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Abstract— This study aimed to isolate and characterize *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) from vegetable and fruit samples collected in Aden Governorate, Yemen. Additionally, it sought to analyze the phytochemical and physicochemical properties of aqueous and ethanolic extracts from mixed-herb {turmeric, *Curcuma longa (C. longa*), ginger, *Zingiber officinale (Z. officinale*), and Indian costus, *Saussurea costus* (*S costus*)}. A cross-sectional study was conducted between August and November 2023, with samples obtained from central markets. Bacterial isolation was performed using selective media: Mannitol Salt Agar (MSA) for *S. aureus* and Eosin Methylene Blue (EMB) agar for *E. coli*. Results revealed a significantly higher prevalence of *S. aureus* (32.07% in vegetables; 14.29% in fruits) compared to *E. coli* (15.09%) in vegetables; undetected in fruits). Phytochemical screening confirmed the presence of bioactive constituents, including flavonoids, alkaloids, terpenoids, and tannins, which likely contributed to antimicrobial effects and emphasizing their therapeutic potential. The total ash content was measured at 5.78%, indicating suitable mineral content for therapeutic applications. The moisture content was 10.76%, which helps reduce microbial growth. Extractive values showed that water is more efficient in extracting polar compounds, while ethanol isolates non-polar compounds. The pH values of both aqueous and ethanolic extracts were slightly acidic, which helps preserve the stability of bioactive compounds. These findings highlight the need for improved agricultural hygiene and storage practices to minimize bacterial contamination in fresh produce. Additionally, the study advocates for further research into the bioactive compounds of plant extracts, optimization of extraction protocols, and exploration of synergistic combinations to enhance their application in food safety and natural antimicrobial therapies. This study underscores the potential of plant-derived compounds in combating foodborne pathogens and supports their integration into targeted strategies for public health, pharmaceutical development, and sustainable agriculture.

Keywords— *Staphylococcus Aureus*, *Escherichia* Coli, Ethanolic and Aqueous Extracts, Phytochemicals, Physicochemical.

I. Introduction

Staphylococcus aureus and *E. coli* are two significant bacterial pathogens that pose considerable health risks to contaminate food products, particularly vegetables and fruits. These bacteria are associated with a wide range of infections and foodborne illnesses, making them a critical focus for public health interventions [1].

Staphylococcus aureus is a Gram-positive bacterium that is part of the normal flora of the skin and mucous membranes in humans and animals [2]. Despite its commensal nature, it can cause severe infections when it breaches host defenses, leading to conditions ranging from mild skin infections to life-threatening diseases like septicemia [3]. The increasing prevalence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), exacerbates its threat to public health [4].

Escherichia coli, a Gram-negative bacterium predominantly residing in the intestinal tract of warm-blooded animals, can also lead to serious health complications. Pathogenic strains like *E. coli* O157:H7 produce Shiga toxins, causing severe gastrointestinal distress and systemic complications such as hemolytic uremic syndrome (HUS), particularly in vulnerable populations [5].

The contamination of vegetables and fruits with these pathogens often arises from poor agricultural practices, including the use of contaminated irrigation water or improper handling during harvest and distribution. *S. aureus* is commonly introduced through human contact, while *E. coli* can infiltrate food through exposure to animal waste or untreated water [6, 7]. Such contamination is a leading cause of foodborne illnesses, necessitating robust prevention and mitigation strategies.

Natural antimicrobial agents derived from plants offer a promising alternative to conventional antibiotics, particularly against antibiotic-resistant strains. Medicinal plants like (turmeric, ginger and Indian costus) have been widely recognized for their antimicrobial properties, attributed to bioactive compounds such as alkaloids, flavonoids, and glycosides [8]. These natural compounds provide a basis for exploring plant-based antibacterial treatments that could improve food safety and reduce dependency on synthetic antibiotics.

This study investigates the microbial contamination of vegetables and fruits in Aden Governorate, Yemen, focusing on the isolation and identification of *S. aureus* and *E. coli*. Additionally, it analyzes the phytochemical and physicochemical properties of mixed-herb extracts from (turmeric, ginger and Indian costus). The research aims to develop natural, plant-based solutions to reduce bacterial contamination in food products, thereby enhancing food safety, improving agricultural practices, and mitigating public health risks associated with foodborne illnesses.

II. Materials and Methods

A. Microbiology Methods and Procedures

1. *Study Area*: Aden, a port city in southern Yemen on the Arabian Peninsula, lies near the eastern entrance to the Red Sea. It is located about 170 km (110 mi) east of the Bab-el-Mandeb strait and to the north of the Gulf of Aden. Due to its strategic coastal position, Aden acts as a vital maritime link between the Red Sea and the Arabian Sea, facilitating connections across Africa, Asia, and the Middle East. Historically, Aden has been a major trading hub since ancient times and was a key port during British colonial rule from 1839 to 1967. Its natural harbor, one of the world's largest, has played a significant role in the region's maritime commerce. As of 2023, Aden's population stands at approximately 1,080,000, making it one of Yemen's largest cities [9].
2. *Sample Collection:* Vegetable and fruit samples, including lettuce, cabbage, Spinach, carrots, potatoes, beets, strawberries, grapes, and watermelon, were collected in the morning from the central market in Al-Shaikh. The samples were then transported to the **Yemen Standardization, Metrology, and Quality Control Organization** for analysis.
3. *Maintenance Media:* Bacterial isolates were preserved in a medium composed of 85% nutrient broth (NB) and 15% glycerol, stored at -20°C to ensure long-term viability.
4. *Gram Staining:* Gram staining was performed to differentiate *S. aureus* (Gram-positive) from *E. coli* (Gram-negative), facilitating initial identification. The procedure involved the following steps: Application of crystal violet dye. Fixation with Lugol's solution. Washing with alcohol. Counterstaining with safranin.
5. *Preparation of Culture Media:* General and selective media, including Blood Agar (BA), MacConkey Agar (MA), and (MSA), were prepared as follows: Media were sterilized via autoclaving to ensure aseptic conditions. Sterility was confirmed by incubating prepared media prior to use. Media were stored under sterile conditions for bacterial cultivation.
6. *Preparation of Fresh Vegetable and Fruit Samples:* Each sample (250 grams) was thoroughly washed with distilled water to remove surface debris. Samples were stored in sterile containers to prevent contamination. Tryptone Soy Broth (TSB) was sterilized by autoclaving and used as an enrichment medium. Serial dilutions of the samples were prepared and incubated at 37°C for 24 hours. Selective media, including MSA, (EMB), and Xylen Xylose Lysine Deoxycholate (XLD), were employed to isolate *S. aureus*, *E. coli*, and other Gram-negative bacteria [10].
7. *Biochemical Testing of Staphylococcus aureus and Escherichia coli:* Biochemical tests were performed to identify and characterize bacterial species based on their metabolic activities:
    a) Catalase Test, differentiates catalase-positive Staphylococcus species from catalase-negative Streptococcus species [11].
    b) Coagulase Test, identifies *S. aureus* by detecting coagulase enzyme activity, which causes plasma coagulation [11].
    c) Oxidase Test, detects cytochrome c oxidase activity, distinguishing oxidase-positive bacteria such as Pseudomonas sp., from oxidase-negative *E. coli* [11].
    d) Methyl Red (MR) Test, confirms stable acid production from glucose fermentation, a characteristic of *E. coli* [12].
    e) Indole Test, detects indole production from tryptophan hydrolysis, with a positive result confirming *E. coli* [13].
    f) Citrate Utilization Test, determines the ability to use citrate as a sole carbon source, distinguishing citrate-negative *E. coli* from other enteric bacteria [14].
    g) DNase Test, identifies DNase activity in *S. aureus*, indicated by a clear zone around the colony [15]. Capsule Staining, highlights bacterial capsules using copper sulfate, confirming capsule presence as a virulence factor [15].
    h) Urease Test, differentiates urease-positive Proteus mirabilis from urease-negative *E. coli* [13].
    i) Sugar Fermentation Test, evaluates glucose and fructose fermentation by *S. aureus* and *E. coli*, indicated by a yellow color change in the pH indicator [16].
    j) Kligler Iron Agar (KIA), assesses carbohydrate fermentation and hydrogen sulfide production, aiding in the identification of *E. coli* [17].

k) Voges–Proskauer (VP) Test, detects acetoin production from glucose fermentation, used to differentiate enteric bacteria [13].

B. Plant Sample Collection and Preparation

1. *Sample Collection*: Dried root samples of (turmeric, ginger and Indian costus) were collected from a herbalist in the Aden Crater area (**Al-Ekbar**). The plant materials were identified and authenticated by **Dr. Othman Al-Hawshabi**, Professor of Taxonomy of Flowering Plants and Flora, at the Biology Department, Faculty of Science, Aden University. After authentication, the roots were processed using traditional methods: Ground into fine powder. Purified to remove impurities. Combined into a homogeneous mixture for subsequent analysis.

2. *Extraction Procedure*: To maximize the range of isolated phytochemicals, a dual extraction method was employed:

   a) *Alcoholic Extraction:* Alcoholic extraction was performed using Soxhlet extraction with 70% ethanol to target alcohol-soluble components. Following established protocols [18, 19, 20]: 40 grams of dried powdered roots were subjected to Soxhlet extraction with 400 mL of 70% ethanol for 6–8 hours. The extract was filtered through sterile gauze and centrifuged, followed by filtration using Whatman No. 1 filter paper. The filtrate was dried in a Petri dish and stored at 4°C for later analysis.

   b) *Aqueous Extraction:* Aqueous extraction targeted water-soluble compounds using distilled water [18, 19], 40 grams of dried powdered roots were combined with 400 mL of distilled water and shaken on a magnetic stirrer at room temperature for 24 hours. The mixture was filtered, centrifuged, and dried in a laboratory oven at 50°C. The dried extract was stored at 4°C until required for further experiments.

3. *Phytochemical Screening:* Phytochemical screening identified bioactive compounds using standard colorimetric tests [21, 22]. Alkaloids were detected using Dragendorff's reagent, where a yellow or orange precipitate indicated their presence. Tannins were identified using 1% $FeCl_3$, resulting in a greenish-black coloration. Saponins were confirmed by the formation of persistent foam after shaking with water. Flavonoids were detected by a yellow color upon the addition of NaOH. Steroids and triterpenoids were identified by heating with H_2SO_4, where a red or pink coloration indicated steroids, while a reddish-brown ring confirmed the presence of triterpenoids.

4. *Physicochemical Analysis*: Determination of Extractive Value, the extractive value measures the quantity of phytochemicals soluble in a specific solvent, reflecting extraction efficiency:

$$Extractive\ value\ \% = \frac{Weight\ of\ the\ extract}{Weight\ of\ the\ air-\ dried\ drug} \times 100$$

Soxhlet extraction (70% ethanol) and distilled water were used for alcoholic and aqueous extracts, respectively [22].

5. *Loss on Drying (Moisture Content):* Moisture content impacts plant stability and preservation:

$$Moisture\ (\%) = \frac{Weight\ before\ dryin\ -\ Weight\ after\ drying\ Weight}{Weight\ of\ the\ sample} \times 100$$

Samples were dried in a pre-heated oven at 135°C, minimizing microbial contamination risks [23].
Ash Content Determination, ash content indicates the purity of plant materials:

$$Ash\ \% = \frac{Ash\ weight}{Weight\ of\ the\ sample} \times 100$$

Samples were heated in a muffle furnace at 500°C until complete combustion yielded white ash, reflecting minimal contamination [24]. Determination of pH, the pH was measured by mixing 5 grams of dried plant powder with 50 mL of distilled water, stirring for 10 minutes, filtering, and recording the pH with a calibrated pH meter [25].

III. Results

A. Total Number of Vegetable and Fruit Samples

The study focused on isolating *S. aureus* and *E. coli* from vegetable and fruit samples to evaluate the antimicrobial effects of aqueous and ethanol plant extracts. The results are summarized as follows in (**Table 1**): Vegetable Samples **(53):** 17 samples (32.07%) tested positive for *S. aureus*. 8 samples (15.09%) tested positive for *E. coli*. 28 samples (52.83%) were excluded. Fruit Samples **(21):** 3 samples (14.29%) tested positive for *S. aureus*. No *E. coli* was detected. 18 samples (85.71%) were excluded.

Table 1: Prevalence of Staphylococcus aureus and Escherichia coli in Vegetable and Fruit Samples

Sample Source	Vegetable Samples	Fruit Samples
Number of Samples	53 (100%)	21 (100%)
Positive Samples	25 (47.17%)	3 (14.29%)
Ignored Samples	28 (52.83%)	18 (85.71%)
S. aureus (Positive)	17 (32.07%)	3 (14.29%)
E. coli (Positive)	8 (15.09%)	0

B. Results of Bacterial Identification

The bacterial identification process confirmed the presence of *S. aureus* and *E. coli* in vegetable and fruit samples. Various growth media were used to isolate and differentiate the bacteria, as outlined below:

1. *Staphylococcus aureus*, (MSA), Yellow colonies indicating mannitol fermentation. Vogel & Johnson Agar (VOG), Black colonies due to tellurite reduction. Blood Agar, White colonies with beta hemolysis (clear zones around colonies). Nutrient Agar, Large white colonies.
2. *Escherichia coli*, (EMB) Agar, Purple to black colonies with a green metallic sheen, indicative of lactose fermentation. MacConkey Agar: Pink colonies with a darker halo due to lactose fermentation. (XLD) Agar: Circular yellow colonies, indicating acid production from xylose fermentation. Blood Agar: Gray colonies with partial hemolysis (alpha hemolysis).

C. Results of Biochemical Tests

Biochemical testing provided crucial insights into the physiological and metabolic characteristics of *S. aureus* and *E. coli* isolated from vegetable and fruit samples (**Table 2**). Capsule Test, *E. coli* was positive, indicating the presence of a polysaccharide capsule. *S. aureus* was negative. Catalase Test, both bacteria tested positive, confirming their ability to decompose hydrogen peroxide. Coagulase Test, *S. aureus* was positive, distinguishing it from other staphylococcal species. *E. coli* was negative. Hemolysis, *S. aureus* showed beta hemolysis (complete lysis of red blood cells). *E. coli* exhibited alpha hemolysis (partial lysis). Oxidase Test, both bacteria were negative, aligning with their facultative anaerobic nature. DNase Test, *S. aureus* was positive, indicating virulence. *E. coli* was negative. Gram Stain, *S. aureus* appeared as Gram-positive cocci (blue). *E. coli* appeared as Gram-negative rods (red). Citrate Utilization, *S. aureus* was positive, showing citrate usage as a carbon source. *E. coli* was negative. Indole Test, *E. coli* was positive, confirming indole production. *S. aureus* was negative. Voges-Proskauer (VP) Test, *S. aureus* tested positive for acetoin production. *E. coli* was negative. Methyl Red (MR) Test, both bacteria were positive, indicating strong acid production from glucose fermentation. Motility Test, *E. coli* was motile, whereas *S. aureus* was non-motile. Hydrogen Sulfide (H_2S) Production, both bacteria were negative for H_2S production.

Sugar Fermentation, both bacteria fermented glucose, but only *E. coli* produced gas. *S. aureus* fermented lactose, mannitol, and sucrose, while *E. coli* showed variability in sugar fermentation. Urease Test, both *S. aureus* and *E. coli* were negative.

Table 2. Biochemical Test Results for Identification of S. aureus and E. coli Isolated from Vegetables and Fruits

Biochemical Test	***Staphylococcus aureus***	***Escherichia coli***
Capsule Test	-	+
Catalase Test	+	+
Coagulase Test	+	-
Hemolysis	Beta (B)	Alpha (∝)
Oxidase Test	-	-
DNase Test	+	-
Gram Stain	Blue (Cocci)	Red (Rod)
Citrate Utilization	+	-
Indole Test	-	+
Voges-Proskauer (VP)	+	-
Methyl Red (MR) Test	+	+
Motility Test	-	+
Hydrogen Sulfide (H_2S)	-	-
Sugar Fermentation		
- Glucose	+	+ (with gas)
- Lactose	+	+
- Fructose	+	-
- Mannitol	+	+
- Xylose	-	+
- Sucrose	+	+
Urease Test	-	-

D. *Isolation of Staphylococcus aureus and Escherichia coli from Vegetables and Fruits*

The study aimed to isolate *S. aureus* and *E. coli* from various vegetable and fruit samples using a six-dilution method across three culture media: MSA, XLD, and EMB. The cultivation process was performed in Tryptone Water for 24 hours, and the procedure was repeated three times to enhance accuracy. The results showed variability in bacterial isolation across different samples and repetitions (**Table 3; 4**).

The isolation rates of *S. aureus* and *E. coli* varied significantly across vegetable types. **Cabbage** showed a *S. aureus* isolation rate of **19.04%** and the highest *E. coli* isolation rate among vegetables at **40.54%**, with undesirable bacteria at **4.21%**.

Lettuce had isolation rates of **16.67%** for *S. aureus* and **35.14%** for *E. coli*, with undesirable bacteria at **9.47%**. **Spinach** presented a moderate *S. aureus* isolation rate of **17.86%** and *E. coli* at **24.32%**, with undesirable bacteria at **12.63%**. **Beetroot** and **potatoes** had the highest *S. aureus* isolation rates at **20.24%**, but *E. coli* was not detected in either sample (0%), while undesirable bacteria were found at **20%**. **Carrots** had the lowest *S. aureus* isolation rate at **5.95%**, with no *E. coli* detected and the highest level of undesirable bacteria at **32.63%**. Fruit samples showed a different contamination pattern. **Watermelon** had the highest *S. aureus* isolation rate at **75%**, with no *E. coli* detected and undesirable bacteria at **30%**. **Grapes** had a *S. aureus* isolation rate of **25%**, with *E. coli* absent and undesirable bacteria at **34%**. **Strawberries** showed no contamination by *S. aureus* or *E. coli*, but undesirable bacteria were present at **36%**, the highest among fruits.

Table 3: Percentage of *Staphylococcus aureus* and *Escherichia coli* in Vegetable Samples

Total count of vegetable samples for all repeats	Type of vegetable sample	*Staphylococcus aureus*	*Escherichia coli*	Ignored bacteria
18 samples of vegetable	Cabbage	19.04%	40.54%	4.21%
	Lettuce	16.67%	35.14%	9.47%
	Spinach	17.86%	24.32%	12.63%
	Beets	20.24%	0	20%
	Carrots	5.95%	0	32.63%
	Potatoes	20.24%	0	20%

Table 4: Percentage of *Staphylococcus aureus* and *Escherichia coli* in Fruit Samples

Total count of fruit samples for all repeats	Type of fruit sample	*Staphylococcus aureus*	*Escherichia coli*	Ignored bacteria
9 samples of fruit	Grapes	25%	0	34%
	Strawberry	0	0	36%
	Watermelon	75%	0	30%

Prevalence of Bacteria in Vegetables. *S. aureus*: The highest prevalence was observed in beetroot, potatoes, and cabbage (~20%), while the lowest detection rate was in carrots (5.95%). This suggests potential contamination due to post-harvest handling practices. *E. coli*: The highest prevalence was recorded in cabbage (40.45%) and lettuce (35.14%), whereas no detection was observed in beetroot, carrots, or potatoes. This absence may be attributed to water contamination sources or inadequate washing procedures. Prevalence of Bacteria in Fruits. *S. aureus*: The highest prevalence was found in watermelon (75%), followed by grapes (25%). No detection was observed in strawberries, indicating better hygienic conditions or natural resistance to bacterial contamination (**Table 5**).

Table 5: Prevalence of *S. aureus* and *E. coli* in Vegetables and Fruits

Sample Type	*S. aureus* (%)	*E. coli* (%)	Other Bacteria (%)
Vegetables	19.04–20.24	24.32–40.54	4.21–32.63
Fruits	25–75	0	30–36

E. Mean Count of S. aureus in Vegetables

The mean count of ***S. aureus*** across dilutions showed a decreasing trend, with the highest mean at 10^{-1} (0.944), progressively declining to **0.389** at 10^{-6}. Significant differences were observed among vegetable types (Table 6). Specifically, beetroot and potatoes exhibited the highest mean counts (0.944), followed by cabbage (0.889) and spinach (0.833). The lowest mean count was found in carrots (0.278), indicating lower susceptibility to contamination.

Table 6: Mean Counts of *S. aureus* in Vegetables

Vegetables	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Mean
Cabbage	1.000	1.000	1.000	1.000	1.000	0.333	0.889
Lettuce	1.000	1.000	1.000	0.667	0.667	0.333	0.778
Spinach	1.000	1.000	1.000	1.000	0.667	0.333	0.833
Beetroot	1.000	1.000	1.000	1.000	1.000	0.667	0.944
Carrots	0.667	0.667	0.333	0.000	0.000	0.000	0.278
Potatoes	1.000	1.000	1.000	1.000	1.000	0.667	0.944

F. Mean Count of Escherichia coli in Vegetables

The mean count of E. coli across dilutions remained stable at 0.444 up to dilution 10^{-4}, then significantly declined to 0.056 at 10^{-6} (**Table 7**). Vegetable-specific findings revealed the highest mean counts in cabbage (0.833) and lettuce (0.722), with moderate counts in spinach (0.500). No detectable E. coli was found in beetroot, carrots, or potatoes.

Table 7: Mean Counts of E. coli in Vegetables

Vegetables	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Mean
Cabbage	1.000	1.000	1.000	1.000	0.667	0.333	0.833
Lettuce	1.000	1.000	1.000	1.000	0.333	0.000	0.722
Spinach	0.667	0.667	0.667	0.667	0.333	0.000	0.500
Beets	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Carrots	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Potatoes	0.000	0.000	0.000	0.000	0.000	0.000	0.000

G. Mean Count of S. aureus in Fruits

Trends Across Dilutions: The highest mean count was observed at dilutions **10^{-1}**, **10^{-2}**, **10^{-4}**, and **10^{-5}** in watermelon (**0.333**), then decreased to **0.000** from **10^{-3}** onward. Fruit-Specific Findings: Watermelon exhibited the highest mean count (**0.333**), followed by grapes (**0.111**). No detectable *S. aureus* was found in strawberries (**Table 8**).

Table 8: Mean Counts of *S. aureus* in Fruits

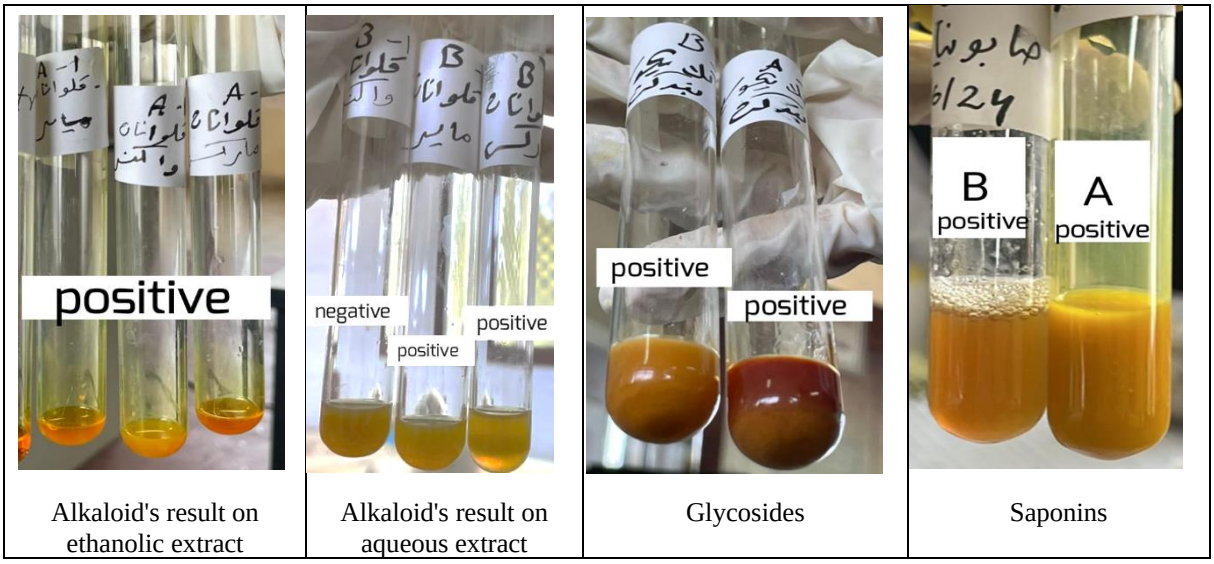
Fruits	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Mean
Grapes	0.333	0.333	0.000	0.000	0.000	0.000	0.111
Strawberries	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Watermelon	0.667	0.667	0.333	0.333	0.000	0.000	0.333

H. Phytochemical Analysis of Ethanolic and Aqueous Extracts

Ethanolic extract (**Table 9; Fig. 1**), showed a broad range of bioactive compounds, including alkaloids, flavonoids, terpenoids, glycosides, tannins, resins, and carbohydrates. Amino acids and fucoidans were absent, indicating ethanol's inefficiency in extracting these polar compounds. Aqueous extract, contained polar compounds such as fucoidans and amino acids, which were absent in the ethanolic extract. Showed presence of glycosides, flavonoids, tannins, terpenoids, and carbohydrates, albeit at possibly lower concentrations.

Table 9: Phytochemical composition of ethanolic and aqueous extracts

Phytochemical	Ethanolic Extract	Aqueous Extract
Alkaloids	Positive (brown precipitate)	Positive (no brown precipitate)
Glycosides	Present	Present
Saponins	Present	Present
Flavonoids	Present	Present
Flavanones	Present	Present
Tannins	Present	Present
Resins	Present	Present
Fucoidans	Absent	Present
Terpenoids	Present	Present
Amino Acids	Absent	Present
Carbohydrates	Present	Present



Alkaloid's result on ethanolic extract | Alkaloid's result on aqueous extract | Glycosides | Saponins

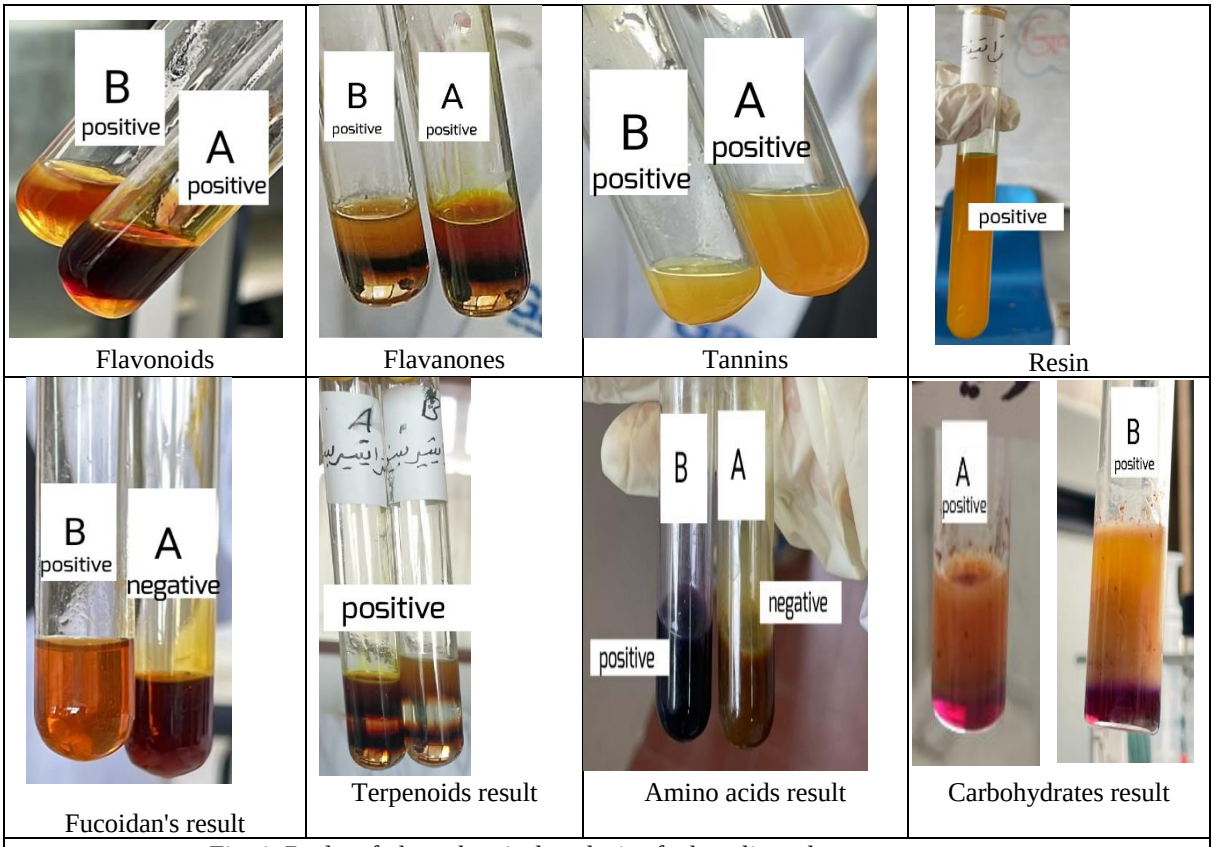


Fig. 1. Reslts of phytochemical analysis of ethanolic and aqueous extracts.
A= Ethanolic extract, B= Aqueous extract

I. Physiochemical Properties of Plant Extracts

Total Ash Content measured value: 5.78%. Indicates moderate mineral content and purity, suitable for therapeutic applications. Moisture content, measured value, 10.76%. Moderate moisture reduces microbial growth and preserves bioactive compounds. Extractive values, aqueous extract, 15.96%. Ethanolic extract, 14.87%. Highlights water's efficiency in extracting polar compounds and ethanol's ability to isolate non-polar compounds. pH values, aqueous extract, 5.113 (slightly acidic). Ethanolic extract, 5.37 (slightly acidic). Acidity preserves bioactive stability, beneficial for pharmaceutical formulations.

Table 10: Summary of Physiochemical Properties

Parameter	Value	Significance
Total Ash Content	5.78%	Indicates mineral content and purity.
Moisture Content	10.76%	Ensures stability and prevents microbial growth.
Aqueous Extract Value	15.96%	Efficient extraction of polar compounds.
Ethanolic Extract Value	14.87%	Efficient extraction of non-polar compounds.
Aqueous Extract pH	5.113	Suitable for preserving bioactive.
Ethanolic Extract pH	5.37	Suitable for stable formulations.

IV. Discussion

A. Importance of the Study

This study contributes to improving food safety in Yemen by exploring natural antimicrobial alternatives to combat bacterial contamination in vegetables and fruits. In a country facing challenging agricultural conditions, including limited access to clean water and proper sanitation, the findings emphasize the potential of plant-based extracts to reduce the presence of harmful pathogens like *S. aureus* and *E. coli* [26]. By utilizing locally available medicinal plants, this research offers a sustainable, cost-effective solution to enhance food safety, reduce reliance on synthetic antibiotics, and mitigate the public health risks associated with foodborne illnesses.

B. Bacterial Isolation and Identification

Mannitol Salt Agar effectively isolated *S. aureus*, as evidenced by the red-to-yellow color change due to mannitol fermentation. Similarly, (EMB) agar successfully isolated *E. coli*, with its characteristic black colonies and green metallic sheen. These results underscore the importance of selecting appropriate media for bacterial isolation, as MSA and EMB are reliable for *S. aureus* and *E. coli*, respectively [27, 28].

Biochemical tests revealed distinct metabolic characteristics of *S. aureus* and *E. coli*, aiding in accurate identification. Both bacteria tested positive for catalase, indicating their ability to survive in oxygen-rich

environments, while *S. aureus* was differentiated by the coagulase test, marking it as a pathogenic species [29]. The DNase test confirmed *S. aureus's* virulence [30], and sugar fermentation tests highlighted the metabolic versatility of both bacteria, with *E. coli* demonstrating motility and gas production [31]. The profiling of *E. coli* further confirmed its metabolic traits, including positive results for catalase, indole, and lactose fermentation [32], while *S. aureus* exhibited beta-hemolysis, indicating its pathogenic potential.

C. Contamination Levels in Vegetables and Fruits:

The study revealed significant variations in bacterial contamination across different produce types. Cabbage exhibited the highest *E. coli* contamination (40.54%), exceeding global averages reported in similar studies [33], while lettuce showed unexpectedly high *E. coli* levels (35.14%) compared to earlier findings [34]. In contrast, carrots and strawberries demonstrated lower contamination rates, likely due to differences in cultivation practices or surface morphology. Notably, *S. aureus* was detected in potatoes and watermelon, suggesting post-harvest handling as a critical contamination source [35, 36].

These discrepancies highlight the role of agricultural and environmental factors in shaping contamination risks. For instance, the use of untreated irrigation water—a common practice in regions like Yemen and Bangladesh [37, 38]—likely contributed to the elevated *E. coli* levels in leafy greens. Furthermore, Yemen's hot climate may accelerate bacterial proliferation, particularly when combined with poor storage conditions [39].

The WHO estimates that nearly 10% of the global population suffers from foodborne illnesses annually [40], with developing nations disproportionately affected due to inadequate sanitation infrastructure [41]. This aligns with our findings, where contamination rates in Yemeni produce mirror trends observed in other resource-limited settings.

We propose that, adopting treated irrigation water and organic fertilizers to minimize pathogen introduction [42, 43]. Training farmers on post-harvest hygiene protocols, including tool sanitation and temperature-controlled storage [44]. Implementing national food safety standards aligned with WHO guidelines to enhance export competitiveness [45].

D. Phytochemical Analysis of Plant Extracts

Phytochemical analysis of (turmeric, ginger, and Indian costus) extracts revealed various bioactive compounds, with solubility influenced by the solvent used. Ethanolic extracts were rich in alkaloids and glycosides, known for their pharmacological properties, while aqueous extracts effectively extracted polar compounds like amino acids. Phytochemical tests confirmed the presence of flavonoids, glycosides, saponins, tannins, carbohydrates, terpenoids, and resins in both extract types. Notably, alkaloids were present in the ethanolic extracts, while the aqueous extract was negative in Wagner's test, highlighting the solvent's role in alkaloid extraction. Amino acids were exclusive to aqueous extracts, confirming their water-soluble nature. Glycosides, present in both extracts, are beneficial for cardiovascular health, such as lowering blood pressure. Our results are consistent with previous studies identifying flavonoids, glycosides, and terpenoids in these plants [46].

Aqueous extracts showed higher extractive values, indicating a greater concentration of water-soluble compounds, including vitamins and glycosides, which are valuable for therapeutic applications targeting such compounds [47]. Ethanolic extracts, although lower in extractive value, were rich in secondary metabolites like alkaloids and flavonoids, responsible for distinct pharmacological effects [48]. Combining water and ethanol as extraction solvents can provide a more comprehensive profile of active compounds, enhancing therapeutic potential [49].

E. Physiochemical Properties of Extracts

The ash content of 5.78% indicates moderate mineral content and purity, aligning with acceptable standards [50]. The moisture content of 10.76% supports proper storage and handling while minimizing microbial growth [51]. The extractive values of 15.96% for aqueous extract and 14.87% for ethanolic extract highlight the efficiency of water in extracting polar compounds and ethanol in isolating non-polar compounds [52, 53]. Both extracts showed mildly acidic pH values, suitable for preserving bioactive stability [54].

F. Public Health and Economic Implications

Plant extracts can significantly improve food safety by reducing bacterial contamination in vegetables and fruits, offering a natural alternative to chemical preservatives. This approach not only enhances the quality and safety of agricultural products but also reduces the risk of foodborne illnesses, which are a major public health concern, especially in regions with limited access to clean water and proper sanitation [55].

Enhancing food safety yields significant economic benefits by reducing losses from bacterial contamination [56]. Implementing improved hygiene practices, along with safer storage and handling methods, minimizes spoilage of agricultural products, thereby reducing waste and extending the shelf life of fresh produce, which increases its market value. Additionally, improving food safety can enhance the competitiveness of agricultural exports [45]. Products that meet international safety standards are more likely to be accepted in global markets, leading to higher demand and better prices for exporters. Furthermore, reducing foodborne illnesses can lower healthcare costs and prevent productivity losses, contributing to overall economic stability and growth [57].

G. Limitations and Future Directions

One limitation of the current study is the relatively small sample size, which may affect the generalizability of the results. A larger sample size could provide more robust data and a clearer understanding of bacterial contamination in various agricultural products. Additionally, this study focused only on *S. aureus* and *E. coli*, and did not explore other pathogenic bacteria that may also pose risks to food safety, such as *Salmonella*, *Klebsiella* spp., or *Listeria*. Future studies should consider expanding the scope by including a

broader range of bacterial species and increasing the sample size to enhance the validity of the findings. This would help provide a more comprehensive understanding of bacterial contamination in produce and contribute to more effective food safety strategies.

V. Conclusion

This study highlights the potential of plant extracts (turmeric, ginger, and Indian costus) as natural antimicrobial agents to reduce bacterial contamination in Yemeni vegetables and fruits. Key pathogens (*S. aureus* and *E. coli*) were isolated using selective media (MSA and EMB) and differentiated via biochemical tests (e.g., catalase, coagulase). Contamination levels varied significantly, with leafy greens (cabbage, lettuce) showing the highest *E. coli* rates (40.54% and 35.14%), likely due to poor irrigation and handling practices. Phytochemical analysis revealed solvent-dependent extraction efficiencies: ethanolic extracts yielded alkaloids/flavonoids, while aqueous extracts contained polar compounds like amino acids. The extracts' mild acidity (pH 5.1–5.4) and stability support their use as safe, sustainable alternatives to synthetic preservatives. By mitigating contamination, these extracts can enhance food safety, reduce economic losses from spoilage, and improve Yemen's agricultural competitiveness, offering a scalable solution for regions facing similar challenges.

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References

[1] D. C. Zaharia, A. A. Muntean, M. G. Popa, A. T. Steriade, O. Balint, R. Micut, C. Iftene, I. Tofolean, V. T. Popa, C. Baicus, M. A. Bogdan, and M. I. Popa, "Comparative analysis of *Staphylococcus aureus* and *Escherichia coli* microcalorimetric growth," *BMC Microbiol.*, vol. 13, p. 171, 2013, doi: 10.1186/1471-2180-13-171.

[2] Centers for Disease Control and Prevention (CDC), "*Staphylococcus aureus* basics," [Online]. Available: https://www.cdc.gov/staphylococcus-aureus/about/index.html. [Accessed: 2024].

[3] T. A. Taylor and C. G. Unakal, "*Staphylococcus Aureus*," *StatPearls [Internet]*, Treasure Island (FL): StatPearls Publishing; 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK441868/

[4] A. M. Algammal, H. F. Hetta, A. Elkelish, D. H. H. Alkhalifah, W. N. Hozzein, G. E. Batiha, N. El Nahhas, and M. A. Mabrok, "Methicillin-Resistant *Staphylococcus aureus* (MRSA): One Health Perspective Approach to the Bacterium Epidemiology, Virulence Factors, Antibiotic-Resistance, and Zoonotic Impact," *Infect. Drug Resist.*, vol. 13, pp. 3255–3265, 2020, doi: 10.2147/IDR.S272733.

[5] B. Pakbin, W. M. Brück, and J. W. Rossen, "Virulence Factors of Enteric Pathogenic *Escherichia coli*: A Review," *Int. J. Mol. Sci.*, vol. 22, no. 18, p. 9922, 2021, doi: 10.3390/ijms22189922.

[6] J. Kadariya, T. C. Smith, and D. Thapaliya, "*Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health," *Biomed. Res. Int.*, vol. 2014, p. 827965, 2014, doi: 10.1155/2014/827965.

[7] S. M. Gambushe, O. T. Zishiri, and M. E. El Zowalaty, "Review of *Escherichia coli* O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective," *Infect. Drug Resist.*, vol. 15, pp. 4645–4673, 2022, doi: 10.2147/IDR.S365269.

[8] K. Nair, *Turmeric (Curcuma longa L.) and Ginger (Zingiber officinale Rosc.) - World's Invaluable Medicinal Spices: The Agronomy and Economy of Turmeric and Ginger*, 2019, doi: 10.1007/978-3-030-29189-1.

[9] Encyclopaedia Britannica, "Aden," *Britannica*, 25 Mar. 2023. [Online]. Available: https://www.britannica.com/place/Aden. [Accessed: 24 May 2023].

[10] A. O. Eni, L. A. Oluwawemitan, and O. U. Solomon, "Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria," *Afr. J. Food Sci.*, vol. 4, no. 5, pp. 291–296, 2010.

[11] B. M. Al-Joda and A. H. Jasim, "Studying the Efficiency Dependence of CR-39 Detector on the Chamber Height," *J. Univ. Babylon Pure Appl. Sci.*, vol. 29, no. 2, pp. 68–176, 2021.

[12] S. Aryal, "Methyl Red (MR) Test - Principle, Procedure and Result Interpretation," *Microbiology Info*, 2018. [Online]. Available: https://microbiologyinfo.com/methyl-red-mr-test-principle-procedure-and-result-interpretation/

[13] J. F. MacFaddin, *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed., Philadelphia: Lippincott Williams & Wilkins, 2000.

[14] C. R. Mahon, D. C. Lehman, and G. Manuselis, *Textbook of Diagnostic Microbiology*, 5th ed., Elsevier, 2015.

[15] W. C. Winn, *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, Lippincott Williams & Wilkins, 2006.

[16] S. Aryal, "*Staphylococcus aureus* - An Overview," *Microbe Notes*, 2022. [Online]. Available: https://microbenotes.com/staphylococcus-aureus-an-overview/

[17] P. Tille, *Bailey & Scott's Diagnostic Microbiology*, Elsevier Health Sciences, 2017.

[18] J. B. Harborne, *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 2nd ed., London and New York: Chapman and Hall, 1984.

[19] S. S. Handa, P. S. Sumnan, L. Gennaro, and D. R. Dev, *Extraction Technologies for Medicinal and Aromatic Plants*, International Centre for Science and High Technology, UNIDO, 2008.

[20] K. Moothy, T. Punith, R. Vinodhini, and N. Thajuddin, "Antimicrobial activity and qualitative phytochemical analysis of *Punica granatum* Linn (Pericarp)," *J. Med. Plants Res.*, vol. 7, no. 9, pp. 474–479, 2012.

[21] N. R. Farnsworth, "Biological and Phytochemical Screening of Plants," *J. Pharm. Sci.*, vol. 55, no. 3, pp. 225–276, 1996, doi: 10.1002/jps.2600550302.
[22] H. O. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical constituents of some Nigerian medicinal plants," *Afr. J. Biotechnol.*, vol. 4, no. 7, pp. 685–688, 2005.
[23] G. Nasser and H. Ahmed, "Study of Chemical Composition, Antioxidant and Antimicrobial Properties of Two Selected Medicinal Plants," M.Sc. thesis, Univ. of Aden, Yemen, 2022, p. 157.
[24] Anonymous, *Pharmacopoeia of India*, Ministry of Health, Govt. of India, New Delhi, 1996.
[25] E. H. Mansour and A. H. Khalil, "Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties," *Food Chem.*, vol. 69, no. 2, pp. 135–141, 2000.
[26] C. Ingrao, R. Strippoli, G. Lagioia, and D. Huisingh, "Water scarcity in agriculture: An overview of causes, impacts and approaches for reducing the risks," *Heliyon*, vol. 9, pp. e14194, 2023.
[27] S. E. Sharp and C. Searcy, "Comparison of mannitol salt agar and blood agar plates for identification and susceptibility testing of *Staphylococcus aureus* in specimens from cystic fibrosis patients," *J. Clin. Microbiol.*, vol. 44, no. 12, pp. 4420–4423, 2006.
[28] D. J. Leininger, J. R. Roberson, and F. Elvinger, "Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens," *J. Vet. Diagn. Invest.*, vol. 13, no. 3, pp. 273–275, 2001.
[29] T. Foster, "Staphylococcus," in *Medical Microbiology*, 4th ed., S. Baron, Ed. Galveston, TX: Univ. of Texas Medical Branch, 1996, ch. 12.
[30] D. P. Kateete, C. N. Kimani, F. A. Katabazi, A. Okeng, M. S. Okee, A. Nanteza, M. L. Joloba, and F. C. Najjuka, "Identification of *Staphylococcus aureus*: DNase and mannitol salt agar improve the efficiency of the tube coagulase test," *Ann. Clin. Microbiol. Antimicrob.*, vol. 9, p. 23, 2010, doi: 10.1186/1476-0711-9-23.
[31] N. Sultana, D. Mridha, R. Sohel, M. Mahmud, M. Shahid, and H. Ahosanul, "Isolation, identification and antibiogram of verotoxin producing *Escherichia coli* from raw salad vegetables at Jashore, Bangladesh," *Afr. J. Microbiol. Res.*, vol. 15, pp. 401–407, 2021.
[32] P. Singh and A. Prakash, "Isolation of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* from milk products sold under market conditions at Agra Region," *Acta Agric. Slov.*, vol. 92, no. 1, pp. 83–88, 2008.
[33] B. V. Maikai and D. O. Akubo, "Coliform count and isolation of *Escherichia coli* in fresh fruits and vegetables sold at retail outlets in Samaru, Kaduna State, Nigeria," *Niger. Vet. J.*, vol. 39, no. 4, pp. 327–337, 2018.
[34] K. Holvoet, I. Sampers, B. Callens, J. Dewulf, and M. Uyttendaele, "Moderate prevalence of antimicrobial resistance in *Escherichia coli* isolates from lettuce, irrigation water, and soil," *Appl. Environ. Microbiol.*, vol. 79, no. 21, pp. 6677–6683, 2013.
[35] D. Devi and R. Gogoi, "Isolation and identification of microorganisms from some spoiled fruits and vegetables collected from Nagaon, Assam," *Plant Archives*, vol. 21, no. 1, pp. 1779–1782, 2021. [Online]. Available: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.242
[36] P. C. Obinna-Echem and L. Koanyie, "Quality and safety characteristics of minimally processed watermelon (*Citrullus lanatus*) sold in selected locations in Port Harcourt," *Eur. J. Agric. Food Sci.*, vol. 2, no. 3, pp. 1–6, 2020. [Online]. Available: https://doi.org/10.24018/ejfood.2020.2.3.57
[37] P. Mead, L. Slutsker, V. Dietz, L. Mccaig, J. Bresee, C. Shapiro, P. Griffin, and R. Tauxe, "Food-related illness and death in the United States," *Emerg. Infect. Dis.*, vol. 5, pp. 607–625, 1999, doi: 10.3201/eid0505.990502.
[38] A. N. Angelakis, G. Tchobanoglous, A. G. Capodaglio, and V. A. Tzanakakis, "The importance of nonconventional water resources under water scarcity," *Water*, vol. 16, no. 7, p. 1015, 2024, doi: 10.3390/w16071015.
[39] S. Bolan, L. P. Padhye, T. Jasemizad, M. Govarthanan, N. Karmegam, H. Wijesekara, D. Amarasiri, D. Hou, P. Zhou, B. K. Biswal, R. Balasubramanian, H. Wang, K. H. M. Siddique, J. Rinklebe, M. B. Kirkham, and N. Bolan, "Impacts of climate change on the fate of contaminants through extreme weather events," *Sci. Total Environ.*, vol. 909, p. 168388, Jan. 2024, doi: 10.1016/j.scitotenv.2023.168388.
[40] World Health Organization, "Food safety," 2024. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/food-safety.
[41] T. Bintsis, "Foodborne pathogens," *AIMS Microbiology*, vol. 3, no. 3, pp. 529–563, 2017, doi: 10.3934/microbiol.2017.3.529.
[42] S. Mishra, R. Kumar, and M. Kumar, "Use of treated sewage or wastewater as an irrigation water for agricultural purposes—Environmental, health, and economic impacts," *Total Environment Research Themes*, vol. 6, p. 100051, 2023, doi: 10.1016/j.totert.2023.100051.
[43] A. Sharma and R. Chetani, "A review on the effect of organic and chemical fertilizers on plants," *Int. J. Appl. Sci. Eng.*, vol. 5, pp. 677–680, 2020.
[44] H. Chen, A. J. Kinchla, N. Richard, A. Shaw, and Y. Feng, "Produce growers' on-farm food safety education: A review," *J. Food Prot.*, vol. 84, no. 4, pp. 704–716, 2021, doi: 10.4315/JFP-20-320.
[45] S. Henson and S. Jaffee, "Food safety standards and trade: Enhancing competitiveness and avoiding exclusion of developing countries," *Eur. J. Dev. Res.*, vol. 18, no. 4, pp. 593–607, 2006. [Online]. Available: https://doi.org/10.1080/09578810601070753
[46] E. M. Abdallah, K. A. Qureshi, A. M. Ali, and G. O. Elhassan, "Evaluation of some biological properties of *Saussurea costus* crude root extract," *Biosci. Biotechnol. Res. Commun.*, vol. 10, no. 4, pp. 601–611, 2017, doi: 10.21786/bbrc/10.4/2.
[47] S. S. Pawar and M. G. Jadhav, "Determination of extractive value and phytochemical analysis of *Bacopa*

monnieri (L)," 2016. [Online]. Available: https://www.researchgate.net/publication/296701173

[48] V. Varadharajan, U. K. Janarthanan, and V. Krishnamurthy, "Physicochemical, phytochemical screening and profiling of secondary metabolites of *Annona squamosa* leaf extract," *World J. Pharm. Res.*, vol. 1, no. 4, pp. 1143–1164, 2012.

[49] M. Jacotet-Navarro et al., "What is the best ethanol-water ratio for the extraction of antioxidants from rosemary? Impact of the solvent on yield, composition, and activity of the extracts," *Electrophoresis*, vol. 0, pp. 1–11, 2018.

[50] M. A. Buternio, C. Cavaliere, A. L. Capriotti, and C. Crescenzi, "Extending the applicability of pressurized hot water extraction to compounds exhibiting limited water solubility by pH control: Curcumin from the turmeric rhizome," *Anal. Bioanal. Chem.*, vol. 401, no. 9, pp. 2977–2985, 2011.

[51] A. K. Sachan and A. Kuma, "Stability testing of herbal products," *J. Chem. Pharm. Res.*, vol. 7, no. 12, pp. 511–514, 2015.

[52] M. C. Ali et al., "Effective extraction of flavonoids from *Lycium barbarum* L. fruits by deep eutectic solvents-based ultrasound-assisted extraction," *Talanta*, 2019. [Online]. Available: https://doi.org/10.1016/j.talanta.2019.05.012

[53] N. Zahed, R. Esmaeilzadeh Kenari, and R. Farahmandfar, "Effect of different extraction methods on antioxidant properties and encapsulation efficiency of anthocyanin of pomegranate peel," *Food Sci. Nutr.*, vol. 11, no. 7, pp. 3780–3787, 2023. [Online]. Available: https://doi.org/10.1002/fsn3.3362

[54] O. R. Alara, N. H. Abdurahman, and O. A. Olalere, "Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design," *J. King Saud Univ. Sci.*, vol. 32, no. 1, pp. 7–16, 2020. [Online]. Available: https://doi.org/10.1016/j.jksus.2017.08.001

[55] K. Pacyga, D. Ronga, and I. Michalak, "Plant extracts—Importance in sustainable agriculture," *Ital. J. Agron.*, vol. 16, 2021, doi: 10.4081/ija.2021.1851.

[56] M. Focker and H. J. van der Fels-Klerx, "Economics applied to food safety," *Curr. Opin. Food Sci.*, vol. 36, pp. 18–23, 2020. [Online]. Available: https://doi.org/10.1016/j.cofs.2020.10.018

[57] PathSpot Technologies, "Economic impact of foodborne illnesses in the food industry and how to prevent them," 2024. [Online]. Available: https://www.pathspot.com.