American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

The Use of Aqueous Extract of Myrtle Plant Leaves in the Treatment of Chronic Bacterial Vaginosis

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Received: 2024, 15, Dec
Accepted: 2025, 21, Jan
Published: 2025, 10, Feb

Annotation: The current study aimed to evaluate the activity of the aqueous extract of the Myrtle plant (Myrtus communis) in treating patients with chronic bacterial vaginosis. Additionally, its inhibitory effect on aerobic bacteria and Candida isolated from vaginosis infections was studied, and antibiotic susceptibility of the isolates was also assessed.

The results of the diagnoses through aerobic incubation showed that Staphylococcus aureus, Staphylococcus haemolyticus, Escherichia coli, and Candida albicans were the most commonly isolated organisms from the studied vaginosis infections. Antibiotic sensitivity results indicated that all the isolates exhibited patterns of multiple antibiotic resistance. The findings suggest that the aqueous extract of myrtle leaves demonstrated a clear inhibitory effect against all studied microbes at all concentrations, with the effect increasing as the concentration increased. The highest inhibition was observed at a 20% (w/v) concentration, with inhibition zones of 32 mm for S. haemolyticus, 30 mm for S. aureus, 27 mm for E. coli, and 38 mm for C. albicans.

The study included the use of a 20% aqueous extract of myrtle leaves for the treatment of 20 patients with chronic vaginosis, resulting in improvements in most patients

starting from the third day of treatment. Various symptoms continued to disappear thereafter, and all patients achieved complete recovery by the end of the treatment week. This reflects the ability of the extract to alleviate all symptoms associated with the studied vaginosis infections, suggesting its potential as a safe and effective alternative treatment for these infections.

Keywords: Vaginosis, Myrtus communis, Aqueous extracts, Antibiotics.

Introduction

Bacterial vaginosis (BV) is a widespread worldwide infection, affects approximately 26% of women of childbearing age globally causing a significant health problem and economic burden. Therefore, there is a continuing and urgent need to investigate the causes of this infection and appropriate prevention and treatment strategies to reduce the burden of bacterial vaginosis among women. [1] Bacterial vaginosis is a common condition characterized by the presence of foul-smelling vaginal discharge with no obvious inflammation, it occurs due to the growth and increase in anaerobic and aerobic species with simultaneous reduction in the vaginal flora [2]. The common aerobic species are *E.coli, Staphylococci,* group B *Streptococcus* (GBS), and *Enterococcus faecalis*., while The common anaerobic species involved are *Gardnerella vaginalis, Mycoplasma hominis, Bacteroides* spp. and *Mobilincus* spp. *Gardnerella* spp. they are commonly isolated in women with bacterial vaginosis infections [3,4]. Vulvovaginal candidiasis affects approximately 30–50% of women at least once during their lifetime, causing uncomfortable symptoms and limitations in their daily quality of life. Antifungal therapy is not very efective, does not prevent recurrences and usually causes side effects. Therefore, alternative therapies are urgently needed [5,6].

Antibiotic resistance develops as a result of prolonged exposure of bacteria to antibiotics, causing a problem of global public health concern, women with asymptomatic bacterial vaginosis have high recurrence rates due to inadequate treatment, to address this problem, researchers are working to identify new therapeutic strategies, such as anti-biofilm agents and alternative antimicrobial agents, with the aim of improving treatment outcomes, reducing recurrence rates, and preventing the development of antibiotic resistance in the management of BV.[7,8]. The emergence and spread of antibiotic resistance among microbes prompted researchers to discover therapeutic medical alternatives to antimicrobial, and among these alternatives are medicinal plants, There is a clear increase in the diagnosis of medicinal plants on a global level and their use as effective and inexpensive agents in treatment as an alternative to chemical compounds[9].

Medicinal plant components have become an effective source in folk medicine and modern medicine [10], one of these medicinal plants is myrtle *(Myrtus communis*) belonged to Myrtaceae family which consists of 150 genera and 3500 species widely distributed in tropical and Mediterranean regions and is an important medicinal and aromatic plant growing spontaneously from the north-western to the eastern Mediterranean, they are traditionally used as antiseptic, hypoglycemic, anti-inflammatory, anti-hemorrhoidal, and astringent agents for the treatment of candidiasis, healing wounds, diarrhea and dysentery [11]. There is a diverse composition of myrtle leaves that expands its scope of use in different industries, there has been significant progress in the validation of its traditional components, essential oils, and extracts by exploring their properties, including antimicrobial, anticancer, etc.[12]. Myrtle leaves have been shown to possess antibacterial activity against both Gram positive and Gram negative

microorganisms, and the Gram positive microorganisms were found to be the most sensitive [13].

The current study aimed to evaluate the activity of Myrtle leaves aqueous extract to treat patients with chronic vaginosis.

Materials and Methods

Patients:

The current study included thirty one patients suffering from clinical symptoms of chronic vaginosis who visited the obstetrics and gynecology clinic, aged between (18-50) years ، married and single.

Specimen collection:

High vaginal swabs (HVS) were taken from patients by the specialized doctor from the top of the vagina using a colposcope and under sterile conditions, swabs was placed in a tube containing transport media, and cultivated within half an hour [14].

Isolation & Identification:

Specimens were cultivated on nutrient agar (Microxpress/ India) and incubated aerobically at 37 C^{O} for 24 hours, then growing colonies was subjected to gram stain and the gram negative isolates were re-cultivated on MacConkey's agar (Microxpress/ India), then gram positive and negative isolates were diagnosed using VITEK 2 compact system (BioMérieux/France). All isolates preserved at refrigerator (4 C^{O}) until use.

Antibiotic susceptibility test:

The antibiotic susceptibility test for the most frequent isolates was carried out using agar disc diffusion method (Kirby-bauer method) as (3-5) pure colonies of the isolates were transferred to tubes containing normal saline solution, and the bacterial suspension was compared with a(No. 0.5) McFarland tube, which is equivalent to (10^{8} cells/cm^{3}) Then the bacterial suspension was separated by swab on Muller Hinton agar (Microxpress/ India) surface and the plates were left for (5) minutes to dry, then the antibiotic disc (Bioanalyse/Turkish) were fixed using sterile forceps and the plates were incubated at 37 C^{O} for 24 hours, zones of inhibition were measured by millimeter [15].

Preparation of Aqueous extract of Myrtle plant:

Myrtle plant was obtained from Mosul city gardens, and the aqueous extracts of plant leaves were prepared, as 20 g of plant parts were crushed in 100 ml of distilled water (20 % w/v) in an using blender, then the mixture was left in the refrigerator for soaking 24 hours, the mixture filtered through several layers of gauze and then filtered through Buchner funnel using filter paper (Whatman No-1), the extract then sterilized using (0.22 µm) filter paper [16]. Two other concentrations of the same extract were prepared (10% and 5%).

Antimicrobial activity of myrtle leaves aqueous extracts

This test was carried out using agar wells diffusion assay method, as the bacterial isolates were grown in Muller Hinton agar medium and their density was compared with McFarland tube (No.0.5),, and 0.1 ml of the bacterial suspension was spread on the surface of the agar by a swab, then sterile cork borer were used to made wells in the medium with (5 mm) diameter, and (50 µl) of myrtle extract at three prepared concentrations were added to each well,, then the plates were incubated at 37 C^{O} for 24 hours. After incubation, the inhibition zones were measured [17].

Study Cases:

This study was conducted by the specialized doctor and included (20) patients those who accepted to undergo this test, the patients had previously undergone treatment with a number of antibiotics and did not recover, and the most important of these antibiotics were: Ciprofloxacin

500mg, Metronidazole 250mg, Amoxicillin 500mg, Nitrofurantoin100mg, Gentamicin injection 80mg, clavulanic acid 1000mg, Ceftriaxone injection1000mg, Levofloxacin 500mg and Amikacin injection. The patients was prescribed for myrtle aqueous extract (20 % w/v) three times daily. Treatment with myrtle aqueous extract included using it as a water bath and vaginal lotion. All patients were followed up within a week to determine the results of the treatment.

Results:

Table (1) shows the types of microbes isolated and diagnosed in the current study, which included types of bacteria and Candida. It is noted that *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli* and *Candida albicans* constituted the highest percentages, noting that most of the infections were mixed, resulting from two or more types of these microbes.

Table (1) : Microbes isolated and identified in the current study and their percentages.

Microbes	Isolate No.	%
Staphylococcus aureus	11	21.15
Staphylococcus haemolyticus	8	15.38
Staphylococcus saprophyticus	3	5.77
Aerococcus viridans	2	3.85
Escherichia coli	9	17.31
Pseudomonas aeruginosa	1	1.92
Klebsiella pneumoniae	3	5.77
Proteus mirabilis	2	3.85
Candida albicans	13	25
Total	52	100

The results in Table 2 show the sensitivity of the studied isolates (which represented the highest isolation percentages in the current study) to 14 types of antibiotics. With regard to bacteria, a variation in the results of the isolates is noted, and all of them showed high and multidrug resistance, as the isolates of staphylococci showed resistance to nine different antibiotics, while *E. coli* showed resistance to eight of them, and all isolates were sensitive to Meropenem and Imipenem, while *Candida albicans* was sensitive to Fluconazole The only antibiotic used against it in the current study.

Table (2): Antibiotic susceptibility results of the studied isolates

Antibiotic (Conc. µg/disk)	Microbe			
	E.coli	***S.haemolyticus***	***S.aureus***	***Candida albicans***
Piperacillin (PRL) 100	R	R	R	-
Levofloxacin (LEV) 5	S	I	S	-
Meropenem (MEM) 10	S	S	S	-
Cephalexin (CN) 10	S	R	R	-
Ciprpoflexacin (CIP) 10	R	R	R	-
Ofloxacin (OFX) 5	R	R	R	-
Imipenem (IPM) 10	S	S	S	-
Amikcin (AK) 10	S	R	R	-
Fluconazole (F) 100	R	R	R	S
Ceftriaxone (CRO) 30	R	S	R	-
Vancomycin (VA) 30	S	S	I	-
Cefotaxime (CTX) 30	R	R	R	-
Fusidic acid (FA) 10	R	R	I	-
Streptomycin (S) 10	R	R	R	-

S: sensitive, I : intermediate sensitivity, R: resistant, - : Test not performed

The results shown in Table 3 and Figure 1 show the sensitivity of the studied isolates to the aqueous extract of myrtle. It is noted that all isolates were sensitive to this extract at all concentrations, noting that the effect increased with increasing concentration and the highest inhibition was at a concentration of 20% in the case of *Candida albicans* (38 mm), *S.haemolyticus* (32 mm), *S. aureus* (30 mm) *and E. coli* (27 mm).

Table (3) : Myrtle aqueous extracts susceptibility results of the studied isolates (Inhibition zones diameters in mm).

Myrtle extract conc. (w/v)	Microbes			
	E. coli	*S.haemolyticus*	*S. aureus*	*Candida albicans*
5%	17	15	22	33
10%	22	27	25	35
20%	27	32	30	38

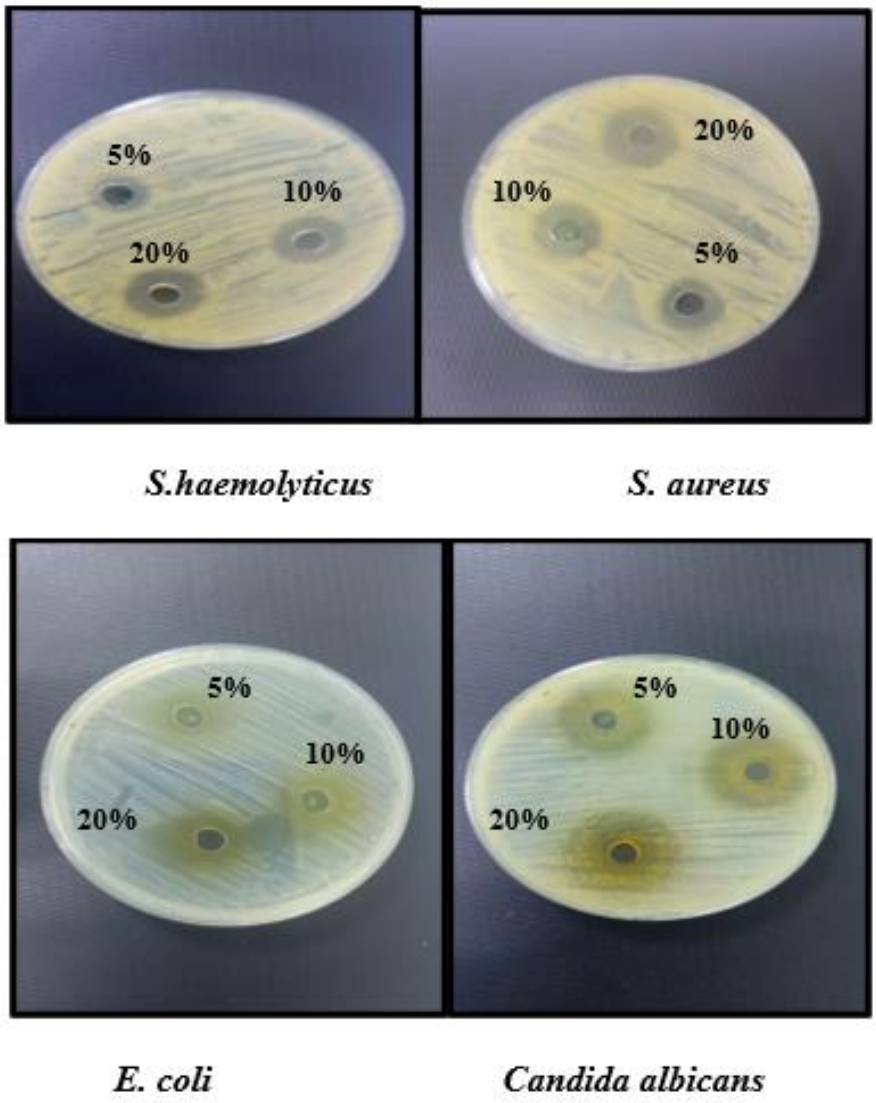

Figure (1) Susceptibility of studied isolates against Myrtle aqueous extracts

The study cases showed excellent results as the treatment method using the Myrtle extract led to improvement in most patients starting from the third day of treatment and the various symptoms continued to disappear after that and all patients achieved complete recovery after reaching the end of the treatment week.

Discussion:

Bacteria are one of the most important causes of vaginosis [18, 19]. The current study focused on the role of aerobic and facultative aerobic bacteria in these infections to use them as a model for the therapeutic effect of aqueous extract of Myrtle plant. The results showed that gram positive bacteria group represented the largest percentage 46.15% compared to gram negative bacteria 28.85%. *Staphylococcus aureus* constituted the largest percentage 21.15% among gram positive bacteria and all isolated bacteria, these bacteria are considered one of the most important types of pathogenic bacteria causing various infections, including vaginal infections [20,21]. *Staphylococcus haemolyticus*, which formed with *Staphylococcus saprophyticus* as coagulase negative bacteria 21.15% of infection the causes, which reflects the increasing and growing role of these types of bacteria in various infections, including vaginal infections [22]. *E. coli* constituted the largest percentage of the Gram negative bacteria in the current results, which confirms the results of many other studies that isolated this bacteria from vaginal infections, it is also a major cause of urinary tract infections, as there are anatomical reasons that increase the chance of infection with this bacteria in females [23]. As for Candida, it constituted 25% of the isolated microbes, and its presence in these infections is common and proven in many other studies[6,24]. It is also worth noting that many of the isolated microbes were present in combination with other microbes in the same sample, whether with bacteria or Candida, and other microbes may be present in these samples, especially with regard to anaerobic bacteria that require special conditions to grow. also there are also many fastidious and uncultured bacterial species that cause bacterial vaginosis[16].

Antibiotic resistance is one of the most important challenges facing the health field, which causes treatment failure and the spread of epidemic diseases. In the current study, the bacterial species isolated in high percentages were selected to test their sensitivity to 13 antibiotics, in addition to testing the sensitivity of *Candida albicans* to fluconazole. The results showed that the types of Staphylococci showed high resistance to the studied antibiotics, reaching 61.5%, while the resistance rate of *E. coli* was 53.8%. The phenomenon of antibiotic resistance has increased significantly as a result of the excessive and random use of these antibiotics, as well as the continuous transfer of this characteristic between bacteria, especially through horizontal gene transfer mechanisms[25, 26]. To eliminate the problem of bacterial resistance to treatments, there is a continuous need to develop drugs and search for different therapeutic alternatives.

Myrtle *(Myrtus communis*), a member of the Myrtaceae family, is a medicinal plant used worldwide. It is widely distributed in the Mediterranean region [11]. It has many medicinal properties, such as antibacterial, antioxidant, anti-inflammatory, and analgesic, etc. The results of our study showed that the aqueous extract of myrtle leaves showed a clear inhibitory effect against all types of microorganism studied as shown in the table (3) and figure (1), This inhibitory effect increases with increasing concentration, as it is noted that the 20% concentration was the most effective. This effect may reflect the content of the myrtle aqueous extract of active substances and compounds extracted from the leaves of this plant, these include polyphenol compounds phenolic acids, tannins, and flavonoids[27,28]. The results of the current study proved the inhibitory effect of the myrtle aqueous extract on bacteria as well as on fungi represented by *Candida albicans*, which was more affected than bacteria. This is considered important because yeast accompanied bacteria in many of the studied samples and sometimes alone.

The clear inhibitory activity of the extract against the studied bacteria or yeast and perhaps its effect on other microbes that have not been studied and that cause vaginal infection may have been reflected in the successful treatment of the patients who underwent the study, although the causes of infection may have been different. From a practical point of view, the use of this extract is suitable because it is safe, easy to prepare, and readily available, in addition to its excellent therapeutic efficacy, which was proven in the current study and for all patients under study.

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