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In-vitro effectiveness of a novel herboclean herbal solution in removing pesticides, heavy metals and microbial agents from fruits and vegetables

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ABSTRACT

In recent years, the incidence of undesirable heavy metals, pesticide residues and microbial contaminants in fruits and vegetables has caused severe worldwide public health concerns. In this investigation, we intended to investigate the in-vitro effectiveness of a novel herboclean herbal solution in eliminating pesticides, heavy metals and microbes from the fruits and vegetables collected from various markets. The collected fruits and vegetables were immersed separately in a beaker containing an already prepared herboclean herbal solution for 30 min. The efficacy of the herboclean herbal solution in removing the pesticides and heavy metals was studied using Liquid Chromatography-Mass Spectrometry (LC-MS), and Inductively Plasma Mass Spectrometry (ICP-MS), respectively. The reduction in microbes was analyzed by the plate count method. From the result, it was clear that the average removal rate of pesticide residues from fruits and vegetables was $89.89 \pm 7.21\%$. In the case of heavy metals average removal rate was $94.74 \pm 9.12\%$. Whereas in the case of microbes, the removal rate was $90.98 \pm 8.38\%$ after washing with Herboclean herbal solution. These results clearly demonstrated that the herboclean herbal solution was very effective in eliminating various pesticides, heavy metals and microbial agents from fresh fruits and vegetables without interfering with their integrity or sensorial parameters. Moreover, this is the first report regarding the efficacy of natural herboclean herbal solutions.

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1. Introduction

Vegetables and fruits are vital constituents of the human diet due to their rich source of several nutrients

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that are essential for human health and are consumed in raw, cooked, peeled or unpeeled forms (1). Even though vegetables and fruits have numerous health benefits, nowadays they are heavily contaminated with several chemicals and biological hazards, including various pesticides, heavy metals and pathogenic



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microbes (2). Pesticides (insecticides, fungicides, herbicides, and others) are widely used to control insect pests, fungal infestations, weed growth, and plant diseases as the world population grows and the demand for food production rises. Hence, the use of pesticides resulted in an increase in the quantity of foods, improved their quality, and protected crops from a reduction in yield. According to FAO statistics, pesticide use is growing every year around the world. Moreover, new varieties of pesticides are launched each year with different applications (3). Furthermore, the practice of misusing and abusing older, more toxic, and environmentally persistent pesticides are still ongoing in a different part of the world (4). Due to these unethical practices, pesticide residues found in soil, water, and air have been widely detected (5). Undue levels of pesticide residues in humans can cause various disorders, which include neurotoxicity, carcinogenicity, reproduction abnormalities, cell dysplasia, etc. Currently, pesticide residues are a key issue, distressing the quality and overall safety of various food commodities, including fruits and vegetables (6). Hence, investigations to remove pesticides from edible crops have been carried out around the world. Simple washing with clean water is the most conventional and direct food processing method to remove various pesticides. This process is commonly practiced as an initial step in cleaning fruits and vegetables to eliminate pesticide residues (7,8). Unfortunately, many pesticides are hydrophobic and water alone was successful in removing various pesticide residues from the surface of fruits and vegetables (9). Despite the implementation of pesticide-use regulations, pesticide residues in fruits and

vegetables remain a grave concern, jeopardizing the overall safety of the food supply.

Food-borne disease outbreaks linked to raw produce consumption have been reported around the world, with a variety of pathogens including *Salmonella* spp., *Shigella* spp., *Escherichia coli* O157, and *Listeria monocytogenes* (10). The global burden of foodborne illnesses is estimated to be 600 million cases per year, with 420000 deaths (10). During cultivation, harvest, transportation, and further processing, vegetables and fruits can become contaminated with enteric bacteria of medical and public health importance. As a result, these organisms have been mentioned in many previous food-borne outbreaks (11).

The interface of pathogenic microbes is thought to occur during the various preharvest and postharvest stages, especially through contaminated water, soil, transportation, or other improper handling methods (12). During food-related outbreaks, enteric bacteria such as *Escherichia coli* and *Salmonella* species can cause serious concerns for humans. Numerous outbreaks of typhoid infections have been linked to eating fruits and vegetables cultivated in contaminated soil, especially sewage (13). Several organizations have proposed conducting a microbiological risk assessment in order to implement appropriate corrective measures to reduce food-borne illness outbreaks caused by contaminated foods. Hence, it is very important to remove the pathogens from the food before consumption.

Heavy metals can be absorbed by plants from the environment and accumulate in higher concentrations in them (14). Heavy metal pollution in the soil is the primary cause of metal bioaccumulation in plants (15).

Meanwhile, as the economy grows and industrialization, urbanization, and agricultural modernization accelerate, so does the risk of polluted farmland (16). Heavy metal contamination on the soil surface is determined by anthropogenic activities, but the geological background also plays a role (17). The most common source of heavy metal exposure in humans is from vegetables and fruits, which account for 90% of metal intake, with the remaining 10% coming from skin contact and inhalation of contaminated dust (14). In general, wastewater comprises substantial amounts of heavy metals and nutrients, which present both benefits and challenges in the production of various agricultural products (18). The clearance of sewage water and industrial wastes has been a key concern in recent years. Furthermore, these wastewaters contaminate agricultural lands where crops such as fruits and vegetables are grown. In addition to this, sewage effluents also contribute to an increase in the level of heavy metals in the soil, especially chromium, nickel, lead, cadmium, etc. (19). The plants can absorb heavy metals via adsorption from contaminated soil or surface deposition from polluted air (20). Once we consume heavy metal contaminated food, it cannot be eliminated from the body and thus accumulates in our vital organs. Based on acute and chronic exposures, this condition causes several diseases and disorders in humans (21).

As previously discussed, washing is a simple and potent method to eliminate pesticides, heavy metals, and various microorganisms from the surface of vegetables and fruits. But washing with clean water alone was not sufficient to eliminate these, especially pesticide residues and heavy metals. Thus, it is

necessary today to develop an effective and safe washing solution to remove pesticides, heavy metals and various microorganisms. The objective of this study was to assess the in-vitro efficacy of an herboclean herbal solution developed by us in eradicating pesticides, heavy metals and microorganisms from fruits and vegetables. To our knowledge, this is the first investigation to use an herbal-based cleaning solution to explore the efficiency in removing surface pesticides, heavy metal residues and microbes from fruits and vegetables. Understanding the effectiveness of herbal-based cleaning solutions in the removal of pesticides, heavy metals and microbes from fruits and vegetables will allow us to develop better approaches to minimize pesticide, heavy metals and microbe exposure from fresh produce.

2. Materials and Methods

2.1. Materials

The standard pesticides were purchased (quinalphos, chlorpyrifos, cypermethrin, imidacloprid, carbendazim, chlorantraniliprole, cyfluthrin & tebuconazole) from Sigma-Aldrich (USA). All other reagents and chemicals used in the present study were of the highest purity. Ultrapure water used in this investigation for the preparation of all solutions was purchased from Merck (India). The growth media used for microbiological analysis were purchased from Hi-Media, Mumbai, India.

2.2. Formula of pesticide mixture

Pesticide standards 1 g/L stock mixture was prepared by mixing 0.010 g of each pesticide in 10 mL water. One mL of the pesticide stock mixture was diluted in

1 L of water for testing purposes. The stock pesticide mixture was stored at -20°C in the dark for further experiments.

2.3. Investigating area

The present investigation was conducted in the three different markets (Chalai, Kattakada, Neyyattinkara) of the Trivandrum district of Kerala, India (Fig. 1).

2.4. Sampling of vegetables and fruits

The vegetable and fruit samples were randomly purchased from the vendors in each market. The triplicate sets of fruits and vegetables were separately collected from Chalai market (cucumber, bitter guard, ladies finger, green chili, apple guava), Kattakada market (carrot, curry leaf, beans, brinjal, mango, grapes) and Neyyathinkara market (cucumber, bitter guard, ladies finger, green chili, apple, guava). Each representative vegetable or fruit sample was made up of ten subsamples of the same commodity drawn at random. To avoid contamination and deterioration, all samples (1 kg each) were labeled, placed in sterile polythene bags in a cooler box, and transported to the laboratory for processing. To determine the efficacy of the herboclean herbal solution, a representative portion (250 g) of the samples was used for various analyses.

2.5. Formulation of herboclean herbal solution

For preparing the herboclean herbal solution, first accurately weigh all the ingredients mentioned in Table 1. Then, the herboclean herbal solution was prepared by blending the herbal powders and extracts by adding natural vinegar. Finally, pure water was added to make up the required volume.

2.6. Immersion of fruits and vegetables in herboclean herbal solution for effective removal of pesticides, heavy metals and microbes

The collected fruits and vegetables (250 g) were carefully placed in a beaker containing the herboclean herbal solution (15 mL herboclean herbal solution diluted in 1 L water) and subjected to occasional mixing for 30 min. After 30 min, the fruits and vegetables were drained using a sieving mesh and rinsed three times with clean water to remove the herboclean herbal solution residue. After cleaning, the fruits and vegetables were left to dry in a clean room for 4 h at ambient temperature. The dried fruits and vegetables were individually packed in plastic zip-lock bags and subjected to various analyses to check their efficacy. Untreated samples were used in order to detect the initial residues of pesticides, heavy metals, and microbial load. The cleaning process for each sample was conducted in triplicate.

2.7. Analysis of pesticide residues in the samples before and after washing with herboclean herbal solution

Two procedures were adopted for checking the efficacy of the herboclean herbal solution in eradicating pesticide residues from vegetables and fruits. First analyzing the pesticide residues in the samples before and after washing with herboclean herbal solution and in the second method, the vegetables and fruits were intentionally spiked with a pesticide cocktail and then subjected to washing with herboclean herbal solution.

2.8. Vegetables and fruits spiked with pesticides

Soaking fruits and vegetables with pesticides was selected as an ideal model for pesticide application since this particular model is similar to a field application. The collected fruits and vegetables were immersed in 2 L of pesticide mixture prepared by mixing 2 mL of the pesticide mixture from the stock solution (1 g/L) for 20 min to ensure the uniform

distribution of pesticides on the surface. After the spiking procedure, the fruits and vegetables were subjected to thorough drying for 24 h at room temperature. Then the samples were subjected to the washing process as mentioned above with an herboclean herbal solution. Samples without washing with the herboclean herbal solution were used as the control.

2.9. Extraction of pesticide residues

For the extraction of pesticide residues, 10 g of macerated vegetable and fruit samples in a triplicate set were used by adopting the QuEChERS protocol (22). The macerated samples (10 g) were separately taken in a 50-mL centrifuge tube and mixed with 10 mL ethyl acetate along with anhydrous MgSO_4 (4 g) and NaCl (1 g). After this, the tubes were subjected to vigorous shaking followed by centrifugation for 10 min at 5000 rpm. Following this, a 1 mL aliquot of each vegetable and fruit extract was treated with MgSO_4 (150 mg), primary secondary amine (PSA) (100 mg) and activated charcoal (10 mg). The extracts were again subjected to shaking, which was followed by rotor spin at 100 rpm for 10 min, followed by centrifugation at 6000 rpm for 10 min. Afterward, the supernatant was carefully collected in a fresh vial and filtered through a nylon syringe filter (0.22 μm). Subsequently, 1 μL of the filtered extract was subjected to LC-MS analysis to check the level of pesticide residues.

2.10. Detection of pesticides through LC-MS analysis

The samples were analyzed using liquid chromatography (Agilent 1200, Santa Clara, CA, USA) coupled with a triple quadrupole mass detector (Agilent 6460) and an Agilent ZORBAX C-18 analytical column with dimensions of 50 mm \times 2.1 mm internal

diameter and 1.8 μm particle size. The sheath gas temperature of the LC-MS was kept at 400°C, whereas the sheath gas flow was 12 L/min. Mobile phases used in LC-MS analysis were deionized water containing 0.1% formic acid (mobile phase A), and acetonitrile and deionized water (95:5, v/v) containing 0.1% formic acid (mobile phase B). The gradient program starts with 10% mobile phase B for 3 min and then increases linearly to 90% mobile phase B over 15 min. Following that, the column was reconditioned for 20 min with 10% mobile phase B. The column temperature was kept constant at 35°C, and the injection volume was 10 μL at a constant flow rate of 0.6 mL/min.

2.11. Analysis of heavy metal in vegetables and fruits after washing with herboclean herbal solution

2.11.1. Sample preparation and digestion of vegetables and fruits

Dried vegetables and fruits (1 g) were taken in a beaker and added to a tri-acid mixture (70% high purity HNO_3 (15 ml), 65% HClO_4 and 70% H_2SO_4 ; 5:1:1) (23). The mixture was then subjected to digestion at 80°C until a transparent solution was obtained. The digested samples were filtered using Whatman No. 42 filter paper after cooling, and the filtrate was diluted to 50 mL with deionized water. Heavy metals such as mercury, arsenic, lead, and cadmium were measured in the filtrate of vegetable and fruit samples using inductively coupled plasma-mass spectrometry (ICP-MS).

2.11.2. ICP-MS

The solutions were carefully transferred to 50 mL Falcon tubes after digestion, and deionized water was added to a final volume of 50 mL. The heavy metal contents in the samples were determined using a

Thermo Fisher Scientific ICP-MS equipped with a nebulizer (iCAPRQ), a Teflon spray chamber, a nickel (Ni) sampling cone, and a platinum skimmer cone (Thermo Fisher Scientific, Waltham, MA, USA). ICP-MS operating parameters were as follows: power: 1500 W; plasma flow gas: 15 L/min; auxiliary gas flow: 0.8 L/min; nebulizer gas flow: 1.0 L/min. All analyses were carried out in triplicate.

2.12. Microbiological analysis of vegetables and fruits after washing with herboclean herbal solution

2.12.1. Processing of samples

Each sample (10 g) was aseptically mixed (1:10) with ringer solution (180 mL) in a sterile conical flask (24). Then, the samples were homogenized in a sterile blender at 6000 rpm for 8 min. Subsequently, the samples were serially diluted up to seven times (10⁻¹-10⁻⁷) in the ringer solution to reduce the load of microorganisms before plating.

2.12.2. Determination of Total viable bacterial count and total yeast and mold count

After serial dilution, the viability was measured using the pour plate method on tryptic soy agar (TSA) and sabouraud dextrose agar (SDA) for estimating the bacterial and fungal counts, respectively. The Tryptic soy agar plates were then incubated at 37°C for 1-3 days for the estimation of bacteria, whereas the Sabouraud dextrose agar plates were incubated at 30°C for 2-5 days for estimating the fungal counts. The number of colony-forming units per gram (cfu/g) was calculated after the incubation period by multiplying the average number of colonies by the dilution factor. All microbial analyses were conducted in triplicate.

2.13. Statistical analysis

The statistical analysis was performed using SPSS software and the data were expressed as the mean± standard deviation. Differences were considered significant when the p-value was <0.05.

3. Results

3.1. Washing with herboclean herbal solution effectively removed the pesticides from the market samples

The fruits and vegetables were washed with an herboclean herbal solution after being collected from the markets. After that, the samples were subjected to pesticide residue analysis. The load of pesticides in the samples was calculated before and after washing and the removal rates of pesticides were determined through comparisons with the initial concentration. From the data, it was clear that grapes, followed by curry leaf, guava, green chili and ladies' finger were heavily contaminated with pesticide residues (Table 2). The results obtained from the pesticide residue analysis clearly demonstrated that the herboclean herbal solution was highly effective in removing the residues of various pesticides from fruits and vegetables (Table 2). The pesticides monocrotophos, imidacloprid, acephate, and bendiocarb were completely removed after washing with the herboclean herbal solution. The reported average removal rate of pesticide residues after cleaning the samples with herboclean herbal solution was 89.89±7.21% (Fig. 2). Moreover, herboclean herbal solution recorded the removal of pesticides in the samples ranging from 76 to 100%. In the collected samples, grapes were heavily contaminated with several pesticides, which were successfully removed by washing with the herboclean

herbal solution. As it is well-reported that simple washing with tap water cannot effectively remove pesticides from fruits and vegetables. Our findings clearly show that the herboclean herbal formulation is very effective in removing various pesticides found in fruits and vegetables.

3.2. Herboclean herbal formulation effectively washed the pesticides spiked on the samples

Herboclean herbal formulation was also very effective in removing the pesticides spiked on the samples (Table 3). The high removal rates were recorded for carbendazim, tebuconazole, bendiocarb, imidacloprid, chlorantraniliprole etc. In addition to this, herboclean herbal solution was effective in removing the pesticides already spiked in the fruits and vegetables. The reported average removal rate of pesticide residues after cleaning the samples with herboclean herbal solution was $84.64 \pm 10.25\%$ (Fig. 2). Moreover, considering the overall performance, herboclean herbal solution has recorded the removal of pesticides in the samples ranging from 53.54 to 100%. It was very tough to eradicate pesticide residues from curry leaves by simply washing them with tap water. Washing the curry leaves with a herboclean herbal solution effectively removed the spiked pesticides (Table 3).

3.3. Herboclean herbal solution effectively removed heavy metals from the collected samples

Herboclean herbal wash significantly removed the heavy metals from the fruits and vegetables collected from various markets. The levels of arsenic and mercury in some collected vegetables and fruits were above the maximum residue limits recommended by the authority.

Interestingly, washing the fruits and vegetables with heavy metals effectively removed the heavy metals (Table 4). In the majority of samples, a 100% reduction in the heavy metals was recorded after washing with the herboclean herbal solution. An average of $94.74 \pm 9.12\%$ removal of heavy metals was recorded after washing with herboclean herbal solution (Fig. 2). Moreover, considering the overall performance, herboclean herbal solution has recorded the removal of heavy metals in the samples ranging from 70.20 to 100%.

3.4. Herboclean herbal solution effectively removed the microbes from samples

In the present investigation, all the collected samples were contaminated with bacteria, yeast and fungi. The microbiological results after treating with an herboclean herbal solution are summarized in Table 5. The herboclean herbal solution showed a significant reduction in the microbial load of fruits and vegetables when compared to the untreated control samples (Table 5). An average of $90.98 \pm 8.38\%$ removal of microbes in the samples was recorded after washing with Herboclean herbal solution (Fig. 2). Moreover, the herboclean herbal solution recorded the removal of microbes from the samples in the range of 72.34 to 100%.

Table 1. Ingredients used for the formulation of herboclean herbal solution

S. No	Ingredients	Quantity (%)
1	<i>Curcuma domestica</i>	10
2	Natural vinegar	25
3	Herbal extract 01*	5
4	Herbal extract 02*	4
5	Herbal powder 01*	3
6	Herbal powder 02*	3
7	Water	50

*Natural edible herbs and its aqueous extracts

Table 2. Effect of herboclean herbal solution in removing pesticides from samples. The values are represented as mean±standard deviation

Samples	Pesticides	Sample before wash	Sample after wash	Loss percentage (%)
Cucumber	Monocrotophos	0.017±0.002	0	100
Bitter gourd	Imidacloprid	0.01±0.004	0	100
Ladies Finger	Methamidaphos	0.014±0.002	0	100
	Acephate	0.13±0.007	0	100
	Bendiocarb	1.31±0.04	0	100
	Monocrotophos	1.6±0.04	0	100
Green chilli	Methamidaphos	0.57±0.04	0.07±0.02	87.91
	Acephate	1.67±0.03	0.4±0.06	76.00
	Imidacloprid	0.13±0.007	0.016±0.003	87.72
	Chlorantraniliprole	0.037±0.003	0.003±0.002	91.89
	Ethion	0.07±0.008	0.014±0.003	80.50
Apple	Carbendazim	-	-	-
Guava	Methamidaphos	0.14±0.005	0.024±0.006	83.40
	Acephate	1.58±0.07	0.105±0.003	93.30
	Bendiocarb	1.74±0.08	0	100
	Monocrotophos	2.29±0.06	0.07±0.008	96.6
Carrot	Imidacloprid	-	-	-
Curry leaf	Carbendazim	0.015±0.002	0.001±0.00	89.13
	Tebuconazole	0.03±0.04	0.006±0.004	84.00
	Chlorpyrifos	0.018±0.001	0.004±0.002	78.57
Beans	Tebuconazole	0.034±0.004	0.003±0.001	89.21
Brinjal	-	-	-	-
Mango	Imidacloprid	0.027±0.005	0.002±0.001	91.46
	Carbendazim	0.019±0.003	0.002±0.001	88.13
Grapes	Methamidaphos	0.10±0.014	0.015±0.004	85.66
	Acephate	0.32±0.04	0.039±0.017	87.87
	Bendiocarb	0.80±0.01	0.127±0.024	84.13
	Monocrotophos	1.17±0.034	0.111±0.010	90.51
	Imidacloprid	0.31±0.04	0.031±0.022	90.03
	Carbendazim	0.80±0.05	0.106±0.010	86.79
	Tebuconazole	1.49±0.07	0.33±0.11	77.40
	Metalaxyl+Metalaxyl-M	0.18±0.04	0.025±0.008	86.58

BDL- Below Detection Limit

Table 3. Effect of washing with herboclean herbal solution in removing the spiked pesticide in the samples. The values are represented as mean±standard deviation. BDL- Below Detection Limit

Sample	Pesticides	Contaminated sample before washing	Contaminated sample after washing	Loss percentage (%)
Cucumber	Chlorantraniliprole	0.104±0.080	0.017±0.010	83.01
	Carbendazim	0.227±0.014	0.016±0.010	92.80
	Tebuconazole	0.277±0.063	0.035±0.018	87.13
	Chlorpyrifos	0.080±0.011	0.020±0.007	74.27
Bitter gourd	Imidacloprid	BDL	BDL	100
	Carbendazim	0.032±0.003	BDL	100
	Tebuconazole	0.072±0.019	BDL	100
Ladies finger	Carbendazim	0.52±0.015	0.014±0.001	73.07
	Tebuconazole	0.294±0.066	0.102±0.023	65.15
Green chilly	Chlorantraniliprole	0.043±0.013	0.003±0.001	92.36
	Carbendazim	0.050±0.014	0.012±0.001	76.00
	Tebuconazole	0.156±0.028	0.022±0.003	85.92
	Chlorpyrifos	0.132±0.031	0.017±0.003	86.61
	Quinalphos	0.055±0.012	0.008±0.001	85.45
Apple	Chlorantraniliprole	0.071±0.005	0.015±0.002	78.97
	Carbendazim	1.306±0.166	0.136±0.015	89.53
	Tebuconazole	0.173±0.015	0.033±0.005	80.96
	Chlorpyrifos	0.091±0.005	0.013±0.002	85.09
	Quinalphos	0.056±0.026	0.024±0.007	56.80
Guava	Methamidaphos	0.146±0.009	0.048±0.005	67.19
	Acephate	1.85±0.078	0.048±0.005	84.68
	Bendiocarb	3.02±0.544	0.356±0.113	88.20
	Monocrotophos	3.516±0.570	0.590±0.074	83.21
	Tebuconazole	0.051±0.017	0.024±0.007	53.54
Carrot	Imidacloprid	0.057±0.005	BDL	100
	Carbendazim	0.311±0.011	0.043±0.018	85.97
	Tebuconazole	0.800±0.050	0.110±0.008	86.21
	Chlorpyrifos	0.057±0.011	0.009±0.001	83.62
	Quinalphos	0.053±0.009	0.013±0.003	75.00
Curry leaf	Methamidaphos	0.174±0.054	0.006±0.002	96.18
	Acephate	1.255±0.056	0.117±0.003	90.62
	Imidacloprid	0.697±0.076	0.133±0.020	80.91
	Chlorantraniliprole	1.800±0.057	0.219±0.114	87.83
	Ethion	0.184±0.032	0.025±0.012	86.23
	Tebuconazole	3.563±0.441	0.524±0.105	85.27
	Chlorpyrifos	1.684±0.102	0.221±0.107	86.82
	Quinalphos	0.42±0.082	0.042±0.019	90.00
	Profenofos	0.035±0.006	0.009±0.001	72.64
Cypermethrin	0.297±0.064	0.018±0.006	93.93	
Beans	Imidacloprid	0.020±0.003	0.003±0.001	85.24
	Carbendazim	0.644±0.076	0.086±0.010	86.65
	Tebuconazole	1.072±0.168	0.111±0.009	89.58
	Chlorpyrifos	0.254±0.092	0.037±0.004	85.30
	Quinalphos	0.101±0.011	0.017±0.006	82.50
	Cypermethrin	0.043±0.009	0.014±0.000	66.41

Brinjal	Chlorantraniliprole	0.062±0.084	BDL	100
	Carbendazim	0.196±0.015	0.011±0.001	94.40
	Tebuconazole	0.088±0.005	0.013±0.001	85.28
	Chlorpyrifos	0.216±0.018	0.055±0.019	74.46
	Quinalphos	0.078±0.016	0.024±0.104	69.06
	Cypermethrin	0.171±0.016	BDL	100
Mango	Imidacloprid	0.054±0.016	0.010±0.002	80.86
	Carbendazim	0.083±0.015	0.017±0.003	78.88
	Tebuconazole	0.089±0.009	0.021±0.008	76.57
	Chlorpyrifos	0.031±0.005	0.006±0.002	80.43
	Quinalphos	0.019±0.001	0.003±0.001	82.45
Grapes	Methamidaphos	0.069±0.017	0.046±0.017	100
	Acephate	0.251±0.013	0.054±0.028	78.19
	Imidacloprid	0.401±0.007	0	100
	Carbendazim	0.645±0.056	0.125±0.010	80.53
	Tebuconazole	2.459±0.205	0.203±0.069	91.73
	Chlorpyrifos	0.032±0.005	0	100
	Metalaxyl+Metalaxyl-M	131.230±4.981	16.33±5.686	87.55
	Bendiocarb	1.370±0.087	0.162±0.055	88.15
	Monocrotophos	1.500±0.121	0.119±0.049	86.71

BDL- Below Detection Limit

Table 4. Effect of washing with herboclean herbal solution in removing the heavy metal residues from the samples. The values are represented as mean±standard deviation. BDL- Below Detection Limit

SL. No	Samples	Heavy metals	Sample before washing	Sample after washing	Loss percentage (%)
1	Cucumber	Arsenic	0.73±0.052	0.15±0.08	78.5
		Cadmium	0.013±0.005	0	100
		Mercury	0.206±0.05	0	100
		Lead	0.37±0.04	0.05±0.03	84.6
2	Bitter gourd	Arsenic	0.86±0.06	0.13±0.02	84.9
		Cadmium	0.016±0.005	0	100
		Mercury	BDL	BDL	100
		Lead	0.60±0.076	0.06±0.03	90.1
3	Ladies Finger	Arsenic	0.88±0.04	0.08±0.04	90.5
		Cadmium	0.04±0.01	0	100
		Mercury	BDL	0	100
		Lead	0.67±0.04	0	100
4	Green chilli	Arsenic	0.76±0.10	0.05±0.03	92.60
		Cadmium	0.05±0.01	0	100
		Mercury	BDL	0	100
		Lead	0.59±0.03	0.13±0.04	77.65
5	Apple	Arsenic	0.84±0.07	0.22±0.02	73.5
		Cadmium	0.02±0.005	0	100
		Mercury	BDL	BDL	100
		Lead	0.85±0.06	0.07±0.04	91.7
6	Guava	Arsenic	1.19±0.20	0	100
		Cadmium	0.01±0.005	0	100
		Mercury	0.19±0.04	0	100
		Lead	0.74±0.11	0.14±0.02	80.3

7	Carrot	Arsenic	BDL	BDL	100
		Cadmium	0.04±0.01	0.01±0.005	71.4
		Mercury	0.13±0.01	0	100
		Lead	BDL	BDL	100
8	Curry leaf	Arsenic	0.43±0.09	0.10±0.02	75.5
		Cadmium	0.08±0.01	BDL	100
		Mercury	BDL	BDL	100
		Lead	0.44±0.07	0.06±0.03	86.4
9	Beans	Arsenic	BDL	BDL	100
		Cadmium	0.01±0.005	BDL	100
		Mercury	0.02±0.01	BDL	100
		Lead	BDL	BDL	100
10	Brinjal	Arsenic	BDL	BDL	100
		Cadmium	0.01±0.005	BDL	100
		Mercury	BDL	BDL	100
		Lead	0.04±0.02	BDL	100
11	Mango	Arsenic	0.01±0.005	BDL	100
		Cadmium	BDL	BDL	100
		Mercury	0.68±0.04	0.20±0.07	70.2
		Lead	BDL	BDL	100
12	Grapes	Arsenic	BDL	BDL	100
		Cadmium	0.05±0.02	0	100
		Mercury	BDL	BDL	100
		Lead	BDL	BDL	100

BDL- Below Detection Limit

Table 5. Effect of washing with herboclean herbal solution in removing the microbes from the samples. The values are represented as mean±standard deviation. BDL- Below Detection Limit. TPC-Total plate count and TYMC- Total Yeast and mold count

Sample	Tested	Contaminated sample before washing	Contaminated sample after washing	Loss percentage (100)
Cucumber	TPC	$17.5 \times 10^5 \pm 25 \times 10^4$	$58 \times 10^3 \pm 20 \times 10^2$	96.68
	TYMC	1200±264	250±50	79.16
Bitter gourd	TPC	$18.3 \times 10^6 \pm 15.2 \times 10^5$	$6.3 \times 10^5 \pm 1.5 \times 10^5$	96.54
	TYMC	$12.3 \times 10^4 \pm 15.2 \times 10^2$	420±91.6	96.59
Ladies finger	TPC	$61.6 \times 10^5 \pm 10.4 \times 10^5$	$76 \times 10^4 \pm 15.8 \times 10^4$	87.67
	TYMC	$19.3 \times 10^4 \pm 25.1 \times 10^3$	1400±360	99.27
Green chilli	TPC	$73 \times 10^5 \pm 11.5 \times 10^5$	$2.4 \times 10^5 \pm 9.8 \times 10^3$	96.71
	TYMC	$55 \times 10^3 \pm 75.4 \times 10^2$	$2.1 \times 10^3 \pm 450$	96.24
Apple	TPC	$24.6 \times 10^2 \pm 503$	256±100	89.59
	TYMC	1700±721	126±25	92.54
Guava	TPC	$60 \times 10^3 \pm 2000$	1433±251	97.61
	TYMC	8066±550	770±182	90.45
Carrot	TPC	$16.8 \times 10^6 \pm 10.4 \times 10^5$	$20 \times 10^3 \pm 3.6 \times 10^2$	99.88
	TYMC	$14 \times 10^5 \pm 15.2 \times 10^4$	$35 \times 10^3 \pm 5.0 \times 10^2$	97.61
Curry leaf	TPC	$65.6 \times 10^7 \pm 60.2 \times 10^6$	$59.6 \times 10^6 \pm 5.5 \times 10^5$	90.91
	TYMC	$78.3 \times 10^4 \pm 12.5 \times 10^4$	$21.6 \times 10^4 \pm 7.6 \times 10^3$	72.34
beans	TPC	$52.3 \times 10^5 \pm 35.1 \times 10^5$	$14.3 \times 10^6 \pm 20.8 \times 10^5$	72.61
	TYMC	$65 \times 10^3 \pm 62.4 \times 10^2$	$15.3 \times 10^3 \pm 37.8 \times 10^2$	76.41

Brinjal	TPC	$61.3 \times 10^4 \pm 32.1 \times 10^3$	$37.3 \times 10^3 \pm 25.1 \times 10^2$	93.91
	TYMC	$25 \times 10^3 \pm 50 \times 10^2$	$53 \times 10^3 \pm 854$	78.80
Mango	TPC	$17 \times 10^4 \pm 26.4 \times 10^3$	$7.6 \times 10^3 \pm 1.9 \times 10^3$	95.50
	TYMC	$80.3 \times 10^3 \pm 21.5 \times 10^3$	$31.3 \times 10^2 \pm 950$	96.09
Grapes	TPC	$17 \times 10^6 \pm 75.4 \times 10^5$	$6.3 \times 10^5 \pm 9 \times 10^4$	96.20
	TYMC	$13.0 \times 10^6 \pm 26.4 \times 10^5$	$7.3 \times 10^5 \pm 10 \times 10^5$	94.30

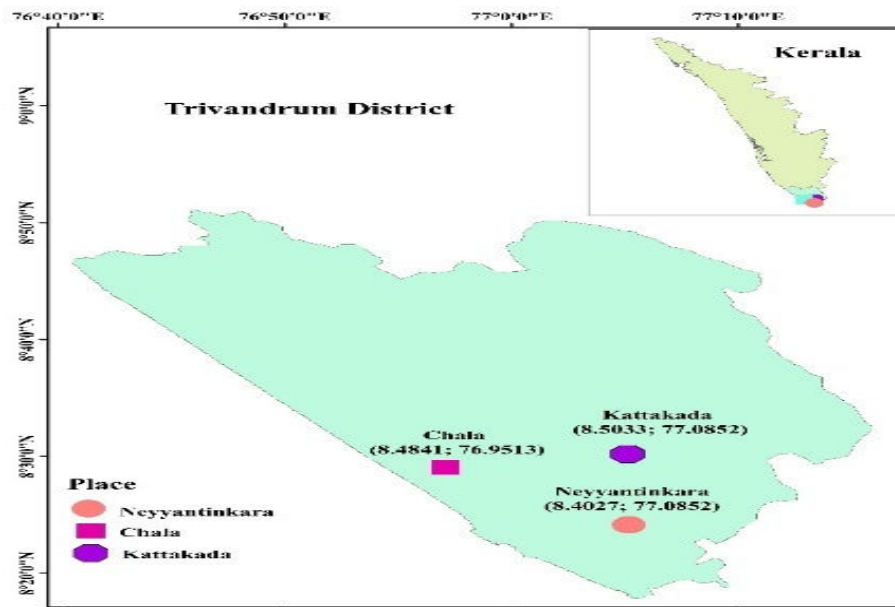


Figure 1. Geographical location of the study area

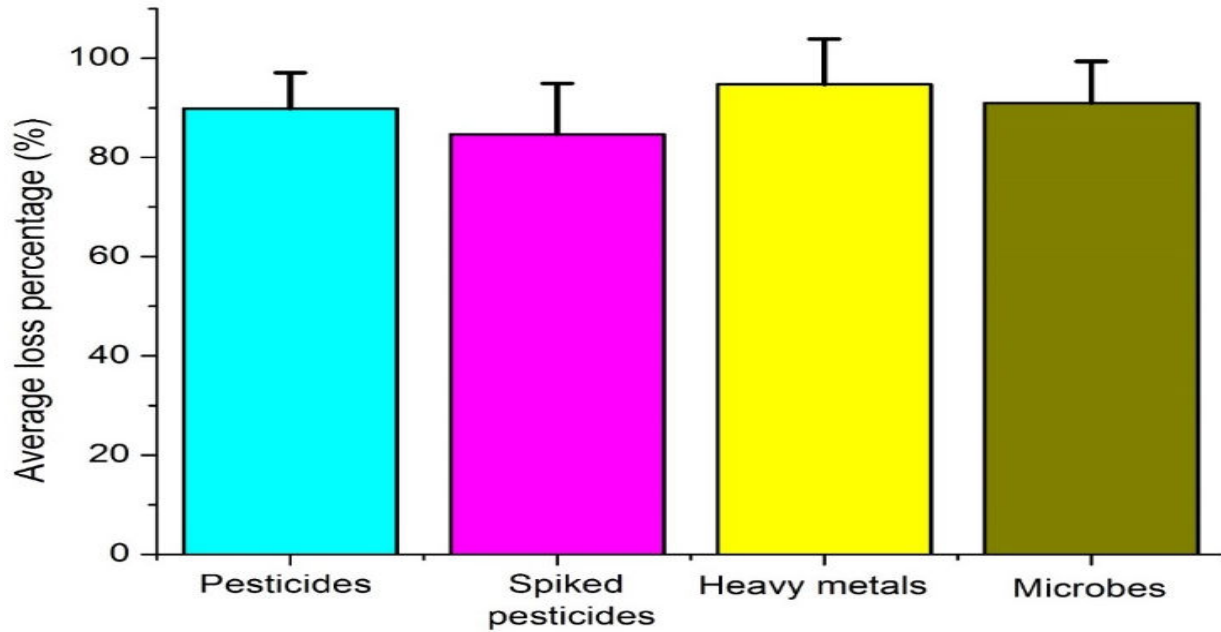


Figure 2. Average loss percentage of pesticides, spiked pesticides, heavy metals and microbes from the fruits and vegetables. The values represented the average overall loss percentage of pesticides, spiked pesticides, heavy metals and microbes present in the samples after washing with herboclean herbal solution.

4. Discussion

Contamination of pesticide residues, heavy metals and pathogenic microorganisms in vegetables and fruits has been measured as a serious food safety problem across the globe in recent years, affecting millions of people. Moreover, the consumption of vegetables and fruits contaminated with higher quantities of pesticide residues and heavy metals will negatively affect the health status of ordinary people. Unfortunately, the level of the aforementioned contaminants in food samples is increasing on a daily basis. Simple washing cannot remove the pesticide residues, heavy metals and microorganisms from vegetables and fruits.

To overcome this issue, we need an effective and cheap washing solution of natural origin with eco-friendly properties.

Hence, we developed a herboclean herbal solution from natural sources to mitigate the above-mentioned problem. The findings of this investigation clearly demonstrated that this herboclean herbal solution is extremely effective in removing pesticide residues, heavy metals, and microorganisms from vegetables and fruits.

Because of the potential long-term negative effects on human health, the presence of pesticide residues in fresh produce has become a health concern for consumers with increased consumption of fresh fruits and vegetables. Hence, the present study was aimed at investigating the efficacy of herboclean herbal solution treatments in eliminating various pesticides commonly found in fruits and vegetables. Early on, certain food processing techniques, such as washing, peeling, blanching, smoking, frying, boiling, and canning, recorded a reduction in the pesticide residue level. It has been well documented that washing with clean

water and cooking does not eradicate or destroy pesticide residues from food commodities (25). Numerous methods have already been reported in the literature for the elimination of pesticide residues from various vegetables and fruits. The removal efficacy of pesticide residues, especially when washed with pure water, is fairly low due to their hydrophobic properties. The use of surfactant solutions may offer a chance to remove a small number of residues, particularly those of a hydrophobic nature, from the surface of fruits and vegetables. Wu and his co-workers reported the effectiveness of tap water, micron calcium solution, alkaline electrolyzed water, ozonated water, active oxygen, and sodium bicarbonate in removing ten distinctive pesticides from a kumquat, cucumber, and spinach (26). In this study, tap water was reported to be less effective in removing pesticides when compared to other methods. Moreover, washing with an active oxygen solution (2%) and micron calcium solution recorded a reduction in the loss of pesticides (26). Among the 10 pesticides tested, pyrethroid recorded the highest removal rate. Furthermore, chlorpyrifos was very hard to eliminate from the samples (26). Yang et al. reported that the coupled free chlorine/ultrasound (FC/US) process was effective in removing three typical pesticides from lettuce (27). Unfortunately, all of these methods are very difficult for the common people to remove pesticides from fruits and vegetables at home. Studies on pesticide removal have shown a trend toward washing fruits and vegetables with surfactants or different kinds of salts, as well as chemicals that have an alkalizing or acidifying effect (28-30).

Yang et al. (2017) reported that surface pesticide residues can be most effectively eradicated by sodium bicarbonate (baking soda, NaHCO_3) solution when compared to either tap water or standard Clorox bleach. This study gives us the information that the standard postharvest washing method using Clorox bleach solution for 2 min is not an effective means to completely remove pesticide residues on the surface of apples (31). In addition to this, recently it was reported that ozone-bubbled water at 3 mg/L was the most efficient treatment to remove fungicide residues in bell peppers (32). In 2021, Srivastava and his co-worker studied the effects of washing with running tap water, soaking in lukewarm water (50–60°C), soaking in solutions of 1% NaCl, 5% NaHCO_3 , 2% CH_3COOH , 0.01% KMnO_4 and three commercial formulations in okra for the decontamination treatment. Here, soaking the okra in 2% acetic acid and then washing proved to be the best decontamination treatments for all the pesticides (33).

Balkan and Yılmaz studied the effect of various washing solutions in removing the pesticides and found that rice vinegar, filtered rice water, carbonated water, NaCl in combination with grape vinegar, hot tap water (40°C), grape vinegar (6% acetic acid), grape vinegar in combination with water, cold tap water (20°C), lemon juice, baking soda water, grape vinegar (8% acetic acid), filtered mint water and grape vinegar (4% acetic acid), respectively effective in removing the pesticides from the vegetables. But all the solutions mentioned above are ineffective in removing all the types of pesticides found in fruits and vegetables (34). In our study, herboclean herbal solutions recorded significant effectiveness in eliminating various

pesticide residues found in the collected samples from the various markets. Interestingly, this solution recorded 100% removal of some major pesticide residues in the fruits and vegetables. Additionally, this process is very simple and can thus be exploited for commercial purposes.

Arsenic, cadmium, chromium, mercury and lead are the most predominant heavy metals that can impose serious risks to human beings even in microgram quantities (35). People's health may be jeopardized if they consume vegetables and fruits contaminated with a cocktail of heavy metals. Heavy metals are really harmful to humans due to their extended biological half-lives, non-biodegradable nature, and capacity to aggregate in vital cellular and organ systems (36-37). In the present study, the herboclean herbal solution significantly removed the heavy metals present in the fruits and vegetables. Earlier, Nirapara fresh wash, a natural formulation, was reported to remove heavy metals from fish without affecting the overall quality of the product (38).

Nowadays, most people eat vegetables after performing household treatments. Thus, it is critical to understand the impact of those treatments on heavy metal levels. Sattar et al. investigated the levels of cadmium, mercury, arsenic, chromium, and lead in vegetables before and after washing with common household chemicals (39). From their studies, they concluded that washing with 10% acetic acid was more effective for heavy metal removal when compared to washing with tap water. Moreover, 5% and 10% sodium chloride were recorded as having heavy metal removal efficiency. Suruchi and Jilani investigated the effect of de-ionized water washing on heavy metal

removal from vegetables (spinach, methi, and coriander) collected from the Agra region of India (40). In this study, mild reductions in the levels of Cd and Pb were recorded in spinach, methi, and coriander. In addition to this, Sattar et al. also studied the effects of washing with tap water, radish solution (4 or 8 %) and ginger solution (4 or 8 %) on the removal of heavy metals in cauliflower, spinach, okra, brinjal, etc. (41). This research group revealed that washing vegetables with ginger containing solution recorded significantly greater removal of heavy metals than the other herbal solutions. Jain et al. reported the effectiveness of various fruit and vegetable peels, including pineapple peels, potato peels, citrus fruit peels, orange peels, pomegranate peels, banana peels, tomato peels, etc. Further, it was discovered that heavy metal removal was influenced by sorbent dose, initial concentration, pH, and temperature. (42).

Using ICP-MS, Bora and his colleagues examined the levels of three toxic trace elements (As, Cd, and Pb) and one microelement (Zn) in fruits and vegetables. They also looked into how washing produce in vinegar (5% and 10% acetic acid) affected the concentrations of the metals. In this study, levels of As and Zn were up to 8% lower for samples washed with 10% vinegar, and levels of Cd were up to 20% lower, while Pb did not show any differences (43).

In our investigation, some of the vegetables and fruits were found to be profoundly contaminated with different heavy metals. The herboclean herbal solution we formulated was very effective in removing the heavy metals from the samples tested. In some fruits and vegetables, the herboclean solution recorded 100% removal. In our formulation, natural vinegar is one of

the major ingredients and the lessening of heavy metals in vegetables and fruits may be due to the variation in pH value. The chemistry of heavy metal solutions, including hydrolysis, complexation by organic and inorganic ligands, redox reactions, precipitation, speciation, and adsorption availability are influenced by the pH of the solution (44). Thus, it can be concluded that this solution can be successfully used to eradicate various heavy metal contents from fruits and vegetables purchased from the market.

Numerous vegetables grow near the ground, where they almost certainly come into contact with soil. Vegetables are likely to be contaminated with a variety of pathogenic microorganisms if the soil has been fertilized with animal manure that hasn't been properly treated or irrigated with contaminated water. It could also be the result of unsanitary farm and food factory workers. Another source of contamination is using contaminated water to rinse and sprinkle the vegetables to keep them fresh. One of the microbiological indicators for food quality is the plate count of aerobic mesophilic microorganisms (45). The presence of various microorganisms affects the overall quality of the samples and some conditions may favor the growth of these microorganisms. Hence, these parameters are useful for indicating whether cleaning, disinfection, and temperature control may be required during industrial processing, transportation, and storage of the samples (45). Numerous strategies, i.e., physical and chemical treatments, have been investigated to remove the pathogenic microbes from fruit and vegetables. The mostly chlorinated solution is used as the decontaminating agent to remove the microbes from the fruit and vegetables. Due to its cost-

effectiveness and ease of handling, chlorine is mostly used in liquid form (46). But heavy use of chlorine may destroy useful microbes in the soil. Moreover, cleaning samples with organic acids, likely acetic acid and citric and sorbic acids, has been shown to reduce microbial load (47,48). Rossi et al. conducted a study to compare the effectiveness of sodium hypochlorite (SH), sodium bicarbonate, and Cinnamomum zeylanicum essential oil as sanitizers on lettuce and Cinnamomum zeylanicum essential oil reported to be effective sanitizing agents, capable of enhancing the microbiological profile of fresh produce (49). In this study, the herboclean herbal solution was very effective in reducing the microbial load from fruits and vegetables. Thus, this solution has immense potential to become an effective decontaminating agent to eliminate microbes from fruits and vegetables. This investigation was conducted without the support of external funding. In this study, we have collected samples from three locations, which is one of the major limitations of the present investigation. In the future, we will investigate the efficacy of the herboclean herbal solution by collecting different varieties of samples from various locations. Further, the sample size can be increased in future investigations. In this study, we have used pesticide dipping techniques to investigate the efficacy. In addition to this, we can adopt other methods to check the efficacy of the herboclean herbal solutions. These limitations can be rectified in the future with the support of funded projects.

5. Conclusion

This investigation was performed to assess the efficacy of the herboclean herbal solution in removing the

pesticide residues, heavy metal contents and microbial load from the fruits and vegetables collected from the three markets located in the Trivandrum district of Kerala, India. The results of the investigation undoubtedly proved that washing fruits and vegetables with a herboclean herbal solution efficiently lessens pesticide residues, heavy metals and microbes in the fruits and vegetables.

The process developed by us is simple and highly effective in removing pesticide residues, heavy metals and microbes and can be adopted by the common people to ensure a healthy diet. Moreover, the herboclean herbal solution was formulated using natural herbal raw materials and is thus safe for the ecosystem. According to the best of our knowledge, none of the wash solutions has the potential to remove pesticides, heavy metals, and microbes. Moreover, this is the first report regarding the efficacy of natural herboclean herbal solutions in removing pesticides, heavy metals and microbes from fruits and vegetables. Further research studies were absolutely necessary to investigate the real mechanism behind the removal of pesticides, heavy metals and microbes.

Conflict of interest

The authors declare no competing interest.

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