Original article:

Screening antibacterial activity of lemon grass oil againt bacteria isolted from salad vegetables

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Abstract

This study was carried out to determine the prevalence of pathogenic bacteria in common salad vegetables sold in local markets of Karachi. Eight samples of salad vegetables sold around the market were analyzed. The highest total viable count was found in Onion (8.00 x 10^8 cfu/g), followed by Cabbage and Beet root (4.00 x 10^8 cfu/g), Radish (3.20 x 10^8 cfu/g), Lettuce (2.40 x 10^8 cfu/g), Tomato (1.60 x 10^8 cfu/g), Cucumber (1.60 x 10^5 cfu/g) and Carrot (1.28 x 10^5 cfu/g). *Staphylococcus aureus, Staphylococcus saprophyticus, Bacillus anthracis, Candida* spp, *Klebsiella pneumoniae,* and *Streptococcus aglactie* were identified. Antibacterial activity of lemon grass oil was observed against the identified organisms. *Bacillus anthracis*, *Staphylococcus aureus* and *Streptococcus aglactie* were sensitive while *Staphylococcus saprophyticus, Candida* spp, and *Klebsiella pneumoniae* were resistant. These results indicate that salad vegetables required effective and feasible sanitation methods to remove pathogens like washing with running water, use of hypochlorite solution, chlorine, and acetic acid (vinegar) for surface decontamination of vegetables.

Keywords: Prevalence, Salad vegetables, Local markets, Lemon grass oil, Decontamination.

Introduction

Salad is a term broadly applied to many food preparations that have a combination of chopped or sliced fresh vegetables. Salad vegetables are consumed without any form heat cure, sometimes without washing, thus, has the possibility of causing food borne ailments (Nwankwo, et al., 2015). Several spates of gastroenteritis have been linked to the consumption of contaminated fresh vegetables and fruits (D.H. and R.H., 2006). Common traditional vegetables used in salad include onions, tomatoes, carrots, cucumber, lettuce, radishes, cabbage, peppermint leaves, lemons, pepper, coriander leaves, beetroot, and armenian cucumber. Vegetables are well known sources of useful nutrients in the form of vitamins, minerals, dietary fibers and other phyto nutrients including flavonoids, carotenoids and phenolic compounds that may reduce the risk of cancer, heart disease and other illnesses (O.U, et al., 2013). These vegetables also contain a great variety of other phytochemicals, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties. These factors provide suitable environment for bacterial and other microbial growth and development (F.M., et al., 2013). Vegetables and salads are rich and relatively cheaper source of vitamins. Consumption of these food sources provides taste, palatability, increases appetite and provides fiber for digestion and prevents constipation (Shahrzad, et al., 2014).

Salad vegetables can be contaminated from different sources, such as soil, water, insects, air, birds,

animals and from equipment during cultivation and marketing them. Generally, vegetables have 10^3 to 10^5 microorganisms/cm^3 or 10^4 to 10^7 microorganisms/g. Enteric pathogens can contaminate vegetables if animal or human wastes and polluted water are used for fertilization and irrigation which may cause illness in human. *Listeria monocytogenes, Salmonella* spp., *Shigella* spp., *Clostridium botulinum, Clostridium perfringens, Bacillus anthracis, Mycobacterium* spp., *Brucella* spp., *Yersinia enterolytica, Klebsiella* spp., and *Escherichia coli* are important pathogens reported to cause food borne infections associated with different vegetables. They may also have different types of molds, such as *Alternaria, Fusarium,* and *Aspergillus* growing on their surface. Pathogens present in contaminated foods may harbour virulence genes, toxins and enzymes, which aids in pathogenesis. Raw vegetables may be contaminated during processing and distribution resulting in the release of plant nutrients which may serve as the potential organic and inorganic substrates for microorganisms. Food borne outbreaks are likely to occur worldwide because of the consumption of contaminated ready to eat salad vegetables (Swagato, et al., 2015). The symptoms of food borne illness range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. Surveillance of foodborne illness is complicated by several factors. The first is underreporting. Although food borne illnesses can be severe or even fatal, milder cases are often not detected through routine surveillance. Second, many pathogens transmitted through food are also spread through water or from person to person, thus obscuring the role of foodborne transmission (Paul S., et al., 1999).

Lemon grass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavor. Herbal tea of lemongrass is used as sedatives, febrifuge and immune stimulant while, lemon grass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence, headaches, blood circulation problems and excessive perspiration due to its antimicrobial and antibacterial activities. It has also been used as carminative, stimulant, emmenagogue, diuretic and antiseptic. Several studies have shown that the lemon grass has antibacterial and antifungal properties. It can be used in cleaning wounds and in food poisoning, staphylococcal infections, and other common infections of the colon, stomach, and urinary tract (Bhoj, at el., 2011).

The aim of this study is to determine the prevalence of pathogenic bacteria in common salad vegetables sold in local markets of Karachi.

Materials and methods

Collection of Samples

8 different samples of salad vegetables (carrot, cucumber, lettuce, cabbage, onion, tomato, radish and beet root) were purchased from local markets of Karachi. Each sample was placed in a separate sterile plastic bag and transported to the laboratory for processing.

Isolation of Microorganisms

Each sample of salad vegetable were washed with running water. Knife and cutting board were cleaned by alcohol swab. Salad vegetables were aseptically weighed and crushed. 5 grams of each homogenized sample was dispensed into a prepared 50 ml of enrichment broth. Samples were incubated at 37^0C for 24 hours. Tenfold serial dilution was prepared from 10^{-1} to 10^{-5} by using saline as a diluent. 1 ml of sample was transferred into a test tube containing 9

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ml of saline. The dilution was done in series. 0.1 ml from the last tube 10^{-5} were taken and spread on the nutrient agar. Plates were incubated at 37^0C for 24 hours. Microbial load of each sample was determined as CFU/g and was calculated by using formula Cfu/g = {(No of colonies / Total dilution of the tube X Volume of inoculums}. Organisms were identified on the basis of morphology (gram staining), biochemical test (Catalase, Coagulase, Citrate utilization, TSI, Urease activity and Oxidase test), and confirmed by sub culturing on the selective and differential media such as (Nutrient agar, MacConkey agar, Blood agar, Mannitol Salt agar, Eosine Methylene Blue agar, and Bismith Sulphide agar).

Antibacterial activity of lemon grass oil against identified bacterial isolates by Agar well diffusion method

Each isolate was inoculated into 3 ml of enrichment broth (Nutrient broth for gram positive and negative organisms, and Brain Heart Infusion Broth for fungi). Incubate it at 37^0C for 24 hours. Observed the turbidity and matched with McFarland 0.5 (tube 5). Isolate were spread on Mueller Hinton Agar. 50 micro liter of lemon grass oil were added in a well on the center of the plate. Plates were incubated at 37^0C for 24 hours. Next day observe the zone of inhibition.

Results and discussion

A total of 8 samples were used to examine the prevalence of pathogenic bacteria in common salad vegetables. In our study we isolate different types of gram positive and gram negative bacteria along with fungi that is *Staphylococcus aureus, Staphylococcus saprophyticus, Bacillus anthracis, Candida* spp*, Klebsiella pneumoniae* and *Streptococcus aglactie* which causes food borne diseases. The overall prevalence of *Staphylococcus aureus* was (37.5 %), *Staphylococcus saprophyticus* was (12.5 %), *Bacillus anthracis* was (12.5 %), *Candida* spp was (12.5 %), *Klebsiella pneumoniae* was (25 %), and *Streptococcus aglactie* was (12.5 %) respectively (Figure 1). Bacterial load (cfu/g) of salad vegetables were showed in (Table 1). Results of biochemical tests of the isolates was presented in (Table 2). We also perform the antibacterial activity of lemon grass oil against the identified food borne pathogens. *Staphylococcus aureus, Bacillus anthracis* and *Streptococcus aglactie* were inhibited by lemon grass oil whereas *Staphylococcus saprophyticus, Candida* spp and *Kelbsiella pneumoniae* were not inhibited by lemon grass oil (Table 3).

Ready-to-eat (RTE) foods include any palatable that is normally consumed in its raw state. Demand for RTE food has led to an increase in the amount and selection of different products available for the consumers. Over the last 30 years there has been at least a 24% of increase in the average amount of fresh vegetable consumed per person in the USA. The number of gastroenteritis outbreaks caused by food borne pathogens after consumption of raw vegetables has increased worldwide. *Salmonella* is the most common cause of disease eruptions associated with lettuce and sprouts, there are other pathogens (Shiga toxin, producing *E. coli* O157, Norovirus) that have been described as relevant microbial hazards. Non tuberculous mycobacteria (NTM) are opportunistic pathogens found in the environment that cause life-threatening infections in humans (Jorge, et al., 2015). Microbial population of vegetable surfaces contains a large number of pathogenic bacteria including members of Enterobactereace like *Escherichia coli (E. coli).* A survey was conducted in three major markets of Rawalpindi, Pakistan. Tomato, lettuce, cabbage and cucumber samples were collected. Each vegetable

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was analyzed as unwashed and washed for total coliforms, fecal coliforms and *E. coli* by FAO (Food Quality Manual). About two hundred and fifty *E. coli* isolates were preserved, serotyped for presence of O157 serotype. Total coliforms, fecal coliforms and *E. coli* count exceeded the permissible limits in most samples. The highest Total coliforms were associated with cabbage. Cucumber was the least contaminated by Total coliforms. *E. coli* was detected in tomato, lettuce, cucumber and cabbage. Washed samples showed reduced bacterial population. Seventy-six isolates of *E. coli* were biochemically characterized and serotyped for O157 antigen (Sadia, et al., 2013).

The results obtained in this study represent the microbiological quality of salad vegetables. We isolate different types of pathogenic bacteria including gram positive, gram negative and fungi which causes food borne illness. The high bacterial ratio is observed in *Staphylococcus aureus* and *Klebsiella pneumoniae* whereas the low bacterial ratio was observed in *Staphylococcus saprophyticus, Bacillus anthracis, Candida* spp, and *Streptococcus aglactie*. Salad vegetables can be contaminated from different sources, such as soil, water, insects, air, birds, animals and from equipment during cultivation and marketing them.

From the above research study, it was concluded that food borne diseases occur as the results of ingestion of foodstuffs contaminated with microorganisms or chemical. The symptoms of foodborne illness range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. It is required to continuously monitor the salad vegetables during cultivation, storage and transportation. Sanitary education through training and workshops to producers, suppliers, and retailers can prevent such problems. However, it is impossible to completely eliminate the microorganisms because it is present everywhere but by taking some safety precautions we can control the spread, growth, and survival of these microorganisms. We can reduce the risk of food borne diseases by maintaining personal hygiene. Sanitation practices may also reduce the risk of these organism.

Table 1. Bacterial Load (cfu/g) of Salad Vegetables.

No of Samples	Types of Salad Vegetables	Microbial Load (cfu/g)
1	Carrot	1.28×10^5
2	Cucumber	1.60×10^5
3	Lettuce	2.40×10^8
4	Cabbage	4.00×10^8
5	Onion	8.00×10^8
6	Tomato	1.60×10^8
7	Radish	3.20×10^8
8	Beet root	4.00×10^8

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Table 2. Results of biochemical tests of the isolates.

Types of Salad Vegetables	Gram reaction	Catalase / Coagulase test	Citrate	TSI		Urease activity	Oxidase Test	Identified Organism
				Slant / Butt	Gas / H_2S			
Carrot	+	+ / +	ND	ND / ND	ND / ND	ND	ND	*Staphylococcus aureus*
Cucumber	+	+ / +	ND	ND / ND	ND / ND	ND	ND	*Staphylococcus aureus*
Lettuce	+	+ / -	ND	ND / ND	ND / ND	+	ND	*Staphylococcus saprophyticus*
Cabbage	+	+ / ND	ND	ND / ND	ND / ND	ND	-	*Bacillus anthracis* and *Candida* spp
Onion	-	ND / ND	+	A / A	- / -	+	ND	*Klebsiella pneumoniae*
Tomato	-	ND / ND	+	A / A	- / -	+	ND	*Klebsiella pneumoniae*
Radish	+	- / ND	ND	ND / ND	ND / ND	ND	ND	*Streptococcus aglactie*
Beet root	+	+ / +	ND	ND / ND	ND / ND	ND	ND	*Staphylococcus aureus*

Note: - = Negative, + = Positive, ND = Not done, A=Acidic reaction, K = Alkaline reaction.

Table 3. Antibacterial activity of lemon grass oil by Agar well diffusion method.

Identified Organism	Zone of Inhibition (mm)	Interpretation
Staphylococcus aureus	20 mm	Sensitive
Staphylococcus saprophyticus	0 mm	Resistant
Bacillus anthracis	25 mm	Sensitive
Candida spp	0 mm	Resistant
Klebsiella pneumoniae	0 mm	Resistant
Streptococcus aglactie	20 mm	Sensitive

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