Antibacterial Potential of Orange Peels Extracts Against Some Bacterial Isolates Associated With Tomato Rot in Jos Metropolis.

[1]*Pandukur, S.G,* [2]*Onyimba, LA,* [3]*Itelima, J.U,*[4]*Plangnan, G.A and*[5]*Dum, V.*

[1,2,4&5]*Department of Science Laboratory Technology and Department of Plant Science and Technology, University of Jos, Nigeria. P.M.B 2084, Jos Nigeria*

Abstract: Study was carried out to determine the antibacterial potential of orange peels extracts against some isolated bacteria associated with tomato rot in Jos. Rotten ripe tomato fruits were aseptically collected from Terminus and Farin-gada markets of Jos metropolis. The plant extract was obtained from dried orange peels by Soxhlet extraction using n-hexane. Agar well diffusion method was used for the antibacterial susceptibility assay and accompanied by Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) determination. The result showed the presence of seven bacterial *(Klebsiella* sp., *Pseudomonas* sp., *Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Erwina* sp. and *Shigella* sp.) isolates with *Klebsiella* sp. having the highest percentage of occurrence (22%) while *Staphylococcus areushad* the lowest percentage occurrence (5%). The result of the susceptibility of the antibacterial test showed *Klebsiella* sp. had the maximum zone of inhibition (8mm) at the concentration of 200mg/ml while *Proteus* sp.showed no zone of inhibition at the same concentration. The differences in the zones of inhibition by the different extract concentrations and the control on the isolates were statistically significant at P= 0.05 across the concentrations on the isolates as compared to the control. The Minimum Inhibitory Concentration values showed that the extract had activity against *Klebsiella* sp. and *Shigella* sp.at 100 mg/ml and 200mg/ml respectively while only *Klebsiella* sp showed a Minimum Bacteriocidal Concentration of 200mg/ml. The result showed that orange peel extract was active but not effective against the tomato spoilage bacteria. Therefore, waste portions of the citrus fruits' peels could be a promising source of antimicrobial variables.

Keywords: Antibacterial potential, Orange peels, tomato rot, Extract.

Introduction

Tomatoes are among the vegetable products cultivated worldwide that has the greatest nutritional value. World tomato production in 2001 was about 105 million tons of fresh fruit from an estimated 3.9 million hectares (FAO, 2005). In Nigeria, tomato accounts for about 18% of the average daily consumption of vegetables (Babalola *et al.,* 2010). This makes it a very important food crop to an average Nigerian, with about 1,107,000 tons of tomato produced in 2008, making Nigeria become the 13th largest producer of tomato in the world that year (FAO, 2010). Despite its importance, tomato fruits have been faced with diseases causing rots which lead to loss of quality and also substantial postharvest loss (Aworth, 1985; Uzeh *et al.,* 2009). There are many disease pest of tomato including bacteria, fungi and many viruses. The bacterial group (*Pseudomonas solanacearum*) is a soil borne bacterium which infects the root and stem of the plant causing sudden disease. During harvest, transportation and storage the biological structure of tomato fruit could be disrupted, serving as a route of entry for opportunistic pathogens. High water level of this fruit provides a conducive environment for these pathogens, reducing its palatability with increased toxicity levels. These changes may be accompanied by alteration in its tastes, smell, appearance or texture (Ugwu *et al.,* 2010).

*Corresponding author:

psgpan1@yahoo.com; Pandukur, S.G

This phenomenon usually referred to as spoilage in tomato. Tomato spoilage would therefore be simply defined as those adverse changes in quality of tomatoes, which are brought about by action of predominantly biological factors and physical factors. Research efforts over the years have helped in the increase in production of tomato but the purpose of obtaining maximum profit will be served only if the increased production is supplemented with the similar efforts to minimize the postharvest losses and enhance shelf life. This has made their microbiological study necessary so as to identify their spoilage organisms so as to find suitable means to minimize post-harvest losses (Frederick, 1983). Various spoilage management methods have been introduced, from field management methods to post harvest methods. High nitrogen in plant tissues generally increases susceptibility to decay, whereas high calcium content reduces postharvest decay on several crops (Conway, 1984, 1989; Janisiewicz *et al.,* 2002). Removing dead and decaying plant matter and other organic material from the crop plant and soil surface will eliminate a major harborage for spoilage microbes as well as other crop pests.

Orange is rich in nutrients, and the peels contain many phytochemicals. *Citrus* essential oils contain large amounts of terpens, aliphatic sesquiterpene, oxygenated derivatives and aromatic hydrocarbons (Merle *et al.,* 2004). The composition of the terpenic mix varies, depending on the examined citrus species to which it owns. The mix of each species is in different proportion, made of limonene, α-pinene, β-pinene, myrcene, linalool and terpinen (Ahmad *et al.,* 2006). Besides, citrus peel essential oil has been

identified to exhibit antibacterial activity (Ayoola *et al.*, 2008; Palakawong *et al.*, 2010; Upadhyay *et al.*, 2010). Previous reports on the antimicrobial potency of peel essential oils of various citrus species against food spoilage bacteria suggest their utilization in food safety (Friedman *et al.*, 2002). Food industries continually researches to reduce the use of chemical preservatives in their products due to increasing pressure of consumers and legal authorities, to either completely remove or to adopt more natural alternatives for the maintenance or extension of product shelf life (Nychas and Tassou, 2000). Antimicrobial potency of peel essential oils of *Citrus senensis* against food spoilage bacteria would suggest their utilization in food safety (Friedman *et al.*, 2002). The exploitation of citrus peel essential oil as antibacterial agents could also provide substitution of the high cost antibiotics which have resulted in increased morbidity and mortality of pathogens. Since there is always an increased attention in bringing waste to wealth from plant products and materials, orange wastes are no exceptions. Suitable methods have to be adopted to utilize them for the conversion into value-added products (Nand, 1998). This study intents to determine the antibacterial activity of orange peel extract on some bacterial causing tomato rot.

Materials and Methods.

Collection of Samples

1500g of the peels of citrus fruit collected from Jos metropolis were cut into small pieces then air dried at room temperature and pounded in a wooden mortar to a coarse form. It was then further pulverized in a blender into powder which yielded 1000g. Spoilt tomatoes (50 each) were picked up respectively from two selected market of Jos; namely, Farin-gada and terminus and placed in separate sterile plastic bags transported to the Pharmacognosis laboratory in the University of Jos for bacterial analysis.

Extraction of Orange Peels Extracts Using Soxhlet extraction using n-Hexane.

The plant extract was determined by the methods of Harbone, (2005).

Determination of Phytochemicals:

Qualitative phytochemical analysis is the process of detecting the bioactive principle present in the extracts with the use of standard phytochemical methods (Edeoga *et al.*, 2005). Preliminary phytochemical studies to determine the presence of phytochemicals was carried out using the method described by Harborne (2005) and Jigna *et al.*, (2005).

Preparation of Plant Extracts Concentrations.

The n-Hexane extract was dissolved in about 10ml of Tween-80, which is a universal solvent with 1g of the dissolved extract further dissolved in 5ml of distilled water to gain a known concentration of 200mg/ml as a standard concentration of aqueous n-Hexane extract which served as stock solution. Different concentrations of extract were prepared such as 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml using double fold dilution method of the prepared stock solution of 200mg/ml concentration.

Method of Isolation

Pour plate method of Bauer (1996) and Onyeagba A. (2004) was adopted to isolate the bacterial species. Samples were washed in sterile water to reduce the microbial load after which sterile forceps were used to pull apart the tomatoes at the site of spoilage, 1g of the monocarp from the advancing margin of the scar were each aseptically transferred into 10mls of sterile normal saline into various beakers according to the number of sample collected. They were allowed to stand in the normal saline for 10 minutes, before being removed with the sterile forceps from the beakers. A ten-fold serial dilution was adopted. Subsequently, 0.1 ml of 10^{-6}(the appropriate dilution) was plated on nutrient agar (pH 5.5) and Mckonkey agar for bacterial growth and enumeration

Preparation of Pure-Culture

For pure culture, distinct colonies were aseptically transferred into newly prepared sterile Nutrient Agar for bacteria by streaking, using sterile wire loop, which was later transferred into sterile Agar slants in Bijou bottles, where the culture was preserved. Samples for identification tests were taken from the agar slants.

Characterization and Identification of Bacterial Isolates.

Bacterial Isolates were characterized and identified using the method of (Cheesbrough; 2006) by staining method, macroscopic examination and biochemical tests. These tests, includes, gram staining, indole test, catalose test, motility test, citrate utilization test, oxidase test, starch hydrolysis test and sugar fermentation tests.

Screening of Orange Peel Extract for Antimicrobial Activity

Agar well diffusion method

Pour plate method of Bauer (1996) was adopted. Holes of 6 mm in diameter were made in the seeded agar using a 6mm cork borer. A drop from each concentrations of extract (about 20 µl) was added into each well using a micropipette on the seeded medium and allowed to stand on the bench for 1h for proper diffusion and thereafter incubated at 37^{0}C for 24h. The resulting inhibition zones were measured in millimeters (mm) using a transparent ruler and values were tabulated.

Determination of Minimum Inhibitory Concentration (MIC)

Determination of Minimum Inhibitory Concentration was done by method of Ibekwe *et al.*, (2001). Eight (8) test tubes were prepared by dispensing 2ml of Peptone water into each tube. 2ml from the stock solution of tested extracts (concentration of 200 mg/ml) was added into the first tube. Then, doubling dilutions were performed by using a sterile needle and syringe. This was repeated up to the sixth (6) test tubes, from which 2ml was taken and discarded into a disinfectant jar. The seventh and eight tubes serve as positive and negative control tubes. The obtained concentration range was from (200 - 6.25) mg/ml, and then 0.2ml of inoculums were added to each tube except a positive control. The orange peel extract with media was used as a positive control and inoculums with media were used as a negative control. The test tubes were incubated at 37^0C for 24h. They were then observed for turbidity, tubes with turbidity indicated growth. MIC was defined as the lowest sample concentration showing no growth (clear) after a period of 24h incubation.

Statistical Analysis

Data was analyzed using R Console Version 3.2.2. Student t-test was used to compare the mean colony forming unit in tomatoes between the two sites. Proportions of occurrence for each organism in relation to collection sites were compared using Pearson's Chi-square test. Also, chi square test was used to compare the proportion of occurrence across organisms. The P-values < 0.05 were considered statistically significant.

Results

The result of the preliminary phytochemical investigation of n-hexane extract of orange peel showed that Steroids were of greatest concentration (+++) in the n- hexane orange peel extract while flavonoids and cardiac glycoside were of moderate concentrations (++) with the least concentration of carbohydrate which appeared only in traces. Alkaloids, Anthraquinones, tannins and saponins were absent in the n- hexane extract of Orange peel extract is shown in table 1.

The result of the bacterial count in the culture of the spoilt tomato samples from each market revealed that the mean bacteria count of spoiled tomato sold in Terminus market of Jos metropolis had a higher mean count than that from Farin-gada market, however, statistically there was no significant difference between bacterial loads of the two markets as clearly shown and presented in table 2.

Table 1: Phytochemical Analysis Of Orange Peel Extracts

SECONDARY METABOLITES	REAGENT	OBSERVATION	RESULT
Alkaloids	Meyer	No precipitation	(-)
Anthraquinones	Borntragers test	No colour change on layer	(-)
Carbohydrate	Molisch	Violet ring	(+)
Cardiac glycoside	Keller Killiani test	Brown ring	(++)
Flavonoids	Acetone/NaoH	Yellow solution	(++)
Saponin	H_2O	No foam	(-)
Steroids	Liebermann-burchard	Blue solution	(+++)
Tannins	$FeCl_3$	No colour change	(-)

Key:
(+) = Present in trace amount
(++) = Present in moderate amount/ concentration
(+++) = Present in high amount/ concentration
(-) = Absent

Table 2: Bacterial count from the Terminus and Farin-gada market sites using Mckonkey agar (MCA)

MEDIA	DILUTIONS	CFU/ml Count For Markets	
		Terminus	Faringada
MCKONKEY AGAR	10-1	1.0×10^3	8.7×10^2
	10-2	6.0×10^3	6.6×10^3
	10-3	4.6×10^4	4.0×10^4
	10-4	3.4×10^5	2.5×10^5
	10-5	1.8×10^6	1.4×10^6
	10-6	9.0×10^6	6.0×10^6
MEAN ± S.E		**$(1.87 \pm 1.45) \times 10^6$**	**$(1.28 \pm 9.69) \times 10^6$**

Table 3: Macroscopic, Microscopic and Biochemical Identification and Characterization of Isolates With Their Individual Frequency of Occurrences

ORGANISM IDENTIFIED	MACROSCOPIC EXAMINATION		MICROSCOPIC (Gram Stain)		BIOCHEMICAL IDENTIFICATION AND CHARACTERIZATION														Frequency of occurrence/ percentage
	OPACITY	COLOUR	Shape	Gram rxn	Catalase	Coagulase	Indole	Urease	Citrate	Oxidase	Motility	Glucose	Sucrose	Lactose	Slope	Butt	Gass	H_2S	
Klebsiella sp	Opaque	Creamy white	ROD	-	+	-	-	+	+	-	+	+	-	-	Y	Y	-	-	28(22%)
Shigella sp	Transparent	Pale	ROD	-	+	ND	-	-	-	-	-	+	-	-	R	Y	-	-	25(19%)
*Erwina*sp	Opaque	Milky	ROD	-	+	ND	+		-	-	+	+		-	Y	Y	+	+	22(17%)
*Salmonella*sp	Transparent	CREAMY WHITE	ROD	-	+	-	-	+	+	-	+	+	-	-	R	Y	-	+	19(15%)
Proteus sp	Transparent	Transparent	ROD	-	+	-	-	+	+	-	+	+	-	-	R	Y	-	-	16(12%)
Pseudomonas sp	Transparent	Creamy light green	ROD	-	+	-	+	+	+	+	+	+	+	+	R	R	-	-	12(9%)
Staphlococcus aureus	OPAQUE	GOLDEN YELLOW	COCCI	+	+	+	+	-	+	-	+	+	+	+	R	Y	-	-	7(5%)

Table 4: Susceptibility Test for Isolates Usjng Extract and a Control

Organism	Extract	Concentration (mg/ml)/Zone of inhibition(mm)							d_f	X^2	P_{value}
	HOP	200	100	50	25	12.5	CONTROL				
							+VE	-VE			
***Klebsiella* sp**		**8**	5	3	0	0	34	0	6	125.56	<0.001
***Erwina*sp**		**3**	0	0	0	0	29	0	6	153.94	<0.001
Shigella sp		**7**	5	0	0	0	37	0	6	157.14	<0.001
***Salmomella*sp**		**5**	3	0	0	0	34	0	6	156.33	<0.001
***Proteus*sp**		**0**	0	0	0	0	28	0	6	168	<0.001
***Pseudomonas*sp**		4	0	0	0	0	30	0	6	154.59	<0.001
S. aureus		3	0	0	0	0	32	0	6	171.6	<0.001

Key:
HOP = n-hexane Orange peel extract
Reference drug/ control = Ciprofloxacin (0.625mg/ml).

Table 4 shows the susceptibility rates of the isolated bacteria from spoilt tomatoes which were associated with the spoilage of fresh tomato using n- Hexane orange peel extract showing different zones of inhibition in relation to the different concentrations of the extract. *Klebsiella* spp showed the highest zone of inhibition (8mm) at a concentration of 200mg/ml while *Proteus* spp showed no zone of inhibition at the same concentration of 200mg/ml. Meanwhile, by statistics, the susceptibility rates across concentrations of the n-hexane extract of orange peel essential oil on each organism showed very high significant difference as compared with the control.

Table 5: Minimum inhibitory Concentration (MIC)

Organism	Extract	Concentration (mg/ml)/Zone of inhibition(mm)							M.I.C
	H.O.P	200	100	50	25	12.5	CONTROL		
							+VE	-VE	
***Klebsiella* sp**		-	-	+	NA	NA	+	-	100
***Erwina*sp**		-	+	NA	NA	NA	+	-	200
***Shigella* sp**		+	NA	NA	NA	NA	+	-	NA
***Salmomellas*p**		+	NA	NA	NA	NA	+	-	NA
***Proteus* sp**		+	NA	NA	NA	NA	+	-	NA
***Pseudomonas* sp**		+	NA	NA	NA	NA	+	-	NA
S. aureus		+	NA	NA	NA	NA	+	-	NA

Key:
HOP = n-hexane Orange peel extract
(-) = No growth
(+) = growth
NA = Not applicable
The MIC values in table 5 showed that the extract was active against *Klebsiella* sp at 100mg/ml and *Erwina*sp at 200mg/ml.

Discussion

Fresh fruits have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms. However, this protection may be eliminated and fruits may become contaminated during their growth in fields or during harvesting; post harvest handling and distribution from where these spoilage organisms get access into the fruits and cause spoilage. The primary causative agents of microbial post-harvest spoilage of tomatoes are the bacteria, yeasts and moulds (Aworth, 1985). Due to the high proliferation rate of bacteria, they are considered highly as a more frequent cause of spoilage of tomato. According to Bukar *et al.* (2010), it was reported that wounded tomato fruits were most liable to microbial infection, hence deterioration is inevitable. A cut on a broken tomato fruit may easily harbor pathogens that may spread and spoil all tomatoes in a lot. The bacterial count recorded for the two markets indicated a high level of spoilage bacterial contamination of the tomato fruit samples. The isolation of these spoilage bacteria due to contamination on the tomato samples was evident of opportunistic contamination from mostly human activity. The mean bacterial counts in the spoiled tomato fruits samples investigated in this study were similar to those obtained in other studies in Nigeria (Chukwuka, 2013, Uzeh*et al.*, 2009; Bukar *et al.*, 2010). The high microbial contamination observed in the fruits in this study may also be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Considering the notoriety of the resistance of *S. aureus* to methicillin, other Penicillin and Cephalosporins (Adeleke and Odelola, 1997), its detection in tomato samples poses a lot of health risk to nourishment seeking consumers even though it's occurrence in this present study within the two major markets of Jos metropolis was very low. Also the high bacteria count could arise from the fact that tomato sellers leave these fruits in the open under the sun where they are heated up and subjected to rotting due to the heat. The presence of seven bacteria isolates (*Klebsiella* sp, *Shigella* sp, *Erwina*sp, *Salmonella* sp, *Proteus* sp, *Pseudomonas* sp, *Staphlococcus aureus*) in this study have previously been isolated from tomato in other studies, both in Nigeria and elsewhere (Ashok *et al.*, 2013; Angela *et al.*,2010).

However this is contrary to the previous work of Duffy,(2003) whoisolated *Bacillus, Listeria, Morgnella, Xanthomonas* and *Lactic acid* bacteria from the spoilt tomatoes. This could be as a result of varietal characteristics, climate influence, geographical and seasonal variations and also various internal and external sources of contamination and agro-technical procedures which include the use of contaminated irrigation water, use of animal and human wastes as fertilizers, improper handling and storage (Effurvwevwere, 2000). Some of the bacteria isolated in this study may be part of the natural flora of tomato or contaminants, from soil, irrigation water, and the environment during transportation, washing/rinsing water or handling by processors (Ofor *et al.*, 2009). *Pseudomonas* sp. is part of the natural flora and is among the most common vegetable spoilage bacteria as well as *Erwina*sp which belongs to the family Enterobacteriaceae; are all associated with plants where they are known to caused plant diseases of the rot and wilt type. These gram negative rods that are related to the genera *Proteus, Serratia, Escherichia, Salmonella* and others and can be indicators of feacal contamination. There was no statistical difference between bacterial loads in the two market sites using the two growths. However, biologically the mean bacterial count in Terminus was higher than that of Faringada market for the both media. This could be due to the high activities of sellers going on in the market which is a mixed market for all commodities; while Faringada is mainly a vegetables market.

The antibacterial effect of the n-hexane orange peel extract on *Klebsiella* sp and highest resistance from *Proteus* sp.was similar to the work by Bukar *et al.*, (2010). Again the work of Obidi *et al.* (2013) stated that "orange oil had a more consistent antimicrobial activity when used along with organic solvent (methanol) than the neat extract (orange essential oil). These all explain the moderate effects of the essential oil of the orange peels on the bacterial isolates in this study.

Conclusion and Recommendation

Fruits and vegetables are very important and have high dietary and nutritional qualities. The importance of these fruits with its nutritional and other importance cannot be over emphasized, as its spoilage often result to wastage of economic resources as well as food poisoning. From the results obtained in this study, several genera of bacteria have been identified as being associated with the spoilage of tomato fruits as well as this spoilage organisms encountered are food borne pathogens. It is also revealed that some spoilage microorganism gained access into these fruits during the processes of cultivating, harvesting, grading and packing and environmental contaminant which have in one time or the other been involved in food poisoning. Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. Based on the antibacterial result in this study, it can be concluded that the waste portions of the citrus fruits' peels could be a promising good source for the eliminations of microorganisms in the environment. The extracts could be used as a drug after proper pharmacological evaluation and clinical trials. Based on the results gained from this study I recommend that concerted efforts should been made by the relevant health workers to discourage or stop the display and sale of spoilt tomato fruits in local markets. Higher

concentrations of extract should be used for further analysis on the antibacterial activity in other to gain a much effective dose to serve as a good substitution for synthetically produced drugs or control.

Future prospects of the current research would include further purification of the extract, further evaluations with the pure compounds for the definite conclusion of the bioactive compounds contributing to the antimicrobial activity, although the nature and number of active components involved in each extract are not clear, however they are promising. This finding can form the basis for further studies to prepare an optimize preparation of the herbal extract.

References

Adeleke, O.E and Odela, H.A. (1997). Plasmid profiles of multiple drug resistant local strain of *Staphylococcus aureus .American Journal of Medical Science*. 1997; 20 pp 111-121.

Ahmad, M.M., Rehman, S.U., Iqbal, Z., Anjumand, F.M., Sultan, J.I. (2006). Genetic variability to essential oil composition in four citrus fruit species. *Pakistant Journal Botany*, 38: 319-324.

Angela, O.E., Ibukunoluwa, A.O. and Oranusi, U. S. (2010) Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria.*African Journal of Food Science*. Vol 14. (5).

Ashok, K., Varun, B., Shikha, V., Gaurav, S. and Sushil, K. (2013): isolation and characterization of microorganisms responsible for different types of food spoilages. *International Journal of Research in Pure and Applied Microbiology*.

Aworth, O.C. (1985). Preservation of perishable food commodities through processing. *Nigerian Food Journal*, 2:2

Ayoola, G.A., Johnson, O.O., Adelowotan, T., Aibinu, E., Adenipekun, A.A., Bello, H.A.B. and Odugbemi, T.O. (2008). Evaluation of the chemical constituents and the antimicrobial activity of the volatile oil of *Citrus reticulate* fruit (tangerine fruit peel) from South West Nigeria. *Africann Journal of Biotechnology*. 7(13): 2227-2237.

Babalola, D.A., Makinde, Y.O., Omonona, B.T., Oyeanmi, M.O (2010). Determinant of post harvest losses in tomato production: A case study of Imoki-Afon local government area of Ogun State. *Journal of Life Physic and Science*. Acta SATECH 3(2): 14-15.

Bauer, A.W., Kirby W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic Susceptibilty testing by a Standardized Single Disk Method. *American Journal of ClinicalPathology*.45: 493-496.

Bukar, A., Uba, A. and Oyeyi, T.I. (2010). Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready - to - eat foods in Kano metropolis, Nigeria. *African Journal of Food Science*. 4(2): 032-036

Chessbrough, M. (2006). District laboratory practice in tropical countries. (2nd Edition) updates Cambridge University press, the water club, Beach Road Grager bay, Cape Town. 8005, South Africa, pp.: 38-39, 62-70; 178-306.

Chukwuka, M. (2013). Isolaion and enumeration of Microorganism associated with spoiled tomato sold in Abakaliki, Ebonyi State. Retrieved from www.doublegist.com/ Isolaion and enumeration of Micro organism associated with spoiled tomato sold in Abakaliki

Conway, W. S. (1984). Preharvest factors affecting postharvest losses from disease. In H. E.Moline (ed.) *Postharvest pathology of fruits and vegetables: Postharvest losses in perishable crops*. (pp. 11–16). University of California Agricultural Experiment Station, Bull. No. 1914 (Pub.NE-87).

Conway, W. S. (1989). Altering nutritional factors after harvest to enhance resistance to postharvest disease. *Phytopathology*, 79, 1384–1387.

Edeoga, H.O., Okwu, D.E. and Mbaeble, B.O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. *Afr. J. of Biotech*, 4: 685-688.

Effurvwevwere, B.J. (2000). Microbial spoilage agents of tropical and assorted Fruits and Vegetables. (AN illustrated References Book). Paragraphic publishing company, Port Harcurt, pp: m1-39.

FAO, (2005). The state of food and agriculture. Food and Agricultural Organisation of the United Nations, Rome, Italy. 2005, FAO Agricultural series.

FAO, (2010). Global hunger declining but unacceptably high: International hunger targets difficult to reach. Economic and social development department, September, 2010. Rome, FAO. Rome.

Frederick, C.R. (1983). Introduction to Microbiology, Charles E. Merril, Columbus, Ohio U.S.A., Pp 511-514.

Friedman, M., Henika, R.P. and Mandrell, E.R. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campilobacterjejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. *Journal of Food Protection*, 65: 1545-1560.

Harborne, J. B. (2005). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis Chapman A & Hall, London; 1973-279.

Ibekwe, V.I., Nnanyere, N.F and Akujobi, C.O. (2001). Studies on antibacterial activity and phytochemical qualities of extracts of orange peels. *International Journal of Environmental Health and Humanity Development*, 2: 41-46.

Janisiewicz, W. J. and Korsten, L. (2002). Biological control of postharvest diseases of fruits.*Annual Review of Phytopathology*, 40, 411 441.

Jigna, P., Darshana, J. and Sumitra, A. (2007). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology* 29: 203-210.

Merle, H., Morn, M., Blázquez, M.A. and Boira, H. (2004). Taxonomical contribution of essential oils in Mandarins cultivars. Biochemistry Systematic Ecology, 32: 491-497.

Nand, K. (1998). Recent advances in the treatment of liquid and solid wastes of food processing.

Nychas, G.J.E. and Tassou, C.C. (2000). Traditional preservative - oils and spices. In : Robinson RK, Batt CA, Patel PD (Ed). Encyclopedia of Food Microbiology Volume.2.Academy Press. London.

Obidi, O. F., Adelowotan, A. O., Ayoola, G. A., Johnson, O. O., Hassan, M. O. and Nwachukwu, S., C. U. (2013). Antimicrobial activity of orange oil on selected pathogens.

Ofor, M.O., Okorie, V.C., Ibeawuchi II., Ihejirika, G.O., Obilo, O.P., Dialoke, S.A. (2009). microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. *Life Science Journal* 1:80-82.

Onyeagba A. (2004). Labouratory guide for microbiology. Crystal Publishers, Okigwe, Imo State pp 95-97.

Palakawong, C., Sophanodora, P., Pisuchpen, S. and Phongpaichit, S. (2010). Antioxidant and antimicrobial activities of crude extracts from mangosteen (*Garciniamangostana*L.)parts and some essential oils. *International Food Research Journal*, 17: 583-589.

Ugwu, O., Chukwuezi, F. O. and Ozougwu, V.E.O. (2010). Microbial Agents of Tomato Spoilage in Onitsha Metropolis, *Advances in Biological Research*. 8 (2): 87-93,

Upadhyay, R.K., Dwivedi, P., Ahmad, S. (2010). Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian Journal Medical Science*, 2: 152-158.

Uzeh, R.E., Alade, F.A. and Bankolé, M. (2009). The microbial quality of pre-packed mixed vegetable salad in some retail outlets in Lagos, Nigeria. *Afr. J. Food Sci.* 3 (9): 270-272.