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# Antimicrobial Effect of Some Plant Extracts on Plant Pathogens that Cause Food Spoilage

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors BAO, TAO and MEF designed the study. Authors TAO and MEF performed the statistical analysis. Authors TAO and MEF wrote the protocol and authors BAO, TAO and MEF wrote the first draft of the manuscript. Authors TAO and MEF managed the analyses of the study. Authors TAO and MEF managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To determine the preservative effect of methanol extracts of *Azadirachta indica, Euphorbia heterophylla* and *Tithonia diversifolia* on fruits as an alternative to chemicals being used for preservation.
**Place and Duration of Study:** The study was carried out at the Department of Biological Sciences, Afe Babalola University, Ado Ekiti between September 2017 and February 2018.
**Methodology:** Pour plate method was used in the isolation of microorganisms from the fruits used and this were pineapple, banana, watermelon pawpaw and orange.
**Results:** Bacteria isolated were *Acinectobacter ursingii, Bacillus subtilis, Bordetella trematum, Klebsiella pneumoniae, Propionibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis*. Fungi isolated were *Aspergillus niger, Fusarium avenaceum, Fusarium oxysporum, Neurospora crassa, Penicillum notatum* and *Rhizopus stolonifer*. Different concentrations of the plant extracts (100 mg/ml, 80 mg/ml, 50 mg/ml and 30 mg/ml) were used on the test organisms and zones of inhibitions were determined which increased with increase in concentration. Methanol extracts of the bark of *Azadirachta indica* and *Tithonia diversifolia* leaves had greater antibacterial

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activity as they reduced the growth all the bacterial isolates especially *Staphylococcus aureus* at 100 mg/ml with a zone of inhibition of $41.00^{c}$ mm and $17.75^{b}$ mm respectively. All the extracts were not active against *Acinectobacter urisingii*. All concentrations of the methanol extract of the bark of *Azadirachta indica* and *Tithonia diversifolia* inhibited the growth of *Aspergillus niger* and *Penicillum notatum* for seven and four days respectively. Only 100 mg/ml of methanol extract of the bark of *Azadirachta indica* inhibited *Fusarium oxysporum* for seven days. The shelf life of watermelon treated with all the extracts was extended for eight days. That of pineapple, pawpaw, banana and orange was extended for four days using all the extracts.

**Conclusion:** These results support the potential use of these plant extracts in the management of diseases caused by tested plant pathogenic organisms.

*Keywords: Shelf life; preservatives; methanol extracts; plants; microorganisms; fruits.*

## 1. INTRODUCTION

Food spoilage is a metabolic process that causes foods and food materials to be undesirable or unacceptable for human consumption due to changes in sensory characteristics [1]. Microbiological damages which leads to safety and quality losses are very important due to two reasons: first, they constitute a hazard for consumers by the possible presence of microbial toxins or pathogenic microorganisms in the product, and second, by economic losses as a result of microbial spoilage [2].

Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides [3]. This is because, the plant extracts are cheap, locally available, non-toxic and easily biodegradable. The use of plant extracts to treat infection is practiced in the most part of the world, especially in developing countries, where there is a dependence on traditional medicine for a variety of diseases [4]. Some plants contain components that are toxic to pathogens and when extracted from the plant and applied on infested crops, are called botanical pesticides or botanicals [5]. The use of natural antimicrobials helps in the reduction of pest and pathogen injury, with the use of resistant varieties or integrated cropping strategies in which plant secondary metabolites may improve crop protection [6]. The use of natural antimicrobials as phytochemicals, organic acids, essential oils, or plant extracts could be a good alternative to ensure food safety [6].

*Azadirachta indica* (*A. Juss*), neem tree is a tropical evergreen with wide adaptability and is known to be resistant to insect infestation like the Mahogany tree [7]. *Azadirachta indica* possesses antibacterial, antifungal, antimalarial, and antiviral properties [8,9]. It also has the ability to affect more than 200 insect species as well as some mites and nematodes [10]. *Euphorbia heterophylla* Linn. (EH) is an annual weed that belongs to the Euphorbiaceae. It is a local medicinal plant commonly known as, spurge weed [11]. The leaves of *Euphorbia heterophylla* is used in traditional medicine practices as a laxative and anti-gonorrhoeal [12]. The latex of the plant has also been reported to be used as an insecticide [13]. *Tithonia diversifolia* is an invasive, annual weed, growing aggressively along road path, abandoned farmlands and hedges all over Nigeria [14]. The extracts from Tithonia plant parts have been reported to protect crops from termites [15] and contain chemicals that inhibit plant growth [16] control insects [17] and acts as a nematicide [18]. *Azadirachta indica, Euphorbia heterophylla* and *Tithonia diversifolia* are all multipurpose plants whose medicinal purposes have been documented and ascertained for a long time.

The preservation of food crops is necessary to prevent and reduce spoilage caused by microorganisms. It also helps to increase the shelf life of food crops and meet the needs of the demands of consumers for safe and natural products without chemical preservatives. Likewise, the use of the high treatment intensities required for microbial inactivation by some physical treatments during processing can cause adverse changes in the sensory or nutritional properties of the food [19]. The emerging non-thermal technologies have been considered expensive in relation to energy use or costly to be practical for use in food processing [20]. The resilience of bacterial spores and the existence of highly resistant microbial subpopulations could also hinder the efficacies of emerging non-thermal technologies [19].

The aim of this study was to determine the preservative effect of methanol extracts of *Azadirachta indica, Euphorbia heterophylla* and *Tithonia diversifolia* on various fruits such as

banana, pawpaw, pineapple, orange and watermelon in order to determine if their shelf life can be increased thus, serving as an alternative to chemical preservatives, physical treatments and non-thermal technologies [21]. The objectives were to isolate and identify bacteria and fungi from some spoilt fruits and assess the shelf-life of fruits treated with methanol extracts.

## 2. MATERIALS AND METHODS

### 2.1 Sampling of Spoilt Fruits

Five types of unwashed and unprocessed spoilt fruits -pineapple (*Ananas comosus*), banana (*Musa* spp), watermelon (*Citrullus lanatus*), pawpaw (*Carica papaya*) and orange (*Citrus sinensis*) were collected in separate plastic carrier bags from different fruit vendors in September, 2017 in Ado-Ekiti, Nigeria and brought to the laboratory for further analysis. Fruits with visible spoilage (discoloration, growth, and odor) which indicated the presence of spoilage were picked.

### 2.2 Isolation of Microorganisms/ Identification and Isolation of Bacterial Isolates

The bacteria were isolated from spoiled fruits by using the pour plate method according to Aneja [22]. One gram of portions on the fruits showing observable changes were picked using sterile inoculating needle and suspended in 10 mls of sterile distilled water. Serial dilution was carried out. 1 ml from each dilution was inoculated into a sterile labelled Petri dishes and nutrient agar was introduced and the plates homogenised and allowed to set. The plates were incubated at 37ºC for 24 hours for bacterial growth. After incubation, the bacterial isolates were subcultured to obtain pure isolates and maintained and stored on nutrient agar slants. Potato dextrose agar plates were inoculated with 1 ml of each diluent and incubated at 28ºC for 7 days for fungal growth and pure fungal isolates were stored on PDA slants for further use. Bacterial isolates were identified based on biochemical characterization and using an online software-Global infectious diseases and epidemiology network (GIDEON) and fungi isolates were identified based on cultural characteristics and morphological examination.

### 2.3 Collection of Plants

Fresh leaves of *Euphorbia heterophylla*, *Tithonia diversifolia* bark and leaves of *Azadirachta indica,* were collected from Federal Polytechnic, Ado-Ekiti, Ekiti State, in September 2017. The plants were authenticated at Ekiti State University Herbarium, Ado-Ekiti and the voucher numbers were obtained; *Azadirachta indica*- UHAE 2018/002, *Euphorbia heterophylla*- UHAE 2018/003 and *Tithonia diversifolia*- UHAE 2018/004. The leaves and bark of the different plants were washed to remove debris and the earth remains and allowed to dry at room temperature. The dried samples were separately blended into fine powder and stored [23].

### 2.4 Soaking

The powdered leaves were weighed using an electronic weighing balance (Ohaus CS5000) and soaked with methanol, in air tight plastic container for 72 h [24]. Two hundred (200) g of *Euphorbia heterophylla* in 1.25 L of methanol, 250 g of *Tithonia diversifolia* in 1.25 L of methanol, 250 g of bark of *Azadirachta indica* was soaked in 1.25 L of methanol and 250 g of leaf of *Azadirachta indica* in 1.25 L of methanol.

### 2.5 Filtration of the Extract

The soaked plant parts were first filtered with a muslin cloth, then with Whatman No 1 filter paper. The filtrates were then concentrated to dryness in a water bath at 45°C. The extracts were stored in a desiccator until needed [23].

### 2.6 Preparation of Extract Concentrations

Stock solutions of the extracts were prepared by weighing 1 g of the extracts and dissolving in 10 ml of Dimethyl sulpho-oxide (DMSO). There after, different concentrations were obtained from the stock solutions.

### 2.7 Antimicrobial Assay

#### 2.7.1 Agar-well diffusion method

The test isolates, *Acinectobacter ursingii*, *Bacillus subtilis*, *Bordetella trematum*, *Klebsiella pneumoniae*, *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were individually grown in nutrient broth for 18 h and afterwards, serial dilution carried out and pour plate method according to Aneja [22] was used. Diluents from 10-3 and 10-5 dilution were used as the inoculum. The antibacterial activities of the crude extracts were determined using the standard agar-well diffusion method [25]. 1 ml of the bacterial inoculum was transferred into a sterile Petri dish and 20 mls of Mueller Hinton Agar (MHA) was poured and

swirled in order to homogenise it. It was allowed to set. Different concentrations of the extracts (100 mg/ml, 80 mg/ml, 50 mg/ml and 30 mg/ml) were introduced into the different bored wells (9 mm cork borer) in each plate. A control using Dimethyl Sulfoxide (DMSO) was also prepared and introduced into the well. The plates were incubated at 37°C for bacteria and observed for zones of inhibition after 24 h. Observed clear zones of inhibition around the wells containing plant extract were measured using a metric ruler [26].

#### 2.7.2 Radial growth for fungi

The antifungal properties of the extracts were tested using the radial growth method as described by Banso et al. [27] on potato dextrose agar. Different concentrations of the plant extracts (100 mg/ml, 80 mg/ml, 50 mg/ml and 30 mg/ml) were introduced separately into sterile plates thereafter, PDA was poured in each plate and swirled gently to homogenise and allowed to set. A sterilize 9 mm cork borer was used to cut into a 7 days old fungus and placed in the centre of each Petri plate and incubated at 28°C for 7 days. Radial growth was measured and recorded at 24 h intervals using a metric ruler. The inhibitory activity of each treatment was expressed as the percent (%) growth inhibition as compared to the negative control (0%) using the following formula:

Growth inhibition (%) = DC – DT / DC x 100 [28].

Where,

DC = Diameter of control and DT = diameter of the fungal colony with treatment.

### 2.8 Shelf-life Testing

This was carried out by spraying 4 mls of 100 mg/ml of the plant extracts on each freshly washed fruit and observed for preservative potentials for a period of eight days. Each fruit had a control which was not treated with the extract. This was used in comparing the rate at which the extract preserved the fruits [29].

### 2.9 Statistical Analysis

The statistical analysis was carried out using One-way Analysis of Variance (ANOVA) using the Statistical Package for Social Science (version 20). The mean values were expressed as mean ± standard deviation (SD) of duplicates. The differences were considered significant at $P \leq 0.05$.

## 3. RESULTS AND DISCUSSION

Five fruit samples were collected and analysed for this study and included, pineapple, pawpaw, water melon, banana and orange. Observable spoilage were seen on these food materials such as discoloration, growth and foul odor which indicated the presence of microorganisms. A total of seven bacterial species were obtained after series of biochemical tests were carried out and included *Staphylococcus aureus* from pawpaw, *Bacillus subtilis* from pawpaw, pineapple, orange and watermelon, *Klebsiella pneumoniae* from pineapple, *Propionibacterium acnes* from orange and watermelon, *Bordetella trematum* from orange, *Staphylococcus epidermidis* from banana and *Acinetobacter ursingii* from banana as presented in Table 1.

The fungi identified after cultural characteristics and morphological examination were, *Neurospora crassa* and *Fusarium avenaceum* isolated from watermelon, orange and pawpaw. *Penicillum notatum* was isolated from orange, *Aspergillus niger* from banana, pawpaw and pineapple, *Fusarium oxysporum* was isolated from banana and pawpaw and *Rhizopus stolonifer* from pawpaw, pineapple and banana. Table 2 shows the morphological description of the fungal isolates.

Table 3 shows the antibacterial activity of the methanol extract of the bark of *Azadirachta indica* against some microorganisms. The extract was effective against all the microorganisms except *Acinetobacter ursingii*. The highest activity zone of inhibition of $32.25^{d}$ mm was observed against *Klebsiella pneumoniae* at 100 mg/ml. The organism with the least zone of inhibition at 100 mg/ml was *Bordetella trematum* at 14.75d mm. All zones of inhibitions for all organisms were significantly different statistically at different concentrations.

Table 4 shows the antibacterial effect of the methanol extract of the leaf of *Azadirachta indica* against some microorganisms. *Bacillus subtillis* showed the highest activity at 100 mg/ml with a diameter zone of inhibition of 18.25d mm. The least activity was observed for *Klebsiella pneumoniae*. At 100 mg/ml, the diameter of zone of inhibition observed was 12.25c mm. The extract did not show any activity on *Acinetobacter ursingii*, *Staphylococcus epidermidis, Acinetobacter ursingii* and *Staphyloccocus aureus.* There was significant difference between all the zones of inhibitions.

**Table 1. Biochemical characteristics and probable bacterial isolates from the spoilt fruits**

| Sample/Isolates | Morphology | Shape | Elevation | Edge | Surface | Chromogenes | Gram stain | Motility | Starch hydrolysis | Catalase | Gas | Methyl Red | Citrate | Butt | Oxidase | Slant | Mannitol | Indole test | $H_2S$reduction | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PW(a) | Cocci | Circular | Raised | Entire | Smooth | Creamy | + | - | - | + | - | + | - | + | - | + | - | - | - | *Staphylococcus aureus* |
| PW(b) | Rod | Circular | Raised | Entire | Dry | Creamy | + | + | - | + | - | + | + | + | - | + | + | - | - | *Bacillus subtilis* |
| PI(a) | Rod | Irregular | Flat | Undulate | Smooth | Creamy | + | + | - | + | - | - | + | + | - | + | - | - | - | *Bacillus subtilis* |
| PI(b) | Rod | Circular | Raised | Entire | Smooth | White | - | - | - | + | - | - | + | - | - | - | + | - | - | *Klebsiella pneumonia* |
| OR(c) | Cocci | Circular | Flat | Entire | Smooth | White | + | - | - | + | + | - | + | + | - | + | + | + | - | *Bordetella trematum* |
| BN(a) | Cocci | Circular | Raised | Entire | Smooth | Creamy | + | - | - | + | - | + | + | + | - | + | - | + | - | *Staphylococcus epidermidis* |
| BN(c) | Cocci | Circular | Raised | Entire | Smooth | Creamy | - | - | - | + | - | + | + | + | - | + | - | - | - | *Acinetobacter ursingii* |
| WM(a) | Rod | Circular | Flat | Entire | Smooth | White | + | - | - | + | - | - | + | - | - | + | - | - | - | *Propionibacterium acnes* |
| WM(b) | Rod | Circular | Raised | Entire | Dry | Creamy | + | + | - | + | - | + | + | + | - | + | + | - | - | *Bacillus subtilis* |

*Key PW = Pawpaw, PI = Pineapple, OR = Orange, BN = Banana, WM = Watermelon, - = Negative, + = Positive*

**Table 2. Texture, colour and morphological features of fungi**

| Fungi | Texture | Colour | Morphological features |
|---|---|---|---|
| *Neurospora crassa* | Fuzzy | Orange | Buff, ropy and conidial band were more or less orange. |
| *Fusarium avenaceum* | Fluffy | White-pink | Microcondia are ovoid in shape. Macroenidia are borne on phialides on branched conidiophores. Septate fusiform, slightly curved and pointed at both ends is present. |
| *Penicillium notatum* | Velvety | Green | Colonies are smooth and ellipsoidal Conidiophores are smooth and short and mycelia arranged irregularly with branches of various lengths. |
| *Aspergillus niger* | Velvety | Black | Conidia heads are large, globose, dark brown, biseriate and rough walled. Smooth walled Conidiophores. |
| *Rhizopus stolinfer* | Thread-like | White | Non-septate mycelia. Sporangiophores smooth walled and ovoid in shape. Sporangia and columella subglobose. |
| *Fusarium oxysporum* | Fluffy | White-orange | Microcondia are ovoid in shape, borne on phialides with branched conidiophores. Septate, slightly curved and pointed at both ends. |

**Table 3. Antibacterial effect of the methanol extract of the bark of *Azadirachta indica* on test organisms**

| Organism | Zones of inhibition (mm)/conc (mg/ml) | | | |
|---|---|---|---|---|
| | 100 | 80 | 50 | 30 |
| *Propionibacterium acnes* | $17.00^{b} \pm 0.00$ | $15.50^{b} \pm 1.41$ | $10.50^{a} \pm 1.41$ | $10.00^{a} \pm 1.00$ |
| *Bacillus subtilis* | $26.00^{b} \pm 0.00$ | $24.75^{b} \pm 0.35$ | $22.75^{a,b} \pm 0.35$ | $17.75^{a} \pm 1.25$ |
| *Bordetella trematum* | $14.75^{d} \pm 0.35$ | $11.75^{c} \pm 0.35$ | $9.50^{b} \pm 0.71$ | $7.25^{a} \pm 0.35$ |
| *Staphylococcus epidermidis* | $20.50^{c} \pm 0.35$ | $16.25^{b,c} \pm 1.00$ | $13.50^{a,b} \pm 0.41$ | $9.50^{a} \pm 0.00$ |
| *Acinetobacter ursingii* | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ |
| *Klebsiella pneumonia* | $32.25^{d} \pm 0.35$ | $30.00^{c} \pm 0.00$ | $28.00^{b} \pm 0.00$ | $16.25^{a} \pm 0.35$ |
| *Staphylococcus aureus* | $41.00^{c} \pm 1.00$ | $32.50^{b} \pm 2.50$ | $34.80^{b} \pm 0.30$ | $25.30^{a} \pm 0.80$ |

*Key: a b c d= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9 mm*

Table 5 shows the antibacterial effect of the methanol extract of leaf of *Euphorbia heterophylla* against some microorganisms. The extract had no activity against *Bacillus subtilis, Staphylococcus epidermidis, Acinetobacter ursingii* and *Klebsiella pneumoniae.* At 100m/ml, zones of inhibition for *Propionibacterium acnes* was $12.25^{d}$ mm which was the highest and *Staphylococcus aureus* $8.81^{d}$ mm which was the lowest. There was significant difference between all the zones of inhibitions.

Table 6 shows the antibacterial effect of methanol extract of leaf of *Tithonia diversifolia* against some microorganisms. The extract did not show any activity on *Staphylococcus epidermidis, Acinetobacter ursingii* and *Klebsiella pneumoniae.* The highest activity observed was against *Staphylococcus aureus* at the different concentrations. The diameter of zone of inhibition at 100mg/ml was $17.75^{b}$ mm. The least activity was observed for *Klebsiella pneumoniae.* At 100 mg/ml, the diameter of zone of inhibition observed was $9.50^{c}$ mm. The zones of inhibitions were not significantly different.

Generally, the bark of *Azadirachta indica* had more antibacterial activity on the bacterial isolates except *Acinetobacter ursingii* which showed no zone of inhibition with all the extracts. Whereas, the extract that had least antibacterial activity on the isolates is *Euphorbia heterophylla* as it showed inhibition on only one isolate, *Propioniumbacterium acnes* and *Staphylococcus aureus.*

Table 7 shows the extract of the bark of *Azadiractha indica* and *Tithonia diverfolia* had the highest activity against *Aspergillus niger* with the bark of *Azadiractha indica* inhibiting the growth of the organism throughout the 7 days at all the concentrations and the leaf of *Tithonia diversifolia* inhibiting the growth of the organism

for 5 days. No growth was observed on the 1st 2 days for the control and all the extracts. The radial growth decreased with increase in concentration of extracts.

Table 8 shows the extract of the bark of *Azadiractha indica* inhibited the growth of *Penicillium notatum* throughout the 7 days at all the concentrations. At 100mg/ml, the leaf extract of *A.indica* inhibited the growth of the fungus till day 6. The radial growth was $4.00_a$±.00mm and was significantly different from the control which was $28.50^f$±0.00.

Table 9 shows the methanol extract at 100 mg/ml of the bark of *Azadirachta indica* inhibited the growth of the organism throughout the experiment. At day four, the radial growth measured was $9.25^a$ mm compared with the control of $33.00^e$ mm at 100 mg/ml of the leaf methanol extract of *Azadirachta indica*. The radial growth of the organism at 100 mg/ml with the methanol extract of *Euphorbia heterophylla* and *Tithonia diversifolia* increased steadily with decrease in concentration from day three till day seven of the experiment and were significantly different at p≤0.05.

**Table 4. Antibacterial effect of the methanol extract of the leaf of *Azadirachta indica* on test organisms**

| Organism | Zones of inhibition (mm)/conc (mg/ml) | | | |
|---|---|---|---|---|
| | 100 | 80 | 50 | 30 |
| *Propionibacterium acnes* | $14.50^c$ ± 0.71 | $11.50^{b,c}$ ± 0.00 | $8.00^{a,b}$ ± 2.83 | $5.25^a$ ± 0.35 |
| *Bacillus subtilis* | $18.25^d$ ± 0.35 | $15.25^c$ ± 0.35 | $14.25^b$ ± 0.35 | $8.20^a$ ± 0.35 |
| *Bordetella trematum* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Staphylococcus epidermidis* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Acinetobacter ursingii* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Klebsiella pneumonia* | $12.25^c$ ± 0.35 | $11.00^b$ ± 0.00 | $6.25^a$ ± 0.35 | $6.25^a$ ± 0.35 |
| *Staphylococcus aureus* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

*Key: a b c d= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9mm*

**Table 5. Antibacterial effect of the methanol extract of leaf of *Euphorbia heterophylla* on test organisms**

| Organism | Zone of inhibition (mm)/conc (mg/ml) | | | |
|---|---|---|---|---|
| | 100 | 80 | 50 | 30 |
| *Propionibacterium acnes* | $12.25^d$ ± 0.35 | $8.25^c$ ± 0.35 | $7.25^b$ ± 0.35 | $0.00^a$ ± 0.00 |
| *Bacillus subtilis* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Bordetella trematum* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Staphylococcus epidermidis* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Acinetobacter ursingii* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Klebsiella pneumonia* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Staphylococcus aureus* | $8.81^d$ ± 0.35 | $11.75^c$ ± 1.06 | $8.25^b$ ± 0.35 | $0.00^a$ ± 0.00 |

*key: a b c d= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9mm*

**Table 6. Antibacterial effect of methanol extract of leaf of *Tithonia diversifolia* on test organisms**

| Organism | Zone of inhibition (mm)/conc (mg/ml) | | | |
|---|---|---|---|---|
| | 100 | 80 | 50 | 30 |
| *Propionibacterium acnes* | $12.50^d$ ± 0.00 | $9.25^c$ ± $0.35^c$ | $8.25^b$ ± 0.35 | $0.00^a$ ± 0.00 |
| *Bacillus subtilis* | $13.75^b$ ± 1.06 | $11.50^b$ ± $1.41^b$ | $0.00^a$ ± 0.00 | $0.00^a$ ± 0.00 |
| *Bordetella trematum* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Staphylococcus epidermidis* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Acinetobacter ursingii* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Klebsiella pneumonia* | $9.50^c$ ± 0.70 | $2.08^b$ ± $0.11^b$ | $0.00^a$ ± 0.00 | $0.00^a$ ± 0.00 |
| *Staphylococcus aureus* | $17.75^b$ ± 1.06 | $10.50^{a,b}$ ± 0.35 | $6.00^{a,b}$ ± 0.35 | $1.83^a$ ± 0.46 |

*Key: a b c d= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9mm*

**Table 7. Radial growth of *Aspergillus niger* using different methanol plant extracts**

| | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| | Control | - | - | $10.00^{d} \pm 0.00$ | $13.00^{d} \pm 0.00$ | $18.00^{e} \pm 0.00$ | $21.00^{d} \pm 0.00$ | $28.50^{e} \pm 0.00$ |
| AL | 100 mg/ml | - | - | $5.20^{a} \pm 0.35$ | $7.20^{a} \pm 0.35^{a}$ | $9.00^{a} \pm 0.00$ | $9.25^{a} \pm 0.35$ | $10.00^{a} \pm 0.00$ |
| | 80 mg/ml | - | - | $7.00^{b} \pm 0.00$ | $9.50^{b} \pm 0.00^{b}$ | $11.25^{b} \pm 0.35$ | $11.25^{b} \pm 0.35$ | $11.25^{b} \pm 0.35$ |
| | 50 mg/ml | - | - | $9.25^{c} \pm 0.35$ | $11.25^{c} \pm 0.35$ | $11.25^{c} \pm 0.00$ | $11.75^{b} \pm 0.35$ | $12.00^{c} \pm 0.00$ |
| | 30 mg/ml | - | - | $16.00^{e} \pm 0.35$ | $16.25^{e} \pm 0.00$ | $16.25^{d} \pm 0.00$ | $17.00^{c} \pm 0.00$ | $19.25^{d} \pm 0.35$ |
| AB | 100 mg/ml | - | - | - | - | - | - | - |
| | 80 mg/ml | - | - | - | - | - | - | - |
| | 50 mg/ml | - | - | - | - | - | - | - |
| | 30 mg/ml | - | - | - | - | - | - | - |
| EH | 100 mg/ml | - | - | $7.25^{b} \pm 0.35$ | $8.25^{a} \pm 0.35$ | $16.00^{d} \pm 0.00$ | $21.00^{d} \pm 0.00$ | $23.25^{d} \pm 0.35$ |
| | 80 mg/ml | - | - | $8.25^{c} \pm 0.35$ | $10.00^{b} \pm 0.00$ | $17.25^{d} \pm 0.35$ | $23.25^{e} \pm 0.35$ | $23.75^{d} \pm 0.35$ |
| | 50 mg/ml | - | - | $10.25^{c} \pm 0.35$ | $20.25^{e} \pm 0.35$ | $21.50^{f} \pm 0.71$ | $25.00^{e} \pm 0.35$ | $24.25^{e} \pm 0.35$ |
| | 30 mg/ml | - | - | $20.00^{e} \pm 0.00$ | $23.25^{e} \pm 0.35$ | $23.25^{f} \pm 0.35$ | $26.25^{f} \pm 0.35$ | $28.25^{e} \pm 0.35$ |
| TH | 100 mg/ml | - | - | - | - | $4.20^{a} \pm 0.35$ | $8.95^{a} \pm 0.07$ | $14.75^{c} \pm 0.35$ |
| | 80 mg/ml | - | - | - | - | $5.00^{a} \pm 0.00$ | $11.25^{b} \pm 0.35$ | $19.25^{d} \pm 0.35$ |
| | 50 mg/ml | - | - | - | - | $8.25^{a} \pm 0.35$ | $11.75^{b} \pm 0.35$ | $22.25^{d} \pm 0.35$ |
| | 30 mg/ml | - | - | - | - | $9.25^{a} \pm 0.35$ | $17.00^{c} \pm 0.35$ | $27.75^{d} \pm 0.35$ |

*Key: a b c d e= means on the same row but with different superscripts are statistically significant ($p<0.05$). Reading in duplicates. Diameter of cork borer 9 mm*
*AL= Azadirachta indica (leaf) EH= Euphorbia heterophylla AB= Azadirachta indica (bark) TH= Tithonia diversifolia*

**Table 8. Radial growth of *Penicillium notatum* using different methanol plant extracts**

| | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| | Control | - | - | $5.50^{d} \pm 0.00$ | $11.00^{d} \pm 0.00$ | $11.00^{d} \pm 0.00$ | $12.50^{d} \pm 0.00$ | $21.50^{d} \pm 0.00$ |
| AL | 100 mg/ml | - | - | - | - | - | - | $4.00^{a} \pm 0.00$ |
| | 80 mg/ml | - | - | - | - | $4.25^{a} \pm 0.35$ | $5.50^{a} \pm 0.00$ | $4.50^{a} \pm 0.00$ |
| | 50 mg/ml | - | - | - | $5.25^{a} \pm 0.00$ | $5.50^{a} \pm 0.35$ | $6.25^{a} \pm 0.35$ | $5.25^{a} \pm 0.35$ |
| | 30 mg/ml | - | - | - | $6.25^{b} \pm 0.35$ | $10.50^{b} \pm 0.35$ | $11.25^{b} \pm 0.00$ | $13.25^{b} \pm 0.35$ |
| AB | 100 mg/ml | - | - | - | - | - | - | - |
| | 80 mg/ml | - | - | - | - | - | - | - |
| | 50 mg/ml | - | - | - | - | - | - | - |
| | 30 mg/ml | - | - | - | - | - | - | - |

| | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| EH | 100 mg/ml | - | - | $4.5^{a} \pm 0.00$ | $5.25^{a} \pm 0.35$ | $9.50^{b} \pm 0.00$ | $17.50^{c} \pm 0.00$ | $21.25^{d} \pm 0.35$ |
| | 80 mg/ml | - | - | $9.25^{b} \pm 0.35$ | $6.25^{b} \pm 0.35$ | $15.25^{c} \pm 0.35$ | $20.00^{e} \pm 0.00$ | $21.50^{d} \pm 0.00$ |
| | 50 mg/ml | - | - | $15.00^{c} \pm 0.00$ | $9.25^{c} \pm 0.35$ | $17.50^{c} \pm 0.00$ | $21.00^{e} \pm 0.00$ | $23.00^{e} \pm 0.00$ |
| | 30 mg/ml | - | - | $15.50^{d} \pm 0.00$ | $11.25^{d} \pm 0.35$ | $21.25^{e} \pm 0.35$ | $21.50^{e} \pm 0.00$ | $24.50^{e} \pm 0.00$ |
| TH | 100 mg/ml | - | - | - | $4.00^{a} \pm 0.00$ | $7.25^{b} \pm 0.35$ | $7.50^{b} \pm 0.00$ | $8.25^{a} \pm 0.35$ |
| | 80 mg/ml | - | - | - | $4.50^{a} \pm 0.35$ | $9.25^{b} \pm 0.35$ | $9.50^{b} \pm 0.00$ | $16.50^{b} \pm 0.00$ |
| | 50 mg/ml | - | - | - | $5.50^{a} \pm 0.35$ | $10.00^{b} \pm 0.00$ | $11.25^{b} \pm 0.35$ | $18.25^{c} \pm 0.35$ |
| | 30 mg/ml | - | - | - | $10.25^{c} \pm 0.35$ | $12.50^{b} \pm 0.00$ | $13.00^{e} \pm 0.00$ | $20.25^{d} \pm 0.35$ |

*Key: a b c d e= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9 mm*
*AL= Azadirachta indica (leaf) EH= Euphorbia heterophylla AB= Azadirachta indica (bark) TH= Tithonia diversifolia*

**Table 9. Radial growth of *Fusarium oxysporum* using different methanol plant extracts**

| | | Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| | Control | - | - | $30.50^{e} \pm 0.00$ | $33.00^{e} \pm 0.00$ | $33.00^{e} \pm 0.00$ | $36.00^{e} \pm 0.00$ | $41.00^{e} \pm 0.00$ |
| AL | 100 mg/ml | - | - | - | $9.25^{a} \pm 0.35$ | $12.50^{a} \pm 0.00$ | $22.00^{a} \pm 0.00$ | $15.00^{a} \pm 0.00$ |
| | 80 mg/ml | - | - | - | $15.50^{b} \pm 0.35$ | $13.50^{b} \pm 0.35$ | $20.25^{b} \pm 0.35$ | $19.00^{b} \pm 0.00$ |
| | 50 mg/ml | - | - | - | $17.25^{c} \pm 0.35$ | $18.50^{c} \pm 0.35$ | $19.00^{c} \pm 0.0$ | $20.25^{c} \pm 0.35$ |
| | 30 mg/ml | - | - | $6.50^{a} \pm 0.00$ | $21.00^{d} \pm 0.00$ | $21.25^{d} \pm 0.35$ | $15.00^{d} \pm 0.00$ | $22.00^{d} \pm 0.00$ |
| AB | 100 mg/ml | - | - | - | - | - | - | - |
| | 80 mg/ml | - | - | - | $8.50^{b} \pm 0.00$ | $10.50^{b} \pm 0.00$ | 13.50 ± 0.00 | 13.50 ± 0.00 |
| | 50 mg/ml | - | - | - | $11.00^{b} \pm 0.00$ | $16.00^{c} \pm 0.00$ | 22.00 ± 0.00 | 22.00 ± 0.00 |
| | 30 mg/ml | - | - | - | $11.50^{c} \pm 0.00$ | $21.25^{d} \pm 0.35$ | 30.00 ± 0.00 | 30.00 ± 0.00 |
| EH | 100 mg/ml | - | - | $12.50^{a} \pm 0.00$ | $18.50^{a} \pm 0.00$ | $18.50^{a} \pm 0.00$ | 20.00 ± 0.00 | $21.00^{a} \pm 0.00$ |
| | 80 mg/ml | - | - | $18.50^{b} \pm 0.00$ | $18.50^{a} \pm 0.70$ | $20.00^{b} \pm 0.00$ | 21.00 ± 0.00 | $23.50^{b} \pm 0.00$ |
| | 50 mg/ml | - | - | $20.00^{c} \pm 0.00$ | $21.50^{b} \pm 0.00$ | $23.00^{c} \pm 0.00$ | 24.50 ± 0.00 | $28.25^{c} \pm 0.00$ |
| | 30 mg/ml | - | - | $21.25^{d} \pm 0.35$ | $23.50^{c} \pm 0.00$ | $24.50^{d} \pm 0.00$ | 28.50 ± 0.00 | $31.00^{d} \pm 0.00$ |
| TH | 100 mg/ml | - | - | $9.00^{a} \pm 0.00$ | $10.00^{a} \pm 0.00$ | $16.25^{a} \pm 0.35$ | 17.00 ± 0.00 | $18.25^{a} \pm 0.35$ |
| | 80 mg/ml | - | - | $10.25^{b} \pm 0.35$ | $17.25^{b} \pm 0.35$ | $20.25^{b} \pm 0.35$ | 20.25 ± 0.35 | $20.25^{a,b} \pm 0.35$ |
| | 50 mg/ml | - | - | $12.25^{c} \pm 0.35$ | $17.20^{b} \pm 0.30$ | $21.25^{c} \pm 0.35$ | 21.50 ± 0.70 | $22.25^{b} \pm 1.76$ |
| | 30 mg/ml | - | - | $17.00^{d} \pm 0.00$ | $20.00^{c} \pm 0.00$ | $24.25^{d} \pm 0.35$ | 25.25 ± 0.35 | $30.25^{c} \pm 0.35$ |

*Key: a b c d e= means on the same row but with different superscripts are statistically significant (p<0.05). Reading in duplicates. Diameter of cork borer 9 mm*
*AL= Azadirachta indica (leaf) EH= Euphorbia heterophylla AB= Azadirachta indica (bark) TH= Tithonia diversifolia*

Fig. 1 shows the treatment of fresh watermelon with methanol extract of *Tithonia diversifolia*. The extract was able to preserve the watermelon all through the eight days of the treatment process when compared with the control that began to spoil by day four with changes in color and splitting of the back of the fruit. By day eight, the control had changed color completely with the back completely destroyed whereas the treated watermelon remained intact.

Fig. 2 shows the treatment of fresh pineapple with methanol extract of the leaf of *Azadirachta indica*. The pineapple was preserved for four days when compared with the control that showed signs of microbial growth and change in color. By day six, the treated pineapple, began showing signs of spoilage with change in color, foul odor, soften back, and these leading to microbial growth. By the eighth day, the treated pineapple had completely changed in color but no foul odor as compared with the control which showed complete signs of spoilage.

Fig. 3 shows the treatment of fresh orange with methanol extract of *Euphorbia heterophylla.* The extract was able to preserve the orange for the whole duration of the experiment as the fruit only shrunk in size. Whereas, on the control, there was evidence of microbial growth which increased until the eighth day of the treatment process.

Five fruits were used for this study. Bacteria isolated and identified from spoilt banana, pineapple, pawpaw, orange and watermelon were, *Acinetobacter ursingii, Bacillus subtillis, Bordetella trematum, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*. Raja et al. [30] reported that *Pseudomonas* sp. and *Bacillus* sp. were dominantly found in both local and super market samples of spoiled vegetables and fruits. In the study conducted by Kumar et al. [31] on thirty 30 spoilt food samples from Paonta Sahib, organisms that were identified and characterized included *Bacillus, Klebsiella, Pseudomonas, Staphylococcus* and *Micrococcus* on the basis of morphology and biochemical reactions. This is in agreement with this study where all these microorganisms were isolated except *Micrococcus* sp.

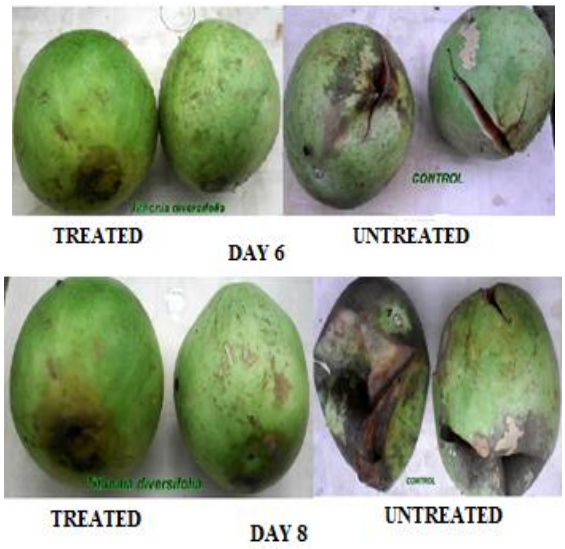


**Fig. 1. Watermelon treated with methanol extract of the leaf of *Tithonia diversifolia***

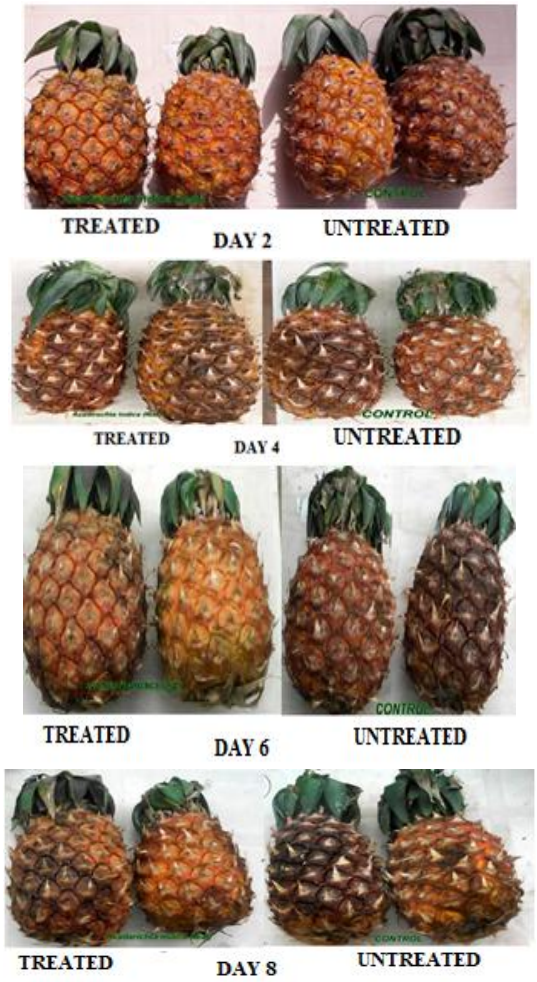


**Fig. 2. Pineapple treated with methanol extract of the leaf of *Azadirachta indica***

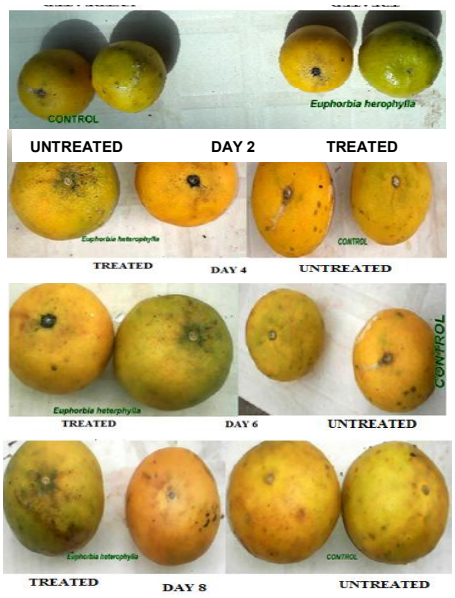


**Fig. 3. Orange treated with methanol extract of the leaf of *Euphorbia heterophylla***

The fungi isolated and identified from banana, pineapple, pawpaw, orange and watermelon were *Aspergillus niger, Fusarium avenaceum, Fusarium oxysporum, Neurospora crassa, Penicillium notatum,* and *Rhizopus stolonifer*. This result is in agreement with the report of Jolaosho et al. [32] who isolated *Rhizopus stolonifer* from sliced packaged pineapple samples in Ogun state. This finding is also in conformity with previous works of Baiyewu et al. [33] and Chukwuka et al. [34] which reported isolation of *Aspergillus niger, Fusarium avenaceum* and *Rhizopus stolonifer* from pawpaw in Nigeria. *Aspergillus niger, Aspergillus flavus, Fusarium solani, Fusarium avenaceum, Penicillium digitatum, Rhizopus stolonifer* and yeast were identified by Mailafia et al. [35] from pawpaw, orange, watermelon, tomato and pineapple in Gwagwalada market, Abuja, Nigeria. These findings are also in agreement with the current study as some of these microorganisms were isolated.

In this study, the choice of the different plants extracts of *Tithonia diversifolia, Azadirachta indica* and *Euphorbia heterophylla* as preservatives for fruits was deliberate because these plants have been shown to act against various microorganisms as well as pests that cause spoilage. The results obtained showed that, methanol extracts of *Azadirachta indica, Euphorbia heterophylla* and *Tithonia diversifolia* possess antimicrobial activities against some common pathogenic microorganisms especially those that cause spoilage of fruits such as *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Propionibacterium acnes, Bordetella trematum, Staphylococcus epidermidis, Neurospora crassa, Fusarium avenaceum, Penicillum notatum, Aspergillus niger, Fusarium oxysporum* and *Rhizopus stolonifer.*

All the extracts had different degrees of antimicrobial activities on the organisms with the bark of *Azadirachta indica* having the highest activity against these organisms as it was able to limit the growth of all the bacteria at the different concentrations except *Acinetobacter ursingii* which did not inhibit its growth. This could be as a result of resistance of *Acinetobacter* spp. to almost all antimicrobial agents that are currently available, including the aminoglycosides, quinolones and broad-spectrum β-lactams as reported by Paton et al. [36].

The methanolic extracts of the leaf and bark of *Azadirachta indica* had high rate of inhibition on all the organisms especially *Staphylococcus aureus, Bacillus subtillis* and *Klebsiella pneumoniae* which were more susceptible to these extracts with increase in the concentrations. The antibacterial properties of *Azadirachta indica* leaves and bark reported in this study is in agreement with the report by Faiza et al. [37]. The extracts of *Azadirachta indica* were also found to be effective against the fungal isolates especially the bark of this plant

which completely inhibited the growth of *Aspergillus niger.* This supports the report from the study conducted by Upasana et al. [38], who found that neem seed extract in methanol was effective against *Aspergillus niger, Fusarium oxysporum* and *Trichoderma resii*.

Also, results from this study shows that methanol extract of *Euphorbia heterophylla* was effective against *Propionibacterium acnes* and *Staphylococcus aureus* at all concentrations used. The inhibition of growth of the test organisms by the extract was dose dependent with the lowest effective concentration being 30 mg/ml with no zones of inhibitions for both organisms. Ross et al. [19] had previously investigated antibacterial activity of the petroleum ether, butanolic and ethanolic extracts of the leaves of *Euphorbia heterophylla* against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis.* The butanolic extract showed a broad spectrum of antibacterial activity against the test organisms at concentrations of 100, 150 and 200 mg/ml. The petroleum ether and ethanolic extracts did not show any antibacterial activity against any of the test organisms. The inhibition of *Staphylococcus aureus* by *Euphorbia heterophylla* also confirms the previous work carried out by [39]. This, therefore, shows that the plant extracts contain compounds that can inhibit the growth of some microorganisms. *Staphylococcus aureus* was more susceptible to the methanol extract of *Tithonia diversifolia* having a zone of inhibition of 17.75b mm at 100 mg/ml. This confirms with the work carried out by Ogundare [40].

With the increase in concentration, the radial growth of fungi decreased. The observation in this study that *Tithonia diversifolia* has antifungal and antibacterial properties are supported by the work of Ogunfolakan [41] who concluded that the leaf extract had promising broad spectrum antimicrobial activity. These results revealed that the antimicrobial activity of the extracts was enhanced by increasing concentration; hence the inhibition activity of the extracts was concentration dependent. This is in agreement with the reports of Ilondu; Chiejina and Ukeh [42,43] that increase in the antifungal activity was observed with corresponding increase in the concentration of plant extracts.

Differences in the degree of preservation by the extracts on the fruits became noticeable by the fourth day after spraying on the fruits, when the fruits started showing signs of spoilage which increased slowly until day eight as compared with the control that decayed more rapidly. This was not the case for watermelon as all the extracts sprayed were able to preserve the fruit for eight days as compared with the control that began spoiling rapidly from day four.

## 4. CONCLUSION

The methanol extracts of *Azadirachta indica, Euphorbia heterophylla* and *Tithonia diversifolia* exhibited a varying degree of activities against spoilage organisms. The leaf of *Tithonia diversifolia* and the bark and leaf of *Azadirachta indica* had the highest inhibitory property on the various spoilage organisms with an increase in concentration as compared the methanolic extracts of the leaves of *Euphorbia heterophylla.*

Fungi and bacteria cause enormous problems in the plant production industry and inadequate control can lead to serious problems in food production. Existing control measures are not enough to deal with emergence or outbreaks of plant fungal and bacterial pathogens. Antimicrobial extracts are proven to not significantly alter the taste, acceptability and perception of the consumer public. Another advantage of antimicrobial extracts is being totally harmless to the environment. It can be considered as a green alternative to synthetic antimicrobials and other postharvest chemical treatments. While food safety is of overall importance, food quality demands by consumers mean that concentration of extracts to be applied to food systems is important from the organoleptic viewpoint as sensory qualities of food products as perceived by consumers are critical commercial factors. Spraying of these plant extracts can be used in preserving and prolonging the shelf-life of fruits.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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