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Antibacterial activity of selected antibiotics and medicinal plants against bacterial isolates from spoiled fruits and vegetables

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Abstract

Microbial contamination is one of the major cause of food spoilage. The consumption of spoiled contaminated food material may lead foodborne infections and illness. In the present study the spoiled fruits and vegetables were examined for bacterial contamination and antibacterial activity of selected antibiotics and methanolic extract of selected medicinal plants was determined against foodborne bacterial isolates. We isolated different bacteria including *Staphylococcus spp*., *Pseudomonas spp*. and *Klebsiella spp.* on the basis of culture characteristics and biochemical behaviour. A total number of 10 bacterial isolates were cultured from spoiled fruits and vegetables. Maximum 05 isolates were of *Staphylococcus spp.* obtained from spoiled food material while 03 and 02 isolates of *Klebsiella spp.* and *Pseudomonas spp.* respectively. Antibacterial potential of antibiotics and medicinal plants were determined using disc diffusion method and agar well diffusion method respectively. Bacterial isolates were found resistant against some of the tested antibiotics. *Staphylococcus* was resistant against kanamicin, ceftazidime and ampicillin while *Pseudomonas* against oxacillin and ciprofloxacin and *Klebsiella* against ceftazidime and oxacillin. Among plants extract, clove exhibited maximum antibacterial activity against *Pseudomonas* isolate while sacred fig exhibited maximum antibacterial activity against *Staphylococcus* and *Klebsiella* isolates. The results of this study suggest that the medicinal plants may be an effective alternate of antibiotics for the treatment of foodborne infections and also for reduction in emergence of bacterial drug resistance.

Keywords: food spoilage, antibacterial activity, antibiotics, medicinal plants, bacterial isolates.

Introduction

Foodborne illness is most commonly caused due to the consumption of contaminated or spoiled food material. Microbial contamination has been established as one of the major cause of food spoiling that may lead food poisoning. Foodborne diseases are one of the remarkable reason of mortality, morbidity and economical loss and worldwide. In concern of global burden of foodborne diseases, one out of ten people was infected due to consuming contaminated food material in every year and children under age of 5 year at of particularly high risk **(WHO's First Ever Global Estimates of Foodborne Diseases Find Children under 5 account for almost one third of deaths, 2016)**. 30% deaths occur in children under the age of five years **(Estimating the Burden of Foodborne Diseases 2023; Mahindroo *et al.,* 2024)**. Pathogenic microbial strains may be transmitted to humans through contaminated food material and cause foodborne infections **(Zhao 2014; Hemalata and Virupakshaiah, 2016)**. In the recent years foodborne infections are counted as one of the major human health problem increasingly worldwide **(Ayana et al., 2015)**. Food materials such as fresh vegetables and fruits may be contaminated with microbial pathogens due to a number of reasons including cropping with contaminated water, farming with faecal material, disposable of industries, improper packaging transportation and distribution, irrigation with wastewater treatment etc **(Jung et al., 2014; Al-Kharousi et al., 2016; Rahman et al., 2021)**. Spoiled and contaminated fruits and vegetables have been established as significant reservoirs for the drug resistant microorganisms that can cause serious infections in humans **(Olanbiwoninu et al., 2024)**. Antibiotic therapy is most commonly used for the treatment of bacterial infections. In almost all of the major foodborne microbial pathogen antibiotic resistance (AR) and multidrug resistance (MDR) have been confirmed which increasing continuously and cause serious problems in effective treatments of foodborne infections and diseases **(Eng et al., 2015; Grudlewska-Buda, 2023)**. The foodborne infections due to antibiotic resistant pathogen reveal the need of alternative of antibiotics for treatment and reduction of chances of emergence of antibiotic resistance. From the last several decades, medicinal plants are used for the treatment of cough, cold, fever, diarrohea, nausea, gastrointestinal infections, and respiratory infections. Medicinal plants exhibit antibacterial, antipyretic, antiviral, antioxidant, antifungal activity as having wide range of phyotoconstituents including terpenoids, phenolic, tannins, alkaloids and flavonoids (**Prabuseenivasan et al., 2006; Ghaffar et al., 2015; Surendra and Roopan, 2016; González-Burgos et al., 2018; Sagar et al., 2020**). Traditionally used medicinal plants has been scientifically established as a natural source of drugs for the treatment of microbial infections and diseases as well as alternative of antibiotics for the solution of drug resistance problem **(Ríos and Recio, 2005; Dinesh et al., 2017; Sagar and Singh 2021; Ifeyinwa, 2022; Sagar et al., 2022**). The aim of present research study to identify the microbial pathogens form spoiled vegetables and fruits samples and determination of effectiveness of selected antibiotics and medicinal plants for inhibition of growth of isolated bacterial isolates that may be beneficial for treatment of foodborne microbial infections.

Materials and Methods

Collection of food samples

A total 07 food samples including fruits i.e.; Banana (*Musa paradisiaca Linn.*), Grapes (*Vitis vinifera*), Mango (*Mangifera indica*), and vegetables i.e.; Bottle gourd (*Lagenaria siceraria*), Bitter gourd (*Momordica charantia*), Capsicum (*Capsicum annuum*), Cauliflower (*Brassica oleracea var.botrytis*) were collected from local market of district Jhansi, Uttar Pradesh, India. All the spoiled food material was subjected for bacterial isolation using standard protocols.

Collection of medicinal plants and extraction

Selected medicinal plants (n=12) **(Table 01)** were collected locally and subjected for air drying at room temperature. Air dried medicinal plant powder were used for methanol extraction. 10 g of dried plant powder was soaked in methanol (100%) and subjected for shaking continuously for three days at the room temperature. After filtration of crude extract through Whatman filter paper No. , the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was evaporated and prepared crude methanol plants extract were preserved at 4°C in air tight bottle for further experiments.

Table No 01 Medicinal plants used in research study

Sr. No.	Plant Name	Botanical Name	Plant part used for extraction
1	Drumstick	*Moringa oleifera*	Leaves
2	Clove	*Syzygium aromaticum*	Buds
3	Neem	*Azadirachta indica*	Leaves
4	Giloy	*Tinospora cordifolia*	Leaves
5	Carom seeds	*Trachyspermum ammi*	Seeds
6	Black pepper	*Piper nigrum*	Seeds
7	Tulsi	*Ocimum tenuiflorum*	Leaves
8	Cardamom	*Elettaria cardamomum*	Pods
9	Sacred fig	*Ficus religiosa*	Leaves
10	Guava	*Psidium guajava*	Leaves
11	Mint	*Mentha piperita*	Leaves
12	Sweet neem	*Bergera koenigii*	Leaves

Antibiotics

Antibiotic disc i.e. streptomycin (10 mcg), kanamicin (30 mcg), oxacillin (1 mcg), ciprofloxacin (5 mcg), pefloxacin (5 mcg), azithromicin (15 mcg), gentamicin (10 mcg), chloramphenicol (30 mcg), tetracycline (30 mcg), ampicillin (10 mcg), ceftazidime (30 mcg), were purchased commercially for this research study.

Isolation of foodborne bacteria from spoiled food samples

The spoiled fruits and vegetables were used for isolation of bacteria using different culture media including Nutrient agar (Himedia), MacConkey agar (HiMedia), Cetrimide agar (HiMedia) and Eosin Methylene Blue agar (HiMedia) at 37˚C for 18-24 hours and further used for biochemical characterization for identification of bacterial spp. using standard protocols.

Biochemical characterization of bacterial isolates

Different essential biochemical test including coagulase test, catalase test, oxidase test, citrate test, methyl red test, SIM/motility, urease test were performed as per the standard protocols for the confirmation of bacterial isolates according to bergey's manual **(Bergey and Holt, 1994**; **Cappuccino and Sherman 2005)**.

Determination of antibacterial activity of selected plant extracts and antibiotics

The bacterial culture was suspended in normal saline solution (0.85%) and turbidity was adjusted with 0.5 McFarland standard to standardize bacterial suspension (10^8 CFU/ml) as per the guidelines of Clinical and Laboratory Standards Institute **(CLSI 2012)**. Mueller Hinton agar (MHA) plate was prepared with standardized

bacterial suspension and the wells (6–8 mm diameter) were made using the sterile cork borer. The methanolic extract of plants was reconstituted at the testing concentration (500 mg/ml) in DMSO (dimethyl sulfoxide). 50 µl of reconstituted methanolic plants extract were poured aseptically in the wells and plates were incubated at 35 (±2°C) for 18–20 hours. The diameter of zone of inhibition (mm) was measured to determine the antibacterial activity of plant extracts. The chloramphenicol and DMSO were used as positive and negative control, respectively. Antibacterial activity of antibiotics was screened using disc diffusion method with standardized bacterial suspension as mentioned above.

Antibiotic disc were placed on MHA plate prepared using bacterial suspension and plates were incubated at 35 (±2°C) for 18–20 hours. The diameter zone of inhibition (mm) was measured to determine antibacterial activity against bacterial isolates.

Results

The different bacteria including *Staphylococcus spp*., *Pseudomonas spp*. and *Klebsiella spp.* were isolated and identified from spoiled fruits and vegetables on the basis of staining, culture characteristics on specific culture media and biochemical behavior. A total number of 10 bacterial isolates were cultured from spoiled fruits and vegetables **(Table 02)**. *Staphylococcus spp.* isolates were confirmed from spoiled vegetables (bittergourd, capsicum) and spoiled fruits samples (mango, banana and grapes). *Staphylococcus spp.* were cultured and identified on Mannitol salt agar (MSA) as *S. aureus* ferment mannitol and Gram positive. *S. aureus* exhibited positive reaction for catalase test, methyl red test, nitrate test, citrate test, coagulase test and fermentative on Manitol. *Pseudomonas* were isolated using cetrimide agar form spoiled banana and cauliflower and Gram negative. *Pseudomonas* isolates exhibited positive reactions for oxidase test, catalase test, citrate test and motility test while negative for coagulase and methyl red test. *Klebsiella* were isolated using MacConkey agar from spoiled vegetables (bittergourd, bottlegourd) and fruit (mango). *Klebsiella* isolates were Gram negative, citrate positive and catalase positive, while negative for oxidase reaction and motility test.

Table No 02 Bacterial isolates from spoiled fruits and vegetables

Sr. No.	Source	Spoiled food sample	Isolated bacterial spp.	Total no. of bacterial isolates	Total no. of bacterial isolates in spoiled vegetables/fruits
1	Vegetables	Bittergourd	*Staphylococcus spp.*	02	05
			Klebsiella spp.		
		Capsicum	*Staphylococcus spp.*	01	
		Bottlegourd	*Klebsiella spp.*	01	
		Cauliflower	*Peudomonas spp.*	01	
2	Fruits	Mango	*Staphylococcus spp.*	02	05
			Klebsiella spp.		
		Banana	*Staphylococcus spp.*	02	
			Peudomonas spp.		
		Grapes	*Staphylococcus spp.*	01	

Antibacterial activity of selected antibiotics was determined **(Figure 01)** against bacterial isolates of *Staphylococcus spp. Klebsiella spp.* and *Pseudomonas spp.* from spoiled vegetables and fruits in which *Staphylococcus* was resistant against kanamicin, ceftazidime and ampicillin while *Pseudomonas* against oxacillin and ciprofloxacin and *Klebsiella* against ceftazidime and oxacillin. Growth inhibitory potential of selected medicinal plants was determined **(Figure 02)** against bacterial isolates. Among the tested medicinal plant extracts, clove exhibited maximum antibacterial activity against *Pseudomonas* isolate while sacred fig exhibited maximum antibacterial activity against *Staphylococcus* and *Klebsiella* isolates. Sweet neem was not found effective against the isolated bacteria.

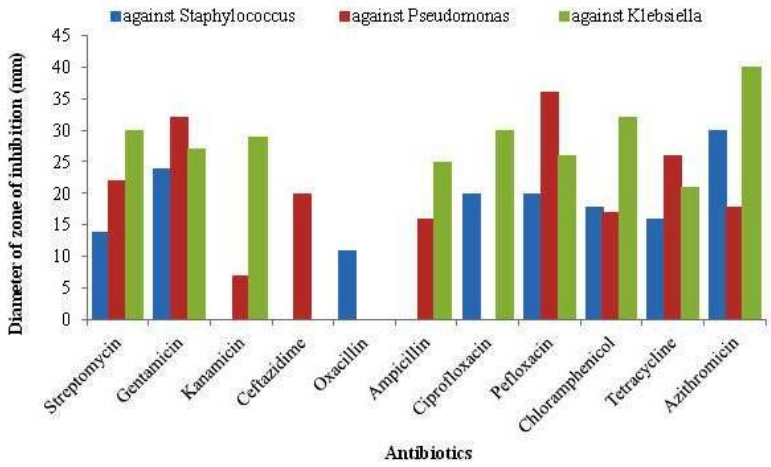

Figure 1 Antibacterial activity (Zone of Inhibition) of antibiotics against isolated bacteria

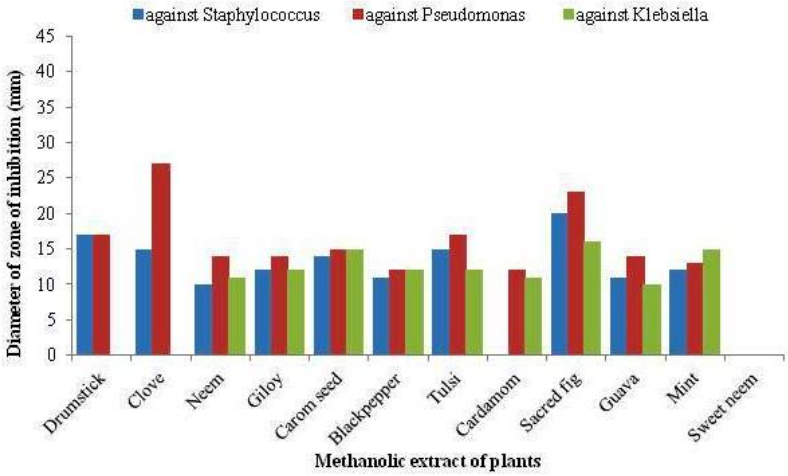

Figure 2 Antibacterial activity (Zone of Inhibition) of medicinal plants against isolated bacteria

Discussion

Consumption of contaminated spoiled food material is one of the major causes of food illness and foodborne diseases and infections. Food material may be spoiled due to the microbial contamination, toxic chemical and other agents. Contaminated foods serve as one of the main reservoir and cause of transmission of pathogenic microorganism. Antibiotics are generally for the treatment of foodborne microbial infections but with the time increasing microbial resistance has become a worldwide problem that limits the effect of conventional antibiotics **(Mandal et al., 2010)**.

As an alternative of antibiotics development of drugs based on the plant component may be an effective solution of microbial drug resistance problem **(Basualdo et al., 2007)**. In present work we isolated different bacterial isolates including *Staphylococcus spp. Klebsiella spp.* and *Pseudomonas spp.* 10 bacterial isolates were cultured from spoiled fruits and vegetables and biochemically characterized. The maximum isolates were of *Staphylococcus spp.* (n= 05) while 03 and 02 isolates of *Klebsiella spp* and *Pseudomonas spp.* respectively obtained from spoiled food material. Similar studies for isolation and characterization of bacterial isolates from spoiled food material was reported previously **(Adesetan et al., 2013: Adekanle et al., 2015; Hemalata and Virupakshaiah, 2016; Hasan and Zulkahar, 2018; Devi and Gogoi, 2021; Olanbiwoninu et al., 2024)**. Fresh fruits and vegetables are the major part of healthy diet for humans which improve their health and immune system as an source of vitamin, minerals, carbohydrates, fibers and protein. As we observed that spoiled fruits and vegetables may be a considerable source of microbial reservoir which may cause infections. In vegetables, maximum bacterial isolates were found from bittergourd including *Staphylococcus* and *Klebsiella* while in fruits, maximum isolates from mango (*Staphylococcus* and *Klebsiella*) and banana (*Staphylococcus* and *Pseudomonas*) **(Table no. 2)**. Bacterial strains including *S. aureus*, *P. aeruginosa* and *K. pneumoniae* are well established cause of various human infections such as hospital acquired infections/ nosocomial infections, pneumonia, respiratory infection, urinary tract infections etc **(Esposito and Leone 2007**; **Zhanel et al., 2008; Agaba et al., 2017)**. Antibiotics are most common weapon for the treatment of such bacterial infections. In this study, the antibiotic sensitivity pattern was determined against isolated bacteria and *Staphylococcus* was found resistant against ampicillin, ceftazidime and kanamicin. Ceftazidime resistant *S. aureus* isolates were also found in previous study **(Olanbiwoninu et al., 2024)**. **Adesetan et al., 2013** reported the antibacterial activity of antibiotics against different bacteria including *K. pneumoniae* and *S. aureus*. Similarly **Srinu et al., 2012** reported effectiveness of selected antibiotic against *Staphylococcus* and *Pseudomonas* isolates. We observed that *Klebsiella* isolates were sensitive to all of the antibiotics except ceftazidime and oxacillin **(Figure 1)**. Previously as similar our study, different bacteria including *Staphylococcus* and *Klebsiella* was isolated from spoiled fruits and their sensitivity to different antibiotics has been reported **(Omorodion and Nwiyege, 2023)**. As similar this study**, Khan and Malik, 2001** reported the bacterial strains isolated from foodstuffs and their antibiotic susceptibility to commonly used antibiotics. Presence of pathogenic microorganism in fruit juice has been reported previously **(Ahmed et al., 2010)**. Similar of this study, drug resistance in *Staphylococcus*, *Pseudomonas* and *Klebsiella* isolated from spoiled food samples against different class of antibiotics were reported by **Hemalata and Virupakshaiah, 2016**. In this study we screened the antibacterial potential of medicinal plants methanolic extract and found significant antibacterial activity of drumstick, clove, carom seed, tulsi and sacred fig against *Staphylococcus* and *Pseudomonas* isolates. The growth of *Klebsiella* significantly inhibited by carom seed, black pepper, mint and sacred fig extract. Previously antimicrobial activity of selected plant extracts against bacterial strains causing food poisoning were determined **(Mostafa et al.,**

2018). The antibiotic sensitivity pattern observed in this study may be helpful for the treatment of infections caused by foodborne pathogens and strongly recommend that effectiveness of considerable antibiotics should be tested *In vitro* before use in treatment of infections to avoid side effect and microbial drug resistance. In previously reported study it was scientifically established that medicinal plant may be used for the treatment of bacterial infections as an alternative of antibiotics and a solution of emergence of microbial drug resistance **(Vuorelaa et al., 2004; Faujdar et al., 2020; Sagar et al., 2024**). Microbial drug resistance is increasing continuously worldwide and antibiotics also shows serious side effect in humans during the treatment. Therefore this is need of time for scientific community to focus on an alternative

approach for safe and effective treatment of microbial infections. In this concern medicinal plants that have a wide range of phytoconsitutents should be explored for treatment as well as reduction in emergence of drug resistance. In this study we screened twelve medicinal plants extract and found impactful results against isolated bacterial strains. We observed some of strains were resistant against tested antibiotics but their growth was inhibited using plant extracts this may due to the presence of wide range of antibacterial phytoconstituents of medicinal plants. Our results reveal the effectiveness of plant extracts for inhibition of growth of bacterial strains isolated from spoiled food materials.

Conclusion

The results of this study revealed that the spoiled fruits and vegetables may be contaminated with different bacterial strains and cause food poising and illness. The plant extract may be explored as a natural alternative of antibiotics that have strong antibacterial potential against different bacterial strains isolated from spoiled food material and a solution of safe and effect treatment of foodborne infections as well as emergence of microbial drug resistance.

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Conflicts of interest

There are no conflicts of interest.

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