OPEN ACCESS
Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) |ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/haya/

Original Research Article

The Preservative Effect of Pawpaw (*Carica papaya*) Seed Extract on Some Selected Food Materials

Adesola M. O, Akande E. A, Adejuyitan J. A*
Department of Food Science and Engineering, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomosho Rd, Nigeria

***Corresponding author:** Adejuyitan J. A
| **Received:** 12.03.2019 | **Accepted:** 25.03.2019 | **Published:** 30.03.2019
DOI:10.21276/haya.2019.4.2.3

Abstract

The use of preservatives in inhibiting and retarding the growth of microorganism responsible for the spoilage or decay of food substance is of great interest. The anti-microbial activities of plant extracts like pawpaw form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies. Therefore, this work was aimed at evaluating the preservative effect of pawpaw (*carica papaya*) seed extract on some selected food materials. T-solo variety of pawpaw was obtained from Akintola Farm, Kinnira, Ogbomoso and the seeds were fermented. Extracts from the fermented seeds were obtained using petroleum ether, n-hexane and hot aqueous solvents. Antioxidative study was carried out on the crude extracts using 1,1- diphenyl-1- picrylhydrazil radical (DPPH) assay. Total Viable Bacteria (TVB) and Total Fungi Count (TFC) of akara, fish, soymilk and dairy milk were carried out**.** The DPPH were 42.14, 33.28, 18.85% for petroleum ether, n-hexane and hot aqueous extracts, respectively. The TVB and TFC of akara, fish, soymilk and dairy milk samples ranged (1.00 -1.42 $x10^4$, 0.60-1.57 $x10^2$; 1.30-2.28$x10^4$, 1.50-2.22$x10^2$; 1.43-2.37$x10^4$, 0.80-2.86 $x10^2$ cfu/g and 1.20-2.60 x 10^4, 1.05 - 1.67 x 10^2 cfu/g), respectively.
Keywords: *Carica papaya*, Total Fungi Count (TFC), DPPH.

INTRODUCTION

Natural additives, such as seeds, spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as food preservatives [1]. Certain natural additives (such as negro pepper, sesame seed, moringa seed and others) prolong the storage life of foods by preventing rancidity through their antioxidant activity and they also possess antimicrobial and antibrowning properties [2, 3]. Natural additives and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of several toxicological studies [1].

The extracts of some seeds have become popular in recent years and attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications [4]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary health care needs [5]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, attention turned to ethnopharmacognosy. Literally thousands of phytochemicals from plants were discovered to be safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrhea, analgesic and wound healing activity were reported. According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries [6]. Pawpaw is a plant with great economic value and its use ranges from nutritional to medicinal. Many scientific investigations have been conducted to evaluate the biological activities of various parts of pawpaw including fruits, shoots, leaves, rinds, seeds, roots and latex [7]. The enzyme papain which is present in the fruit, stem and leave is a proteolytic enzyme which has a wealth of industrial uses; it has milk clotting (rennet) and protein–digesting properties [8]. It can be used to digest helminths (worms) within the belly. Digestive supplements that combine papain and bromelain are used as digestive support for protein digestion [9]. Papain is commonly used commercially in meat tenderisation, in clearing of beer and in the production of chewing gum [10]. Phytochemicals in papain may increase immune system and may also promote the release of natural chemicals that attack tumor cell [11]. Ripe and unripe pawpaw fruits and seeds are active against bacteria which is why many women on the west coast dry the bitter seeds to prepare them as tea. The seeds of *Carica papaya* are medically important in the treatment of sickle cell diseases and

poisoning related disorder [12]. However, several seeds are being wasted and they remain underutilized due to ignorance of consumers on their potentials. Pawpaw seed is one of those seeds that are underutilized, harnessing its preservative potentials will add to catalogue of natural food additives. Therefore, this work is aimed at evaluating the preservative effect of pawpaw seed extract on akara, fish soymilk and dairy milk.

MATERIALS AND METHODS

MATERIALS

Matured ripe fruits of *Carica papaya* (variety T. solo) were obtained from Akintola Farm, a private farm in Ogbomoso. The fruits were identified and authenticated at Department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso. The chemicals and reagents used were of analytical standard. The experiment was carried out in Food Science and Engineering Lipid Laboratory, LAUTECH, Ogbomoso and Food Science and Technology Laboratory FUTA, Akure.

METHODS

Preparation of *Carica papaya* Seeds

The method of Afolabi and Ofobrukweta [13] and Dakare [14] was adopted with some modifications. The raw pawpaw fruit was cut into two longitudinal halves. The seeds were removed and dehulled manually, the hulls were allowed to float and decanted to obtain the seed kernel. The seed kernels were pre-dried in the oven (Model, Uniscope SM9053) at 50 °C for 20 h. The seeds were incubated at 37 ºC in a dark room, allowed to ferment for 72 h and dried in the oven (Model, Uniscope SM9053) at 120 °C for 10 h. It was milled and packaged [14].

Pawpaw Seed Extract Preparation

The extracts were prepared using three solvents which are petroleum ether, n-hexane and aqueous solution.

Antioxidative Assessment of the Extracts

Antioxidative assessment of the crude extract was carried out by determining the free radical scavenging activity in the 1,1- diphenyl-2-picrylhydrazil radical (DPPH) assay.

Determination of the free radical scavenging activity (FRSA) in the 1,1-diphenyl-2 picrylhydrazil radical (DPPH) assay

The antioxidant activity was determined using the DPPH assay as reported by Ansari *et al*., [15]. The DPPH method was used because this technique is easily applicable and widely used. DPPH is a synthetic free radical that accepts an electron or hydrogen to be converted to DPPH stable molecules. The disappearance of the DPPH radical is monitored by decrease in absorbance of solution at 517nm. One (1) g of sample was mixed with 80% methanol (4:l of methanol and water). This was incubated for 1 h at 37 °C in a shaking water bath and centrifuged (3,500 x g at 4 °C) in an Eppendorf centrifuge (Model AJ-IC 03) for 35 min. Ten times dilution of extracts was made with 80% methanol. 100 µl of diluted extract was mixed with l00 µl of DPPH solution (0.1 mjvl) in a 96 well microplates. The mixture was kept in the dark at ambient temperature while the absorbance recorded at 517 nm after 20 min. Blank was made from l00 µl of DPPH and l00 µl methanol. The FRSA was calculated as:

$$\left[1-\left(\frac{A-B}{C}\right)\right] x100$$

Where,

A = is the absorbance of 100 µl of the diluted extract solution mixed with an equal volume of the DPPH solution

B = is the absorbance of 10 µ1 of the diluted extract solution mixed with an equal volume of methanol.

C = is the absorbance of blank sample prepared by mixing 100 µl of the DPPH with an equal volume of methanol: water (4:1). Analysis was done in triplicates.

Preparation of bean cake (akara)

The method of Ajibola and Filani [16] was adopted with some modifications. Beans (500 g) was cleaned and winnowed to remove dirts, stones and metals. It was soaked for 5 mins, after which it was dehulled by rubbing with hands until the loosed coats were floated off in water. The dehulled cotyledons were soaked in water for 15 min to soften the seeds. It was milled to form a paste. The extract was incorporated into bean paste at 1% concentration according to Adedeji and Ade-omowaye [17].

Preparation of soymilk

The method of Ajibola and Filani [16] was adopted with some modifications. 500g of soybean was cleaned and winnowed to remove dirt, stones and metals. It was boiled for 20 min to remove the beany flavor and to enhance easy dehulling. The soybean was cooled dehulled and drained. It was milled, reconstituted with water and sieved. The filtrate was boiled for 30 min after which the extract was added to soymilk at 1% concentration and thoroughly mixed together.

Preparation of fried fish

Method of Abou-Taleb *et al*., [18] was adopted with some modifications. Whole Tilapia fish was obtained from the market, it was cut into small sizes, gills were removed and the fish was cleaned. It was macerated with the extract at 1% concentration for 1 h, then it was deep fried.

Preparation of dairy milk

Dairy milk (Peak Evaporated Milk) was obtained from the market. The can was opened and poured into a jar with a lid; 800ml of milk was thoroughly mixed with the extract at 1% concentration.

Determination of total viable bacteria

Pour plate method was used. Ten (10) ml of each food sample was aseptically poured into sterile bottles and diluted serially in distilled water up to a 10^{-8} dilution. One ml of each of the 10^{-7} and 10^{-8}dilutions were mixed with nutrient agar in Petri dishes. The agar plates were allowed to solidify and incubated at 37 ºC for 24 - 48 h. The colonies that grew were counted and the values were expressed as colony forming units (cfu)/g [19].

Determination of total fungi count

Pour plate method was used. Ten ml of each sample was aseptically poured into sterile bottles and diluted serially in distilled water up to a 10^{-8} dilution. One ml of each the 10^{-7} and 10^{-8} dilutions were mixed with Potato Dextrose agar in Petri dishes. The agar plates were allowed to solidify and incubated at 25 ºC for 72 h. The colonies that grew were counted and the values are expressed as colony forming units (cfu)/g [19].

RESULTS AND DISCUSSION

Antioxidative activities of pawpaw seed extract

The 1,1- diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activities of the extracts is as shown in Table-1. Petroleum ether extract had the highest antioxidant activity of 42.14%, aqueous extract had 33.28% while n-hexane extract had the least antioxidant activity of 18.85%. This result is different from that of Ng *et al*., [20] which reported a research on the influence of different extraction parameters on antioxidant properties of *Carica papaya* peel and seed. It was discovered that the deionized water extract had the highest antioxidant activity in all the extract concentrations. At the highest concentration, the DPPH of 35.9% was recorded using deionized water extract while the lowest antioxidant activity of 1.4% was from n-hexane extract. Variations in antioxidant concentration and scavenging activities may be found in the extract from the same plant materials due to factors like different degrees of exposure of the plant to sun, specie of the plant and other environmental factors. The yields of hydrophilic and lipophilic antioxidants are dependable on the polarity of the solvent used [21].

Table-1: DPPH (%) of Crude Extracts of *Carica papaya* Seeds

Extracts	% DPPH
Aqueous	33.280
n- hexane	18.850
Petroleum Ether	42.140

Total viable bacteria count of akara treated with pawpaw seed extract

Results of the total viable bacteria and total fungi count of akara treated with pawpaw seed extract is presented in Figure-1. On day zero, which was the day of production; Akara Control Sample (ACS) had a Total Viable Bacteria (TVB) count of $1.48x\ 10^4$cfu/g. It increased significantly ($p<0.05$) to $4.10x10^4$cfu/g after two days of storage. On the fourth day there was a further increase in the growth of bacteria to $6.37x10^4$cfu/g. For the Akara treated with Petroleum ether extract Sample (APS) at day zero, the total viable bacteria count was 1.36×10^4 there was a slight decrease in the TVB to 1.22×10^4cfu/g on day two. However, at day four there was a significant decrease ($p<0.05$) to $1.00\ x10^4$ cfu/g this implied that petroleum ether extract was able to eliminate some of the bacteria so it has a bacteriostatic effect. This is similar to the work of Adesokan *et al.,* [22] who recorded a gradual decrease in the total viable count of *Hisbiscus sabdariffa* drink blended with aqueous extract of ginger and garlic over a storage period of four days.

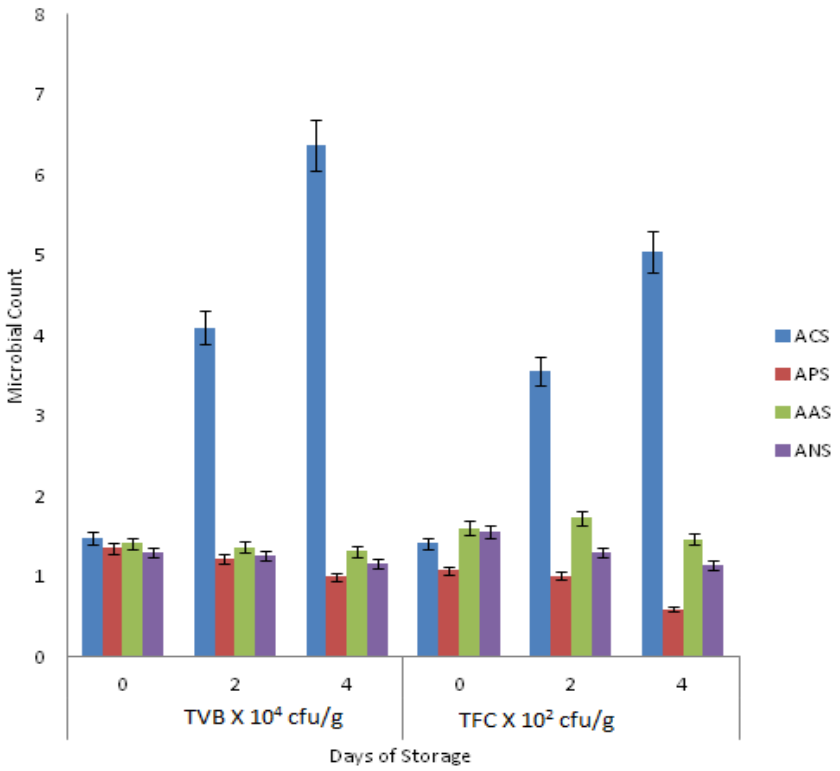

Fig-1: Total Viable Bacteria and Total Fungi Counts of ''Akara'' treated with Pawpaw Seed Extracts

KEY: TVB= Total Viable Bacteria Count, TFC= Total Fungi Count, ACS=Akara Control Sample, APS= Akara treated with Petroleum ether extract Sample, AAS= Akara treated with Aqueous extract Sample, ANS= Akara treated with n-hexane extract Sample

The total viable bacteria of Akara treated with Aqueous extract Sample (AAS) was 1.42 x10^4cfu/g on day zero however, on day two, there was a slight decrease to 1.37x 10^4cfu/g and on day four, the TVB further decreased to 1.32 x10^4cfu/g. This in an indication that aqueous extract of *Carica papaya* seed was able to retard the growth of bacteria. Akara treated with N-hexane Sample (ANS) had an initial TVB of 1.30x 10^4cfu/g on day zero but there was a decrease to 1.26 cfu/g on day two and on day four the value of TVB further decreased to 1.17cfu/g. This establishes that n-hexane extract of *Carica papaya* seed also has a bacteriostatic effect. This is similar to the work of [23] recorded a significant decrease in the total aerobes and total anaerobes of "Wara" that was treated with *Terminalia cattapa* seed extract, *Carica paaya* seed extract and Nisin and stored over a period of three weeks.

Total fungi count of akara treated with pawpaw seed extracts

There was a progressive increase in the Total Fungi Count (TFC) of the control sample on day zero from 1.42x10^2cfu/g to 3.56x10^2cfu/g on day two, and to 5.05 x10^2cfu/g on day four. For Akara treated with Petroleum ether Sample (APS) the TFC was 1.08 x10^2cfu/g on day zero, there was a slight decrease on day two to 1.01x 10^2cfu/g and a significant ($p<0.05$) decrease to 0.6 x10^2cfu/g on the fourth day of storage. This suggests that petroleum ether extract of *Carica papaya* seed has a fungicidal effect, that is, it was able to retard some fungi. Akara treated with Aqueous Extract (AAS) had an initial TFC of 1.08x 10^2cfu/g, there was an increase to 1.73x10^2cfu/g. This may be because the fungi were initially resistant however, on the fourth day there was a decrease to 1.47x10^2 cfu/g. For Akara N-hexane Sample (ANS) the initial TFC was 1.57 x10^2cfu/g there was a gradual decrease to 1.30x 10^2 cfu/g on the second day of storage and a further decrease to 1.15 x10^2 cfu/g on the fourth day of storage which implied that n-hexane extract of *Carica papaya* seed has a fungicidal property.

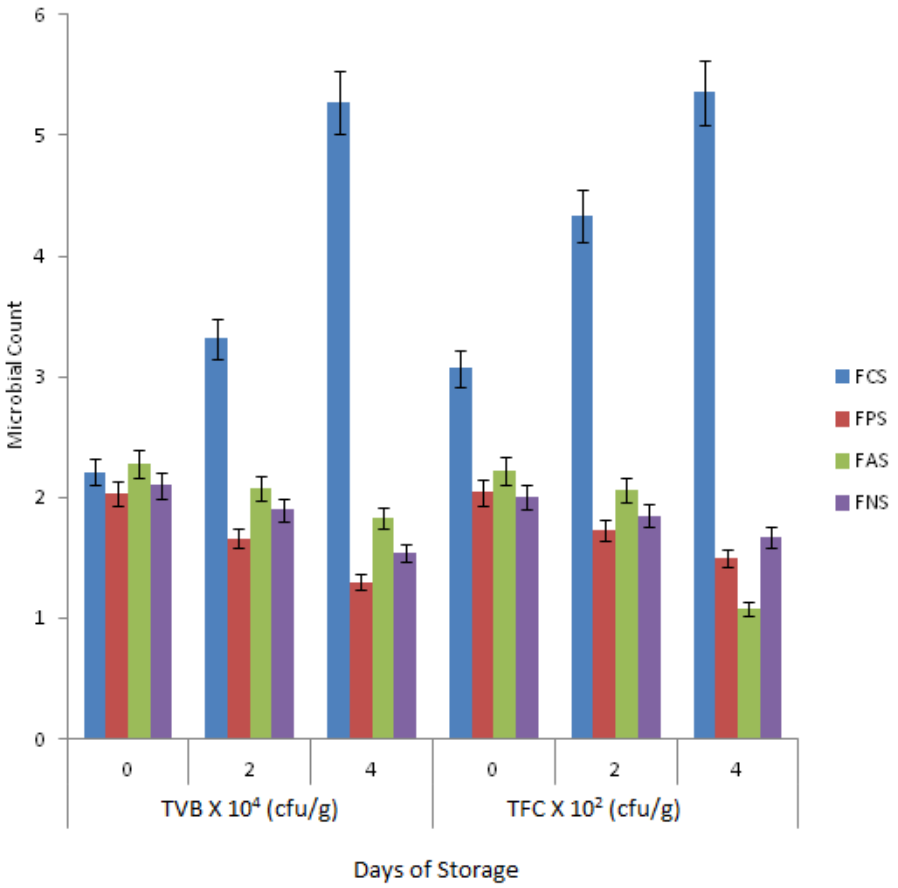

Fig-2: Total Viable Bacteria and Total Fungi Counts of Fish treated with Pawpaw Seed Extracts
Key: TVB=Total Viable Bacteria, TFC= Total Fungi Count, FCS= Fish Control Sample, FPS= Fish treated with Petroleum ether extract Sample, FAS= Fish treated with Aqueous extract Sample, FNS= Fish treated with n-hexane extract Sample

Total viable bacteria of fish treated with pawpaw seed extract

Total Viable Bacteria (TVB) count in day zero of fried Fish Control Sample (FCS) increased significantly ($p<0.05$) from 2.21x 10^4 to 3.32x10^4cfu/g after two days of storage. On the fourth day, there was a further increase in the growth of total viable bacteria to 5.2x10^4cfu/g.

For the Fish sample treated with petroleum ether extract (FPS), in day zero, the total viable bacteria count was 2.03 x 10^4cfu/g, there was a significant decrease in the TVB to 1.66 x 10^4 cfu/g on day two, furthermore, in day four, there was a significant decrease ($p<0.05$) to 1.30 x10^4cfu/g, this implied that petroleum ether extract was able to retard the growth of some of the bacteria so it has a bacteriostatic effect. For Fish-Aqueous extract Sample (FAS), there was also a significant decrease in TVB from 2.28 x10^4cfu/g to 2.08x 10^4cfu/g after two days and on day four there was a further decrease to 1.83x 10^4cfu/g. Fish n-hexane Sample (FNS) recorded a gradual decline from 2.10 x 10^4cfu/g to 1.90 x10^4 cfu/g and a further decrease to 1.54 x10^4 cfu/g. The decreasing trend may be as a result of the potency of the extracts in retarding the growth of viable bacteria in fish and in some cases eliminating the bacteria. Oyelese and Oyedokun [24] carried out a research on the microbial load of some common hot smoked freshwater fish species using different packaging materials. The report shows that the total viable count for Niloticus dropped from 10.6 to 8.4x10^4cfu/g (fresh state- hot smoked) and *M.rume* (9.8 -7.0 x 10^4) cfu/g while *C. gariepinus* slightly increased from 12.4 to 12.6 x 10^4cfu/g over a storage period of 12 weeks.

Total fungi count of fish treated with pawpaw seed extract

The Total Fungi Count (TFC) for the Fish Control Sample (FCS) on day zero was 3.07 x10^2cfu/g it increased to 4.33 x10^2 cfu/g in day two and to 5.36 x10^2 cfu/g in day four. This is because the sample was not treated. However, for the Fish Petroleum ether Sample (FPS) there was gradual decrease in the fungi count from 2.04x10^2cfu/g on day zero to 1.73x10^2cfu/g on day two, there was a further decrease to 1.50 x10^2 cfu/g on day four. This showed that petroleum ether extract of *Carica papaya* seed was effective in

preventing the growth of fungi and it also reduced some of the fungi. The fish aqueous sample also recorded a gradual decrease in the Total Fungi Count (TFC) from 2.22 $x10^2$cfu/g on day zero to 2.06 $x10^2$cfu/g on day two and finally to 1.80 $x10^2$cfu/g on day four. This implies that aqueous extract of *Carica papaya* seed also has a fungicidal effect though not as potent as the petroleum ether extract. Similarly, there was a decrease in fungi count of Fish n-hexane extract Sample (FNS) from $2.00x10^2$ to 1.67 $x10^2$cfu/g after four days of storage. This also indicates that n-hexane extract has a fungicidal property. However, FPS had the least total fungi count which indicated that petroleum ether extract was more effective in eliminating fungi.

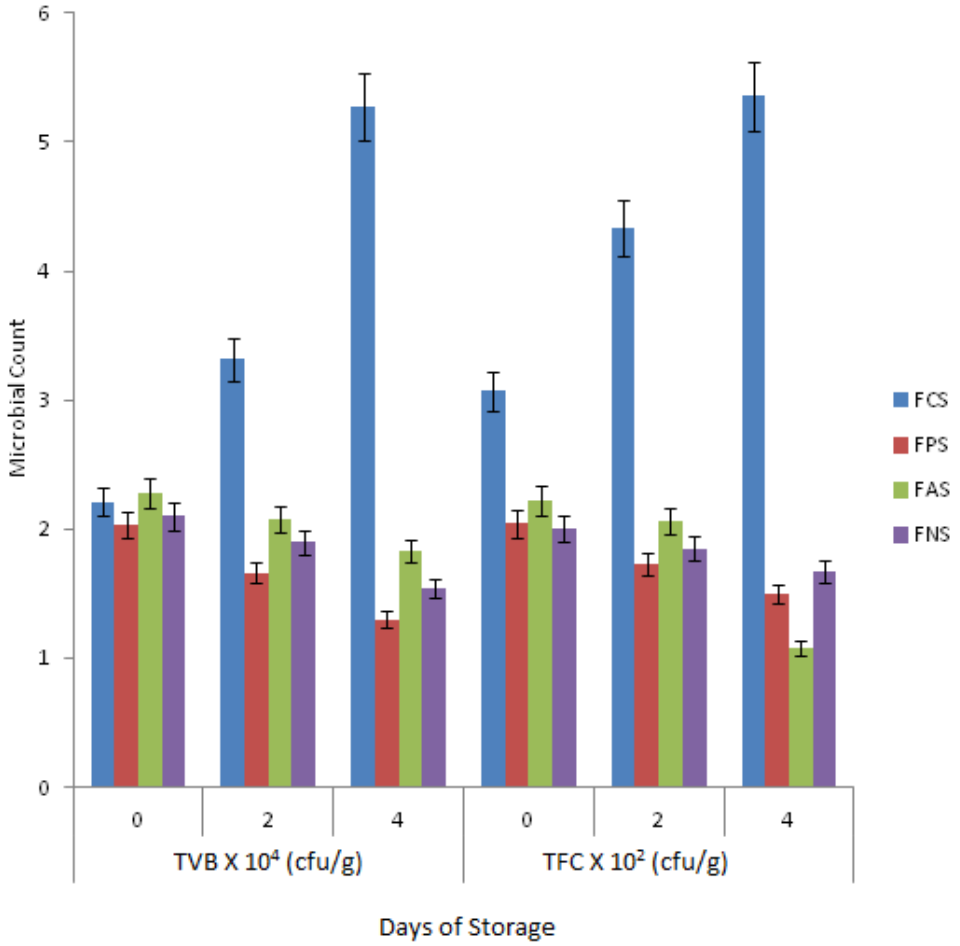

Fig-3: Total Viable Bacteria and Total Fungi Counts of Soymilk treated with Pawpaw Seed Extracts
Key: TVB= Total viable Bacteria, TFC=Total Fungi Count, SCS= Soymilk Control Sample, SPS= Soymilk treated with Petroleum ether extract Sample, SAS= Soymilk treated with Aqueous extract Sample, SNS= Soymilk treated with n-hexane extract Sample

Total Viable Bacteria and Total Fungi Counts of Soymilk treated with Pawpaw Seed Extracts

Total Viable Bacteria count (TVB) of soymilk sample treated with pawpaw seed extracts is shown on Figure-3. The TVB of Soymilk Control Sample (SCS) increased significantly ($p<0.05$) from 2.57x 10^4 s to 6.60 $x10^4$cfu/g after two days of storage, on the fourth day there was a further increase in the growth of total viable bacteria 9.28 $x10^4$cfu/g. For the Soymilk Petroleum ether extract Sample (SPS), there was a significant decrease in the TVB. The TVB decreased from 2.13 x 10^4cfu/g on day zero to 1.70 x 10^4 cfu/g on day two. It decreased further significantly ($p<0.05$) to 1.43 $x10^4$cfu/g on day four. This implied that petroleum ether extract was able to reduce some of the bacteria so it has a bacteriostatic effect.

For Soymilk Aqueous extract Sample (SAS) there was also a significant decrease in TVB from 2.10 $x10^4$ to 1.90 x 10^4cfu/g after two days with a further decrease to 1.72 $x10^4$cfu/g on day four. Soymilk -n-hexane Sample (SNS) recorded a gradual decreased from 2.37 x 10^4 to 1.75 $x10^4$cfu/g and a further decrease to 1.56 $x10^4$cfu/g. The decreasing trend may be as a result of the potency of the extracts in retarding the growth of viable bacteria in soymilk. This is similar to the work of Kabiru *et al.*, [25] who recorded decrease in the total bacteria count of soymilk samples that were

treated with cloves and guinea pepper. The samples were stored for 12days, during the storage period, there was a significant bacterial and fungal growth in the control sample than in the treated sample and combination of cloves and guinea pepper extracts was able to reduce microbial growth effectively than when the extracts were used singly.

Total fungi count of soymilk treated with pawpaw seed extract

The Soymilk Control Sample (SCS) increased in fungi count throughout the four days of storage. The total fungi count increased from 3.90 $x10^2$cfu/g in day zero to 6.27 $x10^2$cfu/g in day two and then to 9.61 $x10^2$cfu/g in day four. This is expected because the control sample was untreated. However, for the Soymilk treated with Petroleum ether Sample (SPS) there was gradual decrease in the fungi count from 2.00 $x10^2$cfu/g in day zero to 1.25$x10^2$cfu/g in the day two and a further decrease to 0.80 $x10^2$cfu/g in day four. This showed that petroleum ether extract of *Carica papaya* seed was effective in preventing the growth of fungi and it also reducing some of the fungi. The soymilk aqueous sample also recorded a gradual decrease in the Total Fungi Count (TFC) from 2.50 $x10^2$ cfu/g on day zero to 2.17 $x10^2$cfu/g on day two and finally to 1.79 $x10^2$cfu/g on day four. This implied that aqueous extract of *Carica papaya* seed also has a fungicidal effect though not as potent as the petroleum ether extract. Similarly, there was a decrease in fungi count of Soymilk N-hexane extract Sample (SNS) from 2.86$x10^2$ to 1.20 $x10^2$ cfu/g after four days of storage. This also indicates that n-hexane extract has a fungicidal property. This result is in agreement with the result obtained by Kabiru *et al.*, [25] who recorded decrease in the total fungi count of soymilk samples that were treated with cloves and guinea pepper.

Total Viable Bacteria Counts of diary milk treated with Pawpaw Seed Extracts

Milk Control Sample (MCS) had a Total Viable Bacteria (TVB) count of 2.60 x 10^4cfu/g in day zero. It increased significantly ($p<0.05$) to 4.33$x10^4$cfu/g after two days of storage and it further increased to 6.82 $x10^4$cfu/g on the fourth day. For the Milk treated with Petroleum ether extract Sample (MPS), the total viable bacteria count was 2.16 x 10^4cfu/g on day zero, there was a decrease in the TVB to 1.82 x 10^4 cfu/g on day two.

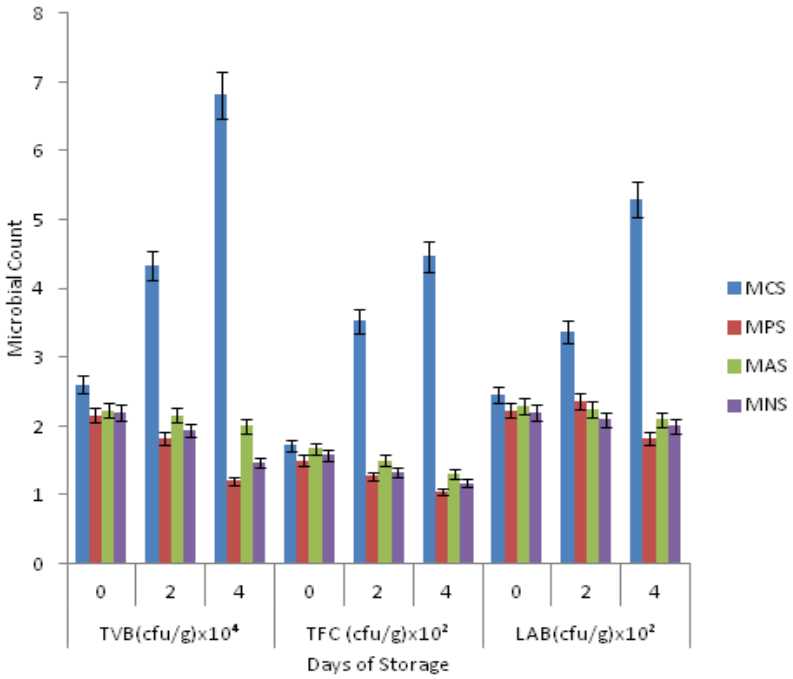

Fig-4: Total Viable Bacteria and Total Fungi Counts of dairy milk treated with Pawpaw Seed Extracts
Key: TVB= Total viable Bacteria, TFC=Total Fungi Count, MCS= Milk Control Sample, MPS= Milk treated with Petroleum ether extract Sample, MAS= Milk treated with Aqueous extract Sample, MNS= Milk treated with n-hexane extract Sample

However, day four recorded a significant decrease ($p<0.05$) to 1.20 $x10^4$cfu/g. This implied that petroleum ether extract was able to reduce some of the bacteria so it is bacteriostatic. This is similar to the the work of Badmos *et al.*, [26] who worked on the effect of crude leaf extracts of *Moringa oleifera* on the bacterial, nutritional and sensory properties of West African soft cheese. The control Cheese increased in bacterial count throughout the three days of storage. It was observed that 3% ethanol extract showed the most significant inhibition of the bacterial population compared with the other treatments. The total viable

bacteria of Milk Aqueous extract Sample (MAS) was 2.23 x10^4cfu/g (on day zero), it decreased to 2.16 x 10^4cfu/g (on day two) it further decreased to 2.00 x10^4cfu/g on day four. This in an indication that aqueous extract of *Carica papaya* seed was able to retard the growth of bacteria. Milk n-hexane Sample (MNS) had an initial TVB of 2.20 x 10^4cfu/ on day zero but there was a decrease to 1.94 x10^4cfu/g (on day two) and a further decrease to 1.47 x10^4cfu/g in the fourth day. This establishes that n-hexane extract of *Carica papaya* seed also has a bacteriostatic effect. This is similar to the work of [23] that recorded a significant decrease in the total aerobes and total anaerobes of "Wara" which was treated with *Terminalia cattapa* seed extract, *Carica papaya* seed extract and Nisin stored for over a period of three weeks without spoilage.

Total fungi of dairy milk treated with pawpaw seed extract

There was a progressive increase in the Total Fungi Count (TFC) in the control sample on day zero (1.72x10^2) cfu/g to 3.53 x10^2 cfu/g (day two) with further increase to 4.47 x10^2 cfu/g on day four. For Milk treated with Petroleum ether Sample (MPS), the TFC was 1.50 x10^2 cfu/g (day zero), there was a slight decrease on day two to 1.27 x 10^2 cfu/g (day two) and a significant ($p<0.05$) decrease to 1.05 x10^2 cfu/g on day four of storage time. There was a progressive decrease in the TFC of the Milk Aqueous extract Sample (MAS) from 1.67 x10^2 cfu/g to 1.50 x 10^2 cfu/g on day two, and 1.30 x10^2 cfu/g on day four. A similar trend was observed in the Milk n-hexane Sample (MNS). There was a gradual decrease in the TFC for MNS from 1.58 x 10^2cfu/g to 1.17 x 10^2cfu/g over a storage period of four days.

Lactic acid bacteria count of milk treated with pawpaw seed extract

The Lactic Acid Bacteria (LAB) count of the control sample (MCS) was 2.45 x 10^2cfu/g on day zero, thereafter was a significant ($p<0.05$) increase to 3.37 x10^2cfu/g on day two and a further increase to 5.30 x10^2cfu/g on the fourth day. Milk treated with Petroleum ether Sample (MPS) had a LAB count of 2.23x 10^2cfu/g there was an initial increase to 2.37 x10^2 cfu/g on day two but on day four a noticeable decrease to 1.83 x 10^2cfu/g was recorded. The initial increase in LAB count may be as a result of some intrinsic conditions that favoured the growth of LAB which was later subdued by the extract. The LAB count for MAS was 2.30 x 10^2 cfu/g on day zero but the value decreased gradually to 2.10 x10^2 cfu/g after the four days of storage. The lactic acid bacteria count of Milk n-hexane extract Sample (MNS) was 2.20 x10^2cfu/g (day zero), it reduced gradually to 2 x 10^2cfu/g on the fourth day of storage. This implied that all the extracts inhibited the growth of lactic acid bacteria but petroleum ether extract of *Carica papaya* seed had the highest inhibitory potency.

CONCLUSION

The results obtained from this study shows that petroleum ether extract had the highest antioxidant activity (39.14%). The microbiological studies revealed that, all the extracts had the potential to retard microbial growth but petroleum ether extract exhibited more antimicrobial activity because samples treated with petroleum ether extract recorded the least bacteria and fungi growth. Therefore, it is a good antimicrobial agent.

REFERENCES

1. Smid, E. J., & Gorris, L. G. M. (1999). Natural Antimicrobials for Food Preservation. In: Handbook of Food Preservation (ed. M.S. Rahman). Marcel Dekker, New York, 285-308.
2. Beuchat, L. R. (1994). Antimicrobial Properties of Spices and their Essential Oils. In: Natural Antimicrobial Systems and Food Preservation (eds. Y.M. Dillon and R.G. Board). CAB International, Oxon, 167-179.
3. Akande, E. A., Olunlade, B. A., & Adesola, M. O. (2014). Effect of Blanching on the Qualities of Negro Pepper. Global Advanced Research. *Journal of Food Science and Technology*, 3(3): 99-102.
4. Vainio, H., & Bianchini, F. (2003). Fruits and Vegetables. ARC, 2.
5. Duraipandiyan, V., Ayyanar, M., & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC complementary and alternative medicine*, *6*(1), 35-44.
6. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *8*(1), 1-10.
7. Onibon, V. O., Abulude, F. O., & Lawal, L. O. (2007). Nutritional and anti-nutritional composition of some Nigerian fruits. *Journal of Food Technology*, *5*(2), 120-122.
8. Chakravarthy, P. K., & Acharya, S. (2012). Efficacy of extrinsic stain removal by novel dentifrice containing papain and bromelain extracts. *Journal of Young Pharmacists*, *4*(4), 245-249.
9. Oyelola, O. (2005). Evaluation of the Hypoglycemic Activity of Trequila Africana Deena (root) in normal and diabetic rats. M.Pharm (Clinical Pharmcy) Dissertation. University of Ibadan Nigeria.
10. Oyeleke, G. O., Isola, A. D., Salam, M. A., & Ajao, F. D. (2013). Evaluation of some chemical composition of pawpaw (Carica papaya) seeds under normal storage ripening. *Journal of Environmental Science, Toxicology and Food Technology*, 18-21.

11. Jari, S. (2009). Papayas are Yummy easy to grow University of Hawaiti-Manoa College of Tropical Agric and Human Resources.
12. Fakeye, T. O., Tijani, A., & Adebisi, O. (2008). A survey of the use of herbs among patients attending secondary-level health care facilities in Southwestern Nigeria. *Journal of herbal pharmacotherapy*, *7*(3-4), 213-227.
13. Afolabi, I. S. (2011). Physicochemical and nutritional qualities of Carica papaya seed products. *Journal of Medicinal Plants Research*, *5*(14), 3113-3117.
14. Dakare, M. A., Ameh, D. A., & Agbaji, A. S. (2011). Biochemical assessment of 'Daddawa'food seasoning produced by fermentation of pawpaw (Carica papaya) seeds. *Pakistan Journal of Nutrition*, *10*(3), 220-223.
15. Ansari, N. M., Houlihan, L., Hussain, B., & Pieroni, A. (2005). Antioxidant activity of five vegetables traditionally consumed by south- Asian migrants in Bradford, Yorkshire, UK. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *19*(10), 907-911.
16. Ajibola, C. F., & Filani, A. (2015). Storage stability of deep-fried cowpea products (akara) incorporated with soy-flour and Aframomum danielli. *British Journal of Applied Science and Technology*, 8(2), 204-212.
17. Adedeji, T. O., & Ade-Omowaye, B. I. O. (2013). The preservative effects of two local Nigerian spices on the shelf life of fried bean cake snacks. *Journal of Nutrition and Food Science*, *3*(2), 188.
18. Abou-Taleb, M., El-Sherif, S. A., & Elhariry, H. (2007). Preservation effect of four plant extracts used to extending the shelf-life of mullet fish fillets during cold storage. *World Journal of Dairy & Food Sciences*, *2*(2), 74-82.
19. Trease, G. E., & Evans, W. C. (2002). Pharmacognosy. WB Saunders Company Ltd. UK.
20. Ng, L. Y., Ang, Y. K., Khoo, H. E., & Yim, H. S. (2012). Influence of different extraction parameters on antioxidant properties of Carica papaya peel and seed. *Research Journal of Phytochemistry*, *6*(3), 61-74.
21. Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y., & Perry, N. B. (2007). Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures. *Food chemistry*, *101*(4), 1417-1424.
22. Adesokan, I. A., Abiola, O. P., Adigun, M. O., & Anifowose, O. A. (2013). Analysis of Quality Attributes of *Hibiscus sabdariffa* (Zobo) Drinks Blended with Aqueous Extract of ginger and garlic. *African Journal of Food Science*, 7(7): 174-177.
23. Adetunji, V. O. (2011). Effect of Packaging Treatments and Storage Conditions on the Survivability of Aerobes and Anaerobes in Vacuum Packaged 'Wara' a Soft White Cheese. *Advance Journal of Food Science and Technology*, 3(4): 289-293.
24. Oyelese, O. A., & Oyedokun, J. O. (2013). Microbial Load (Bacteria, Coliform and Mould Count/Flora) of Some Common Hot Smoked Freshwater Fish Species Using Different Packaging Materials. *Food and Nutrition Sciences*, *4*(12), 1201-1208.
25. Kabiru, Y. A., Makun, H. A., Saidu, A. N., Muhammad, L. H., Nuntah, L. C., & Amoo, S. A. (2012). Soymilk preservation using extracts of Cloves (Syzygium Aromaticum Myrtaceae) and Guinea- pepper(Xylopia Aethiopica Annonaceae). *IOSR Journal of Pharmacy and Biological Sciences*, *3*(5), 44-50.
26. Badmos, A. H. A., El-Imam, A. M. A., & Ajiboye, D. J. (2014). The effect of crude leaf extracts of Moringa oleifera on the bacterial, nutritional and sensory properties of West African soft cheese. *Wayamba Journal of Animal Science*, *1*, 939-946.