Studying the inhibition effect of some food additives against pathogenic bacteria

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Abstract

Latterly, natural products have been used as antibiotics and have proven effective against a large number of microorganisms. The present study included the measurement of antibacterial activities of pomegranate juice or molasses, tamarind molasses, garlic oil with thyme and with chili pepper on the five selected bacteria (2 Gr+ and 3 Gr-) by disk and agar well diffusion assays on the Muller Hinton Agar (MHA) and Blood Agar (BA). Pomegranate and tamarind molasses exhibited a broad spectrum of anti-bacterial activity inhibiting both the groups of bacteria. Pomegranate and tamarind molasses has shown highest antimicrobial activity compared to garlic oil with herbs. Inter-selected of bacterial cultures, the highest antibacterial effect of pomegranate and tamarind molasses by using disk diffusion was recorded against E. coli, Staphylococcus aureus, Streptococcus mutans and Pseudomonas aeruginosa (20,18,20,12 mm) (20,20,20,17 mm) respectively while, when used agar well diffusion assay to tested antibacterial activities of pomegranate and tamarind molasses the result show rather increased inhibition zone of selected bacteria but, bacterial culture of Klepsiella pneumonia recorded resistance against food additives which can used in this study.

Key words: Food additives, Pomegranate molasses, Tamarind molasses, Garlic oil, Antibacterial activity.

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1. Introduction

Medicinal plants contribute at the present time in the treatment of many diseases, where the scientific interest and the increasing demand for these products contributed to encouraging the development of these products, as recent studies have shown that herbal medicines have a prominent role in the field of health and medicine (Gragg and newman, 2001). Medicinal plant products can be used to treat many difficult diseases, with the aim of combining the practices of modern medicine with folk medicine, which can treat many medical problems such as resistance to many pathogens to antibiotics and others (Afolayan, 2003; Olavi Pelkonen et al, 2014). Food additives can be used as anti-microbial preservatives, which inhibit the growth of bacteria and fungi; the food industry has been using a variety of non-antibiotic based antimicrobials, including additives and disinfectants for controlling food borne spoilage and pathogenic microorganisms. Food additives includes preservatives, sweeteners, color additives, spices and flavors, flavor enhancers, emulsifiers, nutrient supplements, texturing agents, acidulants and enzymes. The excessive and inappropriate use of antibiotics in medicine and agriculture has led to the emergence of antimicrobial resistant bacteria. (Meera et al, 2017). Pomegranate (Punicagranatum L.) is consumed both as fresh fruit and fruit juice. It is also being used in the production of jam, wine, liqueur, food coloring agent and flavor enhancer. The kernels are also used as a garnish for desserts and salads (Al-Maiman and Ahmad, 2002). The pomegranate molasses is commonly used in salads and many dishes in Asian countries (Altan and Maskan, 2005; Maskan, 2006). Pomegranate is also a rich source of anthocyanins, elagitanins and other phenolic compounds that have been shown to have antioxidant and anti-tumor activity (Big et al, 2010). There is a range of phytochemical compounds in pomegranate that have showed antimicrobial activity, but most of the researchers have found...
that ellagic acid and larger hydrolyzable tannins, such as punicalagin, have the most important activities. In many cases, the mixture of the pomegranate constituents offers the most advantage (Howell and Souza, 2013). Pomegranate fruits have also been used in traditional medicine to treat dysentery, diarrhea and respiratory diseases (Dey and Hazra, 2015). Recent study indicates that both pomegranate aril and peel extracts have an effective antimicrobial activity, as evidenced by the inhibitory effect on the bacterial growth of two important human pathogens, including \textit{Staphylococcus aureus} and \textit{Escherichia coli}, often involved in foodborne illness (Pagliarulo et al, 2016). In addition, experimental data strongly support the antibacterial activity of pomegranate extracts against oral pathogen such as \textit{S. mutans} (Sabramaniam et al, 2012). Tamarind fruits are used in Indian spices as a tension agent to provide the acidity required in different meals and are widely used in foods around the world. It has been used for centuries as a medicinal plant, its fruits are the most important medicinal part (Gupta et al, 2014). Tamarind fruits have a proven hepato- protection activity associated with the presence of polyhydroxyl compounds, (El-Siddig et al, 2004; Meléndez and Carriles, 2006). Cavalletto and his co-workers reported in 1994 that allicin, the main antimicrobial compound in garlic, rapidly degrades into dialysis sulfide, and that garlic oil and dialysis sulfide (DADS) is the most abundant amount of sulfide in aqueous garlic extracts that have no antimicrobial effect. These early negative results, few studies of the post-antimicrobial activity of garlic oil and component sulfides, and some vegetable sulfur compounds, including DMTS and allele isothiocyanate (AITC), are more effective in inhibiting yeast growth than bacterial growth (Kyung and Fleming, 1997).

2. Materials and Methods
2.1. Isolates and Cultivation
The bacteriology and food microbiology laboratory in Dept. of biology-Science College-Kirkuk University/ Iraq provided “5” selected bacteria (2 Gram+ and 3 Gram-) (these types of bacteria were taken from urinary tract, food poisoning samples, teeth infection, wounds, in Kirkuk Hospital). Bacterial isolates were identified by biochemical diagnostic depending on the method followed by (Collee et al, 1996). Bacteria \textit{E.coli} and \textit{Klepsiella pneumonia} were cultured and growth in Macconkey Agar, \textit{Staphylococcus aureus} in Manitol Salt Agar (MSA), \textit{Streptococcus mutans} in Blood Agar, and \textit{Pseudomonas aeruginosa} in Nutrient Agar (NA) and incubation at 37C for 24 hr.

2.2. Preparation of Food Additives
Pomegranate molasses was prepared traditionally by handpicking, washing, and peeling and the arils with seeds were hand-crushed and then squeezed in order to obtain the juice. The juice was concentrated to 30-35% by heated for 4-5 hours until to be thickly then leave cooled. We used the similar methods to obtain Tamarind molasses without seeds. Garlic oil with herbs purchased from commercial market of the Kirkuk city, Iraq.

2.3. Determination of Antibacterial Activity
The antimicrobial activity of the food additives which used in this study was measured by using agar disk and well diffusion assay in five pathogenic bacteria, two Gr+ bacteria (\textit{Staphylococcus aureus} and \textit{Streptococcus mutans}) and three Gr- bacteria (\textit{Pseudomonas aeruginosa}, \textit{E.coli} and \textit{Klepsiella pneumonia}). According the pour plate method, (1 ml) of each bacterial culture (18-24 hr) was taken to the center of sterile Petri dish and then poured (20 ml) of (MHA) in each plates, which contained bacterial inoculums and mixed well, after the solidification of medium four wells of each plate were made by using cork borer (6mm). Afterward, distribute (20 µl) of food additives (Pomegranate, Tamarind molasses, Garlic oil with thyme and Garlic oil with chili pepper) into each well on the plates and allow it to diffuse for 15-20 minutes at lab temperature. The other assay of testing are disk diffusion method. In this method, standardized inoculums of all each bacteria speared on the surface of culture medium using L-shape then, placed filter paper discs (6 mm), containing the antibacterial agents as requested concentration and incubation under suitable conditions (Mounyr et al, 2016). Commonly, the mechanism of antimicrobial agents is diffuses into the agar medium and inhibits growth of microorganism by measuring the diameters of inhibition zones as shown in (Fig. 1). Table 1 and 2.
3. Result and Discussion

Antibacterial activity of food additives against pathogenic bacteria

The results are shown in fig. 1. Table (1,2) when using pomegranate molasses against \textit{E. coli} bacteria on the medium (MHA) and in the case of the use of Disk and well where a diameter of 20 mm inhibitor was consistent with the results of a studies (Amy and Doris, 2013). (Sheilla et al, 2016) where they found that pomegranate molasses contain antioxidants, organic acids, antibiotics and enzymes such as Isocitric acid, Sulphate, Citric acid, Lactoferrin, Lactoperoxidase acting as antimicrobial (Bige et al, 2010).

The results also showed that pomegranate molasses inhibited \textit{Staphylococcus aureus} bacteria by 18 mm in the case of Disk and 20 mm in the case of well This explains the possibility of using these nutrients in the treatment of various infections caused by these pathogenic bacteria such as skin and pulmonary infections, tonsillitis, middle ear and urinary tract infections. These results are consistent with his findings (Valeria et al, 2019). The results also showed that the pomegranate molasses inhibitor \textit{Streptococcus mutants} by 20 mm in the case of Disk and 25 mm in the case of well on the medium (MHA) and 20 and 22 mm in the case of Disk and well, respectively on the blood agar medium, fig. 2. Table 3 may be them substances that can prevent tooth decay, membrane pneumonia, peritonitis and peritonitis caused by \textit{Streptococcus mutants}, which are rich in various compounds that inhibit bacteria and fungi (flavonoids, phenolic acid, tannins, moreover antioxidant) (Romeo et al,2015). These results are consistent with the results of both researchers (Gianmaria et al, 2017) (Fischer et al, 2011). The results showed that pomegranate molasses inhibited the growth of \textit{Pseudomonas aerogun}’s bacteria 12 mm in the case of disk and 17 mm in the case of well on the medium (MHA). The difference in results in both cases may be due to the correlation of the active inhibitory material of these bacteria in pomegranate molasses with the paper material and less concentration than in the case of well. The results show that pomegranate molasses can be used in the treatment of many infections caused by \textit{Pseudomonas aerogenosa} especially in burns and infections of the middle ear or other infections. These results are consistent with (Amy and Doris, 2013). The results also showed that \textit{Klepsiella pneumonia} was resistant to pomegranate molasses on (MHA) in the case of tablets and pits. This explains the high resistance of these bacteria against the action of many plant extracts because of their possession of the capsule and a distinct cell wall and the presence of sugars that enhanced the presence of the capsule in this bacteria. Some researchers (Udayabdul-Reda et al, 2007) found that the aqueous and alcoholic extract of pomegranate inhibited the growth of \textit{Klepsiella pneumonia} (14,10) mm respectively (fig. 1). The results showed in fig. 1. Table (1,2) that used Tamarind molasses on (MHA) medium by Disk method was inhibited against (\textit{E.coli}, \textit{Staphylococcus aureus}, \textit{Streptococcus mutants Pseudomonas aerogenosa}) respectively (15,20,20,20) mm and well method (18,28,12,26) mm. Fig. 1. This results Enhances the high ability of Tamarind molasses to inhibit these pathogenic bacteria. Tamarind contains ions, minerals, proteins and organic acids such as Ascorbic acid, Tartaric acid, Citric acid, Terpenoids, Tannins, Riboflavin, which can be antioxidants and inhibitors of microorganisms such as bacteria and fungi (Dheerj etal, 2007). These results were consistent with the results of the researchers (jayaprakash et al, 2017) on \textit{E. coli}, \textit{Staphylococcus aureus}, and \textit{Pseudomonas aerogenosa}, where recorded inhibition diameters respectively (22, 16, 15) mm and with the results of the researchers (Gupta et al 2014) on the bacteria \textit{E.coli}, \textit{Staphylococcus aureus}, \textit{Pseudomonas aerogenosa} where recorded inhibition diameters respectively (16,18,13) mm. The present study did not any inhibition results of Tamarind molasses against \textit{Klepsiella pneumonia} due to the presence of the capsule which was enhanced by the presence of different sugars in Tamarind molasses which became the protective agent for bacteria against the action of inhibitors in Tamarind molasses (Dheerj etal, 2007). The results as shown in tables (1, 2) in fig. 1. when using Garlic oil (GO) with thyme, Garlic oil with chili peper on MHA medium and in two methods Disk and well are not inhibited for each of the pathogenic bacteria used in the current study (\textit{E .coli}, \textit{Staphylococcus aureus}, \textit{Streptococcus mutants Pseudomonas aerogenosa}) except when using Garlic oil with peper against \textit{Staphylococcus aureus} were inhibition recorded a 15 mm. This may be due to the presence of oil that have inhibited and neutralized many active and inhibitory free radicals in the garlic plant, such as the Allicin compound in garlic, which inhibits the formation of Acetyl CoA and thus inhibits the construction of DNA, RNA and thus inhibits protein building (Khashan, 2014). Jay et al, 2004 indicated that (GO) did not have strong antibacterial effects because that allicin, the principal
antimicrobial compound of garlic, was rapidly decomposed to diallyl sulfides, and that garlic oil, diallyl sulfides, and aqueous garlic extracts lacking allicin all had no antimicrobial effect. Because of these results, subsequent studies were conducted on the antimicrobial activity of garlic oil (GO) and its active sulfides compounds.

**TABLE 1.** Zone of inhibition (mm) for some food additives against different bacterial isolates (Disk diffusion testing) on MHA media

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pomegranate molasses</td>
</tr>
<tr>
<td>E. coli</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>18</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>20</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>12</td>
</tr>
<tr>
<td>Klepsiella pneumoniae</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 2.** Zone of inhibition (mm) for some food additives against different bacterial isolates (Agar well diffusion testing). On MHA media

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pomegranate molasses (1)</td>
</tr>
<tr>
<td>E. coli</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>25</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>17</td>
</tr>
<tr>
<td>Klepsiella pneumoniae</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 3.** Zone of inhibition (mm) for some food additives against *Streptococcus mutans* on Blood Agar

<table>
<thead>
<tr>
<th>Diffusion samples</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disk diffusion</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Agar well diffusion</td>
<td>22</td>
<td>-</td>
<td>25</td>
<td>-</td>
</tr>
</tbody>
</table>
**Figure 1.** *Streptococcus mutans, Pseudomonas aeruginosa, Staphylococcus aureus, Klepsiella pneumoniae,* and *E.coli* on (MHA), disk and agar well diffusion assay.

**Figure 2.** *Streptococcus mutans,* on Blood agar, disk and agar well diffusion assay

### 4. Conclusion

In recent study suggest that Pomegranate and Tamarind molasses are a much better antimicrobial agents so that to be a natural antimicrobials for food preservation showing large-scale as antibacterial activity against most pathogenic bacteria which used in this study than Garlic oils therefore, these substance can be considered a natural and inexpensive source of food preservation. We conclude in this present study that pomegranate and Tamarind molasses are also rich in phenols which act as antimicrobial agents and have high nutritional values in addition to containing antioxidant of great importance to human health.

### References


