Control of Microbial Load of Fresh Produce Using Phytochemicals

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***Abstract*—Fruits and vegetables form an important part of a healthy diet. This is especially true as nutritionists recommend natural unprocessed food asthe ultimate source of all the goodfibers. However, due to the use of manure and the use of wastewater for irrigation, most leafy productssuch ascarrots, reddish, cabbage, and leafy vegetables have a high load of pathogenic bacteria. This makes the consumption of suchunprocessed food a health concern. Common chemicals used to clear the surface microflora from fresh produce include the use of chlorine and hydrogen peroxide. The presence of these chemicals in trace amounts has harmful effects on health.Fruits of *Garcinia indica*is considered as generally recognized as safe (GRAS) food and are rich in hydroxy citric acid besides many other phytochemicals. Antioxidant activity of the fruit is widely reported. A 10% aqueous extract of *Garcinia indica* was prepared. The extract was completely dried before use. Carrot and lettuce samples were dipped in this solution for 5, 10 and 15 minutes. After the treatment the samples were washed and the wash water was collected for enumeration of bacteria. The control sample of carrot had 10 5 cfu/mL bacterial load whereas the lettuce samples had a mean bacterial count of 10 4 cfu/mL. After the treatment of the samples with the extract for 15 min, the wash water used for lettuce showed 3 log reduction in bacterial count whereas one log reduction was observed in carrot.**

IndexTerms*— Garcinia indica, Lettuce, Carrot, Fresh produce.(keywords)*

I.INTRODUCTION

The increasing public health concern related to the microbial safety of fruit and vegetables has resulted in increased numbers of studies that analyze the efficiency of different methods for reducing the microbial load of fresh produce. [4]. Fresh-cut produce is one of the hottest growing convenience foods because it offers freshness, nutrition, and convenience. Minimally processed fruits and vegetables have increased in popularity because of potential health benefits and a trend toward consumers' desire for "fresh" foods [20]. However, contamination of fresh produce with human pathogens can occur anywhere in their journey from farm to table [24]. The market sales of ready-to-use fresh vegetables have grown rapidly in recent decades because of changes in consumer attitudes, especially consumption of fresh-cut lettuce and carrot due to their use in prepared salads [48].However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for the transmission of human pathogens that were traditionally associated with foods of animal origin.

Despite the increased importance of fresh produce as a vehicle for human pathogens, there is currently limited knowledge about where in the supply chain contamination occurs or about the mechanism by which human pathogens colonize and survive on or in fruits and vegetables [9]. Minimally processed ready-to-eat salads include fresh, washed, and chopped vegetables, and these products are packaged with sealed polymeric films. However, minimally processed fresh-cut vegetables provide a good substrate for pathogenic microorganisms. Several outbreaks of foodborne illness have been traced to minimally processed vegetables [3]. Chlorine is the most widely used sanitizing agent for reducing pathogens on whole and fresh-cut vegetables. The recommended concentrations of chlorine range from 50 to 200 ppm with a contact time of 1–3 min [51]. However, there is a trend in eliminating chlorine from the disinfection process because of the concerns about its efficacy on the product and about the environmental and health risks associated with the formation of carcinogenic halogenated disinfection byproducts. Most of the current investigations have been focused on the search for alternative sanitizers based on assuring the quality and safety of the produce [22].

Fresh produce does not receive any 'lethal' treatment that kills all pathogens prior to consumption. Hence, pathogens introduced at any point of the production chain may be present when the product is consumed. Safe production methods and proper disinfection procedures are therefore critical steps in ensuring the safety of ready-to-eat fresh fruit and vegetables [1]. In the fresh-cut industry, chlorine is commonly used to disinfect produce [11], however, inhibitory, or lethal activity depends on the amount of free available chlorine (in the form of hypochlorous acid, HOCl) and it loses its activity in contact with organic matter and with exposure to air, light, and metals. Furthermore, prolonged exposure to chlorine vapor may cause irritation to the skin and respiratory tract [11]. Therefore, there is much interest in developing safer and more effective sanitizers for fruit and vegetable. Several alternative disinfectants (including hydrogen peroxide, organic acids, and ozone) have been tested to reduce bacterial populations mainly on vegetables [11]. The efficacy of decontamination methods is reflected in the microbiological reduction obtained and, even more important, in the maintenance of this reduction during storage.

An important step in the processing of ready-to-use vegetables is thorough washing , it is desirable if accompanied by rinsing in an antimicrobial solution, to ensure reduction of microbiological load. The effectiveness of disinfection varies greatly with the type and pH of disinfectant, contact time, type of vegetable and the pathogen/microflora present [19].The use of natural antimicrobials such as organic acids, essential oils, plant extracts, andbacteriocinscould be a good alternative to ensure food safety.

Garcinia indica belonging to the family *Guttiferae* (in the mangosteen) is an indigenous tree of India. Also known as "kokum:, it is an important culinary agent and is used as an acidulant for curries by people in India. Studies have shown that (-) -Hydroxycitric acid (HCA) is the major organic acid in kokum leaves and rinds. Hydroxycitric acid is nontoxic as experimental studies have shown that by oral route it did not cause death or systemic or behavioral toxicity even at high dose of 5 g/kg b.wt. Cold Water extraction was used to extract kokum pulp from its dried rinds. This method is often used to maximize, HCA (Hydroxycitric acid) content [8]. Simple practice of washing raw fruit and vegetables removes a portion of pathogenic and spoilage microorganisms, decreasing their initial levels and microbiological activity. Because bacteria cells attach in a relatively short time period to the surface of fruit and vegetables and they tend to locate in protected binding sites, they may escape contact with washing or sanitizing agents, which makes it difficult to remove all cells by vigorous washing or treatment with chlorine. Thus, the success of the washing depends on different factors such as target microorganisms, characteristics of produce surfaces, attachment of cells to produce surfaces, formation of resistant biofilms and internalization of microorganisms, type of washing, exposure time, dose, pH, temperature, etc.Studies have shown that (-)-Hydroxycitric acid (HCA) is the major organic acid in kokum leaves and rinds [8]. Thus, a Hot Water Extract (HWE) of *Garcinia indica* was preparedand used as a dipping solution to study the efficacy of this natural sanitizer to inactivate the pathogens particularly *Escherichia coli* on fresh-cut salad produce.
In this study, we have evaluated the antibacterial efficacy of the widely used plant *Garcinia indica.* The effect of the plant extract is studied on the surface flora of the fresh produce. The following chapters containthe Aims and Objectives, Literature Review, Methodology, Results, and discussions based on the study.

II.AIMS AND OBJECTIVES:

Minimally processed fruits and vegetables have increased in popularity because of potential health benefits and a trend towards consumer's desire for "fresh" foods. The increasing public health concern related to the microbial safety of fruit and vegetables has resulted in increased numbers of studies that analyze the efficiency of different methods for reducing the microbial load of fresh produce. Recently, several outbreaks have been traced to fresh-cut fruits and vegetables that were processed under less than sanitary conditions. Given that fresh-cut products are ready to eat, and are not subjected to further microbial killing steps, the use of effective sanitizing agents during produce washing is necessary to ensure product safety. The recent trend has been for use of natural sanitizers due to the adverse health effect of chemicals ones.Therefore, to eliminate the use of harsh chemical sanitizers, an alternative source of safe, effective and acceptable natural washing sanitizer for the fresh produce needs to be explored.
The study is intended to evaluate the efficacy of the sanitizer prepared from the *Garcinia indica* (Kokum) Hot Water Extract on the surface microflora of the fresh produce.

- Primary screening was performed to detect the presence of *E. coli, which is a commonly associ.*
- Minimum Inhibitory Concentration for *Garcinia indica* was undertaken.
- pH of various concentration of the prepared sanitizing solution was studied.
- Time kill study was undertaken to analyse the time required to kill or remove fraction of the total number of bacteria.
- Artificial inoculation of vegetables using Dip inoculation method was carried out for challenge study.
- The inoculated vegetables were treated with the prepared sanitizer to determine the log reduction in the number of pathogens from vegetable surface.
- Sensory and visual quality of fresh produce before and after sanitizer treatment was evaluated.
- Shelf life and the stability of the extract was also studied.

III. LITERATURE SURVEY:

3.1 Fresh produce:

Tremendous growth in the ready-to-use (RTU) vegetable industry (10% p.a.) has been largely due to increasing demand for fresh, healthy, and convenient foods. A substantial portion of vitamins and minerals in the diet comes from fruit and vegetable consumption [34]. Vegetables and fruits have many similarities with respect to their compositions, methods of cultivation and harvesting as well as storage properties and processing. In fact, many vegetables are considered fruits in true botanical sense. Fruits are those parts of plants that house seeds. Thus, many vegetables like tomato, cucumber, and sweet corn are regarded as fruits.
Vegetables are distinguished from fruits based on their usage. Plant items eaten with the main course of a meal are regarded as vegetables while those consumed alone are considered as fruits. The edible portion of most fruits is the fleshy part of the pericarp. Fruits, in general, are acidic and sugary. They are most grouped in major divisions depending on their botanical structure, chemical compositions and climatic requirements [37]. Minimally processed ready-to-eat salads include fresh, washed and chopped vegetables, and these products are packaged with sealed polymeric films. Consumption of minimally processed and fresh-cut vegetables has increased because of their convenience and their health benefits [3]. Fresh cut fruits and vegetables have been very popular for the bioavailability of numerous vitamins, minerals and other phytochemicals [58]

3.2 Advantage of fresh cut fruits and vegetables:

There is mounting evidence to support the alleviation of many degenerative diseases including cardiovascular disease, cancer and ageing by the consumption of fruit and vegetables [31]. These beneficial health effects of fruit and vegetables have been attributed to the presence of antioxidants that act as receptors of free radicals. Ascorbic acid and β-carotene are the antioxidants present in the greatest quantities in fruit and vegetables. Fresh or fresh cut fruits and vegetables with high levels of vitamins and minerals are an essential part of the world's population's diet [64]. A diet that is rich in fruit and vegetables has been shown to be protective against cancers and chronic illnesses such as coronary heart disease, and the recommended population nutrient intake goal for fruit and vegetable consumption is ~400 g per day [62].

Fresh fruit and vegetables are important components of a healthy and balanced diet; their consumption is encouraged in many countries by government health agencies to protect against a range of illnesses such as cancers and cardiovascular diseases. However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin. Despite the increased importance of fresh produce as a vehicle for human pathogens, there is currently limited knowledge about where in the supply chain contamination occurs or about the mechanism by which human pathogens colonize and survive on or in fruits and vegetables.

3.3 Pathogens of most concern:

Food biological safety is still the top priority of the fresh-cut industry. Although fruits and vegetables are among the safest foods available, pathogens may be present, and a number of outbreaks of foodborne disease have been traced to fresh-cut products. The pathogens most frequently associated with fresh-cut products are discussed below.

***Listeria monocytogenes*:**
Listeria monocytogenes is a pathogenic bacterium that can cause the serious disease "listeriosis". If the organism manages to infect the central nervous system, the disease carries a high mortality rate. One of the factors that makes *L. monocytogenes* particularly difficult to control in fresh-cut fruits and vegetables is its ability to grow at refrigeration temperatures on packaged fresh product. The minimum temperature for growth is reported to be -0.4^{0}C; some strains even seem able to grow down to about - 1.5^{0}C. Growth can occur in the presence and almost absence of oxygen. *L. monocytogenes* has been isolated from minimally processed fresh fruits and vegetables. Previous work has shown that *L. monocytogenes* survives or grows on many processed vegetables, such as ready-to-use lettuce and endive salads. shredded cabbage, and minimally processed artichokes [51] at refrigeration temperatures.

***Escherichia coli* O157:H7:**
Despite the commensal status of most strains, pathogenic strains, particularly enterohemorrhagic *E. coli* O157:H7, is recognized as an emerging foodborne pathogen. Gastroenteritis and hemorrhagic colitis are classical symptoms, while complications including thrombocytopenic purpura and hemolytic uremic syndrome have also been documented [38]. The latter potentially leads to renal failure and death in 3–5 percent of juvenile cases. Fresh vegetables such as lettuce, alfalfa sprouts, and coleslaw have been identified as a vehicle for *E. coli* O157:H7 outbreaks [65]. Researchers have shown these organisms can survive at low pH. In addition, packaging under modified atmosphere has no effect on survival or growth of *E. coli* O157:H7 on shredded lettuce, sliced cucumber, and minimally processed artichokes [52].
***Salmonella*:**
The genus *Salmonella* is composed of over 2700 serotypes. Some pathogenic species of Salmonella include *S. typhimurium, S. enteritidis,S. senftenberg, S. saintaul, and S. montevideo*. Symptoms of salmonellosis include diarrhea, nausea, abdominal pain, vomiting, mild fever, and chills [51]. *Salmonellae* are mesophiles, with optimum temperatures for growth of 35–43°C.
Clostridium botulinum:
The *C. botulinum* species compromises a group of Gram-positive, endospore-forming obligate anaerobic bacteria that form a powerful neurotoxin. Botulism in adult humans is caused by the consumption of food containing the toxin produced by the growth of *C. botulinum.* The severity and fatality rate of botulism have been a significant concern to food processors and consumers since the late 1800s and, as a result, discussion will be restricted to this speciesThe risk of *C. botulinum* on ready-to-eat MAP fresh-cut fruits and vegetables has also been investigated extensively by a number of research groups in recent years [26]. In several surveys,*C. botulinum* has not been detected in fruit and vegetables, but in some investigations a high incidence was found. In a survey of modified-atmosphere-packaged vegetables, one package each of shredded cabbage, chopped green pepper, and Italian salad mix tested positive for type A. A salad mix also contained type A and B spores. The results showed an incidence of 0.36 percent [40].

3.3 Sources of pathogens contaminating the fresh produce:

Although spoilage bacteria, yeasts and molds predominate on raw fruits and vegetables, isolations of pathogenic bacteria, parasites and viruses are not infrequent. This contamination can occur either pre- or post-harvest [13]. Pre-harvest sources include soil, feces, irrigation water, reconstituted fungicides and insecticides, dust, insects, inadequately composted manure, wild or domestic animals and human handling. Human handling can contribute to postharvest contamination along with harvesting equipment, transport containers, insects, dust, rinse water, ice, transport vehicles and processing equipment [13]. Soil is a natural environment for variety of human pathogens including *B. cereus, C. botulinum, and C. perfringens, L. monocytogenes* and *Aeromonas*.Fields that contain animal manure are more likely to be contaminated with enteric pathogens because of their ability to survive in soils for months or years [16]. Feces may naturally contain between 10^2 and 10^5 CFU/g *E. coli* and between 10^2 and 10^7 CFU/g *Salmonella spp*.; slurry between 10^2 and 10^4 CFU/g *E. coli* and *Yersinia spp*., and manure between 10^2 and 10^7 CFU/g *Salmonella* spp. The manure of ruminants (cattle, sheep) and sewage are considered the main sources of *Salmonellaand E. coli* O157:H7. Since *L.monocytogenes* is widely distributed in nature (soil, decaying vegetation), the pathogen is a common contaminant of vegetables, especially root crops [39]. Foodborne pathogens can survive for long periods in animal manure at cool temperatures. *E. coli* O157:H7 survived in bovine manure for over 70d at 5^{0}C [52], but only 49d at 22 or 30^{0}C [52]

3.5 Commonly used chemical sanitizers:

Several sanitizing agents may be used for fruit and vegetable washing with the intention of reducing the risk of microbial contamination, helping in the prevention of postharvest diseases and foodborne illness.

Chlorine (hypochlorite):

In fruits, vegetables and fresh-cut produce, chlorine rinses are frequently used with concentrations varying from 50 to 200 ppm and with typical contact times of less than 5 min [49]. Although chlorine is the most used sanitizer, it is inactivated by organic material and during production can also lead to the liberation of chlorine vapours and formation of chlorinated by-products (DBPs), with potential adverse health effects.[48].

Chlorine dioxide:

As an alternative to chlorine, raw fruits and vegetables can be sanitized with chlorine dioxide (ClO_2) (up to 3 ppm), which has an oxidant capacity 2.5 times greater. In addition, ClO2 does not participate in chlorination reactions that result in harmful by-products [31]. For raw produce, this method is not so efficient at permitted concentration levels. ClO_2 gas has received attention due to its greater penetration ability than liquid.

Several studies showed the efficacy of this gas on produce surface decontamination, particularly against pathogens like *E. coli* O157:H7 and *Listeria monocytogenes* [23] Different factors can influence the lethality of the ClO_2 gas treatment, such as ClO_2 gas concentration, time of exposure, relative humidity, and temperature. ClO_2 is a promising non-thermal technology for reducing pathogenic and spoilage bacteria on fresh produce.

Ozone:

Ozone is a strong antimicrobial agent with high reactivity and penetrability. When used in water, ozone concentrations range from 0.03 to 20.0 ppm. When used in the gas form, the concentration reaches higher doses such as 20,000 ppm [48]. Ozonated water has been commonly applied for sanitation purposes of fresh-cut vegetables achieving some microbial reductions and extending the product shelf-life. Several studies showed that gaseous ozone is generally more effective than aqueous solutions [36]. This treatment was effective against pathogenic and spoilage microorganisms while assuring acceptable product quality.

3.6 Need for natural antimicrobial sanitizing solution:

The use of natural antimicrobials such as organic acids, essential oils, plant extracts, and bacteriocins could be a good alternative to ensure food safety [57]. The use of plant extracts with known antimicrobial properties can be of great significance in food preservation. The value of plants lies in some chemical substances that produce a definite action on the microbiological, chemical and sensory quality of foods, and these phytochemicals have been grouped in several categories including polyphenols, flavonoids, tannins, alkaloids, terpenoids, isothiocyanates, lectins, polypeptides or their oxygen substituted derivatives [17].Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Phytochemicals, especially flavonoids, polyphenols, anthocyanins, and carotenoids, share the major market. Food antimicrobials are chemical compounds or substances that may delay microbial growth or cause microbial death in a food matrix. Antimicrobials are called traditional when they have been used for many years and many countries approve them for inclusion in foods. Although, many synthetic antimicrobials are found naturally (benzoic acid in cranberries, sorbic acid in rowanberries, citric acid in lemons, malic acid in apples, tartaric acid in grapes, etc.), the perception of natural has become important for many consumers. The efficiency of an antimicrobial compound depends on the type, genus, species, and strain of the target microorganism, besides the environmental factors such as pH, water activity, temperature, atmospheric composition and initial microbial load of the food substrate. The antimicrobial nature of phytochemicals is determined by its chemical properties, such as pKa value, hydrophobicity/ lipophilicity ratios, solubility, and volatility [56]. Therefore, it is very important to know the specific characteristics of the food system that needs to be preserved since a high proportion of lipids could limit the effectiveness of some antimicrobial agents [45].

3.7 Plant under study:

Garcinia indica, a plant in the mangosteen family (Clusiaceae), commonly known as *kokum*, is a fruit-bearing tree that has culinary, pharmaceutical, and industrial uses. **Fig 1**. shows the *Garcinia indica* fruit. It is also known as Kokam (Kokum), Amsul, Bidin, Biran, Bhirand, Ratamba in vernacular languages.

Description:

The fruit contains approximately 30% acid calculated as Citric acid. The principal acid was identified as (-)- Hydroxycitric Acid from the fruit rinds and is represented as (-)- HCA. The fruit also has Camboginol, Benzophenone derivatives, Garcinol and iso-garcinol. For the past several years, small and complex molecules have been isolated from the various species of *Garcinia*, which include xanthones and xanthone derivatives. However, the isolation of (-)-hydroxycitric acid [(-)- HCA] from a few species of *Garcinia* and its biological properties have attracted the attention of biochemists and health practitioners.[30].Various species of *Garcinia*contain several secondary metabolites which exhibit a wide range of biological and pharmacological activities such as antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities.

IV. MATERIALS AND METHODS:

4.1 Material:

Fruit rind of *Garcinia indica* was collected from the local market and was authenticated by the Botany Department of Mithibai College, Mumbai. Dried powder was prepared by sun drying the fruit rind. The dried powder was used to prepare the extract. Crude powder of the plant material contains most of the phytochemicals which can be extracted in various solvents.

4.2 Samples:

Fresh carrots and lettuce heads were collected from the local market in sterile bags. The samples were screened for their surface flora and the viable count was enumerated.

4.3 Isolation and identification:

Bacterial isolate of five different strains of *E.coli* were isolated and identified from agricultural samples like carrots and lettuce for the study on culturing on Nutrient Agar and selective media like MacConkey Agar. It was maintained at -20ºC in Nutrient broth supplemented with 15% (v/v) glycerol. When required the cultures were thawed at room temperature. A loopful of culture was transferred to Trypticase Soy broth and incubated at 37ºC for 24h. These were then transferred to Trypticase soy agar slants. Sterile distilled water would be used for preparation of culture suspension. All the media ingredients were obtained from Hi-Media Labs Pvt. Ltd, Mumbai. Based on the cultural and morphological characters, the isolates were inoculated into appropriate biochemicals. The biochemical results were used in identification of the isolates.

4.4 Sample preparation:

Fresh carrots and lettuce heads were taken for this study as the main characteristic rootsystem and leafy vegetables for fresh-cut salads. Once in the laboratory, they were used within 24 hours. Prior to their use, they were stored at 5ºC. The core and outer leaves of whole lettuce heads were removed and discarded. Carrots and lettuce were cut into 3 cm pieces with a sterile stainless-steel knife. Shredded vegetables were homogenized and divided into different batches and stored as 25g portions. .[4]

4.5 Extraction methods:

Preparation of Crude powder:

Crude powder was prepared by sun drying the plant material as explained earlier.

Hot Water Extract (HWE):

Dried powder was added to a beaker containing distilled water. The beaker was heated in a water bath until the volume is reduced to half its original volume. The contents were filtered, and the filtrate was evaporated to dryness. The residue obtained after drying was used as HWE. Hot extraction is more efficient and is used for extractions of all thermostable compounds like Fruit acids, Saponins, Tannins, Sugars, Amino acids, Peptides and Phenolics.

4.6 Study of Minimum Inhibitory Concentration (MIC):

Minimum Inhibitory Concentrations indicates the efficacy of the prepared extract which is used as the vegetable sanitizer. Lower the MIC value, greater is the efficacy. MIC was studied in following way:

Tube Dilution Method:

In this method, various concentrations of the prepared sanitizer were prepared in the tubes containing a suitable medium. A constant volume of the organism was inoculated in the medium and the tubes were incubated at required temperatures. A negative control ensures the sterility of the medium. A loopful from each tube was streaked on a suitable solid medium devoid of any antibiotic after the incubation period. This is done to ascertain bactericidal nature of the sanitizer by observing for the growth.

4.7 pH study:

pH was tested for the different concentrations of the prepared sanitizer i.e., 2%, 4% and 10%. This was done to ensure that the killing of bacteria after sanitizer treatment was not due to the acidity of the fruit rind.

4.8 Time kill study:

Time kill study is used to analyses the fraction of total bacteria killed within the given time interval. The fresh produce was subjected to treatment by sodium hypochlorite(25-200 mg/L) and the prepared sanitizer (1%, 2% and 4%) at various time intervals- 5, 10, 15, 20, 30(minutes). It is the efficacy study between the widely used commercial sanitizer, Sodium hypochlorite and the prepared Hot Water Extract or the sanitizer at various concentrations for different time intervals. Sodium hypochlorite (25-200 mg/L) and the prepared sanitizer (1%, 2% and 4%) were used for time-kill study. The culture used was *E.coli* (10^5 cfu/mL) and studied at various time intervals- 5, 10 , 15, 20, 30 (minutes).

4.9 Artificial inoculation of the vegetables/ Challenge test:

Dip inoculation method was used for challenge study of sanitizer. Samples were placed in a beaker containing inoculum suspension of *E.coli*. 25g of vegetables was dipped in 250mL of inoculum suspension. The beaker was agitated for 30 minutes on the shaker. Following this, spin drying was carried out [24].

4.10 Sanitizer treatment procedure:

Artificially inoculated vegetable pieces as discussed above were dipped into 2%, 5% and 10% aqueous extract of *Garcinia* having sample to wash solution ratio of 1:20 (w/v). Carrot and lettuce were kept submerged for 5, 10 and 15 minutes. The vegetables were then drained and spin dried. Wash water samples were taken for enumeration. A portion of vegetable (25g) was treated in similar manner as explained using sterile water. The sanitizer treated and the water treated vegetables along with untreated sample were liquidized and used for bacterial enumeration[24].

4.11 Evaluation of sensory and visual quality of fresh produce:

Color parameters were evaluated in the vegetables before and after processing. Samples were scored for overall visual quality, where the extremes and center of the interval were represented as follows: 0 'very bad, no characteristic of the product', 5 'limit of acceptance from the consumers point of view', and 10 'very good, very characteristic of the product'. The remaining attributes, flavor and firmness, was evaluated in a 5 point scale, where 5 = fully characteristic of the product, 2.5 = moderate and 0 = not characteristic. Defects of the product, off-odors, browning and dehydration was evaluated as follows: 5 = severe, 2.5 = moderate and 0 = absence [41].

4.12 Shelf life and the stability of the extract:

The sanitizing solution was studied for its stability when left at room temperature and kept in the fridge. At predetermined time intervals, a loopful of sanitizing solution was streaked on suitable solid media to observe for the sterility for a period of about one month. Also, the stability tests were performed. The color, odor, pH value, emulsion stability (signs of separation) was evaluated. For the microbiological stability test, the degree of contamination with bacteria, mould, and yeasts was evaluated.

V.RESULTS AND DISCUSSIONS:

5.1 Study of surface flora:

As presented in **Table 1,** the vegetable samples- Carrot showed an initial bacterial count of 2.38 X10^6 CFU/g and lettuce shows the initial count of 1.02 X 10^6 CFU/g. These results are in agreement with those obtained by [58].

Table 1: Initial viable counts of the vegetable samples

Samples	CFU/g
Carrot	2.38 X 10^6
Lettuce	1.02 X 10^6

(Average of ten samples for each)

5.2 Isolation and Identification:

The isolates were purified using standard biochemical tests. The isolates were cultured on selective media like MacConkey Agar. Based on cultural and morphological characters, the isolates were inoculated into appropriate biochemicals. The biochemical characters were compared with standard biochemical results and the isolates were identified as *Escherichia coli.*

Both vegetable samples showed the presence of *E.coli* on their surface. The isolates identified as *E.coli* were cultured on a selective medium. A total of five isolates were screened on the above-mentioned selective media.

5.3 Determination of Minimum Inhibitory Concentration:

Fresh powder and the Hot Water Extract of the plant material was used for determining the MIC. As displayed in **Table 2,** the crude powder of *Garcinia indica* inhibited all the isolates at a concentration of 10mg/ml. The HWE of *Garcinia indica* also inhibited *E.coli* at 10mg/ml.

Table 2: Minimum Inhibitory Concentration (MIC) in mg/mL

Organism	*G. indica* HWE (mg/mL)
E.coli (Strain 1)	10
E.coli (Strain 2)	10
E.coli (Strain 3)	10
E.coli (Strain 4)	10
E.coli (Strain 5)	10

5.4 pH Study:

pH of the sanitizer was studied for different concentrations- 2%, 4% and 10%. Various acidified compounds have been used as sanitizers, although their mode of action may not be solely due to low pH [18]. pH of 2% was found to be 5 whereas, for 4% and 10%, it was found to be 3.5 and 2 respectively.

Concentration (%)	pH
2	5
4	3.5

10	2

Table 3: pH of the sanitizer

5.5 Efficacy Study of the Hot Water Extract:

The vegetable samples were exposed to 10ml of 2% HWE of *G.indica* for 5, 10 and 15 minutes. The dried crystals obtained after complete drying of the Hot Water Extract which served as the sanitizer has been displayed in **Fig 1.**

Appropriate controls were maintained to establish the initial bacterial count. Water washing control was also maintained to estimate the efficacy for the said time intervals. The results are presented in **Tables 4a and 4b.**

The carrot sample had an initial bacterial count of 10^6 CFU/g. The lettuce sample also showed an initial bacterial count of 10^6 CFU/g. At 15 minutes of water washing for carrot, the water sample provided no log reduction. However, both 10- and 15-minutes sanitizer treatment gave 1 log reduction. At 10 minutes water washing for lettuce one log reduction was observed whereas, the sanitizer treated lettuce showed maximum reduction of 2 log after 10 minutes, 3 log after 15 minutes as displayed in **Graph 1 and 2**.

Water treatment by itself did not cause a significant change in pathogens count. Thereduction of less than 0.5 log CFU/g of *E. coli*, *Salmonella*, and *L. monocytogenes* observed on shredded carrots was similar to that reported previously [24]. Peroxyacetic acid reduced *E. coli* by 1.24 log CFU/g as shown by Ruiz-Cruz et al. (2007). However, the reduction of *E. coli* observed in this study is slightly less than those reported by others.[24]. The results of the study agrees with a recent study that has reported that acidified sodium chlorite at 1200 ppm was capable of reducing *E. coli* O157:H7 and *Salmonella* on spray-inoculated lettuce by more than 3 log CFU/g [56].

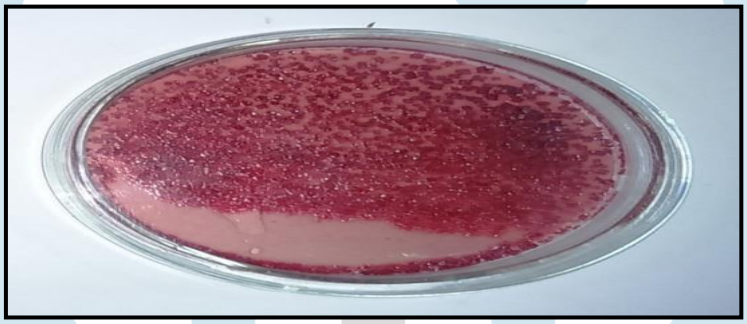


Fig 1: Crystals obtained after evaporating the HWE to complete dryness

Time (minutes)	Control (CFU/g)	Water Washing (CFU/g)	Sanitizer treatment 2% (CFU/g)
5	2.38×10^6	2.1×10^6	2.49×10^6
10			4.30×10^5
15			1.02×10^5

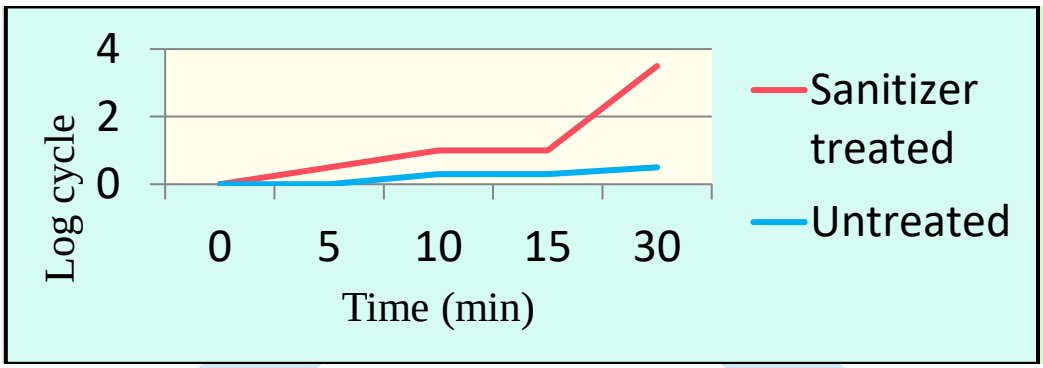
(Average result of ten samples)

Table 4a:Viable count of bacteria from the surface of carrot after sanitizer treatment

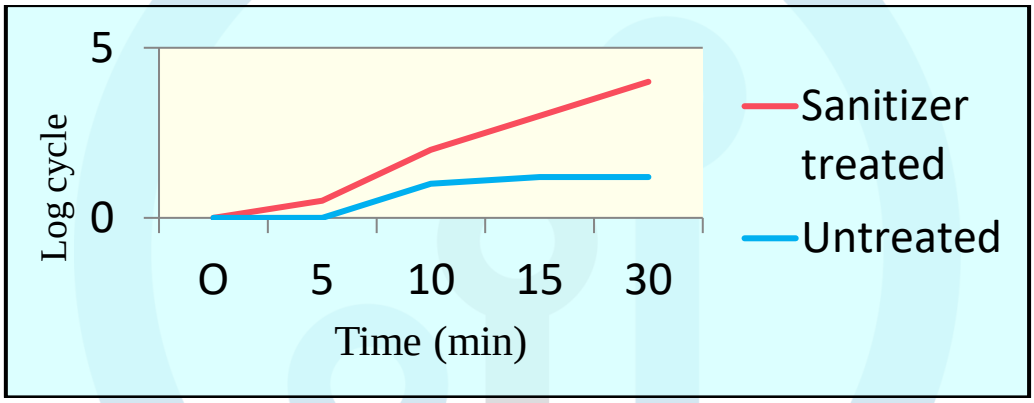
Time (minutes)	Control (CFU/g)	Water Washing (CFU/g)	Sanitizer treatment 2% (CFU/g)
5	1.02×10^6	3.2×10^1	2.2×10^1
10			2.9×10^2
15			1.6×10^3

(Average result of ten samples)

Table 4b: Viable count of bacteria from the surface of lettuce after sanitizer treatment



Graph 1: Log reduction for carrot

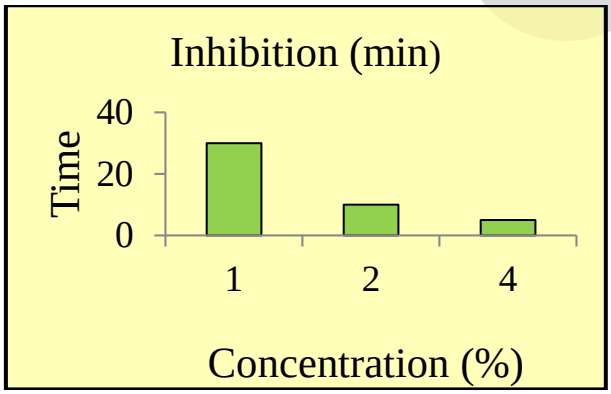


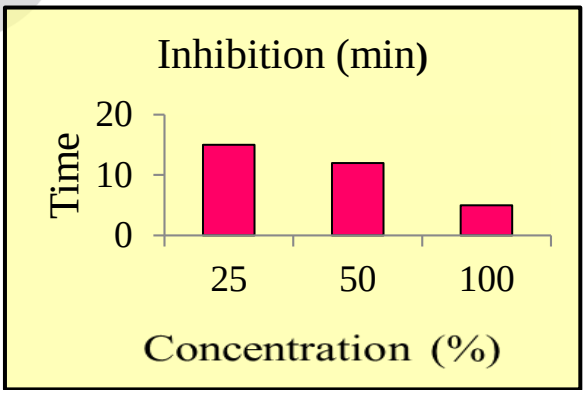
Graph 2: Log reduction for lettuce

5.6 Time kill study:

The time-kill study basically determines the time required to kill the pathogens associated with the fresh produce on treatment with the sanitizer. In other words, it is the analysis based on contact time. The vegetable sample was treated with the widely used commercial sanitizer, sodium hypochlorite at a concentration range of 25-200 mg/L and the prepared sanitizer at the concentration of 1%, 2%, and 4% for different time intervals- 5, 10, 15, 20, 30 (minutes). Also, the commercial sanitizer, Sodium hypochlorite, and the prepared Hot Water Extract or the sanitizer at various concentrations and for different time intervals as mentioned above were compared.

Graph 3, shows the fraction of pathogens and spoilage microorganisms killed on treatment with the prepared sanitizer and sodium hypochlorite at different time intervals. From the graph, it was seen that as the concentration of both the sanitizers increased, the efficacy increased with a decrease in the contact time. Sodium hypochlorite showed maximum reduction at a concentration of 100 mg/L with a contact time of 5 minutes. However, the prepared sanitizer with the same contact time of 5 minutes showed a maximum reduction at a concentration of 4%.The sanitizer under the study is plant-derived and does not have any side effects or the formation of harmful by-products at the concentration used (2%). It eliminates harmful pathogens while maintaining the safety, preservation, and shelf-life of these products.





Graph 3: Time kill graph of the prepared sanitizer (left) and of sodium hypochlorite (right)

5.7 Evaluation of visual and sensory quality of fresh produce:

The vegetable samples carrot and lettuce retained their freshness and firmness after they were treated with 2% sanitizer for 15 minutes as displayed in **Fig 3.** However, when vegetable treatment was carried out with higher concentrations of sanitizer and for longer periods, the vegetable samples could not retain their texture and appeared to be shriveled and limp. The sample was scored as 10 'very good, very characteristic of the product' for the overall visual quality, the remaining attributes, flavor, and firmness, was evaluated to be 5 = fully characteristic of the product. Defects of the product, off-odors, browning, and dehydration were found to be absent.

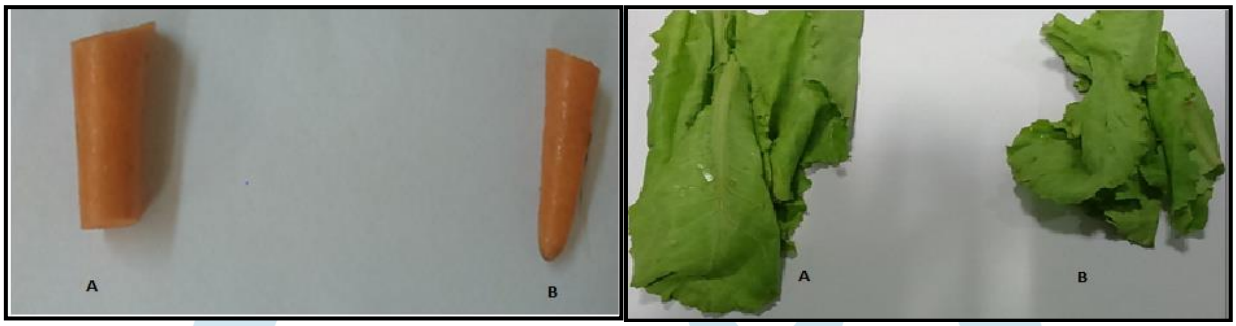


Fig 3: Carrots (left) and lettuce (right) after treatment with the sanitizer. A: Untreated samples B: Sanitizer-treated samples

5.8 Shelf life and the stability of the extract:

Data in **Table 5** shows, that the color of the sanitizer was evaluated to be dark red. On storage for about one month, the color of the sanitizing solution did not change. The odor was found to be pleasant, and the pH of the sanitizing solution did not change and was evaluated to be 5. Also, it did not show any signs of separation thus, maintaining its emulsion stability. All the above-mentioned parameters were maintained over the period of one month.
As the microbiological stability was evaluated, the sanitizing solution was found to be completely sterile when streaked on the appropriate solid media for 15 days and for a month. It was found that the physical parameters and the microbiological stability of the sanitizing solution were maintained when left at room temperature for a period of one month.

Table 5: Stability tests of the extract

Physical Parameters	Observation
Color	Deep red
Odor	Pleasant
pH value	5
Emulsion stability	Stable/ No phase separation

VI. CONCLUSION:

Fresh-cut fruits and vegetables with high levels of vitamins and minerals are an essential part of thediet. However, they are no longer considered low risk in terms of food safety. Present investigation indicates that raw produce like carrots and lettuce have a very high bacterial population on their surface when fetched from the local market.
Commonly used chemical sanitizers have various harmful effects and so, an alternative source of effective sanitizing solution needs to be explored. Traditionally, processors have used water with or without chemical sanitizing agents to rinse fresh-cut and minimally processed produce. Chlorine has been the most widely used sanitizer. However, chlorine has a limited effect in reducing microorganisms on fruit and vegetable surfaces. In addition, concerns have been raised about the residual chlorine by-products that may be generated, such as trihalomethanes, in the wastewater. There has been an increasing interest in the discovery of new natural antimicrobials and disinfectants due to an increase in risk in the rate of infections with antibiotic-resistant microorganisms. Thus,

in this present study, a sanitizer from the *Garcinia indica* Hot Water Extract (HWE) was prepared to improve the microbiological quality of the fresh produce.All the isolates were identified to be *Escherichia coli* based on biochemicalcharacteristics. The HWE of *Garcinia indica* was found to inhibit *E.coli* at 10 mg/mL. pH of various concentrations of the sanitizer was found to be as follows: 2% concentration showed a pH value of 5 whereas, for 4% and 10%, pH values of 3.5 and 2 respectively were observed. 2% concentration of the sanitizer with a treatment time of 15 minutes could reduce microbial load and did not affect the sensory, visual and organoleptic quality of fresh produce. It had no browning effect and maintained the freshness of the treated vegetables. However, brownness and softness of the vegetables was observed at higher concentrations of the sanitizer. Thus, 2% concentration was selected to be appropriate with a contact time of about 15 minutes.

VII. FUTURE PROSPECTS:

Commercial use of the plant-derived natural sanitizer is believed to overcome the potential hazards of chlorine to the environment and human health. Therefore, the sanitizer can be formulated from the HWE of *Garcinia indica* that would serve as a safe, natural, and effective alternative to chemical sanitizers.

The prospects of the study are as follows:

- Modern produce washers should be designed to assist with the use of this sanitizing solution that would besides removing fluids and exudates from cut surface, also improve the microbiological quality of the fresh produce.
- For the future, more studies should be carried out to determine the antimicrobial and synergistic effects of other plant extracts that would control all the parameters whilst maintaining the quality and shelf-life of the minimally processed products.
- Further studies should be elucidated with the different contact time to simulate typical commercial conditions in addition to the other types of fruits, vegetables, and microbial pathogens.

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