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Research Article

Effects of Plant Extracts on Bacterial Isolates from Infections of the Female Genital Tract

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Abstract: Fifty samples of female genital secretions were collected from a group of women whose age ranges from 15 to 50 years old at Al-Batool Hospital for Maternity and Children. The isolates *Escherichia coli, Staphylococcus aureus, Candida albicans*, and *Staphylococcus epidermidis* were found in the fungal and bacterial cultures of the samples, with percentages of (45, 31, 13, 11), respectively. Positive samples were isolated at the highest rate of 87 percent for samples isolated from people aged 30 to 40. In the case of pregnancy, there is an increase in the infection rate with fungal and bacterial species, with a percentage of 60 % compared to women. The results of testing the effect of plant extracts (marjoram, chamomile, thyme, peat, and thuja) on isolated bacterial and fungal species showed inhibition diameters (20, 16, 15.5, 13, 10, 10) mm at concentrations (25 %, 50 %, 75 %, 100 %). We discovered that different concentrations of Marjoram, chamomile, thyme, peat, and thuja extracts (25 %, 50 %, 75 %, 100 %) were associated with increased inhibition rates of fungal and bacterial infection with different diameters (10, 12, 15, 16, 20 mm).

Keywords: *Candida albicans*, Bacterial species, Plant extracts, Infectious diseases.

1. INTRODUCTION

The recurrence of urinary tract infection (UTI) after antibiotic treatment is an indication that the bacteria have developed resistance to the medicine. This necessitates the investigation of an alternate treatment. It is known that a certain proportion of people are now using alternative medications, as a significant number of women are using herbal therapies to boost their immunity, drinking cranberry juice, or consuming probiotics in order to restore their normal vaginal flora. That is occasionally distressed following antibiotic medication [1]. Plants have an essential role in the treatment of fungal and bacterial illnesses. Several studies show that plant extracts (Chamomile and Thyme, Marjoram, Peat, Thuja) have antifungal and antibacterial activity against *Candida albicans* and bacterial infection (*Staphylococcus aureus, Escherichia coli, and Staphylococcus epidermidis*) [2-6].

Candida species are generally known as fungal pathogens in humans. These species are typically commensal yeasts that are a component of the normal microbiota. They settle lightly on human skin, vagina, oral cavity, and gut, but they may change into opportunistic pathogens if they have insufficient host immunity. [7]. The global distribution, versatility in adjusting to novel difficult environments, and variety of *Candida* genus species and straining to dictate that such yeast-like fungi lead to a wide spectrum of infections in susceptible persons [8]. These might include diseases with significant illness but without lethal consequences, like excruciating and scratchy mucocutaneous candidiasis such as candidal vulvovaginitis, oropharyngeal candidiasis, gastrointestinal tract overgrowth, keratitis, or skin and nail mycoses [9]. The *Candida* genus has about two hundred species, with the most common being *C. albicans*, which represent almost half of all identified shallow and systematic candidiasis in people [10]. Commonly, *C. albicans* can be the furthermost cause of Vulvovaginal candidiasis (VVC), followed by *C. glabrata*, *C. krusei, C. lusitaniae,* and *C. parapsilosis* [11]. Vulvovaginal candidiasis is common among

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women who have had antibiotic treatment, are pregnant, or are on hormone replacement therapy [12]. The current study attempts to apply alternative medicines (plant extracts) in the treatment of fungal and bacterial infections due to fungi and resistance of bacteria to antibiotics.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifty swabs were collected using a sterile cotton swab from different locations of the female genital tract of women with inflammation of the female genital tract during visits to Al-Batool Hospital advisory clinics as well as Al-Zahra in Muqdadiya from 12/20/2019 to 8/20/20. The patient was chosen based on the existence of clinical symptoms identified by the specialist and including; the presence of vaginal fluids, odour, ulceration, itching, burning, and fever. The ages of the ladies varied anywhere from 15 to 50.

2.2 Isolation and Diagnosis

The swabs taken from the patients were planted on the culture media (Blood agar, MacConkey agar, Sabouroud dextrose, and Mannitol salt). Dishes were incubated in the incubator at a temperature of 37 °C for 24-28 hours, then growth was checked after that period and then a secondary culture was made of the dishes that showed a positive result. As for the direct examination, the samples were examined directly by making two slides. The first was stained with gram dye, while the second slide was placed on it a drop of physiological solution and was examined under 40v after placing the slide cover on it. All bacterial and fungal isolates were subjected to some biochemical diagnostic tests as well as the gram dye test, studying shapes, colours and edges of colonies growing on nutrient medium, growth on the blood agar medium and hemolysin production [41].

2.3 Preparation of the Hot Aqueous Extract

Extract is prepared by dissolving 50 g vegetable powder in 500 ml of distilled water. Then, boil at a temperature of 100 °C and leave for 10 minutes. Then, filtering through the Whatman No. 1 filter papers that were poured into glass dishes and left in the incubator at a temperature of 37 °C. When power develops, it is kept in the refrigerator until use [42].

2.4 Effect of Plant Extracts on the Growth of Bacteria and Fungi

Agar well diffusion process was employed to observe the effect of aqueous extracts of marjoram, chamomile, thyme, peat, and thuja at concentrations (25, 50, 75, 100) mg/ml on bacterial growth and isolate *C. albicans* yeast. From the female genital tract, Mueller–Hinton agar medium was inoculated with a sterile cotton wick of bacterium containing 108 YS ml and SDA medium that was inoculated with Mycobacterium. Drills were made in the middle of the cultivated culture media using a flint drill and the prepared concentrations of each extract were placed in the amount of (50 milliliters per hole) with the use of distilled water as a control. The diameter of the damping area was measured in mm for each hole, and the average of the three replicates was calculated [43].

2.5 Statistical Analysis

The parameters were expressed as percentage frequencies, and the Pearson-Chi-square test was used to determine if there were significant differences between frequencies. The statistical package SPSS version 25.0 and Excel 2013 were employed to carry out these analyses. A p-value $\leq$ 0.05 was considered significant.

3. RESULTS

3.1 Age Groups and bacterial infections

Data from the current study show significant differences among age groups related to bacterial infections, where the age groups 21-2 and 31-0 years scored the highest percentage (81 % and 87 %) respectively, compared to other groups (table 1).

3.2 Microbial Infections and Women Status

Data from the current study showed a significant difference between expectant and non-pregnant women related to bacterial and fungal infections, where pregnant women scored the highest

Table 1. Frequency and percentage of bacterial infections according to age groups

Age groups (Years)	Total Number	Positive Number	%	p-value
15-20	6	3	50	1
21-25	16	13	81	0.001***
26-30	8	4	50	1
31-40	16	14	87	0.001***
41-50	4	2	50	1
p-value	0.001***			

percentage (60 %) with bacterial and fungal infections than non-pregnant (40 %). Similarly, there is a significant difference between those females who are pregnant and those who are non-pregnant related to *E. coli* infection. Finally, there is no significant difference between pregnant and non-pregnant women related to *S. aureus, S. epidermidis, and C. albcans* infections (table 2).

3.3. Effect of Plant Extracts on the Growth of Bacteria and Fungi

The study's findings showed that there was no statistically significant difference between amounts of Chamomile and Thyme extracts and their ability to inhibit *C. albicans*, *E. coli*, *S. epidermidis*, and *S. aureus* (Figures 1 and 2).

Table 2. Frequency and percentage of bacterial and fungal infections according to woman's status

Woman status	*S. aureus*	*S. epidermidis*	*E. coli*	*C. albicans*	*%*	*p-value*
Pregnant	3	13	4	7	60	0.03*
Non-pregnant	3	7	1	7	40	0.13
Total	6	20	5	14	100	0.003**
p-value	1	0.18	0.002**	1	0.52	

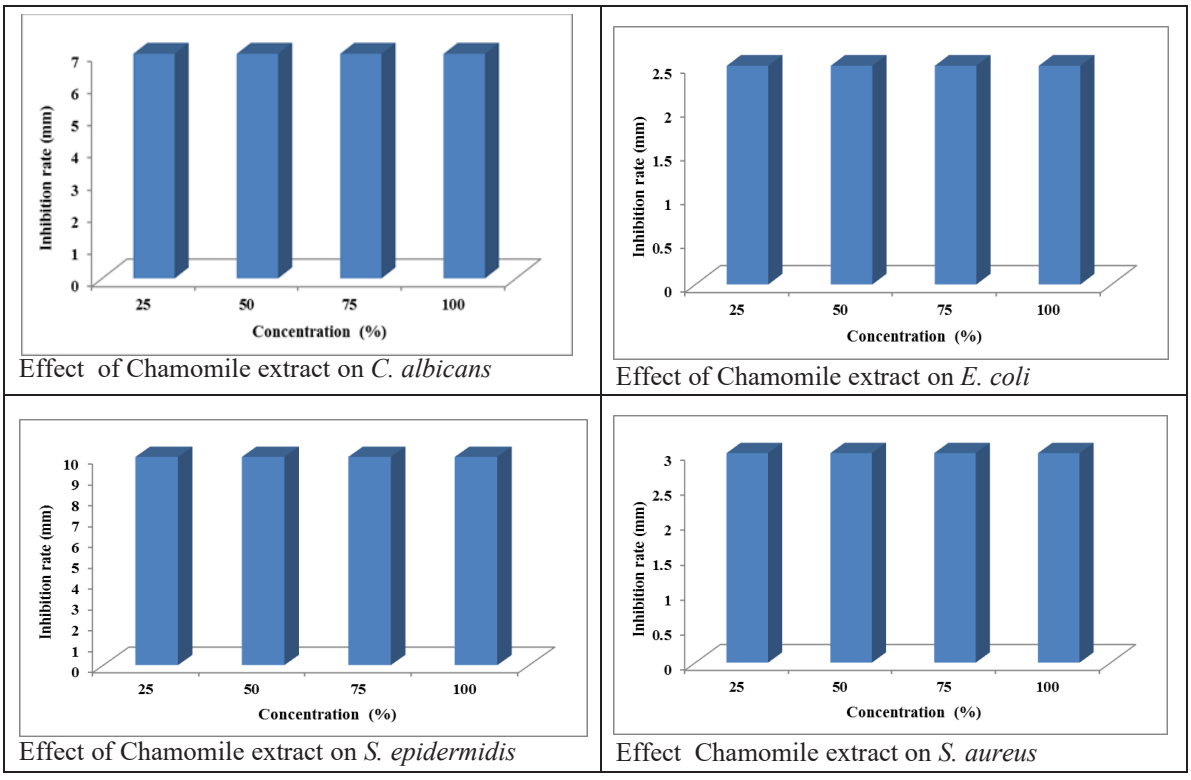
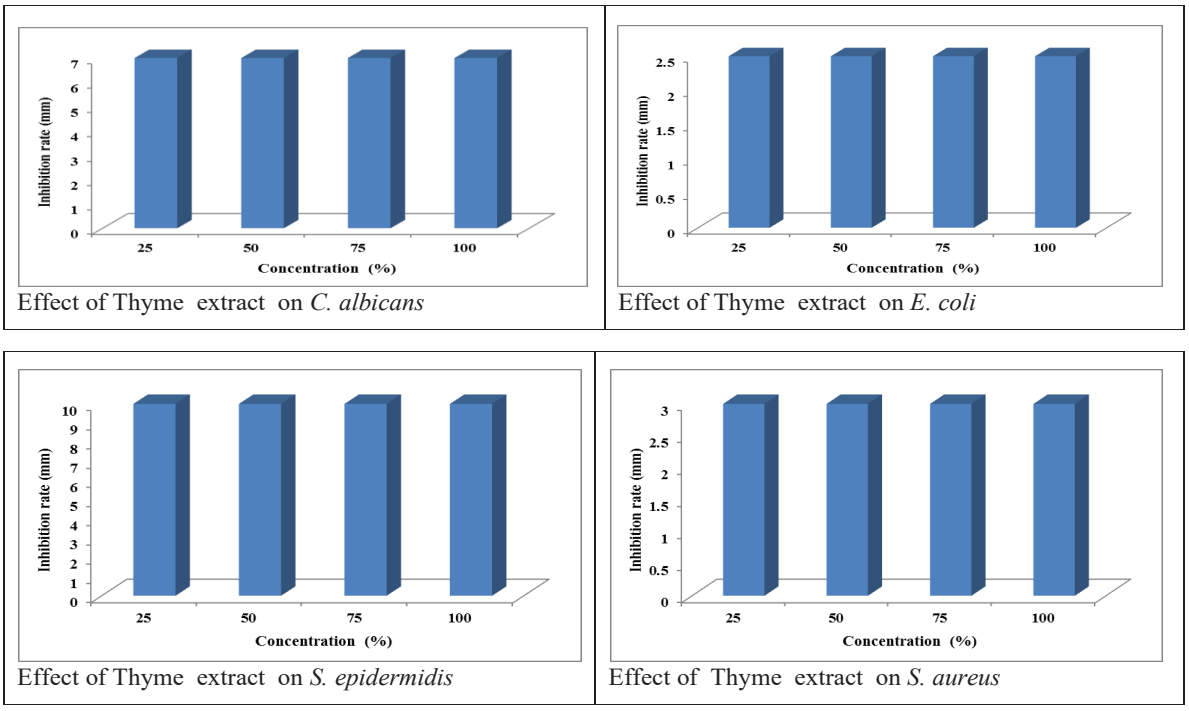


Fig. 1. Effect of Thuja extract on *C. albicans, E. coli, S. epidermidis,* and *S. aureus*

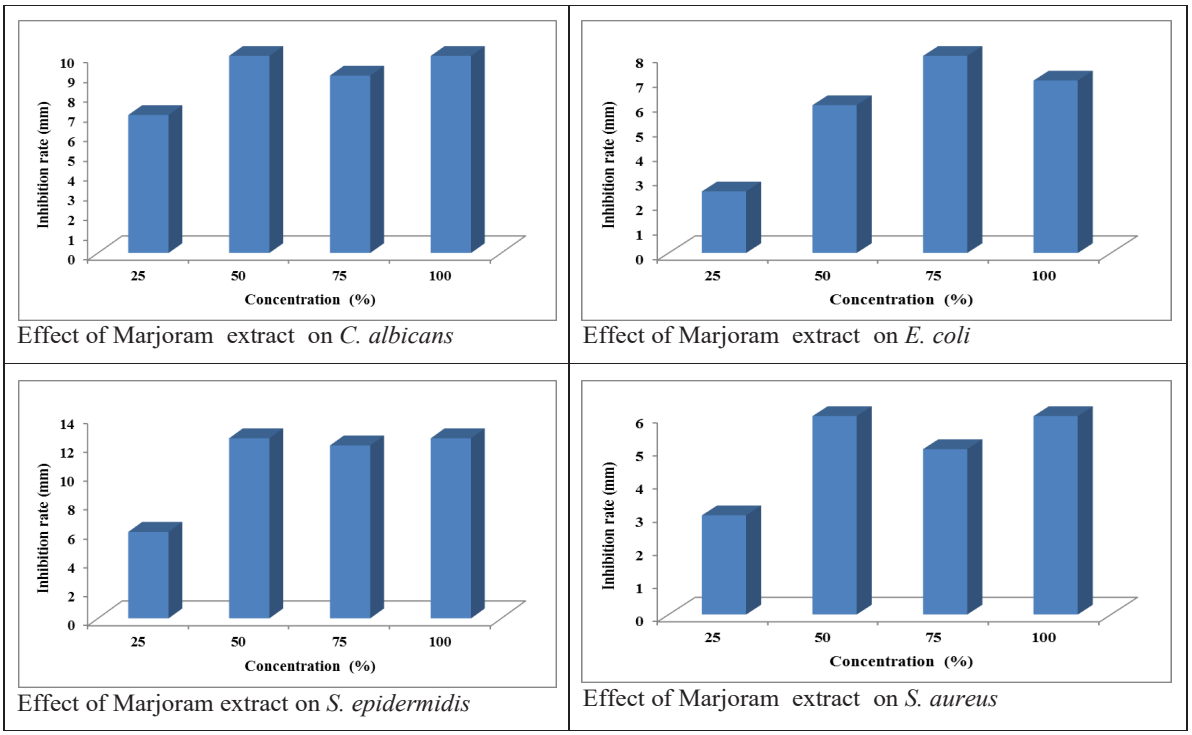


Effect of Thyme extract on *C. albicans* | Effect of Thyme extract on *E. coli*

Effect of Thyme extract on *S. epidermidis* | Effect of Thyme extract on *S. aureus*

Fig. 2. Effect of Thyme extract on *C. albicans, E. coli, S. epidermidis*, and *S. aureus*

The current investigation demonstrated that the inhibition rates of *E. coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* differed significantly depending on the concentration of marjoram extract and that the inhibition rate increased with concentration. Contrarily, our study found no correlation between the amounts of marjoram extract and the pace at which *Candida albicans* were inhibited *(*Figure 3). Similarly, our study showed there is a significant difference among Peat extract concentrations and inhibition rate of *Candida albicans, E. coli, and Staphylococcus*



Effect of Marjoram extract on *C. albicans* | Effect of Marjoram extract on *E. coli*

Effect of Marjoram extract on *S. epidermidis* | Effect of Marjoram extract on *S. aureus*

Fig. 3. Effect of Marjoram extract on *C. albicans, E. coli, S. epidermidis,* and *S. aureus*

epidermidis.

It was discovered that as concentration rose, the inhibition rate rose as well. On the other hand, our research did not discover a connection between Peat extract concentrations and how quickly *Staphylococcus aureus* was suppressed (Figure 4). The study concluded by showing a significant relationship between Thuja extract concentration and inhibition rates of *C. albicans*, *E. coli, S. epidermidis*, and *S. aureus*, finding that the inhibition rate increases as the concentration does (Figure 5).

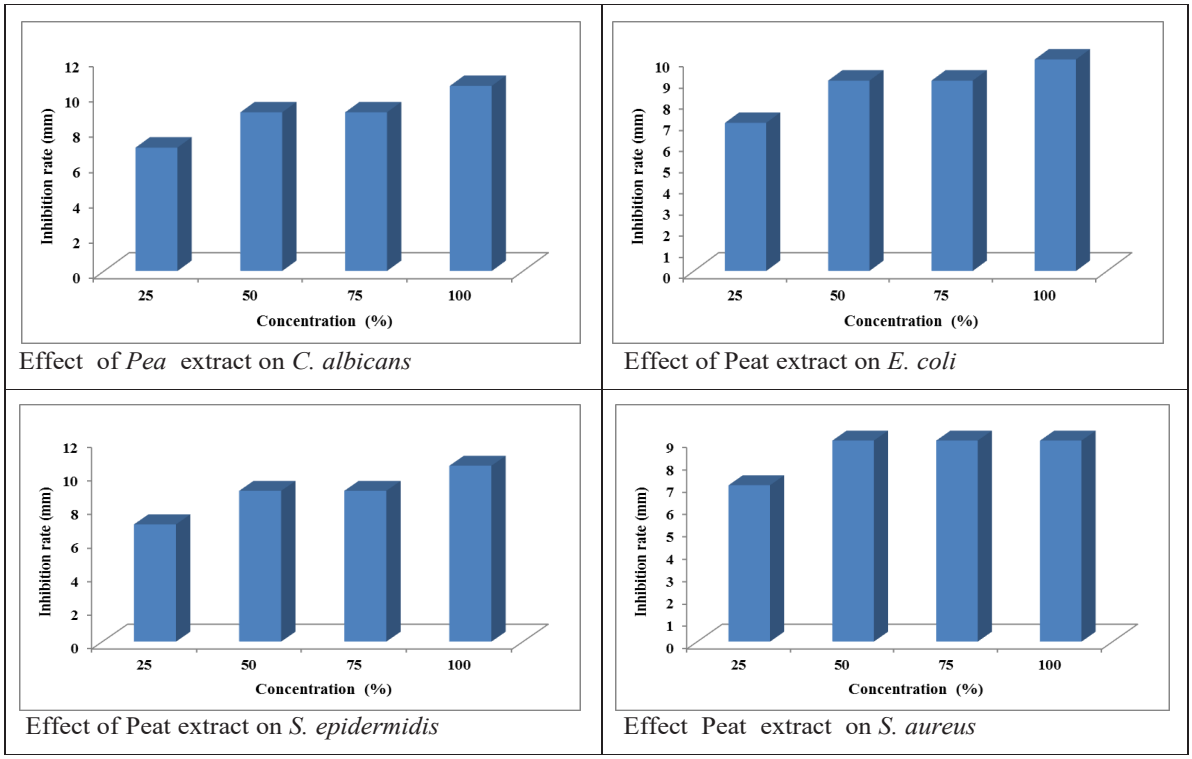


Fig. 4. Effect of Peat extract on *C. albicans, E. coli, S. epidermidis,* and *S. aureus*

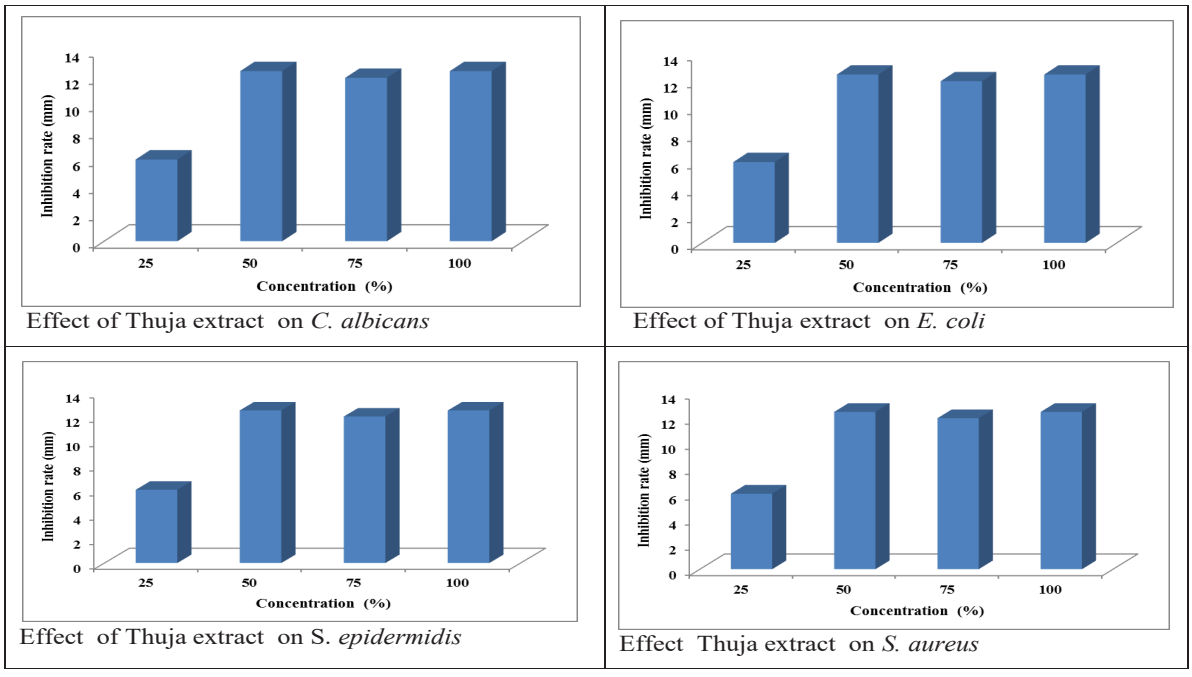


Fig. 5. Effect of Thuja extract on *C. albicans, E. coli, S. epidermidis,* and *S. aureus*

According to the findings, women aged 21-52 and 31-40 years had the highest percentage of bacterial infections (81 percent and 87 percent, respectively) compared to other age groups, and the reason for this could be due to age progression, impaired immune status, multiple births, and sample collection. The high incidence of bacterial infection in women was linked to the pH of the vaginal fluid (pH=4.5), participant age, pregnancy, and HIV infection history. To avoid mother and infant morbidity and mortality, early detection of conditions contributing to bacterial overgrowth on the vaginal wall is critical [13]. Protecting mother and child from morbidity and death is critical [13].

4. DISCUSSION

Bacterial vaginosis is a global public health risk for women, particularly throughout their reproductive years. The age and gender of the patient are important variables in establishing the etiology of UTIs; they can improve the accuracy of identifying the causative uropathogen and provide good information for empiric therapy [14]. Bitew, Abebaw, et al. found that the prevalence of bacteria was linked to a variety of variables, which is consistent with our findings [13]. A total of 413 research participants were tested for bacterial vaginosis, with a 39.5 percent overall prevalence of bacteria that cause bacterial vaginosis. This result is similar to studies carried out in Kenya (43.1 %), Nigeria (33 %), Addis Ababa (41.5 %), and Cameroon (38 %) [15]. Mucci *et al.* found *C. albicans* 4+ positive in pregnant women, which is consistent with our findings [16]. Researchers in Beirut, Lebanon, found a considerable incidence of *C. albicans* and non-*Candida albicans*. Vulvovaginitis is an infection that may be caused by certain Candida strains, and it is more common in pregnant women. *Candida* testing as part of prenatal care is recommended to reduce the risk of a negative neonatal outcome or gestational problems. [17]. *Candida* species were found in much larger numbers in symptomatic pregnant women than in asymptomatic pregnant women. *Candida* infection was substantially linked to those between the ages of 26 and 40. The antifungal medicine amphotericin B was the most sensitive. Multiple drug-resistant *Candida* species were found in high numbers. As a result, symptomatic women should be examined and treated on a regular basis [18]. The prevalence of vulvovaginal candidiasis (VVC) was observed to be higher among symptomatic non-pregnant women of reproductive age in Vietnam in comparison to various other nations. Non-albicans *Candida* species were found in high numbers, and they were typically resistant to antifungal therapies. Antifungal resistance was low in vaginal *C. albicans* isolates. The findings suggest that changes in species distribution and antifungal sensitivity should be monitored and treated on a regular basis [19]. Pregnant and postpartum women are increasingly susceptible to this virus. *S. aureus*, including MRSA, is a prevalent source of healthcare-associated illnesses and community-acquired infections [20]. The carriage of *S. aureus* in mothers is associated with an increased likelihood of *S. aureus* infection in neonates. The prevalence data indicate that there is a higher number of MRSA isolates in comparison to *S. aureus* isolates. This phenomenon may be attributed to the inappropriate use of antibiotics during pregnancy in China, and warrants further investigation. The greater incidence of vaginal isolates in pregnant women compared to nasal isolates implies that pregnant women should prioritize vaginal hygiene. [21]. The study's results indicate a high prevalence of *E. coli* and extended-spectrum-lactamase-producing (ESBL) *E. coli* in pregnant women, which aligns with our own findings. Furthermore, it was observed that the misuse of antibiotics was more prevalent among pregnant females in Europe and Asia as compared to other continents [22].

The findings indicate that certain virulent clones are implicated in *E. coli* bacteremia among pregnant women, however, the severity of the condition, as evidenced by fetal mortality, is primarily linked to bacteremia originating from the genital region [23]. The study findings indicate that there exists a noteworthy prevalence of ESBL carriage among expectant women in Lebanon. In order to accurately assess the potential for transmission, further investigation is warranted, such as conducting longitudinal radiographic studies on expectant mothers and monitoring the postnatal health status of their offspring [17]. Antibiotic-resistant strains can survive in the mother's vaginal tract and be passed on to the baby after birth. Preterm infants in the neonatal care unit are at a notable risk of sepsis caused by Enterobacteriaceae that produce extended-spectrum beta-lactamase (ESBL). In

the case of maternal-neonatal transmission, it was found that identical strains were transmitted vertically from the mother to the newborn [24]. The study's findings indicate that the ethanol extracts of chamomile did not exhibit any antifungal activity against *C. albicans* in both the control and test groups. However, the hydroalcoholic extract of chamomile demonstrated inhibitory and fungicidal effects that were attributed to 70 % ethanol rather than the chamomile itself [2]. In comparison to the untreated biofilm, the utilization of chamomile extract exhibited a significant reduction in the quantity of *Enterobacter cloacae* colony-forming units per milliliter in biofilm. However, it did not demonstrate any impact on the amount of viable DNA in *C. albicans* biofilm. [3]. By using the agar diffusion method, the antimicrobial activity of chamomile essential oil was examined, and it was discovered to have a considerable antibacterial impact, with inhibition zones of 13.33 mm to 40.00 mm in diameter (on *Listeria monocytogenes*) (on *S. aureus*). Chamomile oil has little antibacterial effect against *P. aeruginosa* bacteria. The chemical composition, antibacterial activity, and antioxidant properties of the studied chamomile essential oil, which was extracted from plant material from the Republic of Srpska's northern region, show that it has great phytomedical potential [25]. Chamomile has antibacterial action against a wide range of microorganisms, including *Enterococcus faecalis*, *P.aeruginosa, S. aureus, K. pneumoniae,* and *E. coli* [26]. The ethanolic extract of chamomile leaves showed antibacterial action against MDR *P. aeruginosa* isolates; however, the extract of flowers demonstrated superior activity against MRSA isolates [4]. Chamomile ethanolic flower extract has shown considerable antibacterial action against MRSA isolates. Accordingly, this extract might be a viable alternative to antibiotic therapy and a practical choice for handling infections produced by MRSA and detrimental bacteria [27]. Essential oils utilized at sub-inhibitory concentrations were sequestered in yeast vacuoles, indicating that cell detoxification activated *Candida* defensive systems. Due to their anti-biofilm action, clove and thyme essential oils can effectively inhibit *Candida* sp. colonization of the studied abiotic surfaces [5]. The findings of this study give a wealth of experimental data on the therapeutic effectiveness of thyme essential oils against drug-resistant clinical isolates of *C. albicans*, which might be utilized to build a novel antifungal medication [28]. Some Thyme essential oils exhibit high effectiveness against both developing and stationary phase *S. aureus*, according to the research [6].

Data results are shown. Thyme has a greater antimicrobial effect at the 30 and 40 percent concentrations. These findings demonstrate that these essential oils have powerful antibacterial activities, implying that they might be useful in treating *S. epidermidis* [29]. The essential oils of marjoram were shown to have potent antifungal properties against the *C. albicans* fungus strain [30]. Another study found that Marjoram essential oils are effective against *C. albicans* [31]. The findings will allow for more research into oregano essential oil (*Origanum heracleoticum* L.) as an alternative antibacterial remedy for boosting the treating process in bacterial infections and as an efficient technique of averting the formation of antibiotic-resistant strains [32]. Data results showed the use of Marjoram essential oil to control *E. coli* in salad dressing might be seen as promising and enable lower salt levels in meals to be assimilated [33]. The findings demonstrate the bioactive potential of decoctions of *Satureja montana* and *Origanum majorana* in inhibiting *E. coli* and encourage the creation of innovative formulations with broad antibacterial capabilities based on these medicinal and fragrant plants [34]. The antibacterial concentrations of Marjoram essential oils are utilized to inhibit *S. aureus*. Because of the potential for inductive biofilm formation, aureus biofilms should be approached with caution [35]. Data results showed antimicrobial activities of peat extract against *S. aureus*, *E. coli*, and *Pseudomonas* spp. [36]. Study results showed the most efficient composites against pathogenic microorganisms in peat are those containing a 70 % ethanol solvent. [37]. Except for *E. coli* and *S. typhi*, fresh leaf juice showed strong effectiveness against Gram-negative pathogens. Except for *S. aureus* and *S. epidermidis*, it is similarly potent against Gram-positive bacteria. Only Gram-positive bacteria were inhibited by acetone, methanol, chloroform, and petroleum ether extracts of the leaves [38]. The results of the inhibition zone measurement revealed that the antibacterial activity of Thuja occidentalis extract gets to 29 and 38 mm against *S. aureus* and 23 and 31 mm against *C. albicans* at concentrations of 40 and 50 %, respectively [39]. The most resistant

strain was discovered to be *C. albicans* (MTCC 3018). Except for *C. albicans*, Thuja essential oil displayed remarkable antifungal efficacy against all pathogens [40].

These findings contradict ours, which demonstrated the impact of *C. albicans* inhibition. According to the findings, extracts made from the leaves and seeds of *Thuja occidentalis* can be utilized as a natural remedy for the treatment of different bacterial infections (e.g., *E. coli* and *S. aureus*).

5. CONCLUSION

We conclude that women in age groups 21-25 and 31-40 years scored the high prevalence with UTIs. Bacterial and fungal infections are more incidence in pregnant than non-pregnant women. Chamomile and Thyme extracts have no significant effects on their ability to inhibit *C. albicans*, *E. coli*, *S. epidermidis*, and *S. aureus*. Marjoram extract is preferred in inhibition rates of *E. coli*, *S. epidermidis*, and *S. aureus*, but it does not have significant activity in the inhibition of *C. albicans*. Peat extract concentrations have a significant inhibition rate against *C. albicans*, *E. coli*, and *S. epidermidis*. Finally, Thuja extract concentration and inhibition rates of *C. albicans, E. coli, S. epidermidis,* and *S. aureus*, found that the inhibition rate increases as the concentration does.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

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