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Assessment of the Bio Preservative Efficacy of *Trametes polyzona* (pers.) Extracts on Tomato Fruits

Oduntan, O.O
[1]Fruits Programme, National Horticultural Research Institute,
P.M.B. 5432, Idi-Ishin, Ibadan, Oyo State, Nigeria.

Abstract:- The present study examined the bio-preservative efficacy of *Trametes polyzona* extracts on tomato fruits. Microorganisms associated with the spoilage of tomato fruits were isolated and identified using standard microbiological methods. Pathogenicity test of all the microbial isolates were also done using standard techniques. Qualitative and quantitative phytochemical constituents of *Trametes polyzona* was also assessed. Moreover, antimicrobial effect and preservative potential of the extracts of *Trametes polyzona* on microbial isolates of tomato and the tomato fruits were assessed respectively. The proximate composition of the fresh and preserved tomato fruits were further evaluated and compared after storage. Result obtained from the microbial analysis revealed that *Staphylococcus aureus, Bacillus* spp, *Escherichia coli, Klebsiella* spp, *Lactobacillus* spp, *Shigella* spp, *Pseudomonas* spp, *Micrococcus* spp, *Rhizopus stolonifera, Aspergillus niger, A.terreus, A. flavus,* and *Fusarium* spp were found in the spoilt tomato fruits. Qualitative phytochemical screening showed saponin, tannin, phytate, flavonoid, terpenoid, total phenol, and oxalate available in *Trametes polyzona* extracts, while anthraquinone and phlobatannin were absent. There were variations in the quantity of the phytochemicals present in each extract with values ranging from (0.03 to 9.61mg/g). N-hexane extract of showed inhibitory effect on all the bacterial isolates with zones of inhibition ranging from (2.04 to 27.00mm). The extracts were not able to inhibit the fungal isolates. At the sixth week of storage, tomato fruits preserved with n-hexane extract and kept in the refrigerator were observed to retain their firmness and texture. Moisture content of preserved tomato fruits were lower than fresh tomato fruits, while ash, fibre, protein and carbohydrate content of the preserved tomato fruits was observed to be higher than fresh tomato fruits. Vitamin C content of the preserved tomato fruits (66.41mg/g) was also higher than the fresh tomato fruits (38.58mg/g). In conclusion, N-hexane extracts of *Trametes polyzona* demonstrated the highest inhibitory potential against all the spoilage bacteria associated with tomato fruits, together with a significant preservative potential on tomato fruits compared to other extracts of *Trametes polyzona*. The results from this study revealed that N-hexane may be used to extend the shelf life of tomato fruits and keeping at refrigeration temperature can further help in preserving tomato.

Keywords:- *Bio-Preservative, Phytochemical Screening, Trametes polyzona Extracts, Tomato Fruits, Shelf Life.*

I. INTRODUCTION

Tomatoes may be pear-shaped, elongated, flattened and heart shaped. They are edible, fleshy and reddish when ripe and vary in their acid composition, with white and yellow ones being less acidic. Tomatoes can be used as savoury or flavouring in soups and cooked foods or can be eaten as fruits. It is used in many dishes, salads, sauces and drinks and can also be dried and ground into pancakes (Effiuvwevwere, 2000). According to the Food and Agriculture Organization (FAO, 2013), global tomato production (processing and fresh) reached 162 million metric tons in 2012, while global trade increased to $59 billion. Nigeria is ranked 16th in the world with the estimated production capacity of 1.8 million metric tons and second highest producer in Africa behind Egypt (FAO, 2013).

Tomato has a much lower sugar content than other fruits and is therefore not as sweet, it is rich in vitamins including vitamin A and vitamin C, carbohydrates, proteins, fats, fibres, potassium, phosphorus, iron and lycopene which has many beneficial health effects (Naika *et al*., 2005, Talvas *et al*., 2010). The consumption of tomatoes throughout the world is believed to benefit the heart and other organs. Tomatoes are the primary source of lycopene in many people´s diets (Slimestad and Verheul, 2005). Lycopene is also responsible for the red colour present in the tomato (Sabio *et al*., 2003). Lycopene has been found to prevent prostate cancer, improve the skin's ability to protect itself against the harmful ultra violet rays, decrease the risk of breast, lung, stomach, bladder, uterine, head and neck cancers, protect against neurodegenerative diseases, lower urinary tract infections and reduce thecardiovascular risk associated with type 2 diabetes (Freedman *et al*., 2008; Zhang *et al*., 2009; Shidfar *et al*., 2010; Zdenka *et al*., 2010). It contains large amount of water which makes it more susceptible to spoilage by the action of microorganisms particularly fungi and bacteria (Bai and Lindhout; 2006, Samuel and Orji, 2015).

Spoilage of tomatoes are those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in taste, smell, appearance or texture of the fruits. Ghosh (2009) reported that fungi were the source of spoilage of most of the tomato samples assessed than bacteria. Fungi affecting tomatoes include; *Trichoderma* spp, *Alternariaalternata*, *Fusariumoxysporum*, *Fusariummoniliformis*, *Aspergilliusniger*, *Mucor* spp, *Rhizopusstolonifer*, *Penicillium* spp, *Geotrichum* spp and *Phytophthora* spp (Fatih *et al*., 2005; Akinmusire, 2011; Akintobi *et al*., 2011).

The macrofungus *Trametes* spp (plate 1) is polyporoid white rot fungi widely distributed in various biotopes and have been the subject of many physiological and biochemical studies (Koroleva *et al*., 2002).

Plate 1:- Photography of *Trametes polyzona*

One of the limiting factors that influence tomato economical value is its relatively short shelf life caused by pathogen attack, inadequate handling and poor preservation methods (Samuel and Orji, 2015). In recent decades, use of synthetic preservatives in food preservation and processing has been a common practice to control foodborne microorganisms. However, a public antipathy has developed recently against synthetic chemicals in foods due to consumer anxiety over pesticides residues on food, and also the ecological damage caused by the chemical (Nicholson, 2007). Therefore, discovering and applying natural antimicrobials especially from traditional food materials such as edible mushrooms (macrofungi) has become attractive to researchers and the food industry (Shen *et al*., 2017). Mushrooms are known to contain useful bioactives with potential preservative properties (Oyetayo *et al*., 2010). Although the reports on successful applications of mushroom antimicrobials as food safety control agents are considerably few, and more studies on the use of mushroom antimicrobials for food safety purposes has been increasing significantly (Gao *et al*., 2005; Alves *et al*., 2012). Hence, this present study aim at examining bio-preservative efficacy of *Trametes polyzona* extracts on tomato fruits.

II. MATERIALS AND METHODS

➢ *Collection of tomato fruits*

The study was carried out in Ondo city and Akure metropolis of Ondo state. Tomato fruits were collected from retailers in three different market in Akure namely; Ojaoba market, Isikan market, and FUTA north gate. The samples from each location were collected in a sterile plastic container identified with codes and transferred to Microbiology Postgraduate Laboratory, Federal University of Technology, Akure for analyses.

➢ *Collection of mushroom*

The fruiting bodies of mushroom, *Trametes polyzona* was collected from the FUTA forest, while identity of the mushroom was authenticated at the Department of Microbiology, Federal university of Technology, Akure, (FUTA) Ondo State.

➢ *Culture media*

The culture media used for this research work are Nutrient agar, Nutrient broth, Eosin methylene blue (EMB) agar, MacConkey agar, Salmonella-Shigella agar, Potato dextrose agar and Mueller Hinton agar. Each of the culture medium was prepared and sterilized according to the manufacturer's specification.

➢ *Glass ware*

The glassware used in this research includes petri dishes, test tubes, conical flasks, beakers and Mac Cartney bottles. They were washed, left to dry in the drying cabinet and sterilized in the oven at 160^{o}C for two hours before use.

➢ *Test organisms*

Tomato fruits purchased were left for one week for spoilage to occur. The spoilt tomato fruits were examined microbiologically using standard technique. Microorganisms isolated from these spoilt tomato fruits were stored on slants in the refrigerator.

➢ *Total Bacterial and fungal load*

Total bacterial and fungal counts were determined using standard pour plate technique method. Nutrient agar and potato dextrose agar were used for isolating the bacteria and fungi respectively. Bacteria and fungi were identified using morphological and cultural characteristics as well as biochemical tests as described by APHA (2002) and Fawole and Oso (2004).

➢ *Identification of bacteria isolates*

The identification of bacteria was based on morphological characteristics and biochemical tests which include Gram staining test, Motility test, Spore staining technique, Catalase test, Coagulase test, Methyl red test, Citrate test, Indole test, Oxidase test and Sugar fermentation according to methods of Fawole and Oso (2004).

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- *Identification of Fungal using Lactophenol Cotton Blue Mounts*

Lactophenol cotton blue mounts was carried out on fungal isolates for full identification of the exact fungi based on the size, shape, surface feature of the conidia and hyphae arrangements. (Fawole and Oso, 2004).

- *Pathogenicity test of the isolates*

Pathogenicity test in this study was carried out according to the methods of Chukwuka *et al.* (2010) and Onuorah *et al.*(2015).

- *Drying and extraction of Trametes polyzona*

Trametes polyzona was collected, air dried and pulverised by an electrical mill. The powdered mushroom sample (100 g) was extracted with 2000 ml of 95% ethanol, ethyl acetate, and n-hexane separately in an Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 3 days for extraction with occasional stirring. The filtrate obtained was dried using rotary evaporator.

- *Phytochemical screening of extracts*

The crude of *Trametes polyzona* was subjected to phytochemical screening for the presence of anthraquinones, alkaloids, tannins, saponins, phlobatannins, steroids, flavonoids and cardiac glycosides using standard techniques.

- *Antimicrobial activity of extracts on Isolates*

Culture of the isolates maintained at 4ºC on slopes of nutrient agar were used. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to testtubes of nutrient broth and were incubated without agitation for 24 hours at 36ºC.

- *Shelf life determination*

Tomato fruits were divided into group A and B. Group A represent tomato fruits preserved with *Trametes polyzona* extract of water, ethanol, ethyl acetate and N-hexane, while group B were fruits preserved with only the solvent. Each of the group were subdivided into two; which contain tomato fruits kept in the refrigerator and fruits left at ambient temperature. The period of storage of the fruits so as to determine the shelf life was six weeks.

- *Proximate Estimation*

The moisture, crude protein, crude fibre, crude fat, ash and soluble carbohydrate (by difference) were determined in accordance with AOAC (2012). The proximate analyses were carried out in triplicate.

- *Vitamin C determination*

Tomato juice extract in the range of 1, 2, 5, 10, and 15 ml were pipetted out into a 25ml standard flask followed by 2.1ml of standard potassium chromate, and 3ml of diphenylcarbazide solution. The mixture was diluted with 0.8M nitric acid up to the mark. (Prabha *et al*., 2011)

- *Data Analysis*

All the experiments were carried out in triplicate and data obtained from the study were subjected to One-way analysis of variance (ANOVA) using SPSS version 23. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance.

III. RESULTS AND DISCUSION

- *Microbial load from spoilt tomato fruits*

Bacterial load of the spoilt tomato fruits ranged between 8.00x10^5 and 14.00 x10^5cfu/g while the fungal load ranged between 1.67x10^5 and 3.33 x10^5sfu/g as shown in Fig 1. Bacterial load is higher than fungal load in the tomato obtained from the three markets examined.

- *Morphological and Biochemical Characteristics of Microbial load in tomato samples*

The cultural, morphological and biochemical characteristics of the bacterial and fungal isolates are shown in Tables 1, and 2 respectively. Eight bacteria and five fungi were isolated from the spoilt tomato fruits. They include; *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Klebsiella* spp, *Lactobacillus* spp, *Shigella* spp, *Pseudomonas aeruginosa*, *Micrococcus* spp, *Rhizopus stolonifer*, *Aspergillus niger*, *A. terreus*, *A. flavus*, and *Fusarium* spp.

- *Prevalence of Microorganisms in the markets examined*

Occurrence of microorganisms isolated from the tomato samples shown in Table 3 revealed that Oja -oba Market and Isinkan Market had higher number of microbial isolates than Northgate Market. All the microbial isolates were present in market 2 except *A. terreus*. *Escherichia coli* and *Fusarium* spp were absent in market 2.

- *Weight loss in tomato fruits after pathogenicity test*

Bacterial isolates recorded higher magnitude in weight loss of the tomato fruits than the fungal isolates as shown in Figs 2 and 3 respectively. All the microbial isolates showed significant effect on all the tomato samples.

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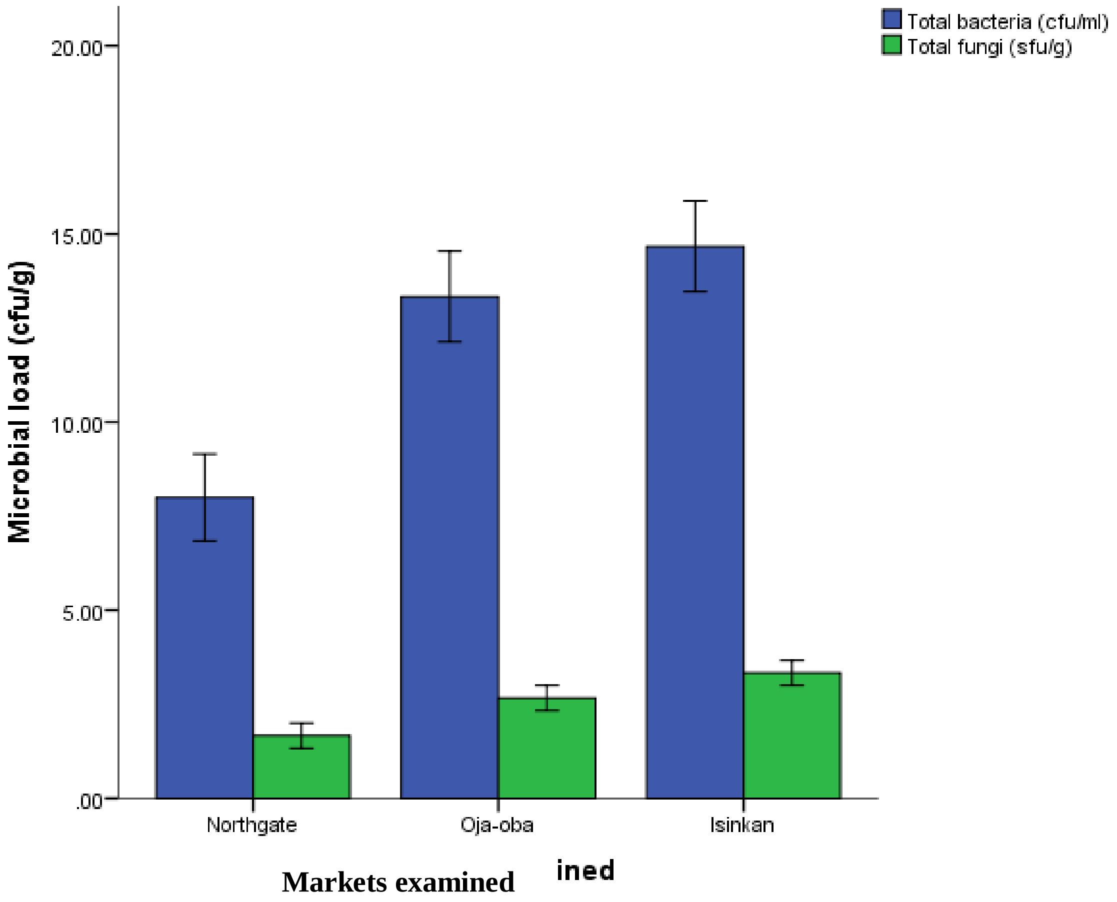

Fig 1:- Microbial load of spoilt Tomato fruits from different Markets

Isolates	**Biochemical characteristics**													
	Gram rxn	**Shape**	**motility**	**Spore formation**	**Catalase**	**coagulase**	**citrate**	**urease**	**Indole**	**oxidase**	**Methyl red**	**glucose**	**lactose**	**Probable organism**
1	+	cocci	-	-	+	+	-	-	-	-	-	-	-	*Staphylococcus aureus*
2	+	Rod	+	+	+	-	-	-	-	-	+	+	-	*Bacillus* spp
3	-	Rod	-	-	-	-	+	-	+	-	+	+	-	*Escherichia coli*
4	-	Rod	-	-	-	-	+	-	-	-	+	+	-	*Klebsiella* spp
5	+	Rod	-	-	-	-	-	-	-	-	-	+	+	*Lactobacillus* spp
6	-	Rod	+	-	-	-	-	-	-	-	-	+	-	*Shigella* spp
7	-	Rod	-	-	-	-	-	-	-	+	-	+	-	*Pseudomonas* spp
8	+	cocci	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus* spp

Table 1:- Biochemical characteristics of bacterial isolates obtained from spoilt tomatoes and characteristics of bacterial isolate

Isolate	Morphological Characteristics	Probable organism
A	White cotton-like mycelia, non-septate hyphae, well developed collumela which is in umbrella-like form.	*Rhizopus stolonifer*
B	Dark-brown conidia, long conidiophores, phialides are borne on brown metulae	*Aspergilus niger*
C	Whitish-pink mycelia, conidial heads are compact and biserate. Conidiophores are smooth and hyaline. Aleurioconida produced directly on the hyphae	*A.terreus*
D	Greenish mycelia, hyphae are septate, roughened and uncoloured conidiophores.	*A. flavus*
E	Reddish-white mycelia, hyphae are septate and hyaline. Phailades are cylindrical conidiophores are medium length	*Fusarium* spp

Table 2:- Morphological characteristics of fungal isolates

Microbial isolates	**Markets examined**		
	S1	S2	S3
Staphylococcus aureus	+	+	+
***Bacillus* spp**	+	+	+
Escherichia coli	-	+	-
***Klebsiella* spp**	-	+	+
***Lactobacillus* spp**	+	+	+
***Shigella* spp**	+	+	+
***Pseudomonas* spp**	+	+	+
***Micrococcus* spp**	+	+	+
Rhizopus stolonifer	+	+	+
Aspergillus niger	+	+	+
A. terreus	+	-	+
A. flavus	-	+	+
***Fusarium* spp**	-	+	-

Table 3:- Occurrence of microorganisms in the Tomatoes obtained from different markets

Key: + = Present
- = Absent
S1 = Northgate Market
S2 = Oja Oba Market
S3 = Isinkan Market

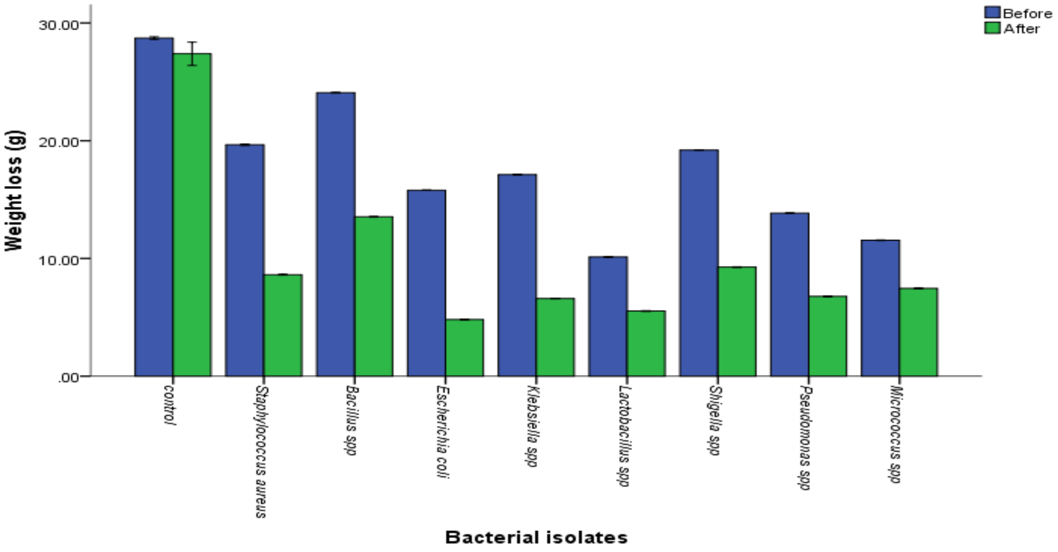

Fig 2: Weight of tomato fruits before and after pathogenicity test with bacterial isolates

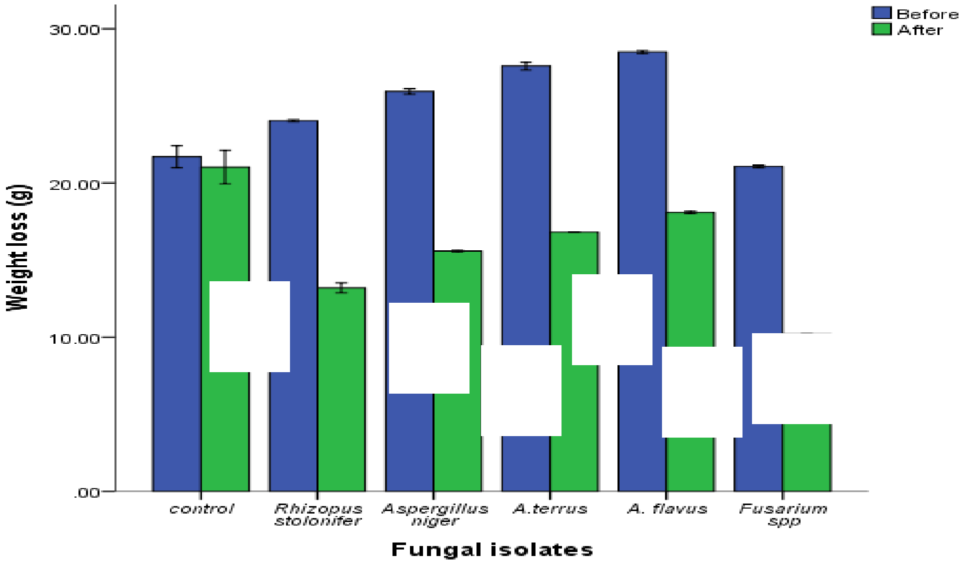

Fig 3:- Weight of tomato fruits before and after pathogenicity test with fungal isolates

➢ *Qualitative phytochemical screening of extracts*

The qualitative analysis of the extract showed that saponin, tannin, flavonoid, and cardiac glycoside were present in all the extracts of *Trametes polyzona* while anthraquinone and phlobatannin were absent (Table 4).

➢ *Quantitative phytochemical screening of extracts*

The quantitative analysis of the extracts showed variation in the phytochemical constituents of *Trametes polyzona* (Table 5).Ethanol and N-hexane recorded significant content of saponin with value of 9.61mg/ml and 7.92mg/ml respectively.

➢ *Antimicrobial activity of Trametes polyzona*

The extract exhibited varying antibacterial effect against all the selected isolates. N-hexane extract of *Trametes polyzona* showed significant ($P<0.05$) inhibitory (for example *Staphylococcus aureus*(10.55 ± 0.05^{b})mm while control has (35.00 ± 2.88^{e})mm and all other bacteria isolated from the spoilt tomato fruits as shown in Table 6. None of the extract showed inhibitory potential on the fungal isolates (Table 7).

➢ *Shelf life extension of Tomato fruits by Trametes polyzona extract*

All the tomato fruits preserved with the extract and kept under refrigerator temperature were still looking wholesome in appearance, texture, colour and firmness after the fourth week of storage while tomato fruits preserved with only solvents showed signs of spoilage after the third week of storage as shown in Tables 7 and 8 respectively.

Phytochemical component	Ethanol	Water	Ethyl acetate	N-hexane
Saponin	+	+	+	+
Tannin	+	+	+	+
Phytate	+	+	+	+
Flavonoid	+	+	+	+
Terpenoid	+	+	+	+
Total phenol	+	+	+	+
Oxalate	+	+	+	+
Anthraquinone	-	-	-	-
Phlobatannin	-	-	-	-

Table 4:- Qualitative screening of *Trametes polyzona*

Extract	Phytochemical constituents						
	Saponin	**Tannin**	**Phytate**	**Flavonoid**	**Terpenoid**	**Total phenol**	**oxalate**
ETHANOL	9.61±0.07^{c}	2.12±0.02^{b}	0.57±0.02^{b}	0.90±0.03^{c}	0.27±0.01^{a}	0.93±0.02^{b}	0.04±0.00^{a}
WATER	4.06±0.75^{a}	2.19±0.09^{b}	1.22±0.00^{c}	0.55±0.05^{b}	0.18±0.00^{a}	1.78±0.09^{c}	0.03±0.00^{a}
ETHYL-ACETATE	5.14±0.33^{a}	0.90±0.00^{a}	0.46±0.01^{a}	3.69±0.16^{d}	1.45±0.02^{b}	0.07±0.01^{a}	0.07±0.01^{b}
N-HEXANE	7.92±0.33^{b}	0.76±0.03^{a}	0.44±0.01^{a}	0.21±0.01^{a}	2.96±0.34^{c}	0.21±0.01^{a}	0.03±0.00^{a}

Table 5:- Quantitative Phytochemical Constituents(mg/g) of *Trametes polyzona*extracts
Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Extract	Zones of inhibition (mm)							
	Staphylococcus aureus	***Bacillus* spp**	***Escherichia coli***	***Klebsiella* spp**	***Lactobacillus* spp**	***Shigella* spp**	***Pseudomonas* spp**	***Micrococcus* spp**
ETHANOL	28.16±1.30^{d}	2.51±0.01^{b}	0.00±0.00^{a}	3.13±0.57^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	5.65±0.05^{b}
WATER	5.23±0.03^{a}	0.00±0.00^{a}	2.30±0.03^{b}	0.00±0.00^{a}	2.02±0.02^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
ETHYL-ACETAE	12.65±0.58^{c}	5.35±0.55^{c}	4.21±0.01^{c}	6.07±0.07^{c}	6.31±0.06^{c}	10.02±0.02^{b}	0.00±0.00^{a}	5.00±0.00^{b}
N-HEXANE	10.55±0.05^{b}	27.00±1.52^{d}	22.66±1.20^{d}	2.04±0.04^{b}	14.00±0.58^{d}	11.66±0.66^{b}	17.66±1.20^{b}	22.66±1.20^{c}
CIPROTAB	35.00±2.88^{e}	26.33±1.85^{d}	23.33±1.20^{d}	21.45±0.05^{d}	22.66±1.20^{e}	25.66±1.20^{c}	21.66±1.76^{c}	23.66±0.88^{c}

Table 6:- Antibacterial properties of extracts of *Trametes polyzona*
Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Extract	Zones of inhibition (mm)				
	Rhizopus stolonifer	***Aspergillus niger***	***A.terrus***	***A.flavus***	***Fusarium* spp**
ETHANOL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
WATER	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
ETHYL-ACETATE	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
N-HEXANE	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Griseofluvin	15.00±1.88^{b}	14.33±1.85^{b}	12.03±1.03^{b}	15.45±0.05^{b}	12.66±1.20^{b}

Table 7:- Antifungal properties of extracts of *Trametes polyzona*
Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

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Weeks observed	Extract	Appearance						Texture						Color						Firmness					
		Amb			Ref			Amb			Ref			Amb			Ref			Amb			Ref		
		S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
	Water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Week 1	Ethanol	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	Ethyl acetate	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	N-hexane	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	Water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Week 2	Ethanol		+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	Ethyl acetate	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	N-hexane	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	Water	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Week 3	Ethanol	-	-		++	++	++	-	-	-	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	Ethyl acetate	-	-	-	++	++	++	-	-	-	++	++	++	+	-	-	++	++	++	-	-	+	++	++	++
	N-hexane	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	-	-	-	++	++	++
Week 4	Water	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	Ethanol	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ethyl acetate	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+
	N-hexane	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 5	Ethanol	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ethyl acetate	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
	N-hexane	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 6	Ethanol	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
	Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N-hexane	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-

Table 8:- Sensory properties of Tomato fruits during preservation with extracts of *Trametes polyzona*

Key: Amb- Ambient temperature, Ref- Refrigeration, S1- Northgate market, S2- Oja-oba market, S3- Isinkan market, (++) = Very good, (+) = Good, (-)= Bad

Weeks observed	Control	Appearance						Texture						Color						Firmness					
		Amb			Ref			Amb			Ref			Amb			Ref			Amb			Ref		
		S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
	Water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Week 1	Ethanol	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	Ethyl acetate	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	N-hexane	-	-	-	++	++	++	-	-	-	++	++	++	-	-	-	++	++	++	-	-	-	++	++	++
	Water	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Week 2	Ethanol	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	Ethyl acetate	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	++
	N-hexane	-	-	-	++	++	++	-	-	-	++	++	++	-	-	-	++	++	++	-	-	-	++	++	++
	Water	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Week 3	Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ethyl acetate	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	N-hexane	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Week 4	Water	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-
	Ethanol	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	Ethyl acetate	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	N-hexane	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 5	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N-hexane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Week 6	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N-hexane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-

Table 9:- Sensory properties of Tomato fruits during preservation with the control (only the solvents)

Key: Amb- Ambient temperature, Ref- Refrigeration, S1- Northgate market, S2- Oja-oba market, S3- Isinkan market, (++) - Very good, (+) –Good, (-) - Bad

➢ *Proximate composition of fresh and preserved tomato fruits*

Tomato fruits preserved with *Trametes polyzona* extractrecorded higher proximate composition than the fresh fruits as revealed in Table 9. Tomato fruit preserved with N-hexane extract of *Trametes polyzona* had significant protein and carbohydrate content with value of 3.26% and 15.33% respectively.

➢ *Vitamin C content of tomato fruits*

Tomato fruits preserved with ethyl acetate recorded the highest vitamin C content as shown in Fig 4, followed by tomato fruits preserved with ethanol.

	Samples	Proximate composition (%)					
		Ash	**Moisture**	**Fibre**	**Fat**	**Protein**	**Carbohydrate**
	S1	0.41±0.00^{c}	63.02±0.03^{cd}	23.91±0.00^{c}	6.03±0.30^{b}	0.67±0.00^{c}	5.94±0.27^{b}
Fresh	S2	0.36±0.00^{b}	63.82±0.57^{d}	20.83±0.00^{a}	5.85±0.41^{b}	0.63±0.00^{a}	8.47±0.15^{c}
	S3	0.31±0.00^{a}	62.02±0.64^{c}	21.94±0.00^{b}	11.97±0.19^{d}	0.66±0.01^{b}	3.08±0.84^{a}
Treated and stored	Ethanol	1.01±0.00^{c}	53.50±0.00^{b}	28.23±0.00^{f}	6.19±0.01^{b}	3.22±0.00^{e}	8.05±0.21^{c}
	Water	0.44±0.02^{c}	58.02±0.02^{bc}	23.91±0.05^{c}	9.07±0.07^{d}	1.51±0.00^{ab}	8.47±0.15^{c}
	Ethyl acetate	1.11±0.00^{d}	52.45±0.00^{ab}	26.76±0.00^{e}	8.30±0.00^{c}	2.51±0.00^{d}	8.83±0.00^{c}
	N-hexane	0.95±0.04^{c}	51.76±0.00^{a}	24.78±0.01^{d}	3.68±0.02^{a}	3.26±0.00^{f}	15.53±0.03^{d}

Table 10: Proximate composition (%)of fresh and stored tomatoes
Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)
S1- Northgate market, S2- Oja-oba market, S3- Isinkan market

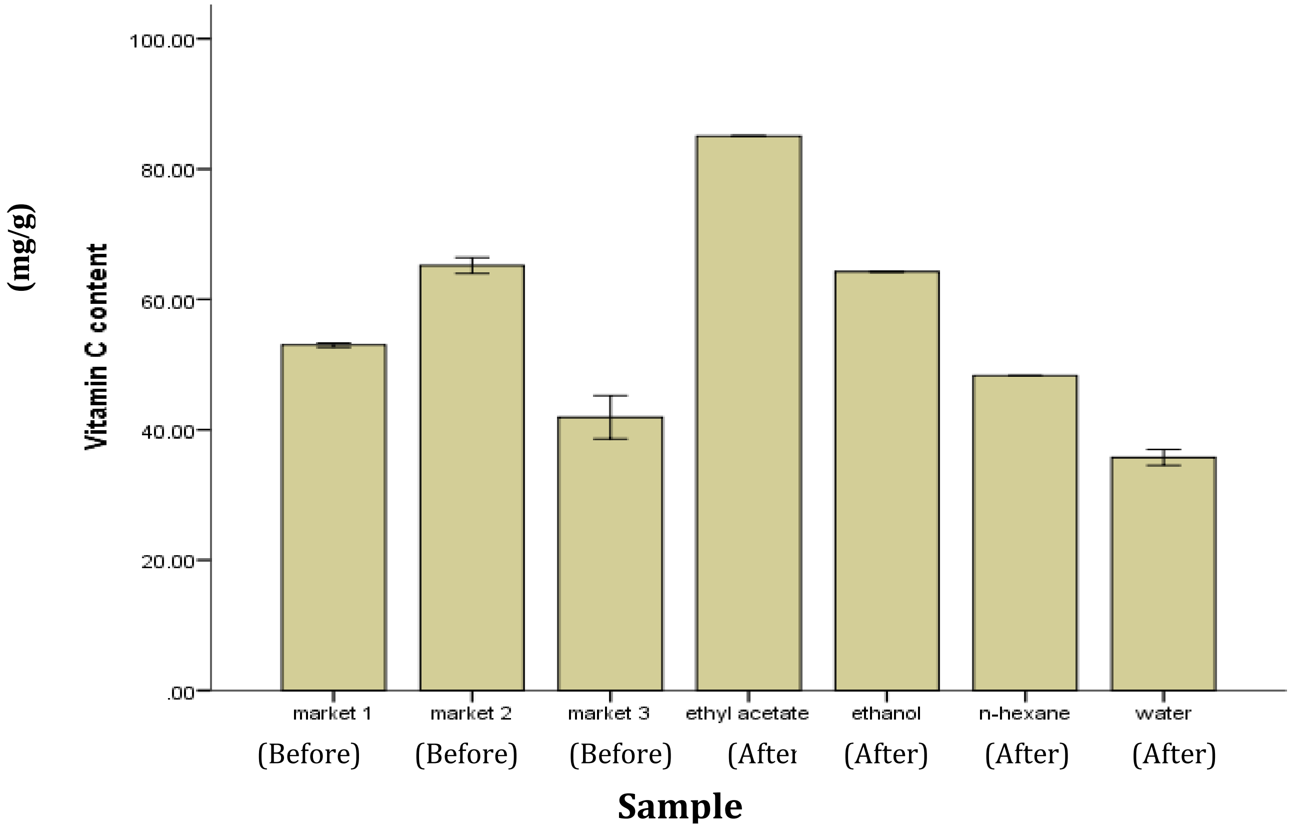

Fig 4:- Vitamin C content of tomato fruits before and after preservation

Microbial load of spoilt tomato fruits showed that bacterial load is higher than fungal load in all the markets examined. Bacterial spoilage of tomatoes may also be attributable to the high water content, environmental conditions, state of handling, state of storage facilities, the microbial load of the handlers and the quality of the tomatoes (Samuel and Orji, 2015).Report on microbial load is comparable to Agbabiaka *et al*. (2015) during microbial examination of tomato fruits from different markets.

A total number of eight bacteria; *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Klebsiella* spp, *Lactobacillus* spp, *Shigella* spp, *Pseudomonas aeruginosa*, and *Micrococcus* spp, were isolated from the tomato fruits. While the five fungi isolated include; *Rhizopus stolonifer*, *Aspergillus niger*, *A. terreus*, *A. flavus*, and *Fusarium* spp. Similar microorganisms were also reported by Oyemaechi *et al*. (2014), during the microbial examination of spoilt tomato fruits in Onitsha metropolis. Report on the occurrence of microorganisms showed that *Staphylococcus aureus*, *Bacillus* spp, *Lactobacillus* spp, *Shigella* spp, *P. aeruginosa*, *Micrococcus* spp, *Rhizopus stolonifera*, and *A.niger* were found on tomato fruits collected from all the markets studied. The presence of *Bacillus* spp, *Staphylococcus aureus*, *Rhizopus stolonifer* and *Aspergillus* spp in the tomato fruits agreed with the findings of Bello *et al*. (2016), who also reported that these microorganisms are responsible for the spoilage of tomato fruits. Fajola (2006) reported that *Rhizopus stolonifer* is a soft rot pathogen of tomato in Nigeria. The mycelium of the fungus can infect adjacent fruits, through mechanical wounds and natural openings, creating a nest of mold growth on diseased fruits (Mahovic *et al*., 2009).

Bartz *et al*. (2009) also reported that certain species of *Pseudomonas* and *Bacillus* can cause soft rot of tomato fruits. Similarly, the presence of enteric organisms such as *E.coli*, *Klebsiella* spp, and *Shigella* spp in the spoilt tomato fruits is an indication that the tomato fruits were exposed to faecal contaminated water or organic manure. Many researchers had reported isolation of similar microorganisms from tomato fruits (Chuku *et al*., 2008; Akinmusire, 2011; Ibrahim *et al*., 2011; Chinedu and Enya, 2014; Wogu and Ofuase, 2014).

It was observed from this study that tomato fruits collected from Oja-oba Market and Isinkan Market had higher number of microbial isolates than Northgate Market. This indicated that tomato fruits sold at Oja-oba Market and Isinkan Market may have not been handled well during transportation and sales than those sold at Northgate Market. This report on the occurrence of microorganisms on tomato fruits agreed with the findings of Bello *et al*. (2016), who reported variation in the occurrence of microorganisms on tomato fruits collected from three different markets.

The result of the pathogenicity test of all the microbial isolates on tomato fruit samples after one week of inoculation showed significant (P>0.05) weight loss with bacterial isolates recording higher magnitude compared with fungi. Changes like wrinkled textures on the fruits and weight lost were observed after one week of inoculation of the spoilage microorganisms.

The Antibacterial assay revealed that N-hexane extract of *Trametes polyzona* showed significant (P>0.05) inhibitory effect on all the bacteria isolated from the spoilt tomato fruits. Ethyl-acetate extract of *Trametes polyzona* showed inhibitory effect on all the bacterial isolates except *Pseudomonas aeruginosa*. Ethanol extract only showed significant (P>0.05) inhibitory effect on *Staphylococcus aureus*. None of the extracts showed inhibitory potential on the fungal isolates.

The result from the Shelf life assessment showed that tomato fruits preserved with the extracts and kept in the refrigerator were acceptable in appearance, texture, color and firmness after the fourth week of storage. N-hexane and ethanol extract of the mushroom were observed to retain the texture of the tomato fruits kept in refrigerator even at the sixth week of storage. The shelf life potential of *Trametes polyzona* extract could be attributed to the presence of phenolic compounds in the extract which have been reported to exhibit an excellent preservative potential on food products containing lipids (Soto *et al*., 2011; Abugria and McElhenney, 2013). Thus, tomato fruits preserved with *Trametes polyzona* extract showed extended shelf life than those preserved with only solvent. Tomato fruits kept in refrigerator temperature also had a longer shelf life than those kept in ambient temperature. It has been reported to play a key role in minimizing food spoilage and waste (James *et al*., 2017). Consequently, tomato fruits preserved with *Trametes polyzona* extract and kept in the refrigerator had longer shelf life when compared with fruits kept in ambient temperature even though preserved with *Trametes polyzona* extract.

Proximate analysis revealed that moisture content of preserved tomato fruits were lower than fresh tomato fruits. Reduction in the moisture content of the preserved tomato fruits makes them less susceptible to spoilage by the action of microorganisms thus, increasing the shelf life of the tomato fruits (Samuel and Orji, 2015). The high moisture content of the fresh tomato fruits provides an enabling environment for proliferation of the microorganisms and hence its spoilage and potential to become health risk to human beings (Agbabiaka *et al*., 2015). Ash and fibre content of the preserved tomato fruits was recorded to be higher than fresh tomato fruits. The ash content is a measure/reflection of the nutritionally important mineral contents present in the food material (Omotosho 2005; Nnamani *et al*. 2009). Therefore, the high ash content recorded in tomato fruits preserved with *Trametes polyzona* extracts indicates that the fruits would provide essential valuable and useful minerals needed for body development (Gemede *et al*., 2015).

Dietary fiber promotes the growth and protects the beneficial intestinal flora. Moreover, high intake of fiber reduces the risk of colon cancer (Dawczynski *et al*. 2007). Fat content of tomato fruits preserved with ethyl acetate extract of *Trametes polyzona* was observed to be higher than other extracts. Fat in di*et al*so serve as a source of energy to man (Olaniyi *et al*., 2010). Protein and carbohydrate content of the preserved tomato fruits was higher than fresh tomato fruits. Although tomato fruits had been reported to be rich in vitamins and minerals. (Oyemaechi *et al*., 2014). Also, tomato fruits preserved with ethyl acetate extract of *Trametes polyzona* had the highest Vitamin C content of all tomato fruits examined. Vitamin C also known as ascorbic acid, is a dietary antioxidants which help to reduce the impact of oxidative stress in human (Giovannucci, 1999; Matteo *et al*., 2010).

IV. CONCLUSION

In conclusion, frequent inspection of the fruits for sale by food inspectors is also recommended, so as to prevent the consumption of contaminated tomato fruits thereby reducing the health hazards posed by some of the opportunistic toxin-producing microorganisms isolated in this study. *Trametes polyzona* extract showed antimicrobial potential which consequently increased the shelf life of the tomato fruits. More so, the nutrient content of tomato fruits preserved with The overall result revealed that *Staphylococcusaureus*, *Bacillus* spp, *Lactobacillus* spp, *Shigella* spp, *Pseudomonas aeruginosa*, *Micrococcus* spp, *Rhizopus stolonifera*, and *Aspergillus niger* were the dominant microorganisms isolated from the tomato fruits. N-hexane extract of *Trametes polyzona* showed inhibitory effect on all the bacterial isolates, while Ethyl-acetate extract of *Trametes polyzona* showed inhibitory effect on all the bacterial isolates except *Pseudomonas aeruginosa*. All the tomato fruits preserved with the extracts and kept in refrigerator temperature were acceptable in appearance, texture, and colour after the fourth week of storage. N-hexane and ethanol extract of the mushroom were observed to preserve the texture of the tomato fruits kept in refrigerator even at the sixth week of storage. Proximate analysis revealed that moisture content of preserved tomato fruits were lower than fresh tomato fruits, protein and carbohydrate content of the preserved tomato fruits was higher than fresh tomato fruits. Moreover Vitamin C content of the preserved tomato fruits was higher than the fresh tomato fruits.

Trametes polyzona extract increased significantly (P>0.05) than the fresh fruits. *Trametes polyzona extract* can therefore be recommended for the preservation of tomato fruits and keeping at refrigerator temperature further enhance the shell life of the fruits.

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