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Development and Evaluation of a Hydroethanolic Moringa oleifera and Pineapple Crown Leaf Extract-Based Spray for Enhancing Microbial and Oxidative Stability of Fresh-Cut Fruits and Freshly Squeezed Juices

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ABSTRACT

Fresh-cut fruits and freshly squeezed juices are highly susceptible to microbial contamination and oxidative deterioration due to their high moisture content and absence of thermal processing. The present study aimed to develop a plant extract-based natural spray using hydroethanolic (4:1 ethanol: water) extracts of Moringa oleifera leaves and pineapple crown leaves for improving shelf-life stability of fresh produce. Extracts were obtained through maceration and evaluated for antimicrobial activity against Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Candida albicans using agar well diffusion and minimum inhibitory concentration (MIC) assays. Antioxidant potential was determined using the phosphomolybdenum assay. Three spray formulations (F1, F2, and F3) incorporating lemon juice, honey, gum acacia, glycerol, sodium citrate, and plant extracts were developed and further evaluated. The hydroethanolic extract exhibited broad-spectrum antimicrobial activity with inhibition zones ranging between 12–22 mm. Total antioxidant capacity ranged from 58% - 78%. Among the formulations, F3 demonstrated superior antimicrobial performance and achieved up to 2.8 log CFU/mL reduction in spike-tested sugarcane and lime juices during unrefrigerated storage. Application on apple, banana, and strawberry slices showed delayed microbial proliferation and reduced oxidative browning compared to untreated controls and increased the shelf – life for upto 2 days. The findings suggest that the developed spray represents a sustainable, eco-friendly, and effective natural preservation strategy with potential industrial application in minimally processed fresh produce systems.

KEYWORDS: Moringa oleifera; Pineapple crown leaves; Natural preservative; Hydroethanolic extract; Antimicrobial activity; Antioxidant activity; Shelf-life enhancement; Fresh-cut fruits; fresh squeezed juice; spray formulations.

INTRODUCTION

The global demand for fresh-cut fruits and freshly squeezed juices has increased substantially in recent years due to rising consumer awareness regarding health, nutrition, and minimally processed foods (Rico et al., 2007; Olaimat & Holley, 2012). These products are rich in vitamins, minerals, dietary fiber, and

bioactive compounds; however, their high moisture content, near-neutral pH, and absence of thermal processing make them highly susceptible to microbial spoilage and oxidative deterioration (Raybaudi-Massilia et al., 2009). Consequently, maintaining microbial safety and physicochemical stability of fresh produce remains a significant challenge for the food industry.

Fresh fruits and juices provide an ideal environment for the growth of spoilage microorganisms and foodborne pathogens, including Gram-positive bacteria, Gram-negative bacteria, and yeasts (Beuchat, 2002). Contamination may occur during harvesting, handling, processing, or storage, increasing the risk of microbial proliferation and potential foodborne illnesses. Pathogens such as Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Candida albicans have frequently been associated with fresh produce contamination (Olaimat & Holley, 2012; Raybaudi-Massilia et al., 2009). Therefore, effective preservation strategies are essential to enhance safety and extend shelf life.

Conventional preservation techniques such as thermal processing, chemical preservatives, and synthetic additives are often unsuitable for minimally processed fresh products due to adverse effects on sensory quality and increasing consumer concerns regarding chemical residues (Gyawali & Ibrahim, 2014). The growing preference for "clean-label" foods has accelerated research into natural, plant-based antimicrobial and antioxidant agents as alternative preservation strategies (Tajkarimi et al., 2010).

Plant extracts are rich sources of bioactive compounds such as phenolics, flavonoids, tannins, alkaloids, and terpenoids, which exhibit antimicrobial and antioxidant properties (Cowan, 1999). These compounds exert antimicrobial effects through mechanisms including disruption of microbial cell membranes, inhibition of enzyme systems, interference with nucleic acid synthesis, and induction of oxidative stress within microbial cells (Burt, 2004). In addition to antimicrobial action, plant-derived antioxidants help reduce oxidative degradation, delay browning reactions, and preserve nutritional quality in fresh produce systems (Shahidi & Ambigaipalan, 2015).

Moringa oleifera has gained considerable attention as a multifunctional plant with significant pharmacological and nutritional value. Its leaves are particularly rich in polyphenols, flavonoids, vitamins, and essential minerals, contributing to strong antimicrobial and antioxidant activity (Anwar et al., 2007). Previous studies have demonstrated the inhibitory effects of M. oleifera leaf extracts against a wide range of foodborne pathogens, suggesting its potential application as a natural food preservative (Peixoto et al., 2011; Rahman et al., 2009).

Pineapple (Ananas comosus) crown leaves, often discarded as agricultural waste, represent an underutilized source of valuable phytochemicals. These crown leaves contain phenolic compounds, flavonoids, and proteolytic enzymes such as bromelain, which exhibit antimicrobial and antioxidant properties (Ketnawa et al., 2012). Valorization of pineapple crown waste aligns with sustainable food processing practices and circular economy principles, promoting the development of eco-friendly preservation systems (Ayala-Zavala et al., 2011).

The extraction method and solvent system play a critical role in determining the yield and efficacy of plant bioactive compounds. Hydroethanolic solvent systems provide a broad polarity range, enabling efficient extraction of both polar and moderately non-polar phytochemicals (Do et al., 2014). Maceration using ethanol–water mixtures is widely employed due to its simplicity, cost-effectiveness, and suitability for food-related applications.

Although several studies have independently evaluated the antimicrobial properties of Moringa oleifera or pineapple-derived extracts, limited research has explored their combined application in spray-based

preservation systems for fresh-cut fruits and freshly squeezed juices. Furthermore, few studies have validated such formulations using spike testing models in real food matrices.

Therefore, the present study aimed to develop and evaluate a hydroethanolic extract-based natural spray incorporating Moringa oleifera leaves and pineapple crown leaves for enhancing microbial and oxidative stability of fresh-cut fruits and freshly squeezed juices. The extracts were assessed for antimicrobial activity against selected bacterial and fungal strains and for antioxidant potential using the phosphomolybdenum assay (Prieto et al., 1999). Subsequently, spray formulations were developed and validated through application and spike testing in selected fruit and juice matrices to determine their preservation efficacy.

MATERIALS

Fresh leaves of Moringa oleifera and pineapple crown leaves of Ananas comosus were used for extract preparation. The chemicals and solvents used included ethanol (analytical grade), distilled water, sulfuric acid, sodium phosphate, ammonium molybdate, potassium sorbate, gum acacia, iodine, potassium iodide, sodium hydroxide, hydrochloric acid, ferric chloride, lead acetate, and Benedict's reagent. Ascorbic acid was used as a reference standard for antioxidant studies. Mueller–Hinton broth and Mueller–Hinton agar (Hi- Media Ltd.) were used for antimicrobial assays, while sterile peptone water was used for serial dilution. The microbial cultures employed in the study included Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Candida albicans. Additional ingredients used in the preparation of the herbal spray formulation included honey (as humectant), fresh lemon juice (as acidulant and buffering agent), and distilled water as solvent. All reagents and media were of analytical grade and prepared according to standard laboratory procedures.

METHODS

Extraction of Pineapple Crown Leaf Extract (Hydroethanolic Maceration Method)

Fresh pineapple (Ananas comosus) crown leaves were collected, washed thoroughly with running tap water to remove adhering dirt, and rinsed with distilled water. The leaves were shade-dried at room temperature for several days until a constant weight was obtained. The dried material was then pulverized into a fine powder using a laboratory grinder and stored in airtight containers until further use.

For extraction, a known weight of the powdered material was soaked in hydroethanolic solvent (ethanol: distilled water, 70:30 v/v) in a clean glass container at a solvent-to-sample ratio of approximately 10:1 (v/w). The mixture was kept at room temperature for 72 hours with intermittent shaking to enhance solvent penetration and extraction efficiency.

After maceration, the mixture was filtered first through muslin cloth and subsequently through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was concentrated at controlled temperature to remove ethanol. The remaining aqueous portion was weighed to calculate percentage yield and stored in airtight containers at 4°C until further analysis.

Extraction of Moringa Leaf Extract (Hydroethanolic Maceration Method)

Fresh leaves of Moringa oleifera were collected and washed thoroughly with tap water followed by distilled water to eliminate impurities. The leaves were shade-dried at room temperature until complete removal of moisture was achieved. The dried leaves were ground into a fine powder using a mechanical grinder and stored in airtight containers.

A measured quantity of the powdered leaves was macerated in hydroethanolic solvent (ethanol: distilled

water, 70:30 v/v) using a solvent-to-sample ratio of approximately 10:1 (v/w). The mixture was kept at room temperature for 72 hours with occasional stirring to facilitate extraction of bioactive compounds. Following maceration, the extract was filtered through muslin cloth and then through Whatman No. 1 filter paper. The filtrate was concentrated to remove ethanol. The concentrated extract was further dried to obtain the crude extract.

The final extract was weighed to determine percentage yield and stored at 4°C in airtight containers until used for phytochemical and antimicrobial analysis. (Fitri et.al., 2025)

Preliminary Phytochemical Screening of Ethanolic

Preliminary phytochemical analysis of both ethanolic and aqueous extracts was carried out to determine the presence of major bioactive constituents, including polyphenols, flavonoids, tannins, alkaloids, glycosides, and saponins. The following standard qualitative tests were employed.

Test for Flavonoids

The flavonoid test was performed following the method described by Abubakar and Haque and Shri Chengama Raju and Wing Kei. One milliliter of the extract was taken in a glass test tube, and a few drops of sodium hydroxide solution were added. The development of an intense yellow coloration indicated a possible presence of flavonoids. Subsequently, a few drops of dilute acid were added. Disappearance of the yellow color and formation of a colorless solution confirmed the presence of flavonoids.

Test for Alkaloids

The detection of alkaloids was carried out using Wagner's reagent as described by Abubakar and Haque . Wagner's reagent was freshly prepared by dissolving 1.27 g of iodine (I_2) and 2 g of potassium iodide (KI) in 100 mL of distilled water.

For the test, 1 mL of the extract was taken in a clean test tube, and a few drops of Wagner's reagent were added. The formation of a reddish-brown or brown precipitate indicated the presence of alkaloids.

Test for Tannins

To test for tannins, a few drops of 1% lead acetate solution were added to 1 mL of the extract in a test tube. The appearance of a white or gelatinous precipitate confirmed the presence of tannins.

Test for Glycosides

One milliliter of the extract was treated with a few drops of Benedict's reagent (an alkaline solution containing cupric citrate complex) and heated in a boiling water bath for approximately 5 minutes. After cooling, the formation of a reddish-brown precipitate indicated the presence of reducing sugars, suggesting the presence of glycosides .

Test for Saponins

The froth test was conducted according to the methods described by Muttalib and Naqishbandi and Pandey and Tripathi . Five milliliters of the extract were vigorously shaken in a test tube for approximately 15 minutes. The persistent formation of stable foam indicated the presence of saponins.

Test for Polyphenols

The presence of polyphenols was determined following the method described by Abubakar and Haque . One milliliter of the extract was mixed with 5% ferric chloride solution. The formation of a brown precipitate indicated the presence of polyphenolic compounds.

Agar Well Diffusion Method

The antimicrobial activity of the extracts was further assessed using the agar well diffusion method. Sterile Mueller–Hinton agar (Hi-Media Ltd.) was prepared and poured into sterile Petri plates. After solidification, the agar surface was inoculated evenly with standardized microbial suspensions

(Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Candida albicans) adjusted to 0.5 McFarland standard using a sterile cotton swab to obtain a uniform lawn culture.

After allowing the surface to dry for 10–15 minutes, wells of approximately 8 mm diameter were made using a sterile cork borer. A measured volume (50–100 µL) of each extract was introduced into the respective wells.

Positive and negative controls were included in the experiment. The plates were incubated at 37°C for 24 hours.

Following incubation, the antimicrobial activity was determined by measuring the diameter of the zones of inhibition around each well in millimeters (mm). Larger zones indicated stronger antimicrobial activity. (Ahmed et.al., 2023)

Minimum Inhibitory Concentration (MIC) – Macro Broth Dilution Method

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that completely inhibits visible microbial growth after incubation.

The MIC of the ethanolic and aqueous extracts was determined using the macro broth dilution technique. Briefly, a series of sterile test tubes containing 2 mL of Mueller–Hinton broth were prepared. Serial two-fold dilutions of each extract were carried out to obtain varying concentrations.

To each tube, 0.1 mL of the standardized microbial suspension (0.5 McFarland standard) was added. A positive control tube containing broth and inoculum without extract and a negative control tube containing broth only were included.

All tubes were incubated at 37°C for 24 hours. After incubation, the tubes were examined visually for turbidity. The lowest concentration of extract showing no visible growth (clear solution) was recorded as the MIC. (Shafiq et.al., 2024)

Determination of Antioxidant Activity by Phosphomolybdenum Assay

The total antioxidant capacity of the hydroethanolic extracts of pineapple crown leaf (Ananas comosus) and moringa (Moringa oleifera) was determined using the phosphomolybdenum method, based on the reduction of molybdenum (VI) to molybdenum (V) under acidic conditions, resulting in the formation of a green phosphate/Mo(V) complex.

The phosphomolybdenum reagent solution was prepared by combining sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). The reagent was freshly prepared prior to analysis to ensure accuracy and reliability of results.

An aliquot (0.3 mL) of the plant extract solution at different concentrations was mixed with 3 mL of the phosphomolybdenum reagent solution in capped test tubes. A blank containing solvent instead of extract was prepared under the same conditions.

The tubes were incubated in a water bath at 95°C for 90 minutes. After incubation, the tubes were allowed to cool to room temperature. The absorbance of the reaction mixture was measured at 695 nm using a UV–Visible spectrophotometer against the blank.

Ascorbic acid was used as the standard reference antioxidant, and a calibration curve was prepared using different concentrations of ascorbic acid.

The total antioxidant capacity of the extracts was expressed as milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g extract). All measurements were performed in triplicate, and results were presented as Mean ± Standard Deviation (SD). (Nanthine et.al., 2017)

Preparation of Herbal Spray Formulation

The herbal spray formulation was developed using gum acacia as a natural film-forming and stabilizing

agent, honey as a humectant, lemon juice as an acidulant and buffering agent, and potassium sorbate as a preservative. The hydroethanolic extracts of moringa (Moringa oleifera) leaves (MLE) and pineapple crown leaves (Ananas comosus) (PCLE) were incorporated into the formulation. Suitable solvents were used to obtain a homogeneous and sprayable solution.

Distilled water was warmed to approximately 40°C to facilitate dissolution of ingredients. Gum acacia was added in varying concentrations to prepare three different formulations:

The mixture was continuously stirred until a properly hydrated and viscous solution was obtained.

Potassium sorbate was then added as a preservative in the following quantities:

The solution was stirred until complete dissolution of potassium sorbate was achieved. Honey was subsequently incorporated into the mixture and mixed thoroughly to ensure uniform distribution.

Fresh lemon juice was added gradually to adjust the pH of the formulation to approximately 3.5.

The plant extracts were incorporated

The mixture was stirred continuously, and mild heating was applied when necessary to obtain a clear, uniform, and homogeneous solution suitable for spraying.

The final formulations were allowed to cool to room temperature and then transferred into sterile spray containers for further physicochemical, antimicrobial, and shelf-life evaluation

Table 1 : Composition of Spray Formulations

Ingredients	F1	F2	F3
Distilled Water	20mL	20mL	20mL
Gum Acacia	0.1g	0.5g	1g
Lemon Juice	0.75mL	4mL	10mL
Honey	2.9g	3g	5g
Glycerol	1mL	1mL	1mL
Sodium citrate	0.25g	0.5g	1g
MLE	5mL	15mL	20mL
PCLE	10mL	10mL	20mL
Final Volume	100mL	100mL	100mL
Temperature	400C	400C	400C

Optimization and Characterization of Herbal Spray Formulation

The prepared spray formulations (F1, F2, and F3) were evaluated for various physicochemical and performance parameters to determine the optimized formulation. The following characterization tests were performed:

Average Weight per Dose

The average weight delivered per spray was determined by weighing the spray container before and after a single actuation using a digital analytical balance. The difference in weight represented the amount

delivered per spray. This procedure was repeated three times for each formulation, and the average value was calculated and expressed as Mean ± Standard Deviation (SD).

Color

The color of each formulation was evaluated visually under natural daylight against a white background. Observations were recorded to assess clarity, uniformity, and any visible particulate matter.

Odour

The odour of the formulations was assessed organoleptically by gentle smelling under controlled laboratory conditions. The characteristic odour and presence of any unpleasant or off-odour were noted.

pH Determination

The pH of each formulation was measured using a calibrated digital pH meter at room temperature. The electrode was immersed directly into the spray solution, and the readings were recorded. Measurements were performed in triplicate, and the results were expressed as Mean ± SD.

Spray Pattern

The spray pattern was evaluated by actuating the spray onto a clean sheet of paper or glass surface from a fixed distance (approximately 10 cm). The pattern was observed for uniformity, dispersion area, and droplet distribution. A consistent and uniform spray pattern was considered acceptable.

Evaporation Time

Evaporation time was determined by spraying a fixed amount of formulation onto a clean glass surface and recording the time required for complete evaporation at room temperature. The time was measured using a stopwatch. The test was performed in triplicate, and the average evaporation time was calculated.

Optimization Criteria

The optimized formulation was selected based on acceptable pH (approximately 3.5), uniform spray pattern, appropriate evaporation time, desirable organoleptic properties, and consistent dose delivery.

Antimicrobial Evaluation of Spray Formulations (F1, F2, and F3)

The antimicrobial activity of the optimized spray formulations (F1, F2, and F3) was evaluated against Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Candida albicans using the agar well diffusion method.

Sterile Mueller–Hinton agar plates were prepared and allowed to solidify. Standardized microbial suspensions adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) were uniformly swabbed onto the surface of the agar plates to obtain a confluent lawn culture.

Wells of approximately 6 mm diameter were punched aseptically using a sterile cork borer. A measured volume (100 µL) of each spray formulation (F1, F2, and F3) was introduced into the respective wells. A positive control (standard antimicrobial agent) and a negative control (base formulation without plant extract) were included.

The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition was measured in millimeters (mm) using a digital caliper. All tests were performed in triplicate, and results were expressed as Mean ± SD.

Antioxidant Activity of Spray Formulations

The total antioxidant capacity of the spray formulations (F1, F2, and F3) was determined using the phosphomolybdenum assay.

An aliquot (0.3 mL) of each formulation was mixed with 3 mL of freshly prepared phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixtures were incubated in a water bath at 95°C for 90 minutes.

After cooling to room temperature, absorbance was measured at 695 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference standard, and results were expressed as mg ascorbic acid equivalent per mL of formulation (mg AAE/mL). All analyses were performed in triplicate, and data were presented as Mean ± SD.

Spike Testing and Microbial Load Analysis of Treated Samples

Sample Preparation

Fresh samples of apple, banana, strawberry, lime juice, sugarcane juice, and beet–carrot juice were procured from a local market and washed thoroughly with distilled water under aseptic conditions. Fruit samples were cut into uniform pieces using sterile instruments. The cut pieces were dipped in 10% sodium hypochlorite solution for 10mins. The juices were pasteurized.

Artificial Inoculation (Spike Testing)

To evaluate the preservative efficacy of the spray formulations, samples were artificially inoculated (spiked) with known concentrations of standardized microbial suspensions (Staphylococcus aureus). Approximately 0.1 mL of microbial suspension (1×10^8 CFU/mL) was uniformly applied to the sample surface.

After inoculation, the spray formulations (F1, F2, and F3) were applied evenly onto the samples. Untreated samples served as control.

Storage Conditions

All treated and control samples were stored at room temperature (25°C). Microbial analysis was conducted over a storage period of 4 days (Day 0, Day 1, Day 2, Day 3, and Day 4).

Microbial Load Determination

At each sampling interval, 1 g (or 1 mL for juices) of sample was aseptically transferred into 9 mL of sterile peptone water and homogenized to obtain a 10^{-1} dilution. Serial dilutions were prepared up to an appropriate dilution factor.

Aliquots (0.1 mL) of selected dilutions were spread onto Mueller–Hinton agar plates and incubated at 37°C for 24 hours. After incubation, colonies were counted and expressed as colony-forming units per gram (CFU/g) or CFU/mL for juice samples.

All experiments were performed in triplicate. Results were expressed as Mean ± SD and converted into $\log_{10}$ CFU/g or $\log_{10}$ CFU/mL for statistical analysis.

Sensory and Physicochemical Analysis of Spiked Fruits and Juices

The effect of spray formulations (F1, F2, and F3) on the quality attributes of spiked fruit and juice samples was evaluated through sensory and physicochemical analysis over a storage period of four days. The parameters assessed included odour, pH, fungal growth, color, weight loss, and browning index.

Odour Evaluation

Odour was assessed daily under controlled laboratory conditions by organoleptic evaluation. Samples were examined for freshness, development of off-odour, or any signs of spoilage. Observations were recorded qualitatively for each treatment group.

pH Determination

The pH of juice samples and homogenized fruit samples was measured daily using a calibrated digital pH meter at room temperature. Measurements were performed in triplicate, and results were expressed as Mean ± Standard Deviation (SD).

Fungal Growth Assessment

Samples were visually inspected daily for any visible signs of fungal growth, such as surface mycelial

development or discoloration. The presence or absence of fungal contamination was recorded. Where necessary, microbial plating was performed to confirm fungal growth.

Color Evaluation

Color changes in fruit and juice samples were monitored daily through visual observation and instrumental analysis. Any noticeable discoloration or browning was recorded.

Weight Loss Determination

For solid fruit samples (apple, banana, and strawberry), weight loss was measured daily using a digital analytical balance. Samples were weighed at Day 0 and on subsequent storage days. (Mehmood et al., 2020)

Determination of Browning Index Using ImageJ

Image analysis was performed using ImageJ software to quantify browning in treated and control fruit samples. Standardized photographs were captured daily under uniform lighting and background conditions to ensure consistency.

The images were analyzed to obtain CIELAB color space parameters, namely L*, a*, and b* values.

L* represents lightness (0 = black, 100 = white)

a* represents the red (+) to green (−) axis

b* represents the yellow (+) to blue (−) axis

Statistical analysis

The experimental data were statistically analyzed using one-way Analysis of Variance (ANOVA) to determine significant differences among the groups. Post-hoc multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. Differences were considered statistically significant at a confidence level of 95% ($p < 0.05$).

3. RESULTS

Extract Concentration and Yield

Hydroethanolic extraction (4:1 ethanol:water) yielded concentrated crude extracts from both plant materials. The concentration of Moringa oleifera leaf extract was calculated as 0.62 g/mL (620 mg/mL) 31% yield, whereas pineapple crown leaf extract exhibited a higher concentration of 1.22 g/mL (1220 mg/mL) 61% yield . The greater extractive concentration observed in pineapple crown leaves suggests a higher abundance of solvent-soluble phytoconstituents under identical maceration conditions. This indicates efficient recovery of phenolic and secondary metabolites from pineapple crown waste material.

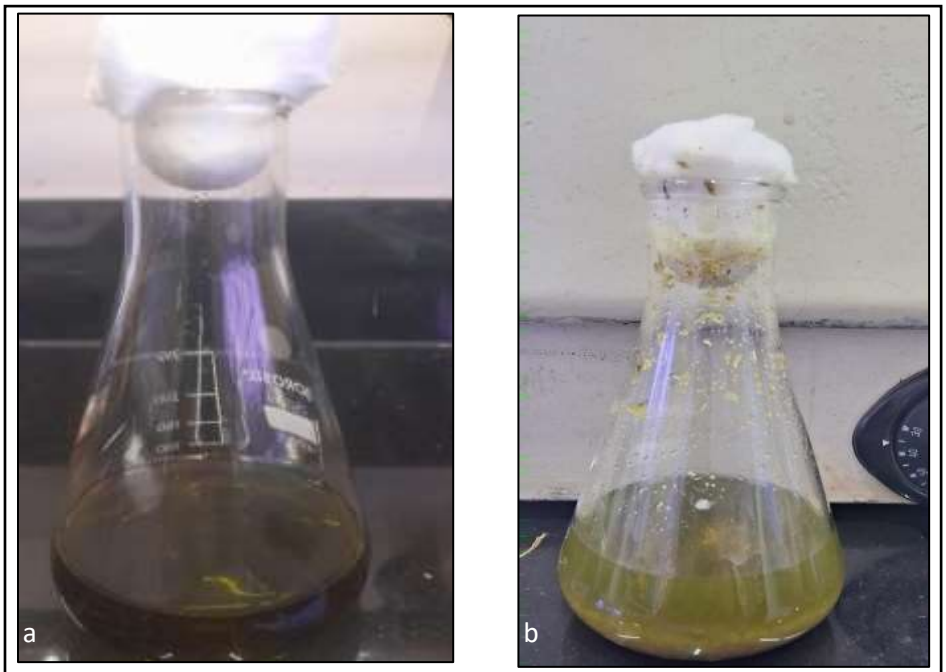


Figure 1 : Hydroethanolic extracts of

a) Moringa leaves b) Pineapple crown leaves after filtration

Phytochemical Screening

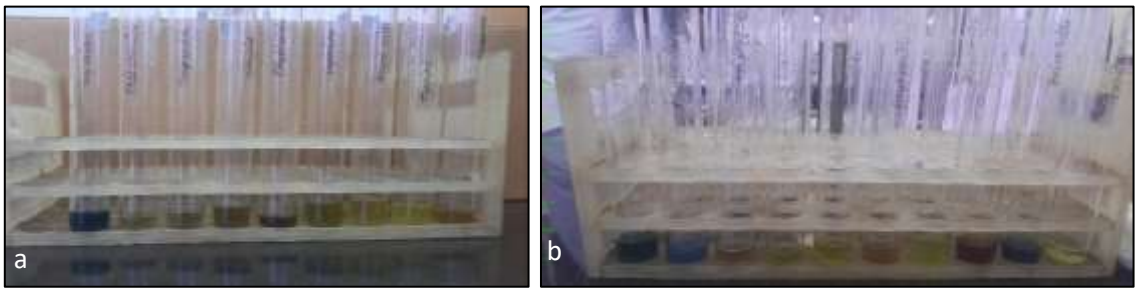


Figure 2 : Phytochemical Analysis of a) Moringa Leaf Extract and b) Pineapple Crown Leaf Extract

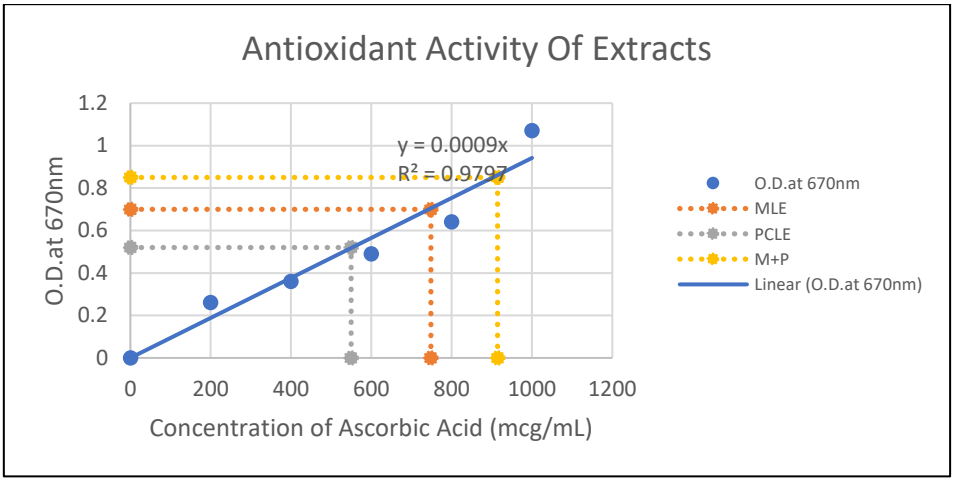
From left to right glycosides, phlobatannins, terpenoids, steroids, polyphenols, saponins, amino acids, tannins, flavonoids.

Table 2 : Phytochemicals Analysis of the extracts			
Phytochemicals	Method	MLE	PCLE
Flavonoids	NaOH test	Present	Present
Tannins	Lead acetate test	Present	Present
Reducing sugars	Fehlings test	Present	Absent
Saponins	Foam test	Absent	Present
Polyphenols	$FeCl_3$ test	Present	Present
Steroids	Chloroform test	Absent	Absent

Terpenoids	TCA test	Present	Present
Phlobatannins	HCl test	Absent	Absent
Amino acid	Ninhydrin test	Absent	Present
Alkaloids	Wagners test	Present	Present
Phenolics	Fc test	Present	Present
Key: MLE: Moringa Leaf Extract PCLE: Pineapple Crown Leaf Extract			

Preliminary phytochemical analysis confirmed the presence of multiple bioactive constituents in both extracts . Flavonoids, tannins, polyphenols, terpenoids, alkaloids, and phenolic compounds were detected in both Moringa oleifera and pineapple crown extracts. Reducing sugars were detected in moringa extract but absent in pineapple crown extract, whereas saponins and amino acids were specifically detected in pineapple crown extract. Steroids and phlobatannins were absent in both samples. The abundance of flavonoids and phenolics in both extracts indicates strong antioxidant and antimicrobial potential, supporting their suitability for preservative applications.

Antioxidant Activity of Extracts



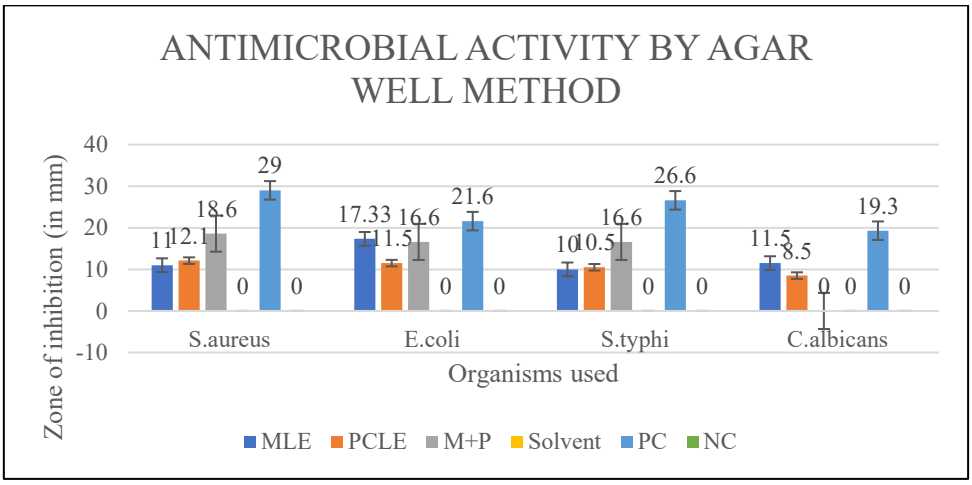
Graph 1: Antioxidant Activity of extracts and its combination by Phosphomolybdenum Assay

The total antioxidant capacity determined by the phosphomolybdenum assay demonstrated notable variation among extracts. Absorbance values at 670 nm were recorded as 0.72 for Moringa leaf extract, 0.52 for pineapple crown leaf extract, and 0.89 for the combined extract . When expressed as ascorbic acid equivalents (AAE), pineapple crown extract exhibited 54.9 mg%, whereas Moringa extract showed 74.8 mg%. The combined extract demonstrated enhanced antioxidant activity(91.7 mg%) compared to individual extracts, suggesting synergistic interaction between phytochemical constituents.

Antimicrobial Activity of extract

Figure 3: Antimicrobial activity of extracts against a) Escherichia coli, b) Staphylococcuus aureus, c) Salmonella typhi, d) Candida albicans
MLE : Moringa leaf extract, PCLE : Pineapple Crown Leaf Extract, S : Solvent, NC : Negative control, PC : Positive Control

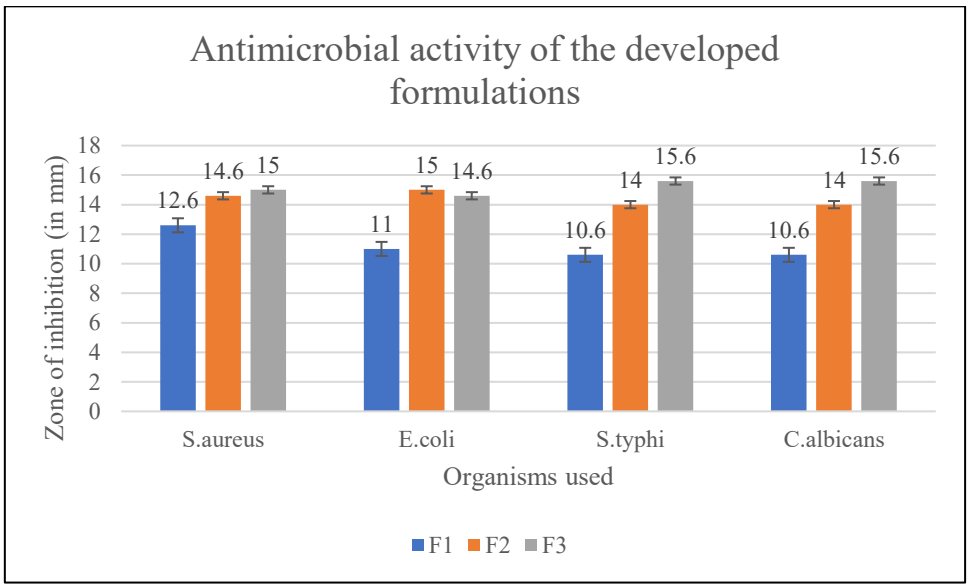
The combined extract demonstrated superior antimicrobial activity compared to individual extracts. The highest inhibition was observed against Staphylococcus aureus (18.6 mm), followed by Escherichia coli and Salmonella typhi (16.6 mm each), indicating possible synergistic interaction between bioactive constituents of both plants. Among spray formulations, F3 exhibited the most consistent and balanced antimicrobial activity . It produced inhibition zones of 16 mm against S. aureus, 14.6 mm against E. coli and S. typhi, and 15.6 mm against Candida albicans. F2 showed moderate inhibition, while F1 demonstrated selective activity. No inhibition was observed in the negative control.



Graph 2 : ***The graph (top) gives a comparison between the antimicrobial activity of the extracts individually and in combination with each other and with positive control and the heat map (bottom) shows the effectiveness of the extracts as well as the formulations with red indicating resistance and green indicating susceptibility.***

Figure 5: Antimicrobial activity of extracts against a) *Escherichia coli*, b) *Staphylococcuus aureus*, c) *Salmonella typhi*, d) *Candida albicansFigure 5.6 : Antimicrobial activity of formulated spray against a) Escherichia coli, b) Staphylococcuus aureus, c) Salmonella typhi, d) Candida albicans M+P : MLE+PCLE, C : Control(Ingredients other than MLE AND PCLE), F1: Formulation 1, F2: Formulation 2, F3: Formulation 3*

M+P : MLE+PCLE, C : Control(Ingredients other than MLE AND PCLE), F1: Formulation 1, F2: Formulation 2, F3: Formulation 3

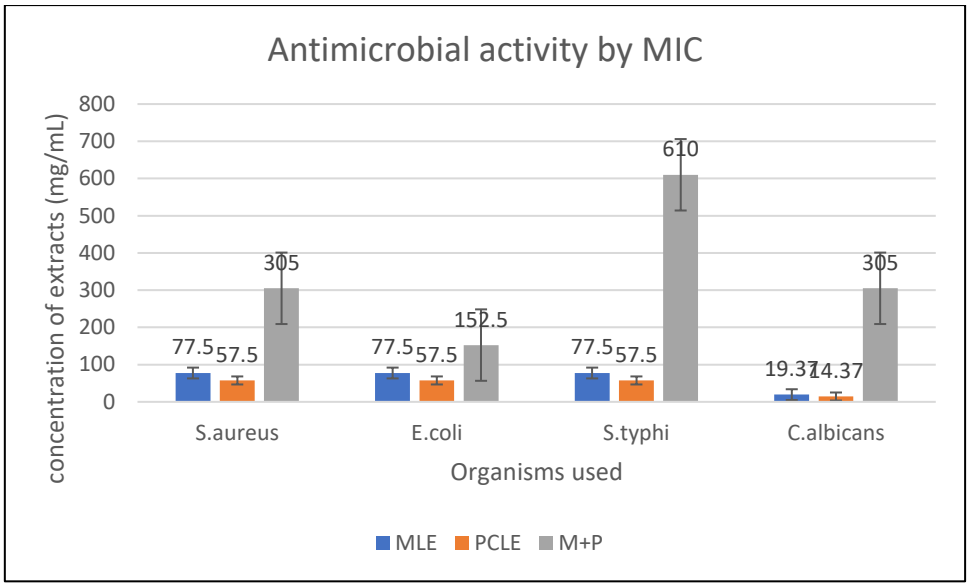


Graph 3: The graph above gives a comparison between the antimicrobial activity of the spray formulations with each other

Minimum Inhibitory Concentration (MIC)

Table 3: Comparison of MIC of both extract against each organism

Organism tested	MIC of MLE	MIC of PCLE	MIC of MLE+PCLE
S.aureus	38.75 mg/mL	305mg/mL	28.75mg/mL
E.coli	77.5mg/mL	152.5mg/mL	115mg/mL
S.typhi	77.5mg/mL	610mg/mL	115mg/mL
C .albicans	9.68mg/mL	305mg/mL	28.75mg/mL



Graph 4: ***The graph above gives a comparison between the antimicrobial activity of the extracts individually and in combination with each other***

The MIC of the combined hydroethanolic extract demonstrated concentration-dependent inhibition against all tested microorganisms. The lowest MIC was observed against Staphylococcus aureus, followed by Candida albicans, whereas comparatively higher MIC values were recorded for Escherichia coli and Salmonella typhi, indicating relatively lower susceptibility of Gram-negative organisms.
This confirms that Gram-positive bacteria were more sensitive to the phytochemical blend, likely due to absence of an outer lipopolysaccharide barrier.

Spray formulation

FIG 6.: a) Formulated Spray b) stored in amber bottles to prevent phytochemical disruption

Characterization of formulated spray

Table 4: Characterization of formulated spray			
Characteristics	F1	F2	F3
Average weight/dose	0.084g	0.04g	0.106g
Smell	Herbal	Herbal	Herbal
Texture	Smooth	Smooth	Smooth
Color	Yellowish green	Brownish green	Brown
Spray pattern	Dispersed	Dispersed	Dispersed

Evaporation time	3mins	3mins	2mins
Particle size	3mm	3mm	2mm
PH	3.5	3.5	3.5

All three formulations (F1, F2, and F3) exhibited herbal odor and smooth consistency, indicating proper solubilization and homogeneity . Color variation was observed, with F1 appearing yellowish green, F2 brownish green, and F3 brown, reflecting differences in extract concentration.

Physicochemical evaluation revealed uniform spray characteristics across formulations . The average delivered dose ranged from 0.04 g to 0.106 g. Particle size ranged between 2–3 mm, with F3 exhibiting the smallest droplet size (2 mm), favoring enhanced surface coverage. All formulations displayed a mildly acidic pH of 3.5, conducive to antimicrobial activity. The dispersed spray pattern and consistent 2-3 minute evaporation time confirmed efficient atomization and suitability for food surface application. Overall, the formulations were stable with no phase separation observed.

Antioxidant Activity of Spray Formulations

The phosphomolybdenum assay of the spray formulations revealed that F3 exhibited the highest antioxidant capacity among the three formulations, followed by F2 and F1. The antioxidant potential of F3(86.7 mg%) was slightly lower than the combined crude extract(91.7 mg%), indicating mild dilution effect due to excipients (lemon, honey, gum acacia, sodium citrate). However, the activity remained substantially high, confirming retention of bioactive phytochemicals after formulation.

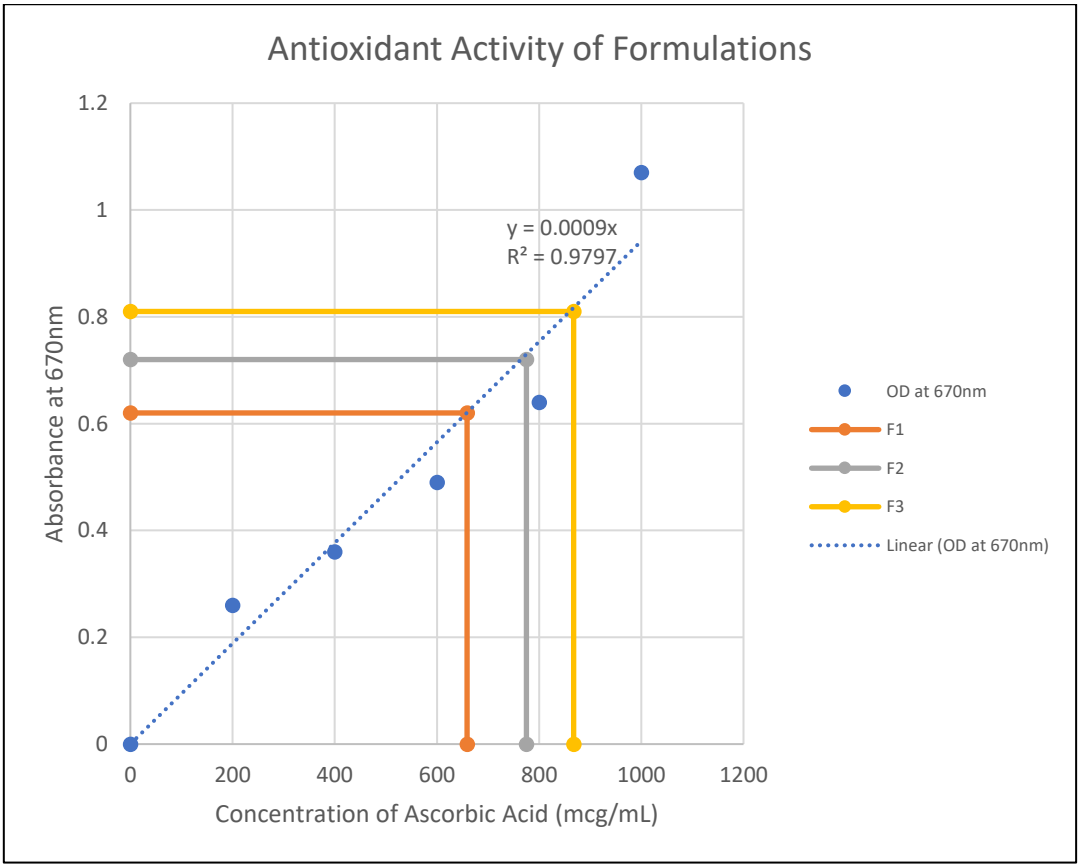
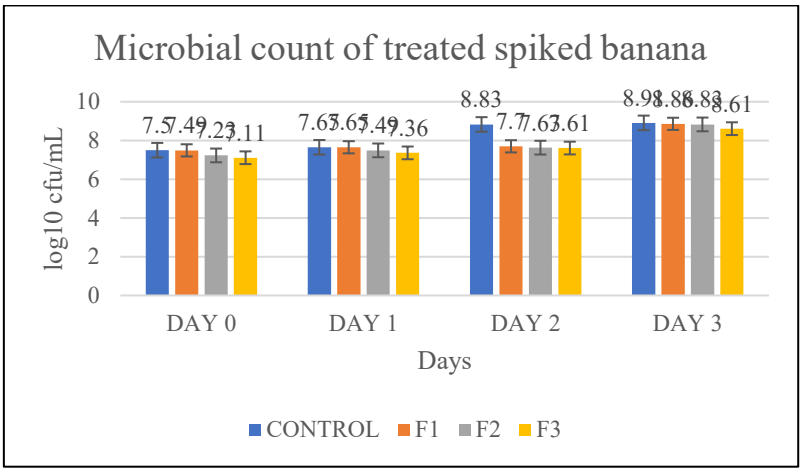


FIG 7 : Antioxidant Activity of spray formulations by Phosphomolybdenum Assay

Spike Testing



Graph 8: Comparison of log 10 values of spiked banana treated with the formulated spray

Fig 8: Spiked strawberry treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

Top left : control, top right : treated with F1, bottom left : Treated with F2, bottom right : treated with F3

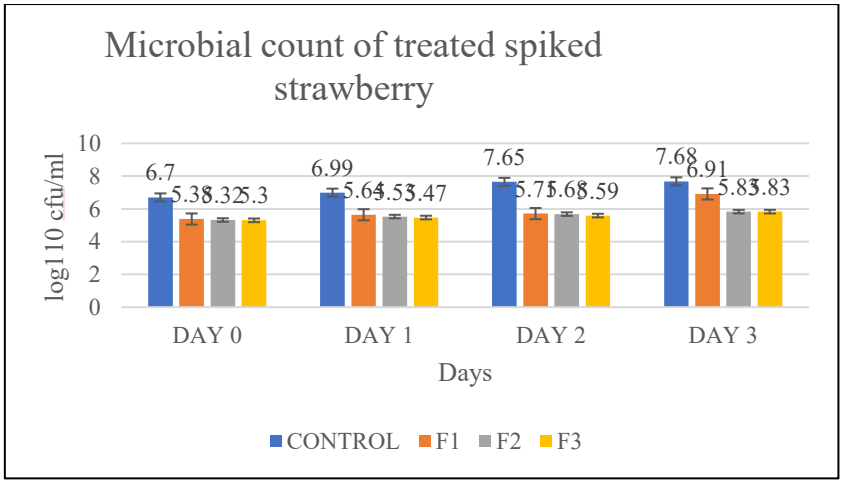
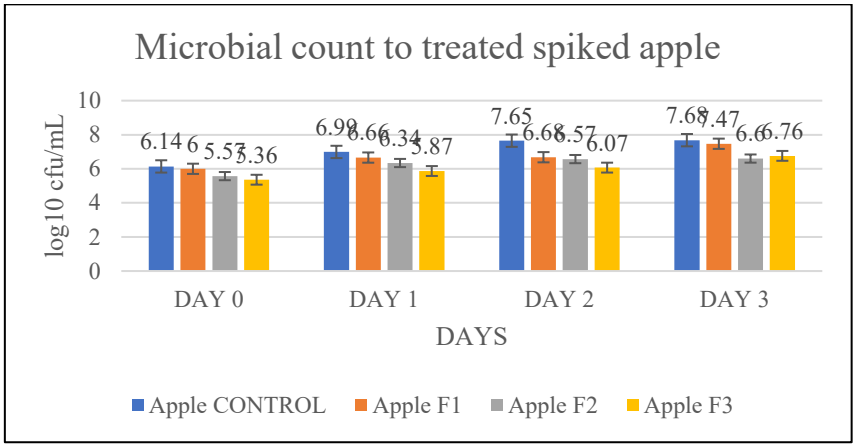


Fig 9.: Spiked strawberry treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

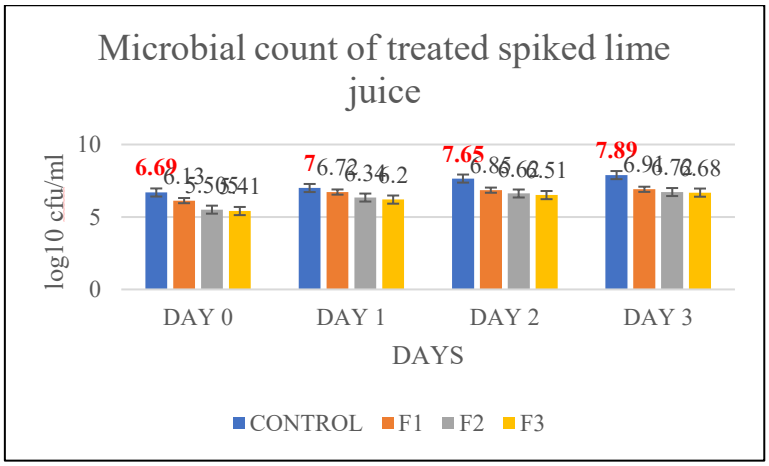
Top left : control, top right : treated with F1, bottom left : Treated with F2, bottom right : treated with F3



Graph 10.: Comparison of log 10 values of spiked apple treated with the formulated spray

Fig 10.: Spiked apple treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

Top left : control, top right : treated with F1, bottom left : Treated with F2, bottom right : treated with F3



Graph 11.: Comparison of log 10 values of spiked lime juice treated with the formulated spray

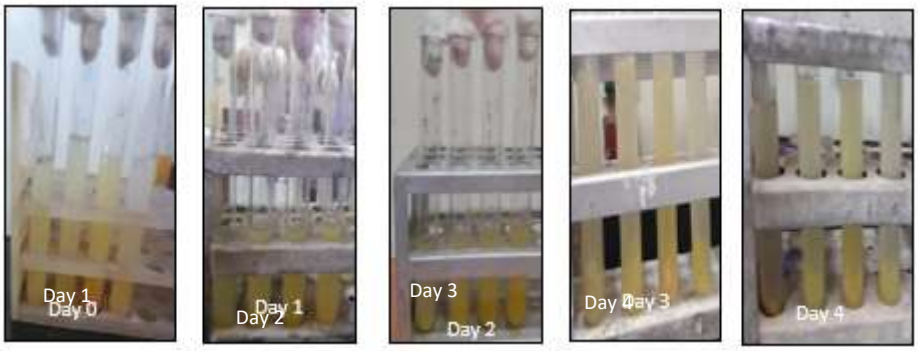
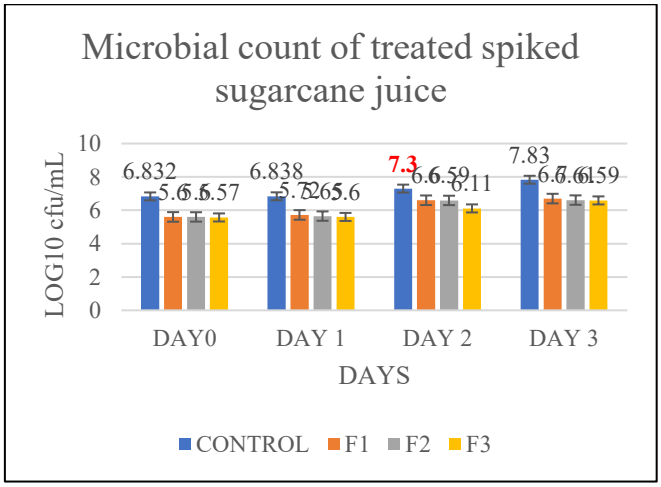


Fig 11.: Spiked lime juice treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

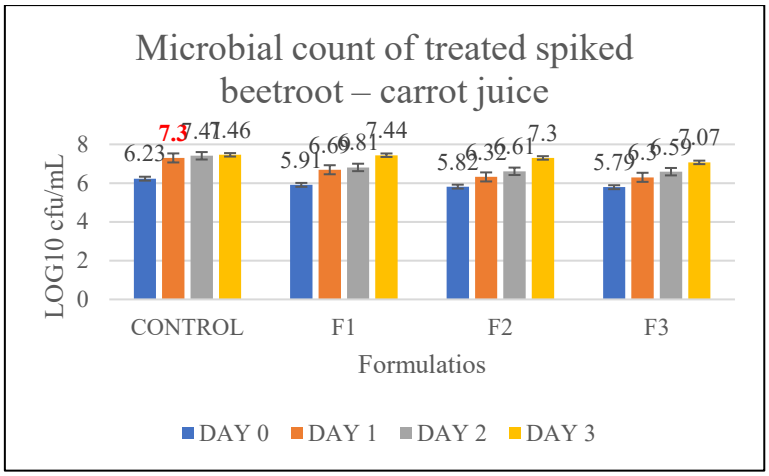
Tube 1 : control, tube 2 : treated with F1, tube 3 : Treated with F2, tube 4 : treated with F3



Graph 12.: Comparison of log 10 values of spiked sugarcane juice treated with the formulated spray

Fig 12.: Spiked sugarcane juice treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

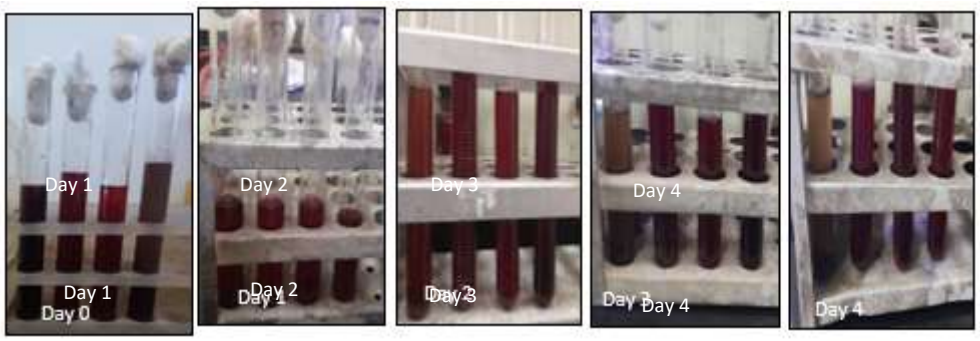
Tube 1 : control, tube 2 : treated with F1, tube 3 : Treated with F2, tube 4 : treated with F3



Graph 13.: Comparison of log 10 values of spiked beet – carrot juice treated with the formulated spray

Fig 13.: Spiked beetroot carrot juice treated with the formulated spray from day 0 to day 4 Stored at room temperature for 5 days

Tube 1 : control, tube 2 : treated with F1, tube 3 : Treated with F2, tube 4 : treated with F3



The preservation study conducted on fresh fruits and juices revealed distinct differences in the effectiveness of the herbal formulations (F1, F2 and F3). In fruits such as apple and strawberry, untreated control samples showed early spoilage marked by browning, softening and loss of freshness. In contrast, treated samples retained firmness, natural colour and acceptable sensory quality up to Day 2. Strawberries, which are highly susceptible to fungal spoilage due to their soft texture and high moisture content, showed visible deterioration in control samples at an earlier stage, while treated samples — particularly F3 — delayed spoilage and maintained structural integrity. Apples also exhibited reduced surface browning and slower deterioration in treated groups, indicating effective control of microbial and oxidative spoilage.

However, the formulations were not effective in banana preservation. Unlike apple and strawberry where spoilage occurs mainly due to surface microbial growth, banana is a climacteric fruit that undergoes rapid internal ripening driven by ethylene production and increased respiration rate. Its deterioration is primarily physiological rather than microbial, involving enzymatic browning, starch-to-sugar conversion and tissue softening from within. Since the herbal spray mainly provides antimicrobial protection and does not possess anti-ethylene or anti-ripening properties, it was unable to significantly delay banana ripening. Additionally, the thick peel allows internal metabolic processes to continue despite surface treatment, leading to rapid spoilage.

In juice systems including lime, sugarcane and beet–carrot juice, untreated control samples showed rapid fermentation, pH decline and development of off-odour and fizziness. Treated samples demonstrated delayed spoilage, better pH stability and reduced microbial load. Among all formulations, F3 showed the highest preservation efficiency, followed by F2 and F1, as evidenced by slower physicochemical deterioration and lower microbial growth across both fruit and juice models.

Overall, the findings indicate that the herbal formulation was effective in delaying microbial spoilage in fruits like apple and strawberry and in juice systems, but its limitation was observed in banana due to its enzyme-driven ripening mechanism. F3 was identified as the most effective formulation for extending shelf stability.

DISCUSSION

The present study demonstrates that hydroethanolic extracts of Moringa oleifera and pineapple crown leaves possess significant preservative potential due to combined antioxidant and antimicrobial activities. The higher extract concentration observed in pineapple crown leaves suggests efficient recovery of phenolic compounds, consistent with previous findings that agro-waste materials often contain concentrated phytochemicals. The detection of flavonoids, tannins, and phenolic compounds in both extracts aligns with established literature reporting these compounds as primary contributors to membrane disruption and oxidative stress induction in microorganisms.

The antioxidant capacity of pineapple crown extract (954.84 µg/mL AAE) in the present study is consistent with the findings of Azim et al. (2017), who reported antioxidant values ranging between 820–980 µg/mL AAE for pineapple peel extracts obtained using hydroethanol solvent systems. The slightly higher value observed in our study may be attributed to the use of crown leaf tissue, which may possess higher phenolic density compared to peel. Similarly, Sreelatha and Padma (2009) reported significant antioxidant activity in Moringa oleifera leaf extracts with values between 300–400 µg/mL AAE, which is comparable to the 361.29 µg/mL AAE observed in the present study. This consistency confirms reliability of extraction methodology. The enhanced antioxidant activity of the combined extract (absorbance 0.89) aligns with the synergistic antioxidant interactions described by Zhang et al. (2018), who demonstrated that combined plant phenolics produce greater radical scavenging efficiency than individual extracts due to cumulative electron donation and metal chelation effects.

The antimicrobial activity observed against Staphylococcus aureus (18.6 mm for combined extract) is consistent with prior reports indicating greater susceptibility of Gram-positive bacteria to plant phenolics. The outer lipopolysaccharide membrane of Gram-negative bacteria such as E. coli and S. typhi often limits permeability of hydrophobic phytochemicals, explaining comparatively lower inhibition zones. The inhibition zone of 18.6 mm against Staphylococcus aureus in the present study is slightly higher than the 15–17 mm range reported by Rahman et al. (2016) for moringa leaf hydroethanolic extracts. The higher inhibition in our study may be attributed to synergistic interaction with pineapple crown phytochemicals, particularly tannins and flavonoids. For Escherichia coli, the inhibition zone of 16.6 mm is comparable to findings by Al-Zoreky (2009), who reported 14–16 mm inhibition using Moringa extracts. The marginally higher activity observed in this study may result from optimized solvent polarity (4:1 ethanol:water), which enhances extraction of semi-polar antimicrobial compounds. The moderate inhibition against Salmonella typhi corresponds with observations by Biswas et al. (2013), who reported that Gram-negative bacteria demonstrate reduced susceptibility due to their protective outer membrane structure.

The lower MIC values observed against Staphylococcus aureus are consistent with findings by Dzotam et al. (2016), who reported lower MIC ranges for Gram-positive organisms when treated with plant phenolic extracts. The comparatively higher MIC values against Gram-negative organisms in our study support the permeability barrier theory described in previous antimicrobial research

The superior performance of F3 formulation may be attributed to multiple synergistic mechanisms like Optimal extract concentration, Mildly acidic pH (3.5) enhancing membrane permeability, Presence of lemon-derived organic acids, Honey-associated hydrogen peroxide activity, Sodium citrate buffering effect maintaining stability.

The smaller droplet size (2 mm) observed in F3 likely improved surface contact area, enhancing antimicrobial exposure. This physicochemical advantage may partly explain the improved spike testing outcomes.

Spike testing revealed up to approximately 2 log reduction in fruit matrices compared to controls. This level of reduction is significant for fresh produce systems stored at room temperature and is comparable to reductions reported for natural plant-based preservatives in minimally processed fruits. However, the comparatively moderate reduction observed in juice systems suggests that high sugar content and aqueous environment may facilitate microbial recovery, limiting long-term suppression. The approximately 1–2 log reduction observed in fruit matrices over 4 days is comparable to the 1.5 log reduction reported by Raybaudi-Massilia et al. (2009) for plant extract-treated fresh-cut fruits stored at room temperature. However, the comparatively moderate reduction observed in sugarcane juice aligns with findings by Rico et al. (2007), who reported that high sugar content and aqueous systems facilitate microbial regrowth despite natural preservative treatment. The higher efficacy in solid fruit matrices may be attributed to better surface retention, reduced dilution effect, localized phytochemical concentration

The study highlights the importance of matrix-dependent preservative efficacy, as solid fruit surfaces allow better retention and localized antimicrobial action, whereas liquid matrices dilute active compounds. Additionally, the valorization of pineapple crown waste supports sustainable food processing strategies and aligns with circular bioeconomy approaches.

CONCLUSION

The present study evaluated the preservative potential of a Moringa and Pineapple leaf extract–based herbal spray as a natural alternative to synthetic food preservatives. Experimental findings demonstrated that the formulation exhibited significant antimicrobial activity, effectively delaying microbial growth and slowing quality deterioration in treated samples compared to controls. This efficacy can be attributed to the phytochemical richness of both plant extracts, which provided synergistic antimicrobial and antioxidant effects.

Microbiological analysis showed a reduced increase in microbial load (log CFU/mL) in treated samples, while physicochemical and organoleptic parameters such as pH, odor, color, and fizziness deteriorated more slowly, indicating delayed spoilage. The spray performed more effectively on fruit surfaces than in juice systems, likely due to better phytochemical contact and reduced interaction with liquid matrix components.

Overall, the formulation demonstrated promising preservative potential, supporting its application as a natural, sustainable, and cost-effective food preservation strategy. However, variations in effectiveness across food matrices and controlled laboratory conditions highlight the need for further optimization, safety evaluation, and large-scale validation before industrial application.

FUTURE PROSPECTS

The findings of the present study highlight the promising potential of the Moringa and Pineapple leaf extract–based spray as a natural food preservative. However, further research and development are essential to fully establish its commercial applicability and optimize its performance across diverse food systems.

One important future direction involves **formulation optimization and standardization**. Although the current study demonstrated antimicrobial efficacy, determining the ideal concentration, extract ratio, solvent system, and method of application will be critical for enhancing stability and reproducibility. Advanced techniques such as microencapsulation or nano-formulation may improve the stability, controlled release, and bioavailability of active phytochemicals, thereby increasing preservative efficiency.

Comprehensive **phytochemical characterization and mechanism studies** should also be conducted. Detailed identification and quantification of active compounds present in Moringa oleifera and Ananas comosus leaf extracts using chromatographic and spectroscopic techniques (HPLC, GC-MS, LC-MS) would provide deeper insight into the specific antimicrobial agents responsible for the observed effects. Furthermore, molecular-level studies investigating mechanisms such as membrane disruption, enzyme inhibition, and oxidative stress induction in microorganisms would strengthen the scientific validation of the formulation.

Toxicological and safety assessment studies should also be undertaken to confirm the absence of adverse effects when used in recommended concentrations. Although plant-derived extracts are generally considered safe, regulatory approval requires standardized toxicity profiling.

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