
17. Huang H, Wang Q, Du T, Lin C, Lai Y, Zhu D, Wu W, Ma X, Bai S, Li Z, Liu L, Li Q (2018) Matrine inhibits the progression of prostate cancer by promoting expression of GADD45B. Prostate 78(5):327-335. doi: 10.1002/pros.23469.
18. Gu YY, Chen MH, May BH, Liao XZ, Liu JH, Tao LT, Man-Yuen Sze D, Zhang AL, Mo SL (2018) Matrine induces apoptosis in multiple colorectal cancer cell lines in vitro and inhibits tumour growth with minimum side effects in vivo via Bcl-2 and caspase-3. Phytomedicine 51:214-225. doi: 10.1016/j.phymed.2018. 10.004.
19. Zhang Y, Yang X, Qiu C, Liu F, Liu P, Liu Z(2018) Matrine suppresses AGE-induced HAEC injury by inhibiting ROS-mediated NRLP3 inflammasome activation. Eur J Pharmacol 822:207-211. doi: 10.1016/j.ejphar.2018.01.029.

20. Liu Z, Zhang Y, Tang Z, Xu J, Ma M, Pan S, Qiu C, Guan G, Wang J (2017) Matrine attenuates cardiac fibrosis by affecting ATF6 signaling pathway in diabetic cardiomyopathy. Eur J Pharmacol 804:21-30. doi: 10.1016/j.ejphar.2017. 03.061
21. Zhao XB, Qin Y, Niu YL, Yang J (2018) Matrine inhibits hypoxia/reoxygenation -induced apoptosis of cardiac microvascular endothelial cells in rats via the JAK2/STAT3 signaling pathway. Biomed Pharmacother 106:117-124. doi: 10.1016/j.biopha.2018.06.003
22. Hardy L, Cerca N, Jespers V, Vaneechoutte M, Crucitti T (2017) Bacterial biofilms in the vagina. Res Microbiol 168(9-10):865-874. doi: 10.1016/j.resmic. 2017.02.001.
23. Swidsinski A, Dörffel Y, Loening-Baucke V, Schilling J, Mendling W (2011) Response of Gardnerella vaginalis biofilm to 5 days of moxifloxacin treatment.FEMS Immunol Med Microbiol 61(1):41–46. doi:10.1111/j.1574- 695X.2010.007 43.x
24. Roilides E, Simitsopoulou M, Katragkou A, Walsh TJ (2015). How Biofilms Evade Host Defenses.Microbiol Spectr 3(3). doi:10.1128/microbiolspec.MB- 0012-2014
25. Verstraelen H, Swidsinski A (2013) The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment. Curr Opin Infect Dis26(1):86–89. doi:10.1097/QCO. 0b013e32835c20cd
26. Patterson JL, Girerd PH, Karjane NW, Jefferson KK (2007) Effect of biofilm phenotype on resistance of Gardnerella vaginalis to hydrogen peroxide and lactic acid.Am J Obstet Gynecol 197(2):170. doi:10.1016/j.ajog.2007.02.027

Figures

Figure 1

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Figure 2

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Figure 3

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