THE ANTIMICROBIAL AND TOXICITY ANALYSIS OF ESSENTIAL OILS USED FOR VAGINAL INFECTIONS

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Declaration

I, Muneebah Haroon Laher, student number 2140432, declare that this Dissertation is my own work and that I contributed in full towards research findings published in the article stated below which are included in my Dissertation.

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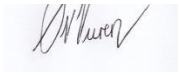
Signature of Primary Supervisor  Date: 30/09/2025

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MH Laher: Drafting, writing, and analysis. Identification of sources, findings and development of overall structure.

Sandy van Vuuren: Review and editing. Ensured the accuracy and clarity of the content.

Ané Orchard: Review and editing. Refinement of literature search strategy and interpretation of the reviewed studies.

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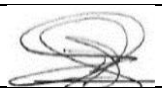
Author 2	Ané Orchard		30/09/2025
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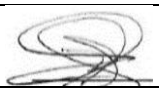
Author 2	Ané Orchard		30/09/2025
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Dedication

In the name of Allah, The Most Gracious, The Most Merciful

"Seek knowledge from the cradle to the grave."- Prophet Muhammad (Peace be upon him)

To my beloved parents, Moulana Haroon and Shehnaaz, whose unwavering faith and support have been my saving grace. I am only because you are.

To my brothers, Qari Muaadh and Haafidh Muhammad Azhar, who may not know the intricacies of my research but have fully supported my endeavours. You are my lifeline.

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To my friends and family who have supported me and endured the tests of my absences, late nights, and everything in between. There are not enough words to adequately express my love and gratitude. May your kindness be rewarded tenfold by Allah.

List of presentations

Appendix A1 (Abstract)

Laher, MH., Orchard, A., and van Vuuren, S.F., The antimicrobial and toxicity analysis of essential oils used for vaginal infections. 2024 Faculty of Health Sciences Research Day on the 5th of September 2024 at the University of Witwatersrand, Johannesburg, (Oral presentation).

Abstract

The increasing resistance of bacterial and fungal pathogens to conventional treatments for vaginal infections, alongside the disruption of protective *Lactobacilli* by many antimicrobials, highlights the urgent need for alternative strategies. Essential oils, long used in traditional medicine and increasingly marketed in over-the-counter formulations, have been cited for their antimicrobial activity and potential microbiota-sparing properties. This study evaluated the antimicrobial efficacy, toxicity, microbiota impact, and biofilm inhibition of essential oils, both individually and in combination. Forty-six essential oils were investigated. These were assessed in terms of antimicrobial activity using broth microdilution, toxicity with the brine shrimp lethality assay, the impact on microbiota determined through *Lactobacillus* broth microdilution assays, and biofilm inhibition. A total of 602 combinations were tested for synergistic interactions. *Citrus aurantifolia* and *Origanum vulgare* showed broad-spectrum antimicrobial activity with non-toxic profiles and minimal inhibition of *Lactobacilli*. Of the 602 combinations, 101 demonstrated bacterial synergy and 48 yeast synergy; 69% were non-toxic, but only 6% spared *Lactobacilli*. The most promising combinations were *Cinnamomum camphora* with *Melaleuca cajuputi*, *C. camphora* with *Ocimum sanctum*, and *Salvia officinalis* with *O. sanctum*. Notably, *C. camphora* with *M. cajuputi* inhibited *Candida albicans* and *Candida glabrata* biofilms by 82% and 72%, respectively, outperforming the individual oils. These findings suggest that selected essential oils and combinations demonstrate promising *in vitro* antimicrobial, microbiota-sparing, and antibiofilm properties. Further preclinical evaluation is warranted to explore their role in developing microbiota-preserving therapies for vaginal infections.

Keywords: essential oils; vaginal infections; synergy; *Lactobacillus*; biofilm inhibition; antimicrobial resistance

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- Thank you to the NRF Thuthuka Fund (Thuthuka grant number 129672), and the Faculty Research Committee (FRC) grant from the University of Witwatersrand for financial assistance.
- To the people I met in my Master's journey and shared a laboratory with, I will hold onto those good memories forever.
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- Thank you to the University of the Witwatersrand for the use of facilities and equipment.

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Equation 1

The ΣFIC values of the combinations were calculated using the following equations:

$$\text{FIC (i)} = \frac{\text{Value of (a *) combined with value of (b *)}}{\text{Value of (a) independently}}$$

$$\text{FIC (ii)} = \frac{\text{Value of (b) combined with value of (a)}}{\text{Value of (b) independently}}$$

The (a*) represents the essential oil compound in combination and (b*) represents the carrier oil.

The FIC index could then be calculated: ΣFIC = FIC (i) + FIC (ii)

Equation 2

The percentage mortality was calculated using the following equation:

$$\%\ \text{Mortality} = \frac{\text{dead shrimp at 24 hours (or 48 hours) (before acetic acid)} - \text{dead shrimp (time 0)}}{\text{dead shrimp (after acetic acid)}} \times 100$$

Equation 3

The percentage inhibition was calculated using the following equation:

$$\%\ \text{Inhibition} = \frac{\text{Optical density (OD) culture control} - \text{OD experimental}}{\text{OD culture control}} \times 100$$

List of abbreviations

ATCC	American type culture collection
BV	Bacterial vaginosis
CFU	Colony forming units
°C	Degrees Celsius
CA	*Candida albicans*
CG	*Candida glabrata*
CK	*Candida krusei*
CT	*Candida tropicalis*
DMSO	Dimethyl Sulfoxide
EF	*Enterococcus faecalis*
FIC	Fractional inhibitory concentration
ΣFIC	The sum of the fractional inhibitory concentration
FMC	Fractional mortality concentration
ΣFMC	The sum of the fractional mortality concentration
GV	*Gardnerella vaginalis*
g	Gram
>	Greater than
≥	Greater than or equal to
hrs	Hours
IC_{50}	Half-maximal inhibitory concentration
INT	*p*-iodonitrotetrazolium violet
LA	*Lactobacillus acidophilus*
LF	*Lactobacillus fermentum*

LG	*Lactobacillus gasseri*
LP	*Lactobacillus plantarum*
LR	*Lactobacillus rhamnosus*
LC_{50}	Lethal concentration to cause 50% mortality
LD_{50}	Lethal dose to cause 50% mortality
<	Less than
≤	Less than or equal to
L	Litre
µg	Microgram
µl	Microlitre
µl/l	Microlitre per litre
µl/mL	Microlitre per mililitre
mg	Milligram
mg/L	Milligram per litre
mg/mL	Milligram per millilitre
mL	Mililitre
MIC	Minimum inhibitory concentration
min	Minutes
PA	*Peptostreptococcus anaerobius*
%	Percentage
SD	Standard deviation
spp.	Species
TV	*Trichomonas vaginalis*
TSA	Tryptone Soya agar
TSB	Tryptone Soya broth
v/v	Volume per volume

VVC	Vulvovaginal candidiasis
w/v	Weight per volume

Dissertation structure

Chapter 1: Introduction

Introduces the study, its significance, objectives, and methodologies.

Chapter 2: Literature review publication

Chapter 3: Experimental publication

Presents results from *in vitro* experiments on essential oils and their combinations, discussing their implications.

Chapter 4: Overview and conclusion

Summarizes key findings, contributions, and limitations, suggests future research recommendations, and concludes the study.

Chapter 1 – Introduction

1.1 Background

Vaginal infections represent a significant health issue for women worldwide, contributing to increased gynaecological consultations, psychological distress, and substantial morbidity (Mora, 2019). These infections are influenced by various factors such as pregnancy, douching, irrational antibiotic use, and certain sexual practices that disrupt the vaginal ecosystem (Artym and Zimecki, 2021). These disruptions may lead to an overgrowth of pathogens, resulting in infections (Willems et al., 2020; Abou Chacra et al., 2022). The leading causes of vaginal infections are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and *Trichomonas vaginalis* (Svobodová et al., 2015; Ibragimova, 2019; Leclair and Stenson, 2022). Symptoms of these infections include malodour, pain, vaginal discharge, itching, and inflammation, which can adversely affect reproductive health and psychological well-being, thereby impacting the overall quality of life (Calinescu et al., 2021).

1.2 Treatment, resistance, and maintaining a healthy vaginal microbiome

Current therapeutic options that are first line, which include Metronidazole for treating BV and Fluconazole for treating VVC, are increasingly challenged by the rise of antimicrobial resistance (Jodar et al., 2023; Akinosoglou et al., 2024), diminishing the efficacy of these standard treatments. Biofilm formation is a survival strategy and a resistance mechanism for pathogenic micro-organisms that provides enhanced protection compared to their planktonic counterparts (Muzny and Sobel, 2022). In the context of vaginal infections, biofilm formation is a critical factor, especially in cases of BV and VVC, contributing to the persistence and high recurrence of these infections (Muzny and Sobel, 2022).

The vaginal microbiome plays a crucial role in maintaining vaginal health, with a healthy vagina predominantly consisting of lactic acid-producing *Lactobacillus* species (Deka et al., 2021). An ideal therapy should inhibit pathogens while preserving commensal *Lactobacillus* spp., and this

selectivity is underexplored for natural antimicrobials. These beneficial bacteria help maintain an acidic environment that inhibits the growth of pathogenic organisms (Deka et al., 2021). However, current therapeutic approaches and resistance mechanisms often disrupt this delicate balance, leading to dysbiosis and recurrent infections (Deka et al., 2021). Therefore, restoring and preserving the population of *Lactobacilli* is essential for effective treatment and long-term vaginal health.

1.3 The use of essential oils in vaginal infections

Essential oils have been recognized for their antimicrobial properties since the early 20th century and have been shown to exhibit a broad-spectrum antimicrobial activity against a wide range of bacteria and fungi (Hili, 2001; Puškárová et al., 2017). Numerous studies have demonstrated the potential of essential oils as a novel treatment strategy for inhibiting microbial growth and biofilm formation (Herman and Młynarczyk, 2015; Machado et al., 2017; Karpiński, 2020; Tomičić et al., 2022; Koursaoui et al., 2023). Studies also demonstrate that essential oils have noteworthy antimicrobial activity and a non-interfering or stimulatory effect on the natural microbiota. For vaginal infections, however, many essential oils and associated pathogens have been neglected (Ouwehand et al., 2010; Valdivieso-Ugarte et al., 2021). While there is a wealth of layman literature on the use of essential oils, there is a lack of scientific studies.

1.4 Essential oil combinations

Essential oils are frequently used in combination rather than in isolation to enhance their therapeutic effects (Bounar et al., 2020). Several studies have reported that combining essential oils can result in synergism, highlighting their therapeutic potential (Orchard et al., 2018; Ahmed Khan and van Vuuren, 2021; dos Santos Janotto et al., 2023; Sayed et al., 2024). Other studies have also presented synergistic activity by combining conventional therapies (such as antimicrobials with essential oils), which is a common practice in conventional medicine to enhance therapeutic outcomes. Combination therapy may lead to a dose reduction in the amount required of the antibiotic, thereby reducing adverse effects and combating resistance (Yap et al., 2014; Bunse et al., 2022; dos santos Janotto et al., 2023). Different interactions can occur,

including synergy, yet the research on the antimicrobial efficacy of essential oil combinations, particularly against vaginal pathogens, is limited (Khan et al., 2012).

1.5 Toxicity

A common misconception is that essential oils are non-toxic and harmless (Leigh-de Rapper et al., 2021). However, studies have reported potential side effects and toxicity associated with essential oils (Labiad et al., 2019; Leigh-de Rapper et al., 2021). This is particularly relevant for vaginal infections, as the vaginal mucosa is anatomically more delicate than external skin due to its thin, non-keratinized epithelium, rich vascularization, and high permeability. These features increase susceptibility to irritation and infection, which can be further exacerbated by certain essential oil constituents, such as phenols and terpenes, known to disrupt mucosal barriers at higher concentrations. Side effects that have been reported are dermatitis, mucous membrane irritation, and even systemic toxicity (Bogavac et al., 2022). Therefore, it is crucial to consider the toxicity of essential oils and their combinations in therapeutic applications. To mitigate these side effects and risks, there has been some insight into the quenching effect of essential oil combinations. Quenching leads to a reduction in the overall toxicity while maintaining or enhancing therapeutic effects and this results in reduced side effects. For instance, certain combinations have shown reduced cytotoxicity while maintaining antimicrobial efficacy, making it safer for treating infections (Yap et al., 2014; Bunse et al., 2022). This further emphasizes the importance of studying essential oils and combinations to optimize their safety and efficacy in sensitive applications like vaginal infections.

1.6 Problem statement

The frequent occurrence of vaginal infections and the diminishing effectiveness of conventional treatments are due to the growing resilience of pathogenic micro-organisms. Additionally, the dysbiosis that occurs because of current therapies and lifestyle factors also highlights the urgent need for innovative alternatives. Essential oils are presented as a plausible option, however, there is a scarcity of studies that scientifically validate the use of essential oils and their combinations in treating vaginal infections. Additionally, the vaginal microbiota has often been overlooked and

warrants attention concerning agents investigated for antimicrobial activity. Few studies consider other antimicrobial mechanisms, such as biofilm formation and their impact on the microbiota, necessitating the need for an investigation that considers the various aspects of vaginal infections, allowing for a more holistic perspective.

1.8 Research question

What is the antimicrobial activity of essential oils used in vaginal infections? What is their effect on commensal microbiota, and what toxicity do the essential oils display?

1.9 Aim

This study aims to investigate the antimicrobial activity and toxicity of essential oils, both alone and in combination, against pathogens causing vaginal infections and determine the inhibitory effects on the natural bacteria colonizing the vagina.

1.10 Objectives

- To source and procure essential oils from a reputable supplier, ensuring the provision of detailed composition data to confirm their purity.
- To investigate the antimicrobial activity of selected essential oils against pathogens associated with vaginal infections and natural microbiota using the broth minimum inhibitory concentration (MIC) microdilution method.
- To assess the interactive profile of essential oil combinations against pathogens associated with vaginal infections and natural microbiota by calculating the fractional inhibitory concentration (ΣFIC) index related to the MIC studies.
- To evaluate the toxicity of essential oils and their combinations using the brine shrimp lethality assay.
- To assess the ability of selected essential oils and combinations using the crystal violet staining assay to inhibit selected biofilms

Chapter 2 – Literature review

Laher, MH., Orchard, A., van Vuuren, S., 2025. Essential oils used for vaginal infections: A review**.** *Journal of Essential Oil Bearing Plants*.

(submitted 29th September 2025).

Proof of submission to journal is provided in Appendix D.

Essential oils used for vaginal infections: A review

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Abstract

This comprehensive review examines the intersection between aromatherapeutic recommendations for the use of essential oils in the treatment of vaginal infections and the scientific evidence supporting their efficacy. The review provides an analysis of the effects of essential oils and their combinations on key vaginal pathogens, their role in biofilm disruption, and their impact on the microbial colonizers of the vaginal tract. Notably, many pathogens remain understudied within the context of the three most common vaginal infections: bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. The data analysis revealed that only 14% of essential oils have been investigated across all three infections, as most studies (70%) focus on bacterial vaginosis and vulvovaginal candidiasis. The study of essential oil combinations was challenging largely due to the reliance on common names in aromatherapeutic sources, which often lack botanical specificity. Preliminary studies demonstrated promising antibiofilm activity, though the

limited number of studies presents a challenge for drawing definitive conclusions. Several essential oils demonstrated a capacity to preserve beneficial *Lactobacillus* species, integral to vaginal health, but there is a significant gap in research regarding their effects on the full spectrum of vaginal microbial colonizers. These findings highlight the scarcity of selectivity-focused EO studies and support the rationale for investigating synergistic, microbiota-sparing EO combinations.

Keywords

Vaginal infections, essential oils, dysbiosis, antibiofilm

INTRODUCTION

Vaginal infections are commonly diagnosed in women of reproductive age[1]. Vaginal discharge, odour and itching are common symptoms of vaginal infections, although light vaginal bleeding or spotting may also occur[2]. The most common infections clinically diagnosed are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomonal vaginitis (TV)[3,4]. These vaginal infections may cause health complications such as pelvic inflammatory disease, cervicitis, preterm labour, low birth weight, miscarriages, and chorioamnionitis if left untreated[2].

Bacterial vaginosis (BV) occurs when there is an imbalance within the vaginal microbiota. This imbalance occurs as a result of a reduction in the lactic acid-producing *Lactobacilli* spp., leading to the dominance of various pathogenic anaerobic bacteria in the vaginal tract[5]. Bacterial vaginosis has a high recurrence rate, with 50% of women experiencing a return of symptoms within the first twelve months of treatment, making it difficult to treat[6,7]. The pathogens more frequently associated with bacterial vaginosis are described in Table 1, with *Gardnerella vaginalis* being the most common pathogen[8-11]. Healthy women may be colonized by *G. vaginalis* strains with lower pathogenic potential, while strains with higher pathogenic potential may cause infection[7]. The adherence of *G. vaginalis* to the vaginal epithelium allows for biofilm formation, where other BV-causing bacteria such as *Atopobium vaginae* can become established[8].

Vulvovaginal candidiasis (VVC) is a yeast infection of the vagina caused by *Candida* spp. It is the second most diagnosed vaginal infection after BV, with approximately three-quarters of women experiencing at least one episode of VVC during their reproductive years [12.] Vulvovaginal candidiasis has been identified as a global issue of concern due to its association with sexually

transmitted infections, particularly the Human Immunodeficiency Virus (HIV), as well as upper genital tract infections[13].

Candida albicans is the leading cause of VVC. *Candida albicans* is a member of the normal human microbiota and asymptomatically colonises the vaginal lumen in about 10%-15% of women. An overgrowth may lead to infection[13,14]. Infection with another *Candida* spp. (Table 1), especially *C. glabrata* appears to be increasing[14]. Fungal vaginal infection caused by non-*C. albicans* spp. is predominant in recurrent infections[13,15]. Vulvovaginal candidiasis significantly reduces the quality of life of implicated women, resulting in psychological, social, and sexual consequences[13].

Trichomonas vaginitis (TV) or trichomoniasis is classified as a severe vaginal infection caused by a sexually transmitted flagellate protozoan known as *Trichomonas vaginalis*[4]. This parasite causes a reduction in the number of *Lactobacilli* spp. colonizing the vagina while promoting the growth of anaerobes, leading to epithelial damage, thus further predisposing women to other genital infections[16]. The pathogens associated with vaginal infections are summarised in Table 2.1.

Table 2.1. Pathogens associated with vaginal infections

Micro-organism	Pathogenesis	Reference
	Bacteria	
Atopobium vaginae	Implicated in bacterial vaginosis.	17,18
Bacteroides ureolyticus	A resident of a bacterial vaginosis environment.	19,20
Enterococcus faecalis	Bacterial vaginosis secondary bacteria, uropathogenic and moves to the vagina due to proximity.	21,22
Gardnerella vaginalis	A normal resident of the vaginal ecosystem that largely increases when *Lactobacilli* become unbalanced.	19,20 19,20
Mobiluncis curtisii	Anaerobic bacteria that cause bacterial vaginosis.	19,20
Mycoplasma hominis	Implicated in bacterial vaginosis.	
Peptostreptococcus anaerobius *P. asacharolyticus*	Frequently discovered in bacterial vaginosis.	20 23
Prevotella bivia	Anaerobic bacteria causing bacterial vaginosis.	20,24
P. disiens	Micro-organism causing bacterial vaginosis.	25,26
P. melaninogenica	Anaerobic bacteria causing bacterial vaginosis.	20,26
Ureaplasma urealyticum	Present in high concentration in women with bacterial vaginosis.	27,28
	Yeasts	
Candida albicans	Present in immunocompromised patients, which results in infection.	29,30

Candida glabrata *Candida krusei* *Candida tropicalis* *Candida parapsilosis*	An opportunistic pathogen causing vulvovaginal candidiasis.	[31]
	Parasite	
Trichomonas vaginalis	A parasite causing vaginal infections.	[19]

Treatment strategies and limitations

The prevalence of microbial resistance to the available antimicrobials leads to recurrences of vaginal infections and makes microbial eradication increasingly difficult. Current treatments that are available such as metronidazole and clindamycin, are becoming less effective due to the increased resistance of vaginal pathogens[32.] *Candida* is one of the most plaguing organisms to display resistance[33]. Studies have shown that the high incidence of *Candida* spp. limits treatment options [34,35]. The need for novel alternative treatments to combat resistance is further emphasized by the resistance of *C. krusei* and *C. glabrata* to fluconazole and *Candida auris* to many antifungals[36.]

Conventional drug therapies are available in different formulations, administered locally and systemically, due to the decreasing efficacy of treatment. However, the locally administered dosage forms have low retention in the vaginal lumen as well as low distribution throughout the vaginal tract, leading to recurrences of these infections, thus causing patient non-compliance[16].

Recent treatment strategies include finding an effective alternative treatment that can achieve complete remission of the infection. Another consideration for newer treatments should be the maintenance and restoration of the vaginal ecosystem. Upon administering conventional medication, the subsequent destruction of the microbiota is of high concern[37]. This was observed in a clinical trial where Metronidazole, used in the treatment of BV, showed a decline in the *Lactobacilli* spp., which are integral to maintaining vaginal homeostasis[37]. In addition to resistance and the microbiome, another consideration during the search for new strategies is biofilm formation, especially in BV and VVC[38]. A biofilm is described as a community of micro-organisms that are attached to a surface and supported by a self-made polymer matrix[39]. Biofilm formation is a survival method and presents better outcomes for the micro-organisms[40].

Biofilms compose 65% of all microbial infections and are highly resistant to conventional therapies[39]. The hallmark of BV is a structured biofilm that consists primarily of *G. vaginalis*[41]. *Candida* is also responsible for biofilm formation. These studies show the implication of vaginal

pathogens in biofilm formation. In-depth biofilm studies are lacking[41] and further investigative studies are imperative[38].

The vaginal microbiome refers to the microbial composition of the vaginal tract[42]. The composition differs vastly between women and fluctuates from birth to menopause[43]. The vaginal microbiome plays an essential role in the maintenance of vaginal health by preventing infections[44]. Approximately 70-90% of the vaginal bacterial species that colonise healthy women of reproductive age are *Lactobacilli*[11]. In contrast, pathogenic micro-organisms such as anaerobic bacteria and *Candida* spp. may be present in lower concentrations[43]. The *Lactobacillus* spp. most commonly found colonizing a healthy vagina are *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus jensenii,* and *Lactobacillus iners*[44]. The prevalence of these species differs with race and ethnicity[45.] Apart from racial diversity, the vaginal microbiome may vary due to the menstrual cycle, pregnancy, genetics, diabetes, age, and menopause[46]. These internal factors may cause changes to the microbial composition of the vaginal tract, leading to dysbiosis, or an imbalance in the microflora[47]. Increased oestrogen levels lead to the thickening of the vaginal epithelium and result in the accumulation of glycogen, which stimulates the proliferation of *Lactobacilli*[48].

Lactobacilli protect the vaginal environment from pathogenic infections via several mechanisms. The *Lactobacilli* do this by releasing antimicrobial compounds, such as lactic acid, which inhibit pathogenic growth, as well as bio-surfactants, which prevent the binding of pathogens to the vaginal epithelium, thus hindering biofilm formation[11]. *Lactobacilli* also use microbicidal proteins to remove pathogenic micro-organisms and compete with pathogens for nutrients[11]. The *Lactobacillus* genus maintains the normal vaginal pH, which varies between 3.8 and 4.5, through the production of lactic acid and by producing hydrogen peroxide, a bacteriostatic compound that can lower pH and create an unfavourable condition for pathogens[43].

Table 2.2 summarizes the different types of *Lactobacilli* spp., which colonize the vaginal tract as well as their different roles in the protection of the vagina against pathogens. Alternative treatments that take the natural microbiota into account should be considered based on these attributes.

Table 2.2. Microbial colonizers of the vagina

***Lactobacillus* spp.**	**Contribution to the vagina**	**Reference**
L. acidophilus	Maintenance of acid pH of the vagina, anti-adhesion factors, the release of bacteriocins lethal to pathogens and immune modulation effects.	[49,50]

Lactobacillus spp.	Contribution to the vagina	Reference
L. crispatus	Normal vaginal flora prevents the invasion of pathogens.	[20]
L. fermentum	In combination with *L. rhamnosus* can reduce colonisation by pathogens.	[51]
L. gasseri, L. iners	Normal vaginal flora prevents the invasion of pathogens.	[20]
L. jensenii		
L. plantarum	Attaches to vaginal epithelial cells and prevents the invasion of *C. albicans.*	[52]
L. reuteri	Potent antagonism against *C. glabrata.*	[53]
L. rhamnosus	Combined with *L. acidophilus* prevents infections of the urogenital tract.	[54]

The use of essential oils for the treatment of vaginal infections

Natural products, such as essential oils, have gained particular interest due to their antimicrobial activity against vaginal pathogens and due to their decreased propensity to hinder the growth of certain *Lactobacilli*[16,55].

Essential oils are often used in healthcare, and numerous studies have reported essential oils' broad-spectrum antimicrobial activity against bacteria, fungi, and parasites[56-60]. These contain active terpenoids, phenols, and aldehydes with documented *in vitro* activity against vaginal pathogens and biofilms, warranting further evaluation [56-60]. In addition, several essential oils have also shown potential in sustaining the vaginal microbiota and have been reported to display antibiofilm activity[61-63]. This highlights essential oils as a plausible treatment option due to the emerging resistance and dysbiosis resulting from conventional treatments. However, it is important to note that essential oils are often used in combinations and rarely in isolation to enhance their effects, and this must be an area of consideration[57].

This study aimed to examine essential oils recommended for use in vaginal infections and to document any research affecting the natural vaginal microbiota

METHODS

Essential oils relevant to the study were identified in the aromatherapeutic literature, which is used by the public. The essential oils that were identified (Table 2.3) were examined for antimicrobial inhibition evidence pertaining to antimicrobial pathogens associated with the vaginal canal. Search engines PubMed, Scopus, and ScienceDirect up until July 2024 were used. The filters included each of the vaginal pathogens (Table 2) or ‘vaginal microbiota’, including the *Lactobacilli* spp. (Table 1) and ‘essential oils’ including the scientific or common names (Table 3), ‘volatile oils’ or

'essential oil combinations' including the common names listed in (Table 4). The articles were screened for relevance, inclusion and exclusion criteria were determined, abstracts were read, and full articles were included in the study based on the PRISMA Guidelines (Supplementary Data, Figure S1).

Inclusion criteria

To collate scientific evidence with the layman's aromatherapeutic literature for the treatment of vaginal infections, the inclusion criteria were broad. Inclusion criteria included the following;

i. Books
ii. Randomized controlled trials
iii. Review articles
iv. Research articles
v. Conference proceedings
vi. Neat whole essential oils and not constituents
vii. Planktonic or biofilm studies only

Exclusion criteria

Publications and or other information were excluded for the following reasons;

i. Plants prepared as organic or aqueous extracts
ii. Irrelevance to vaginal infections
iii. Diffusion assays. Numerous publications document inconsistencies associated with the disc diffusion methodology when investigating essential oils[59,64]
iv. Inability to access the full publication after making every effort for retrieval
v. If the publication was in any language other than English
vi. Studies on formulations containing essential oils

Data analysis

The data was then extracted and analysed. All the information was recorded in Microsoft Excel version 16.77.1.

Use of essential oils for vaginal infections

A total of 47 different sources of the aroma-therapeutic layman literature were consulted, from which 52 commercial essential oils were recommended for vaginal infections (Table 2.3). Yeast infections are the most cited infection for which essential oils are used, according to the layman aromatherapeutic literature. *Melaleuca alternifolia* (tea tree oil) is the essential oil that was the most frequently cited within the layman's aromatherapeutic literature for use in BV, VVC, and TV, followed by *Lavandula angustifolia* and *Thymus vulgaris*. In terms of exhibiting both antifungal and antibacterial activity, the essential oils most cited in the layman aromatherapeutic literature were *Lavandula angustifolia* (Lavender) and *Origanum vulgare* (Oregano).

Table 2.3. Essential oils used to treat vaginal infections, as identified in aroma-therapeutic literature

Scientific name	Common name	Indications/ pathogens stated in the aromatherapeutic literature	Ref.
Backhousia citriodora F.Muell.	Lemon myrtle	*Candida albicans*	65
Boswellia carteri Birdw.	Frankincense	*Candida albicans* and *Candida tropicalis*	57,66
Cedrus atlantica (Endl.) G.Manetti ex Carrière	Cedarwood	Leucorrhoea, vaginal pruritus, genital infections	57,66
Cinnamomum camphora (L.) J.Presl	Camphor	Relieves irritation of the sexual organs	67
Cinnamomum zeylanicum Garcin ex Blume	Cinnamon	*Candida* species	65
Citrus bergamia Risso	Bergamot	*Candida* species, vaginal thrush	65
Commiphora myrrha (T.Nees) Engl.Friedrich Ludwig Nees and Adolf Engler	Myrrh	*Candida* (species not specified), thrush	66,67
Coriandrum sativum L.	Coriander Seed	Candidiasis	68
Cymbopogon citratus (DC.) Stapf	Lemongrass	*Candida albicans*, Candidiasis	68,69
Cymbopogon martini (Roxb.) Will.Watson	Palmarosa	*Candida albicans*	27,70
Eucalyptus citriodora Hook.	Lemon eucalyptus	Vaginitis	71
Eucalyptus globulus Labill.	Eucalyptus	*Candida albicans*, genital infections	57,69
Eucalyptus radiata Sieber ex DC.	Eucalyptus	Leucorrhoea	71
Eugenia caryophyllata Thunb. (syn. of *Syzygium aromaticum* (L.) Merr. & L.M.Perry)	Clove	*Candida albicans*	27,69
Foeniculum vulgare Mill.	Fennel seed	*Candida albicans*	65

Scientific name	Common name	Indications/ pathogens stated in the aromatherapeutic literature	Ref.
Helichrysum angustifolium (Lam.) DC.	Curry Plant	*Candida* (species not specified)	67
Helichrysum italicum (Roth) G.Don	Immortelle	*Candida albicans*	65
Hyssopus officinalis L.	Hyssop	Leucorrhoea	66,67
Jasminum officinalis L.	Jasmine	Genital discharge	71
Juniperus communis L..	Juniper	Vaginal pruritus, leucorrhoea	57,66
Laurus nobilis L..	Laurel	Gynaecological fungal infection	71
Lavandula angustifolia, Mill.	Lavender	Thrush, vaginal infection	57
Lavandula latifolia Medik.	Lavender spike	Gynaecological fungal infection	71
Lippia alba (Mill.) N.E.Br. ex Britton & P.Wilson	Bushy matgrass	*Candida albicans*	69,72
Matricaria recutita L.	German Chamomile	Genital irritation, vaginal pruritis	57
Melaleuca alternifolia Cheel	Tea tree	Candidiasis, *Candida albicans*, vaginal yeast infection, trichomoniasis, *Prevotella* spp. and *Peptostreptococcus* spp.	68,69,73
Melaleuca cajuputi Powell	Cajput	*Candida albicans*	67
Melaleuca quinquenervia (Cav.) S.T.Blake	Niaouli	Gynaecological infections	71
Melissa officinalis L.	Melissa	*Candida albicans*	69
Mentha spicata L.	Spearmint	Leucorrhoea	67
Mentha piperita L.	Peppermint	*Candida albicans*	69
Ocimum gratissimum L.	Basil, African	*Candida albicans*	65
Ocimum tenuiflorum L.	Basil, holy	*Candida* strains	65
Origanum majorana L.	Majoram	Leucorrhoea	66
Origanum vulgare L.	Oregano	Thrush	71
Pelargonium graveolens Charles Louis L'Hér.	Geranium	*Candida* (species not specified)	65
Pinus-larix nigra Marshall	Austrian pine	*Candida albicans*	69
Pinus sylvestris Lour.	Pine	Leucorrhoea	74
Pinus palustris Mill.	Longleaf pine	Leucorrhoea	66
Pimenta racemosa (Mill.) J.W.Moore	Indian Bay tree	*Candida albicans*	69
Piper longum Blume.	Long pepper	*Candida albicans*	66
Piper cubeba L.f.	Cubeb	Leucorrhoea	66
Pistacia lentiscus L.	Mastic	Leucorrhoea	66
Rosa damascena Mill.	Damask Rose	Leucorrhoea	66
Rosmarinus officinalis L.	Rosemary	*Candida albicans* (it is not specified), thrush	69,71
Santalum album L.	Sandalwood	Leucorrhoea	57
Santolina chamaecyparissus L.	Santolina	*Candida albicans*	69

Scientific name	Common name	Indications/ pathogens stated in the aromatherapeutic literature	Ref.
Styrax benzoin Carl Linnaeus (Hayne) P.W.Fritsch.	Benzoin	Leucorrhoea	[74]
Thymus vulgaris L.	Thyme	*Candida* species	[65]
Vetiveria zizanioides Stapf	Vetiver	*Candida albicans*	[69]

Essential oil combinations

Essential oils are rarely used alone and are often used in combination to achieve synergistic treatment regimens[75]. To interpret the interaction in a 1:1 combination between the oils, the fractional inhibitory concentration or ΣFIC index is used [76]. The FIC index is determined as, $\Sigma FIC = FIC^{(a)} + FIC^{(b)}$, where the FIC of oils a and b are the mean MIC values of the combined oils (a+b) divided by the MIC of each oil alone, thereafter the FIC of a and b are added[76].The ΣFIC are classified as synergistic (≤0.5), additive (>0.5-1.0), indifferent (>1.0-<4.0) or antagonistic (≥4.0)[76, 77]. Table 4 summarises essential oil combinations recommended to treat vaginal infections as identified in the layman's aroma-therapeutic literature. *Melaleuca alternifolia* has the greatest number of recommendations (22 recommendations) for combination with other recommended essential oils (Table 2.4).

Table 2.4. Essential oil combinations recommended to treat vaginal infections as identified in the layman's aroma-therapeutic literature

Scientific name	Common name	Essential oils recommended in combination (common names only) *	Ref.
Cinnamomum camphora (L.)	Camphor	Basil, Cajuput, Chamomile, Lavender, Melissa.	[67]
Citrus bergamia Risso	Bergamot	Sandalwood, Benzoin, Clove, Frankincense, Galbanum, Lavender, Patchouli.	[67,78]
Eucalyptus citriodora Hook.	Lemon eucalyptus	Cedarwood, Cypress, Juniper, Lavender, Palmarosa, Patchouli, Petitgrain, Pine, Rosemary, Sandalwood, Thyme.	[71]
Eucalyptus globulus Labill.	Eucalyptus	Cedarwood, Cypress, Lavender, Lemon, Marjoram, Pine, Tea tree, Thyme.	
Helichrysum angustifolium Planellas	Immortelle	Bergamot, Chamomile, Geranium, Frankincense, Lavender, Mandarin, Orange, Petitgrain, Rose, Rosewood, Yarrow.	[67]
Jasminum officinale L.	Jasmine	Bergamot, Black pepper, Clove, Coriander, Geranium, Ginger, Lemon, Lime, Mandarin, Neroli, Orange, Palmarosa, Patchouli, Petitgrain, Rose, Sandalwood, Ylang ylang.	[67,71]
Matricaria chamomilla var. recutita (L.) Grierson	German chamomile	Clary sage, Lavender, Lemon, Marjoram, Rose.	[57]
Melaleuca alternifolia Cheel	Tea tree	Cypress, Eucalyptus, Geranium, Juniper, Lavender, Lemon, Myrrh, Niaouli, Petitgrain, Pine, Rosemary, Cinnamon, Clove, Cypress, Eucalyptus, Ginger, Lavender, Lemon, Mandarin, Orange, Rosemary, Thyme.	[67,71]
Melaleuca quinquenervia (Cav.)	Niaouli	German and roman Chamomile, Clary sage, Eucalyptus, Geranium, Juniper, Lavender, Lemon, Pine, Rosemary, Sandalwood, Vetiver.	[71]
Origanum vulgare L.	Oregano	Bergamot, Clary sage, Cypress, Eucalyptus, Frankincense, Geranium, Ginger, Grapefruit, Hyssop, Lemon, Lime, Mandarin, Myrtle, Neroli, Orange, Palmarosa, Pine, Rosemary, Vetiver, Tangerine.	[71]
Salvia officinalis L.	Sage	Basil, Bergamot, Cedarwood, German and roman Chamomile, Cypress, Eucalyptus, Frankincense, Geranium, Grapefruit, Juniper, Lavender, Lemon, Lime, Mandarin, Orange, Petitgrain, Pine, Tangerine, Tea tree, Thyme.	[71]

*Common names mostly mentioned in layman literature

Despite the popularity of essential oils and their recommendations, the scientific evidence has not been reviewed in depth against vaginal pathogens or the beneficial microbiota. There are also limitations because of common names being used as opposed to scientific names, which makes collating evidence-based recommendations challenging. Thus, this study will critically review the aroma-therapeutic literature in tandem with the scientific literature that discusses and investigates the use of essential oils in the treatment of vaginal infections and their effect on the vaginal microbiota.

RESULTS AND DISCUSSION

Description of scientific studies

Following the first database search 2984 articles were screened. A total of 298 duplicates were removed, reducing the articles to 2786. First, the titles were screened by two independent reviewers. Thereafter, the abstracts were scanned and read, and more articles were removed due to not meeting the inclusion criteria. A final number of 513 articles was read and reviewed, as shown in Supplementary Figure S1.

Bacterial vaginosis

The plant essential oils studied against pathogens associated with bacterial vaginosis were compiled and summarized in Table 2.5. Despite *G. vaginalis* being the pathogen most associated with bacterial vaginosis, it is not the most studied pathogen. The urogenital pathogen, *E. faecalis* was the most studied bacterial pathogen. Due to the vast number of studies available, these were not included in Table 2.5, but the studies that were found as per the inclusion criteria can be found in Supplementary Data-Table S1.

Table 2.5. *In vitro* studies of essential oils studied against pathogens associated with BV

Strain number	Essential oil	Results	Reference
	Gardnerella vaginalis		
Clinical strain	*Artemisia princeps* Pamp.	MIC[a] = 0.06 µm/l	[79]
Clinical strain	*Melaleuca alternifolia*Cheel	MIC = 0.06% (v/v)	[73]

Strain number	Essential oil	Results	Reference
ATCC 14018	*Thymbra* Griseb.	MIC = 0.31 µl/ml	[80]
ATCC 49145	*Trachyspermum ammi*Sprague	MIC = 0.06 mg/ml MBC[b] = 0.125 mg/ml	[81]
N/A[c]	*Zataria multiflora* Boiss.	MIC = 0.1% (v/v)	[82]
	Peptostreptococcus anaerobius		
ATCC 27337	*Angelica archangelica* L.	MIC = 2.25 % (v/v)	[83]
ATCC 27337	*Backhousia citriodora* F.Muell.	MIC = <0.12 mg/ml	[84]
ATCC 27337	*Calophyllum inophyllum* L.	MIC = 5 mg/ml	[84]
ATCC 27337	*Citrus bergamia* Risso	MIC = ≤ 0.06-0.5 mg/ml	[83]
ATCC 27337	*Echinophora spinosa* L.	MIC = 2.25% (v/v)	[83]
ATCC 27337	*Juniperus communis* L.	MIC = 5 mg/ml	[84]
ATCC 27337	*Lavandula angustifoli* Mill.	MIC = < 0.12 mg/ml	[84]
ATCC 27337	*Melaleuca alternifolia* Cheel	MIC = 0.06% (v/v)	[85,86]
ATCC 27337	*Melissa officinalis* L.	MIC = <0.06 mg/ml	[87]
ATCC 27337	*Origanum majorana* L.	MIC = <0.06 mg/ ml	[84]
ATCC 27337	*Pimpinella anisum* L.	MIC = <0.12 mg/ml	[84]
ATCC 27337	*Rosmarinus officinalis* L.	MIC = < 0.06 mg/ml	[84]
ATCC 27337	*Thymbra capitata* Griseb.	MIC = 0.63 µl/ml	[80]
ATCC 27337	*Zingiber officinale* Roscoe	MIC = <1.2 mg/ml	[84]
	Prevotella bivia		
Clinical strain	*Backhousia citriodora* F.Muell.	MIC = 0.12-4 mg/ml	[84]
Clinical strain	*Calophyllum inophyllum* L.	MIC = 20 mg/ml	[84]
Clinical strain	*Citrus bergamia* Risso and Poit.	MIC = >2 mg/ml	[84]
Clinical strain	*Juniperus communis* L.	MIC = >20 mg/ml	[84]
Clinical strain	*Lavandula angustifoli* Mill.	MIC = >2 mg/ml	[84]
Clinical strain	*Melaleuca alternifolia* (Maiden and Betche) Cheel	MIC = <0.12 mg/ml	[86]
Clinical strain	*Melissa officinalis* L.	MIC = 1 mg/ml	[87]
Clinical strain	*Origanum majorana* L.	MIC = >2 mg/ml	[84]
Clinical strain	*Pimpinella anisum* L.	MIC = >2 mg/ml	[87]
Clinical strain	*Rosmarinus officinalis* L.	MIC = 1 mg/ml	[84]
Clinical strain	*Zingiber officinale* Roscoe	MIC = >20 mg/ml	[84]
	Prevotella disiens		
Clinical strain	*Melaleuca alternifolia* Cheel	MIC = >2 mg/ml	[86]
	Prevotella melaninogenica		
ATCC 700524	*Chrysopogon zizanioides* (L.) Roberty	MIC = 50 µg/ml MBC = 50 µg/ml	[88]
Clinical strain	*Melaleuca alternifolia* Cheel	MIC = 1 mg/ml	[86]
	Bacteroides ureolyticus		
ATCC 25285	*Backhousia citriodora* F.Muell.	MIC = <0.12 mg/ml	[84]
ATCC 25285	*Juniperus communis* L.	MIC = >20 mg/ml	[84]

Strain number	Essential oil	Results	Reference
ATCC 25285	*Lavandula angustifolia* Mill.	MIC = 1 mg/ml	[84]
Clinical strain	*Melaleuca alternifolia* Cheel	MIC = >2 mg/ml	[86]
ATCC 25285	*Melissa officinalis* L.	MIC = 0.25 mg/ml	[84]
ATCC 25285	*Origanum majorana* L.	MIC = 1 mg/ml	[84]
ATCC 25285	*Pimpinella anisum* L.	MIC = 0.25 mg/ml	[84]
ATCC 25285	*Rosmarinus officinalis* L.	MIC = 1 mg/ml	[84]
ATCC 25285	*Zingiber officinale* Roscoe	MIC = 10 mg/ml	[84]
	Mobiluncus curtsii		
ATCC 35241	*Thymbra capitata* Griseb.	MIC = 0.16 µl/ml MBC = 0.31 µl/ml	[80]
	***Fannyhessea vaginae* (*Atopobium vaginae*)**		
ATCC BAA-55	*Thymbra capitata* Griseb.	MIC = 0.16 µl/ml MBC = 0.31 µl/ml	[80]
	Mycoplasma hominis		
Clinical strain	*Cinnamomum zeylanicum* Garcin ex Blume	MBC_{90} = 500 µg/ml	[89]
Clinical strain	*Citrus bergamia* Risso	MIC_{50} = 0.5% (v/v) MIC_{90} = 1% (v/v)	[90]
Clinical strain	*Eugenia hiemalis* var. *marginata* (O.Berg) *D.Legrand*	MIC = 333.33 µg/ml	[91]
Clinical strain	*Melaleuca alternifolia* Cheel	MIC = 0.06–0.12% (v/v)	[92]

[a]MIC- Minimum Inhibitory Concentration; [b]MBC-Minimum Bactericidal Concentration; [c]N/A-Not provided by authors.

A limited number of studies were found on the antimicrobial activity of essential oils against *Mobiluncus* spp., *Bacteroides* spp., *Peptostreptococcus anaerobius Peptostreptococcus asaccharolyticus, Prevotella* spp., and *Ureaplasma urealyticum*. These pathogens have been neglected despite their clinical importance in vaginal infections. Out of numerous essential oils studied against *E. faecalis*, 26% of the essential oils recommended by the aroma-therapeutic literature correlated with scientific literature. *Melaleuca alternifolia* is the essential oil most studied (73%) against BV-associated pathogens. This is followed by *Juniperus communis, Origanum majorana,* and *Rosmarinus officinalis,* which are each studied against 36% of the relevant pathogens. Other frequently studied essential oils that demonstrate a noteworthy antibacterial effect are *Lavandula angustifolia, Cinnamomum zeylanicum, Cymbopogon citratus,* and *Thymus vulgaris*. These are recommended in both aroma-therapeutic and scientific literature. While the MIC values in Table 2.5 indicate that essential oils such as *Melaleuca alternifolia, Thymbra capitata,* and *Origanum majorana* exhibit potent *in vitro* activity against BV-associated

pathogens, translating these results to clinical efficacy requires caution. Achieving comparable concentrations in the vaginal environment may be challenging due to solubility, diffusion, and formulation limitations. *Melaleuca alternifolia*, the most studied essential oil, demonstrated activity against multiple pathogens, including *E. faecalis*, yet achieving effective concentrations in the vaginal environment may be limited by formulation, solubility, and mucosal tolerance. Other frequently studied oils, such as *Juniperus communis*, *Origanum majorana*, *Rosmarinus officinalis*, *Lavandula angustifolia*, *Cinnamomum zeylanicum*, *Cymbopogon citratus*, and *Thymus vulgaris*, also show *in vitro* efficacy; local irritation at higher concentrations has not been established in a clinical setting.

Vulvovaginal candidiasis

The essential oils studied against pathogens associated with vulvovaginal candidiasis were compiled and summarised (Table 2.6). *Candida albicans* was found to be the most studied yeast pathogen. In contrast, *C. krusei* and *C. tropicalis* are among the least studied species in the context of vaginal infections.

Upon reviewing the essential oils recommended against *C. albicans* by aroma-therapeutic literature, it was discovered that 66% of the essential oils had been investigated in scientific literature, with less attention given to the other non-*C. albicans* spp. (16% of essential oils were studied against *C. glabrata*, 14% were studied against *C. parapsilosi*s, 6% were studied against *C. tropicalis*, and a mere 2% were studied against *C. krusei* within the scientific literature that correlated with the layman aromatherapeutic literature).

Table 2.6. Essential oils studied against *Candida albicans, Candida krusei, Candida glabrata, Candida tropicalis,* and *Candida parapsilosis*

Strain number	Essential oil	Results	Reference
	Candida albicans		
Clinical strain	*Allium haemanthoides* Boiss. ex Regel	MIC[a] = 0.155 µl/ml	[93]
Clinical strain	*Ammoides verticillata* (Desf.) Briq.	MIC = 500 µg/ml	[94]
Clinical strain	*Anethum graveolens* L.	MIC = 625 µg/ml	[95]
Clinical strain	*Apium graveolens* L.	MIC = 1% (v/v)	[73]
Clinical strain	*Artemisia sieberi* Besser	MIC = 1.21 µl/ml	[73]
Clinical strain	*Boswellia carterii* Birdw.	MIC = 1% (v/v)	[73]
Clinical strain	*Calamintha nepeta* Willk.	MIC = 1.25 µl/ml	[96]

Strain number	Essential oil	Results	Reference
Clinical strain	*Cananga odorata* (Lam.) Hook.f. and Thomson	MIC = 1% (v/v)	[73]
Clinical strain	*Cedrus atlantica* (Endl.) Manetti ex Carrière	MIC = >2% (v/v)	[73]
Clinical strain	*Cinnamomum aromaticum* Nees	MIC = 0.03% (v/v)	[73,97]
Clincal strain	*Cinnamomum cassia* (L.) J. Presl	MIC = 0.125% (w/v)	[98]
Clinical strain	*Cinnamomum zeylanicum* Garcin ex Blume	MIC = 64 μg/ml	[95]
Clinical strain	*Citrus medica f. aurantifolia* (Christm.) M.Hiroe	MIC = 2% (v/v)	[73]
Clinical strain	*Citrus* × *aurantium* L.	MIC = 1% (v/v)	[73]
Clinical strain	*Citrus bergamia* Risso	MIC = 1% (v/v)	[73]
Clinical strain	*Citrus limon* (L.) Osbeck	MIC = 2% (v/v)	[73]
Clinical strain	*Citrus madurensis* Lour.	MIC = 2% (v/v)	[73]
Clinical strain	*Citrus paradisi* Macfad.	MIC = 1% (v/v); 0.0039% (v/v)	[73,99]
Clinical strain	*Commiphora myrrha* (T.Nees) Engl.	MIC = >2% (v/v)	[73]
ATCC 10231	*Coriandrum sativum* L.	MIC = 0.25% (v/v); 0.11 μl/ml	[61,73]
Clinical strain	*Thymbra capitata* (L.) Cav.	MIC = 128 μg/ml	[96]
Clinical strain	*Cucurbita pepo* Lour.	MIC = >2% (v/v)	[73]
Clinical strain	*Cuminum cymimum* Wall.	MIC = 50 μl/ml	[101]
Clinical strain	*Cupressus sempervirens* L.	MIC = >2% (v/v)	[73]
Clinical strain	*Cymbopogon citratus* (hort. ex DC.) Stapf	MIC = 0.06% (v/v)	[73,97]
Clinical strain	*Cymbopogon flexuosus* (Nees ex Steud.) W.Watson	MIC = 0.125% (w/v)	[98]
Clinical strain	*Cymbopogon martini* Stapf	MIC = 0.06% (v/v); 63 μg/ml	[73,95]
IP 4872	*Cymbopogon nardus* (L.) Rendle	MIC = 0.12% (v/v)	[73,100]
Clinical strain	*Cymbopogon winterianus* Jowitt	MIC = 1000 μg/ml	[95]
Clinical strain	*Daucus carota* L.	MIC = 2% (v/v)	[73]
Clinical strain	*Dittrichia graveolens* (L.) Greuter	MIC = 2 μl/ml	[102]
Clinical strain	*Duguetia lanceolata* A.St.-Hil.	MIC = 60 μg/ml	[95]
ATCC 90028	*Eucalyptus borealis* (C.A.Gardner) D.Nicolle	MIC = 8% (v/v)	[103]
IP 4872	*Eucalyptus camaldulensis* Dehnh.	2.5 mg/ml	[100]
IP 4872	*Eucalyptus citriodora* Hook.	MIC = 5 mg/ml	[100]
ATCC 90028	*Eucalyptus globulus* Labill.	MIC = >8% (v/v)	[103]
ATCC 90028	*Eucalyptus loxophleba* Benth.	MIC = 8% (v/v)	[103]
ATCC 90028	*Eucalyptus kochii subsp. plenissima* (Gardner)	MIC = 8% (v/v)	[103]
Clinical strain	*Eucalyptus polybractea* R.T.Baker	MIC = 1% (v/v)	[73]
Clinical strain	*Eucalyptus radiata* Sieber ex DC.	MIC = 2.5% (w/v)	[98]
Clinical strain	*Syzygium aromaticum* (L.) Merr. & L.M.Perry.	MIC = 0.5% (w/v)	[98]
ATCC10231	*Foeniculum vulgare* Mill.	MIC = 0.5% (v/v) MIC = 0.78 %	[73,104]
Clinical strain	*Gaultheria procumbens* L.	MIC = 0.25% (v/v)	[73]

Strain number	Essential oil	Results	Reference
Clinical strain	*Hymenocrater longiflorus* Benth.	MIC = 240 µg/ml	96
ATCC 10231	*Jasminum grandiflorum* L.	MIC = >100 g/l	106
ATCC 24433		MIC = >100 g/l	
ATCC 10231	*Jasminum sambac* (L.) Aiton	MIC = 1% (v/v)	107
Clinical strain	*Juniperus communis* L.	MIC = 2% (v/v)	73
IP 4872	*Lantana camara* L.	MIC = 2.5 mg/ml	100
ATCC 14053	*Laurus nobilis* L.	MIC = >4% (v/v)	99
Clinical strain	*Lavandula angustifolia* Mill.	MIC = 512 µg/ml	73
		MIC = 0.5% (v/v)	96
		> 2% (v/v)	108
Clinical strain	*Lavandula binaludensis* Rech.f.	MIC = 3.90 - 11.71 mg/ml	109
Clinical strain	*Lavandula intermedia* Emeric ex Loisel	MIC = 2% (v/v)	108
ATCC 14053	*Lavandula latifolia* Medik	MIC = 0.25% (v/v)	99
Clinical strain	*Lavandula luisieri* (Rozeira) Rivas Mart.	MIC = 1.25-2.5 µl/ml	96
Clinical strain	*Lavandula multifida* L.	MIC = 0.32 µl/ml	96
Clinical strain	*Lavandula angustifoli* Mill.	MIC = 2.4 µl/ml	93
ATCC 14053	*Lavandula stoechas* L.	MIC = 1.56 mg/ml	110
ATCC 60193	*Leucodon sciuroides* (Hedw.) Schimp.	MIC = 711 µg/ml	105
IP 4872	*Lippia multiflora* Moldenke	MIC = 5 mg/ml	100
Clinical strain	*Macadamia integrifolia* Maiden & Betche	MIC = >2% (v/v)	73
ATCC 14053	*Melaleuca alternifolia*Cheel	MIC = 0.5% (v/v)	58
		2% (v/v)	73
		MIC = 0.03-8%	99
Clinical strain	*Melaleuca cajuputi* Roxb.	MIC = 1% (v/v)	73
Clinical strain	*Melaleuca quinquenervia* (Cav.) S.T.Blake	MIC = 0.25% (v/v)	73
ATCC 14053	*Melissa officnalis* L.	MIC = 0.39 mg/ml	110
Clinical strain	*Mentha mozaffarianii* Jamzad	MIC = 0.39 µg/ml	96
Clinical strain	*Mentha* × *piperita* L.	MIC = 0.5% (v/v)	73
ATCC 14053		MIC = 0.39 mg/ml	110
		MIC = 12.5-100 µg/ml	111
Clinical strain	*Mentha pulegium* L.	MIC = 1.25 µl/ml	96
		MIC = 1.150 mg/ml	112
ATCC 14053	*Mentha spicata* Crantz	MIC = 0.12% (v/v); 2% (v/v)	73, 99
ATCC 10231	*Mentha suaveolens* Ehrh.	MIC = 0.39 g/l	106
ATCC 24433		MIC = 0.39 g/l	106
Clinical strain	*Micromeria inodora* (Desf) Benth.	MIC = 1000 µg/ml	96
Clinical strain	*Monarda fistulosa* L.	MIC = 1% (v/v)	108
Clinical strain	*Monarda didyma* L.	MIC = 0.25% (v/v)	108
Clinical strain	*Nepeta asterotricha* Rech. f.	MIC = 500-2000 µg/ml	96
Clinical strain	*Nepeta cataria* L.	MIC = 0.125 µl/ml	96
Clinical strain	*Ocimum basilicum* L.	MIC = 0.5% (v/v); 1.56 g/ml	73,113
Clinical strain	*Ocimum americanum* L.	MIC = 0.04% (v/v)	95
ATCC 90028	*Ocimum gratissimum* L.	MIC = 1.56 g/ml	113
ATCC 90028	*Ocimum kilimandscharicum* Guerke	MIC = 6.25 mg/ ml	113
Clinical strain	*Oenothera biennis* L.	MIC = >2% (v/v)	73

Strain number	Essential oil	Results	Reference
Clinical strain	*Origanum boissieri* Ietsw.	MIC = 125-250 μg/ml	96
Clinical strain	*Origanum hirtum* Link	MIC = 0.25% (v/v)	108
Clinical strain	*Origanum glandulosum* Desf.	MIC = 250-500 μg/ml	94
Clinical strain	*Origanum marjorana* L.	MIC = 0.25% (v/v); 1.0% (v/v)	73,97
ATCC 14053	*Origanum vulgare* L.	MIC = 0.12% (v/v); 0.05% (w/v)	73,98
		MIC = 0.78 mg/ml	110
		MIC = 25 μg/ml	114
Clinical strain	*Pelargonium asperum* (Curtis) L'Hér.	MIC = 0.45 μg/ml	98
Clinical strain	*Pelargonium graveolens* (L.) L'Hér.	MIC = 0.12% (v/v)	73
Clinical strain	*Pimenta racemosa* (Mill.) J.W.Moore	MIC = 0.12% (v/v)	73
ATCC 14053	*Pimpinella anisum* S.G.Gmel.	MIC = 4% (v/v)	113
Clinical strain	*Pinus sylvestris* Lour	MIC =2% (v/v)	73
Clinical strain	*Piper nigrum* L.	MIC = >2% (v/v)	73
Clinical strain	*Pogostemon cablin* (Blanco) Benth.	MIC = 32 μg/ml	95
Clinical strain	*Pogostemon patchouli* (Blanco) Benth.	MIC = 0.5% (v/v)	73
Clinical strain	*Prunus armeniaca* Blanco	MIC = >2% (v/v)	73
Clinical strain	*Prunus dulcis* D.A.Webb	MIC = >2% (v/v)	73
ATCC 14053	*Rosmarinus officinalis* L	MIC = 1% (v/v); 4% (v/v)	73,99
ATCC 76615		MIC = 1.56 mg/ml	110
Clinical strain	*Saccocalyx satureioides* (Desf.) Benth.	MIC = 500-1000 μg/ml	94
Clinical strain	*Salvia officinalis* L.	MIC = 0.5% (v/v)	73
ATCC 14053		MIC = 12.5 mg/ml	110
Clinical strain	*Salvia sclarea* L.	MIC = >2% (v/v); 0.5% (v/v)	73,97
Clinical strain	*Santalum album* L.	MIC = 0.06% (v/v)	73
Clinical strain	*Santolina chamaecyparissus* L.	MIC = 1000 μg/ml	95
Clinical strain	*Satureja intermedia* Benth.	MIC = 3400 μg/ml	73,95
ATCC 14053	*Satureja montana* L.	MIC = <0.0019% (v/v)	99
Clinical strain		MIC = 0.25% (v/v)	108
Clinical strain	*Syzygium aromaticum* (L.) Merr. and L.M.Perry	MIC = 0.12% (v/v); 0.25% (v/v)	73,97
ATCC 10231	*Tetradenia riparia* (Hochst.) Codd	MIC = 31.2 to 62.4 μg/ml	115
Clinical strain	*Thuja occidentalis var. plicata Mast.*	MIC = 0.05% (w/v)	98
Clinical strain	*Thymbra capitata* (L.) Cav.	MIC = 0.32 μg/ml	98
Clinical strain	*Thymus algeriensis* Boiss.	MIC = 4.697 mg/ ml	112
ATCC 14053	*Thymus capitatus* Hoffmanns. and Link	MIC = 0.015% (v/v)	99
ATCC 14053	*Thymus vulgaris* L.	MIC = 3.125 mg/ml	110
Clinical strain	*Trachyspermum ammi* (L.) Sprague	MIC = 500 μg/ml	95
		MIC = 0.25 μl/ml	116
Clinical strain	*Chrysopogon zizanioides* (L.) Roberty	MIC = 0.12% (v/v)	73
IP 4872	*Xylopia aethiopica* (Dunal) A.Rich.	MIC = 5 mg/ml	100
Clinical strain	*Zataria multiflora* (Boiss.) Jalas	MIC = 0.155 μl/ml	93
Clinical strain	*Zingiber officinale* Roscoe	MIC = >2% (v/v)	73
	Candida krusei		
ATCC 6258	*Melaleuca alternifolia* Cheel	MIC = 0.25% (w/v)	117

Strain number	Essential oil	Results	Reference
Clinical strain	*Mentha australis* R.Br.	MIC = 1.23 µg/ml	[96]
Clinical strain	*Nepeta cataria* L.	MIC = 0.5 µl/ml	[96]
Clinical strain	*Satureja macrosiphon* (Coss.) Maire	MIC = 0.25 µg/ml	[96]
Clinical strain	*Trachyspermum ammi* Sprague	MIC = 4 µl/ml	[96,116]
	Candida glabrata		
Clinical strain	*Ammoides verticillata (Desf.) Briq.*	MIC = 500 µg/ml	[94]
Clinical strain	*Artemisia sieberi* Besser	MIC = 37.4-4781.3 µg/ml	[118]
Clinical strain	*Thymbra capitata (L.) Cav.*	MIC = 1.25 µl/ml	[96]
Clinical strain	*Lavandula angustifolia* Mill.	MIC = >2% (v/v)	[108]
Clinical strain	*Lavandula × intermedia Emeric ex Loisel.*	MIC = 2% (v/v)	[108]
ATCC 66032	*Lavandula stoechas* L.	MIC = 1.67 mg/ml	[110]
ATCC 90030	*Melaleuca alternifolia* (Maiden and Betche) Cheel	MIC = 0.06% (w/v)	[117]
ATCC 66032	*Melissa officinalis* L.	MIC = 0.84 mg/ml	[110]
Clinical strain	*Mentha australis* R.Br.	MIC = 1.02 µg/ml	[108]
ATCC 66032	*Mentha x piperita* L.	MIC = 3.186 mg/ml	[110]
Clinical strain	*Mentha spicata L.*	MIC = 1.25 µl/ml	[96]
Clinical strain	*Monarda didyma* L.	MIC = 0.25% (v/v)	[108]
Clinical strain	*Monarda fistulosa* L.	MIC = 1% (v/v)	[108]
Clinical strain	*Nepeta cataria* L.	MIC = 0.25 µl/ml	[108]
Clinical strain	*Origanum glandulosum* Desf.	MIC = 250-500 µg/ml	[94]
ATCC 66032	*Origanum vulgare* L.	MIC = 1.1 mg/ml	[110]
ATCC 66032	*Rosmarinus officinalis L.*	MIC = 3.15 mg/ml	[110]
Clinical strain	*Saccocalyx satureioides* Roussine	MIC = 500-1000 µg/ml	[94]
ATCC 66032	*Salvia officinalis* L.	MIC = 22.5 mg/ml	[110]
ATCC 90030	*Satureja intermedia* P. Wilson	MIC = 3.8 ± 0.3 mg/ ml MIC = 3.5 ± 0.1 mg/ ml	[110] [135]
Clinical strain	*Satureja macrosiphon* (Coss.) Maire	MIC= 0.7 µg/ml	[108]
Clinical strain	*Satureja montana* L.	MIC= 0.25% (v/v)	[108]
Clinical strain	*Trachyspermum ammi* Sprague	MIC = 0.5 µl/ml	[116]
ATCC 66032	*Thymus vulgaris* L.	MIC = 3.44 mg/ml	[110]
Clinical strain	*Zataria multiflora* Rech. f.	MIC = 0.062 mg/ml	[94]
	Candida tropicalis		
Clinical strain	*Lavandula angustifolia* Mill.	MIC = >2% (v/v)	[108]
Clinical strain	*Lavandula intermedia* Emeric ex Loisel.	MIC = 2% (v/v)	[108]
Clinical strain	*Lavandula multifida* L.	MIC = 0.32 µl/ml	[96]
Clinical strain	*Lavandula viridis* L.	MIC = 1.25 µl/ml	[96]
ATCC 750	*Melaleuca alternifolia* Cheel	MIC = 0.06% (w/v)	[96]
Clinical strain	*Mentha pulegium* L.	MIC = 1.25 µl/ml	[96]
Clinical strain	*Mentha spicata* L.	MIC = 1.25 µl/ml	[96]
Clinical strain	*Monarda didyma* L.	MIC = 0.25% (v/v)	[108]
Clinical strain	*Monarda fistulosa* L.	MIC = 1% (v/v)	[108]
Clinical strain	*Nepeta cataria* L.	MIC = 0.125 µl/ml	[96]

Strain number	Essential oil	Results	Reference
Clinical strain	*Nepeta transcaucasica Grossh.*	MIC = 250 µg/ml	[96]
Clinical strain	*Origanum boissieri Ietsw.*	MIC = 250 µg/ml	[96]
Clinical strain	*Origanum hirtum* Link	MIC = 0.25% (v/v)	[96]
Clinical strain	*Satureja macrosiphon* (Coss.) Maire	MIC = 0.5 µg/ml	[96]
Clinical strain	*Satureja montana* L.	MIC = 0.25% (v/v)	[108]
Clinical strain	*Satureja thymbra* L.	MIC = 0.32 µg/ml	[96]
Clinical strain	*Thymus leptobotrys* Murb.	MIC = 0.32 µg/ml	[96]
Clinical strain	*Thymus × viciosoi* (Pau) R. Morales)	MIC = 0.32 µg/ml	[96]
Clinical strain	*Trachyspermum ammi* Sprague	MIC = 0.25 µl/ml	[116]
Clinical strain	*Ziziphora tenuior* L.	MIC = 1.25 µg/ml	[96]
	Candida parapsilosis		
Clinical strain	*Calamintha nepeta Willk.*	MIC = 0.125% (w/v)	[98]
Clinical strain	*Cinnamomum cassia* (L.) D.Don	MIC = 0.125% (w/v)	[98]
Clinical strain	*Cymbopogon flexuosus* (Nees ex Steud.) Stapf	MIC = 0.5% (w/v)	[98]
Clinical strain	Eugenia caryophyllata Thunb.	MIC = 2.5% (w/v)	[98]
Clinical strain	*Eucalyptus radiata* Sieber ex DC	MIC = >2% (v/v)	[108]
Clinical strain	*Lavandula angustifolia* Mill. .	MIC = 2% (v/v)	[108]
Clinical strain	*Lavandula intermedia* Emeric ex Loisel.	MIC = 0.32 µl/ml	[96]
ATCC 22019	*Lavandula multifida* Burm.f..	MIC = 0.125% (w/v)	[117]
Clinical strain	*Melaleuca alternifolia* Cheel	MIC = 0.125% (w/v) MIC = 1.25 µl/ml	[96, 98]
Clinical strain	*Mentha spicata* L.	MIC = 1.25 µl/ml	[96]
Clinical strain	*Mentha pulegium* L.	MIC = 0.5% (v/v)	[108]
Clinical strain	*Monarda didyma* L.	MIC = 1% (v/v)	[108]
ATCC 22019	*Monarda fistulosa* L.	MIC = 0.031% (w/v)	[119]
Clinical strain	*Myrcia ovata* Cambess.	MIC = 750 µg/ml	[96]
Clinical strain	*Nepeta transcaucasica* (Bieb.) Boiss.	MIC = 125 µg/ml	[96]
Clinical strain	*Origanum boissieri* Ietsw.	MIC = 0.25% (v/v)	[108]
Clinical strain	*Origanum hirtum* Link	MIC = 0.05% (w/v)	[98]
Clinical strain	*Origanum vulgare* L.	MIC = 0.25% (v/v)	[108]
Clinical strain	*Satureja montana* L.	MIC = 0.32 µg/ml	[96]
Clinical strain	*Satureja thymbra* L	MIC = 0.05% (w/v)	[98]
Clinical strain	*Thuja plicata* Donn ex D.Don	MIC = 8 µl/ml	[116]
Clinical strain	*Trachyspermum ammi* Sprague	MIC = 0.32 µg/ml	[96]
Clinical strain	*Thymus leptobotrys* Murb.	MIC = 0.32 µg/ml	[96]
Clinical strain	*Thymus × viciosoi* (Pau) R. Morales	MIC = 0.05% (w/v)	[98]
Clinical strain	*Thymus vulgaris* L	MIC = 1.25 µg/ml	[96]

[a]MIC- Minimum Inhibitory Concentration

Melaleuca alternifolia essential oil has been studied the most against pathogens associated with VVC. The second most studied essential oils are *Lavandula angustifolia* and *Origanum vulgare*. When correlating the most frequently recommended essential oils in layman aromatherapeutic literature with the scientific studies, it was found that *Origanum vulgare* demonstrated effectiveness against multiple *Candida* strains, with MIC values ranging from 0.125 to 1 mg/ml for *Candida albicans*, *C. glabrata*, and *C. tropicalis*. The anti-yeast activity of *Origanum vulgare* is primarily attributed to high levels of carvacrol and thymol. *Thymus vulgaris* exhibited MIC values ranging from 0.0625 to 3.44 mg/ml, showing significant activity against several *Candida* spp. mainly due to the presence of thymol. *Melaleuca alternifolia* showed MIC values between 0.125 and 1 mg/ml, effective against *C. albicans* and non-*C. albicans* spp., with terpinen-4-ol as its primary active compound. The MIC values for the essential oils were higher when tested against *C. parapsilosis* than the other non- *C. albicans* spp., due to this pathogen's extreme resilience. *Lavandula angustifolia* displayed MIC values of 0.5 to 4 mg/ml against *C. albicans* and non- *C. albicans* species, indicating moderate anti-yeast activity.

While *Melaleuca alternifolia*, *Lavandula angustifolia*, *Origanum vulgare*, and *Thymus vulgaris* demonstrate promising *in vitro* antifungal activity against both *C. albicans* and non-*C. albicans* species, translating these results to clinical efficacy presents several challenges. Achieving effective concentrations in the vaginal environment may be limited by solubility, formulation, and mucosal tolerance, especially for more resilient species such as *C. parapsilosis*. The variability in essential oil composition between batches and potential local irritation at higher concentrations further complicates their therapeutic use. Additionally, despite strong activity against *C. albicans,* limited studies on *C. krusei*, *C. tropicalis*, and *C. glabrata* indicate gaps in evidence for non-*C. albicans* pathogens. Therefore, while these essential oils hold potential as complementary interventions for VVC, *in vivo* studies are essential to assess achievable vaginal concentrations, safety margins, and formulation strategies to ensure both efficacy and tolerability.

Parasitic vaginal infections (*Trichomonas vaginitis*)

The plant essential oils studied against *T. vaginalis* were compiled and summarized in Table 2.7.

Table 2.7. Essential oils studied against *Trichomonas vaginalis*

Strain number	Essential Oils	Results	Ref.
Clinical strain	*Aframomum sceptrum* K. Schum.	IC_{50} = 0.12±0.02 µl/ml MLC[a] = 1.72 µl/ml	120
Clinical strain	*Amomum tsao-ko* Crevost & Lemarié	IC_{50} = 22.49 mg/ml after 48 hrs MLC = 44.97 mg/ml after 48 hrs	121
ATCC JH31A	*Artemisia absinthium* L.	Growth Inhibition= 99.1% at 500 µg/ml	47
Clinical strain	*Artemisia ludoviciana* Nutt.	IC_{50} = 64 mg/ml dose significantly reduced parasite proliferation in the first 8 hrs. Parasite was completely dead in the 48th hr	122
T. vaginalis reference strain G3	*Atalantia sessiflora* Guillaumin	IC_{50} = 0.016% (v/v) after 48 hrs IC_{90} = 0.03% (v/v) after 48 hrs MIC[b]= 0.06% (v/v) after 48 hrs	123
Clinical strain	*Bunium persicum* (Boiss.)	IC_{50} = 14.41 mg/ml after 12 hrs IC_{50} = 45.19 mg/ml after 24 hrs	124
Clinical strain	*Coriandrum sativum* L.	MIC = 0.015 g/ml MLC= 0.031 g/ml	125
Clinical strain	*Cymbopogon flexuosus* (Nees ex Steud.)	MIC = 1.5% (v/v)	126
Clinical strain	*Eucalyptus globulus* Labill.	No growth observed after 24 hrs	126
Clinical strain	*Foeniculum vulgare* Mill.	MLC = 13.7 µg/ml	127
Clinical strain	*Hornstedtia bella* Škorničk	IC_{50} of 0.008% (v/v) IC_{90} of 0.016% (v/v) MLC of 0.03% (v/v)	128
Clinical strain	*Lavandula angustifoli* Mill.	Growth Inhibition% = 81.7%	129
Clinical strain	*Leoheo domatiophorus* Chaowasku	IC_{50} = 0.008% (v/v) IC_{90} = 0.016% (v/v) MIC = 0.03% (v/v)	123
Clinical strain	*Limnocitrus littoralis* (Miq.) Swingle	IC_{50} = 0.03% (v/v) IC_{90} = 0.06% (v/v) MLC = 0.12% (v/v)	123
Clinical strain	*Melaleuca cajuputi* Roxb.	MIC = 0.03 to 0.25% at 24 hrs and 0.02 to 0.25% at 48 hrs	130
Clinical strain	*Marrubium vulgare* L.	MIC = 291+-136 µg/ml [146]	131
ATCC 30236	*Nectandra megapotamica* Mez	IC_{50} = 98.7 µg/ml Showed zero viability at 500 µg/ml	132
Clinical strain	*Origanum vulgare* L.	EC[c]100 where total inhibition at 50 µg/ml after 48 hrs	133
Clinical strain	*Ocimum basilicum* L.	No growth observed after 24 hrs	126
Clinical strain		MLC = 30 µg/ml after 24 hrs MLC = 20 µg/ml after 48 hrs MLC = 10 µg/ml after 96 hrs	134
Clinical strain	*Paramignya trimera* (Oliv.) Burkill	IC_{50} = 0.016% (v/v) IC_{90} = 0.03% (v/v) MLC=0.06% (v/v)	123
Clinical strain	*Pistacia lentiscus* L.	MLC = 15 mg/ml after 24 hrs MLC = 10 mg/ml after 48 hrs MLC = 5 mg/ml after 96 hrs	134
Clinical strain	*Rosa damascene* Mill.	IC_{50}= 1.79 mg/ml after 24 hrs IC_{50}= 2.24 mg/ml after 48 hrs	135

Strain number	Essential Oils	Results	Ref.
Clinical strain	*Trachyspermum ammi* Sprague	IC_{50}= 6.11 mg/ml after 72 hrs MIC = 2.24 mg/ml after 48 hrs and 6.11 mg/ml after 72 hrs IC_{50} = 8.08 mg/mL after 12 hrs IC_{50} of 25.81 mg/mL after 24 hrs IC_{50} = 207.4 µg/mL after 24 hrs IC_{50} = 69.47 µg/mL after 48 hrs	81, 124
Clinical strain	*Zataria multiflora* Boiss	Complete inhibition at 0.1-0.0004%	82

[a]MLC- Minimum Lethal Concentration (Minimum Bactericidal Concentration); [b]MIC- Minimum Inhibitory Concentration; [c]EC- Effective Concentration

Of the 30 essential oils recommended for use in aromatherapeutic literature against *T. vaginalis*, 26% were further studied against this relevant pathogen. Most studies were *in vitro* based using clinical strains.

While Table 2.7 summarizes MIC, MLC, IC_{50}, and IC_{90} values of essential oils against T. vaginalis, it is important to interpret these findings with caution in a clinical context. Most studies were conducted *in vitro* using controlled concentrations, which may not directly translate to achievable levels in the vaginal environment. Factors such as local bioavailability, vaginal pH, mucosal absorption, and potential irritation can limit the concentrations of essential oils that can be safely administered. For instance, some of the highly active oils show IC50 values as low as 0.008% v/v but achieving these levels *in vivo* could exceed safety margins. Therefore, while the data demonstrates promising antimicrobial activity, further studies including optimized formulations, pharmacokinetics, and *in vivo* validation are required before clinical recommendations can be made.

Essential oil combinations for vaginal infections

Essential oil combinations that have been previously studied against vaginal pathogens were compiled and summarized in Table 2.8.

Table 2.8. Essential oil combinations studied against vaginal pathogens

Strain number	Essential oil blends	Results	Ref.
	Enterococcus faecalis		
ATCC 7314	*Ammoides verticillata* (Desf.) Briq. / *Satureja candidissima* Briq.	Synergy MIC[a] = 0.3-1.5 µl/ml MBC[b] = 0.4-6.2 µl/ml	[136]
Clinical strain	*Citrus bergamia* Risso / *Citrus* × *sinensis* (L.) Osbeck	Synergy	[137]
Clinical strain	*Eucalyptus globulus* Labill. / *Pimenta pseudocaryophyllus* (Gomes) Landrum	Indifferent effect	[138]
CIP 103907	*Lippia multiflora* Moldenke / *Mentha* × *piperita* L.	Synergy ΣFIC[c] = 0.11	[139]
CIP 103907	*Lippia multiflora* Moldenke / *Ocimum basilicum* L.	Synergy ΣFIC = 0.44	[139]
CIP 103907	*Mentha* × *piperita* L. / *Ocimum basilicum* L.	Synergy ΣFIC = 0.37	[139]
	Candida albicans		
ATCC 10231	*Allium sativum* L. / *Citrus limon* (L.) Osbeck	Additive effect ΣFIC = 0.93	[140]
ATCC 10231	*Allium sativum* L. / *Cymbopogon martinii* (Roxb.) Will. Watson	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Allium sativum* L. / *Melaleuca alternifolia* Cheel	Indifferent effect ΣFIC = 2.33	[140]
ATCC 10231	*Allium sativum* L. / *Pogostemon patchouli* Pellet.	Additive effect ΣFIC = 0.94	[140]
ATCC 10231	*Anthemis nobilis* L. /*Citrus* × *aurantium* L.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 90028	*Artemisia absinthium* L. / *Tanacetum vulgare* L.	Combination inhibited the pathogen. No ΣFIC studies conducted.	[141]
ATCC 10231	*Boswellia carteri* Birdw. / *Cedrus atlantica* G.Manetti	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Boswellia carteri* Birdw. / *Citrus limon* (L.) Osbeck	Additive effect ΣFIC = 0.63	[141]
ATCC 90028	*Cananga odorata* (Lam.) Hook. f. & Thomson / *Citrus aurantium* L., *Citrus reticulata* Blanco / *Pogostemon cablin* (Blanco) Benth.	Combination inhibited the pathogen. No ΣFIC studies conducted.	[141]
ATCC 90028	*Cinnamomum zeylanicum* Blume / *Acorus calamus* L. / *Cinnamomum cassia* (L.) J. Presl / *Boswellia carteri* Birdw. / *Ferula gummosa* Boiss. / *Hyssopus officinalis* L. / *Commiphora myrrha* (T.Nees) Engl. / *Nardostachys jatamansi* (D.Don) DC.	Combination inhibited the pathogen. No ΣFIC studies conducted.	[141]
ATCC 90028	*Cinnamomum zeylanicum* Blume / *Citrus limonum* Risso / *Eucalyptus radiata* Sieber ex DC. / *Rosmarinus officinalis* L. / *Syzygium aromaticum* (L.) Merr. and L.M. Perry	Combination inhibited the pathogen. No ΣFIC studies conducted.	[141]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Cedrus atlantica G.Manetti*/ *Citrus bergamia* Risso and Poit.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cedrus atlantica G.Manetti* / *Juniperus virginiana* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cedrus atlantica G.Manetti* / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cedrus atlantica G.Manetti*/ *Rosmarinus officinalis* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cedrus atlantica G.Manetti*/ *Vetiveria zizanioides* (L.) Nash	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cinnamomum verum* J. Presl / *Boswellia carteri* Birdw.	Indifferent effect ΣFIC = 1.06	[140]
ATCC 10231	*Cinnamomum verum* J. Presl / *Citrus × sinensis* (L.) Osbeck	Indifferent effect ΣFIC = 1.06	[140]
ATCC 10231	*Cinnamomum verum* J. Presl / *Litsea cubeba* (Lour.) Pers.	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Cinnamomum zeylanicum* Garcin ex Blume / *Boswellia carteri* Birdw.	Indifferent effect ΣFIC = 1.25	[140]
ATCC 10231	*Cinnamomum zeylanicum* Garcin Blume / *Citrus sinensis* (L.) Osbeck	Indifferent effect ΣFIC = 1.25	[140]
ATCC 10231	*Cinnamomum zeylanicum* Garcin ex Blume / *Litsea cubeba* (Lour.) Pers.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cinnamomum zeylanicum* Blume / *Boswellia carteri* Birdw.	Indifferent effect ΣFIC = 0.63	[140]
ATCC 10231	*Cinnamomum zeylanicum* Blume / *Citrus sinensis* (L.) Osbeck	Additive effect ΣFIC = 0.78	[140]
ATCC 10231	*Cinnamomum zeylanicum* Garcin ex Blume / *Litsea cubeba* (Lour.) Pers.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Citrus aurantium* L. var. *amara* (flower) / *Citrus bergamia* Risso	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Citrus × aurantium* L. / *Commiphora myrrha* (T.Nees) Engl.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Citrus bergamia* Risso / *Cupressus sempervirens* L.	Additive effect ΣFIC = 1.50	[140]
ATCC 10231	*Citrus bergamia* Risso / *Lavandula angustifolia* Mill.	Additive effect ΣFIC = 3.00	[140]
ATCC 10231	*Citrus limon* (L.) Osbeck / *Cananga odorata* (Lam.) Hook. f. and Thomson	Additive effect ΣFIC = 1.88	[140]
ATCC 10231	*Citrus limon* (L.) Osbeck / *Cymbopogon martinii* (Roxb.) Will. Watson	Additive effect ΣFIC = 0.79	[140]
ATCC 10231	*Citrus limon* (L.) Osbeck / *Styrax benzoin* Dryand.	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Melaleuca alternifolia* Cheel	Synergistic effect ΣFIC = 0.42	[140]
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Pelargonium graveolens* L'Hér.	Additive effect ΣFIC = 1.00	[140]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Pelargonium odoratissimum* (L.) L'Hér.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Citrus limon* (L.) Osbeck / *Juniperus virginiana* L.	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Boswellia carteri* Birdw.	Indifferent effect ΣFIC = 1.13	[140]
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Lavandula angustifolia* Mill.	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Commiphora myrrha* (T.Nees) Engl. / *Pogostemon patchouli* Pellet.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Cymbopogon citratus* (hort. ex DC.) Stapf / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 0.65	[140]
ATCC 10231	*Cymbopogon martinii* (Roxb.) Will. Watson / *Boswellia carteri* Birdw.	Additive effect ΣFIC = 0.69	[140]
ATCC 10231	*Cymbopogon martinii* (Roxb.) Will. Watson / *Cananga odorata* (Lam.) Hook. f. and Thomson	Indifferent effect ΣFIC = 1.75	[140]
ATCC 10231	*Cymbopogon martinii* (Roxb.) Will. Watson / *Pelargonium graveolens* L'Hér.	Additive effect ΣFIC = 0.58	[140]
ATCC 10231	*Coriandrum sativum* L. / *Cymbopogon citratus* (hort ex DC.) Stapf	Additive effect ΣFIC = 0.78	[140]
ATCC 10231	*Cymbopogon martinii* (Roxb.) Will. Watson / *Cedrus atlantica* (Endl.) Manetti ex Carrière	Additive effect ΣFIC = 0.88	[140]
ATCC 10231	*Cymbopogon martinii* (Roxb.) Will. Watson / *Pelargonium odoratissimum* (L.) L'Hér.	Indifferent effect ΣFIC = 1.17	[140]
ATCC 10231	*Eucalyptus globulus* Labill. / *Lavandula angustifolia* Mill.	Indifferent effect ΣFIC = 3.00	[140]
ATCC 10231	*Helichrysum italicum* (Roth) G.Don / *Anthemis nobilis* L.	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Helichrysum italicum* (Roth) G.Don (*immortelle*) / *Matricaria recutita* L.	Additive effect ΣFIC = 0.92	[140]
ATCC 10231	*Kuneza ericoides* (Poir.) Kuntze / *Leptospermum scoparium* J.R.Forst. and G.Forst.	Additive effect	[142]
Clinical strain	*Lavandula binaludensis* Jamzad / *Cuminum cyminum* L.	Combination inhibited the pathogen. No ΣFIC studies conducted.	[109]
ATCC 10231	*Lavandula x intermedia* Emeric ex Loisel. / *Mentha arvensis* L.	Synergistic effect	[143]
ATCC 10231	*Lavandula angustifolia* Mill. / *Abies balsamea* (L.) Mill.	Additive effect ΣFIC = 0.63	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Andropogon muricatus* Retz.	Synergistic effect ΣFIC = 0.45	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Angelica archangelica* L. *(*seed*)*	Additive effect ΣFIC = 0.83	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Angelica archangelica* L. *(*root*)*	Synergy ΣFIC = 0.42	[76]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Lavandula angustifolia* Mill. / *Anthemis nobilis* L	Synergy ΣFIC = 0.33	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Anthemis nobilis* L	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Canarium luzonicum* Miq.	Synergy ΣFIC = 0.25	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cananga odorata* (Lam.) Hook. f. and Thomson (heads)	Additive effect ΣFIC = 0.83	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cananga odorata* (Lam.) Hook. f. and Thomson	Indifferent effect ΣFIC = 1.25	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Carum carvi* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cinnamomum zeylanicum* Blume	Synergy ΣFIC = 0.40	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Citrus aurantium* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Citrus grandis* Osbeck	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Citrus medica limonum* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Citrus × sinensis* (L.) Osbeck	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Commiphora myrrha* (T.Nees) Engl.	Synergy ΣFIC = 0.29	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cupressus sempervirens* L.	Synergy ΣFIC = 0.15	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cymbopogon citratus* (DC.) Stapf	Antagonistic effect ΣFIC = 6.67	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Juniperus virginiana* L. (berries)	Synergy ΣFIC = 0.21	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Cymbopogon nardus* (L.) Rendle	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Daucus carota* L.	Synergy ΣFIC = 0.50	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Eucalyptus globulus* Labill.	Synergy ΣFIC = 0.38	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Foeniculum dulce* Mill.	Synergy ΣFIC = 0.45	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Hyssopus officinalis* L.	Synergy ΣFIC = 0.33	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Juniperus virginiana* L.	Synergy ΣFIC = 0.50	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Laurus nobilis* L.	Additive effect ΣFIC = 0.83	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Litsea cubeba* (Lour.) Pers.	Synergy ΣFIC = 0.19	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Matricaria chamomilla* L.	Indifferent effect ΣFIC = 1.17	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Melaleuca alternifolia* Cheel	Synergy ΣFIC = 0.50	[76]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Lavandula angustifolia* Mill. / *Melaleuca viridiflora* Sol. ex Gaertn.	Additive effect ΣFIC = 0.90	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Mentha piperita* L.	Additive effect ΣFIC = 0.63	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Myrtus communis* L.	Synergy ΣFIC = 0.50	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Ocimum basilicum* L.	Additive effect ΣFIC = 0.67	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Origanum majorana* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Pelargonium odoratissimum* (L.) L'Hér.	Indifferent effect ΣFIC = 1.04	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Pinus sylvestris* L.	Synergy ΣFIC = 0.50	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Piper nigrum* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia/ Pogostemon patchouli*	Synergy ΣFIC = 0.50	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Pogostemon patchouli* Pellet.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 0.73	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Santalum album* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Styrax benzoin* Dryand.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Syzygium aromaticum* (L.) Merr. & L.M. Perry	Additive effect ΣFIC = 0.58	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Tagetes patula* L.	Synergy ΣFIC = 0.42	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Thymus vulgaris* L.	Additive effect ΣFIC = 0.67	[76]
ATCC 10231	*Lavandula angustifolia* Mill. / *Allium sativum* L.	Indifferent effect ΣFIC = 1.25	[140]
ATCC 10231	*Lavandula angustifolia* Mill. / *Anthemis nobilis* L.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Lavandula angustifolia* Mill. / *Matricaria recutita* L.	Indifferent effect ΣFIC = 1.17	[140]
ATCC 10231	*Lavandula angustifolia* Mill. / *Melaleuca alternifolia* Cheel	Additive effect ΣFIC = 0.83	[140]
ATCC 10231	*Leptospermum petersonii* (F.Muell.) Mabb. / *Kuneza ericoides* (A.Cunn.) T.G.Hartley	Additive effect	[142]
ATCC 10231	*Leptospermum petersonii* (F.Muell.) Mabb. / *Leptospermum scoparium* J.R.Forst. and G.Forst.	Additive effect	[142]
ATCC 10231	*Leptospermum scoparium* J.R.Forst. and G.Forst. / *Commiphora myrrha* (T.Nees) Engl.	Additive effect ΣFIC = 0.75	[140]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Leptospermum scoparium* J.R.Forst. and G.Forst. / *Lavandula angustifolia* Mill.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Matricaria recutita* L. / *Citrus aurantium* L. var. *amara* flower	Indifferent effect ΣFIC = 1.17	[140]
ATCC 10231	*Melaleuca alternifolia* Cheel / *Citrus limon* (L.)	Additive effect ΣFIC = 0.57	[140]
ATCC 10231	*Melaleuca alternifolia* Cheel / *Rosmarinus officinalis* L.	Indifferent effect ΣFIC = 3.33	[140]
ATCC 10231	*Melaleuca alternifolia* Cheel / *Santalum album* L.	Indifferent effect ΣFIC = 2.50	[140]
ATCC 10231	*Melaleuca alternifolia* Cheel / *Santalum austrocaledonicum* (Dunal) A.DC.	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Melissa officinalis* L. / *Pelargonium odoratissimum* (L.) L'Hér.	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Melissa officinalis* L. / *Pelargonium graveolens* L'Hér.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Nardostachys jatamansi* (D.Don) DC. / *Citrus reticulata* Blanco	Additive effect ΣFIC = 0.88	[140]
ATCC 10231	*Origanum vulgare* L. / *Commiphora myrrha* (T.Nees) Engl.	Additive effect ΣFIC = 0.92	[140]
ATCC 10231	*Origanum vulgare* L. / *Pelargonium graveolens* L'Hér.	Indifferent effect ΣFIC = 1.38	[140]
ATCC 10231	*Origanum vulgare* L. / *Pelargonium odoratissimum* (L.) L'Hér.	Additive effect ΣFIC =0.92	[140]
ATCC 10231	*Origanum vulgare* L. / *Pinus sylvestris* L	Additive effect ΣFIC = 0.92	[140]
ATCC 10231	*Origanum vulgare* L. / *Rosmarinus officinalis* L.	Indifferent effect ΣFIC = 1.38	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Citrus aurantium* L. var. *amara* flower	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Citrus bergamia* Risso and Poit.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Citrus limon* (L.)	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Citrus paradisi* Macfad.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Citrus sinensis* (L.) Osbeck	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Juniperus virginiana* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Lavandula angustifolia* Mill.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Santalum album* L.	Synergy ΣFIC = 0.50	[140]
ATCC 10231	*Pelargonium graveolens* L'Hér. / *Santalum austrocaledonicum* (Dunal) A.DC.	Synergistic effect ΣFIC = 0.38	[140]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Citrus aurantium* L	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Citrus bergamia* Risso and Poit.	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Citrus limon* (L.)	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Citrus paradisi* Macfad.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Citrus sinensis* (L.) Osbeck	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Juniperus virginiana* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Lavandula angustifolia* Mill.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Santalum album* L.	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Pelargonium odoratissimum* (L.) L'Hér. / *Santalum austrocaledonicum* (Dunal) A.DC.	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Pogostemon patchouli* (L.) Benth. / *Cedrus atlantica* (Endl.) Manetti	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Pogostemon patchouli* (L.) Benth. / *Citrus bergamia* Risso and Poit.	Indifferent effect ΣFIC = 2.00 [161]	[140]
ATCC 10231	*Pogostemon patchouli* (L.) Benth. / *Pelargonium graveolens* L'Hér.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Pogostemon patchouli* (L.) Benth. / *Pelargonium odoratissimum* (L.) L'Hér.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum album* L. / *Boswellia carteri* (Oliv.) Engl.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Santalum album* L. / *Cananga odorata* (Lam.) Hook.f. and Thomson	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Santalum album* L. / *Citrus aurantium* L. var. *amara* flower	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum album* L. / *Citrus bergamia* Risso and Poit.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum album* L. / *Citrus limon* (L.)	Synergy ΣFIC = 0.63	[140]
ATCC 10231	*Santalum album* L. / *Commiphora myrrha* (T.Nees) Engl.	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Santalum album* L. / *Cymbopogon martinii* (Roxb.) Wats.	Indifferent effect ΣFIC = 1.17	[140]
ATCC 10231	*Santalum album* L. / *Lavandula angustifolia* Mill.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Santalum album* L. / *Melaleuca alternifolia* Cheel	Synergy ΣFIC = 0.83	[140]
ATCC 10231	*Santalum album* L./ *Piper nigrum* L.	Indifferent effect ΣFIC = 1.50	[140]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Santalum album* L. / *Piper nigrum* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum album* L. / *Pogostemon patchouli* (L.) Benth.	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Santalum album* L. / *Styrax benzoin* Dryand.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Santalum album* L. / *Vetiveria zizanioides* Stapf.	Synergy ΣFIC = 0.38	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Boswellia carteri* Birdw.	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Cananga odorata* (Lam.) Hook.f. & Thomson	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill./ *Citrus aurantium* L.	Synergy ΣFIC = 0.88	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Citrus bergamia* Risso and Poit.	Synergy ΣFIC = 0.75	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Citrus limon* (L.) Osbeck	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Commiphora myrrha* (Nees) Engl.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Citrus limon* (L.)	Synergy ΣFIC = 0.58	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Commiphora myrrha* (T.Nees) Engl.	Additive effect ΣFIC = 0.75	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Cymbopogon martinii* (Roxb.) Wats.	Indifferent effect ΣFIC = 1.25	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Piper nigrum* L.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Pogostemon patchouli* (L.) Benth.	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Styrax benzoin* Dryand.	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Santalum austrocaledonicum* Vieill. / *Vetiveria zizanioides* (L.) Nash	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Styrax benzoin* Dryand. / *Lavandula angustifolia* Mill.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Syzygium aromaticum* (L.) Merr. &L.M. Perry / *Cinnamomum verum* J. Presl	Additive effect ΣFIC = 0.63	[140]
ATCC 10231	*Syzygium aromaticum* (L.) Merr. & L.M. Perry / *Cinnamomum zeylanicum* Garcin ex Blume	Indifferent effect ΣFIC = 2.00	[140]
ATCC 10231	*Syzygium aromaticum* (L.) Merr. & L.M. Perry / *Cinnamomum zeylanicum* Garcin ex Blume	Additive effect ΣFIC = 0.80	[140]
ATCC 10231	*Syzygium aromaticum* (L.) Merr. & L.M. Perry / *Citrus bergamia* Risso .	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Syzygium aromaticum* (L.) Merr. & L.M. Perry / *Citrus limon* (L.) Osbeck	Indifferent effect ΣFIC = 1.13	[140]

Strain number	Essential oil blends	Results	Ref.
ATCC 10231	*Syzygium aromaticum* (L.) Merr. and L.M. Perry / *Lavandula angustifolia* Mill.	Indifferent effect ΣFIC = 1.50	[140]
ATCC 10231	*Thymus vulgaris* L. / *Citrus bergamia* Risso	Additive effect ΣFIC = 1.00	[140]
ATCC 10231	*Thymus vulgaris* L. / *Citrus limon* (L.) Osbeck	Additive effect ΣFIC = 0.47	[140]
ATCC 10231	*Thymus vulgaris* L. / *Rosmarinus officinalis* L.	Additive effect ΣFIC = 1.00	[140]

[a]MIC- Minimum Inhibitory Concentration; [b]MBC-Minimum Bactericidal Concentration; [c]ΣFIC Fractional Inhibitory Concentration where the interaction classification is based on recommended criteria from literature[77]

Essential oil combination studies were predominantly tested against *E. faecalis* and *C. albicans*. There is thus a plethora of vaginal pathogens (Table 1), for which little to no research is available. Out of the combinations studied against *E. faecalis*, 50% were indifferent to the pathogen, while 50% were found to have an additive effect. Regarding *C. albicans*, 50% of combinations tested had an additive effect, 25% had an indifferent interaction, 22% had a synergistic effect and only 1% was antagonistic.

Table 8 compiles studies on essential oil combinations against vaginal pathogens, primarily *E. faecalis* and *C. albicans*. Similar to the single-oil studies, the MIC, MBC, and ΣFIC values reported are predominantly *in vitro*. Achievable concentrations *in vivo* may be lower than those tested, and interactions between oils may vary depending on formulation, route of administration, and exposure time. While additive and synergistic effects were observed in several combinations (e.g., *Lavandula angustifolia* with multiple oils), some combinations showed indifferent or even antagonistic interactions. Consequently, clinical applicability remains uncertain. Further research integrating safe dosing, local pharmacodynamics, and *in vivo* efficacy is needed to validate these combinations for therapeutic use.

When attempting to collate the scientific validation studies with the recommended essential oil combinations in the layman's aromatherapeutic literature, it was found that there were numerous barriers. Firstly, common names as opposed to scientific names are mainly used within the layman's literature. While the scientific literature is inclusive of common names, there are often references to essential oils bearing the same scientific name and common name, yet the derivation of the oil is from a different source or part of the plant (including flowers, leaves, bark, stems and roots). For example, in the case of a study conducted by de Rapper et al., 2013 two types of *Angelica archangelica* essential oil were combined with *Lavandula angustifolia*, one is obtained

from the seed and the other from the root. These challenges present difficulty in correlating the scientific data with the aromatherapeutic literature. A call for the harmonisation and collaboration between aromatherapists and scientific researchers is in need to develope an evidenced-based database of essential oils used in combination.

The effect of essential oils on the vaginal microbiota

Studies based on the effect of essential oils on the vaginal microbiota were compiled and summarized in Table 9. The optimal outcome is observing little to no inhibition on the *Lactobacillus* spp. as this is indicative of the preservation of the vaginal microbiome. An overview of the results as presented in Table 9. The results have been summarized in Figure 1.

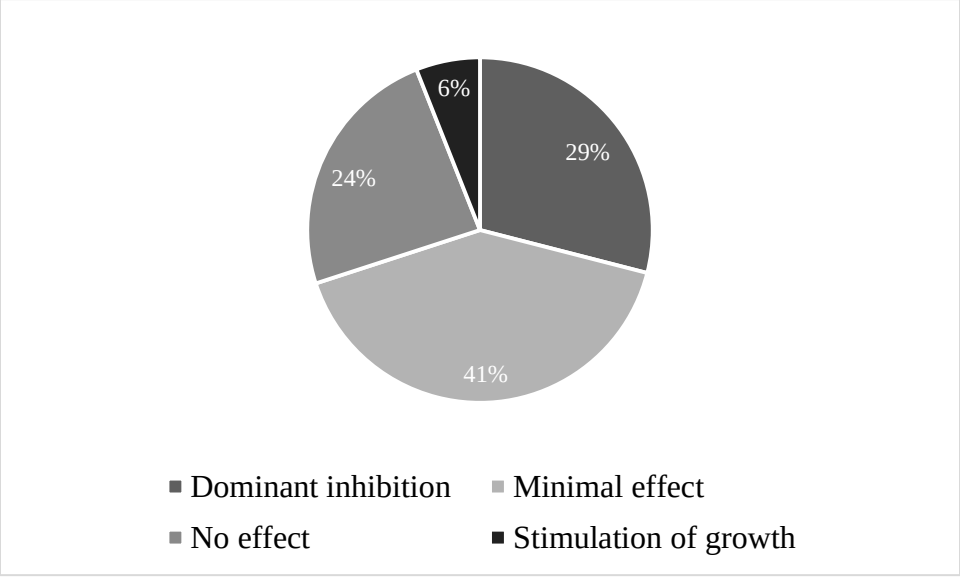

Figure 1. Summary of the effect of essential oils on the vaginal microbiota

Most studies ultimately reveal that a positive effect on the vaginal microbiome was observed (Table 2.9). There are limited studies where all the relevant microbiota are considered for example, certain *Lactobacillus* spp. such as *L. gasseri, L. crsipatus, L. jensenii* have been neglected despite their role in the maintenance of the vaginal ecosystem.

Table 2.9. Effect of essential oils on the vaginal microbiota

Strain number	Essential oil	Results	Reference
		Lactobacillus rhamnosus	
ATCC 53103	*Ammoides verticillata* (Desf.) Briq.	MIC[a]= 0.4 (±) 0.1 µl/ml	[136]
CNCM I-4036	*Cinnamomum zeylanicum* Garcin ex Blume	No inhibitory effect at concentrations of 0.01% and 0.001%	[136]
ATCC 9595		MIC= 0.025%	
ATCC 7469	*Cuminum cyminum* Wall.	No inhibition of *Lactobacillus* spp.	[144]
ATCC 7469	*Eucalyptus globulus* Labill.	Selective antibacterial activity with little to no effect on *Lactobacillus* MIC= 14.8 mg/ml	[138]
ATCC 7469	*Mentha longifolia* (L.) Huds.	MIC = 80 µg/µl MBC[b] = 120 µg/µl	[145]
ATCC 9595	*Malaleuca alternifolia* (Maiden and Betche) Cheel	MIC= 3977.3 µg/µl MIC = 300 µg/µl	[146]
ATCC 9595	*Mentha piperita* L.	Inhibition at a concentration of 0.4%	[147]
ATCC 9595	*Syzygium aromaticum* (L.) Merr. and L.M. Perry (Myrtaceae)	Inhibition at a concentration of 0.2%	[147]
ATCC 9595	*Satureja candidissima* (Poir.) Greuter and Burdet.	MIC = 3.1 ± 0.6 µl/ml	[136]
CNCM I-4036	*Thymus vulgaris* L.	No inhibitory effect at concentrations of 0.01% and 0.001%	[148]
		Lactobacillus acidophilus	
ATCC 53103	*Amygdalus communis* L.	MIC= 0.5 µl/ml	[149]
ATCC 53103	*Azadirachta indica* A. Juss.	*Lactobacillus* spp. inhibited by essential oil MIC= 0.125 µl/ml	[149]
NCTC 1723	*Bidens pilosa* f. *alausensis* Sherff	Limited inhibition on *Lactobacillus* spp.	[150]
ATCC 53103	*Citrus limon* (L.) Osbeck	MIC= 0.25 µl/ml	[149]
ATCC 314	*Echinacea purpurea* (L.) Moench	MIC = 5.86 µl/ml	[151]
ATCC 53103	*Olea europaea* L.	MIC= 0.25 µl/ml	[149]
Clinical strain*	*Origanum vulgare* L.	Delay in the growth but no inhibition on existing *Lactobacillus* spp.	
ATCC 53103	*Psoralea corylifolia* L.	MIC= 0.125 µl/ml	[149]
Clinical strain	*Rosmarinus officinalis* L.	Delay in the growth but not inhibition on existing *Lactobacillus* spp.	
KS400	*Thymbra capitata* Griseb.	*Lactobacillus* spp. demonstrated higher levels of tolerance than *G. vaginalis*, i.e. less inhibition of *Lactobacillus* spp. MIC= 2.50 µl/ml	[63]
ATCC 53103	*Trachiyspirum ammi* Sprague	MIC= 0.125 µl/ml	[149]
ATCC 314	*Zataria multiflora* Boiss.	MIC= 0.08 µl/ml	[151]
		Lactobacillus fermentum	
ATCC 14931	*Citrus* × *sinensis* (L.) Osbeck	Stimulates growth of *L. fermentum*	[55]
ATCC 14931	*Origanum vulgare* L.	Stimulates growth of *L. fermentum*	[55]

Strain number	Essential oil	Results	Reference
ATCC 9338	*Thymus vulgaris* L.	No inhibition observed	[55, 152]
ATCC 14931		Stimulates growth of *L. fermentum*	
		Lactobacillus plantarum	
Lp3547	*Cinnamomum zeylanicum* Garcin ex Blume	No inhibition at 0.01 and 0.001%	[148]
Lp3547	*Syzygium aromaticum* (L.) Merr. and L.M. Perry	No inhibition at 0.01 and 0.001%	[148]
Clinical strain	*Cuminum cyminum* Wall.	Stimulated growth at 0.5, 1.0 and 2.0%	[153]
ATCC 8014	*Citrus paridisi* Macfad.	No inhibition	[154]
ATCC 8014	*Citrus lemon* (L.) Osbeck	No inhibition	[154]
Clinical strain	*Satureja bachtiarica* Bunge	MIC = 25 µl/ml	[155]
Clinical strain	*Satureja Khuzestanica* Jamzad	MIC = 12.5 µl/ml	[155]
Clinical strain	*Satureja bachtiarica* Bunge with *Satureja Khuzestanica* Jamzad	ΣFIC = 0.75	[155]
Lp3547	*Syzygium aromaticum* (L.) Merr. and L.M. Perry	No inhibition at 0.01 and 0.001%	[148]
ATCC 8014	*Thymbra capitata* Griseb.*Thymbra capitata* Griseb.	MIC= 2.50 µl/ml	[63]
(Valdivieso-Ugarte et al., 2021)	*Thymus vulgaris* L.	No inhibition at 0.01 and 0.001%	[148]
		Lactobacillus reuteri	
ATCC 23272	*Origanum vulgare* L.	No inhibition observed	[156]
ATCC 23272	*Rosmarinus officinalis* L.	No inhibition observed	[55]
ATCC 23272	*Thymus vulgaris* L.	No inhibition observed	[55]
DSM20016	*Thymbra capitata* Griseb.	MIC= 2.50 µl/ml	[63]
		Lactobacillus crispatus	
KS116.1	*Thymbra capitata* Griseb.	MIC = 2.50 µl/ml	[63]
		Lactobacillus gasseri	
KS114.1	*Thymbra capitata* Griseb.	MIC = 2.50 µl/ml	[63]
		Lactobacillus jensii	
KS119.1	*Thymbra capitata* Griseb.	MIC = 1.25 µl/ml	[63]

[a]MIC- Minimum Inhibitory Concentration

Only 17% of essential oils that were recommended by the aroma-therapeutic layman could be correlated with scientific literature. However, most, to the best of our knowledge, reported a degree of inhibition on the natural microbial colonizers. There has been an interest regarding essential oils such as *Thymus vulgaris* and *Origanum vulgare* that demonstrated the ability to stimulate the growth of *L. fermentum*. However, there are limited studies that examine how this phenomenon occurs. Another challenge is that there are limited studies that consider the effects of essential oils in combination against various *Lactobacillus* spp., despite essential oils being utilized

as blends in common practice.

Although essential oils exhibit promising antimicrobial activity *in vitro*, their use in sensitive mucosal environments, such as the vaginal mucosa, requires careful evaluation due to potential safety concerns. Oils containing phenolic or terpene compounds, such as thymol, carvacrol, or 1,8-cineole, can cause local irritation, allergic reactions, or mucosal disruption at concentrations close to their minimum inhibitory concentrations (MICs)[73,163]. *In vivo* studies and limited clinical trials indicate that, when properly diluted in suitable carriers such as gels or emulsions, essential oils are generally well tolerated, though adverse effects such as burning, erythema, or mild discomfort may occur at higher concentrations[164,165]. Systemic absorption from topical vaginal application is typically minimal, but repeated or high-dose exposure could increase risk, particularly in sensitive populations. These findings highlight that, while essential oils hold potential as adjunct or alternative therapies for vaginal infections, careful attention to formulation, dose optimization, and rigorous safety evaluation is essential before clinical use.

The effect of essential oils on vaginal biofilms

The effect of essential oils on biofilms is often interpreted by the minimum concentrations of the essential oil required to reduce the biofilm cell formation by 50% ($MBIC_{50}$) (Wijesinghe et al., 2021). Biofilm formation is a contributor towards resistance in vaginal infection, however, limited studies (Table 2.10) focused on neglected pathogens such as *G. vaginalis*, which are heavily implicated in biofilm formation for BV and VVC. More commonly studied was *Candida* spp. The majority of studies (69%) report a significant reduction in biofilm formation and demonstrate notable antibiofilm activity (81%); however, there is a limited number of available biofilm studies on neglected pathogens associated with the vaginal canal. Furthermore, only 28% of aromatherapeutic recommendations have been scientifically evaluated in biofilm studies.

Table 2.10. Effect of essential oils on biofilms associated with the vaginal canal

Pathogen	Essential Oil	Results	Reference
Bacterial vaginosis (BV)			
Gardnerella vaginalis- Clinical strain	*Thymbra capitata* Griseb.	49-91% biofilm mass reduction	[63]
Vulvovaginal candidiasis (VVC)			
C. albicans ATCC 10231 and other isolates	*Foeniculum vulgare* Mill.	For 34 strains, concentrations ranging between 6.25% and 25% there was a 50% biofilm reduction.	[104]
C. tropicalis- Clinical strain	*Cymbopogon citratus* (hort. ex DC.) Stapf,	Resulted in a 45-76% reduction in biofilm formation.	[157]
C. tropicalis- Clinical strain	*Cuminum cyminum* Wall.	≤45% reduction	[157]
C. tropicalis- Clinical strain	*Citrus limon* (L.) Burm.f.	≤45% reduction	[157]
C. tropicalis- Clinical strain	*Cinnamomum verum* J. Presl	≤45% reduction	[157]
C. albicans- Clinical strain	*Cuminum cyminum* Wall.	A biofilm reduction of 48.8% was observed.	[158]
C. albicans- Clinical strain	*Lavandula angustifolia* L.	A biofilm reduction of 42.5% was observed.	[158]
C. albicans- Clinical strain	*Ziziphora tenuior* Falk.	A biofilm reduction of 49.3% was observed.	[158]
C. albicans- Clinical strain	*Cymbopogon citratus* (hort. ex DC.) Stapf	Anti-biofilm activity at 0.5× MIC.	[159]
C. albicans, C. glabrata, C. guilliermondii, C. krusei, C. parapsilosis, and *C. tropicalis*- Clinical isolates, spp. identified	*Melissa officinalis* L., *Origanum vulgare* L. *Lavandula stoechas* L. *Mentha* × *piperita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., and *Thymus vulgaris* L.	*Origanum vulgare* and *Thymus vulgaris* essential oils inhibited biofilm growth by about 90%. *Lavandula stoechas, Mentha* × *piperita*, and *Rosmarinus officinalis* oils inhibited biofilm growth by 75-85%. The weakest antibiofilm activity was observed with lemon balm and *Salvia officinalis* oils, which destroy only 60-70% of biofilm.	[110]
C. albicans- Clinical strain	*Melissa officinalis* L.	Reduced up to 95.36% of the biofilm.	[160]
Candida isolates- Clinical strain	*Origanum vulgare* L.	Reduction of 1 to 5 orders of magnitude (Log CFU/cm^2).	[161]
Clinical strain	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Inhibition at ≤ 0.5× MIC	[159]
Clinical strain- *Candida* isolates	*Thymus vulgaris* L.	Reduction of 0.2 to 2 orders of magnitude (Log CFU[a]/cm^2).	[161]

[a]CFU-Colony forming units

CONCLUSION

Figure 2 provides a comprehensive overview of essential oils with antimicrobial effects on the relevant vaginal pathogens. Among the most studied pathogens were *E. faecalis* and *C. albicans*, likely due to their implication in a variety of infections beyond vaginal conditions. Although

several pathogens have been previously discussed, it is evident that many have been largely neglected in the context of essential oil studies. This is a critical gap, as several of these pathogens are implicated in polymicrobial infections, which can disseminate and result in severe complications [162].

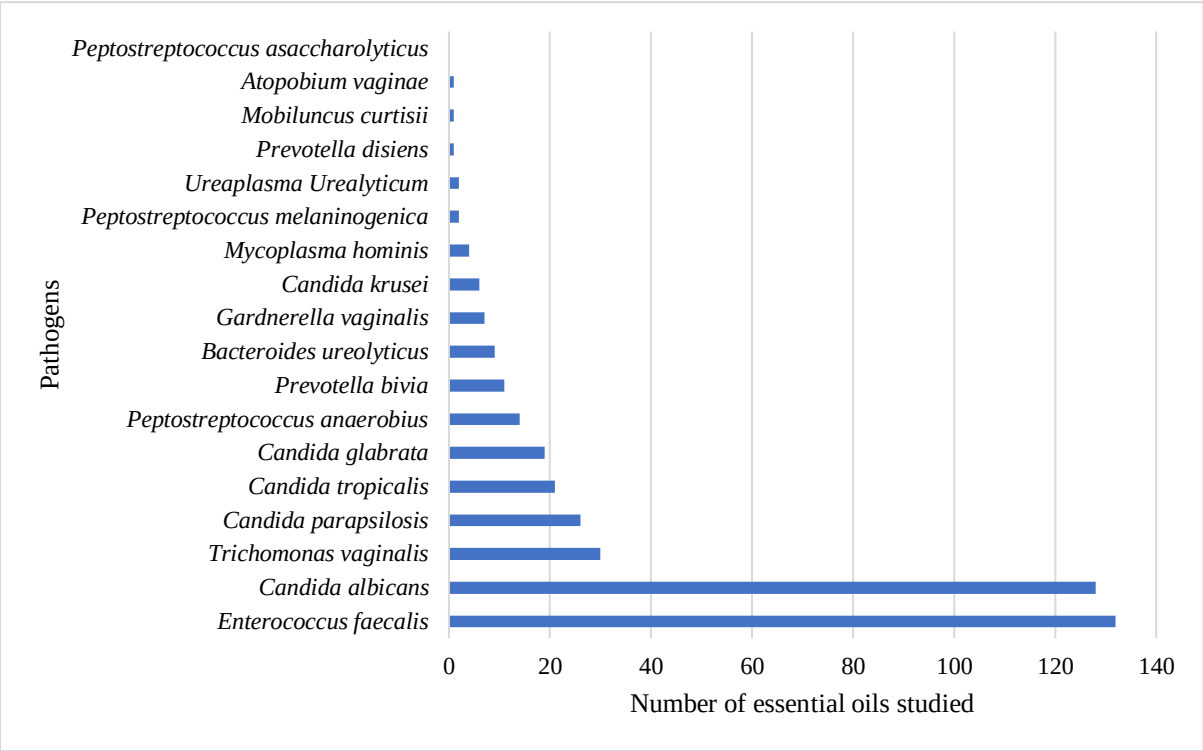

Figure 2. Number of essential oils that have an antimicrobial effect on vaginal pathogens

Only 14% of essential oil recommendations have been evaluated against the pathogens associated with BV, VVC, and TV. A closer examination reveals a significant disparity in the number of studies on *Trichomonas vaginalis*, in contrast to the larger body of work on *C. albicans* (implicated in VVC) and *E. faecalis* (implicated in BV). There is also a disparity between the number of studies on *C. albicans* compared to non-*C. albicans* spp. and this demonstrates that despite the dire implications of non-*C. albicans* spp. in vaginal infections, there is not enough scientific validation that correlates with the aroma-therapeutic recommendations. Consequently, there is a lack of studies that comprehensively address the effects of essential oils on parasites.

Notably, *Origanum vulgare* has demonstrated broad-spectrum antimicrobial properties, exhibiting antibacterial, antifungal, antiparasitic, and antibiofilm activities[73,133]. Similarly, *Melaleuca alternifolia* shows these properties, but the specificity of its effects on different

Lactobacillus spp. remains unclear[85]. In contrast, *Origanum vulgare* appears to have minimal interference with most *Lactobacillus* spp., except *Lactobacillus plantarum*[153.] Interestingly, it has displayed the ability to stimulate the growth of *Lactobacillus fermentum*, which may confer beneficial effects[55]. However, comprehensive studies on the impact of essential oils on the full spectrum of *Lactobacillus* spp. are lacking, with many spp. receiving limited investigation (Table 2). The vaginal microbiome should also be a main area of focus in future studies.

In addition to the under-representation of certain pathogens, only 70% of essential oil recommendations have been studied concerning BV and VVC, and merely 26% against TV. While some studies have explored the effects of essential oil combinations, these account for only 14%, with challenges in data collation arising from the frequent use of common names in the layman's aromatherapeutic literature, which lacks botanical specificity. Furthermore, the potential toxicity of essential oils is often presumed but inadequately investigated, necessitating their consideration in future studies.

Research on the antibiofilm activity of essential oils is also severely limited. Although some essential oils display promising antibiofilm potential, the small number of studies restricts the ability to draw definitive conclusions about their overall efficacy.

Essential oils offer promising potential for the development of novel adjuvants in the treatment of vaginal infections. However, extensive clinical studies are required to generate the evidence-based data necessary for their application. The majority of the cited studies are *in vitro*, which presents substantial limitations for clinical translation. Issues such as bioavailability, formulation design, and the variability in essential oil composition must be considered, as they may significantly alter therapeutic outcomes. Moreover, essential oils are often assessed individually *in vitro*, whereas in practice they may be applied as blends or formulated products, which could modify their activity. A concerted effort to align aromatherapeutic literature with rigorous scientific data is crucial. Collaborative efforts among researchers, healthcare professionals, and aromatherapists will be essential in developing a systematic and reliable database for future research and clinical use.

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DATA AVAILABILITY STATEMENT

Data is available on request.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

Supplementary data: Fig. S1 and Table S1 are provided as supplementary data.

References

1. **Aubyn, G.B. and Tagoe, D.N.A. (2013).** Prevalence of vaginal infections and associated lifestyles of students in the University of Cape Coast, Ghana. Asian Pacific Journal of Tropical Disease, 3(4): 267-270.
2. **Salinas, A.M., Osorio, V.G., Pacha-Herrera, D., Vivanco, J.S., Trueba, A.F., and Machado, A. (2020).** Vaginal microbiota evaluation and prevalence of key pathogens in Ecuadorian women: An epidemiologic analysis. Scientific Reports, 10(1): 18358-18358.
3. **Bitew, A. and Abebaw, Y. (2018).** Vulvovaginal candidiasis: species distribution of *Candida* and their antifungal susceptibility pattern. BMC Women's Health, 18(1): 94-94.
4. **Abdul-Aziz, M., Mahdy, M.A.K., Abdul-Ghani, R., Alhilali, N.A., Al-Mujahed, L.K.A., Alabsi, S.A., Al-Shawish, F.A.M., Alsarari, N.J.M., Bamashmos, W., Abdulwali, S.J.H., Al Karawani, M., and Almikhlafy, A.A. (2019).** Bacterial vaginosis, vulvovaginal candidiasis and trichomonal vaginitis among reproductive-aged women seeking primary healthcare in Sana'a city, Yemen. BMC Infectious Diseases, 19(1): 879-879.

5. **Redelinghuys, M.J., Geldenhuys, J., Jung, H., and Kock, M.M. (2020).** Bacterial vaginosis: Current diagnostic avenues and future opportunities. Frontiers in Cellular and Infection Microbiology, 11(10): 354.
6. **Bagnall, P. and Rizzolo, D. (2017).** Bacterial vaginosis: A practical review. Journal of the American Academy of Physician Assistants, 30(12): 15-21.
7. **Muzny, C.A., Łaniewski, P., Schwebke, J.R., and Herbst-Kralovetz, M.M. (2020).** Host–vaginal microbiota interactions in the pathogenesis of bacterial vaginosis. Current Opinion in Infectious Diseases, 33(1): 59-65.
8. **Onderdonk, A.B., Delaney, M.L., and Fichorova, R.N. (2016).** The Human Microbiome during bacterial vaginosis. Clinical Microbiology Reviews, 29(2): 223-238.
9. **Coleman, J.S. and Gaydos, C.A. (2018).** Molecular diagnosis of bacterial vaginosis: An update. Journal of Clinical Microbiology, 56(9): 10-128.
10. **Chen, X., Lu, Y., Chen, T., and Li, R. (2021).** The female vaginal microbiome in health and bacterial vaginosis. Frontiers in Cellular and Infection Microbiology, 11: 631972.
11. **Abou Chacra, L., Fenollar, F., and Diop, K. (2022).** Bacterial vaginosis: What do we currently know? Frontiers in Cellular and Infection Microbiology. Frontiers Media S.A, 11:672429
12. **Dovnik, A., Golle, A., Novak, D., Arko, D., and Takač, I. (2015).** Treatment of vulvovaginal candidiasis: A review of the literature. Acta Dermatovenerologica Alpina Pannonica et Adriatica, 24(1):5-7.
13. **Aniebue, U.U., Nwankwo, T.O., and Nwafor, M.I. (2018).** Vulvovaginal candidiasis in reproductive age women in Enugu Nigeria, clinical versus laboratory-assisted diagnosis. Nigerian Journal of Clinical Practice, 21(8): 1017-1017.
14. **Willems, H.M.E., Ahmed, S.S., Liu, J., Xu, Z., and Peters, B.M. (2020).** Vulvovaginal candidiasis: A current understanding and burning questions. Journal of Fungi. 2020, 6(1): 2.
15. **Farr, A., Effendy, I., Frey Tirri, B., Hof, H., Mayser, P., Petricevic, L., Ruhnke, M., Schaller, M., Schaefer, A.P.A., Sustr, V., Willinger, B., and Mendling, W. (2021).** Guideline: Vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses, 64(6): 583-602.

16. **Palmeira-de-Oliveira, R., Palmeira-de-Oliveira, A., and Martinez-de-Oliveira, J. (2015).** New strategies for local treatment of vaginal infections. Advanced Drug Delivery Reviews, 92: 105-122.
17. **Bradshaw, C.S., Tabrizi, S.N., Fairley, C.K., Morton, A.N., Rudland, E., and Garland, S.M. (2006).** The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. The Journal of Infectious Diseases, 194(6):828-836.
18. **Mastromarino, P., Vitali, B., and Mosca, L. (2013).** Bacterial vaginosis: a review on clinical trials with probiotics. The new microbiologica, 36(3): 229-238.
19. **Nayagam, A.T., Smith, M.D., Ridgway, G.L., Allason-Jones, E., Robinson, A.J., and Stock, J. (1992).** Comparison of ofloxacin and metronidazole for the treatment of bacterial vaginosis. International Journal of STD & AIDS, 3(3): 204-207.
20. **Forsum, U., Holst, E., Larsson, P.G., Vasquez, A., Jakobsson, T., and Mattsby-Baltzer, I. (2005).** Bacterial vaginosis - A microbiological and immunological enigma. Review article. APMIS, 113(2): 81-90.
21. **Javed, A., Parvaiz, F., and Manzoor, S. (2019).** Bacterial vaginosis: An insight into the prevalence, alternative treatments regimen and it's associated resistance patterns. Microbial Pathogenesis, 127: 21-30.
22. **Serretiello, E., Santella, B., Folliero, V., Iervolino, D., Santoro, E., Manente, R., Dell'Annunziata, F., Sperlongano, R., Crudele, V., De Filippis, A., Galdiero, M., Franci, G., and Boccia, G. (2021).** Prevalence and antibiotic resistance profile of bacterial pathogens in aerobic vaginitis: A retrospective study in Italy. Antibiotics, 10(9): 1133-1133.
23. **Sobel, J.D. (2000).** Bacterial Vaginosis. Annual Review of Medicine, 51(1): 349-356.
24. **Citron, D.M., Tyrrell, K.L., Warren, Y.A., Fernandez, H., Merriam, C.V., and Goldstein, E.J.C. (2005).** *In vitro* activities of tinidazole and metronidazole against *Clostridium difficile*, *Prevotella bivia* and *Bacteroides fragilis*. Anaerobe, 11(6): 315-317.
25. **Spiegel, C.A. (1991).** Bacterial vaginosis. Clinical Microbiology Reviews, 4(4): 485-502.

26. **Austin, M.N., Beigi, R.H., Meyn, L.A., and Hillier, S.L. (2005).** Microbiologic response to treatment of bacterial vaginosis with topical clindamycin or metronidazole. Journal of Clinical Microbiology, 43(9): 4492-4497.
27. **Hili, P. (2001).** The Antimicrobial Properties of Essential Oils. Winter Press: Kent, United Kingdom.
28. **Hunjak, B., Sabol, I., Vojnović, G., Fistonić, I., Erceg, A.B., Peršić, Z., and Grce, M. (2014).** *Ureaplasma urealyticum* and *Ureaplasma parvum* in women of reproductive age. Archives of Gynecology and Obstetrics, 289(2): 407-412.
29. **Rex, J.H., Walsh, T.J., Sobel, J.D., Filler, S.G., Pappas, P.G., Dismukes, W.E., and Edwards, J.E. (2000).** Practice guidelines for the treatment of candidiasis. Clinical Infectious Diseases, 30(4): 662-678.
30. **Silva, R.A.d., Antonieti, F.M.P.M., Röder, D.V.D.d.B., and Pedroso, R.d.S. (2021).** Essential Oils of *Melaleuca*, *Citrus*, *Cupressus*, and *Litsea* for the management of infections caused by candida species: A systematic review. Pharmaceutics, 13(10): 1700-1700.
31. **Pekmezovic, M., Kaune, A.-K., Austermeier, S., Hitzler, S.U.J., Mogavero, S., Hovhannisyan, H., Gabaldón, T., Gresnigt, M.S., and Hube, B. (2021).** Human albumin enhances the pathogenic potential of *Candida glabrata* on vaginal epithelial cells. PLOS Pathogens, 17(10): e1010037-e1010037.
32. **Coudray, M.S. and Madhivanan, P. (2020).** Bacterial vaginosis—A brief synopsis of the literature. European Journal of Obstetrics & Gynecology and Reproductive Biology, 245: 143-148.
33. **Arastehfar, A., Gabaldón, T., Garcia-Rubio, R., Jenks, J.D., Hoenigl, M., Salzer, H.J.F., Ilkit, M., Lass-Flörl, C., and Perlin, D.S. (2020).** Drug-resistant fungi: An emerging challenge threatening our limited antifungal armamentarium, in Antibiotics, 9(12):877.
34. **Surain, M.P., Aggarwal, N., Dhiman, R., and Kumar, V. (2016).** *Santalum album*: clinical aspects for treatment of *Candida* infections. International Journal of Pharmaceutical Sciences and Research, 7: 2813-2829.

35. **Yassin, M.T., Mostafa, A.A., Al-Askar, A.A., and Bdeer, R. (2020).** *In vitro* antifungal resistance profile of *Candida* strains isolated from Saudi women suffering from vulvovaginitis. European Journal of Medical Research, 25(1): 1-9.
36. **da Silva, R.A., Antonieti, F.M.P.M., Röder, D.V.D.d.B., and Pedroso, R.D.S. (2021).** Essential oils of *Melaleuca*, *Citrus*, *Cupressus*, and Litsea for the management of infections caused by *Candida* species: A systematic review. Pharmaceutics, 13(10):1700.
37. **Ruiz-Perez, D., Coudray, M.S., Colbert, B., Krupp, K., Kumari, H., Stebliankin, V., Mathee, K., Cook, R.L., Schwebke, J., Narasimhan, G., and Madhivanan, P. (2021).** Effect of metronidazole on vaginal microbiota associated with asymptomatic bacterial vaginosis. Access Microbiology, 3(5): 000226.
38. **Muzny, C.A. and Schwebke, J.R. (2015).** Biofilms: An underappreciated mechanism of treatment failure and recurrence in vaginal infections. Clinical Infectious Diseases, 61(4): 601-606.
39. **Muhammad, M.H., Idris, A.L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X., and Huang, T. (2020).** Beyond risk: Bacterial biofilms and their regulating approaches. Frontiers in microbiology, 11: 928.
40. **Hongyue, D. and R, L.C. (2015).** Microbial surface colonization and biofilm development in marine environments. Microbiology and Molecular Biology Reviews, 80(1): 91-138.
41. **Cavagnetto, A.R., Premo, J., Coleman, Z., and Juergens, K. (2022).** Accuracy and idea consideration: A study of small-group interaction in biology. CBE life sciences education, 21(1): https://doi.org/10.1187/cbe.21-03-0067.
42. **Chaban, B., Links, M.G., Jayaprakash, T.P., Wagner, E.C., Bourque, D.K., Lohn, Z., Albert, A.Y.K., van Schalkwyk, J., Reid, G., Hemmingsen, S.M., Hill, J.E., and Money, D.M. (2014).** Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle. Microbiome, 2(1): 23-23.
43. **Alhabardi, S.M.A., Sherif, E., Bahieldin, A., and Rizk Al-Hindi, R. (2021).** The composition and stability of the vaginal microbiome of healthy women. Journal of the Pakistan Medical Association, 29: 1-20.

44. **Chee, W.J.Y., Chew, S.Y., and Than, L.T.L. (2020).** Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. Microbial cell factories, 19(1): 203.

45. **Tortelli, B.A., Lewis, W.G., Allsworth, J.E., Member-Meneh, N., Foster, L.R., Reno, H.E., Peipert, J.F., Fay, J.C., and Lewis, A.L. (2020).** Associations between the vaginal microbiome and *Candida* colonization in women of reproductive age. American Journal of Obstetrics and Gynecology, 222(5): 471.e1-471.e9.

46. **Gupta, P., Singh, M.P., and Goyal, K. (2020).** Diversity of vaginal microbiome in pregnancy: Deciphering the obscurity. Frontiers in Public Health, 8: 326.

47. **Martínez-Díaz, R.A., Ibáñez-Escribano, A., Burillo, J., Heras, L.d.l., Prado, G.d., Agulló-Ortuño, M.T., Julio, L.F., and González-Coloma, A. (2015).** Trypanocidal, trichomonacidal and cytotoxic components of cultivated *Artemisia absinthium* Linnaeus (Asteraceae) essential oil. Memórias do Instituto Oswaldo Cruz, 110(5): 693-699.

48. **Nunn, K.L. and Forney, L.J. (2016).** Unraveling the dynamics of the human vaginal microbiome. The Yale Journal of Biology and Medicine, 89(3): 331-337.

49. **Collins, E.B. and Hardt, P. (1980).** Inhibition of *Candida albicans* by *Lactobacillus acidophilus*. Journal of Dairy Science, 63(5): 830-832.

50. **Reid, G. and Burton, J. (2002).** Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes and Infection, 4(3): 319-324.

51. **Reid, G., Charbonneau, D., Erb, J., Kochanowski, B., Beuerman, D., Poehner, R., and Bruce, A.W. (2003).** Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunology & Medical Microbiology, 35(2): 131-134.

52. **De Seta, F., Parazzini, F., De Leo, R., Banco, R., Maso, G.P., De Santo, D., Sartore, A., Stabile, G., Inglese, S., Tonon, M., and Restaino, S. (2014).** *Lactobacillus plantarum* P17630 for preventing *Candida* vaginitis recurrence: a retrospective comparative study. European Journal of Obstetrics & Gynecology and Reproductive Biology, 182: 136-139.

53. **Chew, S.Y., Cheah, Y.K., Seow, H.F., Sandai, D., and Than, L.T.L. (2015).** Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exhibit strong antifungal

effects against vulvovaginal candidiasis-causing *Candida glabrata* isolates. Journal of Applied Microbiology, 118(5): 1180-1190.

54. **Bertuccini, L., Russo, R., Iosi, F., and Superti, F. (2017).** Effects of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* on bacterial vaginal pathogens. International Journal of Immunopathology and Pharmacology, 30(2): 163-167.
55. **Ouwehand, A.C., Tiihonen, K., Kettunen, H., Peuranen, S., Schulze, H., and Rautonen, N. (2010).** *In vitro* effects of essential oils on potential pathogens and beneficial members of the normal microbiota. Veterinární medicína, 55(2): 71-78.
56. **Farrar, A.J. and Farrar, F.C. (2020).** Clinical Aromatherapy. Nursing Clinics of North America, 55(4): 489-504.
57. **Curtis, S. (1996).** Essential oils. Aurum Press: London, United Kingdom.
58. **Carson, C.F., Hammer, K.A., and Riley, T.V. (2006).** *Melaleuca alternifolia* (tea tree) oil: A review of antimicrobial and other medicinal properties. Clinical Microbiology Reviews, 19(1): 50-62.
59. **Orchard, A. and van Vuuren, S. (2017).** Commercial essential oils as potential antimicrobials to treat skin diseases. Evidence-Based Complementary and Alternative Medicine, 2017: 1-92.
60. **Wińska, K., Mączka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A., and Szumny, A. (2019).** Essential oils as antimicrobial agents—Myth or real alternative? Molecules, 24(11): 2130-2130.
61. **Bogavac, M., Karaman, M., Janjušević, L., Sudji, J., Radovanović, B., Novaković, Z., Simeunović, J., and Božin, B. (2015).** Alternative treatment of vaginal infections *in vitro* antimicrobial and toxic effects of *Coriandrum sativum* L. and *Thymus vulgaris* L. essential oils. Journal of Applied Microbiology, 119(3): 697-710.
62. **Spisni, E., Petrocelli, G., Imbesi, V., Spigarelli, R., Azzinnari, D., Donati Sarti, M., Campieri, M., and Valerii, M.C. (2020).** Antioxidant, anti-inflammatory, and microbial-modulating activities of essential oils: Implications in colonic pathophysiology. International Journal of Molecular Sciences, 21(11): 4152-4152.
63. **Machado, D., Gaspar, C., Palmeira-de-Oliveira, A., Cavaleiro, C., Salgueiro, L., Martinez-de-Oliveira, J., and Cerca, N. (2017).** *Thymbra Capitata* essential oil as

potential therapeutic agent against *Gardnerella vaginalis* biofilm-related infections. Future Microbiology, 12(5): 407-416.

64. **Kalemba, D. and Kunicka, A. (2003).** Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10(10): 813-829.
65. **Rhind, J.P. (2012).** Essential oils: A Handbook for Aromatherapy Practice 2. Original Edition. Vol. 2. Jessica Kingsley Publishers, London, United Kingdom.
66. **Lawless, J. (1995).** The Encyclopedia of Essential Oils: The Complete Guide to the Use of Aromatic Oils in Aromatherapy, Herbalism, Health, and Well Being. Element Books: United States of America.
67. **Sellar, W. (2005).** The Directory of Essential Oils. 2nd ed. Random House UK Limited, London, United Kingdom.
68. **Harding, J. (2008).** The Essential Oils Handbook. 1st ed. Duncan Baird Publishers Ltd.: London, United Kingdom.
69. **Buckle, J. (2003).** Clinical Aromatherapy: Essential Oils in Practice, Second Edition. Churchill Livingstone: London, United Kingdom.
70. **Duarte, M.C.T., Figueira, G.M., Sartoratto, A., Rehder, V.L.G., and Delarmelina, C. (2005).** Anti-*Candida* activity of Brazilian medicinal plants. Journal of Ethnopharmacology, 97(2): 305-311.
71. **Kovač, M. (2011).** A Quick Guide to Essential Oils. Aromadelavnice, Slovenia.
72. **Oliveira, D.R., Leitão, G.G., Santos, S.S., Bizzo, H.R., Lopes, D., Alviano, C.S., Alviano, D.S., and Leitão, S.G. (2006).** Ethnopharmacological study of two *Lippia* species from Oriximiná, Brazil. Journal of Ethnopharmacology, 108(1): 103-108.
73. **Hammer, K.A., Carson, C.F., and Riley, T.V. (1999).** Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86(6): 985-990.
74. **Sellar, W. (1992).** The Directory of Essential Oils. C. W. Daniel Company Ltd: London, United Kingdom.
75. **Bassolé, I.H.N. and Juliani, H.R. (2012).** Essential oils in combination and their antimicrobial properties. Molecules, 17(4): 3989-4006.
76. **de Rapper, S., Kamatou, G., Viljoen, A., and van Vuuren, S. (2013).** The *in vitro* antimicrobial activity of *Lavandula angustifolia* essential oil in combination with other

aroma-therapeutic oils. Evidence-Based Complementary and Alternative Medicine, 2013: 1-10.

77. **van Vuuren, S. and Viljoen, A. (2011).** Plant-based antimicrobial studies – methods and approaches to study the interaction between natural products. Planta Medica, 77(11): 1168-1182.
78. **Harding, J. (2002).** A Guide to Essential Oils. 1st ed., Parragon: Bath, United Kingdom.
79. **Abad, M.J., Bedoya, L.M., Apaza, L., and Bermejo, P. (2012).** The *Artemisia* L. genus: A review of bioactive essential oils. Molecules, 17(3): 2542-2566.
80. **Rosca, A.S., Castro, J., Sousa, L.G.V., França, A., Cavaleiro, C., Salgueiro, L., and Cerca, N. (2022).** Six bacterial vaginosis-associated species can form an *in vitro* and ex vivo polymicrobial biofilm that is susceptible to *Thymbra capitata* essential oil. Frontiers in Cellular and Infection Microbiology, 12: 824860.
81. **MehriArdestani, M., Aliahmadi, A., Toliat, T., Dalimi, A., Momeni, Z., and Rahimi, R. (2020).** Evaluation of antimicrobial activity of *Trachyspermum ammi* (L.) Sprague essential oil and its active constituent, thymol, against vaginal pathogens. Traditional and Integrative Medicine, 27:49-58.
82. **Mahboubi, M. (2018).** Systematic review: The potency of *Zataria multiflora* Boiss in treatment of vaginal infections. Infectio, 22(2): 76-83.
83. **Fraternale, D., Genovese, S., and Ricci, D. (2013).** Essential oil composition and antimicrobial activity of aerial parts and ripe fruits of *Echinophora spinosa* (*Apiaceae*) from Italy. Natural Product Communications, 8(4): 1934578X1300800431.
84. **Kędzia, A., Hołderna-Kędzia, E., and Wiśniewska, J. (2018).** Activity of lavender oil (*Oleum Lavandulae*) on anaerobic bacteria. Postępy Fitoterapii, 19(3): 170-175
85. **Hammer, K.A., Carson, C.F., and Riley, T.V. (1999).** *In vitro* susceptibilities of *Lactobacilli* and organisms associated with bacterial vaginosis to *Melaleuca alternifolia* (tea tree) oil. Antimicrobial Agents and Chemotherapy. 1999, American Society for Microbiology, 43(1):196.
86. **Ziółkowska-Klinkosz, M., Kedzia, A., Meissner, H.O., and Kedzia, A.W. (2016).** Evaluation of the tea tree oil activity to anaerobic bacteria-*in vitro* study. Acta Poloniae Pharmaceutica, 73(2): 389-94.

87. **Kędzia, A. and Hołderna-Kędzia, E. (2019).** Effectivenes *in vitro* melissae oil (*Oleum Melissae*) against anaerobic bacteria. Postępy Fitoterapii, 20(4): 244-250.

88. **Oliveira, T.A.S., Vieira, T.M., Esperandim, V.R., Martins, C.H.G., Magalhães, L.G., Miranda, M.L.D., and Crotti, A.E.M. (2022).** Antibacterial, antiparasitic, and cytotoxic activities of chemical characterized essential oil of *Chrysopogon zizanioides* roots. Pharmaceuticals, 15(8): 967-967.

89. **Sleha, R., Mosio, P., Vydrzalova, M., Jantovska, A., Bostikova, V., and Mazurova, J. (2014).** *In vitro* antimicrobial activities of cinnamon bark oil, anethole, carvacrol, eugenol and guaiazulene against *Mycoplasma hominis* clinical isolates. Biomedical Papers, 158(2): 208-211.

90. **Furneri, P.M., Mondello, L., Mandalari, G., Paolino, D., Dugo, P., Garozzo, A., and Bisignano, G. (2012).** *In vitro* antimycoplasmal activity of *Citrus bergamia* essential oil and its major components. European Journal of Medicinal Chemistry, 52: 66-69.

91. **Zatelli, G.A., Zimath, P., Tenfen, A., Mendes de Cordova, C.M., Scharf, D.R., Simionatto, E.L., Alberton, M.D., and Falkenberg, M. (2016).** Antimycoplasmic activity and seasonal variation of essential oil of *Eugenia hiemalis* Cambess. (Myrtaceae). Natural Product Research, 30(17): 1961-1964.

92. **Reichling, J., Schnitzler, P., Suschke, U., and Saller, R. (2009).** essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – An overview. Complementary Medicine Research, 16(2): 79-90.

93. **Katiraee, F., Ahmadi Afshar, S., Rahimi Pirmahalleh, S.F., and Shokri, H. (2017).** *In vitro* antifungal activity of essential oils extracted from plants against fluconazole-susceptible and -resistant *Candida albicans*. Current Medical Mycology, 3(2): 1-6.

94. **Benziane, M.Y., Bendahou, M., Benbelaïd, F., Khadir, A., Belhadef, H., Benaissa, A., Ouslimani, S., Mahdi, F., and Muselli, A. (2023).** Efficacy of endemic Algerian essential oils against single and mixed biofilms of *Candida albicans* and *Candida glabrata*. Archives of Oral Biology, 145: 105584-105584.

95. **Zida, A., Bamba, S., Yacouba, A., Ouedraogo-Traore, R., and Guiguemdé, R.T. (2017).** Anti- *Candida albicans* natural products, sources of new antifungal drugs: A review. Journal de Mycologie Médicale, 27(1): 1-19.

96. **Potente, G., Bonvicini, F., Gentilomi, G.A., and Antognoni, F. (2020).** Anti-*Candida* activity of essential oils from *Lamiaceae* plants from the Mediterranean area and the Middle East. Antibiotics, 9(7): 395-395.

97. **Rusenova, N. and Parvanov, P. (2009).** Antimicrobial activities of twelve essential oils against microorganism of veterinary importance. Trakia Journal of Sciences, 7(1): 37-43.

98. **Kozics, K., Bučková, M., Puškárová, A., Kalászová, V., Cabicarová, T., and Pangallo, D. (2019).** The effect of ten essential oils on several cutaneous drug-resistant microorganisms and their cyto/genotoxic and antioxidant properties. molecules, 24(24): 4570-4570.

99. **Bona, E., Cantamessa, S., Pavan, M., Novello, G., Massa, N., Rocchetti, A., Berta, G., and Gamalero, E. (2016).** Sensitivity of *Candida albicans* to essential oils: are they an alternative to antifungal agents? Journal of Applied Microbiology, 121(6): 1530-1545.

100. **Baba-Moussa, F., Adjanohoun, A., Kifouli, A., Djimon, G., D, A., L, K., Moudachirou, M., Koffi, A., P, B., Kotchoni, S., Fatiou, T., and Baba-Moussa, L. (2012).** antimicrobial properties and phytochemical profiling of essential oils extracted from traditionally used medicinal plants in Benin. International Journal of Natural Product Science, 2: 1-11.

101. **Abd Ellah, N.H., Shaltout, A.S., Abd El Aziz, S.M.M., Abbas, A.M., Abd El Moneem, H.G., Youness, E.M., Arief, A.F., Ali, M.F., and Abd El-hamid, B.N. (2021).** Vaginal suppositories of cumin seeds essential oil for treatment of vaginal candidiasis: Formulation, *in vitro*, *in vivo*, and clinical evaluation. European Journal of Pharmaceutical Sciences, 157: 105602-105602.

102. **Mahboubi, M. (2011).** Chemical composition, antimicrobial and antioxidant activities of *Dittrichia graveolens* (L.) Greuter essential oil. Kerba polonica, 57(3): 20-31.

103. **Aldoghaim, F., Flematti, G., and Hammer, K. (2018).** Antimicrobial activity of several cineole-rich Western Australian eucalyptus essential oils. Microorganisms, 6(4): 122-122.

104. **Bassyouni, R.H., Wali, I.E., Kamel, Z., and Kassim, M.F. (2019).** Fennel oil: A promising antifungal agent against biofilm forming fluconazole resistant *Candida albicans* causing vulvovaginal candidiasis. Journal of Herbal Medicine, 15: 100227-100227.

105. **Cansu, T.B., Özdemir, T., Batan, N., Yayli, B., Karaoglu, S.A., and Yayli, N. (2014).** Essential oil analysis and antimicrobial activity of *Neckera complanata* (Hedw.) Huebener and *Neckera crispa* Hedw. (Neckeraceae) grown in Turkey. Asian Journal of Chemistry, 26(7): 2005-2008.

106. **Pietrella, D., Angiolella, L., Vavala, E., Rachini, A., Mondello, F., Ragno, R., Bistoni, F., and Vecchiarelli, A. (2011).** Beneficial effect of *Mentha suaveolens* essential oil in the treatment of vaginal candidiasis assessed by real-time monitoring of infection. BMC Complementary and Alternative Medicine, 11(1): 18-18.

107. **Abdoul-Latif, F.M., Edou, P., Eba, F., Mohamed, N.H., Ali, A.A., Djama, S.O., Obame, L.C., Bassolé, I.H.N., and Dicko, M.H. (2010).** Antimicrobial and antioxidant activities of essential oil and methanol extract of *Jasminum sambac* from Djibouti. African Journal of Plant Science, 4: 38-43.

108. **Di Vito, M., Smolka, A., Proto, M.R., Barbanti, L., Gelmini, F., Napoli, E., Bellardi, M.G., Mattarelli, P., Beretta, G., Sanguinetti, M., and Bugli, F. (2021).** Is the antimicrobial activity of hydrolates lower than that of essential oils? Antibiotics, 10(1): 88-88.

109. **Minooeianhaghighi, M.H., Sepehrian, L., and Shokri, H. (2017).** Antifungal effects of *Lavandula binaludensis* and *Cuminum cyminum* essential oils against *Candida albicans* strains isolated from patients with recurrent vulvovaginal candidiasis. Journal de Mycologie Médicale, 27(1): 65-71.

110. **Karpiński, T.M., Ożarowski, M., Seremak-Mrozikiewicz, A., and Wolski, H. (2023).** Anti-*Candida* and antibiofilm activity of selected *Lamiaceae* essential oils. Frontiers in Bioscience-Landmark, 28(2): 28-28.

111. **Bogavac, M.A., Perić, T.M., Mišković, J., and Karaman, M. (2022).** Antimicrobial and toxic effects of *Boswellia serrata* Roxb. and *Mentha piperita* Linn. essential oils on vaginal inhabitants. Medicines, 9(12): 62-62.

112. **Houda Benabed, K., Gourine, N., Ouinten, M., Bombarda, I., and Yousfi, M. (2017).** Antioxidant and antimicrobial activities of the essential oils of three Algerian *Lamiaceae* species. Current Nutrition and Food Science, 13(2): 97-109.

113. **Anand, A., Mohan, M., Haider, S.Z., and Sharma, A. (2011).** Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand (India). Int. J. Pharm. Pharm. Sci., 3: 223-225.

114. **Karaman, M., Bogavac, M., Radovanović, B., Sudji, J., Tešanović, K., and Janjušević, L. (2017).** *Origanum vulgare* essential oil affects pathogens causing vaginal infections. Journal of Applied Microbiology, 122(5): 1177-1185.

115. **Gazim, Z.C., Amorim, A.C.L., Hovell, A.M.C., Rezende, C.M., Nascimento, I.A., Ferreira, G.A., and Cortez, D.A.G. (2010).** Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of *Tetradenia riparia* (Hochst.) Codd in Southern Brazil. Molecules, 15(8): 5509-5524.

116. **Omidpanah, S., Vazirian, M., Hosseinkhani, F., Hamedani, M.P., Hadjiakhondi, A., Hamedani, P., and Manayi, A. (2016).** Antibacterial activity of essential oil of *Trachyspermum ammi* (L.) Sprague ex Turrill against isolated and standard bacteria, in ~ 5 ~. American Journal of Essential Oils and Natural Products, 4(2016): 05-11.

117. **Mondello, F., De Bernardis, F., Girolamo, A., Cassone, A., and Salvatore, G. (2006).** *In vivo* activity of terpinen-4-ol, the main bioactive component of *Melaleuca alternifolia* Cheel (tea tree) oil against azole-susceptible and -resistant human pathogenic *Candida* species. BMC Infectious Diseases, 6(1): 158-158.

118. **Khosravi, A.R., Shokri, H., Kermani, S., Dakhili, M., Madani, M., and Parsa, S. (2011).** Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis. Journal de Mycologie Médicale, 21(2): 93-99.

119. **Cândido, C.S., Portella, C.S.A., Laranjeira, B.J., Silva, S.S.d., Arriaga, A.M.C., Santiago, G.M.P., Gomes, G.A., Almeida, P.C., and Carvalho, C.B.M. (2010).** Effects of *Myrcia ovata* Cambess. essential oil on planktonic growth of gastrointestinal microorganisms and biofilm formation of *Enterococcus faecalis*. Brazilian Journal of Microbiology, 41(3): 621-627.

120. **Cheikh-Ali, Z., Adiko, M., Bouttier, S., Bories, C., Okpekon, T., Poupon, E., and Champy, P. (2011).** Composition, and antimicrobial and remarkable antiprotozoal activities of the essential oil of rhizomes of *Aframomum sceptrum* K.Schum. (Zingiberaceae). Chemistry & Biodiversity, 8(4): 658-667.

121. **Dai, M., Peng, C., Peng, F., Xie, C., Wang, P., and Sun, F. (2016).** Anti- *Trichomonas vaginalis* properties of the oil of *Amomum tsao-ko* and its major component, geraniol. Pharmaceutical Biology, 54(3): 445-450.

122. **Baldemir, A., Karaman, Ü., İlgün, S., Kaçmaz, G., and Demirci, B. (2018).** Antiparasitic efficacy of *Artemisia ludoviciana* Nutt. (Asteraceae) essential oil for *Acanthamoeba castellanii*, *Leishmania infantum* and *Trichomonas vaginalis*. Indian Journal of Pharmaceutical Education and Research, 52(3): 416-425.

123. **Le, N.T., Donadu, M.G., Ho, D.V., Doan, T.Q., Le, A.T., Raal, A., Usai, D., Sanna, G., Marchetti, M., Usai, M., Diaz, N., Rapelli, P., Zanetti, S., Cappuccinelli, P., and Nguyen, H.T. (2020).** Biological activities of essential oil extracted from leaves of *Atalantia sessiflora* Guillauminin Vietnam. The Journal of Infection in Developing Countries, 14(09): 1054-1064.

124. **Siyadatpanah, A., Norouzi, R., Mirzaei, F., Haghirosadat, B.F., Nissapatorn, V., Mitsuwan, W., Nawaz, M., Pereira, M.L., Hosseini, S.A., Montazeri, M., Majdizadeh, M., Almeida, R.S., Hemati, M., Wilairatana, P., and Coutinho, H.D.M. (2023).** Green synthesis of nano-liposomes containing *Bunium persicum* and *Trachyspermum ammi* essential oils against *Trichomonas vaginalis*. Journal of Microbiology, Immunology and Infection, 56(1): 150-162.

125. **Chalechale, A., Zangeneh, M.M., Zangeneh, A., Hamzavi, Y., and Valadkhani, Z. (2019).** Chemical characterization and anti-parasitic property of essential oil of *Coriandrum sativum* leaf against *Trichomonas vaginalis*. Iranian Journal of Pharmacology & Therapeutics, 17(1): 1-7.

126. **Fenalti, J.M., Baccega, B., Bruni, M., Birmann, P., Neves, R., Mello, A., Islabão, Y., Gündel, S., Velho, M.C., Godoi, S.N.d., Ourique, A.F., Acosta, D., Savegnago, L., Stauffert, D., Farias, N.A.D.R., and Oliveira, C.B. (2020).** Evaluation of inhibitory and antioxidant activities of free essential oils and nanoemulsions on *Trichomonas vaginalis*. Disciplinarum Scientia - Ciências Naturais e Tecnológicas, 21(1): 139-153.

127. **Karami, F., Dastan, D., Fallah, M., and Matini, M. (2019).** *In vitro* activity of *Foeniculum vulgare* and its main essential oil component trans-anethole on *Trichomonas vaginalis*. Iranian Journal of Parasitology, 14(4): 631-638.

128. **Donadu, M.G., Trong Le, N., Viet Ho, D., Quoc Doan, T., Tuan Le, A., Raal, A., Usai, M., Marchetti, M., Sanna, G., Madeddu, S., Rappelli, P., Diaz, N., Molicotti, P., Carta, A., Piras, S., Usai, D., Thi Nguyen, H., Cappuccinelli, P., and Zanetti, S. (2020).** Phytochemical compositions and biological activities of essential oils from the leaves, rhizomes and whole plant of *Hornstedtia bella* Škorničk. Antibiotics, 9(6): 334-334.

129. **Ziaei, H., Nadeali, N., Saeedi, M., Soosaraei, M., Jorjani, O., Momeni, Z., and Fakhar, M. (2017).** The effect of lavender essential oil and nanoemulsion on *Trichomonas vaginalis* in vitro. Feyz, 21: 326-334.

130. **Thi Ha Trinh, N., Nu Phuong Anh, T., and Rappelli, P. (2021).** Evaluation of the susceptibility of *Trichomonas vaginalis* isolates to *Melaleuca cajuputi*. International Journal of Multidisciplinary Research and Publications Melaleuca cajuputi, 3(8): 1-4.

131. **Akbari, Z., Dastan, D., Maghsood, A.H., Fallah, M., and Matini, M. (2018).** Investigation of *in vitro* efficacy of *Marrubium vulgare* L. essential oil and extracts against *Trichomonas vaginalis*. Zahedan Journal of Research in Medical Sciences, 20(9): e67003.

132. **Farias, K.S., Kato, N.N., Boaretto, A.G., Weber, J.I., Brust, F.R., Alves, F.M., Tasca, T., Macedo, A.J., Silva, D.B., and Carollo, C.A. (2019).** *Nectandra* as a renewable source for (+)-α-bisabolol, an antibiofilm and anti-*Trichomonas vaginalis* compound. Fitoterapia, 136: 104179-104179.

133. **Dogan, N., Qoran, İ., and Kurkcuoglu, M. (2016).** *In vitro* activity of different 5-nitroimidazole derivatives and essential oils against *Trichomonas vaginalis.* International Journal of Infectious Diseases, 45: 358-359.

134. **Ezz Eldin, H.M. and Badawy, A.F. (2015).** *In vitro* anti-*Trichomonas vaginalis* activity of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil. Journal of Parasitic Diseases, 39(3): 465-473.

135. **Saghafi, F., Mirzaie, F., Gorji, E., Nabimeybodi, R., Fattahi, M., Mahmoodian, H., and Zareshahi, R. (2021).** Antibacterial and anti-*Trichomonas Vaginalis* effects of *Rosa Damascena* Mill petal oil (a persian medicine product), aqueous and hydroalcoholic extracts. BMC Complementary Medicine and Therapies, 21(1): 265-265.

136. **Benyoucef, F. (2020).** Synergistic effects of essential oils of *Ammoides verticillata* and *Satureja candidissima* against many pathogenic microorganisms. Anti-Infective Agents, 18(1): 72-78.

137. **Fisher, K. and Phillips, C. (2009).** The mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*. Journal of Applied Microbiology, 106(4): 1343-1349.

138. **Ambrosio, C.M.S., de Alencar, S.M., Moreno, A.M., and Da Gloria, E.M. (2018).** Evaluation of the selective antibacterial activity of *Eucalyptus globulus* and *Pimenta pseudocaryophyllus* essential oils individually and in combination on *Enterococcus faecalis* and *Lactobacillus rhamnosus*. Canadian Journal of Microbiology, 64(11): 844-855.

139. **Bassolé, I.H.N., Lamien-Meda, A., Bayala, B., Tirogo, S., Franz, C., Novak, J., Nebié, R.C., and Dicko, M.H. (2010).** Composition and antimicrobial activities of *Lippia multiflora* Moldenke, *Mentha x piperita* L. and *Ocimum basilicum* L. essential oils and their major monoterpene alcohols alone and in combination. Molecules, 15(11): 7825-7839.

140. **Orchard, A., van Vuuren, S.F., and Viljoen, A.M. (2019).** Commercial essential oil combinations against topical fungal pathogens. Natural Product Communications, 14(1): 1934578X1901400-1934578X1901400.

141. **Donaldson, J.R., Warner, S.L., Cates, R.G., and Gary Young, D. (2005).** Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. Pharmaceutical Biology, 43(8): 687-695.

142. **van Vuuren, S.F., Docrat, Y., Kamatou, G.P.P., and Viljoen, A.M. (2014).** Essential oil composition and antimicrobial interactions of understudied tea tree species. South African Journal of Botany, 92: 7-14.

143. **Iseppi, R., Tardugno, R., Brighenti, V., Benvenuti, S., Sabia, C., Pellati, F., and Messi, P. (2020).** Phytochemical composition and *in vitro* antimicrobial activity of essential oils from the *Lamiaceae* family against *Streptococcus agalactiae* and *Candida albicans* biofilms. Antibiotics, 9(9): 592-592.

144. **Petretto, G.L., Fancello, F., Bakhy, K., Faiz, C.A., Sibawayh, Z., Chessa, M., Zara, S., Sanna, M.L., Maldini, M., Rourke, J.P., and Pintore, G. (2018).** Chemical

composition and antimicrobial activity of essential oils from *Cuminum cyminum* L. collected in different areas of Morocco. Food Bioscience, 22: 50-58.

145. **Ghasemi, M., Radjabalizade, K., Gharari, K., Yaghoobi, H., and Asgarpoor, D. (2016).** Chemical composition and *in vitro* antibacterial activity of native *Mentha longifolia* essential oil from Ardabil, Iran on *Streptococcus mutans*, *Lactobacillus rhamnosus* and *Actinomyces viscosus*. Infection, Epidemiology and Medicine, 2(1): 11-14.

146. **Diniz-Silva, H.T., Brandão, L.R., de Sousa Galvão, M., Madruga, M.S., Maciel, J.F., Leite de Souza, E., and Magnani, M. (2020).** Survival of *Lactobacillus acidophilus* LA-5 and *Escherichia coli* O157:H7 in Minas Frescal cheese made with oregano and rosemary essential oils. Food Microbiology, 86: 103348-103348.

147. **Moritz, C.M.F., Rall, V.L.M., Saeki, M.J., and Fernandes Júnior, A. (2012).** Inhibitory effect of essential oils against *Lactobacillus rhamnosus* and starter culture in fermented milk during its shelf-life period. Brazilian Journal of Microbiology, 43(3): 1147-1156.

148. **Valdivieso-Ugarte, M., Plaza-Diaz, J., Gomez-Llorente, C., Lucas Gómez, E., Sabés-Alsina, M., and Gil, Á. (2021).** *In vitro* examination of antibacterial and immunomodulatory activities of cinnamon, white thyme, and clove essential oils. Journal of Functional Foods, 81: 104436-104436.

149. **Upadhyay, R., Dwivedi, P., and Ahmad, S. (2010).** Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian Journal of Medical Sciences, 2: 152-158.

150. **Ocheng, F., Bwanga, F., Joloba, M., Softrata, A., Azeem, M., Pütsep, K., Borg-Karlson, A.-K., Obua, C., and Gustafsson, A. (2015).** Essential oils from Ugandan aromatic medicinal plants: Chemical composition and growth inhibitory effects on oral pathogens. Evidence-Based Complementary and Alternative Medicine, 2015: 1-10.

151. **Yazdanian, M., Rostamzadeh, P., Alam, M., Abbasi, K., Tahmasebi, E., Tebyaniyan, H., Ranjbar, R., Seifalian, A., Moghaddam, M.M., and Kahnamoei, M.B. (2022).** Evaluation of antimicrobial and cytotoxic effects of *Echinacea* and *Arctium* extracts and *Zataria* essential oil. AMB Express, 12(1): 75-75.

152. **Micucci, M., Protti, M., Aldini, R., Frosini, M., Corazza, I., Marzetti, C., Mattioli, L.B., Tocci, G., Chiarini, A., Mercolini, L., and Budriesi, R. (2020).** *Thymus vulgaris* L. Essential oil solid formulation: Chemical profile and spasmolytic and antimicrobial effects. Biomolecules, 10(6): 860-860.

153. **Kivanç, M., Akgül, A., and Doğan, A. (1991).** Inhibitory and stimulatory effects of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. International Journal of Food Microbiology, 13(1): 81-85.

154. **Raspo, M.A., Vignola, M.B., Andreatta, A.E., and Juliani, H.R. (2020).** Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. Food Bioscience, 36: 100651-100651.

155. **Hashemi, S.M.B. and Khodaei, D. (2020).** Antimicrobial activity of *Satureja Khuzestanica* Jamzad and *Satureja bachtiarica* Bunge essential oils against *Shigella flexneri* and *Escherichia coli* in table cream containing *Lactobacillus plantarum* LU5. Food Science & Nutrition, 8(11): 5907-5915.

156. **Shazadi, K., Arshad, N., Ambreen, H.S., Riaz, A., and Mehreen, A. (2024).** *In vivo* studies could not confirm *in vitro* prophylactic synergism between Moringa essential oil and *Lactobacillus reuteri* (MT180537). Brazilian Journal of Biology, 84.

157. **Sahal, G., Woerdenbag, H.J., Hinrichs, W.L.J., Visser, A., Tepper, P.G., Quax, W.J., van der Mei, H.C., and Bilkay, I.S. (2020).** Antifungal and biofilm inhibitory effect of *Cymbopogon citratus* (lemongrass) essential oil on biofilm forming by *Candida tropicalis* isolates; An *in vitro* study. Journal of Ethnopharmacology, 246: 112188-112188.

158. **Dolatabadi, S., Salari, Z., and Mahboubi, M. (2019).** Antifungal effects of *Ziziphora tenuior*, *Lavandula angustifolia*, *Cuminum cyminum* essential oils against clinical isolates of *Candida albicans* from women suffering from vulvovaginal candidiasis, Infectio, 23(3): 222-226.

159. **Khan, M.S.A. and Ahmad, I. (2012).** Biofilm inhibition by *Cymbopogon citratus* and *Syzygium aromaticum* essential oils in the strains of *Candida albicans*. Journal of Ethnopharmacology, 140(2): 416-423.

160. **Ranđelović, M., Dimitrijević, M., Otašević, S., Stanojević, L., Išljamović, M., Ignjatović, A., Arsić-Arsenijević, V., and Stojanović-Radić, Z. (2023).** Antifungal activity and type of interaction of *Melissa officinalis* essential Oil with antimycotics against biofilms of multidrug-resistant *Candida* isolates from vulvovaginal mucosa. Journal of Fungi, 9(11): 1080-1080.

161. **Fernandes, L., Costa, R., Henriques, M., and Rodrigues, M.E. (2023).** Simulated vaginal fluid: *Candida* resistant strains' biofilm characterization and vapor phase of essential oil effect. Journal of Medical Mycology, 33(1): 101329-101329.

162. **Gajdács, M., Spengler, G., and Urbán, E. (2017).** Identification and antimicrobial susceptibility testing of anaerobic bacteria: rubik's cube of clinical microbiology? Antibiotics, 6(4): 25-25.

163. **Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., and Fecka, I. (2020).** Thymol and Thyme Essential Oil—New Insights into Selected Therapeutic Applications. Molecules, 25(18): 4125.

164. **Cowan, M.M. (1999).** Plant products as antimicrobial agents. Clinical microbiology reviews, 12(4): 564-582.

165. **Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M.R., Ademiluyi, A.O. and Sharifi-Rad, R., (2017).** Biological activities of essential oils: From plant chemoecology to traditional healing systems. Molecules, 22(1): 70.

Supplementary Data

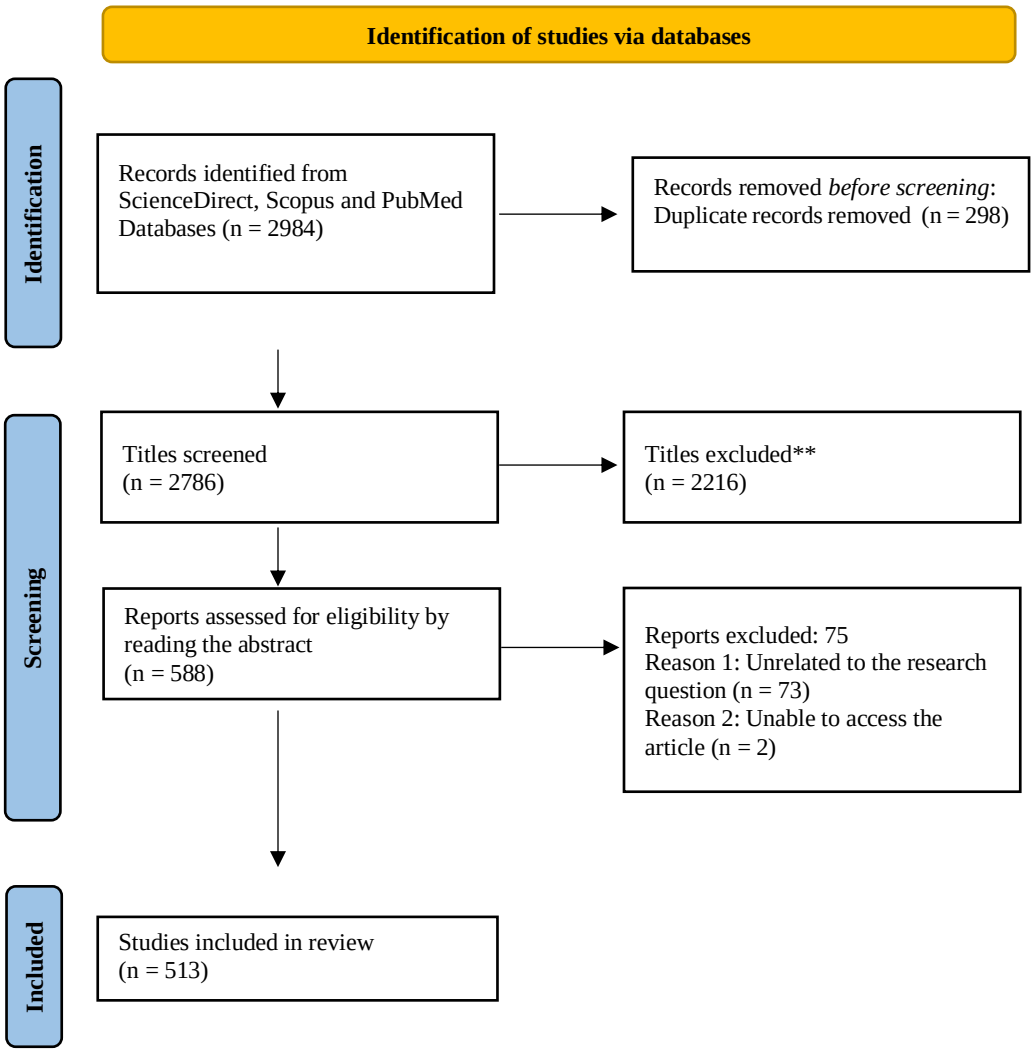

Figure S1: PRISMA diagram illustrating the literature review process (https://prisma-statement.org/prismastatement/flowdiagram.aspx)

Table S1: Table illustrating essential oils tested against *E. faecalis*

Strain number	Essential oil	Results	Ref.
	Enterococcus faecalis		
ATCC 29212	*Achilea milefolium* L.	MIC = 160 µg/ml	[1]
ATCC 7314	*Ammoides verticillate* (L.) Rchb.	Agar dilution method MIC = 1.5 ± 0.3 µl/ml	[2]
NCTC 8213	*Aniba rosaeodora* (Ducke) Meisn.	MIC = 0.5% (v/v)	[3]
NCTC 8213	*Apium graveolens* L.	MIC = 2% (v/v)	[3]
NCTC 8213	*Boswellia carterii* (Oliv.) Herder	MIC = 2 % (v/v)	[3]
MTCC 439	*Callistemon macropunctatus* Cheel	MIC = 8.9 mg/ml	[3]
NCTC 8213	*Cananga odorata* (Lam.) Hook.f. andThomson	MIC = 2% (v/v)	[3]
ATCC 29212	*Cinnamomum aromaticum* Nees	MIC = 0.125% (v/v)	[4]
ATCC 29212	*Cinnamomun zeylanicum* Garcin ex Blume	MIC50 = 50.4 mg/ml	[5]
NCTC 8213	*Citrus aurantifolia* (Christm.) Swingle	MIC = >2% (v/v)	[3]
NCTC 8213	*Citrus aurantium* L.	MIC = >2% (v/v)	[3]
NCTC 8213	*Citrus bergamia* Risso	MIC = >2% (v/v)	[3]
NCTC 8213	*Citrus limon* (L.) Osbeck	MIC = 2% (v/v)	[6]
NCTC 8213	*Citrus madurensis* (L.) N.S. Jodha	MIC = >2% (v/v)	[3]
NCTC 8213	*Citrus paradisi* Macfad.	MIC = >2% (v/v); 25 mg/ml	[3, 7]
ATCC 29212	*Clausena anisate* (Willd.) Benth.	No antibacterial activity	[8]
NCTC 8213	*Commiphora myrrha* (Torr.) Engl.	MIC = 05% (v/v)	[3]
NCTC 8213	*Coriandum sativum* L.	MIC = 05% (v/v)	[3]
NCTC 8213	*Cucurbita pepo* L.	MIC = >2% (v/v)	[3]
ATCC 29212	*Cuminum cyminum* L.	MIC = 50% of EO	[9]
NCTC 8213	*Cupressus sempervirens* L.	MIC = 1% (v/v)	[3]
NCTC 8213	*Cymbopogon martini* (Roxb.) Wats.	MIC = 0.25% (v/v); 0.25 mg/ml	[3, 10]
ATCC29212	*Cymbopogon citratus* (DC.) Stapf	MIC = 0.12% (v/v); 0.125% (v/v)	[3, 4]
ATCC 29212	*Cymbopogon nardus* (L.) Rendle	MIC = 1% (v/v); No antibacterial activity at highest concentration tested	[8]

Strain number	Essential oil	Results	Ref.
ATCC 29212	*Daucus carrot* L.	MIC = 2% (v/v); 0.0975% of EO	[9]
Clinical strain	*Dittrichia graveolens* (L.) W. Greuter	MIC = 0.5 µl/ml	[11]
ATCC 29212	*Dysphania ambrosioides* (L.) Mosyakin and Clemants	MIC = 105 ug/ml	[12]
ATCC 29212	*Eucalyptus borealis* F. Muell.	MIC = 4% (v/v)	[13]
ATCC 29212	*Eucalyptus camaldulensis* Dehnh.	MIC = 5 mg/ml	[8]
ATCC 29212	*Eucalyptus citriodora* Hook.	MIC = No antibacterial activity at the highest concentration tested	[8]
ATCC 29212	*Eucalyptus globulus* Labill.	MIC = 4% (v/v)	[13]
ATCC 29212	*Eucalyptus plenissima* (C.A.Gardner) Brooker	MIC = >8% (v/v)	[13]
NCTC 8213	*Eucalyptus polybractea* F. Muell.	MIC = 2% (v/v)	[3]
ATCC 29212	*Foeniculum vulgare* Mill.	MIC = >2% (v/v); 100% of EO	[9]
NCTC 8213	*Gaultheria procumbens* L.	MIC = >2% (v/v)	[3]
ATCC 29212	*Hertia cheirifolia* Kuntze	MIC = 0.156 mg/ml	[14]
CIP 103907	*Jasminum sambac* (L.) Aiton	MIC = 1% (v/v)	[15]
ATCC 29212	*Juniperus communis* L.	MIC = 2% (v/v); 6.25% of EO	[9]
ATCC 29212	*Lantana camara* L.	MIC = 10 mg/ml	[8]
ATCC 29212	*Laurus nobilis* L.	MIC = 08% of EO	[9]
Clinical strain	*Lavandula angustifolia* Mill.	MIC = > 2% (v/v)	[16]
ATCC 29212	*Lavandula intermedia* Emeric ex Loisel.	MIC = 10 mg/ml	[8]
ATCC 29212	*Lippia multiflora* Moldenke	MIC = 10 mg/ ml	[8]
NCTC 8213	*Macadamia integrifolia* Maiden and Betche	MIC = >2% (v/v)	[3]
NCTC 8213	*Melaleuca alternifólia* Cheel	MIC = 2% (v/v)	[3]
ATCC 29212	*Melaleuca quinquenervia* (Cav.) S.T. Blake	MIC = 1% (v/v); No antibacterial activity at highest concentration tested	[8]
ATCC 49452	*Mentha longifolia* (L.) Huds.	MIC = 1.50 ± 0.18 µg/ml	[17]
ATCC 29212	*Mentha* × *piperita* L.	MIC; MIC50 = 1% (v/v); 11.3 mg/ml	[3, 5]
ATCC 29212	*Mentha pulegium* L.	MIC = 1.035 mg/ml	[18]
ATCC 29212		MIC = 0.3 µl/ml	
NCTC 8213	*Mentha spicata* L.	MIC = 2% (v/v)	[3]
Clinical strain	*Monarda fistulosa* L.	MIC = 2% (v/v)	[16]
Clinical strain	*Monarda didyma* L.	MIC = 0.25% (v/v)	[16]
ATCC 29212	*Myrcia ovata* Cambess.	MIC = 0.031% (w/v)	[6]
ATCC 29212	*Myristica fragrans* Houtt.	MIC =100% of EO	[9]

Strain number	Essential oil	Results	Ref.
ATCC 29212	*Neckera crispa* Hedw.	MIC = No antibacterial activity at highest concentration tested	[19]
ATCC 29212	*Neckera complanate (*Hedw.) Schimp.	MIC = 576.2 µg/ml	[19]
ATCC 29212	*Nepeta mahanensis* (Benth.) Benth.	Agar dilution method MIC = 2.27 mg/ml	[20]
ATCC 29212	*Ocimum basilicum* L.	MIC = 2% (v/v); >10 mg/ml	[8]
ATCC 29212	*Ocimum gratissimum* L.	MIC = 0.625 mg/ml	[8]
NCTC 8213	*Oenothera biennis* L.	MIC = >2% (v/v)	[3]
ATCC 29212	*Origanum hirtum* (L.) Link	MIC = < 0.06% (v/v)	[16]
ATCC 29212	*Origanum marjorana* L.	MIC = 2% (v/v); 1.0% (v/v)	[3, 4]
NCTC 8213	*Origanum vulgare* L.	MIC = 0.25% (v/v	[3]
NCTC 8213	*Pelargonium graveolens* (L.) L'Hér.	MIC = 0.5% (v/v)	[3]
ATCC 29212	*Peperomia pellucida* (L.) Kunth	MIC = 1000 µg/ml	[9]
NCTC 8213	*Pimenta racemosa* (Mill.) J. W. Moore	MIC = 0.5% (v/v)	[3]
ATCC 29212	*Pimpinella anisum* L.	MIC = 2% (v/v)	[9]
NCTC 8213	*Pinus sylvestris* L.	MIC = >2 %(v/v)	[3]
ATCC 29212	*Piper callosum* (L.) Steud.	MIC = 1000 µg/ml	[9]
ATCC 29212	*Piper marginatum* Jacq.	MIC = 500 µg/ml	[9]
NCTC 8213	*Piper nigrum* L.	MIC = 1% (v/v)	[3]
ATCC 29212	*Pistacia khinjuk* Stocks	MIC = 1.875 µg/ml	[21]
Clinical strain	*Pistacia lentiscus* L.	MIC = 0.68 mg/ ml	[22]
NCTC 8213	*Pogostemon patchouli* (Miq.) Benth	MIC = 0.12% (v/v)	[3]
NCTC 8213	*Prunus armeniaca* L.	MIC = >2% (v/v)	[3]
NCTC 8213	*Prunus dulcis* (Mill.) Rchb	MIC = >2% (v/v)	[3]
ATCC 3360	*Rosa alba* L.	MIC = 1.36 mg/ml	[23]
ATCC 29212	*Rosmarinus officinalis* L.	MIC = >2% (v/v); 160 µg /ml	[1]
ATCC 49452	*Ruta graveolens* L.	MIC = 800 µl/ml	[17]
ATCC 29212	*Salvia officinalis* L.	MIC = 2% (v/v); 25 mg/ml	[3, 24]
ATCC 51299	*Salvia potentillifolia* L.	MIC = 64 µg/ml	[25]
ATCC 29212	*Salvia sclarea* L.	MIC = 2.0% (v/v)	[3, 4]
NCTC 8213	*Santalum album* L.	MIC = 0.25% (v/v)	[3]
ATCC 7314	*Satureja candidissima* Boiss.	MIC = 6.2 ± 0.9 µl/ml	[2]
ATCC 29212	*Satureja horvatii* Šilić	MIC = 0.4 µl/ml	[1]
ATCC 29212	*Satureja intermedia* (Ser.) Briq.	MIC = 8.9 ± 0.0 mg/ml	[26]
ATCC 49452	*Satureja macrantha* (Coss.) Briq.	MIC = 20 µg/ml	[17]
Clinical strain	*Satureja montana* L.	MIC = <0.06% (v/v)	[16]

Strain number	Essential oil	Results	Ref.
ATCC 29212	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	MIC = 0.5% (v/v)	[3, 4]
ATCC 29212	*Tetradenia riparia* (Hochst.) Codd	MIC = 31.2 to 62.4 µg/ml	[27]
ATCC 29212	*Teucrium polium* L.	MIC =5 µl/ml	[28]
ATCC 29212	*Thymus algeriensis* Boiss.	MIC = 0.528 mg/ml	[18]
Clinical strain	*Thymus broussonnetii* Boiss.	MIC = 4± 0.001 mg/ml	[29]
Clinical strain	*Thymus capitatus* Hoffmanns. & Link	MIC = 4± 0.001 mg/ml	[29]
ATCC 29212	*Thymus hirtus* Willk. Ex Lange	MIC = 0.05% (v/v)	[26]
ATCC 14506	*Thymus serpyllum* L.	MIC = 6.25 µl/ml	[30]
ATCC 29212	*Thymus vulgaris* L.	MIC; MIC50 = 0,5% (v/v); 21,7 mg/ml	[5, 29]
Clinical strain	*Thymus zygis* L.	MIC = 1.00 mg/ml	[10]
ATCC 29212	*Trachyspermum ammi* Sprague	MIC = 2 µl/ml	[31]
NCTC 8213	*Vetiveria zizanioides* (L.) Nash	MIC = 0.12% (v/v)	[3]
ATCC 29212	*Xylopia aethiopica* (Dunal) A. Rich.	MIC = No antibacterial activity at highest concentration tested	[8]
NCTC 8213	*Zingiber officinale* Roscoe	MIC = >2% (v/v)	[3]

References

1. **Bogojevic, J., Nikolic, M., Markovic, T., Ciric, A., and Markovic, D. (2016).** Analysis of chemical composition of the most efficient essential oils towards *Enterococcus faecalis* referent strain ATCC 29212 and clinical isolates. Lekovite sirovine, (36): 3-25.
2. **Benyoucef, F. (2020).** Synergistic effects of essential oils of *Ammoides verticillata* and *Satureja candidissima* against many pathogenic microorganisms. Anti-Infective Agents, 18(1): 72-78.
3. **Hammer, K.A., Carson, C.F., and Riley, T.V. (1999).** Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86(6): 985-990.
4. **Rusenova, N. and Parvanov, P. (2009).** Antimicrobial activities of twelve essential oils against microorganism of veterinary importance. Trakia Journal of Sciences, 7:37-43.
5. **Rosato, A., Sblano, S., Salvagno, L., Carocci, A., Clodoveo, M.L., Corbo, F., and Fracchiolla, G. (2020).** Anti-biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. Antibiotics (Basel, Switzerland), 9(10):637.
6. **Cândido, C.S., Portella, C.S.A., Laranjeira, B.J., Silva, S.S.d., Arriaga, A.M.C., Santiago, G.M.P., Gomes, G.A., Almeida, P.C., and Carvalho, C.B.M. (2010).** Effects of *Myrcia ovata* Cambess. essential oil on planktonic growth of gastrointestinal microorganisms and biofilm formation of *Enterococcus faecalis*. Brazilian Journal of Microbiology, 41(3): 621-627.
7. **Özogul, Y., Özogul, F., and Kulawik, P. (2021).** The antimicrobial effect of grapefruit peel essential oil and its nanoemulsion on fish spoilage bacteria and food-borne pathogens. LWT, 136: 110362-110362.

8. **Baba-Moussa, F., Adjanohoun, A., Kifouli, A., Djimon, G., D, A., L, K., Moudachirou, M., Koffi, A., P, B., Kotchoni, S., Fatiou, T., and Baba-Moussa, L. (2012).** Antimicrobial properties and phytochemical profiling of essential oils extracted from traditionally used medicinal plants in Benin. International Journal of Natural Product Science, 2: 1-11.
9. **Carvalho, M., Albano, H., and Teixeira, P. (2018).** *In Vitro* antimicrobial activities of various essential oils against pathogenic and spoilage microorganisms. Journal of Food Quality and Hazards Conrol, 5(2): 41-48.
10. **Marinković, J., Ćulafić, D.M., Nikolić, B., Đukanović, S., Marković, T., Tasić, G., Ćirić, A., and Marković, D. (2020).** Antimicrobial potential of irrigants based on essential oils of C*ymbopogon martinii* and Thymus zygis towards *in vitro* multispecies biofilm cultured in *ex vivo* root canals. Archives of Oral Biology, 117: 104842-104842.
11. **Mahboubi, M. (2011).** Chemical composition, antimicrobial and antioxidant activities of *Dittrichia graveolens* (L.) Greuter essential oil. Kerba polonica, 57(3): 20-31.
12. **Kandsi, F., Elbouzidi, A., Lafdil, F.Z., Meskali, N., Azghar, A., Addi, M., Hano, C., Maleb, A., and Gseyra, N. (2022).** Antibacterial and antioxidant activity of *Dysphania ambrosioides* (L.) Mosyakin and Clemants essential oils: Experimental and computational Approaches. Antibiotics, 11(4): 482-482.
13. **Aldoghaim, F., Flematti, G., and Hammer, K. (2018).** Antimicrobial activity of several cineole-rich western Australian *Eucalyptus* essential oils. Microorganisms, 6(4): 122-122.
14. **Majouli, K., Hlila, M.B., Flamini, G., Jannet, H.B., and Kenani, A. (2016).** *In vitro* antibacterial activity of the *Hertia cheirifolia* L. essential oils. Journal of Coastal Life Medicine, 4(11): 865-867.
15. **Abdoul-Latif, F.M., Edou, P., Eba, F., Mohamed, N.H., Ali, A.A., Djama, S.O., Obame, L.C., Bassolé, I.H.N., and Dicko, M.H. (2010).** Antimicrobial and antioxidant activities of essential oil and methanol extract of J*asminum sambac* from Djibouti. African Journal of Plant Science, 4: 38-43.
16. **Di Vito, M., Smolka, A., Proto, M.R., Barbanti, L., Gelmini, F., Napoli, E., Bellardi, M.G., Mattarelli, P., Beretta, G., Sanguinetti, M., and Bugli, F. (2021).** Is the antimicrobial activity of hydrolates lower than that of essential oils? Antibiotics, 10(1): 88-88.
17. **Desam, N.R., Al-Rajab, A.J., Sharma, M., Mylabathula, M.M., Gowkanapalli, R.R., and Mohammed, A. (2017).** Chemical composition, antibacterial and antifungal activities of Saudi Arabian *Mentha longifolia* L. essential oil. Journal of Coastal Life Medicine, 5(10): 441-446.
18. **Houda Benabed, K., Gourine, N., Ouinten, M., Bombarda, I., and Yousfi, M. (2017).** Antioxidant and antimicrobial activities of the Essential oils of three Algerian Lamiaceae species. Current Nutrition and Food Science, 13(2): 97-109.
19. **Cansu, T.B., Özdemir, T., Batan, N., Yayli, B., Karaoglu, S.A., and Yayli, N. (2014).** Essential oil analysis and antimicrobial activity of *Neckera complanata* (Hedw.) Huebener and *Neckera crispa* Hedw. (Neckeraceae) Grown in Turkey. Asian Journal of Chemistry, 26(7): 2005-2008.
20. **Amirzadeh, M., Soltanian, S., and Mohamadi, N. (2022).** Chemical composition, anticancer and antibacterial activity of *Nepeta mahanensis* essential oil. BMC Complementary Medicine and Therapies, 22(1): 173-173.

21. **Alkis Kocturk, S., Nabaz Fisal Fisal, D., and Alma, H. (2021).** Evaluation of antibacterial activity of essential oil from *Pistacia Khinjuk* Stoks gum of Iraqian using broth microdilution. American Journal of Laboratory Medicine, 6(2): 27-27.
22. **Derwich, E., Benziane, Z., and Boukir, A. (2010).** Chemical composition and *in vitro* antibacterial activity of the essential oil of *Cedrus atlantica*. International Journal of Agriculture and Biology, 12: 381-385.
23. **Mileva, M., Kussovski, V., Krastev, D., Dobreva, A., and Galabov, A. (2014).** Chemical composition, *in vitro* antiradical and antimicrobial activities of Bulgarian *Rosa alba* L. essential oil against some oral pathogens. International Journal of Current Microbioly and Applied Sciences, 3(7): 11-20.
24. **Yazgan, H. (2020).** Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. LWT, 130: 109669-109669.
25. **Elif, O.K.s., Gouml zde, O.n.t., and Atila, Y. (2013).** Chemical composition and antimicrobial activity of essential oil of *Salvia potentillifolia* Boiss. Heldr. ex Benth. from Turkey. African Journal of Microbiology Research, 7(16): 1489-1495.
26. **Fredj, M.B.H., Fredj, H., Marzouk, B., Cheraief, I., Boukef, K., and Marzouk, Z. (2007).** Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. Journal of Food, Agriculture and Environment, 5(1): 52-55.
27. **Gazim, Z.C., Amorim, A.C.L., Hovell, A.M.C., Rezende, C.M., Nascimento, I.A., Ferreira, G.A., and Cortez, D.A.G. (2010).** Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from Leaves of *Tetradenia riparia* (Hochst.) Codd in Southern Brazil. Molecules, 15(8): 5509-5524.
28. **Belmekki, N., Bendimerad, N., Bekhechi, C., and Fernandez, X. (2013).** Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria. Journal of Medicinal Plants Research, 7(14): 897-902.
29. **Tagnaout, I., Zerkani, H., Hadi, N., El Moumen, B., El Makhoukhi, F., Bouhrim, M., Al-Salahi, R., Nasr, F.A., Mechchate, H., and Zair, T. (2022).** Chemical composition, antioxidant and antibacterial activities of *Thymus broussonetii* Boiss and *Thymus capitatus* (L.) Hoffmann and link essential oils. Plants, 11(7): 954-954.
30. **Lević, J., Čabarkapa, I., Todorović, G., Pavkov, S., Sredanović, S., Coghill-Galonja, T., and Kostadinović, L. (2011).** *In vitro* antibacterial activity of essential oils from plant family Lamiaceae, in Romanian Biotechnological Letters, 16(2): 6034-6041.
31. **Hassanshahian, M., Bayat, Z. Saeidi, S., Shiri, Y. (2014).** Antibacterial activity of *Trachyspermum ammi* essential oil against human bacterial. International journal of Advanced Biological and Biomedical Research, 2(1): 18-24.

Chapter 3 – Experimental publication

Laher, MH., Orchard, A., van Vuuren, S., 2025. Essential Oils Against Vaginal Pathogens: Efficacy, Toxicity, and Microbiota Preservation**.** *Natural Product Communications*.

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Proof of submission to a journal is provided in Appendix E.

Essential Oils against Vaginal Pathogens: Efficacy, Toxicity, and Microbiota Preservation

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Abstract

The effectiveness of current treatments for vaginal infections is declining due to rising bacterial and yeast resistance, along with adverse effects on the vaginal microbiome. Although essential oils are known for their antimicrobial properties, their potential in treating vaginal infections either as monotherapies or in combination has not been thoroughly explored. This study assessed the antimicrobial efficacy (minimum inhibitory concentrations and biofilm inhibition) and toxicity of 46 essential oils and 86 combinations against both pathogens and beneficial vaginal microbiota (*Lactobacilli* spp.). Among the single essential oils, *Coriandrum sativum* showed the most promising broad-spectrum antimicrobial activity (values as low as 0.13 mg/mL). *Origanum vulgare* and *Myrtus communis* were also of interest, having no toxicity with minimal impact on the vaginal biome (*Lactobacilli* spp.). The combination of *Cinnamomum camphora* and *Melaleuca cajuputi* essential oil demonstrated superior antimicrobial inhibition

(ΣFIC values 0.08-0.35), was non-toxic at both 24 and 48 hr (percentage inhibition of 34.34 ± 6.49 and 46.90 ± 0.79, respectively), and was able to inhibit *Candida* biofilms. These findings suggest that selected essential oils have potential as alternative treatments for vaginal infections, with the ability to inhibit vaginal pathogens, demonstrate low toxicity while not inhibiting the natural microbiota *in vitro.*

Keywords

Vaginal infections, vaginal microbiota, essential oils, biofilm, essential oil combinations, *Lactobacilli* spp, antimicrobial

Introduction

Vaginal infections represent a significant concern in women's health, with implications for both physical and psychological well-being.[1] Among these, bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) are the most prevalent, especially in women during their reproductive years. Bacterial vaginosis accounts for approximately 60% of cases, and at least 75% of women experience an episode of VVC in their lifetime.[2, 3] Added to that, the rates of re-infection within the first six months and year of treatment are incredibly high. [4, 5]

The recurrent nature of vaginal infections complicates treatment as current therapies, including antibiotics and antifungals, are increasingly compromised due to the emergence of resistant micro-organisms.[6] This pattern of resistance exacerbates the cycle of recurrent infections, leading to increased use of conventional medications, contributing to the broader issue of antimicrobial resistance. Additionally, the use of conventional treatments often has a negative impact on the vaginal microbiota, particularly on the *Lactobacillus* species, which are crucial for maintaining the vaginal microbiome.[7] The formation of biofilms by pathogens further complicates treatment, as this enhances the resilience of pathogens implicated in vaginal infections and leads to treatment failure.[8]

Despite the well-documented historical use and current popularity of essential oils in the aromatherapeutic literature, there is a lack of sufficient scientific evidence supporting their efficacy in treating vaginal infections.[4,9,10] Furthermore, studies on the antimicrobial activity of essential oil combinations against vaginal pathogens are limited, despite essential oils being often used in combination to achieve therapeutic synergy.[11-13] There are several essential oils (*Eugenia*

caryophyllata Thunb., *Mentha piperita* L., *Thymus vulgaris* L., *Citrus paradisi* Macf., *Citrus limon* L., *Origanum vulgare* L., *Rosmarinus officinalis* L.) that have been associated with no inhibition (either with planktonic cells or biofilms) of the vaginal microbiota, suggesting a positive effect on the vaginal microbiome.[14-20] Some essential oils (*Citrus sinensis* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L.) have also been reported to stimulate the growth of micro-organisms that are part of the vaginal microbiome.[14]

There is also a mistaken assumption that essential oils, being natural products, are inherently non-toxic; however, several reports do exist emphasizing the toxicity of essential oils.[13, 21, 22] Thus, this necessitates a thorough investigation into their toxicity.

This study aimed to evaluate the antimicrobial activity of essential oils and their combinations against vaginal pathogens. Additionally, the study assessed the effect of essential oils and combinations on the vaginal microbiota and their potential to inhibit biofilms. Additionally, the research addresses the toxicity of essential oils and combinations to help evaluate safety as well as the effects on the vaginal microbiota.

Materials and Methods

Essential Oil Selection and Chemical Analysis

Forty-six essential oils and 86 combinations were selected based on the aromatherapeutic literature accessible to the layman and their reported anti-infective use against vaginal infections.[23-29] As the layman literature does not specify which part of the plant is recommended or very often does not document the scientific name, more than one essential oil species was included for several of the oils, and the part was selected based on essential oil availability. The essential oils used in this study were procured from Scatters Oils (Gauteng, South Africa) and Prana Monde (Belgium). The chemical profiles of the essential oils were obtained from the suppliers of the essential oils.

Preparation of Cultures

The 12 micro-organisms that were investigated were obtained from the American Type Culture Collection (ATCC). Pathogens were selected based on their relevance to BV and VVC, their known relevance in vaginal infections, and their availability. Commonly occurring bacterial

reference strains in the vaginal canal included *Enterococcus faecalis* (ATCC 29212) and *Gardnerella vaginalis* (ATCC 14018). The bacterial reference strain, *Peptostreptococcus anaerobius* (ATCC 27337), was included due to its status as a neglected pathogen. The yeast reference strains included *Candida albicans* (ATCC 10231), *Candida krusei* (ATCC 2159), *Candida glabrata* (ATCC 90030), and *Candida tropicalis* (ATCC 40042). Additionally, microbial colonizers (*Lactobacillus acidophilus* ATCC 314, *Lactobacillus fermentum* ATCC 9338, *Lactobacillus gasseri* ATCC 19992, *Lactobacillus plantarum* ATCC 8014, and *Lactobacillus rhamnosus* ATCC 7469) were chosen due to their relevance in the vaginal microbiome.

Regarding the bacterial culturing, *E. faecalis* was inoculated into Tryptone Soya broth (TSB) (Oxoid) and grown at 37°C for 24 hrs. The fastidious pathogens *G. vaginalis* and *P. anaerobius* were inoculated into Mueller Hinton broth supplemented with 5% sheep's blood and incubated at 37°C in a 5% CO_2 incubator. *Gardnerella vaginalis* required 48 hrs incubation, while *P. anaerobius* required three to five days. All *Candida* cultures were inoculated into TSB and incubated at 37°C for 48 hrs. The *Lactobacillus* colonizers were inoculated into De Man–Rogosa–Sharpe (MRS) broth (Oxoid) and incubated at 37°C for 24 hrs in a 5% CO_2 incubator. Each culture was streaked onto relevant agar to confirm the purity of the culture. The University of the Witwatersrand Human Research Ethics Committee (Reference W-CBP-230405-01) granted a waiver for the use of these micro-organisms.

Minimum Inhibitory Concentration (MIC)

The broth microdilution method was carried out for all 46 essential oils as described by Orchard et al .[30] The 96-well micro-titre plates were prepared by adding 100 µL of the respective broth into each of the wells. The essential oils (diluted in acetone to a concentration of 32.00 mg/mL) were added to the first row of the micro-titre plate at a volume of 100 µL, serially diluted, and then the relevant culture added (100 µL of a 0.5 McFarlands turbidity standard yielding an approximate concentration of 1×10^6 colony forming units (CFU)/mL). This was then added to the wells at a volume of 100 µL. Each micro-titre plate was sealed with a sterile adhesive sealing film to prevent loss of essential oil due to evaporation. The micro-titre plates were incubated at an appropriate temperature for a suitable duration depending on the growth requirements. After incubation, 40 µL *p*- iodonitrotetrazolium violet solution at a concentration of 0.04 mg/mL was added to each

well of the microtiter plate. The presence of a colour change to pink/purple was indicative of the presence of microbial growth. The lowest dilution with no colour change was considered the MIC value for that essential oil. A MIC value of ≤ 1.00 mg/mL was considered noteworthy.[31] For the microbial colonizers, MIC values ≥8 mg/mL were considered noteworthy, as the objective was to ensure little to no inhibition of the microbiota.

Based on the combinations recommended in the aromatherapeutic literature, the essential oils were tested at a 1:1 ratio, where a volume of 100 µL comprised of 50 µL of each oil used.[31] To interpret the interactive profile, the Fractional Inhibitory Concentration index (ΣFIC) was calculated:[11]

$$\text{FIC (i)} = \frac{\text{MIC of (a} *\text{)combined with (b} *\text{)}}{\text{MIC of (a) independently}}$$

$$\text{FIC (ii)} = \frac{\text{MIC of (b} *\text{)combined with (a} *\text{)}}{\text{MIC of (b) independently}}$$

*Where (a) is the MIC of the one essential oil in the combination and (b) is the MIC of the other.

Thereafter, the ΣFIC index was calculated as ΣFIC = FIC (i) + FIC (ii). The ΣFIC for each essential oil combination was interpreted as: ≤ 0.5 as synergy, > 0.5-≤1.0 being additive, > 1.0-≤ 4.0 indicative of indifference, and > 4.0 antagonism.[11] The inverse of this criterion was used when evaluating the natural microbiota where > 4.0 was synergy, > 1.0-≤ 4.0 additive, > 0.5-≤1.0 indifference, ≤ 0.5 antagonism, as exhibition rather than inhibition was favoured for the *Lactobacilli* spp.

A 100 µL volume of each positive, negative, and culture control was included in all assays for each micro-organism. The positive controls were incorporated to ensure antimicrobial susceptibility. The negative control (32.00 mg/mL water in acetone) was used to determine if the solvent exhibited an antimicrobial effect. The culture control in the respective broth was used to ensure that the media supported the growth of the pathogen. All antimicrobial studies were undertaken in triplicate, and the mean and standard deviation were recorded on Microsoft Excel Version 16.77.1.

Brine Shrimp Lethality Assay

The brine shrimp lethality assay is extensively employed as an initial screening method to evaluate the toxicity of various substances, including natural products and essential oils.[32] Following the assay protocol [33], sea salt weighing 32 g was dissolved in 1 L of distilled water. Dried, brine-shrimp (*Artemia franciscana*) eggs (Ocean Nutrition™) weighing 0.5 g were added to the artificial seawater. The eggs were incubated for 18-24 hrs at ambient temperature. Thereafter, 400 µL of saltwater containing live brine shrimp was added to each well of a 48-well microtiter plate. Essential oil (400 µL for single oils and 200 µL of each essential oil for combinations) was added to each well. Toxicity for all samples was tested at a concentration of 1.00 mg/mL. The tests were done in triplicate. The negative control used was salt water at a concentration of 32.00 g/L to ensure the simulated seawater supported growth. The positive control used was 1.6 mg/mL potassium dichromate. After 24 and 48 hrs, the plates were observed under a light microscope (Olympus) at 40 × magnification, and the dead shrimp were counted. Lastly, a lethal dose of 50 µL acetic acid (100% v/v) was added to the percentage mortality was calculated:

$$\%\ \text{Mortality} = \frac{\text{dead shrimp at 24 hours (or at 48 hours)(before acetic acid)} - \text{dead shrimp (time 0)}}{\text{dead shrimp after acetic acid}}\ \text{x } 100$$

For combinations, the fractional mortality was calculated using the same equation as the ΣFIC index.[11] Biological cytotoxicity was considered for a percentage mortality of 50% or greater.[34] The toxicity was deemed as the fractional mortality concentration (FMC). The FMC values were calculated using an adapted version of the ΣFIC index:

$$\text{FMC (i)} = \frac{\%\ \text{Mortality (a}*\text{)combined with (b}*\text{)}}{\%\ \text{Mortality (a) independently}}$$

$$\text{FMC (ii)} = \frac{\%\ \text{Mortality (b}*\text{) combined with (a}*\text{)}}{\%\ \text{Mortalit (b) independently}}$$

*Where (a) is the % mortality of the one essential oil in the combination and (b) is the % mortality of the other.

Thereafter, essential oils and combinations that displayed both noteworthy MIC values and showed no toxicity were further investigated for anti-biofilm activity.

Biofilm Studies

The method,[35,36] was used for the biofilm inhibition studies. Essential oil (100 µL if alone; or 50 µL: 50 µL of each essential oil for combinations) was added into the wells of a 96-well microtiter plate. The essential oils added were in varying concentrations (2.00 mg/mL, 1.00 mg/mL, and 0.50 mg/mL). The concentration range was chosen to determine the lowest concentration that could be utilized to inhibit biofilm formation.

Candida albicans and *C. glabrata* were selected for biofilm study due to their resilient biofilms which is heavily implicated in infections associated with the vaginal microbiome. Additionally, *C. glabrata*, while less studied, is also the second most prevalent pathogenic yeast in humans after *C. albicans.*[37-41] The positive control was Amphotericin B, and the negative control was water in acetone. A standardized solution containing approximately 1×10^6 CFU/mL of the cultures (*C. albicans* and *C. glabrata*) was added into 100 µL within a 96-well sterile microtitre plate and incubated at 37ºC for 4 hrs without shaking. Thereafter, the plates were incubated for a further 48 hrs. The 4-hour incubation period was performed to allow initial adhesion of *Candida* cells to the microtiter plate surface, which is a prerequisite step for biofilm establishment. This was then followed by a further 48-hour incubation period to enable maturation of the biofilm structure. Simply reporting a total of 52 hours would not capture this distinction, as the initial adhesion phase and subsequent biofilm development are discrete and biologically relevant stages in the standard biofilm assay methodology.

Biofilm biomass was then assayed using the modified crystal violet (CV) staining assay, which comprised washing the incubated plates with sterile water and oven-drying them at 60ºC for 45 min, after which the wells were stained with 100 µL of 1% crystal violet and incubated at room temperature for 15 min. To assess biofilm formation, 125 µL of ethanol was added to de-stain the wells. This was then transferred to a new plate, and the biofilm mass was quantified through absorbance at 590 nm using a microplate reader (Universal microplate reader ELX 800). The mean absorbance of the essential oils and percentage inhibition was determined using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Optical density (OD)culture control} - \text{OD experimental X 100 OD culture control}}{\text{(OD)culture control}}$$

Results were interpreted following established criteria.[42] Biofilm inhibition percentages ranging from 0.00% to 49.00% were considered indicative of poor inhibitory activity, while values between 50.00% and 100.00% were classified as demonstrating good biofilm inhibitory activity. The biofilm inhibition of the essential oils alone and in combination was carried out in triplicate, and the average and standard deviation of the values were recorded.

Results and Discussion

Chemical Analysis of Essential Oils

Table 1 provides the major compounds for each of the essential oils that were investigated in this study as obtained from the respective suppliers. It is known that a strong correlation exists between the chemical composition of the essential oils and their antimicrobial activity.[43] Including the chemical profiles is also a means of ensuring quality control; hence, including GC-MS analysis in antimicrobial studies is highly recommended.[44] The majority of the GC-MS data were consistent with previous studies referenced in Table 1; however, some variability was observed. These differences in the essential oils can be attributed to factors such as geographical location, batch, supplier, plant part used, harvest time, maturity, and altitude.[12]

Minimum Inhibitory Concentration of Single Essential Oils

Table 3.1 displays the average MIC values with standard deviation of the essential oils against the various bacterial and yeast pathogen reference strains, as well as the effect on the microbial colonizers (*Lactobacillus* spp.). On average, 35% of essential oils had noteworthy activity against the bacterial species (MIC $\leq$ 1.00 mg/mL), and 36% of essential oils had noteworthy activity against the yeast species.

The essential oils that exhibited broad-spectrum antibacterial activity included *M. officinalis, P. cablin, C. paradisi,* and *C. aurantium*. Those demonstrating broad-spectrum anti-

yeast activity (n=4) were *C. sativum*, *C. martini*, *C. aurantifolia*, *M. communis*, and *O. vulgare*. While *C. aurantifolia*, *C. paradisi*, *O. vulgare*, and *M. communis*, effectively inhibited six out of seven pathogens at a noteworthy MIC value (≤ 1.00 mg/mL). These also did not interfere with most of the *Lactobacilli* spp. growth. Thirteen essential oils had no substantial inhibitory effect on all *Lactobacilli* spp. tested. Best activities (0.13 mg/mL) were observed for *C. sativum* and *C. citratus* essential oils against the *Candida* reference strains.

Table 3.1. Chemical analysis of essential oils where major compounds are ≥10%

Essential oil (Scientific name)	Essential oil (Common name)	GC-MS (Major compounds)[1]	Previous studies (Major compounds)[1]	Reference
Boswellia carteri Birdw.	Frankincense	α- Pinene (44.18%) Limonene (12.22%)	α- Pinene (37.00%) Limonene (19.80%)	45
Cedrus atlantica G.Manetti	Cedarwood	α-Longipinene (41.90%) α-Himachalene (15.90%)	β-Himachalene (28.20%) α-Longipinene (21.26%)	46
Chamaemelum nobile All.	Roman chamomile	Methyl allyl angelate (20.23%)	Isobutyl angelate (37.22%) Methyl allyl angelate (11.85%) 2-Methylbutyl angelate (18.71%)	47
Cinnamomum camphora J. Presl	Camphor	Camphor (27.00%)	Camphor (36.81%)	48
Cinnamomum zeylanicum Garcin ex Blume	Cinnamon leaf	Cinnamaldehyde (56.97%)	Cinnamaldehyde (57.83%- 77.10%)	49, 50
Citrus aurantifolia (Christm.) M.Hiroe	Lime	Limonene (39.10%) α-Terpineol (16.40%) Terpinolene (11.20%)	Limonene (42.6-63.35%) α-Terpineol (16.5%) Terpinolene (11.80%)	51, 52
Citrus aurantium L.	Petigrain	Linalool (41.81%) Limonene (13.91%)	Linalool (43.2-65.97%) Linalyl acetate (0.77%-24.77%)	53
Citrus reticulata (Blanco) M.Hiroe	Mandarin	Limonene (93.8%)	Limonene (92.52-97.3%)	54
Citrus bergamia Risso	Bergamot	Linalool (29.57%) Limonene (29.10%) Linalyl acetate (26.82%)	Linalool (8.80-46.34%) Limonene (17.06%-37.20%) Linalyl acetate (17.69-30.10%)	55, 56
Citrus paradisi Macfad.	Grapefruit	Limonene (95.00%)	Limonene (95.1%)	57
Citrus × *sinesis* L. P. Osbeck	Orange	Limonene (93.5%)	Limonene (93.2%)	58
Commiphora myrrha (T.Nees) Engl.	Myrhh	Furanoeudesma-1,3-diene(49.43%) Curzerene (19.98%)	Furanoeudesma-1,3-diene (18.8%) Curzerene (11.64% - 34.65%) Lindestrene (12.9%)	59 60
Coriandrum sativum L.	Coriander seed	Linalool (29.76%) 2-Decenal (17.78%)	Linalool (36.69-72.35%) γ-Terpinene (0.1-13.6%)	61, 62
Cymbopogon citratus (hort. ex DC.) Stapf	Lemongrass	Geranial (36.99%)	Geranial (32.20%)	63

Essential oil (Scientific name)	Essential oil (Common name)	GC-MS (Major compounds)[1]	Previous studies (Major compounds)[1]	Reference
		Neral (29.67%)	Neral (25.70%)	
Cymbopogon martini Stapf	Palmarosa	Geraniol (77.80%)	Geraniol (76.77-94.03%)	64, 65
			Geranyl acetate (12.4%)	
Eucalyptus globulus Labill.	Bluegum eucalyptus	1,8-Cineole (63.6%)	1,8-Cineole (63.10%%)	66, 67
		α-Terpineol (10.5%)		
Eucalyptus radiata Sieber ex DC.	Narrow-leaved peppermint	1,8-Cineole (65.59%)	1,8-Cineole (65.70%)	68
		α-Terpineol (12.27%)	α-Terpineol (12.80%)	
Eugenia carophyllata C.Thunberg	Clove	Eugenol (70.67%)	Eugenol (75.80%)	69, 70
		Eugenyl acetate (19.21%)	Eugenyl acetate (18.98%)	
			β-Carophyllene (6.00-18.90%)	
Foeniculum vulgare Mill.	Fennel seed	Trans-anethole (80.61%)	Trans-anethole (84.00%)	71
Helichrysum angustifolium DC.	Immortelle	α-Pinene (22.39%)	α-Pinene (29.77%)	72
		γ-Curcumene (14.23%)	γ-Curcumene (18.80%)	73
Hyssopus officinalis L.	Hyssop	β-Pinocamphone (35.08%)	β-Pinocamphone (34.00%)	74
		β-Pinene (15.95%)	β-Pinene (10.10%)	30
Juniperus communis L.	Juniper	α-Pinene (44.00%)	α-Pinene (46.63%)	75
		β-Pinene (10.81%)	*β-Pinene* (10.70%)	76
Laurus nobilis L.	Laurel	1,8-Cineole (54.10%)	1,8-Cineole (55.00%)	77, 58
Lavandula angustifolia Mill.	English lavender	Linalyl acetate (41.89%)	Linalyl acetate (36.70%)	
		Linalool (26.42%)	Linalool (31.40%)	
			β-Carophyllene (10.20%)	
Melaleuca alternifolia Cheel	Tea tree	1,8-Cineole (56.70%)	Terpinen-4-ol (44.60%)	57
Melaleuca cajuputi Banks, Maton & Sm. ex R.Powell	Cajuput	Linalyl acetate (49.80%)	Linalyl acetate (49.80%)	43
		Benzyl alcohol (10.60%)	Benzyl alcohol (10.60%)	
Melaleuca quinquenervia (Cav.) S.T.Blake	Niaouli	1,8-Cineole (43.59%)	1,8-Cineole (0.1-76.00%)	78, 79
		α-Pinene (10.48%)	α-Pinene (13.73%)	
Melissa officinalis L.	Lemon balm	Citronellal (35.6%)	Citronellal (31.1%)	80
Mentha piperita L.	Peppermint	Menthol (44.51%)	Menthol (47.50%)	58
		Menthone (18.19%)	Menthone (18.60%)	
Myrtus communis L.	Myrtle	Estragole (74.20%)	Estragole (74.20%)	43
		Limonene (10.00%)	Limonene (10.00%)	
Ocimum sanctum L.	Holy basil	Estragole (74.61%)	Estragole (68.00%)	81, 43

Essential oil (Scientific name)	Essential oil (Common name)	GC-MS (Major compounds)[1]	Previous studies (Major compounds)[1]	Reference
		Linalool (17.78%)	Linalool (18.40%)	
Origanum majorana L.	Marjoram	Terpinene-4-ol (22.54%)	Terpinene-4-ol (16.80%)	82
		γ-Terpinene (15.14%)		
Origanum vulgare L.	Oregano	Carvacrol (62.73%)	Carvacrol (67.90)	43
			Patchouli alcohol (26.32%)	83, 58
Pogostemon cablin (Blanco) Benth.	Patchouli	Patchouli alcohol (30.80%)	δ- Guaiene (14.60%)	
		δ- Guaiene (18.04%)	α- Guaiene (12.50%)	
		α- Guaiene (14.98%)		
				57
Pelargonium graveolens L'Hér.	Geranium	Dihydrogeraniol (27.77%)	Dihydrogeraniol (26.70%)	
		Geraniol (14.09%)	Geraniol (14.09%)	
Pinus sylvestris L.	Pine	Bornyl acetate (34.8%)	Bornyl acetate (42.30%)	58
		α-Pinene (11.10%)	α-Pinene (11.00%)	
		δ-3-Carene (12.60%)		
Pistacia lentiscus L.	Mastic	Myrcene (20.2%)	Myrcene (36.92%)	84
		α-Pinene (18.70%)	α-Pinene (18.72%)	
		Limonene (14.50%)		
Rosa damascena Mill.	Damask rose	Citronellol (34.06%	Citronellol (28.13%)	85, 43
		Geraniol (14.29%)	Geraniol (18.29%)	
		Nerol (10.20%)		
Rosmarinus officinalis L.	Rosemary	Camphor (19.8%)	Camphor (8.87%)	86
Salvia sclarea L.	Clary sage	Linalyl acetate (66.22%)	Linalyl acetate (69.40%)	87
		Linalool (14.30%)		
Salvia officinalis L.	Sage	Linalyl acetate (58.5%)	Linalyl acetate (58.5%)	43
		Camphor (19.8%)	Camphor (19.8%)	
Santalum album L.	Sandalwood	α-Santalol (32.1%)	α-Santalol (≥43%)	88
		4,5,9,10 Dehydroisolongifolene (12.3%)		
		cis-α-Santalol (11.3%)		
Styrax benzoin (Hayne) P.W.Fritsch	Benzoin	Eugenol (81.90%)	Eugenol (81.90%)	43
		Dimethyl phthalate (72.20%)		

Essential oil (Scientific name)	Essential oil (Common name)	GC-MS (Major compounds)[1]	Previous studies (Major compounds)[1]	Reference
Thymus vulgaris L.	Thyme	Thymol (46.61%) *p*-Cymene (17.38%)	Thymol (44.97%)	89
Vetiveria zizanioides Stapf	Vetiver	Khusenique acid (25.02%)	Zizanol (12.80%)	31

Origanum vulgare had the best broad-spectrum activity, having noteworthy inhibitory MIC values against six out of the seven pathogen reference strains tested and minimal to no inhibition against most of the natural microbiota, highlighting the potential for future *in vivo* studies. The anti-*lactobacillus* effects of *O. vulgare* correspond with several previous studies.[90-92]

Specific MIC values of the essential oils tested against *P. anaerobius* were not found in the literature. The lack of studies may be due to the challenges in growing this fastidious pathogen and the absence of a standardized growth protocol. This gap highlights a broader issue where the clinical relevance of neglected vaginal pathogens is often understudied. Despite these challenges, 33% of the essential oils tested in this study exhibited noteworthy antimicrobial activity against *P. anaerobius*. This finding emphasizes the importance of including neglected pathogens in antimicrobial research, particularly in the context of vaginal infections, where they may contribute to persistent or recurrent symptoms. Addressing these overlooked organisms is essential for developing more comprehensive and effective treatment strategies.

Minimum Inhibitory Concentration of Essential Oil Combinations against Pathogens

A total of 602 essential oil combinations were tested (86 combinations against seven pathogenic and five microbial colonizer reference strains), where the average MIC and ΣFIC values were recorded (Table 3.2). Among the interactions, 101 exhibited synergy, 39 were additive, 88 were non-interactive, and 30 were antagonistic against the bacterial reference strains. Notably, *E. faecalis* was highly susceptible to most combinations, despite showing higher MIC values when investigated with single essential oils. *Gardnerella vaginalis* and *P. anaerobius* showed low levels of susceptibility to the combinations, which emphasises the strain's resilience.

There were 48 combinations that resulted in synergy against the yeast pathogens. Additionally, 127 combinations were additive, 124 were non-interactive, and only 45 were antagonistic. *Candida albicans* was the most susceptible, while *C. glabrata* was the least susceptible pathogen to inhibition by the essential oil combinations. Broad-spectrum synergistic activity was mostly observed where the essential oils *S. officinalis* was combined with *O. sanctum,* and *C. camphora* was combined with *M. cajuputi*. The lowest ΣFIC values and hence the best synergy observed were recorded for *C. bergamia* with *B. carteri* and *C. bergamia* with *C. atlantica* against *E. faecalis* (ΣFIC value of 0.07).

Table 3.2. MIC values (mg/mL) against bacterial and yeast pathogens and beneficial microbiota

Essential Oil	*EF*[1]	*GV*	*PA*	*CA*	*CK*	*CG*	*CT*	*LA*[2]	*LF*	*LG*	*LP*	*LR*
B. carteri	4.00 (± 0.00)	1.20 (± 0.45)	3.67 (± 0.75)	2.33 (± 0.82)	2.67 (± 1.03)	1.60 (± 0.49)	1.31 (± 0.56)	**1.67 (± 0.52)**[3]	**4.00 (± 0.00)**	**>8.00 (± 0.00)**	**2.33 (± 0.82)**	**2.67 (± 1.03)**
C. atlantica	4.00 (± 0.00)	4.67 (± 1.63)	1.50 (± 0.50)	3.00 (± 1.10)	1.83 (± 0.41)	1.60 (± 0.49)	1.50 (± 0.50)	**1.60 (± 0.55)**	1.00 (± 0.00)	**1.60 (± 0.55)**	**1.80 (± 0.45)**	**1.67 (± 0.52)**
C. aurantifolia	**0.50 (± 0.00)**	2.00 (± 0.00)	**0.50 (± 0.00)**	**0.50 (± 0.00)**	**0.75 (± 0.35)**	**0.50 (± 0.00)**	**0.25 (± 0.00)**	0.60 (± 0.22)	**2.00 (± 0.00)**	1.00 (± 0.00)	**4.00 (± 0.00)**	**4.00 (± 1.15)**
C. aurantium	**1.00 (± 0.00)**	**0.46 (± 0.10)**	**1.00 (± 0.00)**	**1.00 (± 0.00)**	2.00 (± 0.00)	**1.00 (± 0.00)**	1.40 (± 0.49)	1.00 (± 0.00)	1.00 (± 0.00)	**2.00 (± 0.00)**	1.00 (± 0.00)	1.00 (± 0.00)
C. bergamia	3.33 (± 1.03)	3.20 (± 1.10)	5.33 (± 2.87)	**1.00 (± 0.00)**	1.33 (± 0.52)	**1.00 (± 0.00)**	1.17 (± 0.37)	**1.33 (± 0.52)**	**1.33 (± 0.52)**	**5.60 (± 2.19)**	**7.00 (± 2.00)**	**8.00 (± 0.00)**
C. camphora	3.67 (± 0.82)	5.33 (± 2.07)	1.67 (± 2.21)	1.67 (± 0.52)	**0.75 (± 0.29)**	1.50 (± 0.50)	4.00 (± 0.00)	**1.17 (± 0.41)**	1.00 (± 0.00)	**>8.00 (± 0.00)**	**3.33 (± 1.03)**	**2.00 (± 0.00)**
C. citratus	1.33 (± 0.52)	4.00 (± 0.00)	1.33 (± 0.47)	**0.13 (± 0.00)**	**0.54 (± 0.25)**	**0.67 (± 0.24)**	2.33 (± 0.75)	0.60 (± 0.22)	1.00 (± 0.00)	**>8.00 (± 0.00)**	**5.33 (± 2.07)**	**8.00 (± 0.00)**
C. martini	1.60 (± 0.55)	4.00 (± 0.00)	1.67 (± 0.47)	**0.25 (± 0.19)**	**0.70 (± 0.27)**	**0.70 (± 0.24)**	**0.30 (± 0.10)**	**1.40 (± 0.55)**	**1.40 (± 0.55)**	0.90 (± 0.22)	**3.60 (± 0.89)**	0.90 (± 0.22)
C. myrrha	1.40 (± 0.55)	5.60 (± 2.19)	2.00 (± 0.00)	2.40 (± 0.89)	1.40 (± 0.55)	2.00 (± 0.00)	3.33 (± 0.94)	**8.00 (± 0.00)**	0.80 (± 0.27)	**>8.00 (± 0.00)**	3.60 (± 0.89)	1.00 (± 0.00)
C. nobile	**1.00 (± 0.00)**	2.00 (± 0.00)	**1.00 (± 0.00)**	4.00 (± 0.00)	**0.58 (± 0.20)**	**0.63 (± 0.22)**	**0.63 (± 0.22)**	0.50 (± 0.00)	**3.50 (± 1.00)**	1.00 (± 0.00)	**4.00 (± 0.00)**	**2.00 (± 0.00)**
C. paradisi	**0.50 (± 0.00)**	**0.75 (± 0.35)**	**0.25 (± 0.00)**	**1.00 (± 0.00)**	1.44 (± 2.38)	**0.38 (± 0.13)**	**0.75 (± 0.25)**	**1.33 (± 0.52)**	**1.50 (± 0.71)**	**2.00 (± 0.00)**	**2.00 (± 0.00)**	1.00 (± 0.00)
C. reticulata	**0.50 (± 0.00)**	2.00 (± 0.00)	2.50 (± 0.87)	2.67 (± 1.15)	2.50 (± 1.00)	**0.67 (± 0.24)**	**0.75 (± 0.25)**	0.45 (± 0.11)	**1.67 (± 0.58)**	**8.00 (± 0.00)**	**>8.00 (± 0.00)**	2.00 (± 0.00)
C. sativum	**1.00 (± 0.00)**	4.00 (± 0.00)	4.00 (± 0.00)	**0.13 (± 0.00)**	**0.13 (± 0.00)**	**0.50 (± 0.25)**	**0.19 (± 0.06)**	0.83 (± 0.26)	0.75 (± 0.29)	0.70 (± 0.27)	2.00 (± 0.00)	1.00 (± 0.00)
C. sinesis	**1.00 (± 0.00)**	**0.88 (± 0.25)**	2.00 (± 0.00)	4.00 (± 0.00)	**0.42 (± 0.14)**	**0.75 (± 0.25)**	**0.75 (± 0.25)**	0.50 (± 0.00)	1.00 (± 0.00)	**>8.00 (± 0.00)**	**>8.00 (± 0.00)**	**4.00 (± 0.00)**
C. zeylanicum	1.67 (± 0.52)	2.17 (± 0.98)	**0.18 (± 0.06)**	**0.95 (± 1.40)**	**0.46 (± 0.10)**	**0.80 (± 0.24)**	1.33 (± 0.47)	0.90 (± 0.22)	**1.20 (± 0.45)**	**2.00 (± 0.00)**	**1.80 (± 0.45)**	**1.40 (± 0.55)**
E. carophyllata	1.33 (± 0.52)	3.33 (± 1.03)	**0.58 (± 0.19)**	**0.38 (± 0.14)**	**0.25 (± 0.00)**	**0.83 (± 0.24)**	1.33 (± 0.47)	**3.00 (± 1.15**)	**1.50 (± 0.55)**	**1.33 (± 0.52)**	**1.67 (± 0.52)**	**1.33 (± 0.52)**
E. globulus	1.33 (± 0.52)	3.00 (± 1.15)	3.20 (± 0.98)	1.33 (± 0.52)	1.40 (± 0.55)	1.80 (± 0.40)	**0.25 (± 0.00)**	**1.20 (± 0.45)**	**1.67 (± 0.52)**	**>8.00 (± 0.00)**	**8.00 (± 0.00)**	1.00 (± 0.00)
E. radiata	2.50 (± 1.00)	4.67 (± 2.73)	>8.00 (± 0.00)	**1.00 (± 0.00)**	1.25 (± 0.50)	1.75 (± 0.43)	1.50 (± 0.50)	**>8.00 (± 0.00)**	**3.00 (± 1.15)**	**3.50 (± 1.00)**	**6.00 (± 2.31)**	**1.20 (± 0.45)**
F. vulgare	8.00 (± 0.00)	3.50 (± 1.00)	8.00 (± 3.42)	**0.75 (± 0.29)**	2.00 (± 0.00)	8.00 (± 0.00)	1.75 (± 0.43)	**1.33 (± 0.52)**	**2.00 (± 0.00)**	**3.50 (± 1.00)**	**2.00 (± 0.00)**	**1.20 (± 0.45)**
H. augustifolium	2.67 (± 1.03)	6.00 (± 2.31)	1.83 (± 0.72)	**0.83 (± 0.26)**	2.33 (± 0.82)	1.67 (± 0.47)	**1.00 (± 0.00)**	1.00 (± 0.00)	**2.67 (± 1.03)**	**2.00 (± 0.00)**	**>8.00 (± 0.00)**	**1.67 (± 0.52)**
H. officinalis	2.00 (± 0.00)	1.75 (± 3.49)	2.00 (± 0.00)	**1.00 (± 0.00)**	1.50 (± 0.58)	1.40 (± 0.49)	1.83 (± 0.37)	**8.00 (± 0.00)**	**2.00 (± 0.00)**	**2.00 (± 0.00)**	**2.33 (± 0.82)**	**1.40 (± 0.55)**
J. communis	2.67 (± 1.03)	6.40 (± 2.19)	1.50 (± 0.50)	1.17 (± 0.41)	2.00 (± 0.00)	2.67 (± 0.94)	4.00 (± 0.00)	**2.80 (± 2.95)**	1.00 (± 0.00)	**5.00 (± 2.45)**	**2.67 (± 1.03)**	**1.67 (± 0.52)**
L. angustifolia	2.33 (± 0.82)	**1.00 (± 0.00)**	2.00 (± 0.00)	1.60 (± 0.55)	1.80 (± 0.45)	2.00 (± 1.00)	**1.00 (± 0.00)**	**3.00 (± 1.15)**	**1.67 (± 0.52)**	**5.33 (± 2.07)**	**6.00 (± 2.19)**	**2.00 (± 1.10)**
L. latifolia	2.00 (± 0.00)	**0.25 (± 0.00)**	**0.92 (± 0.19)**	**1.00 (± 0.00)**	1.50 (± 0.58)	2.50 (± 0.87)	3.00 (± 1.00)	**4.00 (± 0.00)**	1.00 (± 0.00)	**2.00 (± 0.00)**	**3.50 (± 1.00)**	**1.60 (± 0.55)**
L. nobilis	3.00 (± 1.15)	6.00 (± 2.31)	**0.88 (± 0.22)**	1.25 (± 0.50)	1.75 (± 0.50)	1.50 (± 0.50)	1.25 (± 0.43)	**2.67 (± 1.03)**	1.00 (± 0.00)	**>8.00 (± 0.00)**	**3.50 (± 1.00)**	**2.00 (± 0.00)**
M. alternifolia	2.00 (± 0.00)	**0.75 (± 0.29)**	2.00 (± 0.00)	1.33 (± 0.52)	**1.00 (± 0.55)**	1.60 (± 0.49)	**0.25 (± 0.00)**	**1.40 (± 0.55)**	**1.50 (± 0.55)**	**4.00 (± 0.00)**	**5.60 (± 2.19)**	**1.67 (± 0.52)**
M. cajuputi	2.80 (± 1.10)	**0.70 (± 0.27)**	1.60 (± 0.49)	1.33 (± 0.52)	1.80 (± 0.45)	1.80 (± 0.40)	1.83 (± 0.37)	**1.67 (± 0.52)**	1.00 (± 0.00)	**3.20 (± 1.10)**	**3.20 (± 1.10)**	**1.60 (± 0.55)**
M. communis	**0.63 (± 0.25)**	**1.00 (± 0.00)**	8.00 (± 3.88)	**0.67 (± 0.29)**	**0.33 (± 0.14)**	**0.50 (± 0.00)**	**0.67 (± 0.24)**	0.50 (± 0.50)	1.00 (± 0.00)	**2.00 (± 0.00)**	**2.00 (± 0.00)**	**8.00 (± 0.00)**
M. officinalis	**1.00 (± 0.00)**	**0.92 (± 0.20)**	**0.15 (± 0.05)**	**0.83 (± 0.26)**	3.33 (± 1.15)	1.00 (± 0.00)	**0.21 (± 0.06)**	**>8.00 (± 0.00)**	1.00 (± 0.00)	**>8.00 (± 0.00)**	0.50 (± 0.00)	**8.00 (± 0.20)**
M. piperita	1.75 (± 0.50)	**0.25 (± 0.00)**	**1.00 (± 0.00)**	**1.00 (± 0.00)**	1.50 (± 0.58)	1.50 (± 0.50)	1.75 (± 0.43)	0.18 (± 0.07)	1.00 (± 0.00)	1.00 (± 0.00)	**6.00 (± 2.31)**	1.50 (± 0.58)
M. quinquenervia	**1.00 (± 0.00)**	>8.00 (± 0.00)	2.00 (± 0.00)	3.00 (± 1.15)	2.33 (± 0.82)	**0.58 (± 0.19)**	**1.00 (± 0.00)**	**2.80 (± 1.10)**	**>8.00 (± 0.00)**	**2.00 (± 0.00)**	**4.00 (± 0.00)**	**8.00 (± 0.00)**

Essential Oil	*EF*[1]	*GV*	*PA*	*CA*	*CK*	*CG*	*CT*	*LA*[2]	*LF*	*LG*	*LP*	*LR*
O. majorana	2.00 (± 0.00)	>8.00 (± 0.00)	8.00 (± 0.00)	1.17 (± 0.41)	1.40 (± 0.55)	2.40 (± 0.80)	1.67 (± 0.47)	**2.33 (± 0.82)**	**>8.00 (± 0.00)**	**3.60 (± 2.52)**	**8.00 (± 0.00)**	**2.40 (± 0.89)**
O. sanctum	1.67 (± 0.52)	1.80 (± 0.41)	6.00 (± 3.15)	**0.92 (± 0.20)**	**0.80 (± 0.27)**	1.17 (± 0.37)	1.17 (± 0.37)	0.92 (± 0.00)	**1.33 (± 0.52)**	1.00 (± 0.00)	**3.00 (± 1.10)**	**1.83 (± 0.41)**
O. vulgare	1.33 (± 0.52)	**0.50 (± 0.00)**	**0.50 (± 0.00)**	**0.83 (± 0.26)**	**0.25 (± 0.00)**	**0.40 (± 0.12)**	**0.50 (± 0.00)**	**>8.00 (± 0.00)**	1.00 (± 0.00)	**4.00 (± 0.00)**	**2.00 (± 0.00)**	**2.00 (± 0.00)**
P. cablin	**0.63 (± 0.25)**	**0.50 (± 0.00)**	**0.25 (± 0.00)**	1.50 (± 0.58)	2.67 (± 1.15)	1.67 (± 0.47)	**0.50 (± 0.00)**	1.00 (± 0.00)	0.33 (± 0.14)	**2.00 (± 0.00)**	1.00 (± 0.00)	**2.00 (± 0.00)**
P. graveolens	1.50 (± 0.55)	**0.83 (± 0.26)**	1.60 (± 3.00)	**0.75 (± 0.27)**	1.17 (± 0.41)	1.33 (± 0.47)	**0.75 (± 0.25)**	1.00 (± 0.00)	0.83 (± 0.26)	**1.83 (± 0.41)**	**1.80 (± 0.45)**	**1.50 (± 0.58)**
P. lentiscus	2.00 (± 0.00)	**0.50 (± 0.00)**	2.00 (± 0.00)	**1.00 (± 0.00)**	1.40 (± 0.55)	1.75 (± 0.43)	1.50 (± 0.50)	**1.50 (± 0.58)**	**2.00 (± 0.00)**	**>8.00 (± 0.00)**	**8.00 (± 0.00)**	**2.00 (± 0.00)**
P. sylvestris	1.67 (± 0.52)	**1.00 (± 0.00)**	6.67 (± 1.89)	**0.50 (± 0.00)**	**0.83 (± 0.26)**	1.33 (± 0.47)	**0.92 (± 0.19)**	**>8.00 (± 0.00)**	**1.17 (± 0.41)**	**6.00 (± 2.19)**	**2.67 (± 1.03)**	**1.30 (± 0.67)**
R. damascena	2.00 (± 0.00)	1.40 (± 0.55)	**1.00 (± 0.00)**	**1.00 (± 0.00)**	1.83 (± 1.17)	1.40 (± 0.49)	**0.15 (± 0.05)**	**2.17 (± 1.47)**	**1.80 (± 0.45)**	**1.80 (± 0.45)**	**3.50 (± 1.00)**	**2.00 (± 0.00)**
R. officinalis	2.00 (± 0.00)	**0.50 (± 0.00)**	**0.42 (± 3.75)**	0.54 (± 0.25)	2.50 (± 1.00)	1.20 (± 0.40)	1.83 (± 0.37)	**3.00 (± 1.15)**	**1.67 (± 0.52)**	**8.00 (± 0.00)**	**8.00 (± 0.00)**	**>8,00 (± 0.00)**
S. album	**0.19 (± 0.07)**	**0.45 (± 0.13)**	1.67 (± 3.00)	>8.00 (± 0.00)	5.33 (± 2.07)	3.00 (± 1.00)	2.00 (± 0.00)	**3.33 (± 1.03)**	0.83 (± 0.26)	**1.67 (± 0.52)**	0.83 (± 0.26)	**4.00 (± 0.00)**
S. benzoin	1.67 (± 0.52)	**0.90 (± 0.22)**	2.00 (± 0.00)	**0.70 (± 0.27)**	1.50 (± 0.55)	3.00 (± 1.00)	1.80 (± 0.40)	0.35 (± 0.14)	**1.80 (± 0.45)**	**1.75 (± 0.50)**	**6.40 (± 2.19)**	**2.00 (± 0.00)**
S. officinalis	4.00 (± 0.00)	3.00 (± 1.15)	8.00 (± 0.00)	1.50 (± 0.55)	2.33 (± 0.82)	1.60 (± 0.49)	1.33 (± 0.47)	**5.33 (± 2.07)**	1.67 (± 0.52)	**2.00 (± 0.00)**	**>8.00 (± 0.00)**	**2.00 (± 0.00)**
S. sclarea	1.50 (± 0.58)	**1.00 (± 0.00)**	**1.00 (± 0.00)**	8.00 (± 0.00)	>8.00 (± 0.00)	8.00 (± 0.00)	3.00 (± 1.00)	1.00 (± 0.00)	**2.00 (± 0.00)**	**2.00 (± 0.00)**	**2.00 (± 0.00)**	**>8,00 (± 0.00)**
T. vulgaris	2.00 (± 0.00)	1.67 (± 0.52)	**1.00 (± 3.11)**	1.25 (± 0.50)	1.40 (± 0.55)	1.17 (± 0.37)	1.17 (± 0.37)	**4.00 (± 0.00)**	**1.67 (± 0.52)**	**2.00 (± 0.00)**	**3.33 (± 1.03)**	**2.00 (± 0.00)**
V. zizanioides	**0.67 (± 0.26)**	1.67 (± 0.52)	2.00 (± 0.71)	2.50 (± 1.00)	**0.75 (± 0.29)**	1.60 (± 0.49)	**0.23 (± 0.05)**	**1.67 (± 0.52)**	0.42 (± 0.13)	0.50 (± 0.00)	**1.50 (± 0.55)**	0.42 (± 0.13)
Controls												
Ampicillin	0.63 mg/mL	ND[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metronidazole	ND	2.00 µg/mL	4.00 µg/mL	ND	ND	ND	ND	ND	ND	ND	ND	ND
Amphotericin B	ND	ND	ND	1.00 µg/mL	0.63 µg/mL	2.00 µg/mL	0.50 µg/mL	ND	ND	ND	ND	ND
Erythromycin	ND	ND	ND	ND	ND	ND	ND	0.078 µg/mL	0.25 µg/mL	0.50 µg/mL	0.125 µg/mL	1.00 µg/mL
Culture control	+	_+	+	+	+	+	+	+	+	+	+	+

[1]: ***EF***= *Enterococcus faecalis* (ATCC 29212); ***GV***= *Gardnerella vaginalis* (ATCC 14018); ***PA***= *Peptostreptococcus anaerobius* (ATCC 27337); ***CA***= *Candida albicans* (ATCC 10231)*;* ***CK***= *Candida krusei* (ATCC 2159); ***CG***= *Candida glabrata* (ATCC 90030)*;* ***CT***= *Candida tropicalis* (ATCC 40042); ***LA***= *Lactobacillus acidophilus* (ATCC 314); ***LF***= *Lactobacillus fermentum* (ATCC 9338); ***LG***= *Lactobacillus gasseri* (ATCC 19992); ***LP***= *Lactobacillus plantarum* (ATCC 8014) and ***LR***= *Lactobacillus rhamnosus* (ATCC 7469).

[2] **Vaginal microbiota** values shaded in to distinguish between pathogens and microbiota. [3]. The noteworthy inhibitory MIC values for pathogens are in bold, and the MIC values demonstrating little to no inhibition for the *lactobacilli* are also indicated in bold in the shaded area [4]ND not determined as the most appropriate positive control was used against each pathogen.

The same ΣFIC values were also recorded for the combination *O. vulgare* with *O. sanctum* against *P. anaerobius*. The lowest ΣFIC value (0.06) was observed for the combination *M. quinquenervia* with *E. globulus* when tested against *G. vaginalis*.

Minimum Inhibitory Concentration of Essential Oil Combinations when tested against vaginal biota

Table 3.3 shows the average MIC and ΣFIC values of the essential oil combinations tested on the *Lactobacilli* found in the vagina. Regarding the combinations that displayed noteworthy antimicrobial activity against the pathogens (Table 3.4), it is interesting to note that *O. vulgare* with either *C. reticulata, O. sanctum*, or *R. officinalis* did not inhibit most of the *Lactobacilli* spp. In terms of combinations with the best synergistic profiles and overall best activity, *C. camphora* with *M. cajuputi, C. camphora* with *O. sanctum*, and *S. officinalis* with *O. sanctum* did not inhibit the growth of most of the *Lactobacillus* spp. Overall, 33% of essential combinations did not inhibit any of the *Lactobacillus* spp. demonstrating a positive effect on the vaginal microbiome. The vaginal microbiota plays a pivotal role in maintaining urogenital health, primarily through the dominance of beneficial *Lactobacillus* species such as *L. crispatus* and *L. gasseri*. These species contribute to the maintenance of a low vaginal pH and inhibit the proliferation of pathogenic organisms via lactic acid beneficial *Lactobacillus* species such as *L. crispatus* and *L. gasseri*. These species contribute to the maintenance of a low vaginal pH and inhibit the proliferation of pathogenic organisms via lactic acid production and competitive exclusion.[93] Preserving this microbial equilibrium is essential in the development of novel therapeutic agents for vaginal infections.

Essential oils that exhibited noteworthy antimicrobial activity against vaginal pathogens while exerting minimal inhibitory effects on *Lactobacillus* spp. can be identified as promising candidates for further *in vivo* studies. This selective antimicrobial profile supports the potential for therapeutic applications that effectively treat infections without compromising the integrity of the native microbiota.

Table 3.3. Average MIC (mg/mL) and ΣFIC values for essential oil combinations against pathogen reference strains

Essential oil combination		*E. faecalis*		*G. vaginalis*		*P. anaerobius*		*C. albicans*		*C. krusei*		*C. glabrata*		*C. tropicalis*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
C. camphora	*C. nobile*	**0.25 (± 0.00)**[1]	***0.16***[2]	**0.50 (± 0.00)**	***0.17***	**0.75 (± 0.25)**	0.51	2.00 (± 0.00)	0.85	**0.50 (± 0.00)**	0.76	**1.00 (± 0.00)**	1.13	**0.75 (± 0.35)**	0.69
	L. angustifolia	**0.25 (± 0.00)**	***0.09***	**0.75 (± 0.35)**	***0.45***	4.00 (± 0.00)	2.20	2.00 (± 0.00)	1.22	**0.75 (± 0.35)**	0.71	**1.00 (± 0.00)**	***0.50***	**0.75 (± 0.35)**	***0.47***
	M. cajuputi	**0.25 (± 0.00)**	***0.08***	2.00 (± 0.00)	1.62	**0.38 (± 0.13)**	***0.23***	**0.50 (± 0.00)**	***0.34***	**0.38 (± 0.18)**	***0.35***	**1.00 (± 0.00)**	0.61	**0.63 (± 0.53)**	***0.25***
	M. officinalis	**0.25 (± 0.00)**	***0.16***	**1.00 (± 0.00)**	0.64	**0.31 (± 0.11)**	1.14	**1.00 (± 0.00)**	0.90	**0.25 (± 0.00)**	***0.20***	**1.00 (± 0.00)**	0.83	**0.25 (± 0.00)**	0.63
	O. sanctum	**0.25 (± 0.00)**	***0.11***	4.00 (± 0.00)	1.49	**0.38 (± 0.13)**	***0.14***	**0.50 (± 0.00)**	***0.42***	**0.38 (± 0.18)**	***0.48***	**1.00 (± 0.00)**	0.76	**0.38 (± 0.18)**	***0.21***
C. bergamia	*B. carteri*	**0.25 (± 0.00)**	***0.07***	4.00 (± 0.00)	2.29	>8.00 (± 0.00)	>1.89	**0.50 (± 0.00)**	***0.36***	2.00 (± 0.00)	1.13	**1.00 (± 0.00)**	0.81	**0.75 (± 0.35)**	0.61
	C. atlantica	**0.25 (± 0.00)**	***0.07***	4.00 (± 0.00)	1.09	4.00 (± 0.00)	1.96	**1.00 (± 0.00)**	0.67	**1.00 (± 0.00)**	0.63	**1.00 (± 0.00)**	0.51	**0.25 (± 0.00)**	0.51
	E. caryophyllata	**0.25 (± 0.00)**	***0.13***	**1.00 (± 0.00)**	***0.31***	>8.00 (± 0.00)	>7.10	**0.63 (± 0.25)**	1.13	**0.75 (± 0.35)**	1.78	**1.00 (± 0.00)**	1.10	**0.38 (± 0.18)**	***0.30***
	L. angustifolia	**0.38 (± 0.18)**	***0.14***	3.00 (± 1.41)	1.97	1.50 (± 0.50)	0.50	**0.75 (± 0.29)**	0.61	1.50 (± 0.71)	0.98	**1.00 (± 0.00)**	0.75	**1.00 (± 0.00)**	0.93
	P. cablin	**1.00 (± 0.00)**	0.94	**1.00 (± 0.00)**	1.16	8.00 (± 0.00)	16.75	**0.63 (± 0.25)**	0.50	**0.50 (± 0.00)**	***0.28***	**0.50 (± 0.00)**	***0.38***	**0.44 (± 0.13)**	0.62
	S. album	**0.50 (± 0.00)**	1.39	4.00 (± 0.00)	5.07	6.00 (± 2.00)	2.36	**1.00 (± 0.00)**	>0.506	2.00 (± 0.75)	0.94	1.50 (± 0.71)	1.00	**0.50 (± 0.00)**	***0.34***
	S. benzoin	**0.25 (± 0.00)**	***0.11***	**0.50 (± 0.00)**	***0.36***	<0.13 (± 0.00)	<0. 04	**0.25 (± 0.00)**	***0.30***	**0.50 (± 0.00)**	***0.35***	>8.00 (± 0.00)	>5.33	**0.50 (± 0.00)**	***0.35***
E. globulus	*L. angustifolia*	**0.25 (± 0.00)**	***0.15***	0.75 (± 0.35)	***0.50***	3.50 (± 0.87)	1.42	>8.00 (± 0.00)	>5.51	**0.38 (± 0.18)**	***0.24***	**0.75 (± 0.35)**	***0.40***	**1.00 (± 0.00)**	2.50
	M. alternifolia	**0.25 (± 0.00)**	***0.16***	4.00 (± 0.00)	3.33	3.00 (± 1.00)	1.22	2.00 (± 0.00)	>1.50	**0.50 (± 0.00)**	***0.43***	**1.00 (± 0.00)**	0.51	**0.25 (± 0.00)**	1.00
	M. officinalis	**0.38 (± 0.18)**	***0.28***	**1.00 (± 0.00)**	***0.29***	3.00 (± 1.00)	1.51	>8.00 (± 0.00)	>3.32	**1.00 (± 0.00)**	1.28	**0.50 (± 0.00)**	***0.50***	2.00 (± 0.00)	4.43
	O. marjoram	**0.50 (± 0.00)**	***0.31***	>8.00 (± 0.00)	>1. 83	8.00 (± 0.00)	1.75	>8.00 (± 0.00)	>6.43	**0.75 (± 0.35)**	0.50	2.00 (± 2.00)	0.97	2.00 (± 0.00)	4.60
	P. sylvestris	**0.50 (± 0.00)**	***0.34***	8.00 (± 0.00)	5.33	6.00 (± 2.00)	1.39	4.00 (± 0.00)	>5.5	**0.50 (± 0.00)**	***0.48***	**1.00 (± 0.00)**	0.65	**0.50 (± 0.00)**	1.27
	T. vulgaris	**0.25 (± 0.00)**	***0.16***	6.00 (± 2.82)	2.80	8.00 (± 0.00)	5.25	3.00 (± 1.41)	>2.33	**1.00 (± 0.00)**	0.71	**0.75 (± 0.35)**	***0.50***	**1.00 (± 0.00)**	2.43
	B. carteri	**0.50 (± 0.00)**	***0.16***	4.00 (± 0.00)	2.00	8.00 (± 0.00)	3.28	1.00 (± 0.00)	0.82	3.00 (± 1.41)	1.21	**0.75 (± 0.35)**	***0.46***	**1.00 (± 0.00)**	0.88
H. angustifolium	*C. aurantium*	**1.00 (± 0.00)**	0.69	4.00 (± 0.00)	4.68	**0.50 (± 0.00)**	***0.39***	1.50 (± 0.508)	1.65	2.00 (± 0.00)	0.93	**1.00 (± 0.00)**	0.80	**1.00 (± 0.00)**	0.86
	C. bergamia	**0.75 (± 0.35)**	***0.39***	**1.00 (± 0.00)**	***0.24***	**1.00 (± 0.00)**	***0.37***	**0.63 (± 0.43)**	0.69	2.00 (± 0.00)	1.18	2.00 (± 0.00)	1.51	**0.50 (± 0.00)**	***0.46***
	C. nobile	**0.50 (± 0.00)**	***0.21***	3.00 (± 1.15)	1.00	**0.25 (± 0.00)**	***0.19***	**0.75 (± 0.29)**	***0.50***	**0.50 (± 0.00)**	***0.50***	2.00 (± 0.75)	2.19	**0.75 (± 0.35)**	0.97
	C. reticulata	**0.75 (± 0.35)**	0.89	3.00 (± 1.15)	**1.00**	3.00 (± 1.00)	1.42	**1.00 (± 0.00)**	0.79	4.00 (± 0.00)	1.66	**1.00 (± 0.00)**	1.05	**1.00 (± 0.00)**	1.17
	C. sinesis	**0.00 (± 0.00)**	0.69	4.00 (± 0.00)	2.61	8.00 (± 0.00)	4.19	3.00 (± 1.15)	2.18	1.00 (± 0.00)	1.41	**1.00 (± 0.00)**	0.97	**0.38 (± 0.18)**	***0.44***
	L. angustifolia	**0.50 (± 0.00)**	***0.20***	2.5 (± 1.00)	1.46	8.00 (± 0.00)	4.19	1.50 (± 0.508)	1.37	4.00 (± 0.00)	1.97	**1.00 (± 0.00)**	***0.50***	2.00 (± 0.00)	2.00
	P. graveolens	**0.50 (± 0.00)**	***0.26***	2.00 (± 0.00)	1.37	**0.50 (± 0.00)**	***0.29***	**0.83 (± 1.60)**	1.06	**1.00 (± 0.00)**	0.64	**0.75 (± 0.35)**	***0.50***	2.00 (± 0.00)	2.33
	R. damascena	**1.00 (± 0.00)**	***0.44***	**1.00 (± 0.00)**	***0.44***	**0.50 (± 0.00)**	***0.39***	**0.38 (± 0.14)**	***0.41***	2.00 (± 0.00)	0.98	**1.00 (± 0.00)**	0.66	**0.25 (± 0.00)**	0.96
M. alternifolia	*C. aurantium*	**0.25 (± 0.00)**	***0.19***	**1.00 (± 0.00)**	1.75	**0.13 (± 0.00)**	***0.09***	2.00 (± 0.00)	1.75	**0.75 (± 0.35)**	***0.35***	**0.75 (± 0.35)**	0.79	**0.25 (± 0.00)**	0.51
	C. myrrha	**0.50 (± 0.00)**	***0.30***	8.00 (± 0.00)	6.05	**0.25 (± 0.00)**	***0.13***	1.50 (± 0.71)	0.88	1.50 (± 0.71)	0.86	2.00 (± 0.00)	1.00	**0.25 (± 0.00)**	0.50
	C. reticulata	2.5 (± 1.33)	3.13	2.00 (± 0.00)	1.83	3.00 (± 1)	1.35	2.00 (± 0.00)	1.13	2.67 (± 1.15)	1.11	**0.75 (± 0.35)**	0.69	**0.25 (± 0.00)**	0.51

Essential oil combination		*E. faecalis*		*G. vaginalis*		*P. anaerobius*		*C. albicans*		*C. krusei*		*C. glabrata*		*C. tropicalis*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
	C. roman	**0.25 (± 0.00)**	***0.19***	2.00 (± 0.00)	0.63	6 .00 (± 2.00)	4.50	2.00 (± 0.00)	1.00	**1.00 (± 0.00)**	0.51	**1.00 (± 0.00)**	0.98	**0.25 (± 0.00)**	0.67
	C. sinesis	**0.25 (± 0.00)**	***0.19***	1.50 (± 0.71)	1.85	4.00 (± 0.00)	2.00	2.00 (± 0.00)	1.00	**1.00 (± 0.00)**	1.41	**1.00 (± 0.00)**	0.73	0.38 (± 0.18)	0.85
	C. zeylanicum	**0.25 (± 0.00)**	***0.14***	**0.50 (± 0.00)**	***0.45***	**0.25 (± 0.00)**	0.76	2.50 (± 1.00)	2.26	**0.25 (± 0.00)**	***0.33***	**0.50 (± 0.00)**	***0.41***	**0.25 (± 0.00)**	0.51
	E. caryophyllata	**0.25 (± 0.00)**	***0.16***	2.00 (± 0.00)	1.63	**0.50 (± 0.00)**	0.51	**1.00 (± 0.00)**	1.69	1.67 (± 0.75)	3.69	**1.00 (± 0.00)**	0.69	**0.25 (± 0.00)**	0.64
	E. globulus	**0.25 (± 0.00)**	***0.16***	2.00 (± 0.00)	1.67	8.00 (± 0.00)	3.25	4.00 (± 0.00)	3.01	1.50 (± 0.71)	0.96	**1.00 (± 0.00)**	1.06	**0.25 (± 0.00)**	0.67
	J. communis	**0.50 (± 0.00)**	***0.22***	4.00 (± 0.00)	5.08	>8.00 (± 0.00)	>4.29	>8.00 (± 0.00)	>6.43	**1.00 (± 0.00)**	0.94	**0.50 (± 0.00)**	0.83	**0.50 (± 0.00)**	0.65
	L. angustifolia	**0.25 (± 0.00)**	***0.12***	4.00 (± 0.00)	4.67	**0.50 (± 0.00)**	***0.25***	**1.00 (± 0.00)**	0.69	2.00 (± 0.00)	0.98	**1.00 (± 0.00)**	0.51	**0.25 (± 0.00)**	1.00
	M. officinalis	**0.38 (± 0.18)**	***0.23***	3.00 (± 1.41)	2.38	6.00 (± 2.00)	3.76	2.00 (± 0.00)	8.44	1.50 (± 0.71)	1.71	**1.00 (± 0.00)**	0.69	0.38 (± 0.18)	1.00
	M. quinquenervia	**0.38 (± 0.18)**	***0.28***	8.00 (± 0.00)	5.83	8.00 (± 0.00)	4.00	**0.25 (± 0.00)**	***0.14***	1.50 (± 0.71)	0.64	**1.00 (± 0.00)**	0.51	**0.25 (± 0.00)**	0.63
	P. graveolens	**1.00 (± 0.00)**	0.51	8.00 (± 0.00)	5.96	3.00 (± 1.00)	1.69	4.00 (± 0.00)	4.17	3.00 (± 1.41)	2. 01	**1.00 (± 0.00)**	0.92	**1.00 (± 0.00)**	0.67
	P. sylvestris	**0.75 (± 0.35)**	***0.41***	**1.00 (± 0.00)**	1.17	8.00 (± 0.00)	2.60	2.00 (± 0.00)	2.75	1.67 (± 0.85)	1.36	>8. 00 (± 0.00)	>4.50	**0.25 (± 0.00)**	0.50
	R. officinalis	**0.25 (± 0.00)**	***0.13***	**0.75 (± 0.35)**	1.25	6.00 (± 2.00)	8.64	**1.00 (± 0.00)**	1.30	**0.38 (± 0.18)**	0.16	**0.75 (± 0.35)**	0.88	**0.25 (± 0.00)**	0.63
	S. sclarea	**0.25 (± 0.00)**	***0.15***	**0.25 (± 0.00)**	***0.15***	6.00 (± 2.00)	4.50	4.00 (± 0.00)	1.75	1.50 (± 0.71)	0.86	**1.00 (± 0.00)**	0.74	**0.25 (± 0.00)**	0.61
	T. vulgaris	**0.25 (± 0.00)**	***0.13***	**1.00 (± 0.00)**	0.97	2.00 (± 0.00)	1.50	1.50 (± 0.71)	1.16	**0.25 (± 0.00)**	***0.14***	**0.75 (± 0.35)**	0.70	**0.25 (± 0.00)**	0.51
M. quinquenervia	*E. globulus*	3.00 (± 1.15)	2.50	**0.25 (± 0.00)**	***0.06***	8.00 (± 0.00)	3.25	>8.00 (± 0.00)	>4.34	**0.38 (± 0.18)**	0.68	**1.00 (± 0.00)**	1.17	**0.25 (± 0.00)**	0.67
	J. communis	**1.00 (± 0.00)**	0.69	1.50 (± 0.71)	***0.25***	>8.00 (± 0.00)	>4.29	4.00 (± 0.00)	2.38	**0.25 (± 0.00)**	***0.25***	**0.50 (± 0.00)**	0.51	**1.00 (± 0.00)**	2.50
	L. angustifolia	**0.25 (± 0.00)**	***0.18***	**0.50 (± 0.00)**	***0.28***	3.00 (± 1.00)	1.50	4.00 (± 0.00)	1.92	**1.00 (± 0.00)**	***0.48***	**0.50 (± 0.00)**	0.62	**0.75 (± 0.35)**	0.88
	M. officinalis	**0.25 (± 0.00)**	***0.22***	1.50 (± 0.71)	***0.25***	1.50 (± 0.50)	0.94	4.00 (± 0.00)	16.05	3.00 (± 1.41)	0.75	**1.00 (± 0.00)**	1.05	**1.00 (± 0.00)**	0.63
	P. sylvestris	**0.38 (± 0.18)**	***0.30***	**1.00 (± 0.00)**	0.51	2.00 (± 0.00)	0.65	4.00 (± 0.00)	4.67	**1.00 (± 0.00)**	0.39	**0.50 (± 0.00)**	0.51	**1.00 (± 0.00)**	1.00
	R. damascena	**0.25 (± 0.00)**	***0.19***	**1.00 (± 0.00)**	1.06	**1.00 (± 0.00)**	1.44	2.00 (± 0.00)	2.19	**1.00 (± 0.00)**	1.09	**0.50 (± 0.00)**	0.80	**1.00 (± 0.00)**	0.71
	S. album	**0.63 (± 0.53)**	1.96	**1.00 (± 0.00)**	1.17	3.00 (± 1.00)	1.65	>8.00 (± 0.00)	>1.83	2.00 (± 0.75)	1.63	**0.50 (± 0.00)**	0.62	**0.50 (± 0.00)**	0.50
	S. sclarea	**0.38 (± 0.18)**	***0.24***	**0.25 (± 0.00)**	***0.15***	6.00 (± 2.00)	4.50	3.00 (± 1.41)	0.69	1.33 (± 1.41)	0.23	**1.00 (± 0.00)**	1.03	**0.75 (± 0.35)**	0.51
	V. zizanioides	**0.13 (± 0.00)**	***0.16***	4.00 (± 0.00)	1.45	1.50 (± 0.50)	0.75	**1.00 (± 0.00)**	***0.37***	3.00 (± 1.41)	0.92	**0.50 (± 0.00)**	0.64	**1.00 (± 0.00)**	0.77
O. vulgare	*B. carteri*	**0.25 (± 0.00)**	***0.13***	2.00 (± 0.00)	2.83	**0.50 (± 0.00)**	0.51	**1.00 (± 0.00)**	0.82	**0.38 (± 0.18)**	1.00	**0.63 (± 0.25)**	0.98	**0.38 (± 0.18)**	1.19
	C. aurantifolia	**0.25 (± 0.00)**	***0.19***	**0.50 (± 0.00)**	0.63	**0.50 (± 0.00)**	1.00	**0.50 (± 0.00)**	0.80	4.00 (± 0.00)	8.75	**0.50 (± 0.00)**	0.78	**0.75 (± 0.35)**	1.04
	C. bergamia	**0.50 (± 0.00)**	***0.34***	**0.75 (± 0.35)**	0.87	**0.25 (± 0.00)**	***0.27***	**1.00 (± 0.00)**	1.10	**0.38 (± 0.18)**	1.02	1.50 (± 0.71)	2.95	**0.25 (± 0.00)**	0.67
	C. martini	**0.50 (± 0.00)**	***0.44***	**0.50 (± 0.00)**	0.51	**0.25 (± 0.00)**	***0.32***	**0.50 (± 0.00)**	1.30	1.50 (± 0.71)	4.00	**0.50 (± 0.00)**	1.13	**0.38 (± 0.18)**	1.13
	C. paradisi	**0.25 (± 0.00)**	***0.22***	2.00 (± 0.00)	3.33	**0.13 (± 0.00)**	***0.38***	0.75 (± 0.35)	0.83	**0.50 (± 0.00)**	1.19	**1.00 (± 0.00)**	1.75	**1.00 (± 0.00)**	1.43
	C. reticulata	**0.25 (± 0.00)**	***0.34***	**0.50 (± 0.00)**	0.63	**0.13 (± 0.00)**	***0.15***	**1.00 (± 0.00)**	0.79	**0.83 (± 1.60)**	2.02	**0.50 (± 0.00)**	0.81	**1.00 (± 0.00)**	1.67
	C. sinesis	**0.25 (± 0.00)**	***0.29***	**0.75 (± 0.35)**	1.18	**1.00 (± 0.00)**	1.25	**0.33 (± 1.84)**	***0.24***	1.13 (± 0.63)	3.29	**0.50 (± 0.00)**	1.00	**1.00 (± 0.00)**	1.21
	E. globulus	**0.25 (± 0.00)**	***0.19***	**0.50 (± 0.00)**	0.51	**0.50 (± 0.00)**	0.51	**0.38 (± 0.18)**	***0.37***	3.00 (± 1.41)	6.60	**0.50 (± 0.00)**	0.81	**1.00 (± 0.00)**	1.27

Essential oil combination		*E. faecalis*		*G. vaginalis*		*P. anaerobius*		*C. albicans*		*C. krusei*		*C. glabrata*		*C. tropicalis*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
	H. officinalis	**0.25 (± 0.00)**	***0.34***	**1.00 (± 0.00)**	1.29	**0.75 (± 0.25)**	0.94	>8.00 (± 0.00)	>8.82	2.00 (± 0.00)	>4. 13	**1.00 (± 0.00)**	1.31	1.50 (± 0.71)	1.75
	M. communis	**0.25 (± 0.00)**	***0.34***	2.00 (± 0.00)	3.00	**0.25 (± 0.00)**	***0.27***	**0.50 (± 0.00)**	0.67	>8.00 (± 0.00)	>8.82	**0.50 (± 0.00)**	1.28	1.50 (± 0.71)	2.50
	M. officinalis	4.00 (± 0.00)	2.50	**0.75 (± 0.35)**	0.84	**0.25 (± 0.00)**	***0.34***	**0.25 (± 0.00)**	1.11	**0.75 (± 0.35)**	1.77	**0.50 (± 0.00)**	0.76	**1.00 (± 0.00)**	3.00
	O. sanctum	**0.25 (± 0.00)**	***0.16***	**0.50 (± 0.00)**	***0.22***	**0.50 (± 0.00)**	***0.07***	**0.50 (± 0.00)**	0.51	**0.50 (± 0.00)**	1.51	**0.50 (± 0.00)**	0.96	**0.50 (± 0.00)**	0.83
	P. graveolens	**0.25 (± 0.00)**	***0.18***	2.00 (± 0.00)	3.20	**0.50 (± 0.00)**	0.66	4.00 (± 0.00)	5.08	**0.50 (± 0.00)**	***0.42***	4.00 (± 0.00)	2.96	**0.25 (± 0.00)**	***0.20***
	P. sylvestris	**0.50 (± 0.00)**	***0.34***	2.00 (± 0.00)	3.00	4.00 (± 0.00)	4.30	1.50 (± 0.71)	2.40	**1.00 (± 0.00)**	2.20	**0.50 (± 0.00)**	1.00	**1.00 (± 0.00)**	1.67
	R. officinalis	**1.00 (± 0.00)**	0.68	**1.00 (± 0.00)**	2.00	**0.75 (± 0.25)**	0.94	**1.00 (± 0.00)**	1.50	**0.50 (± 0.00)**	2.00	**0.50 (± 0.00)**	1.13	**0.50 (± 0.75)**	0.87
	S. sclarea	**0.50 (± 0.00)**	***0.26***	**0.75 (± 0.35)**	1.13	1.50 (± 0.50)	2.25	**0.75 (± 0.35)**	***0.50***	**1.00 (± 0.00)**	2.60	**0.75 (± 0.35)**	1.22	**0.50 (± 0.00)**	0.77
S. officinalis	*B. carteri*	**0.25 (± 0.00)**	***0.28***	4.00 (± 0.00)	2.33	8.00 (± 0.00)	1.51	**0.50 (± 0.75)**	***0.27***	**1.00 (± 0.00)**	0.64	**1.00 (± 0.00)**	0.69	1.50 (± 0.71)	1.14
	C. atlantica	**0.25 (± 0.00)**	***0.28***	3.00 (± 1.41)	0.82	**1.00 (± 0.00)**	***0.40***	**1.00 (± 0.00)**	***0.50***	**1.00 (± 0.00)**	1.08	**1.00 (± 0.00)**	1.11	**0.50 (± 0.00)**	***0.35***
	C. aurantifolia	**0.25 (± 0.00)**	***0.28***	3.00 (± 1.41)	1.25	**1.00 (± 0.00)**	1.06	**0.75 (± 0.35)**	1.00	2.00 (± 0.00)	0.83	**1.00 (± 0.00)**	1.06	**0.25 (± 0.00)**	0.51
	C. bergamia	**0.25 (± 0.00)**	***0.38***	4.00 (± 0.00)	1.29	**1.00 (± 0.00)**	***0.16***	**0.75 (± 0.35)**	0.63	1.50 (± 0.71)	0.73	**1.00 (± 0.00)**	0.63	**0.75 (± 0.35)**	0.60
	C. nobile	**0.50 (± 0.00)**	***0.31***	2.00 (± 0.00)	0.83	**0.75 (± 0.25)**	***0.42***	1.50 (± 0.71)	0.69	**0.50 (± 0.00)**	***0.29***	**0.50 (± 0.00)**	***0.30***	**1.00 (± 0.00)**	1.17
	C. paradisi	4.00 (± 0.00)	1.25	2.00 (± 0.00)	1.67	**0.50 (± 0.00)**	1.03	**1.00 (± 0.00)**	0.83	>8.00 (± 0.00)	>3.72	1.50 (± 0.71)	0.75	**1.00 (± 0.00)**	1.04
	C. reticulata	**0.50 (± 0.00)**	***0.31***	**0.75 (± 0.35)**	0.51	4.00 (± 0.00)	1.05	4.00 (± 0.00)	2.08	**1.00 (± 0.00)**	1.41	**0.75 (± 0.35)**	0.73	**1.00 (± 0.00)**	1.04
	C. sinesis	**0.50 (± 0.00)**	0.50	**1.00 (± 0.00)**	0.67	4.00 (± 0.00)	1.25	**1.00 (± 0.00)**	***0.46***	2.00 (± 0.00)	1.63	**1.00 (± 0.00)**	0.69	**0.50 (± 0.00)**	***0.50***
	E. globulus	**0.50 (± 0.00)**	***0.21***	4.00 (± 0.00)	1.33	3.00 (± 1.00)	0.66	**0.44 ± 0.13)**	***0.31***	2.00 (± 0.00)	0.80	**1.00 (± 0.00)**	0.63	2.80 (± 1.73)	6.65
	J. communis	**0.25 (± 0.00)**	***0.08***	4.00 (± 0.00)	0.98	8.00 (± 0.00)	3.17	2.67 (± 1.15)	2.03	>8.00 (± 0.00)	>3.94	**1.00 (± 0.00)**	0.51	3.60 (± 1.73)	1.80
	L. angustifolia	**0.25 (± 0.00)**	***0.13***	4.00 (± 0.00)	2.67	1.50 (± 0.50)	**0.47**	4.00 (± 0.00)	2.58	2.67 (± 1.15)	3.04	**0.50 (± 0.00)**	0.50	2.00 (± 0.00)	1.75
	M. alternifolia	**0.25 (± 0.00)**	***0.09***	1.50 (± 0.71)	0.70	2.00 (± 0.00)	0.63	2.00 (± 0.00)	1.42	3.00 (± 1.41)	1.72	1.50 (± 0.71)	1.11	0.38 (± 0.18)	0.89
	M. officinalis	**0.25 (± 0.00)**	***0.28***	4.00 (± 0.00)	1.67	2.00 (± 0.00)	0.88	4.00 (± 0.00)	16.72	1.50 (± 0.71)	1.32	**1.00 (± 0.00)**	1.31	2.00 (± 0.00)	1.18
	O. sanctum	**0.25 (± 0.00)**	***0.16***	**0.50 (± 0.00)**	***0.22***	***0.50 (± 0.00)***	***0.07***	**0.50 (± 0.00)**	***0.44***	**0.50 (± 0.00)**	***0.30***	2.00 (± 0.00)	1.63	**0.25 (± 0.00)**	***0.20***
	P. graveolens	**0.25 (± 0.00)**	***0.28***	**0.50 (± 0.00)**	***0.38***	**1.00 (± 0.00)**	***0.38***	**1.00 (± 0.00)**	1.00	1.50 (± 0.71)	0.84	**1.00 (± 0.00)**	1.63	1.50 (± 0.71)	1.51
	P. sylvestris	**0.50 (± 0.00)**	***0.21***	1.50 (± 0.71)	1.25	2.00 (± 0.00)	0.27	4.00 (± 0.00)	5.33	**0.75 (± 0.35)**	***0.50***	**0.50 (± 0.00)**	***0.31***	**0.50 (± 0.00)**	***0.46***
	T. vulgaris	**0.25 (± 0.00)**	***0.09***	4.00 (± 0.00)	1.17	6.00 (± 0.00)	3.38	2.00 (± 0.00)	1.47	3.00 (± 1.41)	1.72	1.50 (± 0.71)	1.11	**1.00 (± 0.00)**	0.80

[1]Noteworthy MIC values in bold; [2]bold italics for synergistic ΣFIC

Table 3.4. Average MIC and ΣFIC values of selected beneficial microbiota reference strains

Essential oil combination		*L. acidophilus*		*L. fermentum*		*L. gasseri*		*L. plantarum*		*L. rhamnosus*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
C. camphora	*C. nobile*	1.00 (± 0.00)	1.00	**6.00 (± 2.00)**	3.86	**2.00 (± 0.00)**	>1.13	**2.00 (± 0.00)**	0.55	**2.00 (± 0.00)**	1.00
	L. angustifolia	**4.00 (± 0.00)**[1]	***4.00***[2]	**>8.00 (± 0.00)**	***>6.40***	**>8.00 (± 0.00)**	>1.25	**2.00 (± 0.00)**	0.47	**4.00 (± 0.00)**	2.00
	M. cajuputi	1.00 (± 0.00)	1.00	**2.00 (± 0.00)**	2.00	**2.00 (± 0.00)**	>0.44	**3.00 (± 1.41)**	0.92	**2.00 (± 0.00)**	1.13
	M. officinalis	0.38 (± 0.18)	>0.18	**2.00 (± 0.00)**	2.00	**2.00 (± 0.00)**	>0.26	1.00 (± 0.00)	1.15	1.00 (± 0.00)	0.31
	O. sanctum	0.50 (± 0.00)	0.50	**2.00 (± 0.00)**	1.75	1.00 (± 0.00)	>0.56	**2.00 (± 0.00)**	0.63	**2.00 (± 0.00)**	1.05
C. bergamia	*B. carteri*	**1.50 (± 0.71)**	2.21	**3.00 (± 1.00)**	1.50	**3.00 (± 1.41)**	>0.46	**4.00 (± 0.00)**	1.14	**>8.00 (± 0.00)**	>2.00
	E. caryophyllata	0.50 (± 0.00)	0.50	**2.00 (± 0.00)**	1.42	**4.00 (± 0.00)**	1.86	**2.00 (± 0.00)**	0.74	**3.00 (± 1.41)**	1.32
	L. angustifolia	**2.00 (± 0.00)**	2.00	1.00 (± 0.00)	0.68	**2.00 (± 0.00)**	0.37	**4.00 (± 0.00)**	0.62	**4.00 (± 0.00)**	1.25
	P. cablin	0.50 (± 0.00)	0.50	1.00 (± 0.00)	1.89	**2.00 (± 0.00)**	0.68	**2.00 (± 0.00)**	1.14	**3.50 (± 1.00)**	1.09
	S. album	**6.67 (± 2.31)**	8.98	0.50 (± 0.00)	0.49	**2.00 (± 0.00)**	0.78	1.00 (± 0.00)	0.67	**2.00 (± 0.00)**	0.38
	S. benzoin	0.13 (± 0.00)	0.13	1.00 (± 0.00)	0.65	1.00 (± 0.00)	0.38	1.00 (± 0.00)	0.15	0.75 (± 0.35)	0.23
E. globulus	*C. atlantica*	**2.00 (± 0.00)**	2.00	**>8.00 (± 0.00)**	***>6.40***	**2.00 (± 0.00)**	>0.76	**1.50 (± 0.71)**	0.51	**4.00 (± 0.00)**	3.20
	L. angustifolia	**4.00 (± 0.00)**	***4.00***	**>8.00 (± 0.00)**	***>4.80***	**6.00 (± 2.83)**	>0.94	**>8.00 (± 0.00)**	>1.17	**>8.00 (± 0.00)**	***>6.00***
	M. alternifolia	1.00 (± 0.00)	1.00	1.00 (± 0.00)	0.63	**>8.00 (± 0.00)**	>1.50	**>8.00 (± 0.00)**	>1.21	**8.00 (± 0.00)**	***6.40***
	M. officinalis	**>8.00 (± 0.00)**	>9.97	1.00 (± 0.00)	0.80	**>8.00 (± 0.00)**	>1.00	**>8.00 (± 0.00)**	>1.25	**8.00 (± 0.00)**	***4.50***
	O. marjoram	**4.00 (± 0.00)**	2.00	**2.00 (± 0.00)**	>0.73	**>8.00 (± 0.00)**	>1.61	**>8.00 (± 0.00)**	>1.00	**8.00 (± 0.00)**	5.67
	P. sylvestris	1.00 (± 0.00)	>0.48	**>8.00 (± 0.00)**	***>5.82***	**4.00 (± 0.00)**	>0.58	**4.00 (± 0.00)**	1.00	**4.00 (± 0.00)**	***3.54***
	T. vulgaris	**>8.00 (± 0.00)**	***>4.30***	1.00 (± 0.00)	0.60	**3.00 (± 1.41)**	>0.94	**3.00 (± 1.41)**	0.64	**4.00 (± 0.00)**	3.00
H. angustifolium	*B. carteri*	**2.00 (± 0.00)**	2.00	**6.00 (± 2.00)**	1.87	**2.00 (± 0.00)**	>0.63	**>8.00 (± 0.00)**	>0.86	1.00 (± 0.00)	0.49
	C. aurantium	0.50 (± 0.00)	0.50	**2.00 (± 0.00)**	1.37	**3.0 (± 1.41)**	1.50	**2.50 (± 1.00)**	>2.50	1.00 (± 0.00)	0.80
	C. bergamia	**2.00 (± 0.00)**	2.00	**6.00 (± 2.00)**	3.38	**>8.00 (± 0.00)**	>2.71	**2.00 (± 0.00)**	>0.28	**2.00 (± 0.00)**	0.72
	C. nobile	**0.50 (± 0.00)**	0.50	**4.00 (± 0.00)**	3.16	**2.00 (± 0.00)**	1.50	**3.0 (± 1.15)**	>0.76	**2.00 (± 0.00)**	1.10
	C. reticulata	**2.00 (± 0.00)**	2.00	1.00 (± 0.00)	0.49	**>8.00 (± 0.00)**	>2.50	**2.00 (± 0.00)**	>0.25	**1.00 (± 0.00)**	0.55
	C. sinesis	**3.0 (± 1.41)**	***4.41***	**1.25 (± 2.78)**	0.86	**>8.00 (± 0.00)**	>2.50	**3.50 (± 1.00)**	>0.50	**2.00 (± 0.00)**	0.85
	L. angustifolia	**8.00 (± 0.00)**	***8.00***	**>8.00 (± 0.00)**	>3.9	**>8.00 (± 0.00)**	>2.75	**>8.00 (± 0.00)**	>1.34	**1.50 (± 0.71)**	0.82
	P. graveolens	1.00 (± 0.00)	1.00	**4.00 (± 0.00)**	1.32	**2.00 (± 0.00)**	1.05	**3.50 (± 1.00)**	>1.94	1.00 (± 0.00)	0.63
	R. damascena	0.50 (± 0.00)	0.50	**2.00 (± 0.00)**	0.93	1.00 (± 0.00)	0.53	**1.25 (± 0.5)**	***>4.31***	0.50 (± 0.00)	0.27
M. alternifolia	*C. aurantium*	0.25 (± 0.00)	0.25	**4.00 (± 0.00)**	3.33	**2.00 (± 0.00)**	0.75	**2.00 (± 0.00)**	1.18	**2.00 (± 0.00)**	1.60
	C. myrrha	0.75 (± 0.35)	1.10	1.00 (± 0.00)	0.96	**1.5 (± 0.71)**	>0.28	**2.00 (± 0.00)**	0.46	1.00 (± 0.00)	0.80
	C. reticulata	**4.00 (± 2.87)**	***6.87***	1.00 (± 0.00)	0.63	**>8.00 (± 0.00)**	>1.50	**4.00 (± 0.00)**	>0.61	**4.00 (± 0.00)**	2.20
	C. sinesis	1.00 (± 0.00)	1.00	**2.00 (± 0.00)**	1.67	**>8.00 (± 0.00)**	>1.50	**>8.00 (± 0.00)**	>1.21	**8.00 (± 0.00)**	3.40
	C. zeylanicum	0.25 (± 0.00)	0.25	0.38 (± 0.125)	0.28	**2.00 (± 0.00)**	0.75	1.00 (± 0.00)	0.37	1.00 (± 0.00)	0.66
	E. caryophyllata	0.50 (± 0.00)	0.50	**2.00 (± 0.00)**	1.33	**4.00 (± 0.00)**	2.00	**2.00 (± 0.00)**	0.78	**2.00 (± 0.00)**	1.35
	E. globulus	1.00 (± 0.00)	1.00	0.75 (± 0.25)	0.47	**>8.00 (± 0.00)**	>1.50	**>8.00 (± 0.00)**	>1.31	**>8.00 (± 0.00)**	>6.40
	J. communis	**3.0 (± 1.41)**	***4.41***	1.00 (± 0.00)	0.83	**>8.00 (± 0.00)**	>1.80	**>8.00 (± 0.00)**	>2.21	**8.00 (± 0.00)**	**4.79**

Essential oil combination		*L. acidophilus*		*L. fermentum*		*L. gasseri*		*L. plantarum*		*L. rhamnosus*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
	L. angustifolia	1.00 (± 0.00)	1.00	**2.00 (± 0.00)**	1.27	**>8.00 (± 0.00)**	>1.75	**4.00 (± 0.00)**	0.69	**8.00 (± 0.00)**	**4.40**
	M. officinalis	1.00 (± 0.00)	1.00	**2.00 (± 0.00)**	1.67	**>8.00 (± 0.00)**	>1.50	**4.00 (± 0.00)**	0.73	**>8.00 (± 0.00)**	>2.90
	M. quinquenervia	**2.00 (± 0.00)**	2.00	1.00 (± 0.00)	>0.39	**1.50 (± 0.71)**	0.56	**>8.00 (± 0.00)**	>1.71	**8.00 (± 0.00)**	2.90
	P. graveolens	1.00 (± 0.00)	1.00	1.00 (± 0.00)	0.94	**4.00 (± 0.00)**	1.59	**4.0 (± 2.31)**	1.47	**4.00 (± 0.00)**	2.53
	P. sylvestris	1.00 (± 0.00)	>0.42	1.00 (± 0.00)	0.76	**2.00 (± 0.00)**	0.42	**2.00 (± 0.00)**	0.55	**4.00 (± 0.00)**	2.74
	R. officinalis	0.75 (± 0.35)	1.10	**1.67 (± 0.47)**	1.05	**4.00 (± 0.00)**	0.75	**3.0 (± 1.41)**	0.46	**2.00 (± 0.00)**	>0.72
	T. vulgaris	1.00 (± 0.00)	1.00	**1.00 (± 0.00)**	0.63	**4.00 (± 0.00)**	1.50	**2.00 (± 0.00)**	0.48	**2.00 (± 0.00)**	1.10
Melaleuca quinquenervia	*C. roman*	**2.0(± 0.75)**	2.75	**2.00 (± 0.00)**	>0.42	**2.00 (± 0.00)**	1.50	**4.00 (± 0.00)**	1.00	**4.00 (± 0.00)**	1.25
	E. globulus	1.00 (± 0.00)	1.00	1.00 (± 0.00)	>0.36	**6.00 (± 2.83)**	>1.88	**>8.00 (± 0.00)**	>1.50	**6.00 (± 2.83)**	3.38
	J. communis	**>8.00 (± 0.00)**	>2.86	**4.00 (± 0.00)**	>2.25	**8.00 (± 0.00)**	2.80	**>8.00 (± 0.00)**	>2.50	**>8.00 (± 0.00)**	>2.90
	L. angustifolia	**6.67 (± 3.4)**	***10.07***	**2.00 (± 0.00)**	>0.73	**3.0 (± 1.41)**	1.03	**8.00 (± 0.00)**	1.67	**>8.00 (± 0.00)**	>2.50
	M. officinalis	**6.0 (± 2.83)**	8.83	**3.00 (± 1.00)**	>1.69	**2.00 (± 0.00)**	>0.63	**8.00 (± 0.00)**	1.75	**8.00 (± 0.00)**	1.00
	P. graveolens	**6.67 (± 3.4)**	***10.07***	**1.50 (± 0.50)**	>0.99	**3.0 (± 1.41)**	1.57	**8.00 (± 0.00)**	3.22	**4.00 (± 0.00)**	1.58
	P. sylvestris	**8.00 (± 3.00)**	**>1.93**	**>8.00 (± 0.00)**	>1.00	1.00 (± 0.00)	0.33	**2.00 (± 0.00)**	0.62	**4.00 (± 0.00)**	1.79
	R. officinalis	**6.00 (± 2.83)**	8.83	**>8.00 (± 0.00)**	>2.90	**2.00 (± 0.00)**	0.63	**2.00 (± 0.00)**	0.38	**3.00 (± 1.41)**	>0.38
	S. album	1.00 (± 0.00)	1.00	0.38 (± 0.13)	>0.25	**1.00 (± 0.00)**	0.55	1.00 (± 0.00)	0.73	**0.75 (± 0.35)**	0.14
	S. sclarea	**1.50 (± 0.71)**	2.21	**2.00 (± 0.00)**	>0.63	**4.00 (± 0.00)**	2.00	**>8.00 (± 0.00)**	>3.00	**6.00 (± 2.83)**	>0.75
	V. zizanioides	**>8.00 (± 0.00)**	>3.83	0.38 (± 0.13)	>0.47	0.50 (± 0.00)	0.63	0.50 (± 0.00)	0.23	0.50 (± 0.00)	0.63
	B. carteri	1.00 (± 0.00)	>0.36	**1.50 (± 0.50)**	0.94	**2.00 (± 0.00)**	>0.38	**2.00 (± 0.00)**	0.93	**2.00 (± 0.00)**	0.87
Origanum vulgare	*C. aurantifolia*	1.00 (± 0.00)	>0.89	1.00 (± 0.00)	0.75	0.75 (± 0.35)	0.47	**2.00 (± 0.00)**	0.75	**2.00 (± 0.00)**	0.75
	C. bergamia	1.00 (± 0.00)	>3.82	1.00 (± 0.00)	0.88	**2.00 (± 0.00)**	0.43	**2.00 (± 0.00)**	0.64	**2.00 (± 0.00)**	0.63
	C. martini	**1.50 (± 0.71)**	>0.63	1.00 (± 0.00)	0.86	**0.75 (± 0.35)**	0.51	0.75 (± 0.35)	0.29	1.00 (± 0.00)	0.81
	C. paradisi	1.00 (± 0.00)	>0.44	1.00 (± 0.00)	0.83	**2.00 (± 0.00)**	0.75	**2.00 (± 0.00)**	1.00	**1.50 (± 0.71)**	1.13
	C. reticulata	**3.00 (± 0.00)**	>3.52	**1.50 (± 0.50)**	1.20	**3.00 (± 1.41)**	0.56	**2.00 (± 0.00)**	>0.63	**2.00 (± 0.00)**	1.00
	C. sinesis	**1.50 (± 0.71)**	>1.59	1.00 (± 0.00)	1.00	**3.00 (± 1.41)**	>0.57	**3.0 (± 1.41)**	>0.94	**2.00 (± 0.00)**	0.75
	E. globulus	**1.50 (± 0.71)**	>0.72	**2.00 (± 0.00)**	1.60	**2.00 (± 0.00)**	>0.38	**2.00 (± 0.00)**	0.63	**2.00 (± 0.00)**	1.50
	H. officinalis	**1.00 (± 0.00)**	>0.12	**2.00 (± 0.00)**	1.50	**4.00 (± 0.00)**	1.50	**2.00 (± 0.00)**	0.93	**1.00 (± 0.00)**	0.61
	M. communis	0.88 (± 0.25)	>0.94	**1.00 (± 0.00)**	1.00	**1.00 (± 0.00)**	0.38	**2.00 (± 0.00)**	1.00	**1.75 (± 0.50)**	0.55
	M. officinalis	1.00 (± 0.00)	>0.89	**1.50 (± 0.50)**	1.50	**4.00 (± 0.00)**	>0.75	**2.00 (± 0.00)**	0.69	**2.00 (± 0.00)**	0.63
	O. sanctum	1.00 (± 0.00)	1.00	1.00 (± 0.00)	0.70	**2.00 (± 0.00)**	1.00	**4.00 (± 0.00)**	>0.90	**2.00 (± 0.00)**	1.00
	P. graveolens	1.00 (± 0.00)	>0.56	**2.00 (± 0.00)**	2.20	**2.00 (± 0.00)**	0.80	**1.50 (± 0.71)**	0.79	**2.00 (± 0.00)**	1.17
	P. sylvestris	**2.00 (± 0.00)**	>0.26	0.75 (± 0.25)	0.70	**2.00 (± 0.00)**	0.42	**1.50 (± 0.71)**	0.66	**2.00 (± 0.00)**	1.27
	R. officinalis	**2.00 (± 0.00)**	>0.46	1.00 (± 0.00)	0.80	**2.00 (± 0.00)**	0.38	**2.00 (± 0.00)**	0.63	**2.00 (± 0.00)**	>0.63
	S. sclarea	**2.00 (± 0.00)**	>1.13	**2.00 (± 0.00**)	1.50	**2.00 (± 0.00)**	0.75	**8.00 (± 0.00)**	***4.00***	**2.00 (± 0.00)**	>0.63
Salvia *officinalis*	*B. carteri*	**3.00 (± 1.41)**	***4.41***	**2.00 (± 0.00)**	0.85	**2.00 (± 0.00)**	>0.63	**8.00 (± 0.00)**	>2.22	**4.00 (± 0.00)**	1.75
	C. atlantica	**3.00 (± 1.41)**	***4.41***	1.00 (± 0.00)	0.80	1.00 (± 0.00)	0.56	**4.00 (± 0.00)**	>1.36	**2.00 (± 0.00)**	1.10

Essential oil combination		*L. acidophilus*		*L. fermentum*		*L. gasseri*		*L. plantarum*		*L. rhamnosus*	
Essential oil 1	**Essential oil 2**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**	**MIC**	**ΣFIC**
	C. aurantifolia	**1.50 (± 0.71)**	2.21	1.00 (± 0.00)	0.80	**2.00 (± 0.00)**	1.50	**4.00 (± 0.00)**	>0.75	**>8.00 (± 0.00)**	>3.00
	C. bergamia	**8.00 (± 0.00)**	***8.00***	1.00 (± 0.00)	0.68	**>8.00 (± 0.00)**	>2.71	**>8.00 (± 0.00)**	>1.07	**>8.00 (± 0.00)**	>2.50
	C. nobile	0.75 (± 0.35)	1.10	**1.50 (± 0.50)**	0.66	**2.00 (± 0.00)**	1.50	**4.00 (± 0.00)**	>0.75	**2.00 (± 0.00)**	1.00
	C. paradisi	0.50 (± 0.00)	0.50	**1.00 (± 0.00)**	0.63	**4.00 (± 0.00)**	2.00	**2.00 (± 0.00)**	>0.63	**2.00 (± 0.00)**	1.50
	C. reticulata	**6.00 (± 2.83)**	***8.83***	**2.00 (± 0.00)**	1.10	**4.00 (± 0.00)**	1.25	**>8.00 (± 0.00)**	>1.00	**>8.00 (± 0.00)**	***>4.00***
	C. sinesis	**>8.00 (± 0.00)**	***>8.75***	**>8.00 (± 0.00)**	***4.80***	**4.00 (± 0.00)**	>1.25	**8.00 (± 0.00)**	>1.00	**>8.00 (± 0.00)**	>3.00
	E. globulus	**>8.00 (± 0.00)**	***>4.08***	**4.00 (± 0.00)**	2.40	**>8.00 (± 0.00)**	>2.50	**>8.00 (± 0.00)**	>1.00	**4.00 (± 0.00)**	3.00
	J. communis	**>8.00 (± 0.00)**	>2.18	**>8.00 (± 0.00)**	***>6.40***	**>8.00 (± 2.83)**	>2.80	**>8.00 (± 0.00)**	>2.00	**>8.00 (± 0.00)**	***>4.40***
	L. angustifolia	**4.00 (± 0.00)**	***4.00***	1.00 (± 0.00)	0.60	**>8.00 (± 0.00)**	>2.75	**>8.00 (± 0.00**)	>1.17	**>8.00 (± 0.00)**	***>4.00***
	O. sanctum	1.00 (± 0.00)	1.00	1.00 (± 0.00)	0.68	**1.50 (± 0.71)**	1.13	**4.00 (± 0.00)**	>0.92	**2.00 (± 0.00)**	1.05
	P. graveolens	1.00 (± 0.00)	1.00	**2.00 (± 0.00)**	1.80	**>8.00 (± 0.00)**	***>4.19***	**>8.00 (± 0.00)**	>2.72	**8.00 (± 0.00)**	**4.67**
	P. sylvestris	**2.00 (± 0.00)**	>0.32	**>8.00 (± 0.00)**	***>6.40***	**>8.00 (± 0.00)**	>2.67	**>8.00 (± 0.00)**	>2.00	**4.00 (± 0.00)**	2.54
	V. zizanioides	**>8.00 (± 0.00)**	>2.90	0.50 (± 0.00)	0.85	0.50 (± 0.00)	0.56	0.50 (± 0.00)	0.29	0.50 (± 0.00)	0.72

[1]Noteworthy MIC values where microbiota is not inhibited in bold; [2]Noteworthy ΣFIC values where combination did not affect the growth of microbiota in bold italic

Toxicity Studies- Brine Shrimp Lethality Assay

The toxicity profiles of the essential oils were investigated for all essential oils and combinations. Table 3.5 displays the percentage mortality for the essential oils after 24 and 48 hrs. Overall, 62% of individual essential oils and 49% of essential oil combinations were found to be non-toxic, suggesting their preference for selection in therapeutic use.

Table 3.5. Percentage mortality of essential oils

Essential oil	% Mortality 24 hrs	% Mortality 48 hrs
B. carteri	**44.44 (± 30.97)**[1]	69.35 (± 6.55)
C. myrrha	**27.35 (± 0.37)**	**45.98 (± 2.94)**
C. reticulata	**10.48 (± 6.1)**	**36.69 (± 9.77)**
C. atlantica	**28.88 (± 23.65)**	80.56 (± 0.57)
C. aurantifolia	**42.51 (± 1.72)**	**48.87 (± 0.74)**
C. aurantium	**38.64 (± 2)**	73.4 (± 2.17)
C. bergamia	85.96 (± 4.97)	87.98 (± 5.99)
C. camphora	**0.00 (± 0.00)**	**3.52 (± 4.99)**
C. citratus	**18.1 (± 16.82)**	**46.02 (± 2.5)**
C. martini	**37.23 (± 9.15)**	**42.64 (± 5.3)**
C. nobile	**21.87 (± 3.37)**	58.09 (± 2.32)
C. paradisi	**22.21 (± 23.11)**	72.21 (± 19.91)
C. sinensis	**8.09 (± 5.27)**	**31.84 (± 5.44)**
C. zeylanicum	**33.6 (± 4.09)**	**41.32 (± 8.86)**
E. globulus	**0.00 (± 0.00)**	**1.84 (± 1.61)**
E. caryophyllata	71.62 (± 6.64)	78.84 (± 2.47)
H. augustifolium	**47.04 (± 10.09)**	71.43 (± 2.29)
H. officinalis	**8.35 (± 5.96)**	**30.15 (± 4.12)**
J. communis	**0.00 (± 0.00)**	**0.00 (± 0.00)**
L. angustifolia	**29.86 (± 10.31)**	**34.16 (± 9.11)**
M. alternifolia	**9.85 (± 5.32)**	63.5 (± 5.58)
M. cajuputi	65.98 (± 18.21)	77.09 (± 5.23)
M. communis	**9.01 (± 3.73)**	**23.42 (± 5.84)**
M. quinquenervia	63.84 (± 4.07)	66.67 (± 0.82)
M. officinalis	**40.4 (± 8.32)**	70 (± 13.57)
O. majorana	**1.99 (± 2.09)**	**7.69 (± 3.78)**
O. sanctum	**12.85 (± 3.21)**	**35.71 (± 16.07)**
O. vulgare	**12.74 (± 14.94)**	**28.01 (± 8.23)**
P. cablin	67.69 (± 15.59)	80.4 (± 4.56)
P. graveolens	**1.09 (± 1.89)**	**4.51 (± 4.16)**
P. sylvestris	**0.76 (± 1.31)**	**10.78 (± 8.58)**
R. damascena	**0.00 (± 0.00)**	**1.67 (± 2.89)**
R. officinalis	**0.00 (± 0.00)**	**9.13 (± 8.29)**
S. officinalis	**11.81 (± 6.81)**	**47.94 (± 2.14)**
S. album	**44.53 (± 2.46)**	50.73 (± 1.44)
S. benzoin	**7.58 (± 11.09)**	**21.34 (± 12.6)**
S. sclarea	**0.00 (± 0.00)**	**0.00 (± 0.00)**
T. vulgaris	**0.00 (± 0.00)**	**1.01 (± 1.75)**
V. zizanioides	**38.45 (± 4.05)**	54.95 (± 3.26)
	Controls	
Potassium dichromate (+)	100.00	100.00

Essential oil	% Mortality 24 hrs	% Mortality 48 hrs
Saltwater (-)	2.26 (± 0.08)	3.76 (± 1.30)

[1]Non-toxic essential oils in bold

Table 3.6 displays the percentage mortality and ΣFMC values for the combinations that displayed noteworthy antimicrobial synergy. The essential oil combinations that exhibited the most synergistic antimicrobial activity, while not inhibiting the vaginal colonizers to some degree, and exhibited non-toxicity were depicted with synergistic ΣFMC values ≤ 0.50.

Table 3.6. Percentage mortality of essential oil combinations

Essential oil combination		% Mortality		ΣFMC	
Essential oil 1	**Essential oil 2**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
C. camphora	*C. nobile*	**3.25 (± 2.82)**	**30.03 (± 5.68)**	≥0.07	4.53
	L. angustifolia	**42.29 (± 3.53)**	**45.53 (± 2.53)**	6.72	7.14
	M. cajuputi	**34.34 (± 6.49)**	**46.90 (± 0.79)**	≥0.26	6.97
	M. officinalis	**44.572 (± 7.93)**	54.45 (± 4.16)	6.89	8.13
	O. sanctum	**35.55 (± 10.03)**	**42.46 (± 6.82)**	≥1.38	6.63
C. bergamia	*B. carterii*	**39.87 (± 1.00)**	**45.06 (± 1.43)**	0.68	0.58
	E. caryophyllata	51.85 (± 9.32)	58.99 (± 3.74)	0.66	0.71
	L. angustifolia	**42.15 (± 4.21)**	51.27 (± 1.6)	0.95	1.04
	P. cablin	**37.44 (± 17.43)**	57.44 (± 4.57)	0.49	0.68
	S. album	**33.74 (± 8.63)**	**42.85 (± 2.39)**	0.58	0.67
	S. benzoin	**26.81 (± 14.68)**	**42.81 (± 6.02)**	1.92	1.25
E. globulus	*C. atlantica*	**42.27 (± 3.98)**	**46.69 (± 0.69)**	≥ 0.73	12.99
	L. angustifolia	**38.59 (± 3.59)**	**48.1 (± 1.31)**	≥ 0.65	13.78
	M. alternifolia	**0.00 (± 0.00)**	**2.33 (± 6.35)**	**≥ 0.00**	0.65
	M. officinalis	**0.00 (± 0.00)**	**2.42 (± 0.6)**	**0.00**	0.69
	O. marjoram	**32.56 (± 0.82)**	**45.4 (± 1.78)**	≥ 8.16	15.29
	P. sylvestris	**0.00 (± 0.00)**	**1.69 (± 2.94)**	**≥ 0.00**	0.54
	T. vulgaris	**0.00 (± 0.00)**	**0.00 (± 0.00)**	**≤0.00**	**0.00**
H. angustifolium	*B. carterii*	**0.00 (± 0.00)**	**1.67 (± 2.89)**	**0.00**	**0.02**
	C. aurantium	**1.36 (± 2.36)**	**24.12 (± 10.4)**	**0.03**	**0.33**
	C. bergamia	**3.55 (± 6.14)**	**39.55 (± 1.68)**	0.06	**0.50**
	C. nobile	**0.78 (± 1.34)**	**3.05 (± 3.47)**	**0.03**	**0.05**
	C. reticulata	**0.00 (± 0.00)**	**27.35 (± 2.28)**	**0.00**	0.56
	C. sinesis	**1.59 (± 2.75)**	**4.15 (± 4.88)**	**0.11**	**0.09**
	L. angustifolia	**7.75 (± 8.17)**	**32.59 (± 14.2)**	**0.21**	0.71
	P. graveolens	**41.42 (± 1.47)**	**48.15 (± 1.33)**	19.39	5.68
	R. damascena	**0.00 (± 0.00)**	**27.55 (± 7.13)**	**≥0.00**	8.46
M. alternifolia	*C. aurantium*	**37.13 (± 13.23)**	**44.18 (± 8.46)**	2.37	0.65
	C. myrrha	**13.22 (± 10.14)**	**41.16 (± 7.56)**	0.91	0.77

Essential oil combination		% Mortality		ΣFMC	
Essential oil 1	**Essential oil 2**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
	C. reticulata	**0.88 (± 1.52)**	76.89 (± 3.75)	**0.09**	1.65
	C. sinesis	**32.84 (± 3.47)**	**43.56 (± 1.48)**	3.70	1.03
	C. zeylanicum	**19.48 (± 11.00)**	**42.54 (± 6.47)**	1.28	0.85
	E. caryophyllata	**45.94 (± 11.19)**	68.45 (± 2.38)	2.65	0.97
	E. globulus	**9.83 (± 17.02)**	**21.04 (± 17.18)**	**≥ 0.50**	5.89
	J. communis	**24.53 (± 4.89)**	**48.38 (± 0.67)**	≥ 1.25	**0.38**
	L. angustifolia	**36.50 (± 4.17)**	**46.80 (± 4.05)**	2.46	1.05
	M. officinalis	**22.57 (± 7.27)**	**45.71 (± 1.1)**	1.77	0.86
	M. quinquenervia	**29.99 (± 7.04)**	**41.06 (± 2.24)**	1.76	0.63
	P. graveolens	**0.27 (± 0.35)**	**35.88 (± 12.86)**	**0.14**	4.26
	T. vulgaris	**16.89 (± 3.29)**	**46.47 (± 1.53)**	≥ 0.86	23.37
Melaleuca quinquenervia	*C. roman*	**0.95 (± 0.83)**	62.81 (± 9.48)	**0.03**	1.01
	E. globulus	**5.71 (± 9.90)**	93.43 (± 4.51)	**≥ 0.04**	26.10
	J. communis	**33.86 (± 13.32)**	97.37 (± 2.99)	≥ 0.27	≥ 0.73
	L. angustifolia	**20.74 (± 15.95)**	67.03 (± 7.03)	0.51	1.48
	M. officinalis	**20.10 (± 17.24)**	80.56 (± 19)	0.71	1.48
	P. graveolens	**22.99 (± 10.4)**	74.45 (± 13.92)	10.70	8.81
	P. sylvestris	**19.05 (± 3.73)**	**35.70 (± 20.61)**	12.73	1.92
	R. officinalis	**21.28 (± 20.67)**	**42.11 (± 9.96)**	**≥ 0.17**	2.62
	S. album	**8.70 (± 1.72)**	**30.21 (± 10.16)**	**0.17**	0.52
	S. sclarea	**14.57 (± 3.88)**	62.50 (± 10.35)	**≥ 0.11**	≥ 0.50
	V. zizanioides	**19.09 (± 4.58)**	**34.91 (± 5.3)**	**0.40**	0.58
Origanum vulgare	*B. carterii*	**29.05 (± 25.15)**	72.59 (± 4.86)	0.85	1.82
	C. aurantifolia	58.83 (± 27.01)	78.27 (± 6.83)	1.74	2.20
	C. bergamia	**11.24 (± 5.81)**	**38.02 (± 9.45)**	**0.27**	0.89
	C. martini	**41.10 (± 6.59)**	81.32 (± 6.42)	1.29	2.41
	C. paradisi	54.61 (± 10.48)	87.03 (± 11)	2.20	2.16
	C. reticulata	**35.74 (± 11.88)**	63.30 (± 17.1)	2.34	1.99
	C. sinesis	**26.03 (± 23.80)**	65.65 (± 6.34)	2.07	2.20
	E. globulus	**13.92 (± 14.97)**	**20.99 (± 7.72)**	**≥ 0.25**	6.08
	H. officinalis	**49.66 (± 8.78)**	87.51 (± 4.2)	3.86	3.01
	M. communis	**25.26 (± 18.50)**	72.38 (± 3.71)	1.85	2.84
	M. officinalis	52.40 (± 13.34)	90.19 (± 3.98)	2.38	2.59
	P. graveolens	50.72 (± 5.42)	61.72 (± 10.75)	24.11	7.94
	P. sylvestris	**13.78 (± 7.82)**	**29.37 (± 4.28)**	9.34	1.89
	R. officinalis	**49.43 (± 1.19)**	75.10 (± 13.91)	≥ 0.88	5.45
	S. sclarea	**8.45 (± 12.28)**	**19.70 (± 9.19)**	**≥ 0.15**	**≤0.35**
	V. zizanioides	**14.67 (± 8.17)**	**25.33 (± 3.28)**	**0.45**	0.68
Salvia officinalis	*B. carterii*	**0.00 (± 0.00)**	**0.61 (± 1.05)**	0.64	**0.01**
	C. atlantica	**8.85 (± 15.34)**	**32.25 (± 5.86)**	**0.25**	0.54
	C. aurantifolia	**0.00 (± 0.00)**	**1.65 (± 1.47)**	**0.00**	**0.03**
	C. bergamia	**14.00 (± 6.28)**	**47.90 (± 1.57)**	**0.23**	0.77
	C. nobile	**0.00 (± 0.00)**	**0.40 (± 0.7)**	**0.00**	**0.01**

Essential oil combination		% Mortality		ΣFMC	
Essential oil 1	**Essential oil 2**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
	C. paradisi	**0.69 (± 1.20)**	**15.60 (± 9.17)**	**0.02**	**0.27**
	C. reticulata	**7.99 (± 1.75)**	**31.68 (± 4.11)**	**0.46**	0.76
	C. sinesis	**0.00 (± 0.00)**	**2.81 (± 3.17)**	**0.00**	**0.07**
	E. globulus	**0.00 (± 0.00)**	**0.81 (± 1.41)**	**≥ 0.00**	**0.23**
	J. communis	**0.00 (± 0.00)**	**0.48 (± 0.82)**	**≥ 0.00**	**≤0.00**
	L. angustifolia	**0.00 (± 0.00)**	**1.51 (± 1.32)**	**0.00**	**0.04**
	M. alternifolia	**0.00 (± 0.00)**	**0.53 (± 0.00)**	**0.00**	**0.01**
	M. officinalis	**4.58 (± 4.02)**	**16.78 (± 11.68)**	**0.17**	**0.36**
	O. sanctum	**32.14 (± 7.04)**	**48.58 (± 1.42)**	1.59	1.19
	P. graveolens	**3.31 (± 4.61)**	**15.01 (± 8.88)**	1.55	1.82
	P. sylvestris	**0.00 (± 0.00)**	**0.81 (± 1.41)**	**0.00**	**0.05**
	T. vulgaris	**0.00 (± 0.00)**	**0.00 (± 0.00)**	**≥ 0.00**	**0.00**

[1] Non-toxic essential oils shown in bold

Biofilm Inhibition Studies

Candida spp. was selected as a model for biofilm studies (Table 3.7) based on the fact that this pathogen is a major colonizer of the vagina.[4,5] Furthermore, the essential oil selection (*C. aurantifolia, C. camphora, M. cajuputi, M. communis,* and *O. vulgare*) was refined based on the essential oils that displayed noteworthy MIC inhibitory activity (with specific synergistic activity against the chosen pathogens for biofilm studies), did not interfere with the majority of the vaginal microbiota, and were non-toxic. The essential oil, *M. communis,* exhibited negative inhibition values against *C. albicans* biofilms, indicating that it did not suppress but instead enhanced biofilm formation. In contrast, the same essential oil exerted modest inhibitory activity against *C. glabrata*, suggesting a species-dependent effect. The inhibition results for *O. vulgare* were consistent with some previous studies, which reported at least 50% inhibition, except at the lowest concentration.[41,94,95] In another study, the percentage inhibition of *O. vulgare* was reported to be estimated at 90% of *C. albicans* and *C. glabrata* biofilms.[96] The concentration ranges utilized in the previous study featured end breakpoint values that do not align with concentrations optimal for *in vivo* applications. This discrepancy reveals the critical need for selecting appropriate biofilm breakpoints, highlighting the importance of considering these factors when interpreting experimental results. Additionally, the use of different strains, *C. albicans* (ATCC 14053) and *C.*

glabrata (ATCC 66032), may have influenced the observed differences found in previous studies.[96]

Table 3.7. Percentage inhibition (%) of biofilm by essential oils against biofilms formed by *C. albicans* and *C. glabrata*

	C. albicans			***C. glabrata***		
Essential oils	**2 mg/mL**	**1 mg/mL**	**0.5 mg/mL**	**2 mg/mL**	**1 mg/mL**	**0.5 mg/mL**
C. aurantifolia	**83.07 (±0.03)**[1]	**83.49 (±0.02)**	**77.08 (±0.01)**	**86.74 (± 0.02)**	**84.53 (± 0.01)**	**77.56 (± 0.05)**
M. communis	-105.26 (± 0.63)	-67.14 (±0.18)	-64.86 (±1.04)	**52.34 (± 0.12)**	14.33 (± 0.1)	4.92 (± 0.11)
O. vulgare	**56.32 (± 0.01)**	**54.93 (±0.08)**	47.08 (±0.06)	**76.84 (± 0.04)**	**74.73 (± 0.01)**	**66.07 (± 0.02)**
			Controls			
Amphotericin B	**73.90**	**73.83**	**68.08**	**50.00**	48.19	41.16
Water in acetone	33.00	25.32	19.40	17.89	14.38	4.92

[1]Expressed as a percentage (%) where ≥ 50% was regarded as noteworthy inhibitory activity, values in bold.

The essential oil combination having synergy against *C. albicans* and *C. glabrata*, being non-toxic and having non-inhibitory effects on the *Lactobacillus* spp. was selected for biofilm testing (Table 3.8). For both *C. albicans* and *C. glabrata*, the combination of essential oils exhibited a higher percentage of biofilm inhibition compared to the oils used individually. At the lowest concentration, the essential oil combination effectively inhibited the biofilm of both *C. albicans* and *C. glabrata*. To the best of the researcher's knowledge, no studies have reported on the biofilm inhibition of these essential oil combinations.

Table 3.8. Percentage inhibition (%) of biofilm by essential oils against biofilms formed by *C. albicans* and *C. glabrata*

Essential oil/Combination	**Essential oil concentration (mg/mL)**	***C. albicans***	***C. glabrata***
C. camphora	2.00	**62.30 (±0.02)**[1]	**60.00 (±0.002)**
	1.00	**60.25 (±0.00)**	**51.89 (±0.02)**
	0.50	48.00 (±0.01)	**50.56 (±0.01)**
M. cajuputi	2.00	**62.49 (±0.009)**	**70.91 (±0.01)**
	1.00	**55.93 (±0.00)**	**67.88 (±0.04)**
	0.50	**55.41 (±0.007)**	**65.26 (±0.01)**
C. camphora with *M. cajuputi*	2.00	**87.01 (±0.008)**	**75.39 (±0.06)**

	1.00	**82.43 (±0.007)**	**69.06 (±0.06)**
	0.50	**81.85 (±0.006)**	**68.43 (±0.06)**

[1]Values where biofilm inhibition ≥ 50% in bold.

Conclusion

This study investigated the antimicrobial effects, impact on vaginal microbiota, toxicity profiles, and biofilm inhibition activity of 46 essential oils against seven bacterial and yeast pathogen reference strains. The most effective broad-spectrum activity overall was essential oils *C. aurantifolia*, *C. nobile*, *C. paradisi*, *O. vulgare*, and *M. communis* when investigated independently.

Subsequently, 602 combinations were tested, with 25% showing synergy, of which 6% did not inhibit vaginal microbiota and were non-toxic. *Origanum vulgare* and *C. camphora* were involved in the most synergistic interactions. The combinations with the best activity were *C. camphora* with *M. cajuputi*, *C. camphora* with *O. sanctum*, and *S. officinalis* with *O. sanctum*. *Cinnamomum camphora* with *M. cajuputi* displayed the most favourable antimicrobial activity across all parameters, including biofilm inhibition.

The study demonstrated that selected essential oils and their combinations have optimal antimicrobial activity against vaginal pathogens, favourable toxicity profiles, minimal interaction with the vaginal microbiota, and effective biofilm inhibition. These findings may hold relevance in the broader context of antimicrobial resistance and the global burden of recurrent vaginal infections. By identifying potential essential oil-based treatments, this study contributes to the growing body of evidence supporting alternative approaches that could, with further validation, reduce dependence on conventional antimicrobials and support improved reproductive health outcomes.

Author Contributions

MH. Laher: data collection, writing of manuscript

S. van Vuuren; Supervising, conceptualization, financial support, and editing of manuscript
A. Orchard; Supervising, conceptualisation, financial support, and editing of manuscript

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Declaration of Conflicting Interests

The author(s) have declared no potential conflicts of interest with regard to the research, authorships, and/or publication of this article.

Data Availability Statement

All the data relevant to this study have been included in the manuscript.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal participants.

References

1. Jansåker F, Frimodt-Møller N, Li X, Sundquist K. Novel risk factors associated with common vaginal infections: A nationwide primary health care cohort study. *Int J Infect Dis*. 2022; 116:380-386.

2. Coudray MS, Madhivanan P. Bacterial vaginosis—a brief synopsis of the literature. *Eur J Obstet Gynecol Reprod Biol*. 2020; 245:143-148.

3. Subedi A, Upreti MK, Rana JC, Sapkota RP, Thapa Shrestha U. Vulvovaginal candidiasis, an increasing burden to women in the tropical regions attending Bharatpur Hospital, Chitwan. *J Med Mycol*. 2024;34(4):101509.

4. Nazzaro F, Fratianni F, Coppola R, Feo VD. Essential oils and antifungal activity. *Pharmaceuticals*. 2017;10(4):86.

5. Neal CM, Martens MG. Clinical challenges in diagnosis and treatment of recurrent vulvovaginal candidiasis. *SAGE Open Med*. 2022; 10:1-12.

6. Superti F, De Seta F. Warding off recurrent yeast and bacterial vaginal infections: Lactoferrin and lactobacilli. *Microorganisms*. 2020; 8:1-12.

7. Kalia N, Singh J, Kaur M. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: A critical review. *Ann Clin Microbiol Antimicrob*. 2020; 19(1):5.

8. Muzny CA, Schwebke JR. Biofilms: An underappreciated mechanism of treatment failure and recurrence in vaginal infections. *Clin Infect Dis*. 2015; 61(4):601-606.

9. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem*. 2003; 10(10):813-829.

10. Hou T, Sana SS, Li H, et al. Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Biosci*. 2022; 47:101716.

11. van Vuuren S, Viljoen A. Plant-based antimicrobial studies – methods and approaches to study the interaction between natural products. *Planta Med*. 2011;77(11):1168-1182.

12. Orchard A, van Vuuren S. Commercial essential oils as potential antimicrobials to treat skin diseases. *Evid Based Complement Alternat Med*. 2017; 1-92.

13. de Rapper S, van Vuuren SF. Odoriferous therapy: A review identifying essential oils against pathogens of the respiratory tract. *Chem Biodivers*. 2020; 17(6):e2000101.

14. Ouwehand AC, Tiihonen K, Kettunen H, et al. *In vitro* effects of essential oils on potential pathogens and beneficial members of the normal microbiota. *Vet Med*. 2010; 55(2):71-78.

15. Moritz CMF, Rall VLM, Saeki MJ, Fernandes Júnior A. Inhibitory effect of essential oils against Lactobacillus rhamnosus and starter culture in fermented milk during its shelf-life period. *Braz J Microbiol*. 2012; 43(3):1147-1156.

16. Petretto GL, Fancello F, Bakhy K, et al. Chemical composition and antimicrobial activity of essential oils from Cuminum cyminum L. collected in different areas of Morocco. *Food Biosci*. 2018; 22:50-58.

17. Raspo MA, Vignola MB, Andreatta AE, Juliani HR. Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. *Food Biosci*. 2020; 36:100651.

18. Micucci M, Protti M, Aldini R, et al. Thymus vulgaris L. essential oil solid formulation: Chemical profile and spasmolytic and antimicrobial effects. *Biomolecules*. 2020; 10(6):860.

19. Valdivieso-Ugarte M, Plaza-Diaz J, Gomez-Llorente C, et al. *In vitro* examination of antibacterial and immunomodulatory activities of cinnamon, white thyme, and clove essential oils. *J Funct Foods*. 2021; 81:104436.

20. Yazdanian M, Rostamzadeh P, Alam M, et al. Evaluation of antimicrobial and cytotoxic effects of Echinacea and Arctium extracts and Zataria essential oil. *AMB Express*. 2022; 12(1):75.

21. Eisenhut M. The toxicity of essential oils. *Int J Infect Dis*. 2007; 11(4):365.

22. Lanzerstorfer P, Sandner G, Pitsch J, et al. Acute, reproductive, and developmental toxicity of essential oils assessed with alternative *in vitro* and *in vivo* systems. *Arch Toxicol*. 2021; 95(2):673-691.

23. Sellar W. The Directory of Essential Oils. London, UK: C. W. Daniel Company Ltd; 1992.

24. Lawless J. The encyclopedia of essential oils: The complete guide to the use of aromatic oils in aromatherapy, herbalism, health, and well-being. USA: Element Books; 1995.

25. Curtis S. Essential Oils. London: Aurum Press; 1996.

26. Hili P. The Antimicrobial Properties of Essential Oils. Kent: Winter Press; 2001.

27. Buckle J. Clinical Aromatherapy: Essential Oils in Practice. 2nd ed. London: Churchill Livingstone; 2003.

28. Sellar W. The Directory of Essential Oils. 2nd ed. London: Random House UK Limited; 2005.

29. Kovač M. A Quick Guide to Essential Oils. Aromadelavnice; 2011.

30. Orchard A, Sandasi M, Kamatou G, Viljoen A, van Vuuren S. The *in vitro* antimicrobial activity and chemometric modelling of 59 commercial essential oils against pathogens of dermatological relevance. *Chem Biodivers*. 2017; 14(1):e1600218.

31. Orchard A, van Vuuren SF, Viljoen AM, Kamatou G. The *in vitro* antimicrobial evaluation of commercial essential oils and their combinations against acne. *Int J Cosmet Sci*. 2018; 40(3):226-243.

32. Hübsch Z, Van Zyl RL, Cock IE, van Vuuren SF. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. *S Afr J Bot*. 2014; 93:185-197.

33. Sarah Q, Anny F, Misbahuddin M. Brine shrimp lethality assay. *Bangladesh J Pharmacol*. 2017; 12:5.

34. Bussmann RW, Malca G, Glenn A, et al. Toxicity of medicinal plants used in traditional medicine in northern Peru. *J Ethnopharmacol*. 2011; 137(1):121-140.

35. Kharsany K, Viljoen A, Leonard C, van Vuuren S. The new buzz: Investigating the antimicrobial interactions between bioactive compounds found in South African propolis. *J Ethnopharmacol*. 2019; 238:111867.

36. Sandasi M, Leonard CM, van Vuuren SF, Viljoen AM. Peppermint (Mentha piperita) inhibits microbial biofilms *in vitro*. *S Afr J Bot*. 2011; 77(1):80-85.

37. Cuéllar-Cruz M, López-Romero E, Villagómez-Castro JC, Ruiz-Baca E. *Candida* species: New insights into biofilm formation. *Future Microbiol*. 2012; 7(6):755-771.

38. Shinde RB, Raut JS, Karuppayil MS. Biofilm formation by *Candida albicans* on various prosthetic materials and its fluconazole sensitivity: A kinetic study. *Mycoscience*. 2012; 53(3):220-226.

39. Rodrigues CF, Rodrigues ME, Silva S, Henriques M. *Candida glabrata* biofilms: How far have we come? *J Fungi (Basel)*. 2017; 3(1):1-16.

40. Pathak P. *Candida albicans* biofilm: Risks, complications and preventive strategies. In: Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry. 2019; 121-128.

41. Hacioglu M, Oyardi O, Kirinti A. Oregano essential oil inhibits *Candida* spp. biofilms. *Z Naturforsch C J Biosci*. 2021; 76(11):443-450.

42. Shirinda H, Leonard C, Candy G, van Vuuren S. Antimicrobial activity and toxicity profile of selected Southern African medicinal plants against neglected gut pathogens. *S Afr J Sci*. 2019; 115(11-12):1-10.

43. De Rapper SL, Tankeu SY, Kamatou G, Viljoen A, van Vuuren S. The use of chemometric modelling to determine chemical composition-antimicrobial activity relationships of essential oils used in respiratory tract infections. *Fitoterapia*. 2021; 154:105024.

44. Van Vuuren SF. Antimicrobial activity of South African medicinal plants. *J Ethnopharmacol*. 2008; 119(3):462-472.

45. Borotová P, Čmiková N, Galovičová L, et al. Antioxidant, antimicrobial, and anti-insect properties of *Boswellia carterii* essential oil for food preservation improvement. *Horticulturae*. 2023; 9(3):1-15.

46. Mohamed Abdoul-Latif F, Ainane A, Oumaskour K, et al. Effect of *Atlas cedar* essential oil against pathogenic medical bacterial strains—*in vitro* test. *Pharmacol Online*. 2021; 3:857-864.

47. Mollova S, Stanev S, Bojilov D, et al. Chemical composition and antioxidant activity of Roman chamomile (*Anthemis nobilis* L.) essential oil. *Oxid Commun*. 2024; 47(2):264-271.

48. Cheribot Cherif M, Boughendjioua H, Sakhraoui N. Chemical composition of essential oil from the leaves of *Cinnamomum camphora* (L.) J. Presl. *J Phytol*. 2024; 16:94-97.

49. Unlu M, Ergene E, Unlu GV, Zeytinoglu HS, Vural N. Composition, antimicrobial activity and *in vitro* cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). *Food Chem Toxicol*. 2010; 48(11):3274-3280.

50. Kaskoos RA. GC/MS profile and in-vitro antidiabetic activity of *Cinnamomum zeylanicum* Blume bark and *Trachyspermum ammi* (L.) Sprague seeds. *J Essent Oil-Bear Plants*. 2019; 22(2):535-544.

51. Spadaro F, Costa R, Circosta C, Occhiuto F. Volatile composition and biological activity of Key lime (*Citrus aurantifolia*) essential oil. *Nat Prod Commun*. 2012; 7(11):1523-1526.

52. Costa R, Bisignano C, Filocamo A, Grasso E, Occhiuto F, Spadaro F. Antimicrobial activity and chemical composition of *Citrus aurantifolia* (Christm.) Swingle essential oil from Italian organic crops. *J Essent Oil Res*. 2014; 26(6):400-408.

53. Ellouze I, Abderrabba M, Sabaou N, Mathieu F, Lebrihi A, Bouajila J. Season's variation impact on *Citrus aurantium* leaves essential oil: Chemical composition and biological activities. *J Food Sci*. 2012;77(9):

54. Hosni K, Zahed N, Chrif R, et al. Composition of peel essential oils from four selected Tunisian citrus species: Evidence for the genotypic influence. *Food Chem*. 2010; 123(4):1098-1104.

55. Sawamura M, Onishi Y, Ikemoto J, Tu NTM, Phi NTL. Characteristic odour components of bergamot (*Citrus bergamia* Risso) essential oil. *Flavour Fragr J*. 2006; 21(4):609-615.

56. Avila-Sosa R, Navarro-Cruz AR, Sosa-Morales ME, López-Malo A, Palou E. Bergamot (*Citrus bergamia*) oils. 2016;247-252.

57. Orchard A, van Vuuren SF, Viljoen AM. Commercial essential oil combinations against topical fungal pathogens. *Nat Prod Commun*. 2019; 14(1):1934578X1901400.

58. De Rapper S, Kamatou G, Viljoen A, van Vuuren S. The *in vitro* antimicrobial activity of *Lavandula angustifolia* essential oil in combination with other aromatherapeutic oils. *Evid Based Complement Alternat Med*. 2013; 2013:1-10.

59. Perveen K, Bokhari NA, Siddique I, Al-Rashid SAI. Antifungal activity of essential oil of *Commiphora molmol* oleo gum resin. *J Essent Oil-Bear Plants*. 2018; 21(3):667-673.

60. Marongiu B, Piras A, Porcedda S, Scorciapino A. Chemical composition of the essential oil and supercritical CO_2 extract of *Commiphora m*yrrha (Nees) Engl. and of *Acorus calamus* L. *J Agric Food Chem*. 2005; 53(20):7939-7943.

61. Msaada K, Hosni K, Taarit MB, et al. Variations in essential oil composition during maturation of coriander (*Coriandrum sativum* L.) fruits. *J Food Biochem*. 2009; 33(5):603-612.

62. Nejad Ebrahimi S, Hadian J, Ranjbar H. Essential oil compositions of different accessions of *Coriandrum sativum* L. from Iran. *Nat Prod Res*. 2010; 24(14):1287-1294.

63. Tchinda ES, Jazet PMD, Tatsadjieu LN, et al. Antifungal activity of the essential oil of *Cymbopogon citratus* (Poaceae) against *Phaeoramularia angolensis*. *J Essent Oil-Bear Plants*. 2009;12(2):218-224.

64. Smitha GR, Rana VS. Variations in essential oil yield, geraniol and geranyl acetate contents in palmarosa (*Cymbopogon martinii*, Roxb. Wats. Var. Motia) influenced by inflorescence development. *Ind Crops Prod*. 2015; 66:150-160.

65. Ajith M, Pankaj, Shakil NA, Kaushik P, Rana VS. Chemical composition and nematicidal activity of essential oils and their major compounds against *Meloidogyne graminicola* (rice root-knot nematode). *J Essent Oil Res*. 2020; 32(6):526-535.

66. Čmiková N, Galovičová L, Schwarzová M, et al. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*. 2023; 12(5):1-16.

67. Daroui-Mokaddem H, Kabouche A, Bouacha M, et al. GC/MS analysis and antimicrobial activity of the essential oil of fresh leaves of *Eucalyptus globulus*, and leaves and stems of

Smyrnium olusatrum from Constantine (Algeria). *Nat Prod Commun*. 2010; 5(10):1669-1672.

68. Mahumane GD, van Vuuren SF, Kamatou G, Sandasi M, Viljoen AM. Chemical composition and antimicrobial activity of *Eucalyptus radiata* leaf essential oil, sampled over a year. *J Essent Oil Res*. 2016; 28(6):475-488.

69. El-Mesallamy AMD, El-Gerby M, Abd El Azim MHM, Awad A. Antioxidant, antimicrobial activities and volatile constituents of clove flower buds oil. *J Essent Oil-Bear Plants*. 2012;15(6):900-907.

70. Sohilait HJ, Kainama H, Kaihena M. Chemical composition and antioxidant activities of the essential oils of Eugenia caryophyllata from northern Buru Island, Moluccas. *BioResources*. 2023; 18(4):7551-7565.

71. Pouryousef M. Variation in the essential oil constituents in indigenous populations of *Foeniculum vulgare* var. vulgare from different locations of Iran. *J Essent Oil Res*. 2014; 26(6):441-445.

72. Nebrigić V, Terzić M, Đurović S, et al. Influence of drying process on chemical composition, antioxidant and enzyme-inhibitory activity of *Helichrysum italicum* essential oils. *J Herbal Med*. 2023; 40:1-10.

73. Kladar NV, Anačkov GT, Rat MM, et al. Biochemical characterization of *Helichrysum italicum* (Roth) G. Don subsp. italicum (Asteraceae) from Montenegro: Phytochemical screening, chemotaxonomy, and antioxidant properties. *Chem Biodivers*. 2015; 12(3):419-431.

74. Fraternale D, Ricci D, Epifano F, Curini M. Composition and antifungal activity of two essential oils of Hyssop (*Hyssopus officinalis* L.). *J Essent Oil Res*. 2004; 16(6):617-622.

75. Rezvani S. Analysis of essential oil of *Juniperus communis* and terpenoids dried fruits from Golestan of Iran. *Asian J Chem*. 2010;22(2):1652-1654.

76. Kumar A, Yadav LBS, Ahmad J, Dubey N, Puri S. Chemical composition of commercial *Juniperus communis* L. leaf oil. *J Essent Oil-Bear Plants*. 2007; 10(4):310-313.

77. Nasser H, Arnold-Apostolides N. *Laurus nobilis* L., comparative chemical composition and antimicrobial activity of essential oils from fresh leaves, flowers and fruits. 2020; 169-178.

78. Trilles BL, Bombarda I, Bouraïma-Madjebi S, et al. Occurrence of various chemotypes in Niaouli [*Melaleuca quinquenervia* (Cav.) S.T. Blake] essential oil from New Caledonia. *Flavour Fragr J*. 2006; 21(4):677-682.

79. Chao WW, Su CC, Peng HY, Chou ST. *Melaleuca quinquenervia* essential oil inhibits α-melanocyte-stimulating hormone-induced melanin production and oxidative stress in B16 melanoma cells. *Phytomedicine*. 2017; 34:191-201.

80. Rehman SU, Latief R, Bhat KA, Shawl AS, Chandra S. Comparative analysis of the aroma chemicals of *Melissa officinalis* using hydrodistillation and HS-SPME techniques. *Arab J Chem*. 2017; 10:S2485-S2490.

81. Padalia RC, Verma RS. Comparative volatile oil composition of four *Ocimum* species from Northern India. *Nat Prod Res*. 2011; 25(6):569-575.

82. Alizadeh A, Khosh-Khui M, Javidnia K, Firuzi O, Jokar SM. Chemical composition of the essential oil, total phenolic content and antioxidant activity in *Origanum majorana* L. (Lamiaceae) cultivated in Iran. *Adv Environ Biol*. 2011; 5(8):2326-2331.

83. Kusuma HS, Mahfud M. GC-MS analysis of essential oil of *Pogostemon cablin* growing in Indonesia extracted by microwave-assisted hydrodistillation. *Int Food Res J*. 2017; 24(4):1525-1528.

84. Tadjine N, Messgo-Moumene S. Antimicrobial potential of essential oils and their chemical components. *Allelopathy J*. 2024; 61(1):53-62.

85. Dobreva A, Getchovska K, Nedeltcheva-Antonova D. A comparative study of Saudi Arabia and Bulgarian rose oil chemical profile: The effect of the technology and geographic origin. *Flavour Fragr J*. 2020 ;35(5):584-596.

86. Ghavam M. GC-MS analysis and antimicrobial activities of a *Rosmarinus officinalis* L. essential oil from Kashan region (Iran). *Biochem Syst Ecol*. 2022; 105:1-8.

87. Pegard A. Multifactorial evaluation of relaxing properties of *Juniperus virginiana* L. essential oil and comparison with *Salvia sclarea* L. essential oil *Phytotherapie*. 2022; 20(3):97-104.

88. Howes MJR, Simmonds MSJ, Kite GC. Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. *J Chromatogr A*. 2004; 1028(2):307-312.

89. Wesołowska A, Jadczak D. Comparison of the chemical composition of essential oils isolated from two thyme (*Thymus vulgaris* L.) cultivars. *Not Bot Horti Agrobo Cluj-Napoca*. 2019; 47(3):829-835.

90. Rentería PM, Herrera RR, Aguilar CN, Nevárez-Moorillón GV. Microbiological effect of fermented Mexican oregano (*Lippia berlandieri* Schauer) waste. *Waste Biomass Valor*. 2014; 5(1):57-63.

91. Marcial GE, Gerez CL, De Kairuz MN, Araoz VC, Schuff C, De Valdez GF. Influence of oregano essential oil on traditional Argentinean cheese elaboration: Effect on lactic starter cultures *Rev Argent Microbiol*. 2016; 48(3):229-235.

92. Chan CL, Gan RY, Shah NP, Corke H. Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. *Food Control*. 2018; 92:437-443.

93. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *Microb Cell Fact*. 2020; 19:1-15.

94. Müller-Sepúlveda A, Cid C, Jara JA, Díaz-Dosque M, Molina-Berríos A, editors. *Origanum vulgare* L. essential oil effect against *Candida* spp. biofilm formation. 2021; 2021/12.

95. Cid-Chevecich C, Müller-Sepúlveda A, Jara JA, et al. *Origanum vulgare* L. essential oil inhibits virulence patterns of *Candida* spp. and potentiates the effects of fluconazole and nystatin *in vitro*. *BMC Complement Med Ther*. 2022; 22(1):39.

96. Karpiński TM, Ożarowski M, Seremak-Mrozikiewicz A, Wolski H. Anti-*Candida* and antibiofilm activity of selected *Lamiaceae* essential oils. *Front Biosci (Landmark Ed)*. 2023; 28(2):28-28.

Chapter 4 – Overview and Conclusion

Vaginal infections are a significant public health concern, with high recurrence rates and rising antimicrobial resistance diminishing the efficacy of standard therapies. In this context, this study aimed to investigate the antimicrobial activity and toxicity of essential oils, both alone and in combination, against pathogens causing vaginal infections, and to determine their inhibitory effects on the natural vaginal microbiota. The findings highlight essential oils and their combinations as promising candidates for microbiota-preserving interventions, providing a foundation for future research into alternative strategies to manage resistant infections.

4.1 Highlights of the study

Upon investigating the antimicrobial activity of 46 essential oils against seven pathogens associated with vaginal infections and five natural microbiota that predominate the vaginal microbiome, the following was noted:

- The essential oils *M. officinalis* (MIC = 0.15-1.00 mg/mL), *P. cablin* (MIC = 0.25-0.63 mg/mL), *C. paradisi* (MIC = 0.25-0.75 mg/mL), and *C. aurantium* (MIC = 0.46-1.00 mg/mL)exhibited broad-spectrum antibacterial activity.
- Broad-spectrum anti-yeast activity was noted in *C. sativum* (MIC = 0.13-0.50 mg/mL), *C. martini* (MIC = 0.25-0.70 mg/mL), *C. aurantifolia* (MIC = 0.25-0.75 mg/mL), and *O. vulgare* (MIC = 0.25-0.83 mg/mL).
- Overall, *C. aurantifolia*, *C. paradisi*, *O. vulgare*, and *M. communis* exhibited broad-spectrum antimicrobial activity, effectively inhibiting six out of seven pathogens at a noteworthy MIC (1.00 mg/mL) and not interfering with the majority of *Lactobacilli* species making them promising candidates for preserving microbiota integrity. Observed differences in MIC values to previous studies were attributed to factors such as variability in ATCC reference strains, use of clinical strains, and differences in essential oil composition (Sook and Shin, 2007, Abdellatif et al., 2014, Poyil et al., 2022). Essential oils with MIC values consistent with literature typically had major constituent compositions within recommended ranges.

The interactive profile of 602 essential oil combinations against seven pathogens associated with vaginal infections and five natural microbiota using the ΣFIC index was analysed and the following was highlighted:

- The essential oil combinations *O. vulgare* with *C. reticulata*, *O. vulgare* with *O. sanctum, and O. vulgare* with *R. officinalis* demonstrated the most noteworthy antimicrobial activity and did not interfere with most *Lactobacilli* spp. ($n \geq 3$).
- In terms of synergy, the combinations *C. camphora* with *M. cajuputi* (ΣFIC = 0.08-1.62), *C. camphora* with *O. sanctum* (ΣFIC = 0.11-1.49), and *S. officinalis* with *O. sanctum* (ΣFIC = 0.07-1.63), showed synergy against the majority of both bacterial ($n \geq 2$) and yeast ($n \geq 3$) pathogens and did not interfere with the majority of *Lactobacillus* spp. ($n \geq 3$).
- *Enterococcus faecalis* was the most susceptible pathogen to the essential oil combinations despite showing resistance in some of the single essential oil testing (MIC range: 0.50–8.00 mg/mL), highlighting the effectiveness of combinations in overcoming resistance against this pathogen.
- *Salvia officinalis* (34 times) and *O. vulgare* (27 times) were most involved in synergistic interactions.
- The combination of *O. vulgare* with *C. reticulata* is the only combination that did not interfere with all five species of *Lactobacilli,* whilst also displaying noteworthy antimicrobial activity.

The toxicity of essential oils and their combinations using the brine shrimp lethality assay were assessed, and the following observations could be made:

- Overall, 62% of essential oils and 49% of essential oil combinations were non-toxic, suggesting their potential for safe therapeutic use.
- The single essential oils *C. aurantifolia*, *O. vulgare*, and *M. communis* displayed noteworthy antimicrobial activity and did not interfere with the majority of the vaginal microbiota and were also found to be non-toxic.
- The essential oil combinations *C. camphora* with *M. cajuputi*, *C. camphora* with *O. sanctum*, and *S. officinalis* with *O. sanctum* exhibited the most synergistic antimicrobial activity while not interfering with natural vaginal colonizers. These combinations also demonstrated non-toxic activity profiles.

Lastly, the Candida spp. was chosen as a model for biofilm studies due to its role as a resilient vaginal pathogen and colonizer (Neal and Martens, 2022). The selection of essential oils was narrowed based on their notable antimicrobial properties, synergistic effects against the pathogens used in the biofilm assay, with minimal impact on the vaginal microbiota, and displaying no or limited toxicity. The results showed that:

- The majority of the essential oils (75%) demonstrated a concentration-dependent relationship, with higher concentrations resulting in greater inhibition of biofilm formation.
- The essential oils *C. aurantifolia* and *O. vulgare* demonstrated the best biofilm inhibition (≥50%) against *C. albicans* and *C. glabrata*.
- The combination of *C. camphora* with *M. cajuputi* exhibited higher inhibition of biofilm formation compared to individual essential oils.

The overall data analysis revealed that:

- Of the 46 essential oils tested against seven pathogens, 36% showed noteworthy antimicrobial activity, with 13% being non-toxic and not affecting most of the natural microbiota tested.
- The most effective oils in terms of demonstrating noteworthy antimicrobial activity, non-interfering to the commensal microbiota, being non-toxic and showing a high percentage inhibition of yeast biofilm inhibition were *C. aurantifolia*, and *O. vulgare*.
- Among 602 essential oil combinations, 25% exhibited synergy, with 6% being non-toxic and not inhibiting microbiota. The *C. camphora* with *M. cajuputi* combination showed the best overall activity by demonstrating noteworthy antimicrobial activity, not inhibiting the vaginal microbiota and not being toxic. This combination also demonstrated noteworthy biofilm inhibition when tested against selected yeast biofilms.

The study flow and outcomes of the investigation are best summarised in Figure 4.1 and the data summary for the essential oils highlighted are summarized in Table 4.1

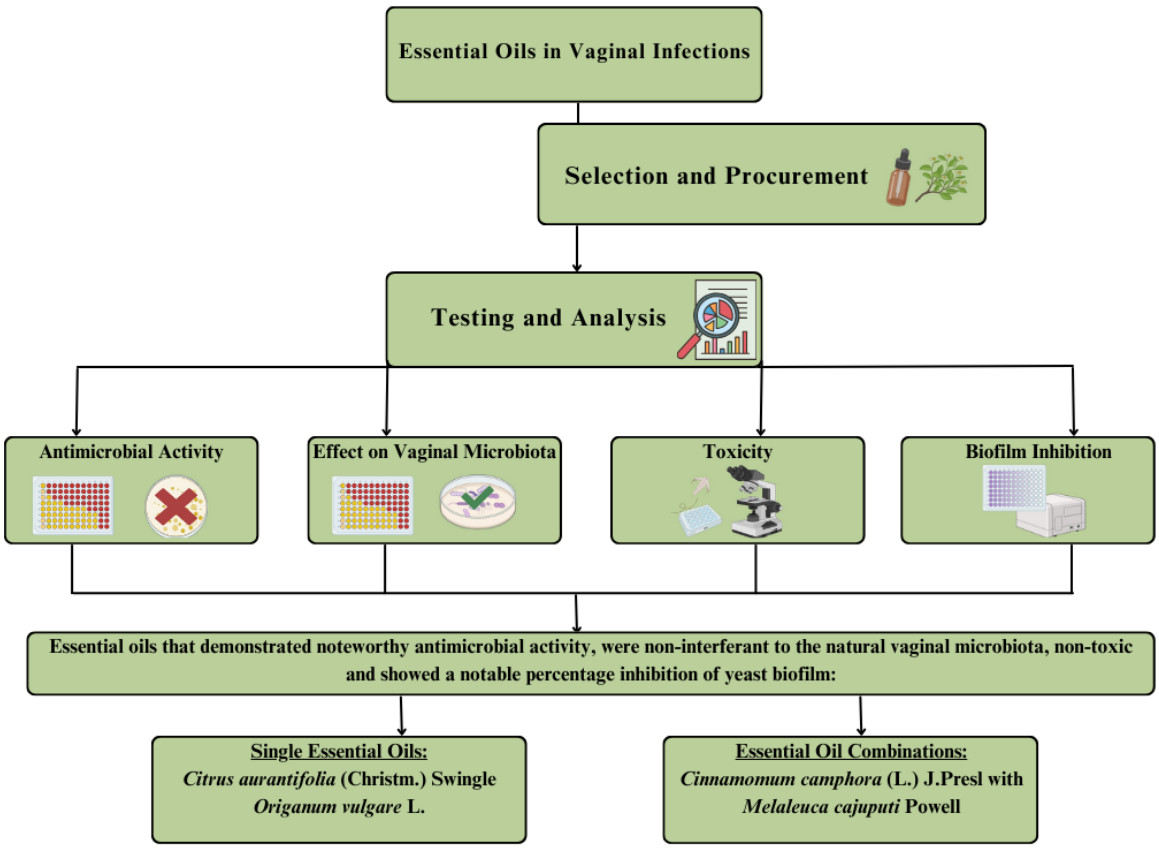

Figure 4.1: **Study flow and highlights**

Table 4.1: Table highlighting the data summary for the key essential oils highlighted in the study outcomes

Essential Oil(s)	Antimicrobial activity (mg/mL)	Effect on Microbiota (mg/mL)	Toxicity (% Mortality)	Biofilm Inhibition (% Inhibition)
C. aurantifolia	MIC = 0.25-2.00	MIC = 0.60-4.00	24 hrs = 42.51 48 hrs = 48.87	77.56-86.74
O. vulgare	MIC = 0.25-1.33	MIC = 1.00->8.00	24 hrs = 12.74 48 hrs = 28.01	47.08-76.84
C. camphora	MIC = 0.75-5.33	MIC = 1.17->8.00	24 hrs = 0.00 48 hrs = 3.52	48.00-60.00
M. cajuputi	MIC = 0.70-2.80	MIC = 1.67-3.20	24 hrs = 34.34 48 hrs = 46.90	55.41-70.91
C. camphora with *M. cajuputi*	MIC = 0.25-2.00 ΣFIC = 0.08-1.62	MIC = 1.00-3.00 ΣFIC = 0.92-2.00	24 hrs = 34.34 48 hrs = 46.90	75.39-87.01

4.2 Limitations and future considerations

A critical limitation of this study was the absence of scientific names and plant parts sourced from the layman's literature, potentially leading to inconsistency in essential oil selection. A way forward is to engage with aromatherapists and encourage the implementation of the use of scientific names and documentation of plant parts used. The use of various laboratory reference strains limits the clinical relevance of the findings. Future studies should incorporate clinical and resistant strains to assess the effectiveness of the promising essential oils studied. Culturing *P. anaerobius* proved challenging due to its fastidious nature. Additional data is urgently needed to evaluate the toxicity of essential oil combinations, particularly in clinical applications. The brine shrimp lethality assay used in this study may not accurately reflect *in vivo* toxicity, necessitating comprehensive toxicity testing in human models. Furthermore, the long-term effects of essential oils on the vaginal microbiota require further exploration, as their prolonged use may impact on the vaginal ecosystem in ways not yet fully understood. Another future consideration to consider is the application of essential oils, which are typically diluted in a carrier or base. It is crucial to account for the interactions between the essential oils and the various potential bases used and their effects on the vaginal microbiota. Lastly, the limited data on biofilm inhibition for some essential oils, especially in combination, highlights the need for further research within this field.

4.3 Conclusion

The essential oils studied herein certainly have the potential to serve as alternative therapies for vaginal infections. The study's novelty lies in evaluating essential oil combinations for antimicrobial activity, toxicity, impact on natural microbiota, and biofilm inhibition. The essential oils *C. aurantifolia*, *O. vulgare*, and the combination of *C. camphora* with *M. cajuputi* showed noteworthy antimicrobial activity, minimal or no toxicity, no disruption to commensal microbiota, and effective biofilm inhibition against *Candida*. The study provides a novel impetus for the use of selected essential oils to combat vaginal infections while keeping the natural biome intact.

References excluding those that are in the publications

Abdellatif, F., Boudjella, H., Zitouni, A. and Hassani, A., 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. *EXCLI Journal*, *13*, p.772.

Abou Chacra, L., Fenollar, F. and Diop, K., 2022. Bacterial vaginosis: what do we currently know?. *Frontiers in Cellular and Infection Microbiology*, *11*, p.672429.

Ahmed Khan, R. and van Vuuren, S.F., 2021. Essential oil combinations against *Clostridium perfringens* and *Clostridium septicum*-the causative agents of gas gangrene. *Journal of Applied Microbiology*, *131*(3), pp.1177-1192.

Akinosoglou, K., Livieratos, A., Asimos, K., Donders, F. and Donders, G.G., 2024. Fluconazole-resistant vulvovaginal candidosis: An update on current management. *Pharmaceutics*, *16*(12), p.1555.

Artym, J. and Zimecki, M., 2021. Antimicrobial and prebiotic activity of lactoferrin in the female reproductive tract: a comprehensive review. *Biomedicines*, *9*(12), p.1940.

Bogavac, M.A., Perić, T.M., Mišković, J. and Karaman, M., 2022. Antimicrobial and toxic effects of *Boswellia Serrata* Roxb. and *Mentha piperita* Linn. essential oils on vaginal inhabitants. *Medicines*, *9*(12), p.62.

Bounar, R., Krimat, S., Boureghda, H. and Dob, T., 2020. Chemical analyses, antioxidant and antifungal effects of oregano and thyme essential oils alone or in combination against selected *Fusarium* species. *International Food Research Journal*, *27*(1), pp.66-77.

Bunse, M., Daniels, R., Gründemann, C., Heilmann, J., Kammerer, D.R., Keusgen, M., Lindequist, U., Melzig, M.F., Morlock, G.E., Schulz, H. and Schweiggert, R., 2022. Essential oils as multicomponent mixtures and their potential for human health and well-being. *Frontiers in Pharmacology*, *13*, p.956541.

Calinescu, G., Bohiltea, R. E., Balan, A. M., Georgescu, T. A., Bacalbasa, N., Balescu, I., Mihai, B. M., Dima, V., Vladareanu, I. T., Salmen, T., Salmen, T., and Grigoriu, C., 2021. Modern investigation of cervico-vaginal infections (from microbiome disorders to pelvic inflammatory disease). *Romanian Medical Journal, 68, pp.*99 -103.

Deka, N., Hassan, S., Seghal Kiran, G. and Selvin, J., 2021. Insights into the role of vaginal microbiome in women's health. *Journal of Basic Microbiology*, *61*(12), pp.1071-1084.

dos Santos Janotto, L., de Melo Nazareth, T., Meca, G., Luciano, F.B. and Evangelista, A.G., 2023. Exploring the efficacy of antibiotic-essential oil combinations: Implications for combating antimicrobial resistance. *Bioresource Technology Reports*, *24*, p.101679.

Herman, A. and Młynarczyk, A., 2015. Essential oils and plant extracts with activity against oral microorganisms: prevention and treatment of oral diseases. *Israel Journal of Plant Sciences, 62*(4), pp.250-257.

Hili, P., 2001. *The Antimicrobial Properties of Essential Oils,* Kent, England. Winter Press.

Ibragimova, D.M., 2019. Features of preoperative preparation of patients for gynecological surgeries (clinical observation*). RMJ. Mother and Child*, *2*(3), pp.213-216.

Jodar, A., Soto, L., Turell, E. and Cortés, J., 2023. Monotherapy with a non-hormonal *Centella asiatica*, hyaluronic acid, and prebiotic-based vaginal gel in women with bacterial vaginosis: Case series. *Clinical Medicine Insights: Case Reports*, *16*, p.11795476231157244.

Karpiński, T.M., 2020. Essential oils of *Lamiaceae* family plants as antifungals. *Biomolecules*, *10*(1), p.103.

Khan, M.S.A., Malik, A. and Ahmad, I., 2012. Anti-candidal activity of essential oils alone and in combination with amphotericin B or fluconazole against multi-drug resistant isolates of *Candida albicans*. *Medical Mycology*, *50*(1), pp.33-42.

Koursaoui, L., Badr, S., Ghanmi, M., Jaouadi, I., Chibani, A., Chahboun, N., Chaouch, A. and Zarrouk, A., 2023. Phytochemical analysis, antioxidant and antimicrobial activity of three *Eucalyptus* species essential oils from the Moroccan Maâmora Forest: *Eucalyptus cladocalyx* F. Muell, *Eucalyptus grandis* W. Hill ex Maiden and *Eucalyptus botryoides* Sm. *Chemical Data Collections*, *48*, p.101101.

Labiad, H., El Jemli, M., Marmouzi, I., Chaouch, A., Ghanmi, M., Satrani, B., Aljaiyash, A.E. and Fadli, M., 2019. Toxicological study and psychotropic activity of the essential oils of *Laurus nobilis* and *Vitex agnus-castus*. *Phytothérapie*, *17*(5), pp.276-282.

Leclair, C. and Stenson, A., 2022. Common causes of vaginitis. *JAMA*, *327*(22), pp.2238-2239.

Leigh-de Rapper, S., Tankeu, S.Y., Kamatou, G., Viljoen, A. and van Vuuren, S., 2021. The use of chemometric modelling to determine chemical composition-antimicrobial activity relationships of essential oils used in respiratory tract infections. *Fitoterapia*, *154*, p.105024.

Machado, D., Gaspar, C., Palmeira-de-Oliveira, A., Cavaleiro, C., Salgueiro, L., Martinez-de-Oliveira, J. and Cerca, N., 2017. *Thymbra capitata* essential oil as potential therapeutic agent against Gardnerella vaginalis biofilm-related infections. *Future Microbiology*, *12*(5), pp.407-416.

Mora, N.O., 2019. Infections of the lower genital tract: vaginal discharge. *Journal of Obstetrics and Gynecology of Venezuela*, *79*(2), pp.98-107.

Muzny, C.A. and Schwebke, J.R., 2015. Biofilms: an underappreciated mechanism of treatment failure and recurrence in vaginal infections. *Clinical Infectious Diseases*, *61*(4), pp.601-606.

Muzny, C.A. and Sobel, J.D., 2022. The role of antimicrobial resistance in refractory and recurrent bacterial vaginosis and current recommendations for treatment. *Antibiotics*, *11*(4), p.500.

Orchard, A., Viljoen, A. and van Vuuren, SF., 2018. Wound pathogens: Investigating antimicrobial activity of commercial essential oil combinations against reference strains. *Chemistry & Biodiversity*, *15*(12), p.e1800405.

Ouwehand, A.C., Tiihonen, K., Kettunen, H., Peuranen, S., Schulze, H. and Rautonen, N., 2010. *In vitro* effects of essential oils on potential pathogens and beneficial members of the normal microbiota. *Veterinarni Medicina*, *55*(2), pp.71-78.

Poyil, M.M., Geddawy, A., Mohideen, A.P. and Raja, K., 2022. Anti-inflammatory and anti-*candidal* activity of *Coriandrum sativum* seed extracts against *Candida albicans* and *Candida tropicalis*. *Gomal Journal of Medical Sciences*, *20*(1), pp.24-29.

Puškárová, A., Bučková, M., Kraková, L., Pangallo, D. and Kozics, K., 2017. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. *Scientific Reports*, *7*(1), p.8211.

Sayed, M.A., Ghazy, N.M., El Sayed, H. and El-Bassuony, A.A., 2024. Synergistic potential of essential oil combinations against *Microsporum*, *Trichophyton*, and *Epidermophyton*. *International Microbiology*, pp.1-17.

Sook, L. and Shin, S.W., 2007. Synergism in antifungal activity against *Candida* and *Trichophyton* species in combination with the essential oil of *Coriandrum sativum* L. and antibiotics. *Natural Product Sciences*, *13*(1), pp.85-89.

Svobodová, L., Lysková, P. and Hamal, P., 2015. Vulvovaginal candidiasis. *Klinicka Mikrobiologie a Infekcni Lekarstvi*, *21*(3), pp.74-81.

Tomičić, Z., Tomičić, R., Kocić-Tanackov, S. and Raspor, P., 2022. Essential oils as antimicrobial and anti-adhesion agents against bacteria *Salmonella Typhimurium* and *Staphylococcus aureus*, and yeasts *Candida albicans* and *Saccharomyces cerevisiae*. *Food and Feed Research*, *49*(2), pp.107-115.

Valdivieso-Ugarte, M., Plaza-Diaz, J., Gomez-Llorente, C., Gómez, E.L., Sabés-Alsina, M. and Gil, Á., 2021. *In vitro* examination of antibacterial and immunomodulatory activities of cinnamon, white thyme, and clove essential oils. *Journal of Functional Foods*, *81*, p.104436.

Willems, H.M., Ahmed, S.S., Liu, J., Xu, Z. and Peters, B.M., 2020. Vulvovaginal candidiasis: a current understanding and burning questions. *Journal of Fungi*, *6*(1), p.27.

Yap, P.S.X., Yiap, B.C., Ping, H.C. and Lim, S.H.E., 2014. Essential oils, a new horizon in combating bacterial antibiotic resistance. *The Open Microbiology Journal*, *8*, p.

Appendix A – Abstract for presentation

The antimicrobial and toxicity analysis of essential oils used for vaginal infections

Muneebah Haroon Laher, Ané Orchard, Sandy van Vuuren

Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa

Current treatment options for vaginal infections are increasingly limited due to bacterial and fungal resistance. Furthermore, these treatments often have adverse effects on the vaginal microbiome. With existing therapies losing effectiveness, there is a drastic need for alternative approaches. Essential oils, known for their antimicrobial properties, have not been extensively studied for treating vaginal infections alone or in combination despite their recommended use by the layman. This study evaluates the antimicrobial activity by determining the minimum inhibitory concentration, (MIC) and toxicity (brine shrimp lethality assay) of 46 essential oils, both individually and in combination, against pathogens causing vaginal infections. These included *Enterococcus faecalis, Gardnerella vaginalis, Peptostreptococcus anaerobius, Candida albicans, Candida tropicalis, Candida krusei, and Candida gasseri*. Additionally, the effects of these essential oils on the natural vaginal bacteria (*Lactobacilli*) were assessed, followed by an interactive assessment using the fractional inhibitory concentration index (ΣFIC) to determine synergistic profiles. The *in vitro* susceptibility tests revealed that the essential oils *Melissa officinalis* exhibited one of the lowest MIC values at 0.16 mg/ml against bacterial pathogens, while *Coriandrum sativum* demonstrated the lowest MIC value at 0.13 mg/ml for fungal pathogens. Notably, *Melissa officinalis* interfered least with the *Lactobacilli*. The combination of *Citrus bergamia* with *Styrax benzoin* demonstrated antimicrobial synergy ($\Sigma FIC \leq 0.5$). Toxicity assays indicated variability, with some essential oils displaying higher toxicity levels than others. These findings suggest that selected essential oils hold promise as alternative treatments for vaginal infections while having minimal effects on the vaginal biome.

Keywords: Vaginal infections; Essential oils; Bacterial vaginosis; Vulvovaginal candidiasis

Appendix B – Ethics waiver

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

05/04/2023

Ref: W-CBP-230405-01

TO WHOM IT MAY CONCERN

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical)

Investigator: Ms MH Laher
Student No. (if appropriate): 2140432
Staff No. (if appropriate):

Supervisor: Professor S van Vuuren

School: Therapeutic Sciences
Department: Pharmacy and Pharmacology
Pharmaceutical Micribiology
Medical School
University

Project title: *The antimicrobial and toxicity analysis of essential oils used for vaginal infections.*

Reason: In vitro laboratory study
No human participants will be involved in the study

Dr CB Penny
Chairperson: Human Research Ethics Committee (Medical)

Research Office Secretariat:
Third Floor, Phillip Tobias Building, corner of St Andrews and York Roads, Parktown, Johannesburg 2193
Postal address: Private Bag 3, Wits 2050
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Website:
https://www.wits.ac.za/research/researcher-support/research-ethics/ethics-committees/

Appendix C – Turnitin report

Thesis Muneebah

ORIGINALITY REPORT

6%	2%	5%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Sandy Van Vuuren, Stephanie de Rapper. "Odoriferous Therapy: Identifying the Antimicrobial Potential of Essential Oils against Pathogens of the Respiratory Tract", Chemistry & Biodiversity, 2020 Publication	3%
2	Mapeka, Tsholofelo Mavline. "An Optimal Polyherbal Formulation with Antimicrobial and Anti-Oxidant Properties.", University of the Witwatersrand, Johannesburg (South Africa), 2024 Publication	1%
3	Lediga, Mahlatse Evah. "Interactive Efficacies Between Fever-Reducing Plants and Silver Nanoparticles", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	1%
4	Submitted to Jacksonville University Student Paper	1%
5	casadesante.com Internet Source	<1%
6	op.niscair.res.in Internet Source	<1%

Appendix D – Literature review publication submission

Dear Sandy van Vuuren,

Thank you for submitting your revised manuscript.

Submission ID	**255858032**
Manuscript Title	**Essential oils used for vaginal infections: A review**
Journal	**Journal of Essential Oil Bearing Plants**

If you made the submission, you can check its progress and make any requested revisions on the Author Portal.

Thank you for submitting your work to our journal.
If you have any queries, please get in touch with journalshelpdesk@taylorandfrancis.com.

Kind Regards,
Journal of Essential Oil Bearing Plants Editorial Office

Appendix E – Experimental publication submission

Dear Dr. Vuuren:

Your manuscript entitled "Essential Oils against Vaginal Pathogens: Efficacy, Toxicity, and Microbiota Preservation" has been successfully submitted online and is presently being given full consideration for publication in Natural Product Communications.

Your manuscript ID is NPX-25-0746.

You have listed the following individuals as authors of this manuscript:
Laher, Muneebah; Orchard, Ané; Vuuren, Sandy van

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://mc.manuscriptcentral.com/npx and edit your user information as appropriate.

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Thank you for submitting your manuscript to Natural Product Communications.

Sincerely,
Abhilasha Shivankar
Natural Product Communications