Original papers

Antimicrobial activity of different plants extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background. Microbial pathogens, mainly bacteria, are a major cause of food spoilage resulting in several foodborne diseases. Food spoilage can be prevented by the application of chemical preservatives in the food industry but such process has harmful effects on human health and causes the introduction of chemicals in several food chains, leading to toxicity and long-term complications. Due to such adverse effects, the need to find natural preservatives that are safer to use, effective and less complicated is increasing.

Objectives. This study is based on plant extracts that play a major role in microbicidal action (the use of natural preservatives is preferred over chemical ones). Antimicrobial action of different plant extracts was assessed using *Staphylococcus aureus* and *Escherichia coli* as experimental bacterial strains.

Material and methods. Ethanolic extracts of different plants like *Punica granatum*, *Acacia catechu* and *Phyllanthus emblica* were highly effective against the both analyzed bacterial strains at a dosage of 10 mg/mL, while the extracts of *Ocimum bacilicum* and *Quercus infectoria* were effective only against *S. aureus* and *E. coli*, respectively.

Results. *Punica granatum* and *Phyllanthus emblica* extracts were found to be the most effective and exhibited bacteriostatic and bactericidal activities against the highly infectious strains of pathogenic bacteria causing food spoilage, with minimum inhibitory concentration (MIC) of 2.5 mg/mL and minimum bactericidal concentration (MBC) of 5 mg/mL.

Conclusions. The plant extracts used in the study were highly effective in reducing bacterial contamination and can be used as an alternative to chemical preservatives to avoid and control foodborne diseases and for preservation of food with no health-related hazards caused by chemicals.

Key words: *Escherichia coli*, *Staphylococcus aureus*, antimicrobial activity, plant extract

Background

There are over 1.8 million deaths worldwide per annum, mostly in young children, due to contaminated edibles, including water. According to the data of the World Health Organization (WHO), 76 million cases of foodborne diseases are recorded worldwide annually, with approx. 5000 deaths. Ingesting nutrition which has been compromised by bacteria, protozoa, toxins, and other contaminants seems to be the most prevalent source of infection and sickness. It is one of the leading causes of illness as well as mortality in poor nations. Bacteriological pollutants, primarily Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, are perhaps the most pronounced risk to public health.[1,2]

Certain bacterial species, such as *Bacillus cereus* and *Staphylococcus aureus*, have indeed been linked to local foodstuff spoilage.[2] Anti-targets have been continuously used in product packaging to prevent the decay of packaged foods.[2,3] Emphasis has been put on healthy, effective and natural food preservatives.[4]

Toxic substances of this kind have proven their efficiency in identifying and mitigating nosocomial infections, but their widespread use has contributed to the establishment of such hazardous substances in food chains, along with a whole slew of adverse environmental effects. Biocompatible chemicals are utilized as organic compounds against pathogenic microorganisms because they are both harmless and edible.[1,6]

Several researches have demonstrated the bioactivity of phytochemicals against disease-causing microorganisms.[7] The antibacterial efficacy of garlic, ginger and guava foliage against a diversity of social infectious agents had been studied, and it was discovered that ginger was perhaps the most efficient towards *S. aureus*, while guava and garlic proved efficacious against most of the microbes studied.[8] Antibacterial property towards *B. subtilis* and *B. cereus* was previously discovered in the foliage of *Syzygium polyanthum*.[9]

Pseudomonas aeruginosa and *Bacillus subtilis* have also been examined, and it was found that perhaps the ethanolic extracts of 4 plants (*Achyranthes aspera*, *Cynodon dacynodon dactylon*, *Lantana camara*, and *Tagetes patula*) are effective against most of the bacterial isolates, with minimum inhibitory concentration (MIC) ranging between 25 mg/mL and 125 mg/mL.[10]

The water-soluble extractions of cinnamon, aloe vera, henna, coriander, myrtle, and chamomile, as well as the hydro-alcoholic extractions of menthol, henna, ginger, chamomile, olive, myrtle, and Christ's thorn, act as their populations grow in small increments in their inhibition zones of approx. 10–28 mm.

Aquil and Ahmad discovered that ethanolic garlic extracts had almost no antibacterial activity against *E. coli* or *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*.[12] In the research by Akinpelu et al., strong antimicrobial effectiveness of butanolic and pure isolates of *Persea americana* infected with *B. cereus* in foodborne illnesses was observed.[13] The minimum bactericidal concentration (MBC) of extracts varied between 3.12 mg/mL and 12.5 mg/mL, and these isolates showed antibacterial activity at doses of 25 mg/mL and 10 mg/mL. Likewise, several of these phytoconstituents have already been tested for antimicrobial effect toward microbial nutrition substances required by the organism to obtain nutrition by catabolism and anabolism.[14] Numerous therapeutic medicinal plants have been shown to counteract foodstuff-related illnesses.

Three therapeutic phytoconstituents utilized in Nigerian traditional system of medicine were shown to have high antimicrobial activity against a broad range of foodborne infections. All had a strong antibacterial effect towards *S. aureus*, *E. coli* and *Salmonella enteritidis*, albeit to a varying extent and often with varying MICs depending on the used plant compounds and microorganisms.[15]

Mikania triangularis, often characterized as "leaf guaco", exhibits antibacterial properties against 5 fungal isolates, 3 bacterium species, as well as *Staphylococcus epidermidis*, *S. aureus*, *B. cereus*, and *P. aeruginosa*, among others.[16] Investigators have also studied the antibacterial efficacy of 8 natural herb isolates against *Listeria monocytogenes*, *B. cereus* and *E. coli*.[17] Methanolic extracts of *Caryophyllus aromaticus* were shown to have the highest antimicrobial effect over *S. aureus* and had been significant against those same microorganisms.[18]

Bioactive compounds from *Myrtus communis* and *Thymus daenensis* are perhaps the most effective, with MIC coefficients ranging from 0.039 mg/mL to 10 mg/mL. Many researchers have investigated and validated the antibacterial effectiveness of *Punica granatum* against germs that cause food spoilage.[19]

Verma et al. computed the antimicrobial properties of tested compounds inhospitable to nutrition pathogenic organisms, including the Citrus, Punica, as well as Allium plant extracts.[20] Thus, every crude extract tested was intrinsically efficacious against different organism like *S. aureus*, *B. cereus*, *S. typhi*, and *E. coli*; however, the *Punica granatum* isolates showed genuinely highest potency with a concentration over 500 mg/mL. Within a concentration between 30 mg/mL and 50 mg/mL, *Punica granatum* peeling ethanolic extracts have been shown to be efficacious towards *Bacillus megaterium*, *S. aureus*, *Micrococcus luteus*, and Gram-negative bacteria such as *E. coli* and *P. aeruginosa*.[21]

Because of the strong antimicrobial properties of *Punica granatum* ethanolic extracts as well as their chunk toward Gram-negative (*E. coli* and *S. typhi*) and Gram-positive bacteria (*S. aureus* and *B. cereus*) causing foodborne illnesses, those certain extracts are used as stabilizing agents in the food processing industry to protect consumers against foodborne diseases.[22]

Several spice formulations used in nutritional supplements are highly effective toward numerous food contamination microorganisms; their antimicrobial effects have been shown by many researchers.[23] For example, cinnamon isolates were shown to be an excellent antimicrobial agent against all tested bacterial strains; ginger, clove and cumin are more effective than cinnamon extracts. Cloves have been found to have antimicrobial properties against Gram-negative bacteria and other diseases.[1,4,24]

According to numerous studies, ethanolic cloves infusion may indeed be helpful against *S. aureus*, *Vibrio parahaemolyticus* and *P. aeruginosa*, but inadequate against *E. coli* and *S. enteritidis*.[25] Clove oil has been shown to be effective against all dangerous microorganisms assessed in several investigations, with perhaps the exception of *Vibrio cholerae*, *Klebsiella pneumoniae* and *S. typhi*, that also have been shown to be impervious to diluted clove extract.[26]

Furthermore, the methanolic extracts of cloves are claimed to be efficacious towards *S. aureus*, *P. aeruginosa* and *E. coli*, with MIC values ranging from 0.1 mg/mL to 2.31 mg/mL.[27] Cumin seeds (*Cuminum cyminum*) aqueous extract has been shown to have antimicrobial effect against some of the Gram-positive and Gram-negative bacteria, exhibiting varying MIC.[3,28]

Cumin extract has been shown to be efficacious towards *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus pumilus*, with MIC values ranging between 6.25 mg/mL and 25 mg/mL,[29] and prescribed dosages ranging from 20 mg/mL to 60 mg/mL.[30]

The effectiveness of 7 ethanolic and aqueous plant extracts was evaluated against several clinically dangerous bacteria. The ethanol extract of *Punica granatum* was shown to be efficacious against all pathogenic microorganisms assayed, with a MIC value of 0.2 mg/mL. *Thymus kotschyana* extract was shown to be efficacious towards *E. coli* and *S. aureus*; however, *Zingiber officinalis* isolate was shown to counteract *P. aeruginosa* and *K. pneumoniae*.[31]

Numerous thyme bioactive compounds have been tested for their bactericidal effectiveness against foodborne infections (*L. monocytogenes*). The prevalence of foodborne diseases have indeed been linked to dangerous microorganisms, notably Gram-negative bacteria like *E. coli*, *S. typhi* and *P. aeruginosa*, and other Gram-positive pathogens like *S. aureus* and *B. cereus*. There is a scarcity of studies in the Arabian region on the viability of *Syzygium aromaticum*, *Thymus vulgaris*, *Punica granatum*, *Zingiber officinale*, and *Cuminum cyminum* against many of the previously mentioned pathophysiological food decomposing bacteria. Therapeutic properties of many bioactive compounds against diseases caused by *S. aureus*, *B. cereus*, *E. coli*, *S. typhi*, and *P. aeruginosa* are now being evaluated in vitro.

Material and methods

Performing the Soxhlet method of alcoholic extraction

Material (galls, peels, heartwood, fruit, and whole plants) from 5 different plant species (Table 1) were obtained from the Universal Biotech Khari Baoli (Old Delhi, India). Plant parts were washed many times to remove any possible impurities. After drying, each plant material was crushed into fine powder that passed through a 100 mm sieve. Approximately 10 g of fine powder was immersed in 100 mL of ethanol and extracted for 48 h with continuous stirring, and then filtered with two-layer muslin cloth, centrifuged for 10 min at 9000 rpm, and finally filtered again using Whatman filter paper (41; Merck Millipore, Mumbai, India). The filtrates were dried using a rotary vacuum evaporator (Hahnshin Scientific, Mumbai, India) under reduced pressure at 60°C and stored in the refrigerator at 5°C. The percentage of yields was measured using the following formula[32]:

$$\text{yields of extract (\%w/w)} = R/S \times 100$$

where R – plant residues extracted weight and S – raw sample of plant weight.

Antibacterial activity of the plant extracts

Bacterial strains

Two strains of bacteria that cause food poisoning were used to test the antibacterial effectiveness of extract of each plant species. In this experiment, we used 1 strain of Gram-positive bacteria (*S. aureus*) and 1 strain of Gram-negative bacteria (*E. coli*). The strains were collected from the Biochemistry Department of Jamia Hamdard (deemed to be university), New Delhi, India.

Table 1. Ethnobotanical data of examined plant species and their extract yield percentage

Plant species	Family	Local name	Common name	Plant part used	Extract pH	Extract yield
Quercus infectoria	Fagaceae	manjakani	Aleppo oak	galls	4.8	1.2%
Punica granatum	Lythraceae	romman	pomegranate	peels	4.5	2.3%
Acacia catechu	Fagaceae	khair	black cutch	heart wood	7.2	2.1%
Ocimum basilicum	Lamiaceae	tulsi	basil	whole plant	7.8	1.98%
Phyllanthus emblica	Phyllanthaceae	amla	Indian gooseberry	fruits	3.9	2.4%

Inoculum preparation

Both strains of bacteria were left overnight for culture in Mueller–Hinton agar medium at 35°C. Using a spectrophotometer, the growth of bacteria was harvested in 5 mL of sterile saline water, and the cell count was diluted to 10^7 CFU/mL at 580 nm.

Antibacterial activity of plants extract

The antibacterial activity of each plant extract was estimated using the disc diffusion method. The residues of plant extract (50 mg) were re-dissolved in 2.5 mL of ethanol. After that, purification was performed with a Millipore filter (0.22 mm; Merck Millipore) and then loaded over a sterile disc of filter paper to reach a final concentration of 10 mg/disc. Then, 15 mL of seeded medium previously infected with bacterial suspension (100 mL of medium/1 mL of 10^7 CFU) was added to 10 mL of Mueller–Hinton agar media in Petri dishes to achieve 10^5 CFU/mL of media.

Sterile filter paper discs with extract of plant concentration (10 mg/mL) were inserted on top of the plates of Mueller–Hinton agar media in Petri dishes. Discs of filter paper containing 5 mg of gentamicin as a positive control were utilized. After this, the plates were kept for 2 h in a fridge at 5°C to allow extract of plant to diffuse before being incubated for 24 h at 35°C. The presence of inhibitory zones was measured using a Vernier caliper, recorded and interpreted as a marker of antibacterial activity.

Determination of minimum inhibitory concentrations of the effective plant extract

After 24 h of incubation, the MIC was characterized by a low concentration of antibacterial agents that prevent microbial growth. By using the method of disc diffusion, the most effective extracts of plants, having strong antimicrobial activities at 10 mg/mL, were modified to calculate their MIC and examine their efficiency in reducing bacterial strains that cause food poisoning. By dissolving 50 mg of plant extracts in 2.5 mL of ethanol, filtering it with a Millipore filter and transferring the required amount to sterile discs, effective plant extracts with different concentrations (1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, 12.5 mg/mL, and 15 mg/mL) were produced (the discs had 8 mm in diameter). Pathogenic strains of cultured bacteria were infused using Mueller–Hinton agar into sterile Petri dishes. Various amounts of extracts were obtained and loaded onto filter paper discs; Mueller–Hinton agar plates were later covered. The plates were then kept at 5°C in the fridge for 2 h and then incubated for 24 h at 35°C. By the help of Vernier caliper inhibition zones were determined and compared to the concentration of the effective extract of the plant.

Determination of minimum bactericidal concentrations of the effective plants extract

Two streaks of plates bearing MIC inhibitory zones with little concentration were collected and cultivated in tryptone soya agar (TSA) plates that displayed limited growth. Next, the plates were put for incubation at 35°C for 24 h. The growth of bacteria was observed in different plant extract concentrations. On freshly infected agar plates, MBC was defined as the extract of plant concentration that did not show any bacterial growth.

Results and discussion

Plants extraction yield

The data for the plants that have been used and their extract percentage yield are presented in Table 1. Ten grams of dried powder plant material allowed for a yield of plant extract ranging from 198 mg to 240 mg. The highest yield of plant extract was obtained from *Phyllanthus emblica* (240 mg) followed by *Punica granatum* (230 mg), while *Quercus infectoria* gave the lowest extract yield (120 mg).

Antibacterial activity of plants extract

To examine the antibacterial activity of plants against food poisoning bacteria, 5 plant species were assessed against a strain of Gram-negative bacteria (*E. coli*) and a strain of Gram-positive bacteria (*S. aureus*). Disc diffusion method was used for the assessment and to find the susceptibility of bacteria. Antibacterial action of plant extracts is presented in Table 2 and Fig. 1. It was found that the used plant extracts were very effective in counteracting the bacterial growth that leads to food poisoning.

Punica granatum was found to be the most effective extract – it has reduced the growth of pathogenic bacteria (*S. aureus* and *E. coli*) at a concentration of 10 mg/mL. *Phyllanthus emblica* was also found effective against both of the pathogenic strains. Varying antimicrobial activity

Table 2. Antimicrobial screening test of ethanolic plant extract (10 mg/mL) against some of the bacterial strains causing food poisoning

Plant species	Inhibition zones [mm]	
	Gram-positive pathogenic bacteria (*S. aureus*)	Gram-negative pathogenic bacteria (*E. coli*)
Quercus infectoria	8.6 ±0.67	0.0 ±0.0
Punica granatum	17.9 ±0.10	14.4 ±0.53
Acacia catechu	16.3 ±0.27	13.1 ±0.27
Ocimum basilicum	15.7 ±0.24	0.0 ±0.0
Phyllanthus emblica	16.5 ±0.32	14.2 ±0.21
Gentamicin (5 µg)	18.5 ±0.21	14.9 ±0.49

Data are means of 3 replicates (n = 3) ± standard error (SE).

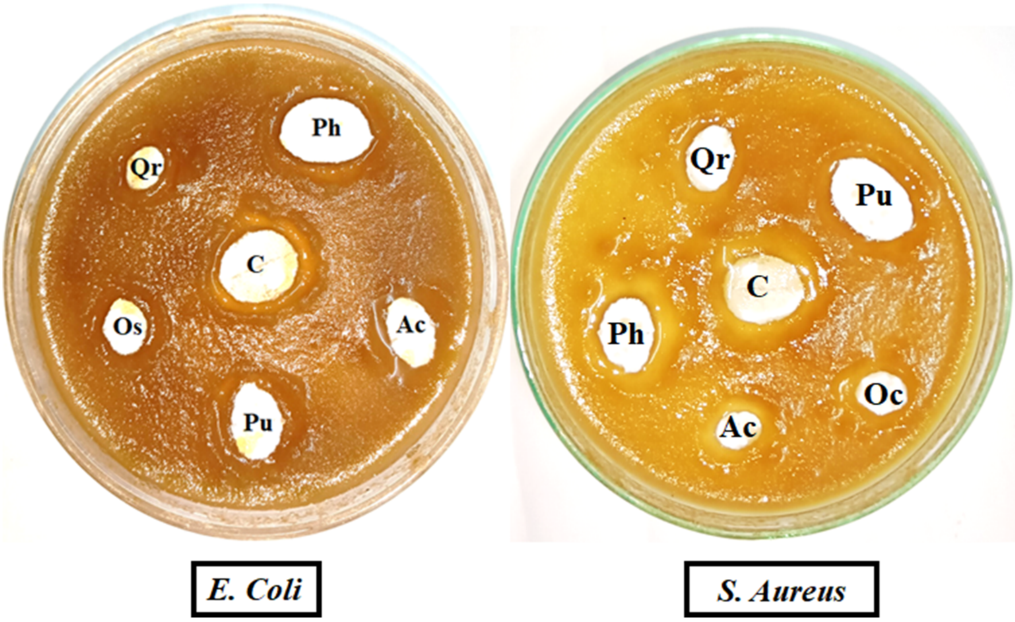

Fig. 1. Growth inhibition of bacterial strains caused by some plant extracts. *Quercus infectoria* (Qr), *Punica granatum* (Pu), *Acacia catechu* (Ac), *Phyllanthus emblica* (Ph), *Ocimum bacilicum* (Os), and C (positive control)

was also shown by other plants against food poisoning bacterial strains. *Acacia catechu* was proved effective against both bacterial strains, while *Ocimum bacilicum* and *Quercus infectoria* only affected *S. aureus* and had a negligible effect on *E. coli* bacteria.

Antimicrobial action of the plant extracts indicates that *E. coli* was the strain most resistant to the used plant extracts. *Punica granatum* and *Phyllanthus emblica* extracts showed highly effective antibacterial action. The experiments were conducted to find the MIC and MBC values of the food poisoning bacteria (*S. aureus* and *E. coli*).

Minimum inhibitory concentration of the effective plants extract

The MIC values of the effective plant extracts (*Punica granatum* and *Phyllanthus emblica*) were calculated using disc diffusion method to find out the bacteriostatic and bactericidal properties of the plant extracts. The concentration of the plant extracts which were found effective are presented in Table 3. The inhibiting effect of *Punica granatum* extract was detected at a concentration of 2.5 mg/mL, with the zone of inhibition at 9.5 mm and 7.5 mm for *S. aureus* and *E. coli*, respectively, while the extract of *Phyllanthus emblica* reduced the growth of bacteria at concentration of 2.5 mg/mL, forming a small inhibition zone of 8.7 mm and 5.6 mm for *S. aureus* and *E. coli*, respectively.

Minimum bactericidal concentrations of the effective plants extract

The MBC can be identified by the absence of bacterial growth streaked from the inhibition zone correlating to the tested strains' lowest MIC. *Punica granatum* was found to have an effective bactericidal activity against

Table 3. Minimum inhibitory concentrations (MICs) of the most effective plant extract against *S. aureus* and *E. coli*

Plant extract	Concentration [mg/mL]	Inhibition zones [mm]	
		Gram-positive pathogenic bacteria (*S. aureus*)	Gram-negative pathogenic bacteria (*E. coli*)
Punica granatum	1.25	0.0 ±0.0	0.0 ±0.0
	2.50	9.5 ±0.60	7.5 ±0.13
	5.0	13.7 ±0.77	10.3 ±0.21
	10.0	17.2 ±0.69	13.7 ±0.27
	12.5	20.7 ±0.32	16.9 ±0.52
	15.0	23.4 ±0.27	19.7 ±0.61
Phyllanthus emblica	1.25	0.0 ±0.0	0.0 ±0.0
	2.50	8.7 ±0.21	5.6 ±0.19
	5.0	12.3 ±0.31	9.7 ±0.21
	10.0	14.9 ±0.41	13.4 ±0.33
	12.5	17.2 ±0.56	16.3 ±0.41
	15.0	19.4 ±0.69	18.2 ±0.52

S. aureus and *E. coli*, having a MBC value of 5 mg/mL, while *Phyllanthus emblica* extract also had an MBC value of 5 mg/mL. Based on that, it can be suggested that *Punica granatum* and *Phyllanthus emblica* can be used to counteract foodborne pathogens and diseases. Bacteria used in this study play a major role in food decomposition and food poisoning. *Staphylococcus aureus* is regarded one of the most common causes of foodborne diseases, while *E. coli* is responsible for producing harmful toxins and other components that play an important role in human gastrointestinal diseases. *Punica granatum* was found effective in inhibiting the growth of all bacterial strains, while *Phyllanthus emblica* extract was found highly effective against *S. aureus* and less effective against *E. coli.*[33] Due to differences in method used for extraction, as well as in components and bacterial strains used in the experiment, a significant variability in MIC of *Punica granatum* could be observed in comparison to other investigations, leading to variation in MIC of different plants extracts. Variations can also be caused by the properties of the chemicals like volatility and the disparities between the chemical constituents. *Phyllanthus emblica* extract was found to be effective with a 10 mg/mL concentration against *S. aureus* and *E. coli*, inhibiting their growth and forming the inhibition zones of 14.9 mm and 13.4 mm, respectively.[34] Some researchers, after studying the plant extracts and their effect on certain bacteria, suggested that plants components like terpenoids, alkaloids and phenolic compounds react with components of bacterial cell membrane and proteins present on it, causing their lysis by inducing an efflux of proton outside the cell or inhibiting important enzymes responsible for synthesis of amino acids.[35] Other study accredited the effect of plant extracts to their hydrophobic properties, which cause the reaction of protein in bacterial cell membrane with mitochondria, leading to lysis and alteration of bacterial structure, and changing its permeability.[36] This study suggests that plant extracts used in this experiment have shown effective antibacterial properties and can also be used as natural preservatives, thereby reducing the application of chemically made preservatives in food industry, which leads to several health hazards.

Conclusions

Various harmful bacteria strains can cause food spoilage. It can be prevented by the application of chemical preservatives in the food industry, but such means have harmful effects on human health and cause the introduction of chemicals in several food chains, leading to toxicity and long-term complications. Due to such adverse effects, natural preservatives that are safer to use, effective and less complicated have to be developed. Existing plant extracts which have shown their potential usefulness (*Punica granatum* and *Phyllanthus emblica*) can be employed as a natural alternative to synthetic antimicrobial agents to prevent food poisoning.

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