Study the Effect of Mango Peel Extract on Pathogenic Bacteria Isolated From Urinary Tract Infection

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KEYWORDS
Urinary tract; Mango peels; Bacterial diagnosis; Plant Extraction; Phenolic compound; Antibacterial.

ABSTRACT
Objective: Urinary tract infection is an infection that affects any part of the urinary system. The urinary system includes the kidneys, ureters, bladder, and urethra. Most infections affect the lower part of the system, the bladder and urethra. The infection may reach the kidneys and develop into kidney failure. This study aimed to find a new natural extract against the germs that cause the disease to help reduce the large number of patients suffering from the disease. Methods: This study included plant extraction for mango peels by used ethanol 70% in soxhlet apparatus. Urine samples were collected from dogs, and samples containing inflammatory cells were isolated and cultured. The cultured bacteria were identified by used morphology of colony, Gram stain and biochemical tests. Biofilm made by used microliter plate method, activity of mango peels extract (MPE) was measured by well agar diffusion method, the concentration of minimal inhibitory (MIC), and the concentration of minimal bactericide (MBC) was measured by broth dilution method, Statistical analysis was used to determine the effectiveness of the extract. Results: Diagnosis for isolated pathogenic bacteria of urine samples which contained inflammatory cells staph aureus, E.coli, pseudomonas. The best Biofilm formation of staph aureus (91.7)% followed by E.coli (83.4), pseudomonas (16.7). The highest effectiveness of mango peels extract activity was recorded against staph aureus (22-26) mm zone of inhibition and (17-19)mm of E.coli ciprofloxacin gave (22-25)mm zone of inhibition of S.aureus and (18-22)mm E.coli . The concentration of minimal inhibitory (2mg/ml) of staph aureus and (4mg/ml) E.coli The concentration of minimal bactericide (4mg/ml) of staph aureus and (8mg/ml) E.coli. The Statistical analysis of inhibition halos of staph aureus (24 ± 0.6) highest than E.coli (18 ± 0.2) of MPE and the Statistical analysis of inhibition halos of staph aureus(23± 0.2) and(20± 0.6) E.coli of ciprofloxacin. Conclusion: Mango peels extract have antibacterial effect against both gram positive and gram negative pathogenic bacteria isolated from UTI. Use of extract is help in treating patients with urinary tract infection, MPE contributing and limiting the progression of the condition.

1. Introduction

The world is suffering from an invasion of pathogenic microorganisms, which causes many diseases. One of the most pathological bacteria that are frequently infected and infect more than one member of the body (E. coli , pseudomonas, Streptocooccus Spp., Staph auras), Infect the respiratory, digestive and urinary , reproductive system and causes many infectious diseases, which vary in severity, they may be simple or acute and sometimes fatal if the condition develops and is not controlled [1]. .Resistance occur in the human (men, women and children) and animals, the disease affects the newborn for both(G+ve,G-ve)bacteria [2].Recently, we have noticed an increase in case of urinary tract infection(UTI) and increasing spread of that diseases in recent times due to the huge spread of pathogenic microorganisms and the observation of repeated infection of the same germ and the lack of response to the treatment allocated to it due to its ability to resist treatment Urge to continue search for antibiotics to limit the spread of these microorganisms and the world's tendencies today for natural remedies extracted from natural materials for easy access and availability in our hands and it has good effectiveness against germ[3] . The rapid development of antibiotic resistance in individual's poses a major threat to health [4].Treatment of infection is essential to preserve the life of the infected person [5] ,not only that, but the recurrence of condition in the same infected person, which calls for finding solution to this annoying problem without causing any collateral damage to the patient and his psychological state [6].Today take mango Extract (ME) to study its effect on UTI because it's one of the tropical fruits that are very beneficial to humans and animals for what he owns minerals, vitamins and enzymes in its composition, whether in its peel or pulp Mango. It's belonging to the family Anacardiaeceae [7] . Mango was uses as antivirus against herpes

virus [8]. and was uses as anti-diabetic [9].And in the 2022 it is used to treat wounds as anti-inflammatory and anti-microbial in the laboratory animals [10] .

2. Methodology

Preparation Mango peel extracts

The mango was taken from the vegetable market and washed with water then peeled to separate the peels from the pulp, the peels were dried and ground by used grinder, "extracted 50 g of ground peels in 250ml of 70%ethanol in the (soxhlet apparatus). After that used rotary evaporated, its crude extract and frozen at 20 C° (until its used) [11].

Pathogenic bacteria

Collection and examination of urine samples

A general urine test was conducted for all samples collected from veterinary clinic for the year 2022, from January to June from the dogs. The urine strip test shows the presence of several signs leukocyte and blood cells, nitrites. The microscopic examination, a count of leukocytes 5 and more per field and presence bacteria are indicates of a UTI [12].

Urine culture

Samples in which inflammatory cells were found during microscopic examination were cultured. The culture is represented positive if have ≥ 10^5 cfu/mL bacterial number. The urine was planted after Prepared standard operation procedure (SOP) in the Laboratory on blood agar, macConkey agar, Mannitol salt agar by sterile loop and incubated for 24h at 37 °C[13].

Bacterial Diagnosis

The colonies that appeared on the culture media were referred to as diagnostic by morphology of the colony, Gram stain and biochemical tests [14].

Biofilm formation assay

The formation of Biofilm made by used microliter plate method, each isolated was separately and Planted in Trypticase Soy Broth(TSB) and incubated it's for 24 h at 37 °C .1:10^2 prepared dilution of suspension,200 microliter of this was inoculated in96-well plat with control negative wells (TSB) only , incubated the plates at 37 °C for 24 h. Three time washed the plates with phosphate buffered saline (PBS) 200 ml , left dry at room temperature and to fix the bacteria used 150 µl of methanol with concentration 99% for each well, then plates drained and dry after that used 1% crystal violet 200 ml for 20 minute , its washed with water and eliminated crystal violet by used 33% acetic acid (200 ml) then incubated for 15 min at 37 °C , after that measured the absorbance at620nm by enzyme-linked immunosorbent assay (ELISA) [15,16].

Antibacterial Activity of mango peel extract

The mango extract activity was measured by well agar diffusion method [17,18,19]. taken 3-4 colonies of the isolated bacteria and emulsified in5ml normal saline and measured turbidity with McFarland 0.5 as turbidity standard. Inoculated the plate surface of a Mueller-Hinton agar by isolated bacteria, sterile tip of micropipette cut wells in the agar and by sterile needle removed it's for each plate of pathogenic bacteria, first well filled with 100µl of 200 mg/ml mango peel extract (20). the second well with 100 µl of 10 mg/ml ciprofloxacin as positive control (21) and third well with distal water as negative control. Incubated the plates for 24 h at 37 °C and measured the inhibition zone in millimeter.

Concentration of Minimal Inhibitory and Concentration of Minimal Bactericide

The concentration of minimal inhibitory (MIC) , the concentration of minimal bactericide (MBC) was measured by broth dilution method (8, 4, 2, 1, 0.5, 0.25, and 0.125) mg/mL concentrations that

were used, added broth of Müller Hinton 50 µL in 96 well plate with100 µL of the stock was added and incubated plate with(1 × 105) UFC/mL concentration for 24h at37◦C [22].

Statistical Analysis

Statistical Analysis in this research was made for (the zone of inhibition of Antibacterial activity) by used Variance Analysis one –way ANOVA and a significance value $p<0.05$ [23] .

3. Results and discussion

After isolation and diagnosis of pathogenic bacteria of urine samples collected from animals who suffered from urinary tract infection, the most frequent bacteria were selected in the samples, which is *staph aureus* [24] and *E. coli* [25] , *pseudomonas* [26] the effect of mango peel extract on it has been studied

Biofilm production

The biofilm result divided into strong (highest rate of biofilm formation) that was gave in *S.aureus* (91.7%) and followed by *E. coli* (83.4%moderate) and *pseudomonas aeruginosa* (16.7% weak).

Activity of Mango peel Extract

Mango peel extract activity was measured by method of agar well diffusion and been compared with ciprofloxacin is appeared in table 1. and it gave results Mango peel extract (22-26)mm zone of inhibition for *S.aureus* and (17-19)mm for *E.coli* ciprofloxacin gave (22-25)mm zone of inhibition for *S.aureus* and (18-22)mm for *E.coli* .

Table 1 Mango peel extract activity (antibacterial activity).

Pathogenic bacteria	Ciprofloxacin	Mango peel extract
S.aureus	(22-25)mm	(22-26)mm
E.col	**(18-22)mm**	**(17-19)mm**

Determined MIC and MBC for Mango Peel Extract

Determined (MIC) and (MBC) appeared in Table 2. measured by the method of broth dilution and results showed the MIC (2mg/ml), MBC (4mg/ml) for *s.aureus and MIC (4mg/ml*), MBC (8mg/ml) for *E.coli*

Table 2 MIC and MBC for MPE on pathogenic bacteria.

Pathogenic bacteria	MIC mg/ml	MBC mg/ml
S.aureus	2	4
E.col	**4**	**8**

Statistical Analysis

The Statistical analysis appeared in Table 3. for (*S.aureus*) given biggest inhibition halos (24 ± 0.6) and (*E.coli*) lowest inhibition zone (18 ± 0.2) and the control positive ciprofloxacin showed inhibition zone (23± 0.2) for (S.aureus) bacteria and (20± 0.6) for (*E.coli*) bacteria.

Table 3. The Statistical analysis for Ciprofloxacin and Mango peel extract activity on *S.aureus* , *E.coli.*

Pathogenic bacteria	Ciprofloxacin	Mango peel extract
S.aureus	**23± 0.2**	**24 ± 0.6**
E.coli	**20± 0.6**	**18 ± 0.2**

Discussion

UTI is a disease from time immemorial to this day harmful and causes major problems whenever the condition is delayed, it can turn into acute kidney inflammation and kidney dysfunction, and may eventually develop into kidney failure if it is not controlled [27] , Today, we notice the tendencies of most of us, or most of our society, towards the use of natural-source antibiotics or natural extracts for

treatment, because they give a result in treatment without affecting the rest of the body's organs and easy access to them[28]. In recent years, most scientists have tended to search for plant chemicals that can be developed to treat diseases[29] .In this study ME showed antibacterial activity against pathogenic bacteria isolated from urine samples for dogs with UTI and compared with ciprofloxacin as positive controlled and gave highest inhibition zone for *S.aureus* ME (24 ±0.6) ,ciprofloxacin (23 ±0.6) and lowest inhibition zone for *E.coli* ME (18±0.6), ciprofloxacin(20±0.6) mm (Mean±SD),this research revealed that the ME has activity against germs that cause UTI, this is due to ME has strong efficacy on most pathogenic bacteria , because it is an important outlet of phenolic compounds, which represent antioxidant activity[28]. Phenolic compounds have the power to eliminate bacteria, viruses and fungi [30]. compared study with a Pakistan results that showed zone of inhibition (mm) of (acetone mango leaf extract AMLE) against S. aureus 18±1.5mm (Mean±S.D) and S. typhi murium 20±1.5mm Mean±S.D [31]. and this study likely Rehab she studied Antimicrobial Effect of peel extracts against bacteria *S.aureus* gave 17mm and E.coli 16 mm zone of inhibition Mangifera indica less effective of which[21] . Victoria showed zone of inhibition for ME against S.aureus (11-17)mm in 120mg/ml [32]. The result of the MIC and MBC for this study was for S.aureus 2mg/ml and MBC 4mg/ml ,MIC for E.coli 4mg/ml and MBC 8mg/ml compared with Pakistan study MIC range from 10-50 mg/ml against S. aureus , and with Andressa study gave MIC between 1.8–7.5 and MBC 15.1, 45.3 mg/ml of ME against *Staphylococcus* spp. [33].All previous studies, the effectiveness of the extract against germs was observed, and the difference in the strength of the ME from one research to another is a result in the difference in the type and species of the bacteria, mechanism of action, difference in the proportions of the natural ingredients of mango and difference in the number and type of phenolic compounds and its concentration found in mango each researcher.

4. Conclusion and future scope

MPE have antibacterial effect against (G+ve) and(G-ve) pathogenic bacteria isolated from UTI dogs , therefore it is recommended to use it to treat UTI because of its therapeutic effects , natural product without harm to the affected person and available.

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