

ANTIMICROBIAL ACTIVITY OF EXTRACT OF PAWPAW (*Carica papaya*) SEEDS ON SOME SELECTED MICRO-ORGANISMS ISOLATED FROM SPOILT TOMATO (*Lycopersicon esculentum*)

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Abstract

The Anti-bacterial and Anti-fungal activities of cold water, hot water and alcoholic extracts of seeds of pawpaw against some selected Micro-organisms isolated from tomato were investigated in this work. The agar well diffusion method was used for testing for the Anti-microbial activity of the extracts. The findings showed that fungi were not sensitive to the extracts at low concentrations of 60 to 100mg/ml unlike at higher concentrations of 300 to 400mg/ml. *Pseudomonas aeruginosa* was observed to be the most sensitive and susceptible bacterium to the extracts at 400mg/ml of aqueous cold, hot and ethanol extract with 18mm, 16mm and 30mm diameter of zone of inhibition. The minimum inhibitory concentration value for ethanol, aqueous cold, aqueous hot extracts ranges between 45mg/ml – 90mg/ml while the minimum bactericidal concentration value for ethanol, aqueous cold, aqueous hot extracts ranges between 22.5mg/ml – 90mg/ml on *Pseudomonas aeruginosa* and *E. cloacae*. The minimum inhibitory concentration value for *Aspergillus flavus* was found to be 90mg/ml for ethanol extract.

Keywords: pawpaw, tomato, antimicrobial, ethanol and water.

Introduction

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey *et al.*, 2011). It is estimated that there are 250,000 to 500,000 species of plants on earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). Many plants species reported to have pharmacological properties as they are known to possess secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids and terpenes which therefore should be utilized to combat the disease causing pathogens (Kamali *et al.*, 2010).

Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Martin, 1995). Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or alcohol extractions and can be followed by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides (e.g., starch) and polypeptides, including fabatin (Zhang and Lewis, 1997) and various lectins, are commonly more effective as inhibitors of pathogen (usually virus) adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents.

Many infectious diseases are treated with chemotherapeutic agents such as antibiotics that inhibit or kill the pathogen while harming the host as little as possible. Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes (Willey *et al.*, 2008).

Haslam *et al.*, (1989) reported that plant extracts and their products are used in many parts of the world as the active principles in herb remedies. They are used locally in the treatment of infections, many centuries before scientific studies were discovered. The discovery of penicillin “the magic drug” led to large scale screening of higher plants for more effective antibiotics.

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Pawpaw (*Carica papaya*) belongs to the family Caricaceae with over 22 species and only one member of the genus *Carica*. In Nigeria, Pawpaw is one of the most popular, cheapest, economically, important fruit tree grown and consumed for its nutritional content (Baiyewu and Amusa, 2005). Pawpaw (*C. papaya*) is short-lived, with a hollow unbranched herbaceous stem. It is dual or multipurpose early bearing, space conserving crop. Papaya may be monoecious or hermaphrodite. Female trees have been found to differ in the amounts of compounds produced. For instance, Phenolic compounds tend to be higher in male plants than female plants.

The papaya seeds are very pungent and peppery making them almost unpalatable. However the seeds seem to have more potent medicinal values than the flesh. Papaya seeds have antibacterial properties and are effective against *E. coli*, *Salmonella*, and *Staphylococcus* infections. Papaya seeds may protect the kidneys from toxin induced kidney failure. Papaya can eliminate intestinal parasites. Cure for piles and typhoid and anti-helminthic and anti-amoebic properties (Aravind et al., 2013). According to the reports of Okeniyi et al., (2007) *C. papaya* fruit and seeds have anti-helminthic and anti-amoebic activities. The seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. It could be used in urino-genital disorder like Trichomoniasis with care to avoid toxicity (Calzada et al., 2007). The seeds irrespective of its fruit maturity stages have bacteriostatic activity on Gram positive and Gram negative organisms which could be used in treating chronic skin ulcer (Musa et al., 2011).

Tomatoes (*Lycopersicon esculentum*) belong to the family Solanaceae (Night shade family). They are seasonal crops and this has made their microbiological study necessary so as to identify their spoilage organisms. Added to their nutritive value, is their medical importance, inspectional effect as well as economic important (Frederick, 1983). Colonization of fungi is a critical phase in the microbial spoilage of post harvested fruits (Baiyewu et al., 2007). The colonization process involves the ability of microorganism (fungi) to establish within the produce (host). Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents. The higher the pH (near neutrality) and moisture contents are associated with their greater predisposition to fungal spoilage. The occurrence of fungal spoilage of fruits is also recognized as a source of potential health hazard to man and animals (Effiuvwevwere, 2000).

Many pathogen comprising bacteria, fungi, viruses and nematodes can attack tomato plants and cause diseases. Some of the more important bacterial diseases and their causal organism are: Bacterial wilt (*Pseudomonas solanaceum*), Bacterial canker (*Corynebacterium michiganense*), Bacterial spot (*Xanthomonas campestris*), Bacterial speck (*Pseudomonas tomatoe*), Bacterial spot (*Xanthomonas tomatoe*), Bacterial soft rot (*Erwinia carotovora*). Thus, the average lives of fresh cut fruits and vegetables are typically 10-14 days (Cantwell and Suslow, 2002).

Justification of Research

This research was conducted to determine the possible effects of extracts of *Carica papaya* seeds on some selected microorganisms isolated from spoilt tomato. In other words, this would be used in determining if the extract has bacteriostatic, bacteriocidal, fungistatic or fungicidal activity on the microorganisms.

The objectives of this research were to:

- (i) Isolate microorganisms involved in tomato spoilage
- (ii) Identify the microorganisms to specie level
- (iii) Determine the antibacterial and antifungal potency of extract of *Carica papaya* seeds in different solvents and at different concentrations, against a spectrum of microorganisms isolated from spoilt tomato.
- (iv) Also focus on the phytochemical potentials of the extracts from *C. papaya* seeds.

Materials and Methods

Collection of plant materials

Ripe *Carica papaya* (Pawpaws) was purchased in abundance from Oja-oba market in Ilorin, Kwara state, Nigeria. The pawpaws were washed with sterile distilled water, sliced with a clean knife and cut into halves for easy collection of the seeds. The seeds were washed with sterile distilled water and air dried. The seeds were later grinded with a clean blender into powder and kept in an airtight glass container in a refrigerator at 4°C until required for use.

Healthy *Lycopersicon esculentum* (Tomatoes) were purchased at Oja-oba market in Ilorin, Kwara state, Nigeria. The Tomato fruits were rinsed with distilled water and kept in the microhumidity chamber to induce spoilage.

Preparation of seed extracts

Aqueous and ethanolic extracts of the pawpaw seeds were prepared.

Aqueous extraction (Cold water)

The seed extract was prepared as described by Peter et al., (2014) with slight modifications. Fifty (50) grams of the powdered seeds was soaked in 500ml of sterile distilled water. The mixture was extracted after agitation on a rotary shaker for 48 hours at 100 revolutions per minute. The extract was filtered with cotton gauze inserted in a

filter funnel. The extract was then air dried using a water bath at 40°C and dissolved in 30% Dimethyl Sulphoxide (DMSO) to form different concentrations of 100, 200, 300, and 400 mg/ml. The standard extract obtained was stored in a refrigerator at 4°C until required for use.

Aqueous extraction (Hot water)

Fifty (50) grams of the powdered seeds was soaked in 500ml of hot sterile distilled water boiled for 30 minutes and agitated on the rotary shaker for 48 hours. The extract was filtered using cotton gauze inserted in a clean filter funnel. The filtrate was air dried using a water bath at 80°C, according to Oyagade *et al.*, (1999). The crude extract was dissolved in 30% Dimethyl Sulphoxide (DMSO) to form different concentrations of 100, 200, 300, and 400 mg/ml. The standard extract obtained was stored in a refrigerator at 4°C until required for use.

Ethanolic Extraction

Fifty (50) grams of the powdered seeds was soaked in 500ml of 70% ethanol and agitated on the rotary shaker at 100 revolutions per minute for 48 hours at room temperature. The content was filtered using cotton gauze inserted in a clean filter funnel and the extract collected was dissolved in 30% Dimethyl Sulphoxide (DMSO) to form different concentrations of 100, 200, 300, and 400 mg/ml. The standard extract obtained was stored in a refrigerator at 4°C until required for use.

Sterility of seed extracts

Each of the above extracts (aqueous and ethanolic extract) was tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on nutrient agar and incubated at 37°C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that the extracts were sterile.

Isolation and maintenance of test organisms

The organisms were isolated from the spoilt tomato sample. The fresh tomato sample was aseptically washed with sterile distilled water carefully to avoid wounding and was sterilized using 70% ethanol. The fruit was placed in a sterile micro-humidity chamber to provide moist environment so as to induce spoilage of the tomato samples for 7 days. For fungal isolation, direct isolation method was used. The diseased portions of the tomato fruit showing different visible growth were carefully picked, each using sterile inoculating needle on already prepared sterile Potato dextrose agar plate. The plates were incubated uprightly at room temperature and the plates were daily observed for fungi growth for 72 hours. The fruit sample was then grounded using a sterile mortar and pestle. Sterile dilutions were carried out by introducing 1ml of the sample with the aid of a sterile syringe into a test tube containing 9ml of sterile distilled water to make a 10^{-1} dilution. This was repeated for four more test tubes containing 9ml of sterile distilled water to make 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions respectively and 1ml was transferred from the 10^{-2} dilution to 10^{-5} dilutions each onto the labelled sterile petri dishes respectively. Molten sterile nutrient agar at 45- 47°C was poured on it, mixed gently by swirling to attain uniformity and allowed to solidify. The plates were incubated at 37°C for 24-48 hours in the incubator. Pure cultures were obtained from the mixed cultures by sub-culturing each distinct colony three times and this was done by aseptically streaking each bacteria colony on sterile Nutrient agar plate, using sterile inoculating loop; Each fungus colony was inoculated using a sterile inoculating needle onto sterile Potato dextrose agar plate, then incubated at 37°C for 24-48 hours and room temperature respectively. The pure cultures obtained were transferred onto Nutrient agar slants for bacteria isolates and Potato dextrose agar slant for fungi isolates. Samples for identification tests were taken from the agar slants. The slants were kept in refrigerator and maintained at 4°C.

Preparation and standardization of Inocula

Preparation and standardization of each isolate was done using the modified method described by Bauer *et al.*, (1966). This was carried out by picking test organism growing on agar slant and then transferred into sterile nutrient broth and incubated for 18-24 hours to grow. Each standardized inoculum was used for antimicrobial test.

Phytochemical screening test for the extract

A small portion of the dry extract was subjected to the phytochemical test using standard procedure (Odebiyi and Sofowora, 1978).

Test for saponins

The seed extracts was shaken well with water and observed for the formation of foam which was stable for 15 minutes indicated a positive result.

Test for tannins

The seed extracts was mixed with basic lead acetate solution and gave white precipitate indicating the presence of tannins.

Test for terpenoids (Salkowshi test)

Two millilitres (2 mls) of the ethanolic extract of seeds was dissolved in 2mls of chloroform and evaporated to dryness. Two millilitres of concentrated sulphuric acid was then added and heated for about 2 minutes.

Development of greyish colour indicated the presence of terpenoids.

Test for alkaloid (Hager's test)

The solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow colour precipitate shows a positive result for the presence of alkaloids.

Test for flavonoids (Ferric chloride test)

The test solution was treated with few drops of ferric chloride solution which resulted in the formation blackish red colour indicating the presence of flavonoids.

Test for glycosides (Liebermann's test)

Two millilitres (2ml) of the extract was dissolved in 2ml of chloroform and then 2ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was added carefully. A colour change from violet to blue to green indicated the presence of glycoside.

Test for carotenoids

One gram (1g) of the sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Antimicrobial Susceptible Testing***Determination of Antibacterial activities of extracts***

The antibacterial effect of extracts of *Carica papaya* seeds on the selected organisms was determined using the Agar well diffusion method described by Alabi *et al.*, (2012) with slight modifications. The bacterial isolates were first cultured in sterile Nutrient broth for 18 hours before use. The isolates were sub-cultured by streaking method using sterile cotton swab stick to inoculate the organisms onto separate solidified sterile Nutrient agar plates and were then labelled accordingly. The plates were cultured at 37°C for 15 minutes after which a sterile 6mm cork borer was used to punch holes (wells) in the inoculated agar. The agar plugs were carefully removed using sterile forceps. The various concentrations: 100mg/ml, 200mg/ml, 300mg/ml, and 400mg/ml of the reconstituted seed extracts were introduced into the wells using sterile 2ml syringes. This was done for each of the concentrations for both Aqueous (hot and cold) and Ethanolic seed extracts. They were then left on the bench for 1 hour for adequate diffusion of the extracts and thereafter were incubated at 37°C for 24 hours. Control experiments were also set up with crude extract used as positive control and sterile distilled water as negative control. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimetre along two axes (i.e. 90° to each other) and the mean of the two readings was then calculated and the diameter of the cork borer (6mm) was removed. The assay was done in triplicates and the zone of inhibition expressed as mean. The presence of these zones around the well was taken as evidence of antibacterial activity of extracts.

Determination of Antifungal activities of extracts

The modified method described by Alabi *et al.*, (2012) was used in determining the antifungal activities of the extracts on the test organisms. Active cultures for the experiments were prepared by seeding a loopful of fungi into Potato dextrose broth and incubated without agitation for 48 hours at room temperature. The disc diffusion method was also used to screen for antifungal properties. Potato dextrose agar plates were inoculated with different fungi culture using sterile cotton swabs and streaked. The plate was incubated at room temperature for 15 minutes. A sterile 6mm cork borer was used to make ditches on each plate and the agar plugs were carefully removed by use of sterile forceps. Each well was filled with different concentrations of the various extracts. The plates were incubated at 25°C for 72 hours and the diameter of the zone of inhibition was measured. Control experiments were also set up with crude extracts and sterile distilled water as positive and negative controls respectively.

Antimicrobial activities of the extracts at lower concentrations

The method described by Alabi *et al.*, (2012) which was slightly modified was used in determining the antimicrobial susceptibility of the extracts at lower concentrations on bacteria and fungi isolates following the same procedure earlier used.

The various concentrations used were 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 160mg/ml, 170 mg/ml, 180 mg/ml and 190 mg/ml. The agar well diffusion method was used to assay the different concentrations of the extracts on bacteria and fungi isolates and incubated at 37°C for 24 hours and room temperature (25°C) for 72 hours respectively. The diameter of the zones of inhibition were recorded and the results were compared with the higher concentrations formerly used. From this result, the concentrations to be used in determining the Minimum inhibitory concentration were derived.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration where no visible turbidity is observed in the test tube. MIC was determined by the standard method of Baker and Breach (1980). Nutrient broth and Potato dextrose broth were prepared and sterilized in an autoclave. One millilitre of the prepared broth was dispensed into sterile test tubes labelled 1-9 using a sterile syringe and needle. Stock solutions of 90 mg/ml were prepared for each of the extracts. Then one millilitre was dispensed into the test tube labelled 1. Subsequently, from tube 1 serial dilution was carried out and one millilitre from tube one was transferred up to tube seven and the one millilitre from tube seven were discarded. Tube eight was control for sterility of the medium and tube nine was for viability of the organisms. An overnight culture of the organisms was prepared in sterile Nutrient broth and Potato dextrose broth. One millilitre of each of the inoculum was transferred into each tube from 1-9 with the exception of tube eight, to which another sterile broth was added. The final concentration of the plant extract in each of the tubes numbered 1-7 after dilution 90, 45, 22.5, 11.25, 5.62, 2.81, 1.40 mg/ml were incubated at 37°C for 24 hours for bacteria isolates and 25°C for fungal isolates at 72 hours and examined for growth. The lowest concentration of the extract in which there was no visible growth is the MIC.

Determination of Minimum Bactericidal Concentration

The MBC of the extract was determined using Adegboye *et al.*, (2008) methods. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto sterile Nutrient agar plate and incubated at 37°C for 48 hours. The MBC was thus taken as the lowest concentration of the extract that did not allow any bacterial or fungal growth on the surface of the agar plates.

Determination of Minimum Fungicidal Concentration

Samples from tubes that inhibited growth in the MIC assay were re-inoculated separately unto sterile potato dextrose agar plate to assay for the fungicidal effect of the extract. The organisms were incubated at room temperature and examined for growth after 7 days of incubation. The lowest concentration of the extract that yielded no growth following sub-culturing on the PDA plate was regarded as MFC in each case (Black, 1996).

Results and Discussion

Phytochemicals present in various *Carica papaya* seed extracts

The Phytochemical screening of the extracts of *Carica papaya* seeds showed the presence of alkaloids, saponins, flavonoids, tannins, glycosides and carotenoids. The phytochemicals found also concurs with those obtained by Mujeeb *et al.*, (2014) in the leaves of *Aegle marmelos* and Khan *et al.*, (2014) in *Cyphostem madigitatum*

Determination of Antibacterial activities of extracts

Antimicrobial effect of the *Carica papaya* seed extracts were tested on four (4) selected organisms including two bacteria isolates and two fungi isolates namely: *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Aspergillus flavus* and *Mucor* sp. Phytochemicals are antimicrobial compounds which have made great contributions for quick and effective management of plant diseases. These phytochemical compounds are known to have inhibitory or cidal effects on microorganisms. From the observations made, higher concentrations of the extracts had more inhibitory effect on the organisms. The findings of this experiment showed that the organisms especially fungi, were not sensitive to the extracts at low concentrations of 60 to 100 mg/ml unlike at higher concentrations of 300 to 400 mg/ml. *Pseudomonas aeruginosa* was observed to be the most sensitive and susceptible bacterium to the extracts (Tables 2, 3 and 4) at 400mg/ml of aqueous cold, hot and ethanol extract with 18mm, 16mm and 30mm diameter of zone of inhibition. This result is in accordance with an earlier report that aqueous extract of *Carica papaya* seeds showed increasing zone of inhibition of *Staphylococcus aureus*, *P. aeruginosa* and *Escherichia coli* with increasing seed extract concentration as 25, 50, 75 and 100 mg/ml (Peter *et al.*, 2014).

Table 1: Phytochemical screening of *Carica papaya* seed extract

Phytochemicals	SEED EXTRACTANT		
	Ethanol	Aqueous cold	Aqueous hot
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	-	-	-
Flavonoids	+	-	-
Alkaloids	+	+	+
Glycosides	-	+	+
Carotenoids	+	+	+

Legend: +, = Presence of the phytochemical compound, -, = Absence of the phytochemical compound

Table 2: Antimicrobial effect of aqueous cold extract of *Carica papaya* seeds on the selected organisms.

Organisms	Zone of inhibition in millimeters			
	Concentrations (mg/ml)			
	100	200	300	400
<i>E. cloacae</i>	-	*2	*6	*16
<i>P. aeruginosa</i>	*2	*5	*12	*18
<i>Aspergillus flavus</i>	-	*2	*8	*10
<i>Mucor sp</i>	-	-	-	-

Legend: -, = Resistant (No zone of inhibition), *, = Zone of inhibition of triplicate result expressed as means

Table 3: Antimicrobial effect of aqueous hot extract of *Carica papaya* seeds on the selected organisms.

Organisms	Zone of inhibition in millimeters			
	Concentrations (mg/ml)			
	100	200	300	400
<i>E. cloacae</i>	*4	*8	*10	*14
<i>P. aeruginosa</i>	*4	*8	*10	*16
<i>Aspergillus flavus</i>	-	*1	*5	*10
<i>Mucor sp</i>	-	-	-	-

Legend: -, = Resistant (No zone of inhibition), *, = Zone of inhibition of triplicate result expressed as means

Table 4: Antimicrobial effect of Ethanol extract of *Carica papaya* seeds on the selected organisms.

Organisms	Zone of inhibition in millimeters			
	Concentrations (mg/ml)			
	100	200	300	400
<i>E. cloacae</i>	*8	*14	*16	*22
<i>P. aeruginosa</i>	*10	*18	*20	*30
<i>Aspergillus flavus</i>	*10	*18	*24	*26
<i>Mucor sp</i>	-	-	*10	*14

Legend: -, = Resistant (No zone of inhibition), *, = Zone of inhibition of triplicate result expressed as means

The ethanol extract of plant parts were more effective than the aqueous extracts. It is likely that the active constituents of the seeds were better extracted with ethanol than with water. The observation was similar to the report of Ezeifeka *et al.*, (2004) that reports the higher antimicrobial activities of ethanol extracts of plant parts than the aqueous extract. The domination of the ethanol extract activity compared to aqueous extracts may confirm the ability of the ethanol to extract active compounds from seeds more than that extracted by water. According to Chandra *et al.*, (2011), n-hexane and ethanol extracts of *Carica papaya* leaves had highest zone of inhibition of *S. aureus* at 17.33 and 15.67 (30mg/disc) respectively while aqueous extract had inhibition of 9

(30mg/disc) also in the case of *Bacillus subtilis*, *E. coli*, and *P. aeruginosa*. In spite of this, almost all the extracts of aqueous cold, hot and ethanol showed appreciable level of potency against the bacteria isolates.

The aqueous extracts of the pawpaw seeds did not have significant effect on the fungal isolates, especially, *Mucor*sp, which was totally resistant to the aqueous extracts (Tables 2 and 3). The result of Table 4 showed that higher concentrations of the ethanol extract of the pawpaw seeds was effective against *Mucor*sp, which might be as a result of the presence of a phytochemical compound soluble or extracted by ethanol and not extracted by water or due to the morphology of the fungus. Aqueous extracts (hot and cold) of *Carica papaya* seeds showed increasing zone of inhibition of *E. cloacae*, *P. aeruginosa* and *A. flavus* with increasing concentrations as 90, 100, 160, 170, 180, 190, 200, 300 and 400mg/ml.

The ethanolic extract of the pawpaw seeds was very effective against the test organisms and was capable of inhibiting *Mucor*sp at high concentrations of 300 and 400mg/ml with 10mm and 14mm diameter of zones of inhibition respectively (Table 4).

Pseudomonas aeruginosa show greater degree of sensitivity at concentrations of 90 - 190mg/ml while *E. cloacae* and *A. flavus* only shows sensitivity at concentration of 190mg/ml and *Mucor*sp was resistant at concentrations of 60 - 190mg/ml for aqueous cold extract of *Carica papaya* seeds. *E. cloacae* and *P. aeruginosa* are the only organisms that show sensitivity at 90 - 190mg/ml while *Aspergillus flavus* and *Mucor*sp was resistant at concentrations of 60 - 190mg/ml for aqueous hot extract of *Carica papaya* seeds. All the organisms were sensitive to ethanolic extract at concentrations of 70 - 190mg/ml except for *Aspergillus flavus* and *Mucor*sp which are sensitive at 90mg/ml to 170mg/ml.

Antimicrobial activity of various extracts on organisms at low concentrations

Table 5: Antimicrobial effect of aqueous cold extract of *Carica papaya* seeds on the tested microorganisms.

Organisms	Zone of inhibition in millimetres							
	Concentrations (mg/ml)							
	60	70	80	90	160	170	180	190
<i>E. cloacae</i>	-	-	-	-	-	-	-	*1
<i>P. aeruginosa</i>	-	-	-	*1	*2	*2	*3	*4
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	*1
<i>Mucor</i> sp	-	-	-	-	-	-	-	-

Legend: -, = Resistant (No zone of inhibition), *, = Zone of inhibition of triplicate result expressed as means

Table 6: Antimicrobial effect of aqueous hot extract of *Carica papaya* seeds on the selected organisms.

Organisms	Zone of inhibition in millimetres							
	Concentrations (mg/ml)							
	60	70	80	90	160	170	180	190
<i>E. cloacae</i>	-	-	-	*2	*4	*5	*6	*7
<i>P. aeruginosa</i>	-	-	-	*1	*3	*4	*5	*7
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	-
<i>Mucor</i> sp	-	-	-	-	-	-	-	-

Legend: -, = Resistant (No zone of inhibition), *, = Zone of inhibition of triplicate result expressed as means

Table 7: Antimicrobial effect of ethanolic extract of *Carica papaya* seeds on the selected organisms.

Organisms	Zone of inhibition in millimetres							
	Concentrations (mg/ml)							
	60	70	80	90	160	170	180	190
<i>E. cloacae</i>	-	*2	*4	*6	*10	*10	*12	*13
<i>P. aeruginosa</i>	-	*2	*4	*8	*13	*14	*16	*16
<i>Aspergillus flavus</i>	-	-	-	*4	*7	*8	*10	*14
<i>Mucor</i> sp	-	-	-	-	-	*2	*3	*5

Legend: -, = Resistant (No zone of inhibition),

*, = Zone of inhibition of triplicate result expressed as means

Aspergillus flavus showed no zone of inhibition for both aqueous cold and aqueous hot extract while *Mucor*sp showed resistance to all the 3 extracting solutions. There was no inhibition at concentrations of 22.5mg/ml of ethanol extract, 45mg/ml of aqueous cold extract, 90mg/ml of aqueous hot extract for *P. aeruginosa* and no inhibition at concentrations of 45mg/ml of ethanol extract, 90mg/ml of aqueous cold extract, 90mg/ml of aqueous hot extract for *E. cloacae*. *Mucor*sp showed no fungicidal activity to all extracting solutions while *A. flavus* showed activity at concentration of 90mg/ml of ethanol extract and no fungicidal activity to aqueous cold and aqueous hot extracts.

In this research, the MIC value for ethanol, aqueous cold, aqueous hot extracts ranges between 45mg/ml – 90mg/ml while the MBC value for ethanol, aqueous cold, aqueous hot extracts ranges between 22.5mg/ml – 90mg/ml on *P. aeruginosa*, and *E. cloacae*. The MIC value for *Aspergillus flavus* was found to be 90mg/ml for ethanol extract.

This concurs with the work of Abalaka et al., (2012) on antibacterial activities of *Azadirachta indica* against *P. aeruginosa*, *K. ozanoe*, *S. aureus* and *E. coli* in which the test organisms have a similar MIC value of 5mg/ml and MBC value of 50mg/ml. This research is a little different from the work of Noumlet al., (2010) on the extract of *Salvadora persica* and *Juglans regia* L. extract on some strains of oral *Candida* where the MIC values of the *Juglans regia* L. against *Candida* strains ranged from 0.006 to 0.195 mg/ml. Two *C. albicans* strains showed a high MIC value (3.125 mg/ml). These results indicate that extracts can contain compounds with therapeutic potential against *Candida* strains and, hence, their possible use as therapeutic agents.

MIC, MBC AND MFC OF EXTRACTS AGAINST TEST ORGANISMS

Table 8: Minimum inhibitory concentrations (MIC) of different concentrations of extracts exhibited by *C. papaya* extracts against the organisms

Organisms	Concentrations of extracts (mg/ml)		
	Ethanol	Aqueous cold	Aqueous hot
<i>E. cloacae</i>	45	90	90
<i>P. aeruginosa</i>	90	90	90
<i>Aspergillus flavus</i>	90	-	-
<i>Mucor</i> sp	-	-	-

Legend: -, = No zone of inhibition

Table 9: Minimum bactericidal concentrations (MBC) of *C. papaya* extracts against some microorganisms.

Microorganism (s)	Concentrations of extracts (mg/ml)		
	Ethanol	Aqueous cold	Aqueous hot
<i>E. cloacae</i>	45	90	90
<i>P. aeruginosa</i>	22.5	45	90

Legend: -, = No inhibition

Table 10: Minimum fungicidal concentrations (MFC) of different concentrations of extracts exhibited by *C. papaya* extracts against the organisms

Organisms	Concentrations of extracts (mg/ml)		
	Ethanol	Aqueous cold	Aqueous hot
<i>Aspergillus flavus</i>	90	NF	NF
<i>Mucor</i> sp	NF	NF	NF

Legend: NF = No Fungicidal activity

Conclusion

The present work has shown that extracts of seeds of *Carica papaya* have antibacterial and antifungal actions in controlling spoilage microorganisms isolated from tomato fruits. There is need to do more work to purify the active principles involved in the antimicrobial actions, to carry out further work in the desirability of using extracts of pawpaw seeds to control spoilage microorganisms in tomato, which if done properly will boost tomato production and improve the lives of farmers, and preserve the organoleptics of the fruit.

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