Original Article

The Effect of Grapefruit Extracts on Causative Agents of Vaginitis among Women in Babylon Governorate, Iraq

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Abstract

Background: Vaginitis is inflammation of the vagina caused by a disruption in the normal vaginal flora. **Objectives:** This research aims to study the inhibitory activity of raw grapefruit juice, alcoholic, and aqueous grapefruit peel extract against the agents causing vaginitis among women in Babylon Governorate. **Materials and Methods:** A total of 177 women attending the Gynaecology and Obstetrical Department at Hilla Teaching Hospital in Babylon Governorate were included in the present study. All patients were complaining of vaginitis. The specimens collected in this study were cultured and diagnosed based on microscopic and cultured characteristics and biochemical tests. Also, an antibiotic susceptibility test for some antibiotics was done; aqueous and alcoholic grapefruit (*Citrus paradise*) peel extracts and juice of grapefruit were used in the study. The antimicrobial activity of both the grapefruit peel extract and raw juice was evaluated against different microorganisms isolated from the high vagina swab of patients presenting with vaginitis. **Results:** Several vaginitis pathogens were isolated in this study: *Staphylococcus aureus* was seen to be common, followed by *Staphylococcus epidermedis*, *Escherichia coli,* then *Enterococcus faecalis, Proteus mirabilis, Klebsiella pneumoniae, Gardnerella vaginalis, Listeria monocytogenes*, and *Actinomyces* spp., In addition, the yeast (*Candida albicans*) is the second leading cause of vaginitis. Tobramycin is the most effective, while Tetracyclin and Metronidazole had no effect. The inhibitory action of alcoholic grapefruit extract and raw juice is more effective than aqueous extract. **Conclusion:** The alcoholic grapefruit peel extract and raw grapefruit juice have an inhibitory effect against all agents under study except *K. pneumoniae*, while the aqueous extract showed less efficacy against the tested microbes.

Keywords: Grapefruit (*Citrus paradise*) extract, inhibitory activity, vaginitis

Introduction

The general expression for the disorders of the vagina is called (vaginitis); it's an inflammatory or infection due to changes in the normal vaginal flora. And it is often described as a polymicrobial syndrome, characterized by a shift in the vaginal flora from a dominant group of lactobacilli to an overgrowth of several vaginitis-causing agents, for example, *Gardnerella vaginalis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.[1-3]

Vulvovaginal candidiasis is regarded as the second most common type of vaginal infection.[4] 50% of women influence from vaginal candidiasis at minimum once.[5] In spite of *Candida albicans* being considered a natural flora, it may be the reason vaginitis for more than 80% of patients[6] and is treated with topical or oral antifungals, according to clinical practice guidelines[7]; vaginitis is the most common cause of odor of the vagina, itching, and discharge, mainly among women of childbearing age.[8,9]

Presently, vaginitis is treated by prescribing antibiotics orally or by injecting them directly into the vagina. The incessant antibiotic treatment is usually recommended; however, this can lead to antibiotic resistance.[10,11] Recently,

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several studies have been conducted on the effect of plant extracts on the outgrowth of microorganisms and, thus, the likelihood of using them in the treatment of some diseases resulting from diverse microbial infections.[12,13] Due to the scarcity of studies on Grapefruit, it was chosen in this study to test its impact on the growth of microorganisms that cause vaginal infections.

Grapefruit is one of the most consumed fruits in the world. Citrus tree of the family Rutaceae, genus: *Citrus* and species: *paradise*. It is a very important fruit, and its production is increasing day by day due to its great importance as medicine, in addition to the various essential oils that it produces, which have economic and consumer importance. And used as an ingredient in cosmetics, perfumes, soaps, and detergents.[14]

Grapefruit (*Citrus paradise*) presents a good exporter of vitamin C, phenolic compounds (phenolic acids, flavonoids, and coumarins), and terpenic substances such as (limonoids and carotenoids). These bioactive compounds are known to act as free-radical scavengers, modulate enzymatic activity, and protect humans from a variety of diseases.[15,16]

Materials and Methods

Study design

A total of 177 high vaginal swabs (HVS) samples were collected from women complaining of vaginitis who attended the emergency and consultant for the obstetrics and gynecology hospital in Babylon Governorate for the period from January to June of 2022. The causative agents of vaginitis were isolated and diagnosed based on microscopic and culture characteristics and biochemical tests according to Koneman's.[17] Nine bacterial isolates were diagnosed and tested in the microbiology laboratory at the College of Medicine, University of Babylon, which included *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. faecalis*, *Listeria monocytogenes*, *G. vaginalis*, *Klebsiella pneumoniae*, *Actinomyces* spp. and *Proteus mirabilis*. In addition to the vaginal *C. albicans* yeast, which is diagnosed by germ tube formation test. Antibiotic susceptibility test was performed on these isolates by disc diffusion method using Mueller Hinton agar and sheep blood agar for fastidious organisms.[18] The antibiotics were tested are Ampicillin (AM10 mg), Tobramycin (TOB 5 mg), Ciprofloxacin (CIP 10 mg), Gentamicin (GEN 30 mg), Nitrofuratoin (NIT 300 mg), Nalidixic acid (NA 30 mg), Chloramphenicol (CHL 30 mg), Tetracyclin (TET 10 mg), Metronidazole (MTZ5 mg) and Co-trimoxazol (CMX 30 mg).

Preparation of grapefruit (*C. paradise*) juice

Raw grapefruit (*C. paradise*) juice was obtained by squeezing this fruit and taking the juice by removing seeds and membranes and filtering through a filter membrane (Millipore) with a diameter of 1.22 mm.[19]

Preparation of alcoholic grapefruit (*C. paradise*) extract

Alcoholic extract of Grapefruit was prepared by using the soaking method, where 20 g of grapefruit peels were crushed and soaked in 200 mL of ethyl alcohol with a concentration of 95% inside a snow bath, and after mixing well, it was left in the refrigerator for 24 h then was filtered through several layers of gauze and filtered again by the suppression Büchner funnel and put in a rotary evaporator device (rotary vacuum evaporator) and using filter paper (Whatman No-1), where this device labors on the basis of evaporation under vacuum pressure and a temperature not exceeding 40°C. After evaporating all the ethanol in the admixture, a thick layer of the extract was formed, which was dried by lyophilization under vacuum pressure by a lyophilizer. The samples were kept freezing in bottles with a tight lid until used in the study. The alcoholic grapefruit peel extract was prepared with a concentration of 200 mg/mL by dissolving 1 g of the extract prepared in the previous step in 5 mL of the DMSO (dimethyl sulfoxide), and the admixture was sterilized by pasteurization method at a temp. Of 62°C for 10 min. This alcoholic extract was then used for the purpose of inhibition test experiments.[19,20]

Preparation of aqueous grapefruit (*C. paradise*) extract

An aqueous extract of *C. paradise* was prepared in this study by crushing 20 g of the *C. paradise* peels in 200 cm^3 of distilled water inside a snow bath and, after shaking the mixture well, put in the refrigerator and left for 24 h for the purpose of soaking, the mixture was filtered through several layers of Gauze and filtrated again during Buechner funnel and using filter paper (Whatman No-1), thus obtaining the raw *C. paradise* extract. Then, the extract was cooled dry under pressure in the drying device, and the samples were kept in freezing bottles with a tight lid until used in the study.

The aqueous grapefruit peel extract was prepared at a concentration of 200 mg/mL by dissolving 1 g of the extract prepared in the previous step in 5 mL of distilled water. The extract was sterilized using a membrane filter of 0.22 M to prevent the passage of germs through it. Sterile aqueous extract of grapefruit peel was used for inhibition test experiments.[19,20]

Antimicrobial Activity Of Raw Grapefruit (*C. paradise*)

The antimicrobial activity of raw grapefruit (*C. paradise*), alcoholic, and aqueous grapefruit peel extract was conducted using the well agar diffusion assay.[21] Well diffusion assay method was followed, as bacterial isolates were cultivated in BHI broth medium, and their density was compared with a MacFarland tube number 0.5, equivalent to 10 cells/ mL. 0.1 was transferred from the

bacterial suspension to the Muller Hinton Agar and published using cotton swabs, and each plate was cultured with a type of bacteria tested in the study, and then drilling was carried out with a diameter of 5 mm on the surface of the culture medium using a sterile cork punch, and added to each pit (50 µl) of the raw Grapefruit juice, alcoholic and aqueous *C. paradise* peels extract prepared at a concentration of (200 mg/ mL). The dishes were incubated under ideal conditions at a temperature of 37°C and for a period of 24 h. After incubation, inhibition areas were observed, and inhibitory activity was recorded by measuring the diameter of the clear inhibition zone around the well with the ruler.

Ethical approval

The study was conducted according to the ethical principles originating in the Declaration of Helsinki. It was performed with patients' verbal and written consent before sampling. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number 137 on June 7, 2022, to get this approval.

Results

Distribution of cases

Vaginal discharge is a symptom that often prompts women to consult a physician in order to determine the presence of infection. However, much vaginal infection, particularly bacterial vaginosis, is nearly asymptomatic. Women were subjected to this study according to some clinical cases. All women patients complained of vaginitis, itching with or without abnormal vaginal discharge. The main clinical feature among women 177 patients was vaginal discharges 95 (53.67 %), followed by vulval itching 54 (30.50 %) and pruritus 28 (15.83%), as shown in Table 1.

Not all the HVS results revealed positive results, as 75(42.37%) of the HVS results over the period yielded no growth [Table 2].

Several types of bacteria were isolated from women under investigation, as shown in Table 3, *S. aureus* 32(31.37%) was common among women, followed by *Staphylococcus epidermedis* 18 (17.64%). The presence of G. vaginalis diagnosed bacterial vaginosis (nonspecific vaginitis); this bacteria was isolated from women at a rate of 6 (5.88%). Moreover, *E. coli* and *E. faecalis* were 15 (14.73%) and 8 (7.84%). The rate of both *L. monocytogenes* and *Actinomyces* spp. bacteria was 5(4.90%), *K. pneumoniae* and *P. mirabilis* bacteria also isolated women at a rate of 6 (5.88%) and 7 (6.86%), respectively. In addition to 71 isolates of *C. albicans*. Moreover, vaginal discharge is a symptom that often leads women to consult a physician to determine the presence of infection. However, much vaginal infection, particularly bacterial vaginosis, is nearly asymptomatic.

Table 1: Distribution of symptoms among women with vaginitis

Symptoms	No.	(%)
Vaginal discharge	95	53.67
Vulval itching	54	30.50
Pruritus	28	15.83
Total no.	177	100

Antibiotic susceptibility of bacterial isolates

Table 4 shows a variation in the susceptibility of the studied isolates to these antibiotics. It was found that *S. aureus, S. epidermidis, E. coli, G. vaginalis,* and *E. faecalis* were sensitive to ciprofloxacin, gentamicin, nitrofuratoin, naldixic acid, tobramycin, and cotrimoxazol, but resistant to ampicillin, chloramphenicol, tetracycline, and metronidazole. *L. monocytogenes*, *K. pneumonia*, and *P. mirabilis* showed resistance to all of these antibiotics except for the Tobramycin, which was sensitive to it. The result indicated that Tobramycin was the most effective, while Ampicillin, Chloramphenicol, Tetracyclin, and Metronidazole had no effect.

Antimicrobial activity of grapefruit (*C. paradise*)

Upon detection of the effect of peel extracts of *C. paradise* on the growth of isolated bacteria in this study, the results are shown in Table 5.

The alcoholic extract of *C. paradise* peels exhibited a stronger growth inhibition effect on *E. coli* and *P. mirabilis* (33 and 31 mm), respectively. It also showed an inhibitory effect on S. aureus and S. epidermidis growth, where the

Table 2: Results of high vaginal swabs (HVS) samples from women with vaginitis

Bacterial isolates	No.	%
Positive growth	102	96.23
Negative growth (No growth)	4	3.77
Total	106	100

Table 3: The number and percentage of bacteria isolated from women with vaginitis

Bacterial isolates	No. of patients	%
S. aureus	32	31.37
S. epidermidis	18	17.64
E. coli	15	14.73
E. faecalis	8	7.84
P. mirabilis	7	6.86
G. vaginalis	6	5.88
K. pneumoniae	6	5.88
L. monocytogenes	5	4.90
Actinomyces spp.	5	4.90
Total	102	100

Table 4: Antibiotics susceptibility test against bacterial isolates under study

Antibiotic	*S. aureus*	*S. epidermidis*	*E. faecalis*	*L. monocytogenus*	*G. vaginalis*	*E. coli*	*K. pneumoniae*	*P. mirabilis*
CIP	S	S	S	R	S	S	R	R
AMP	R	R	R	R	R	R	R	R
GEN	S	S	S	R	S	S	R	R
NIT	S	S	S	R	S	S	R	R
NAL	S	S	S	R	S	S	R	R
CHL	R	R	R	R	R	R	R	R
TET	R	R	R	R	R	R	R	R
MTZ	R	R	R	R	R	R	R	R
TOB	S	S	S	S	S	S	S	S
CMX	S	S	S	R	S	S	R	R

Table 5: Antibacterial activity of grapefruit juice, alcoholic, and aqueous peel extract against microbial isolates under study (inhibition zone diameters in mm)

Microorganisms	Raw grapefruit juice	Alcoholic extract	Aqueous extract
S. aureus	14	27	0
L. monocytogenes	0	26	0
S. epidermidis	12	25	0
E. coli	20	33	7
G. vaginalis	12	17	0
E. faecalis	10	22	5
Actinomyces spp.	15	19	0
P. mirabilis	18	31	0
K. pneumoniae	0	0	0
C. albicans	10	24	0

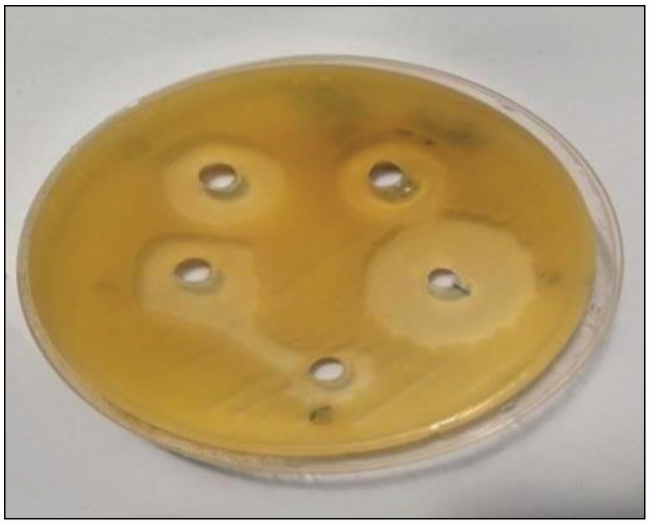

Figure 1: Antimicrobial activity of the alcohol extract of Grapefruit (*C. paradise*) peels on *S.epidermidis*

inhibition zone diameter was 27 and 25 mm [Figure 1]. The diameters of the inhibition of this alcoholic extract towards *L. monocytogenes, E. faecalis*, *G. vaginalis,* and *Actinomyces* spp. (26, 22,17, and19 mm), respectively, while *K. pneumoniae* showed resistance to this extract. So, it was found that the aqueous extract of *C. paradise* peels did not show an inhibition effect toward all isolated bacteria except *E. coli* and *E. faecalis*, which showed sensitivity to this extract (7 and 5mm), respectively. Regarding raw *C. paradise* juice, it was noted from the results that the *E. coli* showed high sensitivity to this extract, as the diameter of the inhibition zone reached (20 mm), whereas *L. monocytogenes* and *K. pneumoniae* showed absolute resistance to this extract, which means that this extract does not have inhibitory efficacy towards these isolates, while it possesses inhibitory capacity towards *S. aureus*, *S. epidermidis*, *E. faecalis*, *G. vaginalis*, and *Actinomyces* spp. as the diameters of the inhibition areas reached (14, 12, 10, 12, and 15mm), respectively. Raw *C. paradise* juice also has good inhibiting efficacy against *Proteus mirabilis* (18 mm). Also, the *C. paradise* peel extract had a clear inhibiting effect against the isolated natural vaginal flora under study represented by the *C. albicans* yeast. The alcoholic extract of the *C. paradise* peels showed a clear inhibiting activity against *C. albicans* (24 mm). At the same time, the aqueous extract of *C. paradise* peels did not show an inhibition effect. Returning to the results in Table 5, it was found that raw *C. paradise* juice had good inhibitory action against *C. albicans* yeast (10 mm) [Figure 2].

Figure 2: Antimicrobial activity of the alcohol extract of Grapefruit (*C. paradise*) peels on *C. albicans* yeast

Discussion

Vaginitis represents a vaginal infection. It is caused by several types of bacteria and *C. albican*s due to sexual activity with poor personal hygiene.[22] Antibiotic susceptibility test shows that Tobramycin was the most effective against bacteria under study, followed by Ciprofloxacin, Gentamicin, Nitrofurantoin, Naldixic acid, and Cotrimoxazol, while Ampicillin, Chloramphenicol, Tetracyclin and Metronidazole had no effect [Table 1]. The difference in antibiotic susceptibility test results is similar to the other studies.[23,24]

Not all the HVS results give positive results (growth); several reasons might explain this. Patients used antibiotics before taking the sample, or maybe because of another type of noncultivable inflammatory agent that needs other techniques to identify it, such as viruses, chlamydia, mycoplasma, and others.[25,26] Several types of bacteria were recovered from women under investigation; the presence of these bacterial species in the vagina is due to an imbalance in the natural flora of the vagina, as in the case of the absence of the flora represented by lactic acid bacilli. *Candida* species were the most prevalent organisms isolated in this study. This result is in line with and similar to reports from other studies.[27] The presence of yeasts in the genital tract is a natural fluoride, but it is an opportunistic organism when the body's resistance is weak or as a result of taking antibiotics abundantly as yeasts are active, proliferate, and cause infections in these places.[28]

In view of the increasing interest in medicinal plants and herbs in recent years, by using them as main sources for the production of medicines or as a source of active substances that enter into the composition of medicine; therefore, the current research was studied the inhibitory effect of raw Grapefruit juice, the alcoholic and aqueous extract of Grapefruit peels, showed the different effect on the bacteria. The results of this study also showed that alcoholic and aqueous peel extract and raw juice used in this study were more effective in Gram-negative bacteria than in Gram-positive bacteria; that is, the positive bacteria have a stronger resistance than negative bacteria against these grapefruit extract, and the reason for this may be due to the nature of cell wall.[29,30]

It was observed from the results shown in Table 2 that all types of bacteria studied were sensitive to grapefruit (juice, alcoholic, and aqueous extracts of peel) except *K. pneumoniae*, which shows stronger resistance to them; this may be due to the difficulty of penetrating the cell walls and capsules of these bacteria.[31,32] The alcoholic extract of grapefruit peel has a clear inhibitory activity against *C. albicans*.[33]

The superiority of the inhibitory effect of the alcoholic extract in this study to the inhibitory impact of the aqueous extract may be explained on the basis that the ethanol alcohol is able to dissolve a number of active substances and collect them in the raw extract in sufficient quantities to induce the inhibitory action or may explain that the active components of the plant may be dissolved in alcohol and not in the water, the alcoholic extract will be rich in the main active compounds of the plant that have antimicrobial activity.[34]

The results showed that the raw citrus fruit juice (grapefruit) has a high-to-moderate inhibitory activity against these microbes, which may be due to the low PH of the medium, which leads to the inhibition of bacterial growth. The inhibitory activity of the Grapefruit extract is due to one or more of the compounds of medicinal importance, including phenolic compounds (flavonoids, phenolic acids, and coumarins) and terpenic substances such as (limonoids and carotenoids), as these compounds have the ability to penetrate the bacterial cell wall.[35,36]

Conclusions

The results of our study showed that alcoholic Grapefruit peel extract and raw grapefruit juice had an inhibitory effect against all vaginitis cases causative agents under study except *K. pneumoniae*, while the aqueous grapefruit peel extract showed less efficacy against the microbes that were tested. Therefore, it is represented as an antiseptic antiseptic to reduce and control infections caused by microorganisms.

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Conflicts of interest

There are no conflicts of interest.

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