



## Assessment of the Preservative Efficacy of *Azadirachta indica* A. Juss. and *Psidium guajava* L. Leaf Extracts on *Capsicum annuum* L. (Bell Pepper)

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### Authors' contributions

This work was carried out in collaboration between both authors. Author JFO designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author VOO supervised the study. Both authors read and approved the final manuscript.

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### ABSTRACT

This study assessed the preservative efficacy of *Azadirachta indica* (Neem) and *Psidium guajava* (Guava) leaf extracts on *Capsicum annuum* (Bell pepper). The phytochemical compositions of *A. indica* and *P. guajava* leaf extracts were determined using standard methods. Phytochemical analysis revealed the presence of saponnin, anthraquinone tannin, steroid, terpenoid, flavonoid and glycosides in ethanol extracts of *Azadirachta Indica* A. Juss. and *Psidium guajava* L. leaves and tannin, terpenoid, flavonoid and glycosides are present in n-hexane extracts of the selected plants. Among the phytochemicals identified, Terpenoid was the highest in value (25.79mg/g) and saponnin has the least value (3.27 mg/g). The ethanolic extracts of Guava had the highest inhibition against the growth of *Staphylococcus aureus* (35.00±1.15 mm). The n-hexane extracts of Neem leaves had the lowest inhibition against the growth of *Staphylococcus* sp (8.00±0.57 mm). Ethanolic extract has the highest antifungal effect against *Saccharomyces cerevisiae* (58.66±0.90 mm). Based on the findings of this study, it can be concluded that the ethanol extracts of *Azadirachta indica* A. Juss. and *Psidium guajava* L. leaves are more effective than the n-hexane

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extracts of *P. guajava* and *A. indica* leaves because it effectively inhibited the growth of the microorganisms. The *Capsicum annuum* L. fruits coated with selected plant extracts were preserved for a longer period than the uncoated fruits. All these results therefore suggest that the leaf extracts of *P. guajava* and *A. indica* can be used as bio preservatives for the extension of the shelf life of *C. annuum*.

**Keywords:** *Capsicum annuum*; *Azadirachta indica*; *Psidium guajava*; preservative; efficacy.

## 1. INTRODUCTION

Bell pepper (*Capsicum annuum*) also known as sweet pepper is a solanaceous vegetable and it is popular for its delicious taste, pleasant flavour and nutritional quality [1]. The most common colours of bell pepper are green, yellow, orange and red. Bell pepper contain antioxidants and bioactive compounds such as ascorbic acid, carotenoids, flavonoids and polyphenols [1]. Consumption of bell pepper is associated with a significantly reduced risk of cancer and cardiovascular diseases [2]. According to the 2007 statistics, Nigeria is the 7th largest producer of Bell pepper in the world with the production rate of 723,000 metric tonnes. Bell peppers are perishable and the causes of postharvest losses can generally be ascribed to mechanical injury that lead to bacterial and fungal infections. Bell peppers and other vegetables are prone to microbial spoilage because of their succulent nature. It is caused by microorganisms like fungi (moulds, yeasts) and bacteria. It is estimated that 20% of fruits and vegetables harvested for human consumption are lost through microbial spoilage [3]. The high water content of fruits and vegetables favours growth of spoilage bacteria, moulds and yeasts. They spoil fruits and vegetable by growing on it and producing substances that changes the colour, texture and odour of the food. These organisms are rarely harmful to humans, but bacterial contamination is often more dangerous because the food does not always look bad, even if it is severely infected [4]. Bell pepper is susceptible to fungal infections caused by *Botrytis cinerea* and *Alternaria alternata* [4]. The rate of postharvest deterioration depends on several external factors, including storage temperature, relative humidity, air speed, atmospheric composition (concentrations of oxygen, carbon dioxide, and ethylene), and sanitation procedures [5]. Bell peppers are very sensitive to mishandling and improper storage conditions, and can quickly be damaged by very low or high postharvest temperature. Utilization of proper harvest and postharvest handling

methods are essential for producing the high quality pepper with maximizing market value and better shelf life.

Control of bell pepper disease has been by application of synthetic chemicals. However, these days, consumers request less use of chemicals because most of the chemicals being used for crop protection are reported to pose a serious threat to human health and they have residual effect. Furthermore, synthetic chemicals are expensive and inaccessible to indigenous farmers in Nigeria. All these factors have led to research for safer and more acceptable alternatives. One of the alternative methods is the use of extracts from natural plant products [6]. Plant extracts have number of active ingredients that inhibit the growth of microorganisms and also prevent spoilage [7]. These botanical extracts are residue free and safe from consumption point of view as compared to chemical preservatives that may be toxic to living beings [8]. *Azadirachta indica* is a medicinal and also a non-toxic plant which possesses excellent antimicrobial properties [9]. Tijjani et al. [10] reported the antibacterial properties of *Azadirachta indica* extract and neem oil against pathogenic micro-organism such as *Salmonella*, *Staphylococcus* and *Vibrio*.

In several studies, *P. guajava*, showed significant antibacterial activity against common food-borne diarrhea-causing bacteria such as *Staphylococcus* species, *Shigella* species, *Salmonella* species, *Bacillus* species, *Escherichia coli*, *Clostridium* species and food spoilage bacteria such as *Pseudomonas* species [11].

The assessment of the preservative efficacy of *Azadirachta indica* and *Psidium guajava* leaf extracts are therefore aimed at in this research. This is to serve as a safe and effective alternative method to extend the shelf life of bell pepper so as to reduce or eliminate postharvest loss by the farmers, traders and consumers.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Green and red variety of Bell peppers (*Capsicum annum*) was purchased at shasha market, Ondo state Nigeria. *Azadirachta indica* and *Psidium guajava* leaves was gotten within Federal University of Technology Akure campus and their identity was authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State.

### 2.2 Microbiological Analysis of the Samples

Stock solution was prepared by cutting a small segment of the bell pepper and dissolving the sample in peptone water. Serial dilution was prepared by using the stock solution. Three and five-fold serial dilution was performed. 1ml of the diluent was dispensed into the petri dishes aseptically and prepared molten agar and other selective and differential media was poured into the petri dishes. Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe agar was used to isolate lactic acid bacteria (12). The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 25°C for 48 to 72 hours. De Man Rogosa sharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The pure colonies were characterized based on biochemical and morphological observations according to the methods of [12].

### 2.3 Molecular Identification of Bacteria

Extraction of DNA using CTAB method was done according to [13]. Polymerase chain reaction analysis was run with a universal primer for fungi called Internal Transcribed Spacer (ITS1 and ITS4) while bacteria was run with a universal primer called 16S rRNA. The amplicon was further purified before the sequencing. The sequences obtained for its isolates were identified using Basic Local Alignment Search Tool (BLAST) on National Centre for Biotechnology Information (NCBI) database.

### 2.4 Preparation of *Azadirachta indica* and *Psidium guajava* Leaf Extracts

The collected leaves were washed in distilled water, dried and ground. Ethanolic and N-hexane

leaf extracts were prepared using standard methods as described by [14]. The crude extracts were obtained by soaking 100 grams of each dried powdered plant in 1000 mL of Ethanol and N-hexane separately for 72 hrs, and sieved with a muslin cloth. The extract was further concentrated by using a rotary vacuum evaporator at 45-50°C and stored.

### 2.5 Phytochemical Screening of Leaf Extracts

Qualitative and quantitative screening was carried out on *Azadirachta indica* and *Psidium guajava* leaf extracts using standard procedures as described by [15].

### 2.6 Evaluation of Preservative Effect of *Azadirachta indica* and *Psidium guajava* Leaf Extracts on Healthy Bell Peppers under Different Storage Conditions

The Bell pepper fruits were coated with the ethanolic and n-hexane extracts of Guava and Neem leaves. The Bell pepper fruits were then arranged on rubber plates and kept at room (32°C) and refrigeration temperature (4°C). Two varieties of Bell pepper fruits were used for each treatment. Bell pepper treated with hydrogen peroxide was used as positive control while uncoated Bell pepper fruits were used as negative control. Shelf life of Bell pepper fruits were evaluated by counting the number of days bell pepper fruits were showing signs of wholesomeness which was evaluated based on appearance and spoilage of fruits.

### 2.7 Antibiotic Susceptibility Profile

Antibiotic susceptibility testing of extracts on isolates was performed using the Kirby Bauer disk diffusion method [16] and interpretation according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [17].

### 2.8 Determination of Antibacterial and Antifungal Activities of Extracts

#### 2.8.1 Standardization of inoculum

Freshly prepared nutrient and potato dextrose broth was inoculated with test Bacteria and Fungi, then incubated for 24 h at 37°C and at 25°C for 48 h respectively. A 0.2 mL aliquot from the cultured broth was aseptically dispensed into

20 mL of freshly prepared nutrient and potato dextrose broth and incubated for 2 to 3 h at 37°C for Bacteria and 25°C for Fungi to standardize to 0.5 McFarland standard of Barium sulphate solution which is equivalent to  $1 \times 10^6$  CFU [12].

### 2.8.2 Antimicrobial assay of crude extracts

The assay was conducted using agar-well diffusion method [18]. 100 mg/ml concentration of both ethanol and n-hexane extracts of *Psidium guajava* and *Azadirachta indica* were reconstituted by dissolving in 5 ml each of 30% v/v dimethyl sulfoxide (DMSO). 10 µl of the Standardized inoculums of each test microorganisms was uniformly spread onto sterile Mueller Hinton and potato dextrose agar plate respectively. The plates were allowed to gel and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. With a micropipette, 50 µl of the test extracts was placed into each well. The plates were left on the bench for 30 min to allow the extract to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 h for bacteria and 25°C for 48 hours for fungi.

### 2.8.3 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the leaf extracts

The Minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the extracts were performed by using agar dilution technique as described by [19]. The Minimum inhibitory and minimum fungicidal concentrations (MIC/MFC) of the extracts were performed by using agar dilution technique as described by [20]. The lowest dilution of the tested leaf extracts to inhibit growth (no turbidity in the tube i.e. no growth is visually observed) was considered as the MIC value of the extract against the tested bacteria and fungi. The least concentration that did not show any growth was considered as the MBC/MFC value of the leaf extracts against the bacterial and fungal isolates.

### 2.9 Statistical Analysis

All the treatments were carried out in triplicates and the data obtained were analysed using analysis of variance (ANOVA). Means were separated using Duncan's New Multiple Range Test at 95% confidence level using Statistical

Packages for the Social Sciences (SPSS) version 22.0. Differences between means were considered significant at  $P \leq 0.05$ .

## 3. RESULTS

### 3.1 Identification and Frequency of Occurrence of Bacteria Isolated from *Capsicum annum*

Table 1 shows the details of sugar fermentation and biochemical characteristics of the bacterial isolates. Table 2 shows the frequency of occurrence of bacteria isolated from red and green bell pepper. Both *Staphylococcus aureus* and *Bacillus subtilis* were isolated in red and green bell pepper and each has a frequency of occurrence of 14.3% while *Enterobacter aerogenes*, *Ralstonia solanacearum*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Citrobacter freundii* were present in red bell pepper and *Bacillus cereus*, *Klebsiella oxytoca* and *Bacillus megaterium* were present in green bell pepper with a frequency of occurrence of 4.8% each.

### 3.2 Identification and Frequency of Occurrence of Fungi Isolated from *Capsicum annum*

Table 3 shows the cultural and morphological characteristics of the fungal isolates. Table 4 shows the frequency of occurrence of fungi isolated from red and green bell pepper. *Pichia kluyveri* was isolated in red and green bell pepper and each has the highest frequency of occurrence of 40% while *Geotrichum candidum* and *Mucor mucedo* were isolated from red bell pepper and they have a frequency of occurrence of 16.7% and 8.3% respectively. Also, *Aspergillus niger* (16.7%) was isolated in green bell pepper.

### 3.3 Molecular Identities of Isolated Microorganisms from Bell Pepper

The comparison between the microorganisms identified using cultural methods and molecular methods was shown in Table 5. The bacteria *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Micrococcus luteus* were molecularly identified as *Pseudomonas aeruginosa*, *Proteus alimentarium* and *Micrococcus endophyticus* respectively, while the Fungi, *Mucor mucedo* and *Pichia kluyveri* were *Mucor circinelloides* and *Pichia kudriavzevii*.

Table 1. Morphological and biochemical characteristics of bacteria isolated from *Capsicum annum* (red and green bell pepper)

Isolate no	Gram reaction	Cell Shape	Catalase	Motility	Coagulase	Citrate	Lactose	Sucrose	D-Mannitol	Maltose	Fructose	Glucose	Indole	Urease	H <sub>2</sub> S	Starch hydrolysis	Probable Identity
1	+	Rod	+	+	NT	-	A	A	AG	A	AG	A	-	NT	-	+	<i>Bacillus cereus</i>
2	+	Rod	+	+	NT	+	-	-	A	A	AG	-	-	-	-	+	<i>Bacillus subtilis</i>
3	+	Rod	+	+	NT	+	A	A	AG	A	AG	AG	-	-	-	+	<i>Bacillus subtilis</i>
4	+	Rod	+	+	-	+	A	A	A	A	AG	A	-	+	+	NT	<i>Staphylococcus spp</i>
5	+	Rod	-	+	-	-	-	-	AG	-	AG	AG	-	NT	+	-	<i>Clostridium spp</i>
6	+	Cocci	+	-	+	+	A	A	AG	A	AG	A	-	+	-	NT	<i>Staphylococcus aureus</i>
7	+	Cocci	+	-	-	-	-	-	A	A	AG	-	+	+	-	-	<i>Micrococcus luteus</i>
8	-	Rod	+	+	-	-	A	A	-	-	AG	A	+	+	+	-	<i>Proteus vulgaris</i>
9	+	Cocci	+	-	+	+	A	A	AG	A	AG	A	-	+	-	NT	<i>Staphylococcus aureus</i>
10	+	Cocci	-	-	-	-	AG	AG	AG	AG	AG	AG	NT	-	-	-	<i>Streptococcus mutans</i>
11	+	Cocci	+	-	+	+	A	A	AG	A	AG	A	-	+	-	NT	<i>Staphylococcus aureus</i>
12	-	Rod	+	+	NT	+	A	A	-	A	A	AG	-	-	-	NT	<i>Enterobacter aerogenes</i>
13	-	Rod	-	+	NT	-	-	-	AG	A	AG	AG	-	-	-	NT	<i>Ralstonia solanacearum</i>
14	-	Rod	+	+	-	+	-	-	A	-	+	A	-	-	-	-	<i>Pseudomonas aeruginosa</i>
15	-	Rod	+	+	NT	+	+	+	AG	A	AG	A	-	+	+	+	<i>Citrobacter freundii</i>
16	-	Rod	+	-	NT	+	+	+	AG	A	AG	A	+	+	-	NT	<i>Klebsiella oxytoca</i>
17	+	Cocci	+	+	-	+	-	-	+	A	A	AG	-	+	+	NT	<i>Staphylococcus spp</i>
18	+	Rod	-	+	-	-	AG	AG	AG	A	A	AG	-	-	+	-	<i>Clostridium spp</i>
19	+	Rod	+	+	NT	+	+	+	AG	A	AG	AG	-	-	-	+	<i>Bacillus subtilis</i>
20	+	Rod	+	+	-	+	+	+	AG	A	AG	A	-	-	+	+	<i>Bacillus licheniformis</i>
21	+	Rod	+	+	-	+	-	-	AG	-	AG	A	-	+	-	+	<i>Bacillus megaterium</i>

Keys: (+) Positive (-) Negative (AG) Acid and Gas produced (A) Acid Produced (NT) Not tested

**Table 2. Frequency of occurrence of some bacterial isolates from red and green bell pepper**

Bacterial Isolates	Red Bell pepper	Green Bell pepper	Frequency of occurrence (%)
<i>Staphylococcus aureus</i>	+	+	14.3
<i>Proteus vulgaris</i>	-	+	4.8
<i>Bacillus subtilis</i>	+	+	14.3
<i>Bacillus megaterium</i>	+	-	4.8
<i>Enterobacter aerogenes</i>	+	-	4.8
<i>Bacillus cereus</i>	-	+	4.8
<i>Micrococcus luteus</i>	+	-	4.8
<i>Citrobacter freundii</i>	+	-	4.8
<i>Ralstonia solanacearum</i>	+	-	4.8
<i>Pseudomonas aeruginosa</i>	-	+	4.8
<i>Klebsiella oxytoca</i>	-	+	4.8

**Table 3. Cultural and Morphological characteristics of fungi isolated from *Capsicum annum* (green and red bell pepper)**

Isolate no	Morphological Characteristics	Microscopy	Probable identity
1	White to cream-coloured, smooth and glabrous.	Predominantly small, elongated to ovoid blastoconidia, 2.5x 4.5 µm	<i>Pichia kluyveri</i>
2	Colonies exhibit moderately rapid growth, producing off-white to cream coloured colonies with a butyrous texture with a velvety, suede-like or ground glass/matt appearance.	They produce hyaline (clear) septate hyphae which show dichotomous branching (7µ-11µ wide).	<i>Geotrichum candidum</i>
3	Granular, flat, often with radial grooves, yellow at first but quickly becomes bright to dark yellow green with age. Reverse plate colour is cream	Conidia heads are typically radiate, later splitting to form loose columus (mostly 300 µ-400 µm in diameter).	<i>Aspergillus flavus</i>
4	The surface appearance is usually described as velvety to powdery. The colony colour is usually a green, blue green, grey green, often with a white edge. The reverse plate colour is usually a pale cream to yellow.	Septate hyaline hyphae (1.5 to 5 µ in diameter), simple or branched conidiophores.	<i>Penicillium chrysogenum</i>
5	Colonies are floccose, pale greyish brown and grow poorly at 37°C.	Sporangia are spherical, varying from 20-80 µm, with small sporangia	<i>Mucor mucedo</i>
6	Colonies grow floccose, at first whitish, later becoming avellaneous to buff-brown, reverse pale, becoming peach-coloured	Conidia on aerial conidiophores (blastoconidia) are usually borne singly	<i>Fusarium incarnatum</i>

**Table 4. Frequency of occurrence of some fungal isolates from red and green bell pepper**

Fungal isolates	Red Bell pepper	Green Bell pepper	Frequency of occurrence (%)
<i>Pichia kluyveri</i>	+	+	40
<i>Geotrichum candidum</i>	+	-	16.7
<i>Mucor mucedo</i>	+	-	8.3
<i>Aspergillus flavus</i>	-	+	8.3
<i>Penicillium chrysogenum</i>	-	+	8.3
<i>Aspergillus niger</i>	-	+	16.7
<i>Fusarium incarnatum</i>	-	+	8.3

**Table 5. Comparison between biochemical and molecular identities of bacteria, yeast and mould isolated from red and green bell pepper**

Biochemical identities	Molecular identities	Accession number of close relative	Similarity (%) with close relative
<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	NR117679.1	99.89
<i>Micrococcus luteus</i>	<i>Micrococcus endophyticus</i>	NR044365.1	99.78
<i>Proteus vulgaris</i>	<i>Proteus alimenterium</i>	NR163665.1	99.89
<i>Mucor mucedo</i>	<i>Mucor circinelloides</i>	MH855669.1	98.23
<i>Pichia kluyveri</i>	<i>Pichia kudriavzevii</i>	MN371886.1	99.78

### 3.4 Phytochemical Composition of *Azadirachta indica* and *Psidium guajava* Leaf Extracts

The qualitative phytochemical composition of *Azadirachta indica* and *Psidium guajava* leaf extracts are recorded in Table 6. Saponnin, anthraquinone tannin, steroid, terpenoid, flavonoid and glycosides were present in ethanolic extracts of *P.guajava* and *A.indica* leaves while tannin, terpenoid, flavonoid and glycosides were present in n-hexane extracts of *P.guajava* and *A.indica* leaves.

The quantitative phytochemical composition of *A.indica* and *P.guajava* leaf extracts is presented in Table 7. The ethanolic leaf extracts of both the plants confirmed the presence of tannins, saponins, flavonoids, steroids, terpenoids and glycosides. Among the reported phytochemicals, terpenoids ( $25.79 \pm 0.01^d$ ) and glycosides ( $25.11 \pm 0.01^d$ ) are recorded in highest values in *P. guajava* and *A. indica* respectively. In hexane extracts saponin and steroids are totally absent in both the plants and other phytochemicals also recorded in minimum quantity than ethanol

extract. Among the two plants, the ethanolic leaf extract of *P. guajava* yields maximum quantity of tested phytochemicals except steroids. (Sentence revised by reviewer).

### 3.5 Antibacterial Activities of *A. indica* and *P. guajava* Leaf Extracts

The *in-vitro* antibacterial activities of ethanolic and N-hexane extracts of guava and neem leaves against seventeen (17) bacteria is shown in Table 8. All extracts exhibited antibacterial activities against all tested bacteria used in this study. The minimum inhibitory concentration/minimum bactericidal concentration of ethanolic and n-hexane extracts of guava and neem is presented in Table 9. The minimum inhibitory concentration of ethanolic extracts of *A.indica* and *P.guajava* leaf against all bacteria ranged from 12.5 to 100 mg/ml and minimum bactericidal concentration ranged from 50 to >100 mg/ml. The minimum inhibitory concentration of n-hexane *A.indica* and *P.guajava* against all test bacteria ranged from 12.5 to 100 mg/ml and minimum bactericidal concentration ranged from 50 to >100 mg/ml.

**Table 6. Qualitative phytochemical composition of n-hexane and ethanolic extracts of *Azadirachta indica* (neem) and *Psidium guajava* (guava)**

Phytochemicals	NNH	GNH	GE	NE
Alkaloid	-	-	-	-
Saponnin	-	-	+	+
Tannin	+	+	+	+
Phlobatanin	-	-	-	-
Steroid	-	-	+	+
Anthraquinone	-	-	-	+
Terpenoid	+	+	+	+
Flavonoid	+	+	+	+
Cardiac glycoside				
Legal test	+	+	+	+
Lieberman test	-	-	+	+
Salkowski test	+	+	+	+
Keller killiani	+	+	+	+

Key: (+) Present - Absent (NE) Neem Ethanolic Extract (GE) Guava Ethanolic Extract (NNH) Neem N-Hexane Extract (GNH) Guava N-Hexane Extract

**Table 7. Quantitative phytochemical composition of n-hexane and ethanolic extracts of *Azadirachta indica* (neem) and *Psidium guajava* (guava)**

Extract	Phytochemicals					
	Tannin (mg/g)	Saponnin (mg/g)	Flavonoid (mg/g)	Steroid (mg/g)	Terpenoid (mg/g)	Glycosides (mg/g)
N-Hexane Neem	5.19±0.00 <sup>d</sup>	0.00±0.00 <sup>a</sup>	9.48±0.01 <sup>a</sup>	0.00±0.00 <sup>a</sup>	14.04±0.01 <sup>a</sup>	17.74±0.02 <sup>b</sup>
N-Hexane Guava	3.51±0.01 <sup>a</sup>	0.00±0.00 <sup>a</sup>	11.49±0.01 <sup>b</sup>	0.00±0.00 <sup>a</sup>	19.46±0.01 <sup>b</sup>	6.94±0.02 <sup>a</sup>
Ethanol Guava	9.45±0.00 <sup>d</sup>	13.63±0.11 <sup>c</sup>	15.10±0.01 <sup>d</sup>	3.71±0.01 <sup>b</sup>	25.79±0.01 <sup>d</sup>	20.26±0.01 <sup>c</sup>
Ethanol Neem	5.61±0.00 <sup>c</sup>	3.27±0.10 <sup>b</sup>	12.51±0.01 <sup>c</sup>	6.20±0.01 <sup>c</sup>	22.36±0.01 <sup>c</sup>	25.11±0.01 <sup>d</sup>

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

**Table 8. Antibacterial effect of *Azadirachta indica* and *Psidium guajava* leaf extracts on bacterial isolates**

Name of isolates	Extracts			
	EN	EG	NHG	NHN
	Zone of inhibition (mm)			
<i>Bacillus subtilis</i> (CR1)	14.33±0.66 <sup>b</sup>	13.66±0.88 <sup>b</sup>	9.66±0.33 <sup>a</sup>	12.66±0.33 <sup>b</sup>
<i>Bacillus subtilis</i> (DNG)	12.00±0.00 <sup>a</sup>	17.00±1.15 <sup>b</sup>	13.00±0.57 <sup>a</sup>	11.00±0.57 <sup>a</sup>
<i>Staphylococcus</i> spp (GNR1)	10.33±0.33 <sup>c</sup>	14.33±0.33 <sup>d</sup>	0.00±0.00 <sup>a</sup>	8.00±0.57 <sup>b</sup>
<i>Staphylococcus aureus</i> (CG2)	13.66±0.88 <sup>a</sup>	16.66±0.88 <sup>b</sup>	12.33±1.20 <sup>a</sup>	12.00±0.57 <sup>a</sup>
<i>Staphylococcus aureus</i> (TR4)	17.33±0.33 <sup>b</sup>	21.00±0.57 <sup>c</sup>	13.67±1.20 <sup>a</sup>	14.66±0.33 <sup>a</sup>
<i>Bacillus</i> spp (TR1)	21.66±0.66 <sup>b</sup>	16.66±0.88 <sup>a</sup>	16.66±1.20 <sup>a</sup>	16.66±0.66 <sup>a</sup>
<i>Bacillus cereus</i> (TG1)	33.66±0.88 <sup>d</sup>	21.00±0.57 <sup>b</sup>	17.33±1.45 <sup>a</sup>	28.00±0.57 <sup>c</sup>
<i>Staphylococcus aureus</i> (TG3)	19.33±0.33 <sup>b</sup>	35.00±1.15 <sup>c</sup>	14.33±0.88 <sup>a</sup>	15.00±0.57 <sup>a</sup>
<i>Bacillus megaterium</i> (CR2)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Micrococcus endophyticus</i> (TR3)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Bacillus licheniformis</i> (TG2)	28.33±0.88 <sup>b</sup>	15.00±0.00 <sup>a</sup>	26.33±0.88 <sup>b</sup>	26.00±1.15 <sup>b</sup>
<i>Streptococcus mutans</i> (TG5)	12.66±0.66 <sup>b</sup>	13.66±0.33 <sup>b</sup>	9.33±0.33 <sup>a</sup>	10.00±0.57 <sup>a</sup>
<i>Staphylococcus</i> spp (GNR2)	20.67±0.67 <sup>c</sup>	16.33±0.33 <sup>b</sup>	11.66±0.88 <sup>a</sup>	16.33±0.33 <sup>b</sup>
<i>Clostridium</i> spp (TR2)	0.00±0.00 <sup>a</sup>	12.33±0.66 <sup>c</sup>	9.67±0.33 <sup>b</sup>	9.33±0.33 <sup>b</sup>
<i>Ralstonia solanacearum</i> (TR6)	16.33±0.33 <sup>b</sup>	13.00±1.15 <sup>b</sup>	13.67±0.88 <sup>ab</sup>	12.66±0.88 <sup>a</sup>
<i>Citrobacter freundii</i> (GNR3)	19.67±0.33 <sup>b</sup>	17.67±0.33 <sup>ab</sup>	16.67±1.20 <sup>a</sup>	17.33±0.88 <sup>ab</sup>
<i>Enterobacter aerogenes</i> (TR5)	0.00±0.00 <sup>a</sup>	28.00±0.00 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
<i>Proteus alimentorum</i> (CG1)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	9.33±0.33 <sup>b</sup>
<i>Klebsiella oxytoca</i> (GNG)	0.00±0.00 <sup>a</sup>	14.33±0.66 <sup>c</sup>	10.00±0.57 <sup>b</sup>	0.00±0.00 <sup>a</sup>
<i>Pseudomonas aeruginosa</i> (TG4)	23.66±0.67 <sup>c</sup>	29.66±0.33 <sup>d</sup>	21.00±0.57 <sup>b</sup>	19.00±0.57 <sup>a</sup>
<i>Clostridium</i> spp (GER2)	32.33±0.88 <sup>c</sup>	28.33±0.88 <sup>a</sup>	26.33±1.45 <sup>a</sup>	33.66±1.85 <sup>b</sup>

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Keys: ED Neem Ethanolic Extract EG Guava Ethanolic Extract NHD Neem N-Hexane Extract NHG Guava N-Hexane Extract

Tables 10 and 11 shows the susceptibility pattern of isolated bacterial species to commercial antibiotic discs. Ethanolic extracts of *P.guajava* had the highest zones of inhibition which ranged from 13.00 to 35.00 mm and this compared favourably with the conventional antibiotics discs used. N-hexane extracts of *P.guajava* had the lowest zones of inhibition which ranged from 9.00 to 27.00 mm. Although some bacteria were resistant to the extracts but most of the bacteria are susceptible.

### 3.6 Antifungal Activities of *A. indica* and *P. guajava* Leaf Extracts

The *In-vitro* antifungal activities of ethanolic and N-hexane extracts of guava and neem leaves against *Pichia kudriazevii*, *Geotrichum candidum*, *Mucor circinelloides*, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Aspergillus*, *Fusarium incarnatum* and *Pichia kyuleri*. The minimum inhibitory concentration/mimimum fungicidal concentration

of ethanolic and n-hexane extracts of guava and neem is outlined in Table 13. The minimum inhibitory concentration of ethanolic extracts of *A.indica* and *P.guajava* leaf against all test fungi ranged from 12.5 to 100 mg/ml and minimum fungicidal concentration ranged from 50 to >100 mg/ml.

The susceptibility pattern of isolated fungal species to commercial antifungal agents is shown in Table 14. All extracts exhibited antifungal activities against all test bacteria used in this study. Ethanolic extracts of *A.indica* had the highest zones of inhibition which ranged from 20.00 to 59.00 mm and this compared favourably with the conventional antifungal drugs (ketoconazole, itraconazole and fluconazole) used. N-hexane extracts of *P.guajava* had the lowest zones of inhibition which ranged from 20.00 to 35.00 mm. All the fungal isolates were susceptible to the leaf extracts but some were resistant to the antifungal agents.

#### 4. DISCUSSION

Plants serve as vegetables and are used in the preparation of food nutritive seasoning. Apart

from its nutritive value, plants have been found to contain bioactive metabolites with potentials to inhibit the growth of microorganisms [21]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids. These compounds from herbs, spices, and plant extracts have been shown to possess antimicrobial properties against a wide range of harmful microorganisms. Thus, there has been increased interest in the antimicrobial properties of plant-derived products for their potential use as alternatives to synthetic preservatives. Plant antimicrobials have proven to be relatively safe and could be used to extend the shelf life of foods and quality of fruits during their storage in order to overcome food safety issues. The actions of these agents deal with the decrease of moisture and the improvement of the general appearance and quality of the products during storage as reported by [22]. Many postharvest diseases originate from the field where pathogens attack growing and mature produce before their harvest. This study showed that a number of microorganisms are associated with post-harvest decay of Bell pepper fruits in storage. Some of the microorganisms isolated from Bell pepper include *Bacillus subtilis*,

**Table 9. Minimum Inhibitory concentration/minimum bactericidal concentration of the leaf extracts against bacteria isolated from *Capsicum annum* (red and green bell pepper)**

Test organisms	Ethanolic extract of <i>A. Indica</i> (mg/ml)		Ethanolic extract of <i>P. guajava</i> (mg/ml)		N-Hexane extract of <i>A. indica</i> (mg/ml)		N-Hexane extract of <i>P. guajava</i> (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus subtilis</i>	50	>100	50	>100	50	>100	100	>100
<i>Bacillus subtilis</i>	25	100	12.5	50	50	>100	50	>100
<i>Clostridium</i> spp	NA	NA	12.5	50	50	>100	50	>100
<i>Staphylococcus</i> spp	12.5	50	50	>100	12.5	100	12.5	100
<i>Ralstonia solanacearum</i>	25	100	12.5	50	25	100	25	>100
<i>Staphylococcus aureus</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Citrobacter freundii</i>	12.5	50	25	100	25	>100	12.5	100
<i>Staphylococcus aureus</i>	12.5	50	25	100	25	>100	50	>100
<i>Bacillus subtilis</i>	12.5	50	25	100	12.5	100	25	100
<i>Enterobacter aerogenes</i>	NA	NA	100	>100	NA	NA	NA	NA
<i>Proteus alimentorum</i>	NA	NA	NA	NA	50	>100	NA	NA
<i>Bacillus cereus</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Klebsiella oxytoca</i>	NA	NA	50	>100	NA	NA	50	>100
<i>Staphylococcus aureus</i>	12.5	50	12.5	50	25	100	25	>100
<i>Bacillus megaterium</i>	NA	NA	NA	NA	NA	NA	NA	NA
<i>Micrococcus endophyticus</i>	NA	NA	NA	NA	NA	NA	NA	NA
<i>Bacillus licheniformis</i>	12.5	50	50	>100	12.5	100	12.5	100
<i>Staphylococcus</i> spp	50	>100	50	>100	50	>100	NA	NA
<i>Streptococcus mutans</i>	25	100	25	100	50	>100	50	>100
<i>Pseudomonas aeruginosa</i>	12.5	50	12.5	50	25	>100	12.5	100
<i>Clostridium</i> spp	12.5	50	12.5	50	12.5	100	12.5	50

Key: NA-Not Active, MIC-Minimum Inhibitory concentration, MBC-Minimum Bactericidal concentration

**Table 10. Susceptibility pattern of gram positive bacteria isolates to commercial antibiotics**

Name of isolates	Antibiotics discs									
	Z	AM	R	CPX	S	SXT	E	PEF	CN	APX
	<b>Zone of inhibition (mm)</b>									
<i>B.cereus</i> (TG1)	20.33±0.88 <sup>c</sup>	0.00±0.00 <sup>a</sup>	20.00±1.52 <sup>c</sup>	24.33±0.66 <sup>de</sup>	26.33±1.45 <sup>e</sup>	20.33±0.66 <sup>c</sup>	0.00±0.00 <sup>a</sup>	16.33±0.33 <sup>b</sup>	23.66±0.88 <sup>d</sup>	0.00±0.00 <sup>a</sup>
<i>B.subtilis</i> (CR1)	21.33±0.88 <sup>bc</sup>	20.00±0.00 <sup>b</sup>	21.00±1.00 <sup>bc</sup>	21.33±0.88 <sup>bc</sup>	23.66±0.88 <sup>c</sup>	23.00±1.15 <sup>c</sup>	13.66±0.66 <sup>a</sup>	23.33±0.88 <sup>c</sup>	23.66±0.88 <sup>c</sup>	19.00±0.57 <sup>b</sup>
<i>B.licheniformis</i> (TG2)	23.33±0.88 <sup>d</sup>	14.33±0.66 <sup>a</sup>	19.33±0.66 <sup>bc</sup>	21.00±1.00 <sup>c</sup>	20.00±0.57 <sup>c</sup>	24.00±1.00 <sup>d</sup>	17.33±0.33 <sup>b</sup>	20.66±0.88 <sup>c</sup>	19.00±0.57 <sup>b</sup>	14.00±1.00 <sup>a</sup>
<i>Staph. Sp</i> (GNR1)	20.33±1.20 <sup>d</sup>	0.00±0.00 <sup>a</sup>	14.00±0.57 <sup>b</sup>	20.00±0.57 <sup>d</sup>	18.33±0.88 <sup>cd</sup>	20.00±1.15 <sup>d</sup>	0.00±0.00 <sup>a</sup>	13.33±0.88 <sup>b</sup>	17.33±0.66 <sup>c</sup>	0.00±0.00 <sup>a</sup>
<i>B.megaterium</i> (CR2)	19.00±0.57 <sup>cde</sup>	17.33±0.66 <sup>bc</sup>	20.66±0.66 <sup>ef</sup>	20.33±0.66 <sup>ef</sup>	22.33±0.88 <sup>f</sup>	17.66±0.33 <sup>cd</sup>	15.33±0.88 <sup>ab</sup>	19.66±0.66 <sup>de</sup>	13.66±0.88 <sup>a</sup>	13.33±0.58 <sup>a</sup>
<i>Clostridium sp</i> (TR2)	6.00±0.57 <sup>b</sup>	9.66±0.33 <sup>c</sup>	16.66±0.88 <sup>e</sup>	17.33±0.66 <sup>ef</sup>	23.00±0.57 <sup>g</sup>	0.00±0.00 <sup>a</sup>	7.00±0.57 <sup>b</sup>	14.33±0.33 <sup>d</sup>	18.66±0.88 <sup>f</sup>	0.00±0.00 <sup>a</sup>
<i>S.aureus</i> (TG3)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	20.66±0.33 <sup>e</sup>	4.00±0.57 <sup>b</sup>	0.00±0.00 <sup>a</sup>	19.00±0.57 <sup>d</sup>	7.00±0.57 <sup>c</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
<i>M. endophyticus</i> (TR3)	21.00±0.57 <sup>c</sup>	0.00±0.00 <sup>a</sup>	18.33±0.66 <sup>c</sup>	22.00±0.57 <sup>c</sup>	20.00±0.57 <sup>c</sup>	20.00±0.00 <sup>c</sup>	20.66±0.88 <sup>c</sup>	13.66±0.88 <sup>b</sup>	14.00±3.05 <sup>b</sup>	0.00±0.00 <sup>a</sup>
<i>P.alimentorium</i> (CG1)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	18.00±0.57 <sup>e</sup>	16.00±0.57 <sup>d</sup>	4.00±0.57 <sup>b</sup>	13.00±1.00 <sup>c</sup>	20.00±0.57 <sup>f</sup>	19.66±0.88 <sup>f</sup>	0.00±0.00 <sup>a</sup>
<i>Clostridium sp</i> (GER2)	9.00±0.57 <sup>b</sup>	12.66±0.88 <sup>c</sup>	13.33±0.88 <sup>c</sup>	23.33±0.66 <sup>e</sup>	18.66±0.88 <sup>d</sup>	7.33±0.88 <sup>b</sup>	4.33±0.33 <sup>a</sup>	18.33±0.88 <sup>d</sup>	22.00±0.57 <sup>e</sup>	0.00±0.00 <sup>a</sup>
<i>Strep.mutans</i> (TG5)	12.33±0.88 <sup>c</sup>	0.00±0.00 <sup>a</sup>	13.00±0.57 <sup>c</sup>	13.33±0.88 <sup>c</sup>	13.66±0.33 <sup>c</sup>	16.66±0.88 <sup>d</sup>	2.00±0.00 <sup>b</sup>	12.66±0.33 <sup>c</sup>	20.00±0.57 <sup>e</sup>	2.33±0.33 <sup>b</sup>

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05). Key: Key: CN- Gentamycin 10µg, S- Streptomycin 30µ g, PEF- Pefloxacin 10µg, SXT-Septrin 30µg, CPX- Ciprofloxacin 10µg, AM- Amoxicillin 30µg, APX-Ampliclox 30µg, E- Erythromycin 10µg, Z-Zinnacef 20µg, R Rocephin 25µg

**Table 11. Susceptibility pattern of gram negative bacterial isolates to commercial antibiotics**

Antibiotic discs	Selected isolates				
	<i>Enterobacter aerogenes</i> (TR5)	<i>Ralstonia solanacearum</i> (TR6)	<i>Klebsiella oxytoca</i> (GNG)	<i>Pseudomonas aeruginosa</i> (TG4)	<i>Citrobacter freundii</i> (GNR3)
	<b>Zone of inhibition (mm)</b>				
CPX	22.00±0.57 <sup>cd</sup>	23.00±1.15 <sup>c</sup>	17.00±0.57 <sup>def</sup>	14.00±0.57 <sup>a</sup>	19.33±0.88 <sup>e</sup>
AM	14.33±0.66 <sup>a</sup>	14.00±0.57 <sup>a</sup>	9.00±0.57 <sup>b</sup>	20.66±0.33 <sup>d</sup>	15.00±0.00 <sup>cd</sup>
AU	19.66±0.33 <sup>cd</sup>	21.33±0.33 <sup>c</sup>	16.66±0.88 <sup>de</sup>	16.66±0.88 <sup>b</sup>	15.00±1.15 <sup>cd</sup>
CN	14.33±0.33 <sup>a</sup>	13.66±0.88 <sup>a</sup>	13.66±0.66 <sup>c</sup>	13.66±0.66 <sup>a</sup>	9.33±0.33 <sup>b</sup>
PEF	20.00±0.57 <sup>cd</sup>	23.33±0.88 <sup>c</sup>	17.33±0.33 <sup>ef</sup>	18.00±1.15 <sup>bc</sup>	13.33±0.88 <sup>c</sup>
OFX	14.00±0.57 <sup>a</sup>	19.00±0.57 <sup>b</sup>	15.33±0.33 <sup>cd</sup>	20.00±0.00 <sup>cd</sup>	10.33±0.88 <sup>b</sup>
S	17.33±0.66 <sup>b</sup>	17.00±0.57 <sup>b</sup>	0.00±0.00 <sup>a</sup>	16.66±0.66 <sup>b</sup>	0.00±0.00 <sup>a</sup>
SXT	19.33±0.33 <sup>c</sup>	22.66±0.88 <sup>c</sup>	14.66±0.33 <sup>c</sup>	13.33±0.88 <sup>a</sup>	0.00±0.00 <sup>a</sup>
CH	14.00±0.57 <sup>a</sup>	17.66±0.66 <sup>b</sup>	18.66±0.88 <sup>f</sup>	15.66±0.88 <sup>ab</sup>	17.00±0.57 <sup>d</sup>
SP	21.00±0.00 <sup>de</sup>	22.66±0.33 <sup>c</sup>	18.00±0.00 <sup>ef</sup>	16.66±0.88 <sup>b</sup>	14.00±0.57 <sup>c</sup>

Data are presented as Mean± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Keys: CN- Gentamycin 10µg, S- Streptomycin 30µ g, PEF- Pefloxacin 10µg, OFL- Tarivid 10µg, SXT-Septrin 30µ g, CH- Chloramphenicol 30µg, SP- Sparfloxacin 10µg, CPX- Ciprofloxacin 10µg, AM- Amoxicillin 30µg, AU-Augmentin 30µg

**Table 12. Antifungal effect *Azadirachta indica* and *Psidium guajava* leaf of extract on fungal isolates**

Name of isolates	Extracts			
	EN	EG	NHG	NHN
	<b>Zone of Inhibition (mm)</b>			
<i>Pichia kudriazevii</i> (DNG1)	38.33±0.88 <sup>d</sup>	23.33±0.88 <sup>b</sup>	32.66±1.45 <sup>c</sup>	19.00±0.57 <sup>a</sup>
<i>Geotrichum candidum</i> (DNR1)	33.33±0.88 <sup>b</sup>	25.33±0.33 <sup>a</sup>	34.66±1.15 <sup>b</sup>	23.00±1.00 <sup>a</sup>
<i>Saccharomyces cerevisiae</i> (GNR2)	58.66±0.88 <sup>c</sup>	39.66±0.33 <sup>b</sup>	37.00±0.57 <sup>ab</sup>	35.00±0.10 <sup>a</sup>
<i>Pichia kluyveri</i> (DER)	44.33±0.33 <sup>b</sup>	58.00±1.15 <sup>d</sup>	34.33±1.20 <sup>a</sup>	51.00±0.57 <sup>c</sup>
<i>Mucor circinelloides</i> (TR2)	20.33±0.33 <sup>b</sup>	14.33±0.66 <sup>a</sup>	20.00±1.15 <sup>b</sup>	12.00±0.57 <sup>a</sup>
<i>Aspergillus flavus</i> (TG1)	31.00±0.57 <sup>b</sup>	38.66±0.88 <sup>c</sup>	27.33±0.88 <sup>a</sup>	42.00±1.15 <sup>d</sup>
<i>Penicillium chrysogenum</i> (GEG)	33.66±0.88 <sup>b</sup>	36.66±0.88 <sup>c</sup>	29.00±0.57 <sup>a</sup>	34.67±0.66 <sup>b</sup>
<i>Geotrichum candidum</i> (DNR2)	29.33±0.33 <sup>b</sup>	41.33±0.88 <sup>c</sup>	25.66±0.66 <sup>a</sup>	28.00±1.15 <sup>ab</sup>
<i>Aspergillus niger</i> (DEG)	27.00±0.57 <sup>a</sup>	57.33±1.45 <sup>c</sup>	26.00±0.57 <sup>a</sup>	51.67±0.88 <sup>b</sup>
<i>Aspergillus niger</i> (TG2)	23.33±0.88 <sup>c</sup>	20.33±0.33 <sup>b</sup>	24.66±0.66 <sup>c</sup>	18.00±0.00 <sup>a</sup>
<i>Fusarium incarnatum</i> (TG3)	24.67±0.88 <sup>b</sup>	18.67±0.88 <sup>a</sup>	23.00±0.88 <sup>b</sup>	17.67±0.88 <sup>a</sup>
<i>Geotrichum candidum</i> (TR1)	25.00±0.00 <sup>a</sup>	41.33±0.88 <sup>c</sup>	24.66±0.66 <sup>a</sup>	38.00±1.15 <sup>b</sup>

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Keys: ED Neem Ethanolic Extract EG Guava Ethanolic Extract NHD Neem N-Hexane Extract NHG Guava N-Hexane Extract

**Table 13. Minimum inhibitory concentration/minimum fungicidal concentration of the leaf extracts against fungi isolated from *Capsicum annum* (red and green bell pepper)**

Test organisms	Ethanolic extract of <i>A. indica</i> (mg/ml)		Ethanolic extract of <i>P. guajava</i> (mg/ml)		N-Hexane extract of <i>A. indica</i> (mg/ml)		N-Hexane extract of <i>P. guajava</i> (mg/ml)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
<i>Pichia kudriazevii</i>	12.5	50	12.5	50	12.5	100	25	>100
<i>Geotrichum candidum</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Geotrichum candidum</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Saccharomyces cerevisiae</i>	25	100	25	100	12.5	100	12.5	100
<i>Mucor circinelloides</i>	50	>100	50	>100	50	>100	100	>100
<i>Aspergillus flavus</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Penicillium chrysogenum</i>	12.5	50	12.5	50	12.5	100	12.5	100
<i>Pichia kudriazevii</i>	12.5	50	12.5	50	12.5	100	25	>100
<i>Aspergillus niger</i>	25	100	12.5	50	25	>100	12.5	100
<i>Aspergillus niger</i>	25	100	12.5	50	25	>100	12.5	100
<i>Fusarium incarnatum</i>	25	100	12.5	50	25	>100	25	>100
<i>Geotrichum candidum</i>	12.5	50	12.5	50	25	>100	12.5	100

MIC-Minimum Inhibitory concentration, MBC-Minimum Fungicidal concentration

*Micrococcus luteus*, *Staphylococcus aureus*, *Citrobacter freundii*, *Aspergillus niger*, *Geotrichum candidum* and *Saccharomyces cerevisiae*. Ijato et al. [23] isolated similar microorganisms from Tomato fruits.

The present study shows that bell pepper fruits coated with neem leaf and guava leaf extracts had reduced decay compared with the uncoated bell pepper fruits. The ability of Neem and guava leaf extracts to decrease the decay level of bell pepper is an indication that Neem leaf and guava

leaf extracts can serve as a possible alternative in the prevention of bell pepper decay by spoilage microorganisms. This observation is in agreement with the reports of [24] who reported that extract from medicinal plants like *Allium sativum* (cloves), *Azadirachta indica* (leaves), *Mentha arvensis* (leaves) and *Psoralea Corylifolia* were found to be most effective in preserving plant fruits from attack by pathogenic and environmental factors. The ability of Neem and guava leaf extracts to minimize the decay of bell pepper fruits in this study can be attributed to

the fact that the Neem and guava leaf extracts contain bioactive compound that suppressed the activity of certain bacteria and fungi that cause spoilage of bell pepper.

Shelf life of the varieties of bell pepper fruits considered in this study was quite significant. During this study, it was found that spoilage of bell pepper fruits during storage increased with an increase in storage duration, though the intensity was influenced greatly by various treatments. However, bell pepper that was treated with neem leaf and guava leaf extracts at refrigeration temperature significantly has increased shelf life as seen in the number of days it took for complete spoilage of the fruits to occur compared to the treated bell pepper stored at room temperature. The result in this study is similar to the findings of [25] who reported that treating tomato fruits with Neem significantly increased their shelf life. Irokanulo et al. [26] also noted that tomato fruits treated with the powders of *Moringa oleifera* plant parts had an extended storage life. Bell pepper fruits coated with Neem leaf and guava leaf extracts showed low post harvest decay. Among the varieties of bell pepper fruits used for this research, the green variety recorded the least decay.

Phytochemical analyses revealed the presence of saponnin, anthraquinone, tannin, steroid, terpenoid, flavonoid and glycosides in ethanolic extracts of Guava and Neem leaves while tannin, terpenoid, flavonoid and glycosides were present in n-hexane extracts of Guava and Neem leaves which agree with the works of previous researchers [27]. The presence of these phytochemicals constituents in the plant extracts are the reasons they have antimicrobial activity. The analysis of the plant extracts revealed the presence of phytochemicals which are known to exhibit preservative, medical and physiological activities. In this study, the value for saponins, tannins, terpenoids, and flavonoids contents differed from one leaf extract to another. This reveals the fact that, the phytochemicals that are present in a leaf extract depend on the solvent used for extraction.

All extracts exhibited antibacterial activity against most of the test bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Micrococcus endophyticus*, *Streptococcus mutans*, *Proteus alimenterium*, *Pseudomonas aeruginosa* and

*Ralstonia solanacearum*) used in this study. Ethanolic extracts of Guava leaf had the highest zones of inhibition and this compared favourably with the conventional antibiotics discs used while N-hexane guava extracts had the lowest zones of inhibition. Although some bacteria were resistant to the extracts but most of the bacteria are susceptible. This result is in agreement with the work of [28]. The inhibitory activity of n-hexane and ethanol crude extracts of *Azadirachta indica* might be due to the presence of higher concentration of phytochemicals (bioactive substance) and probably the n-hexane and ethanol could be good solvents that support the inhibitory activity of these test strains. The concentration of bioactive compounds are good determinant of microbial susceptibility. When the concentration of a bioactive compounds is high, there might be better possibility of a higher and better zones of inhibition (ZOI). The present study revealed that *Azadirachta indica* leaf extract possessed good antibacterial and antifungal activity, confirming the potential of bioactive compounds in neem leaf and rationalizing the use of this plant in primary health care [29]. The results of the present study shows similarities to the findings of [30] who investigated the antimicrobial activity of *Psidium guajava* leaf extract, the results showed that both aqueous and ethanolic extracts of guava leaf inhibited the growth of the bacteria and fungi tested but the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms. In a similar study, n-hexane and aqueous extract of *Azadirachta indica*, inhibited *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus pyogenes* and *Staphylococcus aureus* [31]. Furthermore, all extracts exhibited antifungal activities against all test fungi (*Pichia kudriavzevii*, *Geotrichum candidum*, *Aspergillus flavus*, *Fusarium incarnatum*, *Aspergillus niger*, *Mucor circinelloides* and *Saccharomyces cerevisiae*) used in this study. Ethanolic neem extracts had the highest zones of inhibition and this compared favourably with the conventional antifungal drugs used while N-hexane guava extracts had the lowest zones of inhibition. Although some fungi were resistant to the extracts but most of the fungi were susceptible. Pandey et al. [32] has also demonstrated the antifungal properties of *Psidium guajava* leaves extracts against spoilage organisms. In line with this research report also, Biswas et al. [33] has reported that *Psidium guajava* extract are effective against Gram-negative and Gram-positive.

**Table 14. Susceptibility pattern of fungal isolates to commercial antifungal agents**

Name of isolates	Ketoconazole	Itraconazole	Fluconazole
<i>Pichia kudriazevii</i> (DNG1)	0.00±0.00	0.00±0.00	0.00±0.00
<i>Geotrichum candidum</i> (DNR1)	19.66±0.88 <sup>a</sup>	20.33±0.33 <sup>a</sup>	19.66±0.88 <sup>a</sup>
<i>Saccharomyces cerevisiae</i> (GNR2)	33.00±1.15 <sup>ab</sup>	35.00±2.08 <sup>b</sup>	28.66±0.66 <sup>a</sup>
<i>Pichia kluyveri</i> (DER)	25.00±1.15 <sup>a</sup>	27.67±1.45 <sup>a</sup>	31.67±0.33 <sup>b</sup>
<i>Mucor circinelloides</i> (TR2)	0.00±0.00	0.00±0.00	0.00±0.00
<i>Aspergillus flavus</i> (TG1)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	11.67±0.33 <sup>b</sup>
<i>Penicillium chrysogenum</i> (GEG)	0.00±0.00	0.00±0.00	0.00±0.00
<i>Geotrichum candidum</i> (DNR2)	29.33±0.66 <sup>a</sup>	33.33±0.88 <sup>b</sup>	30.67±0.88 <sup>ab</sup>
<i>Aspergillus niger</i> (DEG)	54.00±1.00 <sup>b</sup>	50.33±1.66 <sup>b</sup>	44.33±0.66 <sup>a</sup>
<i>Aspergillus niger</i> (TG2)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	31.67±0.88 <sup>b</sup>
<i>Fusarium incarnatum</i> (TG3)	33.66±0.88 <sup>b</sup>	37.66±1.20 <sup>c</sup>	23.66±0.88 <sup>a</sup>
<i>Geotrichum candidum</i> (TR1)	22.33±1.45 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Data are presented as Mean± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

The MIC/MBC of ethanolic and n-hexane extracts of neem and guava leaf against all test bacteria ranged from 12.5 to 100 mg/ml and minimum bactericidal concentration ranged from 50 to >100 mg/ml. This result is similar to the findings of [34] who investigated the antimicrobial activity of *Azadirachta Indica* (neem) leaf, bark and seed extracts.

## 5. CONCLUSION

Based on the results from this study, it can be concluded that ethanolic and N-hexane extracts of Neem and Guava leaves were able to extend the shelf life and quality of bell pepper fruits beyond their normal shelf life. This research has provided baseline information on the use of plant leaf extracts in post-harvest preservation of fruits. This may be a safe alternative to the use of synthetic chemicals for post-harvest preservation of bell pepper fruits. Ethanolic and N-hexane extracts of Neem and Guava leaves were found to also possess antibacterial and antifungal activities against spoilage organisms isolated from bell pepper. The antimicrobial activity can be attributed to the phytochemicals that are present in the leaf extracts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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