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Original article

A effects plant extract shows novel sanitising against *Escherichia coli* on romaine lettuce and cucumber

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Background: Diarrhoeal diseases affect approximately 550 million people and account for 230,000 deaths every year globally. Children in particular are at risk of foodborne diarrhoeal diseases, with 220 million falling ill and 96,000 dying every year. In 2017, an outbreak of diarrhoea caused by *Escherichia coli (E. coli)* occurred as a result of consuming contaminated/ill-washed Romaine lettuce in the USA. Consumption of ready-to-eat vegetables (RTEV) is increasing around the globe.
Objective: The current study sought to assess the efficacy of extracts of selected African medicinal plants [*Lannea edulis* (Sond.) Engl. var. *Edulis* (*L. edulis*), *Cassia abbreviata* Oliv. (*C. abbreviata*), *Solanum incanum* (*S. incanum*) and *Grewia bicolor* (*G. bicolor*)] on removal of *E. coli*, an indicator of faecal contamination from *Lactuca sativa* L. var. *longifolia* (*L. sativa*) (Romaine lettuce) leaves, and *Cucumis sativa* L. (*C. sativa*) (cucumber) surfaces.
Methods: Two cm^2 square sections of lettuce and cucumber epidemal tissues were soaked in extracts of the medicinal plants (10^{-1} of original extracts) for 1 hr. Washing of the RTEV with sterile water caused significant reductions in *E. coli* loads on lettuce or cucumber ($P < 0.01$) surfaces compared to unwashed controls.
Results: Extracts from the medicinal plants caused reductions in *E. coli* loads on all both RTEV (load reduction by at least 2 log colony forming units (CFU)/cm^2) ($P < 0.01$) without changes in surface morphology and colour. Use of extracts from *L. edulis*, *C. abbreviata*, *S. incanum* and *G. bicolor* have potential for use in sanitisation of RTEV, especially in killing, inhibiting or removing pathogenic *E. coli* that are transmitted via these fresh foods and products. While washing with *G. bicolor* resulted in significant reductions of *E. coli* on cucumber and lettuce surfaces, its efficacy was lowest compared to the rest of the treatments; hence, it is the least favourable of the selected medicinal plants. Soaking in the extract preparations did not lead to significant changes in odor, texture or taste of the vegetables.
Conclusion: Use of extracts from these plants is suggested as a possible way to sanitise RTEV to reduce occurrence of, or contain outbreaks of diseases that are borne on these vegetables.

Keywords: *Cucumis sativa*, *Lactuca sativa*, romaine lettuce, cucumber, diarrhoea, medicinal, inhibition, WASH.

Diarrhoeal diseases affect approximately 550 million people and account for 230,000 deaths every year; more than half of the global occurrence of foodborne diseases. Children in particular are at risk of foodborne diarrhoeal diseases, with 220 million falling ill and 96,000 dying every year. [(1)] Populations of low-income countries around the globe are frequently affected by the outbreaks of pathogenic *Escherichia coli* (*E. coli*). [(2)] Populations of high income countries are not spared from the food-borne pathogens, however. A number of outbreaks of diarrhoea implicated on consumption of *E. coli* contaminated Romaine lettuce have been reported in USA. [(3 - 5)]

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Hygiene requirements, hazard analysis and critical control points (HACCP) manual and processing of raw vegetables must be done under the following conditions: (i) raw vegetables must be washed or cleaned to remove soil and other contamination; (ii) water used for washing, rising or conveying food products must be of sanitary quality; (iii) water must not be reused for washing, rinsing or conveying if contamination of food may result;and, (iv) raw materials shall not contain levels of microorganisms that may produce food poisoning or other disease, or they shall be pasteurised or otherwise treated during manufacturing operations so that the product will not be adulterated. [6] While these guidelines are present, erratic cases of food poisoning emenating from vegetables remains a concern. [7] Vegetables that are cooked or cured prior to packaging or consumption carry a reduced risk of pathogen transmission than do ready-to-eat products. [8] To reduce risks of transmission of pathogens through raw vegetables, care must be given to reduce levels of contamination with pathogenic microorganisms.

While it is a standard practice to wash vegetables prior to consumption, risks of transmssion of pathogens remain high. [9] Ready-to-eat vegetables are a normal part of Western diets but not in most low-income countries. Despite the fact that ready-to-eat vegetables (RTEV) are widely consumed in the developed world, coupled with the availability of clean water to wash the produce, RTEV are often implicated for outbreaksof food-borne pathogens. [10] The emergence of a RTEV eating habit in low income countries, especially as salads and in sandwiches, is thought to be linked to a sustained high burden of diarrhoea. [11] Although tap-water is often clean and potable in many developed countries, the efficacy of this water alone in inhibition, killing or removal of bacteria from vegetables is low. [12] As for low income countries, unavailability of potable water poses an added risk to consumption of RTEV since wash waters may be adding pathogens rather than removing them. Chlorine or sodium hypochlorite are widely used to clean RTEV. [13] However, chlorinated compounds, when used on RTEV result in the formation of trihalomethanes, which are linked to cancer and adverse reproductive outcomes. [14] Chemical sanitisers have another drawback in that they often lead to off flavors and odors if retained at the surface of these products. [13] Recent research by Min SC, *et al.* [15] yielded a novel plasma based method of inhibiting packaged Romaine lettuce. This method has potential, its downside is that installation of plasma generating machinery is costly. [15] Hence, we derived a sanitisation method from nature – relying on extracts from medicinal plants.

The method described here has its strength that the extracts are edible and they do not change the texture, cosmetic appeal and organoleptic properties of washed vegetables. Additionally, energy requirements can be reduced since the method can rely on unheated, chlorinated, water in places where domestic water is of such quality. This project was undertaken to establish a plant extract based method for sanitising RTEV against *E. coli*.

Materials and methods

The method to obtain the substances tested that contains the sub-protocols: (i) extraction of crude extracts from selected Southern African plant species; (ii) methods to develop efficaceous plant extract based formulations; (iii) method of soaking RTEV to kill, inhibit and/or remove pathogens; and, (iv) a rinsing step to remove residual plant extractsthat can affect the organoleptic properties of RTEV and residual and microorganisms. Plant materials used consisted of leaves, bark and roots of mature plants drawn from *Aloe* spp., *Cassia abbreviata* Oliv. (*C. abbreviata*), *Grewia bicolor* (*G. bicolor*), *Lannea edulis* (Sond.) Engl. var. *Edulis* (*L. edulis*), *Solanum incanum* (*S. incanum*), *Strychnos spinosa* (*S. spinosa*) and *Ximenia caffra* (*X. caffra*) (Table 1). These were collected from the Chivhu and Bindura Districts of Zimbabwe. Ready-to-eat cucumber (*Cucumis sativus* L.), tomatoes (*Solanum lycopersicum* L) and lettuce (*Lactuca sativa* L.) were purchased from Bindura Vegetable Market and Bindura Supermarket.

The plants contain several phytoactive chemicals. The formulations and methods developed were based on traditional knowledge and use with the aim of reducing counts of enterobacteriae, particularly *E. coli*, on the surfaces of RTEV. The plants are widely available in the Southern African biome. Formulations and methods would be low cost and innovative ways to sanitise RTEV in domestic and industrial settings where RTEV are frequent vehicles for the spead of diarrhoeal pathogens.

Table 1. Plants used to obtain extracts.

Scientific name	Local common name	Part used	Use	Reported pharmacological use
Ximenia caffra Sond. var. *caffra*	Munhengeni/Mutengeni (Shona), Sourplum (English), Umthunduluka (Ndebele)	Roots and leaves	Diarrhoea and infertility	Flavonoids, phenolic and tannins with antimicrobial activities [16]
Aloe species	Gavakava (Shona), Aloe (English)	Leaves	Gonorrhoea and constipation	Alkaloids, phenolic compounds and antioxidant activity [17]
Strychnos spinosa Lam.	Mutamba - mun'ono (Shona) Spiny monkey-orange (English) Umhahli/Umngono (Ndebele)	Roots and fruits	Abdominal pains	Sterols and triterpenoids [18]
Lannea edulis (Sond.) Engl. var. *edulis*	Mutsambatsi/Tsombori (Shona), Intakubomvu (Ndebele), Wild grape (English)	Roots	Bilharzia (schistomiasis), diarrhoea and gonorrhoea	flavonoids, tannins, alkylphenols (cardonol 7 and cardonol 13) and dihydroalkylhexenones [19]
Cassiaabbreviata Oliv.	Muremberembe/Muvheneka (Shona), sihaqa (Ndebele), Long-tail cassia (English)	Roots	Constipation, diarrhoea and gonorrhoea	Polyphenolics, particularly proanthocyanidins, epicatechin, epiafzelechin, epiguibourtinidol, and entcassiaflavan monomers and their dimers, trimers [20]
Solanum incanum sensu auct. pro parte	Poison apple/Snake apple/ Sodom apple/Thorn apple/Bitter apple (English), Munhomboro/ Munhundurwa (Shona), Intume/ Umdulukwa (Ndebele)	Fruits	Rash, ringworm, skin infections and warts	Flavonoids, phenolic acids including chlorogenic acid 5-(3''-(3', 4'- dihydroxyphenyl) acryloyloxy)-2, 3, 4-trihydroxypentanoic acid [5- caffeoyl-2, 3, 4-trihydroxypentanoic acid], alkaloids, tannins, triterpenoids, glycosides, steroids, resins and saponins [21-22]
Grewia bicolor Juss.	Mutongoro (Shona), Umhlampunzi/ Umpumpulwane (Ndebele), White-leaved raisin (English)	Roots	Diarrhoea	Beta-sitosterol, beta-sitosterol esters, triterpenes (lupeol and betulin) triterpene esters, beta-sitosterol-glucoside, alkaloids (harman, 6-methoxyharman and 6-hydroxyharman) [23]

The method to obtain the plant based sanitisers involves soaking of RTEV for 1 to 3 hr. This method relies on use of ordinary household containers to wash vegetables. The protocol describes an efficaceous method of cleansing RTEV prior to processing including packaging, serving and consumption of RTEV. The method included: (i) extraction of crude extracts from selected Southern African plant species; (ii) developing a protocol to develop efficaceous plant extract based formulations for removal, killing or inhibiting of diarrhoeal pathogens; and, (iii) a rinsing method used in conjunction with the formulations to rid vegetables of residual extracts and diarrhoeal pathogens from surfaces or lodgements on RTEV. A spray nozzle can be applied to supply clean water during the rinsing phase. The rinse water can be ordinary tap water (where safety is monitored) or pasteurised (boiled) to kill pathogens.

The plants were selected on the basis of traditional uses and extracts were obtained based on traditional use. One hundred grams (g) of the air-dried plant materials were immersed into 100 mL of distilled water and left for 24 hr. The extraction procedure consisted of macerating medicinal plant tissues using a pestel and mortar, or with a blender followed by soaking in distilled water for 24 hr to obtain extracts. Any extracts that encouraged growth of microorganisms as shown by Gram staining were declared not efficaceous and therefore discarded. The extracts were evaporated in a Sohxlet apparatus at room temperature at room temperature until a dry residue of extraxt was obtained. The dried extract was weighed and resuspended in a known volume of diluent to achieve initial concentrations of 100 - 200 mg/mL to obtain a stock solution. The stock was diluted to the minimum inhibition concentration (MIC) (defined as the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after overnight incubation) of each extract [(25)] to concentrations between 17.4 and 1,595 μg/mL to determine level needed to kill pathogens during treatment. The vegetable pieces were soaked in the extract solutions for 1h after which they were rinsed twice with clean/sterile water.

The study was carried out in 2 stages. In the first vegetable pieces (2 × 2 cm squares) were soaked in extract or control solutions (sterile Ringers solution or sodium hypochlorite) for 1 to 3 hr. The pieces were rinsed twice in sterile Ringer's solution to remove extract residue. The pieces of the RTEV were placed onto soild MacConkey Agar in Petri dishes. Care was taken to ensure that complete prints of the RTEV piece sufaces were made on the agar. The experiment was in triplicate plates per treatment. Plates were incubated for 48 hr at 35°C.

Counts on cucumber and/or lettuce are presented as mean ± standard error or mean (SEM). Differences in efficacies of the treatment were compared using one-way (treatments) analysis of variance (ANOVA) using Turner's [(25)] an online tool: https://goo.gl/iA1EYp. $P < 0.05$ was considered statistically significant in all analyses. Concentrationsbelow MIC were all found to lead to insignificant reductions in CFU/cm^2 of vegetable surface; hence, they were left out from the analyses.

Results

Evaluation of antimicrobial effects of formulations

Reduction of *E. coli* counts on cucumber and lettuce surfaces

The vegetables were deliberately contaminated with *E. coli* (an environmental strain). The *E. coli* was grown overnight in MacConkey broth at 37^{o}C. The concentration of the *E. coli* culture was adjusted to 10^6 CFU/mL using an internally established protocol where an optical density of 0.2 equates to 1.6 × 10^8 CFU/mL when measured at 600 nm. 6.25 mL of the *E. coli* culture at the original concentration were diluted into 1L of Ringers solution to achieve a concentration of 1 × 10^6 CFU/mL. Contamination was achieved by soaking the vegetables in a spiiked Ringers solution for 1 hr. The vegetables were then dried at room temperature over 2 hr cut sections were then obtained; they were subsequently soaked in extract solutions (test) or left unsoaked (negative control).

The results show that the vegetables had the following initial loads of *E. coli*: (i) cucumber: 7.1 $\log_{10}$/cm^2 ; and, (ii) letuce: 5 $\log_{10}$ CFU/cm^2. Soaking in- and rinsing with sterile water did not yield significantly different reductions in the counts of *E. coli* per cm^2 of cucumber or lettuce surfaces. However, soaking in a 10^{-1} solution of *Aloe* spp., *C. abbreviata*, *G. bicolor*, *L. edulis*, *S. incanum*, *S. spinosa*, *X. caffra* or combinations of these extracts resulted in significant reductions in the counts of *E. coli* per cm^2 surfaces of each RTEV. These results suggest that *S. spinosa*, *S. incanum*, *L. edulis* and *Aloe* species had the strongest sanitising effects.

When effects of the extracts were considered between lettuce vs cucumber (Table 2), only *G. bicolor* indicated significantly greater sanitisation against *E. coli* on cucumber than on lettuce. Washing of lettuce with sterile water yielded significantly lower CFU of *E. coli* than cucumber with the same treatment (*P* = 0.035).

When effects of the extracts (or control treatments) were considered (Table 3), the results showed washing of cucumber with sterile water, vinegar, sodium hypochlorite or extracts resulted in significant reductions in the counts of *E. coli* (CFU/cm^2) compared to not washing (*P* < 0.01).

When effects of the extracts (or control treatments) were considered (Table 4), the results showed washing of cucumber surfaces with vinegar, sodium hypochlorite or extracts (single or combined) resulted in significant reductions in the counts of *E. coli* (CFU/cm^2) compared to washing with sterile water (*P* < 0.01).

When effects of the extracts (or control treatments) were considered (Table 5) the results showed washing of cucumber surfaces with *C. abbreviata* resulted in significant reductions in the counts of *E. coli* (CFU/cm^2) compared to washing with the rest of the extracts (*P* < 0.01). Additionally, washing with *L. edulis*, *S. spinosa*, *C. abbreviata*, *S. incanum*, *X. caffra* and/or *Aloe* spp., resulted in significant reductions in the loads of *E. coli* on cucumber surfaces than with *G. bicolor* (*P* < 0.001). However, as shown in Table 4 and Table 5, washing of cucumber surfaces with *G. bicolor* led to significant reductions in the counts of *E. coli* compared to unwashed or washing with sterile water.

The results for loads of *E. coli* on lettuce after washing with the same treatments as above yielded comparable results (Figure 1).

This example shows that soaking and washing of cucumber or lettuce leaves with sterile water yielded a significant reduction in the loads of *E. coli* compared to the unwashed (*P* < 0.01) (Table 3). Soaking of cucumber or lettuce in vinegar, sodium hypochlorite and extracts from all selected plants or their combinations (vs the unwashed/soaked in tap-water) yielded significant reductions in the *E. coli* loads on the surfaces.

Soaking of vegetable sections in vinegar, sodium hypochlorite or extracts of *C. abbreviata*, *S. incanum*, *G. bicolor* or combined extracts from the three plant species for 1 hr also (vs the unwashed/soaked in tap-water) yielded significant reductions in the *E. coli* loads. Notably, soaking in vinegar or sodium hypochlorite (8 µl/L) resulted in greater reductions in the loads of *E. coli* from the surfaces of the fruits and vegetables.

Duration time after dipping

The bacterial survival after dipping of fruits or vegetables in the formulations was evaluated to assess the best time for the treatments compared to a water-only wash. An aqueous composition of the present method was used in a 1:10 dilution with water.

Table 2. Pairwise comparison (ANOVA) of inhibitory efficacies of each treatment or control on loads of *E. coli* on cucumber vs lettuce surfaces (mean CFU/cm^2). Extracts were tested at respective MIC (µg/mL).

Treatment	F	*P* - value
UWC vs UWL water	105	0.0070
GBC vs GBL	33	0.0350
SWC vs SWL	30	0.0394

UWC denotes unwashed cucumber.
UWL denotes unwashed lettuce.
GBC denotes cucumber washed with *G. bicolor* extract.
GBL denotes lettuce washed with *G. bicolor* extract.
SS denotes sum of squares.
MS denotes mean square.
df denotes degrees of freedom.
F is the F value, a statistical test of significance between means (Kao *et al.*, 2008).

Table 3. Pairwise comparison (ANOVA) of inhibitory efficacies of extract of plant extracts or control treatment vs (unwashed) on loads of *E. coli* on cucumber surfaces (mean CFU/cm^2). Extracts were tested at respective MIC (μg/mL).

Treatment	F	*P* - value
UW* vs sterile water	125	0.0054
UW vs vinegar	3,083	< 0.001
UW vs sodium hypochlorite	3,264	< 0.001
UW vs combined extracts	1,493	0.0001
UW vs G. bicolor	1,617	0.0001
UW vs G. bicolor	1,617	0.0001

* UW denotes unwashed.

Table 4. Pairwise comparison (ANOVA) of inhibitory efficacies of extract of plant extracts CFU/cm^2). Extracts were tested at respective MIC (μg/mL).

Treatment	F	*P* - value
SW vs sodium hypochlorite	1,875	0.0000
SW vs vinegar	1,745	0.0001
SW vs combined	3,264	< 0.001
SW vs*C. abbreviata*	1,608	< 0.001
SW vs*G. bicolor*	1,617	0.0001
SW vs*S. spinosa*	1,508	0.0001
SW vs*L. edilus*	1,392	0.0001
SW vs *X. caffra*	1,481	0.0001
SW vs*Aloe*spp.	1,348	0.0001

* SW denotes sterile water

Table 5. Pairwise comparison (ANOVA) of inhibitory efficacies of plant extracts (pairwise) on loads of *E. coli* on cucumber surfaces (mean CFU/cm^2). Extracts were tested at respective MIC (μg/mL).

Treatment	F	*P* - value
C. abbreviata vs *G. bicolor*	1,608	0.0001
C. abbreviata vs *S. spinosa*	1,617	0.0001
C. abbreviata vs *L. edulis*	1,508	0.0001
C. abbreviata vs *X. caffra*	1,392	0.0001
C. abbreviata vs *L. edulis*	1,508	0.0001
C. abbreviata vs *X. caffra*	1,392	0.0001
G. bicolor vs *Aloe* spp.	1,481	0.0001
S. spinosa vs *L. edulis*	0.0190	0. 9929
S. spinosa vs *X. caffra*	27	0.0447
S. spinosa vs *Aloe* spp.	3	0. 4032
L. edulis vs *X. caffra*	45	0.0233
L. edulis vs *Aloe* spp.	4	0. 3285
X. caffra vs *Aloe* spp.	73	0.0117

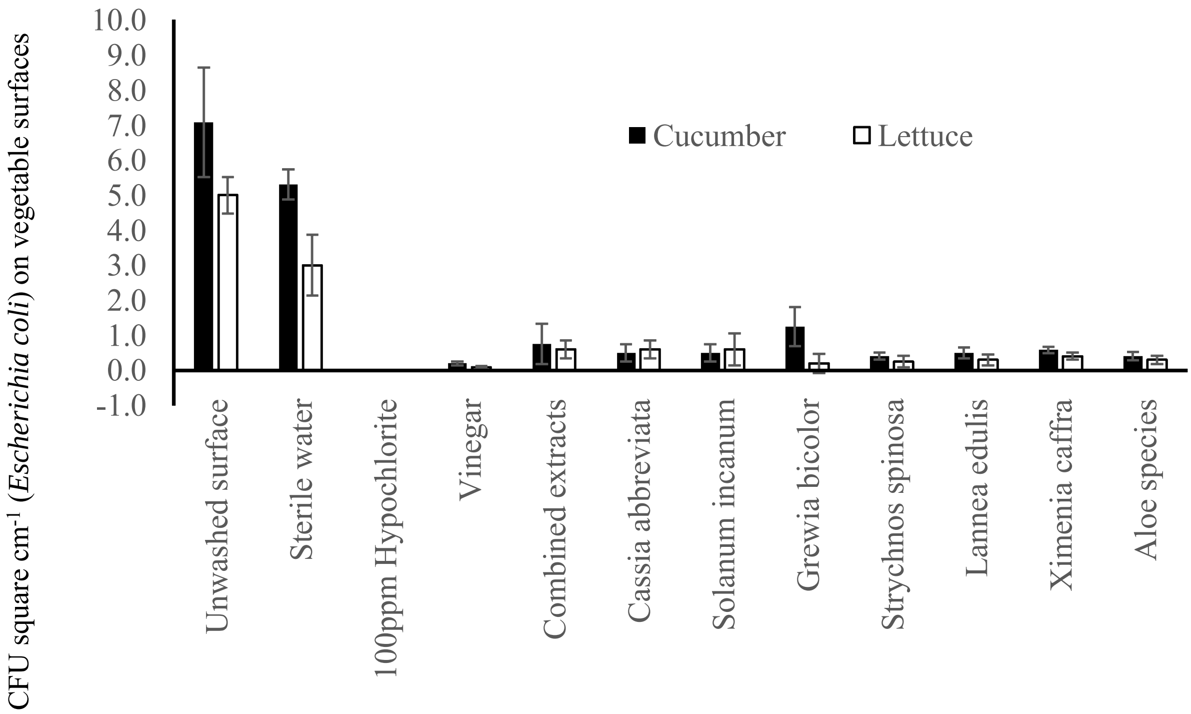

Figure 1. Reduction of presumptive *E. coli* counts on lettuce and cucumber surfaces.

The results of the experiment, not shown here, demonstrated that soaking for times less than 30 min were not effective enough to inhibit or kill *E. coli* or other species present on the foods. Soaking of the RTEVin formulations for periods longer than 1 hr reduced the loads of microorganisms at the surfaces of the products by at least 12 times (7.0×10^5 CFU/cm^2 to 4×10^3 CFU/cm^2 or approximately 2 log CFU reduction/cm^2). Soaking in sodium hypochlorite (8 µl/L) resulted in total eradication of all *E. coli* on the fruits an vegetables within 30 min. Therefore the duration of soaking of the fruits and vegetables in the plant extracts must be done for periods greater than 1h for significant reductions in pathogenic bacterial loads, especially *E. coli.*

Discussion

Our results demonstrate that soaking and washing of lettuce leafand cucumber surface squares with either tap or sterile water was not effective enough to reduce the loads of *E. coli* on the surfaces to safe levels compared to the unwashed. When efficacies of the individual treatments were considered on the two vegetables (main effects), only treatment with *G. bicolor* was shown to be marginally significant in sanitising cucumber of *E. coli* than lettuce. Based on these results, it is clear that soaking and rinsing of RTEV with water (tap or sterlised) alone are not effective in ridding the foods of *E. coli*. A recent study by Uhlig *et al.* [12] reported the same finding. In their study, Uhlig E, *et al.* (2017) demonstrated that washing of lettuce using a a high flow tap water (8 L/min) could reduce the loads of microorganisms by a statistically significant margin. Additionally, Uhlig E, *et al.* (2017) study further demonstrated that the removal, killing or inhibition was more efficient when they considered total aerobic counts (reduction by 80%), but reduction of *Enterobacteriaceae* count was lower (68%) and that of contaminating *E. coli* was not significantly reduced. The current study shows that soaking in vinegar, sodium hypochlorite and extracts from all selected plants or their combinations of these vegetables must have inhibited, killed or removed *E. coli* cells from the surface and lodgements at the surface of the fresh produce. The current study also demonstrated that inhibition, killing or removal of *E. coli* was significantly higher than that of total aerobic bacteria (results are not shown).

The plants that were tested in this study are known to be rich in phenolic acids, flavonoids, tannins, terpenes, triterpenes, saponins and other phytonutrients. The following phenolic acids, as contained in these plants, are known to have antimicrobial activities: coumaric acid MIC = 1 mg/ml against methicillin resistant *Staphylococcus aureus* (*S. aureus*), vanillic acid (MIC = 0.5 mg/ml against methicillin resistant *S. aureus* [27], ferulic acid (MIC of 1. 5 mg/ml against *E. coli* [28] and chlorogenic acid. [29] Some of these compounds reportedly kill bacteria by disrupting the cell walls, for example chlorogenic acid. [29] The presence of these compounds with known antimicrobial activities explains the antimicrobial activities shown herein.

Applications of the method

The compositions of the present method can be used to sanitise any food product, particularly the ready to eat varieties. This method refers to “unprocessed or minimally processed” fruit and vegetable products, usually single-ingredient foods or single fruit or vegetable products that become part of multi-ingredient foods that have undergone no or slight modifications. Their sanitiation effect can also be employed on any ready-to-eat fruits (RTEF) including apples, grapes, peaches, peppers and tomatoes and RTEV including all garden produce that is consumed fresh or with minimal processing (for example lettuce, cucumber, coleslaw, baby carrots, cabbage, cauliflower and broccoli florets and sliced celery). As for safety purposes, ready to eat food products must be sanitised with clean/portable water. However, effective sanitisation requires more than just water. Use of plant extracts from medicinal plants, being shown to be edible, are ideal. While extracts from plants such as *S. incanum* are known to be poisonous as a result of its high content of cytostitic saponins [30 - 31], their safety as a sanitiser of RTEV requires further investigation. Overall, the use of extracts from these plants is preferred since the use of chemical disinfectants such as chlorine and sodium hypochlorite is widely opposed due to the uncharacteristic taste, odours and textures that result. Besides, most chemical disinfectants are not edible.

A proposed plant extract based fruit and vegetable sanitising procedure

Pre-washing and soaking

Before processing, fruits and vegetables are picked from source or from their market. Fruits and vegetables can be washed with water to remove soil before entering a kitchen or a processing line. In the kitchen or processing plant, fruits and vegetables are typically sanitised with clean water before addition of flavourants and condiments and then serving or packaging for sale. The method requires inclusion of a soaking step, typically for at least 30 min (up to 3 hr) in a vat/dish containing the formulation. This allows the antimicrobial ingredients time to inhibit/kill the resident microorganisms. Following the soaking step the fruits and vegetables are allowed to drain, then rinsed twice with clean/portable water. Rinsing ensures that the residual extracts are removed. The residual extracts may impart off flavours, odours, textures and other off qualities on the fruit and vegetables. Organoleptic evaluation of unspiked and washed cucumber and lettuce cut pieces using a panel of 10 naïve tasters and 10 trained testers revealed no significant changes in odour, texture or taste of the vegetables after the treatments. The sanitised fruits and vegetables must now be processed with clean (sanitised) hands and equipment to limit the reintroduction of pathogenic and other microorganisms into the products. The method does not guarantee sterility of the products, but microbial loads would be reduced for extended shelf life and enhanced microbial safety.

Packaging and spraying

A preferred packaging method incorporating this method is one where fruits and vegetables are washed whole with clean water, soaked in the formulation, rinsed in clean water and drained. Fruits and vegetables are then sprayed with the formulation described in this method prior to sealing of packages to prevent. Application of the antimicrobial composition by spray can be accomplished using a manual spray wand application, an automatic spray of food product moving along a production line using multiple spray heads to ensure complete contact or other spray means. Upon sealing, the packaging can be agitated by physical/slowly turning product. Agitation increases the spread of sprayed formulation to increase the killing of micro-organisms.

Antimicrobial formulations

The present method provides antimicrobial, herbal compositions which combine high antimicrobial efficacy and safety. The compositions are particularly well suited for use in sanitisation of fruits and vegetables or any other food product in any setting including farms. private residences, kitchens and food processing industry.

Throughout this specification, quantities are defined by ranges, and by lower and upper boundaries of ranges. Each lower boundary can be combined with each upper boundary to define a range. The lower and upper boundaries should each be taken as a separate element. In one embodiment, the antimicrobial compositions of the present method comprise crude extracts from *S. spinosa*, *L. edulis*, *X. caffra*, *C. abbreviata*, *S. incanum*, *G. bicolor* and *Aloe* species (Table 6). Extracts from these plants of others that are rich in phenolic acid, flavonoids, tannins, saponins and other phytonutrients, or preparations made from synthetic equivalents of these compounds, have antimicrobial activities. Typically, compounds found in these plants can be isolated and enriched, for example, at least about 90% pure, 95% pure, 97%, or 99% pure. Phenolic acids derived from these plants include, but are not limited to, gallic acid, protocatechilic acid, *p*-hydroxybenzoic acid, 3-hydroxybenzoic acid, gentisic acid, *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid.

Table 6. Formulations of sanitising solutions.

Formulation	MIC (µg/ml)
L. edulis	100.0
S. spinosa	110.0
X. caffra	125.0
C. abbreviata	152.0
S. incanum	1595.0
G. bicolor	17.4
Aloe species□	143.0
Combined extracts*	18.9

□*Aloe* species can be any of the following: *A. greatheadii* Sch□nland var. *greatheadii*, *A. chabaudii var.chabaudii*, *A. munchii*, *A. zebrina* Baker, *A. spicata* L. f. .
*Combined extracts described here are comprised of extracts from *L. edulis*, *S. spinosa*, *X. caffra*, *C. abbreviata*, *S. incanum*, *G. bicolor*, *A. greatheadii*, *A. chabaudii*, *A. munchii*, *A. zebrina* and/or *A. spicata.*

Flavonoids princpally fall into 6 classes, namely flavanols, anthocyanidins, flavanones, flavones, flavonols and isoflavones.

Tannins, principally existing as condensed tannins are classified as procyanidins, propelargonidins, prodelphinidins, profisetinidins, proteracacinidins, proguibourtinidins, prorobinetidins, profisetinidin and luteoforolor.

Formulations made from specific compounds (natural compounds that are at least 80% pure or their artificial derivatives) that exist in the plants specified above or other plants relying on the principles of the current method can also be used. The compounds include those listed above or any other that are found within the plants listed as follows: flavonoids, tannins, alkylphenols, terpenoids, triterpenoids, sterols, secoiridoids, alkaloids, polyphenolics, glycosides, steroids, saponins, anthraquinones and others found in African plants.

Conclusion

Extracts of *L. edulis*, *C. abbreviata*, *S. incanum* and *G. bicolor* have strong inhibitory effects, and therefore they have the capacity to significantly reduce the number of viable cells of *E. coli* on the surfaces of RTEV Romaine lettuce and cucumber. Additionally, we have shown that the extracts have no negative impact on organileptic properties of the RTEV. The extracts have potential use for sanitisation of RTEV, especially in killing, inhibiting or removing pathogenic *E. coli* that are transmitted via these fresh foods and products.

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Conflict of interest

The authors, hereby, declare no conflict of interest.

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