The Antimicrobial Activity of Plant Extracts from *Punica granatum*, *Camellia*, and *Prosopis farcta* on Some Antibiotic Resistant Bacterial Species

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Abstract

Medicinal plants play important role in the development of therapeutic agents, for curing diseases. The study was conducted to evaluate the antibacterial activity of aqueous and ethanolic extracts of three medicinal plants; pomegranate (*Punica granatum*), black tea (*Camellia)* and Kharnoob (*Prosopis farcta*) against some antibiotic resistant isolates The agar-well diffusion method was used for the determination of the antibacterial activity of the extract at different concentration (25,50,100, and 200) mg/ml. The bacteria, isolated from infected wound and diarrheal stool, then identified using routine cultural, morphological, and biochemical testes, The Kirby-Bauer antibiotic susceptibility assay showed that the selected bacterial isolates demonstrated was high resistance to most common used antibiotics, and different multidrug resistance patterns had been seen. The results showed that aqueous and ethanolic extracts from the peels of *P. granatum* peels had strong activity against bacterial isolates from wound infections caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus,* and *Escherichia coli*. In addition, the aqueous and ethanolic extract of *P. granatum* pulp, and black tea leaves demonstrated high antibacterial activity against a number of bacterial isolates obtained from diarrheal stool

samples, including; *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. In contrast extract from *P. farcta* didn't show any antimicrobial activity. It can be concluded that the aqueous and ethanolic extracts of *P. granatum* (pulp and peel), and black tea had broad spectrum of antibacterial activity against different bacterial species, but ethanolic extracts were more active than aqueous extracts. Moreover, the action of these extracts increased with increasing their concentration. Hence these plant may be used in developing novel, and economic therapeutic agents for treatment of wounds, and gastrointestinal tract disorders causing bacterial strain. Further investigations on the antimicrobial activity of pomegranate, and black tea, against other pathogenic bacteria, are recommended.

Key words: Medicinal plants, Pomegranate, Black tea, Antibiotics, Bacteria.

الفعالية الضد ميكروبية للمستخلصات النباتية (الرمان و الشاي الاسود والخرنوب) على بعض انواع الجراثيم المقاومة للمضادات الحياتية

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الخلاصة

تلعب النباتات الطبية دوراً هاماً في تطوير الأدوية لعلاج الأمراض. أجريت الدراسة لتقييم الفعالية الضدمايكروبية للمستخلصات المائية والكحولية (إيثانول) لثلاثة نباتات طبية لقشرة ولب الرمان والشاي الاسود وثمرة الخرنوب ضد بعض انواع البكتيرية المعزولة من النماذج السريرية والمقاومة للمضادات الحياتية. وأستعملت طريقة الأنتشار بالحفر (agar-well diffusion method) للتراكيز (25، 50، 100، 200) ملغم/مل. تم الحصول على العزلات البكتيرية من خمجات الجروح وعينات البراز من المرضى المصابين بالإسهال، ثم شخصت هذه العزلات بإستخدام الطرق التقليدية الزرعية والمظهرية والكيموحيوية المعروفة لتشخيص البكتيريا. أظهر فحص (Kirby-Bauer) للحساسية تجاه المضادات الحياتية بأن هناك مقاومة عالية من قبل العزلات المختارة تجاه معظم المضادات الحياتية المعروفة الإستخدام. ولوحظ نماذج متعددة للعزلات المتعددة المقاومة. وبينت النتائج فعالية عالية للمستخلصات المائية والكحولية لقشور الرمان على البكتيريا المعزولة من خمجات الجروح

بينما كان **(***P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. saprophyticus*, and *E. coli* and *E. coli* **)** للمستخلصات المائية والكحولية للب الرمان والشاي الأسود تأثيراً على عالياً على البكتيريا (*E. coli*, *K. pneumoniae*, and *P. mirabilis*) المعزولة من عينات البراز للمرضى المصابين بالإسهال. بالمقارنة، لم يكن للمستخلص المائي والكحولي لثمرة الخرنوب أي تأثير ضد العزلات قيد الدراسة. نستنتج بأن المستخلص المائي والكحولي لقشرة ولب الرمان بالاضافة الى الشاي الأسود لهم تأثير ضد مايكروبي واسع المدى تجاه انواع مختلفة من البكتيريا، لكن فعالية المستخلص الكحولي أعلى من المستخلص المائي، إضافة الى ذلك فإن فعالية هذه المستخلصات تزداد بزيادة تراكيزها، عليه فإن هذه النباتات ممكن إستخدامها لإنتاج ادوية حديثة، أمنة وإقتصادية لعلاج إضطرابات الجهاز الهضمي والجروح الناتجة من الاصابات البكتيرية. يوصى بدراسة الفعالية الضدمايكروبية للرمان والشاي الأسود ضد أنواع أُخرى من الجراثيم المرضية.

الكلمات المفتاحية: النباتات الطبية، الرمان، الشاي الاسود، الجراثيم، للمضادات الحياتية.

Introduction

The undiscriminating use of antimicrobial therapy has led to an increase in microbial resistance to these drugs, therefore there is a demand to find out new, safe, and more effective antimicrobial agents with alternative modes of actions as accessories to the common antibiotics [1] Medicinal plants have important role in the development of potent therapeutic agents for curing infectious disease [2]. The most beneficial bioactive constituents of plants are steroid, terpenoids, carotenoid, flavonoids, alkaloid, tannins, and glycosides which are considered a valuable starting material for drug development. Hence consumption of specific doses of these components can reduces the risk of many infectious and noninfectious disorders. Aqueous and alcoholic extracts of *Punica granatum*, black tea had shown antimicrobial activity on various pathogenic bacteria [3, 4, 5].

The different part of *P. granatum*, including; flowers, fruits, fruit peels, seed, dried bark of steam, and root commonly used to treat various diseases. Some investigators reported the anti-fungal and antibacterial activity of (peels, seeds, juices, and whole fruits) extracts on seven gram positive, gram negative bacterial strains and five fungal strains [6, 7]. The activity of ethanolic extract from *P. granatum* pericarp against different strains of enterohemorrhagic *E. coli* O157:H7 [8] was reported. Furthermore, aqueous extract of *P. granatum* showed high

effect on *K. pneumoniae*, *S. aureus*, *Bacillus subtilis*, and *Bacillus megaterium*, with moderate activity against *P. aeruginosa* as reported by other researchers [6].

All the components of *P. granatum* peels were reported to have therapeutic properties, and their antibacterial effects *in vitro* had been investigated [9, 10]. There are a number of reports on clinical uses of tea (*Camellia sinensis*) against various diseases such as cancer, and diabetes with promising results [11]. Moreover, the antibacterial activity of black tea which is a major source of theaflavins, and thearubigins had been reported by many investigators [5, 12, 13, 14].

The present study aimed to isolate and identify some bacteria from infected wounds, and stool samples, then determine their antibiotic susceptibility pattern to some commonly used antibiotics, the antibacterial activity of *P. granatum* peels on some multidrug resistant bacteria isolated from wound infection, together with the inhibitory effect of *P. granatum* pulp, *Camellia*, and *P. farcta* fruits on Gram negative bacteria isolated from stool of patient with diarrhea have been invistigated.

Materials and methods

Sample collection, isolation, and identification of bacterial isolates

A total of 58 wound, and stool samples were collected from in and out patients suffering from wound and diarrhea attending Tikrit Hospital in Tikrit city during the period from December 2015 to April 2016. The wound samples were collected using disposable sterile swabs, whereas diarrheal stool samples were collected in sterile containers, then transferred promptly to the laboratories for culturing in Brain heart infusion broth, on blood agar, nutrient agar and MacConkey agar, then incubated at 37ºC for 24 hours. The grew colonies were further sub cultured on other selective media as cetrimide, and mannitol salt agar. The pure colonies were tested with routine microscopic and biochemical testes for identification of bacteria according to Bergey's manual of determinative bacteriology [15]. The cultures were maintained on nutrient agar medium slants. Inoculated slants were grown in an incubator at 37 ˚C for 24 hr. After that the slants were stored at 4˚C in a refrigerator for short term preservation and sub cultured every 15 days.

Antibiotic susceptibility test

Twenty-four bacterial isolates were selected randomly for determining their susceptibility to 14 antibiotics, using the disc diffusion (Kirby Bauer) method. The antimicrobial agents tested were Amikacin (AK; at 10 and 30mg), Ampicillin (Am;10mg), Amoxicillin (AMC; 30 mg), Cefotaxime (CTX; 30 mg), Ceftriaxone (CRO; 30mg), Chloramphenicol (C; 10mg), Ciprofloxacin (CIP; 30mg), Gentamycin (GM; 10 mg), Levofloxacin (LEV; 5 mg), and Trimethoprim/sulfamethoxazole (ME; 10 mg). The diameters of inhibition zones were measured and interpreted according to [16].

Preparation of plants' extracts

The plants extracts were prepared according to the method described previously [17, 18] with some modifications. The *P. granatum*, black tea and Kharnoob (*P. farcta)* were purchased from local markets in Tikrit city. The peel and pulp of *P. granatum*, black tea and *P. farcta* fruits were washed with distilled water, dried in dark, then grounded into fine powders using house hold electric blender. Later, 40g of peel and pulp of *P. granatum*, Black tea leaves and *P. farcta* fruits were weighed, then transferred separately to conical flask containing 160ml of cold distilled water, and 95% of ethanol for aqueous and alcoholic extract respectively, then allowed to soak at ambient temp. for 72 hours. The extracts were filtered using whatman No.1 filter papers. The filtrates were concentrated and dried using electric oven at 40 ºC for 24 hours. Later 1g of each aqueous extracts dissolved separately in 5ml of sterile distilled water, to obtain a standard solution, 200mg/ ml in concentration and sterilized by Millipore filter 0.2 µm pore size. While, for preparing standard alcoholic solution; 1g of ethanolic extract dissolved in 5ml of Dimethyl sulphoxide, then sterilized by pasteurization at 62 ºC for 10minute.From standard solution, other diluents; 25,50, and100 mg/ml were prepared.

Results and discussion

Out of 58 wound and diarrheal samples 43 bacteria were isolated. They were belonging to five genera (*Escherichia*, *Staphylococcus*, *Proteus*, *Pseudomonas*, and *Klebsiella*). Concerning the prevalence, *E. coli* demonstrated the highs rate (27.5%) followed by *S. epidermidis*, *S. aureus*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, and *S. saprophyticus*, were 13.7%, 8.6%, 8.4%, 5.1%, 5.1%, 5.1 % respectively. As revealed in Table 1 and 2, the Kirby-Bauer antibiotic susceptibility assay showed a high resistance of the 24 selected isolates to the most of the antibiotics used in the current study, in addition some different multi resistance (at least two classes of antibiotics) patterns were demonstrated. Earlier report by Santhanamari et al. [19] showed that 92 isolates of *S. aureus* and *P. aeruginosa* were resistant to almost all the ten frequently prescribed antibiotics except Vancomycin (for *S. aureus*). The overuse of antibiotics together with the length of time over which they have been used have caused the emergence of resistant organisms, leading to morbidity and mortality [20]. The extracts of pulp and peel of *P. granatum*, black tea (*Camellia)* and Kharnoob (*P. farcta)* were tested for their antibacterial activity against some multidrug resistance isolates of gram positive and gram negative bacteria isolated from clinical samples.

Table 1: The antibiotic susceptibility pattern of bacteria isolated from diarrheal stool.

Bacterial Name	AK 10 mg	AK 30 mg	AM 10 mg	AMC 30 mg	CTX 10 mg	CTX 30 mg	CRO 30 mg	C 10 mg	CIP 30 mg	GM 10 mg	LEV 5 mg	NOR 10 mg
P. aeruginosa-1	R	I	R	R	R	S	S	R	R	R	I	I
P. aeruginosa-2	I	S	R	R	R	R	R	R	R	R	R	R
P. aeruginosa-3	R	I	R	R	R	S	S	R	R	R	I	I
P. mirabilis-1	S	R	R	R	R	R	R	R	S	R	R	R
P. mirabilis-2	R	I	R	R	R	R	R	R	S	I	S	S
P. mirabilis-3	S	S	R	R	R	R	I	R	S	S	S	S
K. apneumoniae-1	R	R	R	R	R	R	R	R	R	R	R	R
K. pneumoniae-2	I	S	R	R	R	R	R	R	R	R	R	R
E. coli-1	R	R	R	R	R	R	R	R	I	R	I	I
E. coli-2	I	S	R	R	R	I	S	R	R	I	I	S
E. coli-3	I	S	R	R	R	I	S	R	R	I	I	S
E. coli-4	S	R	R	R	R	R	R	I	S	R	R	R
E. coli-5	S	S	R	S	S	I	R	S	S	S	R	S
E. coli-6	S	S	R	R	R	R	I	I	S	S	S	S

Table 2: The antibiotic susceptibility pattern of bacteria isolated from wound infections

Bacterial Name	AK 10 mg	AK 30 mg	AUG 10- 20 mg	AMC 30 mg	C 10 mg	CIP 30 mg	GM 10 mg	LEV 5 mg	ME 10 mg	NOR 10 mg
S. epidermides-1	R	I	R	R	R	R	R	R	R	R
S. epidermides-2	R	S	R	R	R	R	S	R	R	I
S. epidermides-3	I	R	R	R	R	R	S	S	R	S
S. epidermides-4	S	S	R	R	R	S	S	S	R	S
S. epidermides-5	R	S	R	R	R	S	S	S	R	R
S. saprophyticus	R	R	R	R	R	R	R	R	R	R
S. aureus-1	R	R	R	R	R	R	R	R	R	R
S. aureus-2	S	S	S	R	R	S	S	S	R	S
S. aureus-3	S	S	R	R	I	S	S	S	R	S
S. aureus-4	I	S	R	R	I	S	S	S	R	S

As shown in Table (3), the maximum antibacterial activity of aqueous extract of *P. granatum* at a concentration of 200mg/ml was detected against *P. aeruginosa*-1 and 2 with a diameter of inhibition zone (DIZ) of 23.5 and 17.5 mm. Regarding *S. aureus* the DIZ was 22 mm. Whereas DIZ of two isolates of *E. coli*-1 and *E. coli*-2 were 18, and 19.5 mm respectively while *S. saprophyticus* had DIZ of 18 mm. The DIZ of 17.5, and 13 mm were recorded for two isolates of *S. epidermides*-2, and *S. epidermides*-2 respectively. In contrast the concentration of 100 mg/ml of *P. granatum* peels aqueous extract had less inhibitory activity against the tested bacterial isolates. However, the maximum inhibitory activity was found against *E. coli*-1 with DIZ of 20 mm, followed by *S. aureus*, *P. aeruginosa*-1, *S. epidermides*-2, *S. epidermides*-1 with DIZ of 19, 15, 10, and 4.5 mm. respectively. In contrast the concentration of 25, and 50 mg/ml of aqueous extract of *P. granatum* had no inhibitory effect against any species of the tested bacteria. This finding may be attributed to the low concentration of active constituents in these extracts. Antibacterial properties of *P. granatum* pericarp (peels) extracts (hot aqueous, methanolic and ethanolic) were evaluated [10] against *E. coli*, *P. aeruginosa and S. aureus* using agar well diffusion method. Hot aqueous, methanolic and ethanolic extracts of *P. granatum* pericarp showed an average inhibitory zone diameter of 23.3, 22.3 and 24.5 mm. respectively. The major active substances (phytocompounds) in the pomegranate extracts

including phenols, tannins, and flavonoids which may be responsible for the antimicrobial activity [7].

Table 3: Effect of aqueous extract of *Punica grantaum* against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from wound infections

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
S. aureus	22	19	0	0
S. saprophyticus	18	0	0	0
S. epidermidis -1	13	4.5	0	0
P. aeruginosa-1	23.5	15	0	0
E. coli -1	18	20	0	0
P. aeruginosa-2	17.5	0	0	0
S. epidermidis -2	17.5	10	0	0
E. coli -2	19,5	0	0	0

Table (4) shows the results of inhibitory effect of ethanolic extract *P. granatum* peels against some gram positive, and gram-negative bacteria isolated from wound infection. The highest inhibitory activity of the above extracts at a concentration of 200 mg/ml was against *S. saprophyticus* with DIZ of 25 mm, followed by *E. coli*-1, *P. aeruginosa*-2, *S. epidermides*-1, *P. aeruginosa*-1, and *S. aureus* with DIZ of 23,21.5, 20, 19 and 16, mm respectively. While the concentration of 100 mg/ml of ethanolic extracts of *P. granatum* had maximum effect on *E. coli*-1 with DIZ of 20 mm, followed by *S. epidermides*-1, *S. aureus*, *S. saprophyticus*, *P. aeruginosa*-1, with DIZ of 15, 14, 13 and 11 mm respectively. However, the concentration of 50 mg/ml of the same extract inhibited *E. coli*-1 with DIZ of 6 mm. The antibacterial activity may be due to the presence of metabolic toxins or broad spectrum antibiotic compounds in the *P. granatum* peels which act against both gram positive and gram-negative bacteria. This is in accordance with previous report by Khan and Hanee [10] who showed antibacterial activity of alcoholic and aqueous extracts of *P. granatum* peels against *E. coli*, *P. aeruginosa*, and *S. aureus*. Moreover, the extracts of *P. granatum* peels were used for the treatment of respiratory diseases, and in the preparation of therapeutic formulae [21].

Table 4: Effect of ethanolic extract of *Punica grantaum* peels against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from wound infections.

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
E. coli-1	23	20	6	0
S. saprophyticus	25	13	0	0
P. aeruginosa-1	19	11	0	0
S. aureus	16	14	0	0
S. epidermides-1	20	15	0	0
P. aeruginosa-2	21.5	0	0	0

Table (5) reveals that the aqueous extract of *P. granatum* pulp at a concentration of 200 mg/ml inhibited the growth of two isolates of *Klebsiella* species (*K. pneumoniae*-1 and *K. pneumoniae*-2) with DIZ of 24.5, and 18 mm respectively. Also affected two isolates of *Proteus* species (*P. mirabilis*-1, and *P. mirabilis*-2) with DIZ of 20.5, and 14 mm respectively. Whereas two isolates of *E. coli* (*E. coli*-4, and *E. coli*-5) were inhibited with DIZ of 16.5, and 14.5, mm respectively. The results of earlier study showed that the water extract of pomegranate pulp (fresh and dried) showed maximum zone of inhibition against *S. aureus* and *E. coli* [4].

Also, another study has proved that pomegranate possess different therapeutic properties and has been used in traditional medicine to treat diarrhea and intestinal parasites [22].

Table 5: Effect of aqueous extract of *Punica grantaum* pulp against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from diarrheal stool

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
E. coli-4	16.5	0	0	0
P. mirabilis-1	20.5	17.5	0	0
K. Pneumonia-1	24.5	21.5	0	0
P. mirabilis-2	14	0	0	0
E. coli-5	14.5	0	0	0
K. pneumonia-2	18	14,5	0	0

Table (6) reveales the results of inhibitory activity of ethanolic extract of *P. granatum* pulp against some isolates of gram negative bacteria. The maximum effect of this extract at a concentration of 200 mg/ml was found against *K. pneumonia*-1 as the DIZ was 26.5 mm,

followed by *P. mirabilis*-1, *E. coli*-4, and *P. mirabilis*-2, in which DIZ were 25, 24.5, 16 mm respectively. While the concentration of 100 mg/ml of the same extract had lower inhibitory effect against the selected isolates, since the higher effect exist against isolate of *K. pneumoniae*-1, *E. coli*-4, and *P. mirabilis*-1. The DIZ were 22.5, 21.5, and 17.5 mm respectively. In contrast, the concentration of 50 mg/ml the extract inhibited only one isolate of *K. pneumonia*-1 with DIZ of 11.5mm. However, the extract at a concentration of 25 mg/ml didn't show any antibacterial activity.

Table 6: Effect of ethanolic extract of *Punica grantaum* pulp against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from diarrheal stools.

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
K. pneumoniae-1	26.5	22.5	11.5	0
P. mirabils-1	25	17.5	0	0
E. coli-4	24.5	21.5	0	0
P. mirabilis-2	16	0	0	0

These results showed that alcoholic extracts of *P. granatum* peel, and pulp were more effective against bacterial isolates than their counterpart of aqueous extracts. This finding was in agreement with Barzani et al. [3] who reported an antibacterial activity (*in vitro and in vivo*) of aqueous and alcoholic extracts of *P. granatum* peels against some burn infections bacteria. As well as Dahha et al. [7] concluded that the broad spectrum of antimicrobial activity of methanolic extract of *P. granatum* against both bacteria and fungi was clearer than aqueous extract. In addition Ahmed and Beg [23] reported that alcoholic extracts of *P. granatum* fruit showed antibacterial activity towords *S. aureus*, *E. coli*, and *Sh. dysenteriae*. The inhibitory activity of aqueous and alcoholic extract of *P. granatum* on the growth of gram positive and gram negative bacteria might be due to the presence of one or more of chemical components in this plant as tannin, which had effect on the nature of protein in bacteria. The effects of tannin was concluded on cells which deduced by the involvement of metabolic enzymes and functional proteins on the tannin–protein interaction, which might be related to the altered functions of the cell metabolism [24]

Table (7) illustrates the effect of aqueous extract of black tea on the growth of Gram negative bacteria which belong to the family Enterobacteriaceae. The highest inhibitory effect of above extract at a concentration of 200 mg/ml was against *E.coli* -5, with the DIZ of 16.5 mm. followed by isolates *P. mirabilis*-2 and *K. pneumonia*-1 with a DIZ of 14.5, and 13.5 mm. respectively. Whereas the lower inhibitory effect of the extract was against other isolates; *E. coli*-4, and *K. pneumonia*-2, with DIZ of 9 and 8 mm respectively. Concerning the concentration of 100 mg/ml of aqueous extract of black tea, it affect the growth of only *K. pneumonia*-1, and the DIZ was 14 mm. However, the concentration of 50, and 25 mg/ml of the same extract had no any inhibitory effect against the tested bacterial isolates.

Table 7: Effect of aqueous extract of black tea against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from diarrheal stools

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
P. mirabilis-2	14.5	0	0	0
P. mirabilis-1	0	0	0	0
E. coli-4	9	0	0	0
E. coli-6	0	0	0	0
K. pneumoniae-1	13.5	14	0	0
E. coli-5	16.5	0	0	0
K. pneumoniae -2	8	0	0	0

In regard to ethanolic extract of black tea, Table (8) revealed thae the concentration of 200 mg/ml had a maximum inhibitory effect against *K. pneumonia*-1 and the DIZ was 25 mm. moreover, the minimum inhibitory activity of the same extract was against *P. mirabilis*-1, and *P. mirabilis*-2 with DIZ of 15 mm. while the concentration of 100 mg/ml of the ethanolic extract of black tea produced DIZ of 10.5, and 16 mm against *P. mirabilis*-1 and *K. pneumonia*-1, respectively. However, 50 and 25 mg/ml of the above extract didn't express any effect against the bacterial isolates under investigation.

Table 8: Effect of ethanolic extract of black tea against bacterial isolates (expressed as the diameter of the inhibition zone in millimeter) from Diarrheal stool

Bacterial species	200mg/ml	100mg/ml	50mg/ml	25mg/ml
P.mirabilis-2	15	0	0	0
E. coli-5	16	0	0	0
K. pneumonia-1	25	16	0	0
P.mirabilis-1	15	10.5	0	0

The finding of the present study indicates that ethanolic extract had better inhibitory activity on the growth of bacteria than aqueous extract of the tea. This might be due to the ability of alcohol to dissolve the active components in the tea, whereas water not able to do so. The ethanolic and methanolic extracts of different tea samples showed significant antibacterial activity against *Staphylococcus, Streptococcus, Bacillus*, and *E. coli* isolates from soil samples of various locations [5]. Furthermore, the results of the current investigation revealed that the antibacterial efficacy of black tea extracts was depending on the bacterial species and this finding is in agreement with the finding of Taguri et al. [25] who concluded that the antimicrobial potency of poly phenols contents in the tea depends on tested bacterial species. Also, the antimicrobial effect of tea may be contributed to other bioactive compounds as alkaloids, that interfere with division of cells, while the flavonoid possess antiglycosyle activity that can inhibit adherence of glycosyle transferase (GFT) activity and bacterial growth by their strong iron-binding capacity. Moreover, the role of catechins which damage bacterial cell membrane [26]. In contrast *P. farcta* didn't showed any antimicrobial activity. Its' effect limited to change the stool consistency, since it has high capacity of attracting water molecules from feces and this may be attributed to lignin content in this plant [27]. It is concluded that the extracts of pulp and peel of *P. granatum*, and black tea had broad spectrum of antibacterial activity against different bacterial species including multidrug resistant isolates of gram positive and gram negative bacteria isolated from clinical samples. However, ethanolic extracts were more active than aqueous extracts and the action of these extracts increased with increasing their concentration. Hence, these plants may be used in developing novel, safe, and economic therapeutic agents for the treatment of wound infection and gastroenteritis caused by bacterial

strains. Further investigation on the antimicrobial activity of pomegranate, and black tea, against other pathogenic bacteria, are recommended.

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