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Antibacterial Effect of some Fruit peels against Bacteria Causing Urinary Tract Infection.

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ABSTRACT

The emergence of multidrug-resistant bacteria has become a serious health threat, afterward; interests in alternative medicine have been increased rather than traditional antibiotic medications. This study tries to found the new antibacterial agents against the bacteria causing the urinary tract infections. We prepared aqueous extract from fruit peels extract, evaluated its functional ingredients and tested its antibacterial activity against the most common bacteria causing urinary tract infection, gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*), gram-positive pathogens (*Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*). The highest levels of functional ingredients are found in banana, pomegranate, orange, lemon, and apple respectively and the lowest levels in watermelon, kiwi, and papaya respectively. The antibacterial activity came in the same trend of the functional ingredients, as the highest antibacterial activity against all tested bacteria was in banana, pomegranate, orange, lemon and apple respectively and the lowest antibacterial activity was in watermelon, kiwi, and papaya respectively. The present study showed significant antimicrobial activity of banana, pomegranate, orange, lemon and apple against the tested microorganisms. This antibacterial activity is related to the high content of functional ingredients especially alkaloids, phytate and saponins. This antibacterial activity could lead to new options for the treatment of infectious diseases and emerging drug resistance.

Keywords: Antioxidant; alkaloid; saponin; tannin; total glycosidic cyanide

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INTRODUCTION

Urinary tract infections (UTIs) are the infections of the urethra, bladder, and kidneys. These infections are considered as the common causes of urethritis, cystitis, pyelonephritis, and glomerulonephritis. Bacteria are the most common causes of UTIs, especially in the urethra and bladder [1]. UTI can cause morbidity and health care expenditures in all the ages. These infections effect is high in the low resource developing countries because of the lack of awareness [2]. UTIs are mainly treated with fluoroquinolones, which result in a rapidly spread of bacteria resistant to quinolone in many countries. In addition, these bacteria are endemic in many parts of the world [3]. The emergence of multidrug-resistant bacteria has become a foremost health threat, afterward, interests in alternative medicine have been increased rather than traditional antibiotic medications [4].

The peels of fruits are considered as agro wastes. These agro wastes are produced in a big amount during household usage and food industry processes. These wastes pose a serious threat to the environment and also are highly prone to microbial spoilage. These wastes should be controlled and used in a useful way [5]. Many researchers reported the effectiveness of fruit peels extract as antimicrobial or anticancer agents because they contain high amount of various mixture of phytochemical constituents [6-8].

The present study aimed to assess the antibacterial activity of the fruit peels aqueous extracts on most commonly bacteria causing the UTTs. Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Gram-positive pathogens such as, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*.

MATERIALS AND METHODS

Extract preparation

The fruits were cleaned and peeled, dried in an oven at 60°C till constant weight. The dried peels ground into fine powder using an electric blender. The fine powder sample (100 g) was extracted in 50 ml water for 24 h using a shaker at 30ºC, and then the extract was filtered. The filtrates were stored at 4°C until use [7].

Functional ingredients

The tannin, glycosidic cyanide, steroid, saponin and alkaloid contents of the extract determination was performed according to Harbourne [9] and modified by Trease and Evans [10]. Phytate content was measured using the method of Vaintraub and Lapteva [11]. Total flavonoid content was determined with the aluminum chloride colorimetric assay [12]. Phenols were estimated according to the method of Julkunen-Tiitto [13].

Antibacterial Test

The extracts were individually tested against gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*), gram-positive pathogens (*Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*). These bacteria were taken from Cairo Microbiological Research Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Shobra Khayma, Cairo, Egypt.

The antibacterial activity of extracts was tested against the previous mentioned pathogenic bacterial species with disc-diffusion method [14]. The bacterial suspensions were spread over the surface of nutrient agar plates. Then, sterilized paper discs of 6-mm in diameter soaked in extracts till saturation were put on the surface of the nutrient agar media with appropriate distance separating them from each other. The inoculated plates were incubated at 37 °C for 24 h, after incubation the zone inhibition of was observed and its diameters were measured.

Minimum inhibitory concentration (MIC)

Antibacterial activity of the dried extracts was tested by conventional broth dilution assay against all the tested bacteria. MIC of the extracts was determined using standard inocula of 1 × 106 CFU/mL [15]. The dried extracts were dissolved in sterilized distilled water, and adjusted to serial dilutions in brain heart infusion broth. The prepared broth put in tubes each containing 10 mL were injected with 100 µL inoculum and incubated for 24 h at 37 °C. After incubation, the turbidity in the samples tubes was measured spectrophotometrically OD600. MIC was defined as the minimum extract concentration causing reduction in the turbidity. Negative control was conducted using the same volume of distilled water instead of the tested extract.

RESULTS AND DISCUSSION

Plant secondary metabolites are very important for mankind through providing dyes, fibers, glues, oils, waxes, flavoring agents, perfumes, insecticides, herbicides and are sources of new natural drugs for many diseases. Fruit peels are from the important natural sources for natural pharmaceutical components [7, 16]. In the present study as shown in Table 1, the highest levels of phytochemical components are found in banana, pomegranate, orange, lemon, and apple respectively and the lowest levels in watermelon, kiwi, and papaya respectively.

The efficiency of these phytochemical components as antibacterial agents is well reported [8, 17]. The results of present work are in the same line of the previous fact as shown in Table 2 and Table 3 the highest antibacterial activity against all tested bacteria was in banana, pomegranate, orange, lemon and apple respectively and the lowest antibacterial activity was in watermelon, kiwi, and papaya respectively. Water melon extract only shows weak antibacterial activity against *Escherichia coli and Klebsiella pneumonia*. The inhibition zone diameters were only 0.956 and 1.28mm respectively. In case of kiwi extract the inhibited bacteria was *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zone diameter 6.48 and 3.28mm respectively. Papaya extract showed antibacterial activity against the tested gram positive bacteria only (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia*), while it did not show any antibacterial against the gram negative one (*Staphylococcus saprophyticus , Enterococcus faecalis, Streptococcus agalactiae*).

Antimicrobial properties of extracts are related to its phenol compounds. The high phenol substances are in the extract, the high its antimicrobial activity is. The efficiency of phenols as antibacterial agent is due to their ability to inhibit bacterial enzymes through reactions with sulfhydryl groups in the proteins [18]. The phytochemical analysis of the peels revealed that secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that are known to have antimicrobial properties [19]. The antibacterial activity of tanins could be correlated to their ability to complex with bacterial proteins and polysaccharides through both covalent and non-covalent interactions [18]. The antimicrobial activity of *Olea* sp. growing in Albaha region in Saudi Arabia is related to its high content of tannins flavonoids, steroids, terpenoids, and coumarins [20]. Terpenoids inhibits microbial respiratory oxygen enzymes uptake and oxidative phosphorylation [19]. The results of the present study reveal a great role of alkaloids, phytate and saponins as antibacterial agents. The present results showed that the lowest antibacterial activity was in watermelon, kiwi, and papaya respectively (Table 2 and 3), interestingly the content of alkaloids, phytate and saponins in these peels extract were zero. This is correlated with other studies showing the effectiveness of alkaloids, phytate and saponins as antimicrobial agents [21-23]. Alkaloids are structurally different compounds, its antimicrobial activity is attributed to different mechanisms such as affecting bacterial cell division therough inhibiting nucleic acid synthesis [24]. Alkaloids also cause respiratory inhibition and enzyme inhibition in bacteria [25]. Another mechanism of the antibacterial activity of alkaloids is bacterial membrane disruption [26]. It is found also those alkaloids affecting virulence genes in bacteria [27]. Zhou et al. [22] reported the effectiveness of phytic acid as antibacterial agent due to its ability to damage the bacterial cell membrane. Phytic acid is considered as an effective bacterial membrane-permeabilizing agent [28]. Saponins are great substances play a critical role in decreasing the resistance of the bacterial stress to antibiotics. Saponins interact with the lipid in the bacterial membranes forming Lipid-saponin complexes and so increase the permeability of bacterial membranes. These complexes promote antibiotic uptake to inherently resistant bacteria cells [21].

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Table 1: Functional ingredients (Total alkaloid, total tannin, total saponin, total steroid, total glycosidic cyanide, total phytate, total phenolic and total flavonoid (µ/ g dry wt) in fruit peels extracts

Parameter	Alkaloids	Flavonoids	phenols	Steroids	Phytate	Saponins	Tannins	Glycosidic cyanide
Apple	8.89±0.892	400.89±0.782	530.19±0.102	18.37±0.098	22.19±0.891	20.89±0.675	16.02±0.192	9.061±0.098
Banana	16.28±0.672	450.89±0.0923	570.94±0.078	22.93±0.009	39.48±0.382	33.92±0.182	24.45±0.754	13.78±0.897
Guava	3.17±0.781	280.78±0.702	350.29±0.319	9.472±0.623	7.293±0.782	15.50±0.092	9.348±0.013	4.092±0.009
Kiwi	-	100.92±0.991	130.19±0.009	5.034±0.093	-	-	3.921±0.150	1.029±0.341
Lemon	10.89±0.669	410.28±0.885	550.32±0.279	19.00±0.002	28.00±0.673	22.45±0.992	21.71±0.453	9.435±0.614
Mango	4.28±0.021	320.91±0.939	370.28±0.724	11.02±0.182	19.28±0.009	17.56±0.467	12.89±0.554	5.293±0.09
Orange	11.89±0.562	420.98±0.028	550.78±0.428	19.38±0.003	28.39±0.932	23.89±0.733	22.01±0.109	10.53±0.745
Papaya	1.03±0.561	220.78±0.759	300.89±0.0782	6.294±0.519	-	10.78±0.672	5.938±0.097	2.102±0.0675
Pomegranate	12.93±0.882	440.29±0.892	560.39±0.990	21.02±0.378	30.29±0.094	30.89±0.892	23.91±0.341	11.02±0.0971
Watermelon	-	70.29±0.0821	120.83±0.038	1.257±0.316	-	-	1.102±0.111	0.782±0.003

Values are given as means of 3 replicates ± standard error.

Table 2: Inhibition zone diameter (mm) of the peels extract on the different tested bacteria

Bacterial Strain	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Streptococcus agalactiae*
Apple	17.85±0.724	20.33±0.783	18.28±0.462	25.38±0.782	16.29±0.719	3.02±0.567	11.78±0.563
Banana	19.48±0.492	27.37±0.993	22.38±0.921	29.37±0.672	30.18±0.563	11.28±0.782	29.31±0.738
Guava	16.93±0.563	18.37±0.271	7.92±0.462	7.59±0.772	3.90±0.562	N.A.	7.19±0.662
Kiwi	6.48±0.768	N.A.	3.28±0.456	N.A.	N.A.	N.A.	N.A.
Lemon	19.16±0.782	28.19±0.893	21.88±0.456	29.36±0.782	29.38±0.092	11.02±0.784	30.21±0.562
Mango	17.59±0.229	19.39±0.452	19.37±0.562	N.A.	8.27±0.193	4.01±0.116	9.29±0.672
Orange	19.53±0.564	27.78±0.566	23.27±0.562	29.07±0.489	31.29±0.432	12.93±0.674	29.67±0.882
Papaya	10.03±0.673	8.99±0.571	5.37±0.872	9.54±0.662	N.A.	N.A.	N.A.
Pomegranate	18.83±0.283	26.98±0.382	21.97±0.927	28.89±0..567	32.48±0.893	10.38±0.928	31.28±0.782
Watermelon	0.956±0.467	N.A.	N.A.	1.28±0.842	N.A.	N.A.	N.A.

Values are given as means of 3 replicates ± standard error.

Table 3: MIC (mg/ml) of the peels extract on the different tested bacteria

Bacterial Strain	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Streptococcus agalactiae*
Apple	5.05±0.039	7.92±0.893	5.49±0.573	13.78±0.267	32.89±0.673	78.89±0.782	3.89±0.673
Banana	2.35±0.489	1.89±0.094	1.56±0.892	1.78±0.648	1.56±0.572	3.28±0.834	1.37±0.267
Guava	9.81±0.382	20.27±0.568	37.28±0.784	49.56±0.552	73.67±0.842	N.A.	88.29±0.282
Kiwi	12.89±0.578	N.A.	93.0±0.726	N.A.	N.A.	N.A.	N.A.
Lemon	2.96±0.488	2.09±0.783	2.59±0.662	2.89±0.278	1.98±0.278	2.98±0.783	1.67±0.278
Mango	6.291±0.672	5.94±0.672	29.07±0.924	N.A.	59.28±0.652	90.27±0.782	55.49±0.392
Orange	1.93±0.092	1.67±0.289	1.28±0.094	1.93±0.921	2.09±0.828	3.01±0.772	1.32±0.842
Papaya	7.29±0.382	23.78±0.562	66.38±0.892	57.38±0.568	N.A.	N.A.	N.A.
Pomegranate	2.17±0.192	1.07±0.367	2.38±0.578	2.78±0.391	1.67±0.718	3.53±0.719	1.56±0.984
Watermelon	78.29±0.574	N.A.	N.A.	69.28±0.572	N.A.	N.A.	N.A.

Values are given as means of 3 replicates ± standard error.

CONCLUSION

The present study showed significant antimicrobial activity of banana, pomegranate, orange, lemon and apple against the tested microorganisms. This antibacterial activity is related to the high content of functional ingredients especially alkaloids, phytate and saponins. This antibacterial activity could lead to new options for the treatment of infectious diseases and emerging drug resistance.

Declarations Statements:

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article

Competing interests

We declare that we have no competing interests.

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Authors' contributions

The authors contributed equally in this work

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