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Evaluating the Antimicrobial Efficacy of Apple Cider Vinegar on Bacteria and Fungi Isolated from Vaginitis

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Keywords: ACV, bacteria, fungi, Vaginitis.

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Abstract:

Vaginitis is a prevalent medical illness that affects a substantial proportion of women, mostly attributed to the excessive proliferation of bacteria and fungi. The objective of this study was to assess the antimicrobial effectiveness of Apple Cider Vinegar against bacteria and fungi that were obtained from cases of vaginitis. 50 vaginal swabs were collected from vaginitis patients at private clinics in Kirkuk City, Iraq (10 pregnant women and 40 non-pregnant women). The research included the isolation and identification of bacterial and fungal isolates. All isolates performed the Minimum Inhibitory Concentration and disc diffusion techniques tests. Different concentrations of apple cider vinegar (20%, 30%, 40%, 50% and 100%) were tested against the isolates and Standard antibiotic drugs were used as positive controls. The results of 50 patients were examined, showing that 22 (44%) had bacterial vaginitis and 39 (78%) had fungal vaginitis. Vaginitis was more prevalent in pregnant women compared with non-pregnant. Staphylococcus aureus recorded 14 (24.9%) out of the 54 bacterial isolates, followed by Echerichia coli, Group B- Streptococcus, Klebsiella pneumoniae, Staphylococcus epidermidis and Pseudomonas aeruginosa respectively. whereas Candida albicans recorded 30 (61.2%) out of the 49 fungal isolates, followed by Candida tropicalis and Candida parapsilosis, respectively. The findings showed a range of sensitivity to apple cider vinegar among the isolated samples. The efficacy of apple cider vinegar was found to be greater against fungal isolates compared to bacterial isolates, with a higher effectiveness observed

against gram-negative bacteria in comparison to gram-positive bacteria. The highest MIC values were found for Gram-positive bacteria, which recorded 125 (µg/mL), followed by Gram-negative bacteria, 62.5 µg/ml. While the lowest MIC values for the isolated fungi were (31.25 µg/ml). A comparison to traditional antimicrobial drugs has highlighted ACV's potential as an alternative treatment for vaginitis. According to these findings, ACV may be an effective treatment choice for vaginitis.

Keywords: ACV, bacteria, fungi, Vaginitis.

تقييم الفعالية المضادة للميكروبات لخل التفاح على البكتريا والفطريات المعزولة من التهاب المهبل

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الخلاصة:

التهاب المهبل هو مرض طبي منتشر يصيب نسبة كبيرة من النساء، ويعزى معظمه إلى التكاثر المفرط للبكتريا والفطريات. الهدف من هذه الدراسة هو تقييم الفعالية المضادة للميكروبات لخل التفاح ضد البكتريا والفطريات التي تم الحصول عليها من حالات التهاب المهبل. تم جمع ٥٠ مسحة مهبلية من مريضات التهاب المهبل في العيادات الخاصة في مدينة كركوك، العراق، (١٠ نساء حوامل و٤٠ امرأة غير حامل). تضمن البحث عزل وتشخيص العزلات البكتيرية والفطرية. أجريت على جميع العزلات اختبارات التركيز المثبط الأدنى وتقنيات انتشار القرص. تم اختبار تراكيز مختلفة من خل التفاح (٢٠٪، ٣٠٪، ٤٠٪، ٥٠٪، ١٠٠٪) ضد العزلات وتم استخدام المضادات الحيوية القياسية كعناصر تحكم إيجابية. تم فحص نتائج ٥٠ مريضة، وأظهرت أن ٢٢ (٤٤٪) مصابات بالتهاب المهبل البكتيري و٣٩ (٧٨٪) مصابات بالتهاب المهبل الفطري. وكان التهاب المهبل أكثر انتشاراً عند النساء الحوامل مقارنة بغير الحوامل. سجلت بكتريا المكورات العنقودية ١٤ عزلة (٢٤,٩٪) من أصل ٥٤ عزلة بكتيرية، تلتها، *Group B- Streptococcus, Echerichia coli Klebsiella pneumonia, Staphylococcus, epidermidis* و *Pseudomonas aeruginosa* على التوالي. بينما سجلت المبيضة البيضاء ٣٠ عزلة (٦١,٢٪) من أصل ٤٩ عزلة فطرية، تلتها *Candida tropicalis* و *Candida parapsilosis* على التوالي. وأظهرت النتائج مدى الحساسية لخل التفاح بين العينات المعزولة. اذ سجلت خل التفاح فعالية أكبر ضد العزلات الفطرية مقارنة بالعزلات البكتيرية، مع ملاحظة فعالية أعلى ضد البكتريا سالبة الكرام مقارنة بالبكتريا موجبة الكرام. تم تسجيل أعلى قيم لل *MIC* للبكتريا موجبة الكرام، والتي بلغت ١٢٥ (ميكروجرام/مل)، تلتها البكتريا سالبة الكرام، ٦٢,٥ ميكروجرام/مل. في حين كانت أقل قيم لل *MIC* للفطريات المعزولة (٣١,٢٥ ميكروجرام/مل). وقد سلطت المقارنة مع الأدوية التقليدية المضادة للميكروبات الضوء على إمكانات استخدام خل التفاح كعلاج بديل لالتهاب المهبل. وفقا لهذه النتائج، قد يكون خل التفاح خيار علاج فعال لالتهاب المهبل.

الكلمات المفتاحية: خل التفاح، بكتريا، الفطريات، التهاب المهبل.

1. Introduction:

Vaginitis, often known as external genital inflammation, is an inflammation of the vaginal mucosa. Itching, burning, irritation, discharge, and discomfort are common side effects of the inflammation. It's one of the most common causes of doctor visits among females [1]. Many different things, including irritants, hormones, foreign bodies, sexually transmitted diseases, and infections, can cause vaginitis. All of these factors have the potential to cause discomfort for sick women [2]. Bacteria, fungi, parasites, and viruses can all contribute to the development of infected vaginitis, in addition to Chlamydia, Staph aureus, Group B streptococcus (GBS), E.coli, Klebsiella pneumonia, Listeria monocytogenes, Acinetobacter spp., and Neisseria gonorrhoea are the most common bacteria that cause vaginitis [3]. Candida albicans, an opportunistic polymorphic fungal species that is a leading cause of vulvovaginal candidiasis (VVC), which causes major quality-of-life issues for women around the world, can also cause vaginitis. VVC is characterized by burning, itching, and redness of the vulva and vaginal mucosa, often accompanied by a thick white vaginal discharge. About 75% of all women will experience VVC at some point in their life [4]. Antimicrobial drug resistance is an established and pervasive issue worldwide. There has been a rise in a variety of infections that are resistant to different kinds of antimicrobials [5]. Warns that the development of resistance to antimicrobial drugs could threaten our ability to treat many diseases that are currently treatable. Because of this, natural antimicrobials have attracted a lot of attention in recent years to be used for immune-compromised and high-risk patients [6]. Apple cider vinegar has been the subject of extensive research into its pharmacological qualities and applications as a natural product that is its growing usage in the pharmaceutical, food, and cosmetic industries, and its antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, anti-hypertensive, immune-stimulating, and anticancer properties are only some of the many biological effects demonstrated by apple cider vinegar [7]. It has a long history of usage in alternative medicine. Numerous applications have been found for it due to the widespread acceptance of its purported antimicrobial, antiseptic, and antiviral properties [8]. The aim of this study is to evaluate the antibacterial and antifungal efficacy of apple cider vinegar for vaginitis isolates

2. Material and Methods:

1- Patients and Specimen Collection: 50 vaginal swabs have been obtained from vaginitis patients at private clinics in Kirkuk city, Iraq. Including 10 pregnant women and 40 non pregnant women. Each sample was inoculated onto blood agar plate, chocolate agar, nutrient

agar, McConkey agar and Sabouraud's Dextrose-Agar. The plates were incubated at 37°C for 24 hours, and Sabouraud's Dextrose agar plates were incubated at 27°C for 48 hours.

2- Identification of bacterial and fungal isolates: Colony morphology, Gram stain, and biochemical testing were used to diagnose bacteria in the patient's vaginal swabs, and the API 20 test was utilized for verifying the isolate's identity **[9]**. According to Murray *et al*., fungi were diagnosed using a series of tests based on their microscopic and cultural characteristics as well as biochemical tests **[10]**.

3- Preparation of Apple Cider Vinegar extract: The natural apple cider vinegar utilised in this research was acquired from a local supermarket. At 0.12 atm and -82 °C, the water content of a 1000 mL sample of vinegar was completely evaporated using a freeze dryer (Christ, Germany). After being freeze-dried, the sample of apple cider vinegar yielded 18 gm. A 1 mg/mL stock solution was prepared using distilled water, and then the solutions were sterilised by filtering them through a 0.45 µm filter **[11]**.

4- Inoculum Preparation: Candida spp. was incubated at 27°C for 48 hours, while all bacterial strains were incubated at 37°C for 24 hours **[12, 13]**. By transferring the bacteria and fungi in 0.9% sterile saline solution and adjusting to a 0.5 McFarland standard for each, the inoculum were standardised to contain around 10^8 cfu/mL for bacteria and 10^7 cfu/mL for Candida spp **[14, 15]**.

5- Minimum Inhibitory Concentration Test: The broth dilution method was used to determine the minimum inhibitory concentration (MIC) **[16, 17]**. The concentration range was obtained by making serial 2-fold dilutions, from (500 to 15.62) µg /mL. The (MIC) has been defined as the lowest concentration of extract required to inhibit the growth of bacteria and fungi. A tube without apple cider vinegar was considered as a positive control, while a tube with only broth was considered as a negative control. Triplicates of every test were done. Finally, the MIC for each isolated organism was recorded.

6- Preparation of concentrate solutions: Different concentrations of ACV extract (20%, 30%, 40%, 50% and 100%) were prepared **[18]**.

7- Disk diffusion method: Antibacterial and antifungal activity of ACV was measured using this method. In brief, sterile paper discs (6 mm) were saturated with 10 µl of each concentrate of ACV and then placed on inoculation plates (nutrient agar for bacteria and Sabouraud agar for fungus) that had already been prepared. Plates with bacteria were incubated at 37°C for 24

hours, while those with fungi were incubated at 27°C for 48 hours. Diameters of the microbial growth inhibitory zone were clearly measured in millimetres. However, fluconazole (25 µg/disc) and clindamycin (2µg /disc) have been employed as positive controls for fungi and bacteria, respectively. Every test was performed in triplicate [19].

8- Statistical analysis: Calculations of means, standard deviation and percentages were performed in SPSS version 18 and Microsoft excel 2013.

3. Results:

Numbers and percentages of bacteria and fungi isolated from vaginitis patients: 50 vaginitis specimens were cultured during the study period. 10 samples were of pregnant women and 40 samples were of non-pregnant women. Bacterial vaginitis included 22 (44%) samples, distributed among 15 (37.5%) non-pregnant women and 7 (70%) pregnant women. While fungal vaginitis included 39 (78%) samples, distributed among 31 (77.5%) non-pregnant women and 8 (80%) pregnant women. As shown in **Table (1).**

Table (1): Numbers and percentages of (bacterial and fungal) vaginitis in pregnant and non-pregnant women

Microorganisms	Non pregnant women (40)	Pregnant women (10)	Total no. (50)
Bacteria	15 37.5%	7 70%	22 44%
Fungi	31 77.5%	8 80%	39 78%

Vaginitis can be caused by a wide variety of microbes, including bacteria and fungi, and often results from an imbalance or interference between the types of organisms that cause inflammation and the normal vaginal flora [20]. Pregnant women had a higher rate of vaginitis (fungal and bacterial infections) than other women. Vaginitis is the most frequent genital infection in pregnant and breastfeeding women [21, 22], because estrogen stimulates and activates the growth and integration of the vaginal epithelial membrane, a normal level of estrogen is necessary to maintain the vaginal balance and its resistance to microbial infections. This is because estrogen is a potent anti-inflammatory [23]. According to Dybas *et al*., study most cases of vaginitis have been reported by pregnant women [24]. As for bacteria, 54 isolates were isolated from infected women: 14 (25.9%) *Staphylococcus aureus* isolates, 11(20.4%) *Echerichia coli*, 9 (16.7%) Group B- Streptococcus, 8 (14.8%) *Klebsiella pneumoniae*, 7 (13%) *Staphylococcus epidermidis*, and 5 (9.3%) *Pseudomonas aeruginosa* isolates. In addition, 49

fungal isolates were isolated from infected women, including 30 (61.2%) *Candida albicans*, 11(22.4%) *Candida tropicalis*, and 8 (16.3%) *Candida parapsilosis* isolates. As shown in **Table (2).**

Table (2): The percentage of bacteria and fungi that cause vaginitis

Bacteria	**No. (54 Isolates)**	**%**
Staphylococcus aureus	14	25.9%
Echerichia coli	11	20.4%
Group B- Streptococcus	9	16.7%
Klebsiella pneumoniae	8	14.8%
Staphylococcus epidermidis	7	13%
Pseudomonas aeruginosa	5	9.3%
Fungi	**No. (49 Isolates)**	**%**
Candida albicans	30	61.2%
Candida tropicalis	11	22.4%
Candida parapsilosis	8	16.3%

The results of the current study agreed with Razzak, *et al.'s*, study, as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, Group B- Streptococcus, *Klebsiella pneumonia*, and *Echerichia coli* were isolated, where they found that the presence of probiotics, which stimulated the production of defence components, correlated inversely with the organism that caused vaginitis **[25]**. Therefore, Antibiotics for vaginitis treatment must be carefully chosen so as not to eliminate the beneficial probiotics that play an essential role in keeping the vagina and its environment in a healthy order. The current study also agreed with the results of Hussein's study, *Candida albicans* that had the largest percentage of Candida species isolated from vaginitis, followed by *Candida tropicalis* and *Candida parapsilosis* **[26]**.

Minimum Inhibition Concentration value and antimicrobial activity of apple cider vinegar: The MIC test is commonly used to determine the minimum concentration of apple cider vinegar that inhibits isolates from vaginitis patients. The highest MIC values were found for gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and Group B-Streptococcus,), all of which were 125 (µg/mL).

Gram-negative bacteria recorded 62.5 (µg/mL) for (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*). While the lowest MIC values were found for the isolated fungi (31.25 µg /mL) for (*Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*), as shown in **Table (3)**.

Table (3): MIC values for apple cider vinegar against isolates (µg/mL)

Isolates	500 (µg/mL)	250 (µg/mL)	125 (µg/mL)	62.5 (µg/mL)	31.25 (µg/mL)	15.62 (µg/mL)	+ve control	-ve control
Staphylococcus aureus	-	-	-	+	+	+	+	-
Echerichia coli	-	-	-	-	+	+		
Group B-Streptococcus	-	-	-	+	+	+		
Klebsiella pneumoniae	-	-	-	-	+	+		
Staphylococcus epidermidis	-	-	-	+	+	+		
Pseudomonas aeruginosa	-	-	-	-	+	+		
Candida albicans	-	-	-	-	-	+		
Candida tropicalis	-	-	-	-	-	+		
Candida parapsilosis	-	-	-	-	-	+		

The antimicrobial properties of apple cider vinegar were tested on all isolates, and the results are presented in **Table (4)**.

Table (4): The (Mean ± SD) of Diameter zones of inhibition (mm) at different concentrations of apple cider vinegar by the desk diffusion method.

Bacterial Isolates	20%	30%	40%	50%	100%	Control Clindamycin (2 µg/disc)
Staphylococcus aureus	-	-	3±1.6	6.5±1.4	8±2.4	20 ±9.1
Echerichia coli	-	0.5±0.4	2±1.2	9±5.1	14±3.4	14±3.5
Group B-Streptococcus	-	-	1.5±0.6	7±3.5	9±4.2	16±5.1
Klebsiella pneumoniae	-	3±1.4	6.5±2.3	10±3.2	13±4.1	14±3.4
Staphylococcus epidermidis	-	0.5±0.3	3±1.4	6±2.3	8.5±3.2	17±4.1
Pseudomonas aeruginosa	1±0.4	4±2.1	6.5±3.2	9±5.2	13±4.2	15±4.3
Fungi Isolates	**20%**	**30%**	**40%**	**50%**	**100%**	**Control fluconazole (25 µg/disc)**
Candida albicans	7±2.3	9±3.2	13±3.4	15±4.1	19±7.1	25±9.2
Candida tropicalis	8±2.3	10±3.3	13.5±4.1	15±4.2	17±4.3	23±7.2
Candida parapsilosis	6±2.1	8±2.4	12±3.4	13±4.1	16±4.4	20±5.1

*Values are the mean ± standard error of 5 replicates

In this study, apple vinegar was found to have significant antibacterial activity against the vaginitis isolates (as shown in Table 4). Apple cider vinegar acts on bacteria by penetrating their cell wall and destroying their DNA, which prevents them from reproducing **[27]**. Apple cider vinegar showed antibacterial activity from (13±4.1) – (14±3.4) mm in gram-negative bacteria and from (8±2.4) – (9±4.2) mm in gram-positive bacteria. Zones of inhibition were

found to be significantly larger for gram negative bacteria compared to gram positive bacteria, Peptidoglycan layers around Gram-positive bacteria were many times thicker than those surrounding Gram-negative bacteria. Long anionic polymers called teichoic acids made up primarily of repetitions of glycerophosphate, glucosyl phosphate, or ribitol phosphate, served as the threads between the peptidoglycan layers. Furthermore, Gram-positive microbes had a wide range of proteins on their surfaces, some of which were comparable to proteins found near Gram-negative species and might offer further antimicrobial action **[28]**. The current study agreed with the results of Kalaba *et al*.'s, study, which had reported an inhibitory zone of 11.33 mm for *Staphylococcus aureus* and of 13.00, 14.00, and 12.66 mm for *Enterobacter kobei*, *E. cloacae*, and *E. coli*, respectively **[7]**. Apple cider vinegar had been shown to be more effective against isolated fungi than against isolated bacteria. Inhibition zones at 100% concentration were between (16±4.4) and (19±7.1) mm in diameter. Some authors claimed that the widespread use of conventional antifungals for the treatment of systemic and superficial infections had contributed to the development of resistant strains among people at risk of contracting a disseminated Candida spp. Infection **[29]**. The objective of this study was not to ascertain the mechanism via which apple cider vinegar exerted its effects. However, according to other scientists, acetate had been shown to inhibit 14-lanosterol-demethylase, Toxic concentrations of acetic acid that could build up inside a cell because it could pass through intact cell membranes and reached its molecular target. As a result of this process, H+-ATPase got activated, consequently, the acidity of the medium increased, which was toxic to fungi **[30, 31]**. Flow cytometer was used in another study to show that prolonged exposure to high concentrations of acetic acid loss of cell integrity **[32]**. The results of the current study agreed with the findings of Ousaaid *et al*., and Patole *et al.'s*, Apple cider vinegar which recorded antibacterial and antifungal activity **[33, 34]**.

4. Conclusion:

This study found that Apple cider vinegar was efficient against vaginitis-causing bacteria and fungus. This finding suggests that ACV may be used as a safe and effective natural remedy for vaginitis. More research is needed to establish the mechanism by which ACV produces its antimicrobial properties and evaluate its therapeutic efficacy.

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