Full Length Research Paper

A study of the antimicrobial activity of Psidium guajava L. and Lawsonia inermis leaf extracts against some foodborne pathogens

Nagwa T. Elsharawy¹,²* and Afra Mohammed Baghdadi¹

¹Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia.
²Department of Food Hygiene, Faculty of Veterinary Medicine, New Valley University, Egypt.

Received 5 January 2022; Accepted 21 February 2022

The study aims to determine the antibacterial activity of Psidium guajava and Lawsonia inermis leaf extracts against some foodborne pathogens. The leaf samples were collected, rinsed, dried, ground, and then stored. The leaf powder was soaked in ethanol and boiled in distilled water. Then, it was filtered. The antibacterial activity of P. guajava and L. inermis leaf extracts against Salmonella enteritidis, Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, and multidrug-resistant S. aureus (MRSA) was studied. Microbial enzymes were examined including Amylase, Protease, and Lipase. The results showed that the most effective extract against all the tested microorganisms was Psidium guajava ethanol extract. Similar results were almost detected in L. inermis water extract. Although P. guajava water extract had wider inhibition zone, its effect was limited against E. coli, S. aureus, and MRSA. In conclusion, the results obtained provide valuable knowledge for food preservation. There is need for further research to better extract high concentrations of P. guajava and L. inermis extracts.

Key words: Antibacterial agents, guava, Henna, Salmonella enteritidis, Staphylococcus aureus, Escherichia coli.

INTRODUCTION

The most recent research seeks to overcome microbial resistance to traditional drugs consisting of natural bioactive compounds and their derivatives obtained from plants which have antimicrobial effects against different pathogenic microorganisms including foodborne microorganisms (Vaou et al., 2021).

Guava leaf (Psidium guajava L.) is obtained from one of the most common tropic fruit trees with about 133 genera and 3,800 species worldwide. It belongs to Magnoliophyta phylum, Magnoliopsida class and Myrtaceae family. It is commonly used for traditional medicine to treat many digestive and respiratory disturbances due to its richness in vitamins and minerals such as phosphorus, calcium, pectin, tryptophan, lysine plus its antimicrobial polyphenolic compounds such as flavonoids, saponins, eugenol, tannins, and triterpenoids (Seo et al., 2014; Naseer et al., 2018).

Henna (Lawsonia inermis) is also one of the most common dying plants related to Lythraceae family grown in most tropical countries all over the world. This plant
leaf contains amino acids and fixed oils. It also has high concentrations of many medicinal ingredients such as triterpenoids, alkaloids, lignins and flavonoids which have antimicrobial effect in accelerating wound healing. Furthermore, it has sedative, cardio-inhibitory, anti-hemorrhagic and hypotensive effects (Parimalam et al., 2021).

Salmonella species (Salmonella Enteritidis (SE) and Salmonella Typhimurium (ST)) and Escherichia coli are Gram negative common foodborne microorganisms mainly found in the intestines of animals and humans. They contaminate food and water through fecal matter. They are considered as the second common cause of gastrointestinal upsets. Over 90,000 infected cases are reported annually in Europe. The infection signs are abdominal cramps, fever and diarrhea within few hours of consumption of contaminated and undercooked meat, fish, chicken or their products. Recovery mostly occurs spontaneously within few days (The Standard of the National Committee for Clinical Laboratory Standards, 2012).

The most global Gram positive microorganisms are Staphylococcus species. Especially, Staphylococcus aureus is the bacterium that spreads most in the skin, mucus membranes, urogenital and upper respiratory systems of healthy animals and people. S. aureus (MRSA) strain has resistance to methicillin and several antibiotics. It is commonly transported via contaminated high salt tolerance meat, chicken, milk and fish or their products. Its high virulence makes it to secrete seven toxins leading to food poisoning. However, its methicillin resistance is not due to its ability to produce enterotoxins which cause food poisoning. The symptoms of illness caused by this strain appear within few minutes after eating food contaminated by the heat stable toxins like mild gastroenteritis. People mainly recover within three days after being infected (da Silva et al., 2019; Le et al., 2021). Most foodborne infectious agent is diagnosed by stool and blood samples, although almost all of these microorganisms become resistance to almost all antibiotics especially in suppressed immune patients. The major problem caused when the infectious agents do not respond to traditional antimicrobials is high cost of treatment using orthodox medicines, including their side effect and the patients’ inability to work. These motivated the researchers to look for new antimicrobials. This study aims to determine the antibacterial activity of ethanol and aqueous leaf extracts of guava and henna against some clinical Gram negative and Gram positive microorganisms including some foodborne pathogens.

**MATERIALS AND METHODS**

**Plant extracts preparation**

Random fresh guava (P. guajava) and henna (L. inermis) green leaf samples were collected from guava trees from different Jeddah fruit farms, Jeddah, Saudi Arabia. The samples collected were stored in labeled plastic bags and then transported to the laboratories of College of Science, University of Jeddah. The leaves of the samples were rinsed, dried, grinded by blender and then sieved by 1 mm aluminium sieve. It was stored inside labeled air tight bottles until used (Seo et al., 2014).

**Methods of extractions**

About 25 g of the leaf powder was soaked in 100 ml ethanol (>99.5%) and boiled in distilled water inside an aluminium flask to prevent light exposure and evaporation. It was stirred in a shaker incubator at 70 rpm for 3 days under sterile conditions to obtain 25% concentration. The solution obtained was centrifuged at 4,000 rpm/25°C for 10 min. The supernatant was filtered by Whatman No. 1 filter paper and then stored at 4°C until it was used (Seo et al., 2014).

**Antibacterial activity**

Fresh guava (P. guajava) and henna (L. inermis) green leaves were extracted for use against sex strains of microorganisms: S. Enteritidis (ESBL700613), S. aureus (ATCC25923), S. Typhimurium (ATTC14028), Escherichia coli (NCTC9001), pathogenic E. coli (MC-SC376609), and MRSA (ATCC43300) using the well diffusion technique. Muller Hinton was used to streak the microorganisms and then were punched by a sterile borer of 5 mm diameter to impede six antibiotics discs (50 mg/mL) as follows: Cefoxitin (FOX), Cephalothin (KF), Cotrimoxazole (TS), Gentamicin (GM), Augmentin (AUG) and Ampicillin (AP)* [Mast Group/ MASTRING-S] (Figure 1). They were compared with the discs of the different plants’ leaf extracts. Three plates were prepared from the same extracts and then incubated at 37°C/24 h. Then the halo/clear zone of each plate was measured by a ruler to estimate the inhibition zone by millimeters. The average of the three readings (The Standard of the National Committee for Clinical Laboratory Standards, 2012) was calculated.

**Microbial enzymes’ examination**

This was performed to know the effect of the plant extracts on the microbial enzymes.

**Amylase assay:** About 25 g of starch agar medium was suspended in 1000 ml distilled water. About 4 mm of bacterial culture was cut on the labeled plate and then incubated at 37°C/24 h with a drop of iodine solution for 30 s. The color of the medium changed because amylase is a starch hydrolyzing enzyme (Ross, 1976).

**Protease assay:** Skim milk agar plate was suspended in about 51.5 g of 1000 ml distilled water. The bacterial culture was inoculated separately. There were clear zones (zone of hydrolysis) around the bacterial colonies after being incubated at 25°C for 48 h (Ali, 1992).

**Lipase assay:** This was done by the addition of 2.5% agar, 2% Tween 20-80 and 0.01% Victoria Blue B (or other indicators). The different tested microorganisms were grown at 30°C in a circular well of about 1 cm diameter). Lipolytic microorganisms were picked out from the culture plates (Samad et al., 1989).

**Statistical analysis**

The statistical program, SPSS version 16 for window, was used to
Table 1. Antimicrobial activity of *Psidium guajava* L. and *Lawsonia inermis* leaf extracts (cm).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>Psidium guajava</em> L.</th>
<th><em>Lawsonia inermis</em></th>
<th>FOX</th>
<th>KF</th>
<th>TS</th>
<th>GM</th>
<th>AUG</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanolic extract</td>
<td>Water extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>4.5</td>
<td>0.0</td>
<td>1.8</td>
<td>4.0</td>
<td>3.0</td>
<td>2.5</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.0</td>
<td>6.5</td>
<td>1.8</td>
<td>2.0</td>
<td>3.0</td>
<td>0.0</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>4.0</td>
<td>0.0</td>
<td>1.8</td>
<td>2.3</td>
<td>3.4</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>4.0</td>
<td>7.3</td>
<td>1.6</td>
<td>2.0</td>
<td>2.5</td>
<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Pathogenic <em>E. coli</em></td>
<td>2.5</td>
<td>0.0</td>
<td>2.7</td>
<td>1.4</td>
<td>2.7</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>MRSA</td>
<td>2.5</td>
<td>3.0</td>
<td>1.2</td>
<td>1.2</td>
<td>2.5</td>
<td>0.0</td>
<td>2.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

determine the means (at the significance level of *P*<0.05), standard error, and analysis of variance (ANOVA) with one way. Statistical significance was tested at the 5% level of significance in this study (SPSS 16, 2007).

RESULTS

Comparison between the antimicrobial activity of *P. guajava* L. leaf extracts and *L. inermis* leaf extracts

Table 1 and Figure 2 show the *in vitro* inhibitory effects of the water and ethanol extracts in comparison with commercial antibiotics. The most effective extract against all the tested microorganisms was the ethanol extract of *P. guajava* (4.5-2.5 cm). Almost similar results were obtained from the water extract of *L. inermis* (4.0-1.2 cm). Although the water extract of *P. guajava* had wider inhibition zone, its effect was limited against *E. coli* (7.3 cm), *S. aureus* (6.5 cm) and MRSA (3.0 cm). While, the ethanol extract of *L. inermis* ranged from 2.7 to 1.2 cm. The study results showed there was higher inhibition effect against Gram negative microorganisms than Gram positive species.

Effects of *P. guajava* L. leaf extracts and *L. inermis* leaf extracts on microbial enzymes

Figure 3 shows the comparison of the *in vitro* inhibitory effect of the water and ethanol extract. The most effective extract against all the tested microbial enzymes was the water extract of *P. guajava*. Almost similar results were detected in the water extract of *L. inermis*. Although, the ethanol extracts of *P. guajava* and *L. inermis* had almost similar inhibition effect against the tested microbial enzymes. All the herbal extracts had higher inhibition effect against lipase followed by amylase and then protease enzymes.
DISCUSSION

This study examines the antibacterial effect of *Psidium guajava* and *Lawsonia inermis* in overcoming the side effects of commercial used antibiotics and the resistance of foodborne pathogens to antibiotics. The study estimates the aqueous and ethanol extracts of *Psidium guajava* and *Lawsonia inermis* against the most common foodborne pathogens which resist almost all commercial antibiotics. It also examines the effects of the different plant extracts on the bacterial enzymes. The results obtained revealed that the most effective extract against all the tested microorganisms was the ethanol extract of *Psidium guajava*; it has higher inhibition effect against the tested Gram negative microorganisms than Gram positive tested species. On the other hand, the most effective extract against all the tested microbial enzymes was the water extract of *Psidium guajava*. Almost similar results were obtained from the water extract of *Lawsonia inermis*. Although, the ethanol extracts of *Psidium guajava* and *Lawsonia inermis* showed almost similar inhibition effect against the tested microbial enzymes. All the herbal extracts had higher inhibition effect against lipase followed by amylase and then protease enzymes.

According to Santhoshkumar et al. (2014), the aqueous and ethanol extracts of *Psidium guajava* leaves have patent effect against *S. aureus*. Samiha et al. (2017) reported that *Psidium guajava* leaf extract has effective antimicrobial activity against *S. aureus* strains. Puntawong et al. (2012) detected the antibacterial effect of *Psidium guajava* leaf ethanol extract against Gram negative and positive microorganisms, especially against gastrointestinal pathogens. Naseer et al. (2018) reported the high effect of *Psidium guajava* leaf extract against *Salmonella* spp., *E. coli* and *S. aureus*. Antibacterial effect of *Psidium guajava* refers to the presence of quinone compounds which can adhere to bacterial cell polypeptides and attach to bacterial enzymes which inhibit the growth of microorganisms. Another antibacterial agent derived from *Psidium guajava* containing flavonoids, saponins, and tannins has great
inhibitory effect against S. aureus and E. coli and other microorganisms’ enzymes (Díaz-de-Cerio et al., 2017).

Nearly similar results were obtained by Elmanama et al. (2011) who recorded the antimicrobial activity of L. inermis that can combine with proteins and carbohydrates which are found in bacterial cell wall and inactivate bacterial enzymes. Santhamari et al. (2011), Borade et al. (2011) and Pereira et al. (2010) recorded the effectiveness of L. inermis against S. aureus, E. coli, Bacillus species, Klebsiella pneumonia, Proteus species, Pseudomonas aeruginosa, Salmonella spp. and MRSA. Arun et al. (2010) observed it has less effect on Gram negative microorganisms. On the other hand, Habbal et al. (2010) stated that the high potency of the ethanol extract of L. inermis is considered as a promising broad spectrum antibacterial agent. The effectiveness of L. inermis is because it contains flavonoids, alkaloids, cardenolides, saponins and other active substances (Santhamari et al., 2011).

**Conclusions**

This study has shown that the aqueous and ethanol extracts of P. guajava and L. inermis leaves possess good inhibitory antimicrobial effect. The results obtained provided valuable knowledge that natural antimicrobial agents have potential applications in food and pharmaceutical industries for controlling foodborne pathogens. More and further research needs to be done to better extract and use high concentrations of P. guajava and L. inermis leaf extracts.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The article was fully funded by the authors.

**REFERENCES**


