Antimicrobial activity of garlic and Pomegranate Peel extracts against some pathogenic bacteria

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Abstract. The extracts of garlic and pomegranate peel were used against *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, E. coli, Klebsiella pneumoniae, Acinetobacter baumannii*.

The aqueous extracts of garlic and pomegranate peel were prepared and tested in vitro for evaluation antibacterial activity by agar well diffusion method. The antibiotics (imipenem, augmentin, ceftriaxone, levofloxacin, amikacin, gentamycin, trimethoprim– sulfamethoxazole, clindamycin, piperacillin) susceptibility were examined against tested bacteria by Kirby-Bauer disc diffusion method.

The results revealed the inhibitory effect of extracts of garlic and pomegranate peel against the isolated bacteria. The inhibition zones caused by the garlic extracts were much higher than pomegranate peel that indicated the potency of the active components in garlic. The antibiotics activity test exhibited these bacteria were susceptible to antibiotics imipenem and resistant to augmentin and ceftriaxone. Whereas, the *Klebsiella pneumoniae* was more resistant to antibiotics than other tested bacteria. The present study indicated that the plant extract has very important role for prevention pathogenic bacteria and can be used for control causative agent.

Keywords: Garlic, Pomegranate Peel, Extracts, Bacteria

Introduction

One of the major reasons of death across the world is the infectious diseases triggered by pathogenic bacteria, and this problem is getting more serious with the emerging of bacterial strain that possess the ability to resist antibiotics (Wilson et al., 2002). The problem of antibiotic

resistance is increasing around the globe, which necessitate searching for alternatives for the classical antibiotics to overcome this problem (Kuok et al., 2017). Many methods had been investigated to overcome this problem such as: photodynamic therapy, antimicrobial peptide, quorum sensing as well as the plant extracts of plants that has a medical or pharmaceutical properties which can provide a trust worthy alternative for classical antibiotics (Megha, 2014).

It worth mention that traditional or folk medicine which depend on plants parts and extracts that are known to have a medical or pharmaceutical potential was used by about 80% of the population across the world, the modern science is traying to further investigated the abilities of these plants and how to invest them (Nascimento et al., 2000). For this study the antimicrobial activity of two plants were studied, garlic and pomegranate peel extract respectively. Garlic (*Allium sativum*) is a well-known plant used to treat variety of infections, it was used by ancient Egyptians, Greek and Chinese civilizations in treating diarrhea and intestinal disease (Gebreyohannes and Gebreyohannes, 2013). Garlic has a great benefit to human due to its contents since it contains allicin (which is responsible for the garlic odor, vitamins A and C, Zn, Mn, Se and Ge (Tyagi et al., 2013) and many more components as illustrated in Table 1.

Table1: Phytochemical compounds content in garlic

Content of Garlic	References
Cysteine, Cysteine sulfoxides, Glutathione, Glucosinolates and Bioactive compounds (Alliin, Ajoenes, Allyl sulfides and 1,2-vinyldithiin)	Martins et al., 2016
Protein, Crude fiber, Volatile oil, Carbohydrate, Vitamin C, Selenium, Zinc, Alliin and glutamyl-(S)-allyl-L-cysteine	Mariam et al., 2016
Amino acids, Manganese, Potassium, Calcium, Phosphorus, Magnesium, Sselenium. Sodium, Iron, Zinc, Copper, some Vitamin, Allyl disulfide, Allyl trisulfide, Alliin and Ajoene	Mardomi, 2017

Pomegranate (*punica granatum*) is widely used in folk medicine, it contains flavonoids which consist 0.2-1% of their fruit. Pomegranate was used from ancient times to treat cough, sore throat, urinary tract diseases as well as heart disease and modern medicine suggested that it can be used to treat cancer as well (Kumar et al., 2012). The compounds content of pomegranate peel are illustrated in Table 2.

Table 2: Phytochemical compounds content in pomegranate peel

Content of Pomegranate Peel	References
Carbohydrates, Glycosides, Proteins, Reducing sugars, Amino acids, Tannins, Phenolic compounds, Sterols, Alkaloids, Saponins and Flavonoids	Farag et al., 2014
Gallic acid, Punicalin, Caffeic acid, Punicalagin, Ellagitannins, Pelletierine alkaloids, Ellagic acid, Luteolin, Quercetin and Kaempferol	Sreekumar et al., 2014
Protein, Glucose, Fructose, Fiber, Tannins, Flavonoids, other Phenolic Compounds, Ellagic acid derivatives, Punicalagin, Gallic acid, Chlorogenic acid and Coumaric acid	Spilmont et al., 2015
Phenolic compounds (like Gallic acid, Punicalagins, Epigallocatechin galate, Catechin, Qquercetin, Rutin, Anthocyanidins and other Flavonoids) dietary Fibers; Neutral sugars (Aravinose, Xylose) Protein, Fat, Carbohydrate, Phenolic - Bioactive compounds (Vanillic acid, *p*-Coumaric acid, Caffeic acid, Gallic acid, Ferulic acid, and Syringic acid)	Charalampia and Koutelidakis, 2017
Protein, Crude fiber, Fat, Total phenol content, Antioxidant, Carbohydrate, Calcium, Potassium, Sodium, Phosphorous, Iron, Zinc, Manganese and Copper	Ranjitha et al., 2018
Alkaloids, Phenolic compounds, Flavonoids, Glycosides, Saponins, Protein, Carbohydrates, Sodium, Calcium, Magnesium, Potassium, Phosphorus, Iron, Zinc, and vitamin C	Sharma et al., 2018

In order to further investigate the effectiveness of these plants extracts against disease causing agent they were tested against a group of G-ve and G+ve bacteria isolated from clinical samples. The first G-ve bacteria in this study was *Pseudomonas aeruginosa* which is responsible for many pulmonary infections and directly involved with hospital related infections (Alhazmi, 2015). Another G-ve bacteria is *Proteus mirabilis* which can cause a several infections but the most known infection related to this bacterium was catheterized urinary tract infection (Armbruster et al., 2018). Also, *Klebsella penumoniae* and *Acinetobactor baumannii* are both G-ve bacteria which greatly involved with hospital tools and equipment infections, *K. penumoniae* represent an opportunistic, greatly invasive bacteria that can cause diseases that led to death, such as pyogenic liver abscess, necrotizing fasciitis and sever pneumonia. It also worth mention that *A. baumannii* show a multi drug resistance (Li et al., 2014; Lee et al., 2017). The last representative of G-ve bacteria in this study was *E. coli* which is a normal flora of the human intestine but some strains of this bacteria can be pathogenic and cause diarrhea (Makvara and Krilov, 2015). The G+ve bacteria representative in this study was *Staphylococcus aureus* which

can present in the environment as commensal form or as pathogen to human, this bacterium can cause endocarditis, osteoarticular, skin and pulmonary infections respectively (Tong et al., 2015).

Many studies had been carried out to study the antimicrobial activity of varieties of plants extracts against different types of bacteria with different degree of success [Nascimento et al., 2000; Mostafa et al., 2018; Duško et al., 2006; Ushimaru et al., 2007]. This study was carried out to investigate the antimicrobial activity of the garlic and the pomegranate peel extract against a group of pathogenic bacteria isolated from clinical samples in comparison with some known synthetic antibiotics.

Materials and methods

Preparation of plants extracts

Fresh garlic and mature pomegranate fruits were collected from a local market in Hillah city. The garlic was peeled and meshed in a blender at ratio 1:5 with distilled water (20%). The peels of pomegranate were manually separated from with the seeds and air dried at room temperature. Then, dried peels were cut to small piece and crushed by electrical grinder to obtained fine powder for preparation of the aqueous extract. The powder was mixed at ratio 1:5 with distilled water (20%). The aqueous suspensions were tested against different pathogenic bacteria isolated from clinical samples .

Pathogenic Bacteria

Both Gram negative (G-ve) bacteria (*Pseudomonas aeruginosa*, *Proteus mirabilis, E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*) and Gram positive (G+ve) bacteria (*Staphylococcus aureus*) were isolation from clinical samples (sputum, nasal swab, blood and urine) and identify in Al- Jemhoury Hospital Central laboratory at Babylon Province. It worth mention that reason for the uneven number of G+ve and G-ve bacteria depend completely on the bacteria presented in the samples.

Antimicrobial Activity of Plant Extracts

The antibacterial activity of the plant extracts was determined by using the agar-well diffusion method (NCCLS, 1993). Muller Hinton agar medium was prepared and poured in the Petri dishes, when the agar is solidified the medium was inoculated and swabbed with bacterial suspension of approximately 10^6 CFU/mL by using a sterile cotton swab. The wells were

prepared by punching with a 6 mm diameter standard sterile cork borer made up of stainless steel and the wells were filled up with 50 µL of plants extract. The plates were incubated at 35°C for 20 – 24 h. The antimicrobial activity was examined by measuring the diameter of the inhibition zone. All the tests were performed in triplicate and then average was taken.

Determination of Antibiotics Activity

In order to estimate the antibiotics susceptibility on tested bacteria, the method of Kirby-Bauer disc diffusion was conducted (CLSI, 2018). The bacterial inoculum (approximately 10^6 CFU/mL) was uniformly spread by using sterile cotton swab on a sterile Petri dish Muller Hinton agar. The antibiotic disks as standard were placed by sterile forceps and gradually busied to contact with the agar. The examined include the following antibiotics: imipenem (concentration 10 µg/disc), Augmentin (concentration 30 µg/disc), ceftriaxone (concentration 30 µg/disc), levofloxacin (concentration 30 µg/disc), amikacin (concentration 30 µg/disc), gentamycin (concentration 10 µg/disc), trimethoprim-sulfamethoxazole (concentration 30 µg/disc), clindamycin (concentration 30 µg/disc and uses only against G+ve bacteria), piperacillin (concentration 30 µg/disc and uses only against G-ve bacteria). Each test was performed in triplicate and then averaged. The plates were incubated for 20 hours at 35℃. The antibiotics activity was determined according to the diameter of the obviously visible of inhibition zones around antibiotics and plants extracts discs respectively. A diameter average of inhibition zone was measured in millimeters for three replicates.

Results and Discussion

Throughout the world infectious diseases is the main cause for death and this is getting worse by the emerging of bacteria strains that had an antibiotic resistance. In order to overcome this problem, the new trends in microbiology is directed toward finding a natural, cheap and available alternatives for the classical antibiotics, which can be achieved by using plant extracts.
The results of this study show that the plants extracts have a different effect for each bacterium as shown in Tables 3 and 4 respectively, and we well explain the effects of antibiotics and the used plants extracts on each bacterium separately.

For *Pseudomonas aeruginosa* the inhibition zone was in the following sequence: the highest inhibition zone was (25 mm) by the imipenem, (20 mm) by both levofloxacin and amikacin, followed by garlic extract with (18 mm) inhibition zone diameter and the lowest was

(13 mm) caused by pomegranate peel extract. While the bacterium was resistant to all of gentamycin, trimethoprim-sulfamethoxazole, piperacillin, augmentin and ceftriaxone with no inhibition zone. The garlic extract shows a good ability in controlling the growth of *P. aeruginosa*, the results of this study agree with the finding of (Ali et al., 2011) which found that garlic had a good antimicrobial against *P. aeruginosa*. Contrarily the pomegranate peel extract exhibits a poor activity in controlling the growth of this bacterium, this disagree with results of (Nozohour et al., 2018) which found that pomegranate extract was highly effective against *P. aeruginosa*.

The results for the *Proteus mirabilis* show that the inhibition zone diameter was in the following order: the largest inhibition zone was (25 mm) with imipenem, (23 mm) with garlic extract, (22 mm) with levofloxacin, (18 mm) with amikacin and the lowest was (10 mm) with pomegranate peel extract. This bacterium was resistant for gentamycin, trimethoprim-sulfamethoxazole, piperacillin, augmentin and ceftriaxone. The garlic extract was highly effective in suppress the growth of this bacterium and that go along with findings of (Alyasari et al., 2018) which found that the aqueous garlic extract was effective in inhibiting the growth of *P. mirabilis* with an inhibition zone diameter of (28 mm). Whilst the pomegranate peel extract show a moderate ability in overcoming the growth of this bacterium and that agree with results of (Zain alabidin and Ahmed, 2015) who found that different strains of *P. mirabilis* show different sensitivity to the pomegranate extracts and also the effect of the extracts was determine by its concentration, different extracts preparation show different inhibition ability.

The growth of *Staphylococcus aureus* was affected variously by the antibiotic and plants extracts. The largest inhibition zone in diameter was (23 mm) by garlic extract, and imipenem and clindamycin with (20 mm) for each of them and the smallest inhibition zone in diameter was (7 mm) with pomegranate peel extract. This bacterium was resistant to levofloxacin, amikacin, gentamycin, trimethoprim-sulfamethoxazole, piperacillin, augmentin and ceftriaxone respectively. Garlic extract show very good ability to overcome the growth of *S. aureus*, this agrees with findings of (Daka, 2011) which stated that garlic extracts can have both bacteriostatic and bactericide effect on *S. aureus*. The pomegranate peel extract had a low ability in reducing the growth of this bacterium. Although; that (Nozohour et al., 2018) had demonstrated that pomegranate extract had an antibacterial effect on *S. aureus*.

Whereas the effect on the growth of *E. coli* was as follow: imipenem caused the highest inhibition zone with (25 mm) in diameter, the garlic extract cause (13 mm) inhibition zone, pomegranate with (4 mm) and the smallest inhibition zone was caused by gentamycin with (2 mm). *E. coli* was resistant to levofloxacin, amikacin, trimethoprim-sulfamethoxazole, piperacillin, augmentin and ceftriaxone respectively. The garlic was moderately effective against this bacterium, the garlic extracts can have both deterrent restrainer to the growth of resident bacteria (Hossein et al., 2014). the pomegranate had a low ability to overcome the growth of *E. coli* and it was much lower than the result reported by (Hassan et al., 2018) which find the inhibition zone was (15 mm) in diameter.

The growth of *Klebsiella pneumoniae* was affected in the subsequent order: (26 mm) by trimethoprim-sulfamethoxazole, (23 mm) by levofloxacin, (22 mm) by garlic extract, (20 mm)by both amikacin and imipenem, (10 mm) by gentamycin, and the lowest was (9 mm) by the pomegranate peel extracts in the diameter of the inhibition zone respectively . this bacterium was resistant to piperacillin, augmentin and ceftriaxone. The garlic extract shows a good ability in reducing the growth of *K. pneumoniae*, this agree with the findings of (Khalegi et al., 2017) which find that garlic extract has a good antibacterial potential. whilst the bacterium was less sensitive to the pomegranate peel extract.

The last bacteria used in this study was *Acinetobacter baumannii*, the effect of the plants extracts and the antibiotics on this bacterium was as follow: the largest inhibition zone in diameter was recorded with (26 mm) caused by imipenem, (23 mm) by levofloxacin, (22 mm)by garlic extracts, (21 mm) by amikacin and the smallest inhibition zone was (12 mm) by the pomegranate peel extracts. This bacterium was resistant to gentamycin, trimethoprim-sulfamethoxazole, piperacillin, augmentin and ceftriaxone respectively. The garlic extract was highly against this bacterium and that agree with the findings of (Jazani et al., 2007) which found that *A. baumannii* sensitive to a low concentration garlic extract. Though, the pomegranate peel extract was moderately effective against this bacterium.

At the end some important notes most be highlighted here, generally throughout the work the garlic extract shows good antibacterial activity against all isolated bacteria in this study. And the effectiveness of the garlic could be attributed to the presence of allicin which act by inhibiting synthesis of RNA and partial inhibition of protein and DNA syntheses (Feldberg et al., 1988).

The pomegranate peel was less effective than past studies and that can be explain by the fact that the effectiveness of the extract depend on the species of the plant used and the type of the solvent used in the preparation of the extract. And that can be explain by the fact that most of the past study used the alcoholic extracts of the pomegranate rather than the aqueous extract and since the water can only extract tannins, while alcohol can extract tannins, alkaloids, polyphenols and flavonoids so the aqueous extracts is generally less effective than alcoholic extract(Al-Humndu and Farj, 2010). The reason for using the aqueous extracts rather than using the alcoholic extracts in this study is that we want to test the effectiveness of these plant against different pathogenic bacteria by an extract than can be prepared and used by non-scientific (ordinary) persons at home.

The results of this study suggested that both garlic and pomegranate extracts can play an important role in controlling many pathogenic bacteria in the future. Some antibiotics such as imipenem may have a good ability to control the growth of theses pathogenic bacteria even so more these antibiotics been more effective than the tested extracts but on the other hand these antibiotics have a more serious side effects on human health. In general, a very promising antibacterial agent can be derived from plants that can play very important role in controlling the growth of many multi-drug resistant bacteria in the near future.

Table 3. Diameter of zone of inhibition (mm) of Garlic and Pomegranate Peel extracted against pathogenic bacteria

Bacteria isolate	**Inhibition Zone**	
	Garlic	**Pomegranate Peel**
Pseudomonas aeruginosa	18 mm	3 mm
Proteus mirabilis	23 mm	10 mm
Staphylococcus aureus	23 mm	7 mm
E. coli	13 mm	4 mm
Klebsiella pneumoniae	18 mm	9 mm
Acinetobacter baumannii	22 mm	12 mm

Table 4. Antibiotics Activity of antibiotic against pathogenic bacteria (inhibition zone in mm)

Bacteria Isolate	**LEV**	**AK**	**GN**	**SXT**	**CD**	**PRL**	**IMP**	**AMC**	**CRO**
Pseudomonas aeruginosa (G-ve)	20	20	0	0	-	0	25	0	0
Proteus mirabilis (G-ve)	22	18	0	0	-	0	25	0	0
Staphylococcus aureus (G+ve)	0	0	0	0	20	-	20	0	0
E. coli (G-ve)	0	0	2	0	-	0	25	0	0
Klebsiella pneumoniae (G-ve)	23	20	10	26	-	0	20	0	0
Acinetobacter baumannii (G-ve)	23	21	0	0	-	0	26	0	0

LEV: Levofloxacin, AK: Amikacin, GN: Gentamycin, SXT: Trimethoprim-Sulfamethoxazole, CD: Clindamycin (against G+ve), PRL: Piperacillin (against G-ve) IPM: Imipenem, AMC: Augmentin, CRO: Ceftriaxone

Conclusion

According to the result, the garlic and peel extracts have prospective as antimicrobial against pathogenic bacteria. Hence, they can be used in the control of infections caused by some types of antibiotic resistant bacteria. However, the garlic extract shows a more effective antibacterial activity than the pomegranate peel extract. Data obviously exhibit the importance of plant extracts in treatment of resistant bacteria, which are becoming a threat to human health. Therefore, the garlic available in meal is very important to the health of human. The determination of the nature substances as the antimicrobial active could be helpful for the future investigations leading the development of new antibacterial agents from plant base rather than synthetic antibiotics.

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