Quest Journals
Journal of Research in Pharmaceutical Science
Volume 10 ~ Issue 5 (2024) pp: 57-63
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Effects of Polynesian Arrow Root Leaf and Stem Extracts on Shelf-Life of Fresh Pepper

JOSEPH UAA JULUKU, INNOCENT OKONKWO OGBONNA*, GRACE MWUESE GBERIKON
Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi.
**Corresponding author*

Abstract
Pepper belongs to the family of solanacease, produced and consumed in many countries and is prone to microbial spoilage. The aim of this study is to assess the effect of Tacca leontopetaloidesleaf and stem extracts on shelf-life of fresh pepper fruits. Microorganisms were isolated and identified using standard microbiological techniques. Extraction of Tacca leontopetaloides leaf and the stem were done using distilled water, ethanol and n-hexane as solvents. The extracts were assayed for phytochemical and antimicrobial activities. Shelf life study of pepper treated with the extracts was performed with untreated as control. The bacterial genera isolated were Staphylococcus, Bacillus, Escherichia, Proteus, Klebsiella and Shigella while the predominant fungal isolates were Aspergillus, Penicillium, Mucor and Saccharomycesspp. Phytochemical activities showed that alkaloids and cardiac glycosides were present in both leaf and stem using all the solvents whereas phenol and quinones were absent. Pepper samples treated with extracts of Taccaleontopetaloides showed extension of shelf life in the range of hexanehigher than ethanol, and ethanol higher than aqueous. The studyconcludes that fresh pepper susceptible to microbial attack can be inhibited most withn-hexane extracts of Taccaleontopetaloides.
Keywords: *leaf and stem extracts, pepper, phytochemical activity, shelf-life, Tacca leontopetaloides.*

***R**eceived 05 May, 2024; **R**evised 15 May, 2024; **A**ccepted 17 May, 2024 © The author(s) 2024.*
P**ublished with open access at **www.questjournals.org

I. INTRODUCTION

Fresh pepper is easily deteriorated by microorganisms. This is because fresh pepper contains high moisture content in addition to other nutrients needed for microbial growth. After harvesting, the sensorial and nutritional quality of fresh pepper from origin begins to decline because of the pepper deterioration and microbial growth [1]. Pepper is prone to deterioration rapidly, they have a very short shelf life due to their high moisture content, in addition to other nutritional ingredients [2].

Use of chemicals in the preservation of foods goes with its attendant implications. In view of the health and environmental effects and high cost of chemicals in the control of spoilage by microorganisms and other environmental factors that are contributing to the deterioration of the fresh pepper, many researchers are seeking alternative methods to put the menace under control. Bio-preservation is hereby proposed with *Tacca leontopetaloides*as the bio-control agent.

This study is set to assess the effect of extracts of *Tacca leontopetaloides* (polynessian arrow root) leaf and stem extracts the control of microbial biodegradation of fresh pepper in Benue State, Nigeria.Microbial deterioration is by far the most important reason for food insecurity in developing countries of the world like Nigeria. Microbial deterioration produces food loss due to microbial spoilage before and after harvest which is the major reason for food scarcity and calls for concern. Control of food spoilage using the conventional physical and chemical methods have not yielded the desired fruits. There is a need to seek alternative and environmentally friendly methods of controlling microbial deterioration of pepper.The aim of the present work is to study the effects of *Tacca leontopetaloides* (Polynesian arrow root) leaf and stem extracts on shelf-life of fresh pepper.

II. MATERIALS AND METHODS

The study was carried out in Makurdi the capital of Benue State, located in central Nigeria and part of the middle belt region of the central Nigeria. The city is situated on the south bank of the Benue River. In 2016, Makurdi and surrounding areas had an estimated population of 365,000. The major ethnic groups in Makurdi are Tiv, Idoma, lgede, Jukum, Hausa and Etulo. Benue state is predominantly an agricultural area specializing in cash crops and subsistence crops. Fig. 1 is map of Benue state showing the study area.

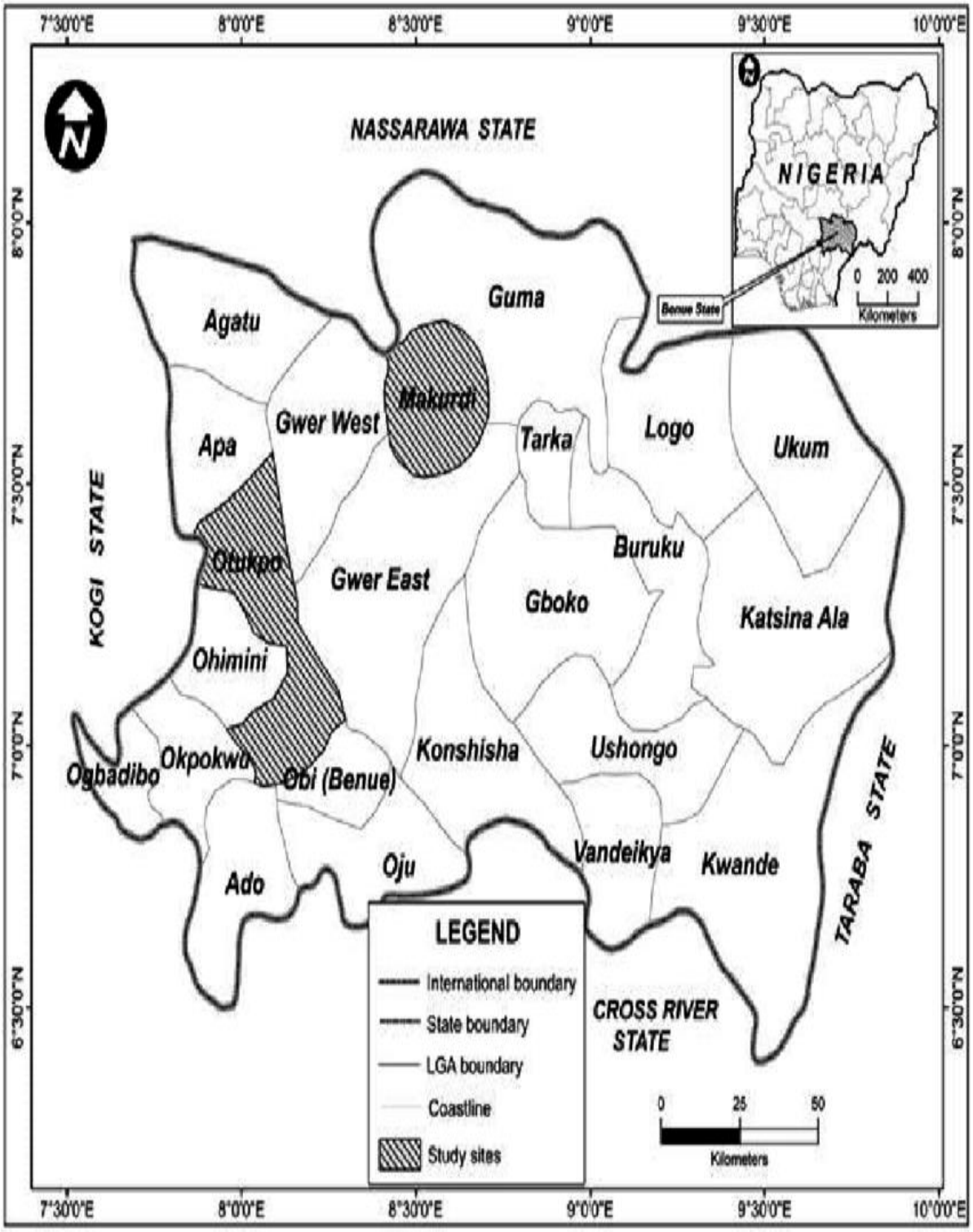

Fig1:Map of Benue state showing the study area

2.1 Collection of pepper samples and identification

A total of 100 fresh samples of pepper were purchased randomly from Abeda and Ugba areas of logo local government, the samples were purchased from pepper producing areas and also from the vendors within the locations. The samples were aseptically collected using sterile polythene bags and transported to Botany Department for identification after which they were taken to microbiology laboratory in Microbiology Department, Joseph Sarwuan Tarka University, Makurdi for analysis.

2.2 Collection of plant materials

The leaf and the stem samples of *Tacca leontopetaloides* were aseptically collected from Adikpo in Kwande local government area of Benue state using sterile polythene bags and transported to Botany Department for identification before being taken to microbiology laboratory in Microbiology Department Joseph Sarwuan Tarka University, Makurdi for analysis.

2.3 Sample preparation and extractions.

The leaf and the stem samples of Polynesian arrow root were washed with water to remove dirt and sand after which it was dried under shade. The dried plant materials were pound to fine powder using a sterile mortar and pestle. Cold extraction technique was used in this study and the solvents used were distilled water, ethanol and n-hexane. In this method, 100 g of leaf and stem powder were soaked in 500 ml of each of the solvents in the ratio of 1: 5 and allowed to stand for 72 hours. The mixture was filtered with a clean and dried filter cloth to remove the debris and the filtrates were further filtered through a filter paper. After the filtration, the filtrates were concentrated to dryness using a water bath at 45 °C to obtain the extract [3].

2.4 Deterioration of fresh pepper after a few days of harvest (post-harvest)

One hundred (100 g) grams of fresh pepper were bought from each of the locations, Abeda and Ugba, and brought to the laboratory for analysis. All the pepper samples were washed with distilled water after which, they were kept on the laboratory bench and allowed to stay for three months with daily observations.

2.5 Media preparation

The media used for the isolation of the bacteria pathogens were Nutrient Agar and MacConkey Agar while Potato Dextrose agar was used for fungi. All the media were prepared according to their individual manufacturers' instructions and were sterilized using autoclave at 121 °C for 15 minutes. After removal from the autoclave, the media was poured into the sterile Petri-dishes and allowed to solidify completely. They were incubated over night at 37 °C to check for complete sterility before further use. In the case of fungi, the media was incubated at 25 °C for 7 days.

2.6 Isolation and identification of organisms from pepper

Serial dilution technique was used in the isolation and identification of the organisms. In this technique, one gram (l g) each of the rotten pepper was taken and added to test tubes containing nine millilitres (9 ml) of sterile distilled water and mixed thoroughly after which it was diluted serially to the fifth test tube using a sterile Pasteur pipette. The inoculum was taken from the dilution factors of 10^{-2} and 10^{-4} and dispensed into sterile Petri-dishes. The molten nutrient agar was added to the bacterial plates while potato dextrose agar was added to the fungal plates, mixed properly and then allowed to solidify. The cultured plates were incubated for 24 hours at 37 °C, for bacteria and 25 °C for 7 days for fungi. The cultured plates were then observed for the growth and morphology. Colony counts were recorded in colony forming unit per millilitre (CFU/g). In each of the cultured plates, a colony was picked at random and sub cultured onto prepared agar slants and stored as stock culture. The stock cultures or slants were cultured on selective media including Mannitol Salt agar (MSA), Eosin Methylene Blue agar (EMBA) and *Shigella* Salmonella agar (SSA), the stock cultures were used to perform all the biochemical tests. For fungi test, the isolates were examined with Lacto Phenol Cotton blue stain to review different structures including the hyphae and shapes.

2.7 Qualitative phytochemical screening

2.7.1 Test for alkaloids (Mayer's Test).

One millilitre (1 ml) of the extract was measured into a test tube and a little amount of dilute hydrochloric acid and Mayer's reagents were added to the solution. The formation of a white precipitate indicated the presence of alkaloids [4].

2.7.2 Test for Phenol

A few drops of ferric chloride solution were added to 2 ml of the extract in a test tube. The appearance of bluish-green colour indicated the presence of phenol.

2.7.3 Test for Quinones

One millilitre (1 ml) of the extract was mixed with concentrated Sulphuric acid. The appearance of the yellow coluor formation signified that Quinone was present.

2.7.4 Test for Saponins (Frothing Test)

Three millilitres (3 ml) of the extract was mixed with equal volume of distilled water and the mixture was shaken vigorously. A copious lather formation was noticed which indicated the absence of saponins [5].

2.7.5 Test for Tannins

Three drops of 0.1 % ferric chloride was added to one millilitre (I ml) of the extract. A brownish- green or bluish-black colour indicated the presence of tannins.

2.7.6 Test for Cardiac Glycosides [6].

A 25 ml of dilute tetra oxo sulphate VI (H_2SO_4) acid was added to five millilitres (5 ml) of the plant extracts in 100 ml flask. It was boiled for 15 minutes, cooled and neutralized with 10 % sodium hydroxide (NaOH). Then, Fehling solutions A and B were added to the neutralized solution and a brick red precipitate of reducing sugar indicated the presence of glycosides.

2.8 Effect of *T. leontopetaloides* Leaf and Stem Extracts on Fresh Pepper.

In this study, 100 g of fresh pepper purchased from selected vendors at Abeda and Ugba markets were taken to the microbiology laboratory in the Department of Microbiology JOSTUM for analysis.

The samples analysed were grouped into A and B. The group A was washed with sterile distilled water while that of group B was soaked into solutions of ethanol, distilled water and hexane leaf and stem extracts of the plant. The group B pepper was allowed to stand in the extracts for 6 hours after which it was removed and both the two groups (A and B) were kept on the bench top in the laboratory and observed for three months with daily observation.

2.9 Biochemical Tests for Identification of Bacteria Isolates

Oxidase test

A piece of Whatman No. 1 filter was placed in a clean Petri-dish, drops of freshly prepared oxidase reagent was added. Using a sterile glass rod, a colony of the test organism was removed and smeared on the filter paper. A blue-purple colour within 15 seconds was an indicator of oxidase positive [7].

2.9.1 Catalase Test

A drop of Hydrogen peroxide (H_2O_2) was placed on a bacterial colony on a slide. The presence of catalase was shown by bubbles immediately [7]. This indicated the presence of *Streptococcus* species.

2.9.2 Coagulase Test

A drop of distilled water was placed on a slide, a colony of the isolate, bacteria was dropped on it and was mixed with a loopful of blood plasma. Agglutination or clumping of cells was an indication of coagulase positive test.

2.9.3 Indole Test

A 3 ml quantity of sterile peptone water was put in bijou bottle with organism and incubated in an incubator at 37 °C for 48 hours. Then 0.5 ml of Kovac's reagent was added, shaken gently. A red colour in the surface layer within 10 minutes was an indication of the indole positive.

2.10 Gram staining

A drop of normal saline was placed on a grease free microscopic slide. A loopful of the bacterial colony was added on to it and smeared on the slide. The smear was allowed to dry by passing it through the Bunsen burner flame, three times. The slide was covered with gram's iodine and was allowed to stay for 1 minute and rinsed with distilled water, safranin stain was added, drained, dried and viewed microscopically using X 100 objective lens with oil immersion. Gram positive stained bluish/black while Gram negative organisms were stained red.

2.11 Preparation of concentration of plant extract

The concentrations were prepared using the method described by Udochukwu*et al*. [8]. One gram (l g) each of ethanol, Aqueous and hexane extracts were added to 20 ml of DMSO to give a concentration of 50 mg/ml. The antimicrobial activity of the extracts was carried out using this concentration.

2.12 Test organisms

Two Gram positive (*Staphylococcus*spp and *Bacillus*spp) and four Gram negative (*Proteus*spp, *E. coli*, *Shigella*spp and *Klebsiella*spp) isolates. Other sources were *Aspergillus*spp, *Penicillium*spp, and *Mucor* were used in this study.

2.13 Statistical Analysis

Data obtained from the study were analysed using SPSS Version 20. Results were presented as means, percentages and table.

III. RESULTS

The result in Table 1, shows the phytochemical constituencies of the ethanol, aqueous and hexane extracts of *Tacca leontopetaloides*. Alkaloids and cardiac glycosides were present in both the root and stem extracts. Phenol, quinone and saponins were absent. Table 2 shows the total colony counts and the total fungal counts of the isolates from the deteriorated pepper samples. From the Table, the range of total colony counts was 9.7 x 10^4 and 2.04 x 10^5 CFU/g for Abeda location. In Ugba location, the range of colony counts was 1.96 x 10^5 CFU/g and 2.66 x 10^5 CFU/g. The range of total fungal counts were 1.6 x 10^4 propagules/g and 4.8 x 10^4 propagules/g for Abeda and 2.6 x 10^4 propagules/g and 5.4 x 10^4 propagules/g for Ugba. In Table 3 shows the results of morphology and biochemical characteristics of the isolates from the deteriorated pepper samples. Most of the isolates were rod shaped and catalase positive. More than half of the isolates were Gram-negative. Table 4 shows the result of the macroscopic and microscopic characteristics of the fungal isolates from deteriorated pepper. The fungal isolates were *Aspergillus* spp., *Penicillium* spp. Mucor and yeasts.

Table 5, shows the percentage distribution of bacterial isolates from deteriorated pepper obtained from Ugba and Abeda, Logo Local Government Area of Benue State. The predominant bacterial isolates were *Bacillus*, *Staphylococcus*, *Proteus* and *E. coli*. Table 6 presents the percentage distribution of fungal isolates obtained from deteriorated pepper from Ugba and Abeda, Logo Local Government Area of Benue State. *Aspergillus* was the highest fungal isolate with a % occurrence of 29.45 %.

Table 1: Qualitative phytochemicals of leaf and stem extracts of *T. leontopetaloides*

Test	Leaf	Stem
Alkaloids	+	+
Tannins	+	–
Cardiac glycosides	+	+
Phenol	–	–
Quinons	–	–
Sapomins	+	–

Table 2: Microbial Count of Isolates from Deteriorated Pepper (CFU/g)

Samples (Location)	Total colony counts (TCC) (CFU/ml)	Total fungi count (Propagules)
	1.56×10^5	3.2×10^4
Abeda	1.38×10^5	4.8×10^4
	2.04×10^5	2.1×10^4
	9.7×10^4	1.6×10^4
	1.58×10^5	2.2×10^4
	2.66×10^5	4.4×10^4
Ugba	2.24×10^5	3.6×10^4
	1.96×10^5	5.2×10^4
	2.08×10^5	2.6×10^4
	2.26×10^5	5.4×10^4

Table 3: Morphological and Biochemical Characteristics of Bacterial Isolates

Colony colour	Colony shape	Elevation	Morphology	Gram's reaction	Motility	Cat.	Cit.	Urea	Indole	H_2S	Suspected bacteria
Cream	Circular	Raised	Cocci	+	+	+	+	-	-	-	Staph spp
White	Irregular	Flat	Rod	+	+	+	+	-	-	-	*Bacillus*spp
Pale	Circular	Raised	Rod	-	+	+	+	+	-	+	*Proteus*spp
Pink	Circular	Raised	Rod	-	+	+	-	-	+	-	*E. coli*
Pale	Circular	Raised	Rod	-	-	+	-	-	+	-	*Shigella*spp
Mucoid Pink	Circular	Raised	Rod	-	-	+	+	+	-	-	*Klebsiella*spp

Table 4: Microscopic and Macroscopic Characteristics of Fungal Isolates

Macroscopic	Microscopic	Fungi Isolates
Velvety filament which sporulate into black powdery spores.	Long septate hyphae with conidiophores bearing brown spores	*Aspergillus*spp
Green velvety filament which sporulates slowly	Septate hyphae with cylindrical spores	*Penicillium*spp
White and woody aerial growth that darkness as it sporulates	Non-septate hyphae with straight sporangiophore and many spherical spores	*Mucor*spp
Flat smooth, moist and glistering colonies that grow rapidly	Multilateral budding with absence of hyphae	*Saccharomyces*

Table 5: Percentage Distribution of Bacteria Isolates from Deteriorated Pepper obtained from Ugba, Logo Local Government Area of Benue State

Organisms	Number of organisms isolated	Percentage of Occurrence (%)
*Staphylococcus*spp	20	17.39
Bacillus spp	25	21.73
*Proteus*spp	20	17.39
E. coli	20	17.39
*Shigella*spp	18	15.65
Klebsiella	12	10.43

Table 6: Percentage Distribution of Fungi Isolates from Deteriorated Pepper collected from Abeda, Logo Local Government Area of Benue State

Organisms	Number of organisms isolated	Percentage of Occurrence (%)
Aspergillusniger	38	29.45
*Penicillium*spp	35	27.13
Mucor piriformis	36	27.90

Results of pepper samples treated with the leaf and stem extracts of *T. leontopetaloides* with a view to extending their shelf life is presented in Table 7. There was no microbial growth on the samples treated with ethanolic stem extract, hexane leaf extract and hexane stem extract in the first 9 weeks of incubation. Distilled water treated pepper, aqueous leaf extract and aqueous stem extracts of *T. leontopetaloides* had growth from the first week of incubation.

Table 7: Pepper samples treated with leaf and stem extracts of *Tacca leontopetaloides*dissolved in water, alcohol and hexane solvents

Duration	DW	AQL	AQS	ETHL	ETHS	HEXL	HEXS
Week 1	10	3	4	0	0	0	0
Week 2	20	3	5	0	0	0	0
Week 3	26	8	6	0	0	0	0
Week 4	140	11	11	0	0	0	0
Week 5	145	11	11	0	0	0	0
Week 6	150	11	12	0	0	0	0
Week 7	160	11	12	0	0	0	0
Week 8	200	10	13	0	0	0	0
Week 9	250	11	14	0	0	0	0
Week 10	300	20	20	1	1	0	0
Week 11	320	35	30	2	2	1	1
Week12	450	35	40	2	3	1	1

KEYS: AQL - Aqueous Leaf, AQS - Aqueous Stem, ETHL - Aqueous Leaf, ETHS - Ethanol Stem, KEXL - Hexane Leaf, HEXS -Hexane Stem

IV. DISCUSSION

This study revealed the presence of bacteria and fungi in high number as the agents of deterioration of fresh pepper. This is probably because of the high moisture contents, oil or fats, crude fibre, organic acids, mineral, vitamins and other important nutrients that support the feeding and the growth of the organisms which as a result, produce enzymes that degrade the tissues of the fruits [9]. Due to the low pH value, fruits are susceptible to attacks by mostly fungi than bacteria. The main cause of spoilage of fresh pepper based on the findings of the research is fungi, *Aspergillus niger* (29.45%), *Mucorpiriformis* 27.9% and *Penicillium*spp 27.13%. The bacterial agents include *Staphylococcus*spp and *E. coli* each having 17.39%, *Klebsiella*spp 17.39%, *Proteus* 15.65% and *Shigella*spp 10.43%.

Ghosh [10] noted that fruits are acidic in nature which favours fungi growth and development than bacteria. The moisture contents of raw pepper in approximately 87% (wet basis) and it contains totally 46.2 Kilocalories 1.5 g of Proteins, 9.4 g of Carbohydrates, Vitamin A 4666 IU, Vitamin C 190 mg. This is probably why it is prone to microbial spoilage [2]. Fungi are mostly the cause of fresh pepper spoilage than bacteria [11]. The presence of the great number of organisms in fresh pepper spoilage may also be because of the favourable conditions for growth and the fungal spores presence in the environment. This study also revealed that *Staphylococcus* aureus, *Bacillus*spp are responsible for fresh pepper spoilage.

The study revealed the presence of phytochemicals such as alkaloids, tannin, saponins, phenols, and flavonoids in *Tacca leontopetaloides* leaf and stem extracts. The presence of these phenolic compounds in these extracts indicates that this plant can serve as antimicrobial agents. This is because phenol and phenolic compounds have been extensively used in disinfection and remains the standard with other fungicides are compared [12]. Okwu[6]reported that phytochemicals exhibit a wide range of biological effect as a consequence of the antioxidant properties as reported. It has also been reported that alkaloids, saponins and tannins in various

antibiotics are used in treating common pathogenic strains, phenolic compounds act as electron donors and are readily oxidized to phenolate ion or quinine, an electron acceptor. Okwu[13] reported that the use of these extracts is important as they prevent infections. Hexane solvent has antimicrobial activity against the biodeteriogens of fresh pepper. Hexane extracts showed more antimicrobial properties followed by ethanolic and aqueous extracts in the present study. This may probably be as a result of high quantity of phytochemicals such as phenols, alkaloids, tannins, saponins and flavonoids found in the extracts compared to ethanol and aqueous extracts. Many studies indicated that the antimicrobial potency of *Tacca leontopetaloides* is as a result of phenolic compounds [14], which are lipid-soluble phenol compounds isolated from the *Tacca leontopetaloides* leaf and stem. The study revealed that the ethanolic extracts has greater microbial activity than its aqueous extracts as indicated by the zones of inhibition. The difference in concentration could be due to the nature of solvent used for extraction [15].

V. CONCLUSION

Pepper samples treated with extracts of *Taccaleontopetaloides* showed extension of shelf life in the range of hexane higher than ethanol, and ethanol higher than aqueous. For the purpose of shelf-life, fresh pepper should be treated with the extracts *Taccaleontopetaloides* extracts immediately after post-harvest so as to be able to avoid biodeterioration which will subsequently result into spoilage and damage of the pepper.

REFERENCES

[1] Shafur-Rahman, M. (2007). Handbook of food preservation. Boca raton, Fl:Rc Press: Taylor and Francis Group LL.

[2] Young K. D. Kyang RC. Hye-Jeong Y. (2015). Dai-jaj Gochujang (Korean red pepper paste): A Korean ethnic sauce, its role and history. *Journal of Ethnic Foods*. 2(1): 29-35

[3] Hamis, P. (2016). Microbial contamination of traditional liquid herbal medical products marketed in Mwanza city: magnitude and risk factors. *Pan African Medicinal Journals*, 23(1).

[4] Sofowora, A. (1993). Medicinal Plants and traditional medicines in West Africa.

[5] Evans, W. C. (1989). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.

[6] Okwu D.E. (2005). Phytochemical, and mineral content of Nigeria medicinal plants *Int. J. Mol. Med. Adv. Sci*, 1: 375-381.

[7] Cheesebrough, M. (2006). District Laboratory practice in tropical countries. Part 2. Cambridge University Press. PP. 143 – 147.

[8] Udochukwu, U; Omoje, F. J; Uloma, J.S.F; Oseiwe, D. (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimumgratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *Amer. J. Res. Communication* 3(5):225-235.

[9] Maddox, D.A. (1998). Implications of new Technologies for seed health testing and the world wide movement of *Seed Science Research*, 8:277-284.

[10] Ghosh, A. (2009) Identification of microorganisms responsible for spoilage tomato (*Lycopersiconesculentum*) fruit. *Journal of phytology* 1(16:414-416).

[11] Akinmusire, O. O., Omomowo, I. O. and Usman, I. M. (2014). Evaluation of the phytochemical properties and antifungal activities of ethanol extract of Allium sativum. *International Journal of Current Microbiology and Applied Sciences*, *3*(10), 143-149.

[12] Doherty, V.F, Olaniran O. O. and Kanif, U. C. (2010). Antimicrobial activities of Afra-momummelagueta (Alligator pepper). *International Journal of Biology*. 2(2): 126-131.

[13] Okwu, D.E (2004). Phytochemical and vitamins content of indigenous species of South Eastern Nigeria. *Journal of Sustainable Agricultural Environment* 6:3034

[14] Hossian, M. A. Shah, I. V. I. D., Sang, S. V. and Sakari, M. (2011). Chemical composition and antibacterial properties of the essential oils and crude extracts of merremiaborneesis. *Journal of king Saud University* of *Science* 24: 243-249.

[15] Singh, G., Kapoor, I.P., Shingh, P., Heluani, C.S. and de Lamposona, M.P. (2008) Chemistry, antioxidant and antimicrobial investigations on essential oil and Oleoresins of Zingiber Officinale. *Food and Chemistry and Toxicology* 46:3295-3302.