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Antimicrobial Effects and Phytochemical Analysis of Jatropha curcas Bark and Root Extracts Bacteria Counts Isolated from Spoilt Okra (Abelmoschus esculentus)

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Abstract

The antimicrobial activity of ethanolic and aqueous extracts of *Jatropha curcas* was tested against some spoilage microorganisms associated with okra. The bacterial isolates were *Serretia marcenscens, Staphylococcus aureus and Micrococcus luteus* at concentration of 0.1%, 1.0%, 2.5%, 4.5%, 5.5%, 7.0%, 8.5%, 10.0% on the bacteria counts using food poison method. The results showed that both the ethanolic and the aqueous extracts were found effective in reducing but the ethanolic extract was more effective against the following bacterial isolates: *Serretia marcescens*, *Staphylococcus aureus*, *Micrococcus luteus*. They exhibited resistance to aqueous extract and were more susceptible to higher concentrations of 10% for the ethanolic extract. The ethanolic and aqueous extracts of this plant were analyzed phytochemically and tested against different microorganisms isolated from spoilt okra to know how effective this extracts were in reducing bacterial count obtained from spoilt okra for 8weeks at different concentration per week. Phytochemical analysis of *Jatropha curcas* root and back extracts showed the presence of many secondary metabolites including steroids, alkaloids, saponins, flavonoids, glycosides, tannins and phenols.

Keywords: Antimicrobial, Ethanolic, Aqueous, Metabolites, Extracts.

1. Introduction

A medicinal plant is any plant which one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical (Rajesh *et al*., 2008). Despite the fact that antibiotics are inhibitory to pathogenic microorganisms, Ajayi and Akintola (2010) reported that a large number of antibiotics that have been discovered are now useless in the treatment of infection due to selective toxicity apart from the problem of microbial resistance.

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In view of this and other problems associated with the use of antibiotics, scientists have reverted to the initial form of chemotherapy, which involves the use of natural plant and plant products, which are usually regarded as medicinal plants (Ncube *et al*., 2008).

Some of these plants metabolites possess important therapeutic properties which can have been utilized in the treatment of human and other animal diseases worldwide. For example, the roots of *Zanthoxyllus* species which were used for cleaning teeth and for the treatment of toothache, urinary problem and throat infection, contain chemical with anaesthetic and antimicrobial properties (Gill and Akinwumi, 1986; Oyagade *et al*., 1999). The use of herbal medicine could be attributed to its affordability, accessibility, availability and even distribution of health personnel between rural and urban areas. Various infections such as, typhoid and paratyphoid fever, malaria, diarrhoea, wound infection, just to mention but few, are treated by the use of varieties of herbal preparation by traditional medical practitioners in Nigeria (Olukoya *et al.,* 1993). *Jatropha* species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994). *Jatropha curcas* commonly called physic nut, purging nut or pig nut.

Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Oliver-Bever, 1986). Fagbenro-Beyioku (1998) investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999). *Jatropha* is known as the physic or purging nut for its use as purgative/laxative, and is widely known as medicine for treatment of a variety of ailments. Preparations of all parts of the plant, including seeds, leaves and bark, fresh or as a decoction, are used in traditional medicine and veterinary purposes (Duke, 2002).

Okra (*Abelmoschus esculentus*) one of the most popular vegetables in Nigeria, is a very nutritious crop, containing protein, dietary fibre, vitamins, minerals and unsaturated fatty acids. Its protein is of very high quality, with a high content of lysine, tryptophan and sulphur-containing amino acids (Pavlos *et al.,* 1991). It is a member of the mallow family and is locally known as “Atuur” in Tiv, “Ikpoho” in Idoma “Okuru” in Ibo “Ila” in Yoruba and “Kubewa” in Hausa. It also has a high content of unsaturated fatty acid e.g. linoleic acid, which makes okra seed oil desirable. The vitamin content of fruits and vegetables is nutritionally superior when compared to many cereals and leguminous crops.

The aims and objectives of this research were: (i) to isolate microorganism associated with spoilage of okra, (ii) to test for the effectiveness of root and stem extracts of *Jatropha curcas* plant on the count of microorganisms isolated from spoilt okra using bacterial count method.

2. Materials and Methods

2.1 Sterilization of material

Glasswares were washed and treated as described by Fawole and Oso (2004).

2.2 Collection of Plant Materials

Roots and barks of *Jatropha curcas* plant were collected in clean polythene bags. The root and the bark were washed with distilled water sliced/cut into pieces and air-dried. Fresh and healthy okra fruits were bought from Ipata market Ilorin, Kwara State. The fruit were collected in a clean sterile polythene bag. Moistened cotton wool was placed at the bottom of a sterile transparent rubber and the okra fruit were placed on the moistened cotton wool, covered and stored at room temperature for 7days for spoilage to occur. Changes in the physicochemical parameters were observed as the fruit deteriorated the fruit then was taken for laboratory analysis.

2.3 Preparation of Extracts

Aqueous, ethanolic of the roots and barks of the plant was prepared. The plant roots and bark were grounded with mortar and pestle.

2.4 Aqueous extract

The aqueous extracts were obtained by using an adaptation of the method described by Okigbo and Omodamiro (2006) and Okigbo and Mmeka (2008) was used with slight modifications.

2.5 Ethanolic extract

The method described by Okigbo and Omodamiro (2006) was also employed.

2.6 Preparation of extract for the antimicrobial test using bacteria counts method

The antimicrobial activity of the bark and root extracts of *Jatropha curcas p*lant using Food Poisoned Technique (Das *et al*., 2010).

2.7 Qualitative and Phytochemical screening of Jatropha extract

The crude extracts of cashew powders was subjected to phytochemical tests using standard procedures to identify the constituents present as described by Herbune (1973), Odebiyi and Sofowora (1978) and Tiwari *et al.* (2011). The methods were deployed in order to detect the presence of plant constituents such as steroids, glycosides, terpenoids, alkaloids, saponins, phenols, tannins and flavonoids in the plant's extract.

3. Results and Discussion

The cultural, morphological and biochemical characteristics of microorganisms isolated from spoilt okra are represented in Table 1. Altogether three bacteria species were identified- they include *Serretia marcesens, Staphylococcus aureus and Micrococcus luteus.*

Table 1: The colonial, cellular and biochemical characteristics of bacteria isolates

Colonial morphology						**Cellular morphology**						**Biochemical tests**												**Tentative identity**
																		Sugar utilization						
Surface	Optical characteristics	Elevation	Edge	Colour	Size	Cell arr.	Cell shape	motility	Grams reaction	Spores	Capsule	Citrate utilization	Catalyse	Oxidase	Coagulase	Indole	Methyl red	Glucose	Sucrose	Lactose	Starch hydrolysis	Oxygen relationship		
Smooth	Transparent	Raised	Lobate	Pale colour	Small	Chain	Rod	+	+	-	-	+	+	-	-	-	-	A&G	A&G	-	-	Fan		*Serretia marcescens*
Transparent	Opaque	Flat	Entire	Golden yellow	Small	Single	Rods	-	-	-	-	-	+	-	+	-	+	A	A	A	-	Ae		*Staphylococcus aureus*

Smooth	Opaque	Flat	Entire	Yellow	Small	Irregular cluster	Cocci	-	+	-	-	+	+	-	-	-	-	A	A & G	A& G	-	AR	*Micrococcus luteus*

- = Negative, + = Positive, AR= Acid formation from fermentation, Fan= Facultative anaerobes, A = Acid only, A and G = Acid and Gas production.

3.1 Phytochemical screening

The phytochemicals identified in *Jatropha curcas* bark and root were-Glycosides, Terpenoids, Saponins, Phenol, Alkaloid, Tannis and Flavonoids which are presented in Table 2.

Table2: Phytochemical screening of *Jatropha curcas* bark and root extracts.

Extract	**Saponin**	**Tannis**	**Alkaloid**	**Phenol**	**Flavonoid**	**Terpenoid**	**Glycoside**
Ethanolic Bark Extract	++	+	++	-	++	-	+
Aqueous Back Extract	++	+	+	-	+	-	-
Ethanolic Root Extract	+	-	+	+	-	-	+

Aqueous							
Root	+	-	+	-	+	-	+
Extract							

KEY: + = Slightly present, ++ = Present, - = Absent**.**

3.2 Antimicrobial test on bacterial counts

The result of changes in the total bacterial counts of spoilt okra with different concentrations of aqueous and ethanolic extract of *Jatropha curcas* for week one is depicted in Figure 1.The antimicrobial activity of the aqueous bark extract on the bacterial counts against *Serretia marcenscens, Staphylococcus aureus and Micrococcus luteus* for week one was less effective due to the low concentration of the extracts. As the concentration increased from week 2 to week 5 as shown in Fig 2-5, the bacterial counts reduced. At week 6, there was a slight increase in the bacterial counts for the test organisms. The antimicrobial activity of the aqueous bark and root extracts was found to reduce bacterial counts from week 2 to week 5. There was a slight increase in the bacterial counts on the 6^{th}, 7^{th} and 8^{th} week.

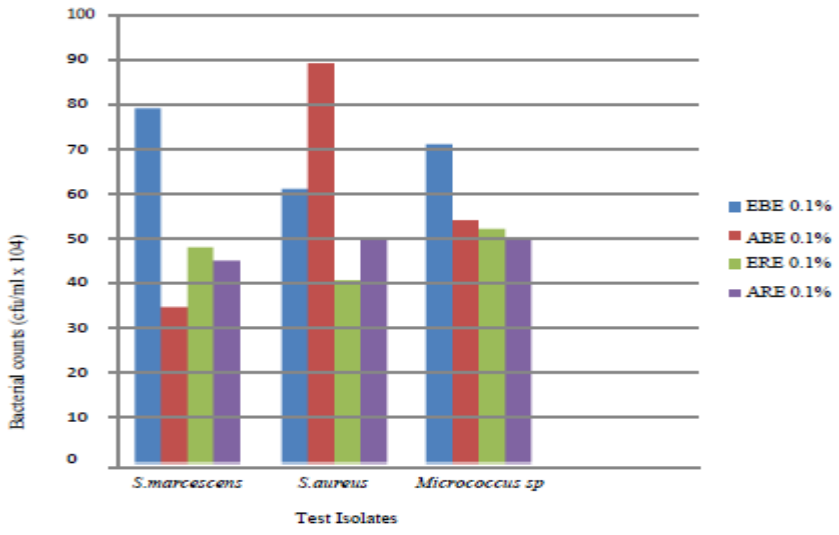

Figure 1: The antimicrobial actions of different concentration of extracts on microorganisms isolated from spoilt okra for first week.

The antimicrobial activity of the aqueous bark extract on the bacterial counts against *Serretia marcenscens, Staphylococcus aureus and Micrococcus luteus* for week one was less effective due to the low concentration of the extracts. As the concentration increased from week 2 to week 5 as shown in Fig 2-5, the bacterial counts reduced.

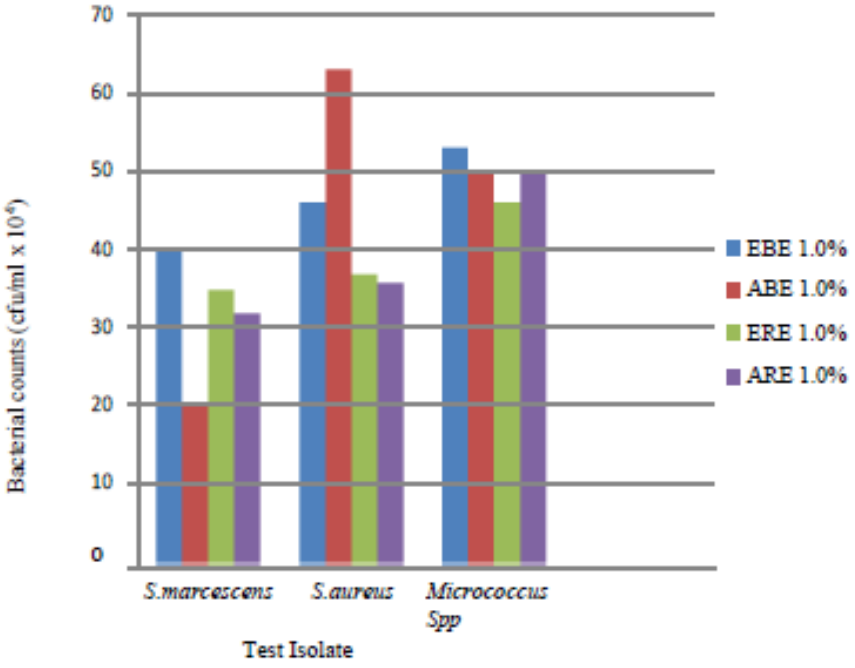

Figure 2: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for second week.

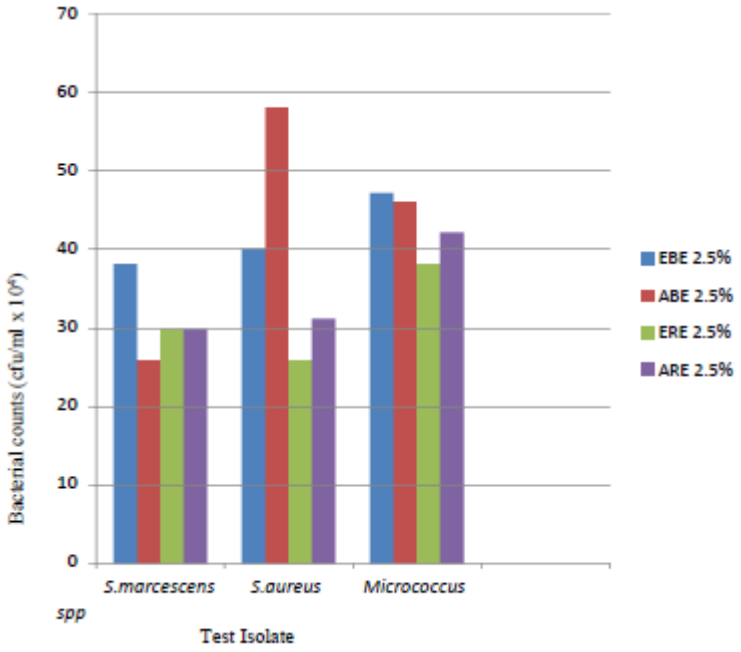

Figure 3: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for third week.

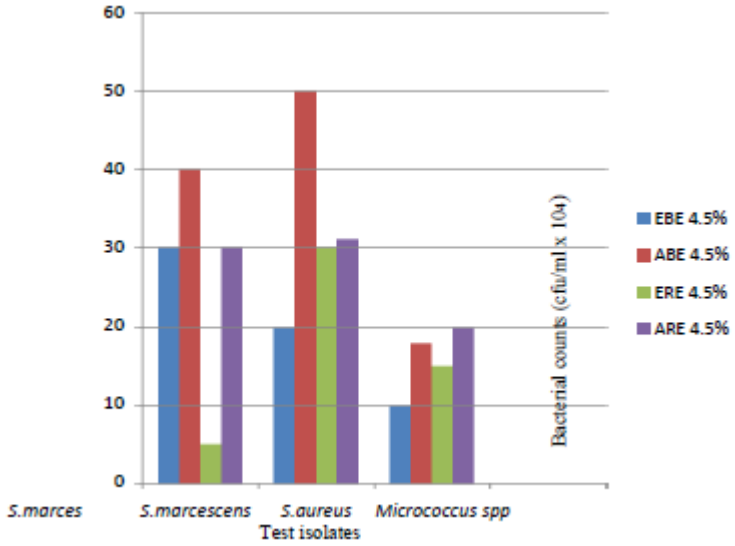

Figure 4: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for fourth week.

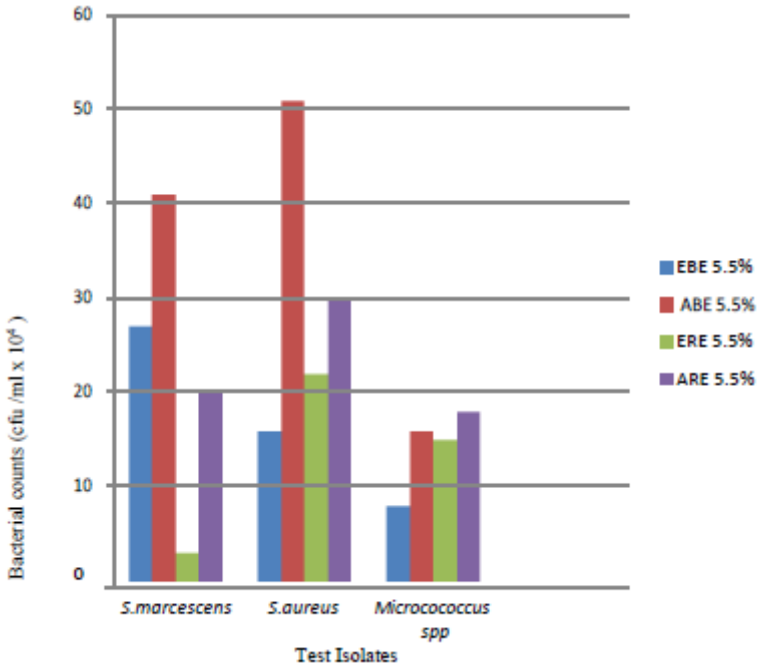

Figure 5: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for fifth week.

At week 6, there was a slight increase in the bacterial counts for the test organisms. The antimicrobial activity of the aqueous bark and root extracts was found to reduce bacterial counts from week 2 to week 5. There was a slight increase in the bacterial counts on the 6^{th}, 7^{th} and 8^{th} week. The antimicrobial activity of the ethanolic bark extracts showed a reduction of bacterial counts from the 1^{st} to 8^{th} week on the test organisms. The antimicrobial activity of the ethanolic root extracts showed a reduction of bacterial count from the 1^{st} to 8^{th} week on the test organisms. This showed the effectiveness of the ethanolic extracts was higher than that of the aqueous extract. The antimicrobial activity on microbial counts is shown in Figure 1-8.

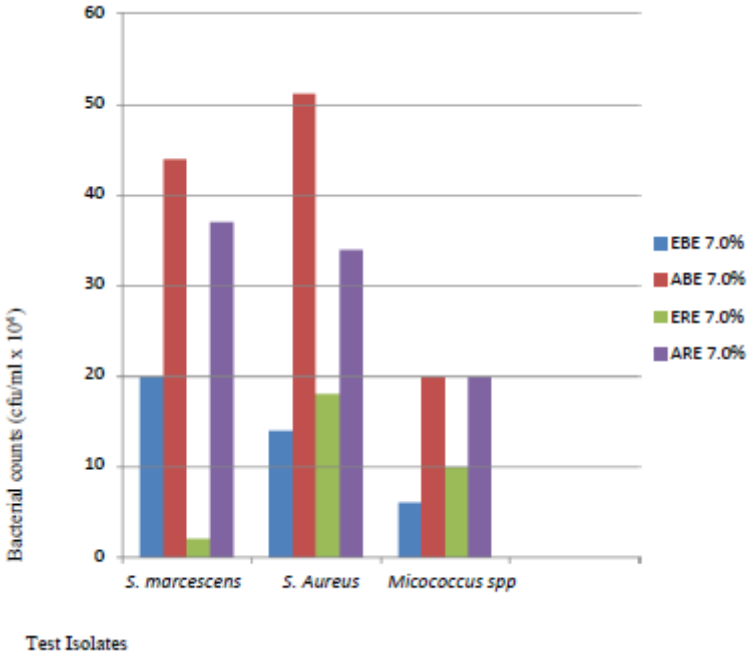

Figure 6: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for sixth week.

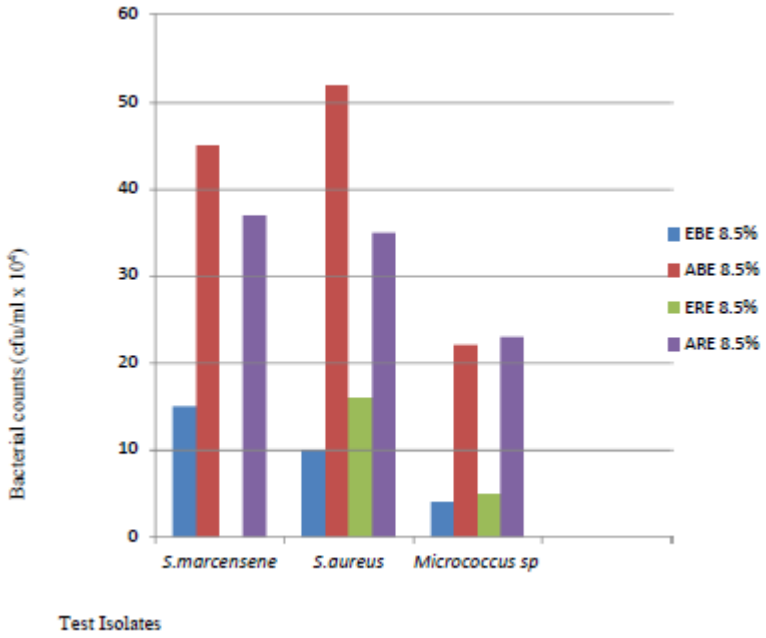

Figure 7: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for seventh week.

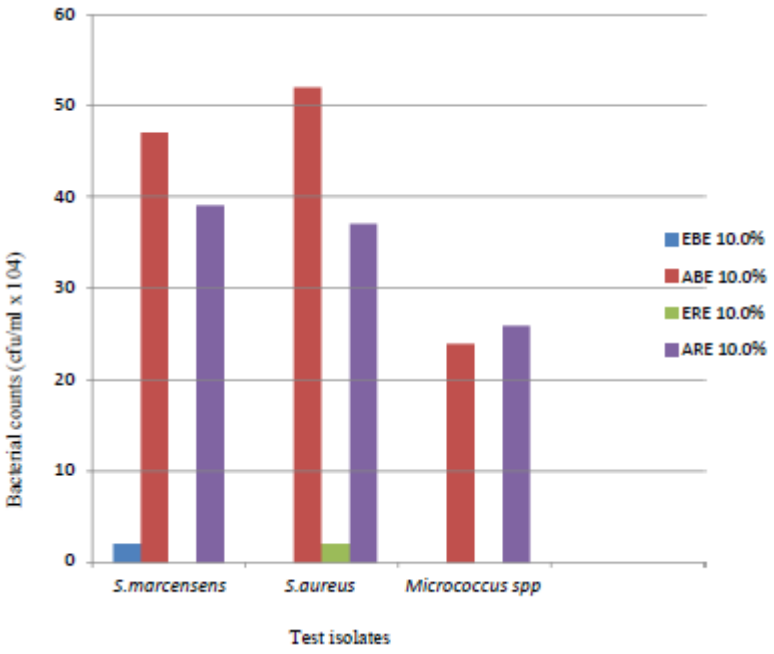

Figure 8: The antimicrobial actions of different extract concentration on microorganisms isolated from spoilt Okra for eighth week.

KEY: EBE = Ethanolic Bark Extract, ABE = Aqueous Bark Extract, ERE = Ethanolic Root Extract, ARE = Aqueous Root Extract.

3.3 Discussion

In the course of this research, three bacteria were isolated from the spoilt okra which included Serretia marcesens, Staphylococcus aureus and Micrococus luteus. All bacteria isolates were pathogenic to the okra causing soft rot and decaying of the fruit. The ethanolic extract was found to be more effective than the aqueous extract due to its greater extractive power. Additionally, ethanol was found to penetrate the cellular membrane quickly to extract the intracellular ingredients from the plant material, while water was found a better medium for the occurrence of the micro-organisms as compared to ethanol, this finding was reported by Tiwari *et al.* (2011).

From the 1st to 6th week, the ethanolic bark extract was found to reduce the bacterial counts at 0.1% to 7.0% concentrations, this might be due to the acclimatization of the microorganism to the presence of the extract in their environment. At the 7thand the 8th

weeks there was drastic reduction in the microbial counts for *Serratia marcescens* from 1.5 X 10^5CFU/ml to 3 X 10^4CFU/ml. At 10% concentration, there was drastic reduction in microbial growth with no growth of *Staphylococcus aureus* and *Micrococcus luteus*. For the aqueous bark extract from the 1st to 5th week, it was observed to be effective from 0.1 to 5.5% concentration and the bacterial counts were found to reduce. For *Serretia marcescens*, there was a reduction from 3.5 X 10^5 to 2.0 X 10^5 CFU/ml. For *Staphylococcus aureus*, there was a reduction from 5.0 X 10^5 to 3.0 X 10^5 CFU/ml while for *Micrococcus* spp., there was a reduction from 5.6 to1.8 X10^5 CFU/ml.

From the 1st to 8th week, the ethanolic root extract was found to be highly effective against the test organisms from 0.1-10% concentration by reducing the microbial counts drastically. At 10% concentration, there was no count of *Serretia marcescens* and *Micrococcus* spp. This might be due to the effectiveness of ethanolic root extracts. This concurs to the findings of Mahadevappa *et al*. (2011) that *Jatropha curcas* root was effective against some urinary diseases caused by *Serretia marcescens.*

From the 1st to 5th week, the aqueous root extract was observed to be effective from 0.1 to 4.0% concentrations against the test organisms (*Serretia marcescens, Staphylococcus aureus* and *Micrococcus luteus*). There was an increase in the bacterial counts from 5.5 to 8.5 % concentrations, and at 10% concentration there was a slight decrease which could be due to the increase in the activity of the extract as its concentration increased. Just *et al*. (1998) reported that saponins exhibited anti-inflammatory activity. While phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitated the entry of toxic material or leakage of vital constituents from the cell (Onwuliri *et al*., 2005). Dathak and Iwu, (1991) found out that flavonoids inhibited the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins. Lipophilic flavonoids have been found to disrupt cell wall integrity (Kurtz *et al.*, 1994) or microbial membranes (Tsuchiya *et al*., 1996) at low concentrations.

The contribution of this research to the advancement of knowledge is that *Jatropha curcas* plant extract can be used in the treatment of bacterial and fungal infections. The use of *Jatropha curcas* plant extracts on diseased okra fruit will be a prospective method in control

of spoilage of tomato fruits and can serve as a means in preserving the shelf life of this important fruit crop. This approach to plant disease management will be economical, cost effective and with less environmental risk, as phytochemicals have little or no residual effect compared to synthetic fungicides. The results obtained in this research work agrees to the results obtain by Jackie et al.,2016 and Aiyelaagbe *et al*. (2007) which both studies shows the use of *Jatropha curcas* plant extracts have high efficacy and antimicrobial effect against isolated pathogenic microorganisms which includes *S. typhi*, *E. coli*, *K. pneumoniae*, *E. aerogenes*, and *L. monocytogenes.*

4. Conclusion

This research has revealed the potential of both aqueous and ethanolic extract of root and bark of *Jatropha curcas* in the reduction of microorganism isolated from spoilt okra fruit. The extracts, if further purified and the appropriate concentrations to be used safely are determined, they may be employed in the control of spoilage of vegetables and the prolongation of their shelf-life. This approach to plant disease management will be economical, cost effective and with less environmental risk, as phytochemicals have little or no residual effect compared to synthetic fungicides. They might serve the desired purpose with lesser side effects that are associated with synthetic antimicrobials.

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