

Full Length Research Paper

Phytochemical screening and antibacterial properties of selected Nigerian long pepper (*Capsicum frutescens*) fruits

Bello, I.*, Boboye, B. E. and Akinyosoye, F. A.

Department of Microbiology, Federal University of Technology, P. M. B. 704, Akure, Nigeria.

Received 19 November, 2014; Accepted 31 August, 2015

Five varieties of Nigerian long pepper (*Capsicum frutescens*) fruits (var. *baccatum*, *chacoense*, *fingerh*, *maxima* and *minima*) were studied for their antibacterial activities. Methods described by Association of Official Analytical Chemists (AOAC) were employed to determine the phytochemicals in the pepper fruits. The *Capsicum frutescens* fruits showed antibacterial activity (10.33 - 32.66 mm zones of inhibition) against all the tested pathogenic bacteria. The minimum inhibitory concentrations for the peppers range between 7.8 and 37.5 mg/ml. Pepper var. *minima* had the highest MIC (37.5 mg/ml) while pepper var. *fingerh* exhibited the lowest MIC (7.8 mg/ml) against *Pseudomonas aeruginosa* and *Salmonella typhi*, respectively. The flavonoids, tannins, alkaloids and saponins found present in the extracts were quantified as 0.0019-0.0043, 0.0084-0.0146, 6.57-15.78 and 15.67-26.29 mg/ml, respectively. From this study, it was concluded that the long peppers have antibacterial effect on the tested food borne pathogens; even the less pungent *C. frutescens* var. *fingerh* showed antibacterial activity. These peppers when consumed raw could prevent elaboration of infection that the test pathogenic bacteria could cause if consumed in foods.

Key words: *Capsicum frutescens*, phytochemical, antibacterial, long pepper fruits, food, pathogens.

INTRODUCTION

Pepper, the plant used in this research, belongs to the genus *Capsicum* in the family Solanaceae (Dutta, 2001). It is cultivated and consumed worldwide, particularly in the African and Asian countries. The five long peppers used in this project are rich in vitamins, especially A, B and E. They are also good sources of essential minerals such as magnesium, zinc, iron, phosphorous and potassium (Otinola et al., 2010). They have been used for thousands of years as spices in food to enhance the

flavor, color and aroma of food. Long pepper fruits are added at a substantial quantity to produce a characteristic taste of cuisine in Nigeria and other parts of the world. In addition to boosting flavor, they are also known for their preservative and medicinal value (Oni, 2011; Otunola et al., 2010).

In view of the fact that long pepper fruits are commonly consumed in Nigeria and because some infectious microorganisms are developing resistance towards

*Corresponding author. E-mail: bellife2010@yahoo.co.uk.

existing commercial antibiotics, it is important to source for antimicrobial agents that can be used to prevent or cure infections caused by some bacteria. Hence, this study was aimed at investigating the antibacterial properties of the widely consumed *Capsicum frutescens* varieties in South-Western part of Nigeria.

Chemical composition of *Capsicum* fruits

The sharp taste of *Capsicum* peppers is due to a mixture of seven related alkaloids of which capsaicin is the most prevalent. Capsaicinoids are mainly found in the seeds and placental area (where seeds attach to the ovary wall) of the pepper fruits (Dong, 2000). The capsaicin content is negligible in sweet bell peppers but found in high concentrations (30,000 - 50,000 SHU) in hot chili or jalapeno peppers that even handling or cutting the peppers can irritate the skin (Nwokem et al., 2010).

The substances responsible for the pungency are the capsaicinoid alkaloids. They are characterized by a high biological activity and their pharmacological, neurological and dietetic activities are well known. When used at low levels in the regular diet, they significantly decrease serum, myocardial and aortic total cholesterol levels (Nwokem et al., 2010). Capsaicin is so potent that it can be tasty in concentrations as low as 1 part per million (Dong, 2000). It is now established that pepper is an important source of capsaicin.

Pepper also contains carotene, mineral elements, vitamins A and C. Vitamins A, C, and Carotene facilitate the growth and function of human beings, while minerals help the body withstand stress, cold and stimulate mucous that protects intestinal lining from ulcer. Minerals, especially trace elements: iron, manganese, lead, cobalt, chromium, cadmium, zinc and copper are classified as essential and unessential that are necessary in biochemical reactions that take place in the body of human beings (Showemimo and Olarewaju, 2004). So peppers play vital role in the proper functioning of various systems within the human body.

Antimicrobial and medicinal potentials of *Capsicum* fruits

A survey of the Mayan pharmacopoeia revealed that the tissues of *Capsicum* spp. are included in a number of herbal remedies for a variety of ailments of probable microbial origin (Cichewicz and Thorpe, 1996). Bioactive amides capsaicin from the fruits was assayed for activity and a high concentration of capsaicin (200-300 mg/ml), only retarded the growth of *E. coli* and *Pseudomonas solanacearum* whereas the growth of *Bacillus subtilis* was strongly inhibited (Dorantes et al., 2000). The antibacterial effects of capsaicin from Korean hot peppers on *B. subtilis*, *B. cereus* and *Sarcina lutea* have also been

reported (Soetarno et al., 1997). The plain and heated extracts were found to exhibit varying degrees of inhibition against *B. cereus*, *B. subtilis*, *Clostridium sporogenes*, *C. tetani* and *Streptococcus pyogenes* (Cichewicz and Thorpe, 1996).

Capsicum baccatum, *C. chinense*, *C. frutescens* and *C. pubescens* varieties were tested for their antimicrobial effects against fifteen bacterial species and one yeast species. Two pungent compounds found in *Capsicum* species (capsaicin and dihydrocapsaicin) were also tested for their antimicrobial effects. The plain and heated pepper extracts were found to exhibit varying degrees of inhibition against *B. cereus*, *B. subtilis*, *Clostridium sporogenes*, *C. tetani* and *Streptococcus pyogenes* (Soetarno et al., 1997).

In fact, a later study has shown that capsaicin strongly inhibits the growth of *B. subtilis* as aqueous and ethanolic extracts of black pepper have been screened for antibacterial activities against Penicillin G resistant strain of *Staphylococcus aureus*, *B. cereus* and *B. subtilis* (George et al., 2010). Thus, *Capsicum* extracts do appear to have some valuable antimicrobial activity.

The antioxidant properties of *Capsicum* species have been well documented (Oboh and Rocha, 2006).

MATERIALS AND METHODS

Pepper fruits

Five different varieties (var. minima ("ata eye"), var. maxima ("ata funfun"), var. chacoense ("ekanna asa"), var. baccatum ("ata wewe") and var. fingerh ("ata sombo") of matured and apparently healthy fruits of *C. frutescens* were used in this project (Plate 1). The *C. frutescens* var. maxima ("Ata funfun") and *C. frutescens* var. minima ("Ata eye") were collected from a farm site at Ayede Ogbese, Ondo State, Nigeria. *Capsicum frutescens* var. fingerh ("Ata sombo"), *C. frutescens* var. baccatum ("Ata wewe") and *C. frutescens* var. chacoense ("Ata ekanna asa") were sourced from "Shasha" market in Akure, Nigeria. The scientific identity of the pepper fruits was ascertained in the Department of Crop Science and Production, Federal University of Technology, Akure. The fruits were transported in sterile cellophane bags to the laboratory for immediate investigation.

Test pathogenic bacteria

The human pathogenic bacteria used for this study were: *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Enterococcus aerogenes* and *Proteus vulgaris*. They were provided by the Public Health Laboratory in Akure, Ondo State, Nigeria. Identities of the pathogens were authenticated in the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Preparation of culture media and inoculum

The media used in this study were: standard plate count agar (SPCA) and nutrient agar (NA). According to manufacturer's specifications, appropriate quantity of each medium was weighed, dissolved in distilled water and sterilized by autoclaving at 121°C for



Plate 1. Physical appearance of the five Varieties of Nigerian Long Pepper Fruit used for this research. Source: This Research.

15 min. The sterilized agar medium was allowed to cool to about 45°C before pouring to prevent steam formation on the cover of Petri dishes.

Preparation of pepper extracts

Preparation of extracts from Pepper samples

The pepper extracts were prepared according to the method described by George et al. (2010). One hundred grams of each pepper fruit was weighed and rinsed thoroughly with sterile distilled water and the fruits were surface sterilized by soaking them in 70% ethanol for 5 min and sodium hypochlorite (12.5% w/v) for 15-20 min. The fruits were rinsed again in sterile distilled water to wash off any residual solution of the sterilizer. Each sample was blended with 150 ml of the extracting solvent (distilled water, absolute ethanol and acetone) with the aid of an electric blender. The extract

was filtered with the aid of a sterile muslin cloth having 1 mm pore size. The filtrate was used as fresh pepper extract for antibacterial test.

Dried pepper extract was prepared from each fruit using the same procedure employed for fresh pepper extract. The fruits were dried at 60°C for 72 h. It was blended into powdery form with the aid of an electric blender and 100 g of the dried pepper extract was soaked in 150 ml of the extracting solvent (sterile distilled water, ethanol and acetone) for 72 h. The extracts were filtered with the aid of a sterile muslin cloth (pore size 1 mm). The filtrate was concentrated using rotary evaporator.

Determination of amount of extract in yield of solvent

This was carried out to know the effect of the extracting solvents on the yield of the pepper extracts after extraction. Amount of extract recovered after extraction was calculated using the following

formula:

$$\frac{\text{weight of dried extract after extraction}}{\text{Initial weight of plant part before extraction}} \times 100$$

Phytochemical screening

The phytochemicals present in the pepper fruit extracts were determined quantitatively according to the methods of AOAC (2002) and Edeoga et al. (2005).

Antimicrobial testing

Preparation of test microorganisms

Bacterial pathogens and isolates used were grown on nutrient agar at 37°C for 24 h. Each bacterium was inoculated from the NA culture into fresh nutrient broth and incubated at 37°C for 24 h.

Assay for antimicrobial activity of the pepper extract

Antimicrobial activity of each pepper extract was determined by agar well diffusion method (George et al., 2010). A 0.1ml of each pathogenic bacterium was aseptically drawn into a petri dish; molten NA was poured and allowed to solidify. Wells of 6mm each were bored in the agar with sterile cork borers. Pepper extracts in concentrations of 100 to 250 mg/ml were prepared by suspending 1 to 2.5g of the concentrated dried extracts into 10 ml of Tween20. The extracts were filter-sterilized using a Millipore filter (0.5 µm) and introduced (0.5 ml) aseptically into the well in the NA. The NA plates were incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured in millimeters. Tween 20 was used as negative control while Amoxicillin (250 mg) was used as a positive control. The experiment was carried out in triplicates.

The same procedure was carried out for the fresh pepper extracts. Aliquot (0.5 ml) of each pepper extract was introduced into each of the bored wells in NA previously seeded with test pathogenic bacterium. The extracting solvent was used as the negative control and amoxicillin (250 mg) used as the positive control.

Determination of minimum inhibitory concentrations (MIC) of the pepper extracts

The agar diffusion and tube dilution technique described by George et al. (2010) were employed to determine the MIC of the pepper extracts. For the agar diffusion method, concentrations of each extract ranging from 25 to 75 mg/ml were prepared. The various extract concentrations (0.5 ml) were introduced aseptically into bored holes in previously inoculated NA. The medium was incubated at 37°C for 24 h. Agar well that serve as negative control contained Tween 20. The test was carried out in replicate. The MIC was obtained by taking the least concentration that showed inhibitory effect on the tested bacteria pathogens.

Tube dilution technique was used to determine the MIC of the fresh pepper extracts. Different concentrations ranging from 50 - 150 mg/ml of the extracts were introduced into test tubes containing sterile nutrient broth. The broth tubes were inoculated with each test pathogen separately and incubated at 37°C for 24 h. The test was also replicated. The MIC was taken as the concentration that showed the lowest bacterial population when the tube content was re-cultured on NA.

Table 1. Effect of extracting solvents on the yield of the pepper fruit extracts.

Solvent	Sample	Yield (%)
Water	A	47.85 ± 0.20 ^a
	E	42.50 ± 0.67 ^b
	W	40.65 ± 1.00 ^b
	S	52.75 ± 0.35 ^b
	F	43.95 ± 0.20 ^a
Ethanol	A	48.40 ± 0.15 ^a
	E	40.85 ± 0.67 ^a
	W	51.20 ± 0.65 ^a
	S	51.80 ± 0.10 ^a
	F	53.00 ± 0.65 ^b
Acetone	A	46.90 ± 0.53 ^b
	E	40.25 ± 0.68 ^a
	W	43.70 ± 1.30 ^b
	S	45.00 ± 0.95 ^c
	F	43.10 ± 1.00 ^c

Values are mean ±std of replicates. Values with the same alphabet across column are not significantly different at P = 0.05. A, *C. frutescens* var. chacoense ("Ata Ekanna Asa"); E, *C. frutescens* var. minima ("Ata Eye"); W, *C. frutescens* var. baccatum ("Ata Wewe"); S, *C. frutescens* var. fingerh ("Ata Shonbo"); F, *C. frutescens* var. maxima ("Ata Funfun").

Statistical analysis

The experiment was carried out in triplicates. Data obtained were analyzed using one way analysis of variance and means were compared by Duncan's Multiple Range Test using (SPSS 17.0 version).

RESULTS AND DISCUSSION

Effect of solvent type and extract concentration on the antibacterial activities of selected varieties of *Capsicum frutescens*

The percentage yield of the extracting solvents ranged from 40.25% (acetone extract of *C. frutescens* var. minima) to 53% (ethanol extract of *C. frutescens* var. minima) (Table 1). The yields of pepper fruits extracted with water ranged from 40.65 (*C. frutescens* var. baccatum) to 52.75% (*C. frutescens* var. fingerh). Among the solvents used to extract the peppers, ethanol yielded the highest amount of extracts from the pepper fruits besides *C. frutescens* var. minima and *C. frutescens* var. fingerh. Apart from *C. frutescens* var. baccatum, acetone

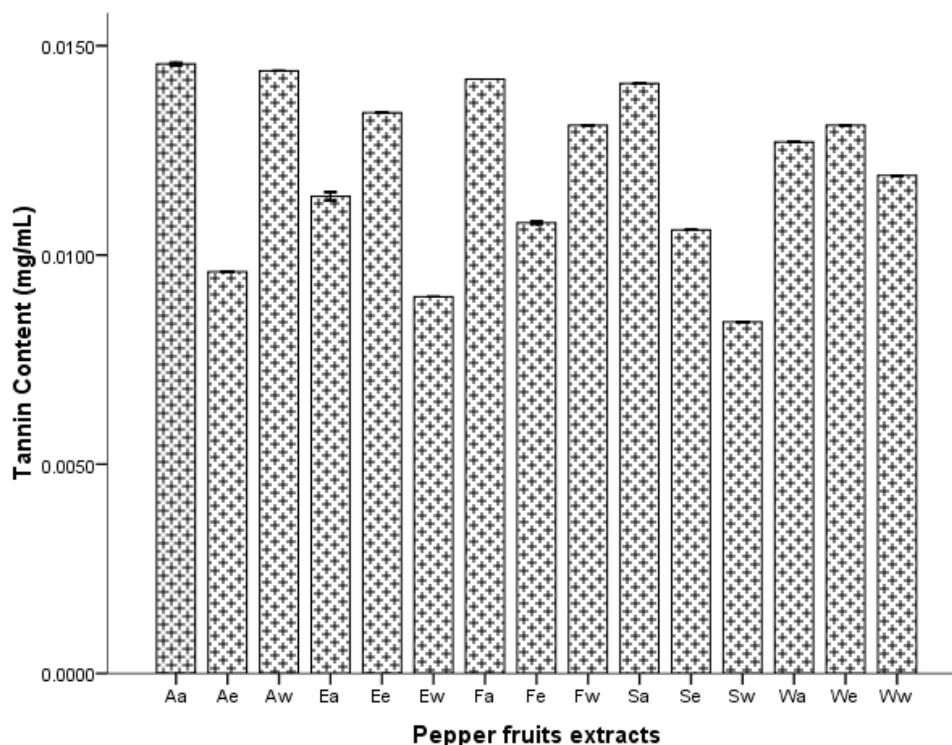


Figure 1. Tannin contents of the pepper fruit extracts. a, Acetone extract; e, ethanolic extract; w, water extract. A, “Ata Ekanna Asa” (*C. frutescens* var. chacoense); E, “Ata Eye” (*C. frutescens* var. minima); F, “Ata Funfun” (*C. frutescens* var. maxima); S, “Ata Shonbo” (*C. frutescens* var. fingerh); W, “Ata Wewe” (*C. frutescens* var. baccatum).

extracted the lowest amounts of extracts from the peppers with a range of 40.25 (var. minima) to 46.9% (var. chacoense) (Table 1).

The phytochemical screening of the pepper fruits extracts revealed that all the pepper varieties investigated contained alkaloid, tannin, saponin and terpenoid. The extracts also contained flavonoid and cardiac glycosides.

The tannin content ranged from 0.0084 to 0.0146 mg/ml (Figure 1). The acetone extract of the pepper variety chacoense had the highest amount (0.0146 mg/ml) of tannin while the lowest content (0.0084 mg/ml) was observed in the water extract of fingerh. Flavonoid content in the pepper ranged from 0.0019 (in acetone extract of var. fingerh) to 0.0043 mg/ml (methanol extract of baccatum pepper) (Figure 2). The concentrations of alkaloid in the pepper varieties were higher than the amounts of other phytochemicals tested in this work (Figure 3). The alkaloid contents ranged from 15.67 to 26.29 mg/ml. The *C. frutescens* var. fingerh contained more of this alkaloid (22.56 to 26.29 mg/ml) than other pepper fruit varieties. The lowest amount (15.67 mg/ml) of alkaloid was obtained in the acetone extract of *C. frutescens* var. maxima.

The saponin content ranged from 6.566 mg/ml in water extract of var. fingerh to 15.781 mg/ml in water extract of var. chacoense (Figure 4), with the highest and lowest

concentrations obtained in the water extracts of “Ata Ekanna Asa” and “Ata Shonbo” respectively. The acetone extracts of *C. frutescens* var. minima (“Ata Eye”) and *C. frutescens* var. maxima (“Ata funfun”) appeared to contain lower amounts of saponin than water and ethanolic extracts of the respective varieties of the long pepper.

The extract obtained with different solvents, showed different inhibitory activities against each pathogen tested (Figures 5-9). The highest inhibitory activity of *C. frutescens* var. fingerh (“Ata shonbo”) was observed against *E. coli* with mean zone of inhibition that ranged from 20 to 32.66 mm, followed by *S. typhi* with zone of inhibition values that ranged from 15.66 to 29.33 mm at 250 mg/ml (Figure 5). The least inhibitory activity was exhibited by the fingerh against *P. aeruginosa* at 100 mg/ml with the zone of inhibition measuring 8 mm.

C. frutescens var. chacoense (“Ata Ekanna Asa”) exhibited the highest inhibitory activity against *Serratia marcescens* with zones of inhibition that ranged from 16.66 to 32.66 mm, followed by *E. coli* with zones of inhibition of 18 to 26.33 mm at 100 and 150 mg/ml (Figure 6). The lowest inhibitory activity of this pepper was observed against *E. aerogenes* with zones of inhibition measuring 12.66 mm. *E. aerogenes* was more inhibited than *E. coli* at 250 mg/ml.

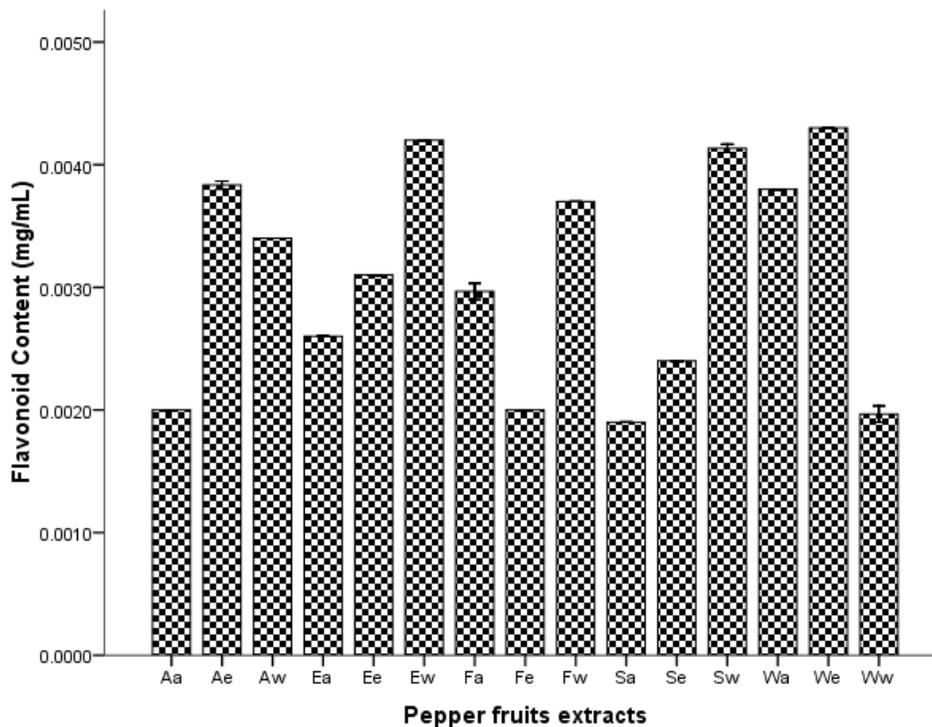


Figure 2. Flavonoid contents of the pepper fruit extracts. a, Acetone extract; e, ethanolic extract; w, water extract. A, "Ata Ekanna Asa" (*C. frutescens* var. chacoense); E, "Ata Eye" (*C. frutescens* var. minima); F, "Ata Funfun" (*C. frutescens* var. maxima); S, "Ata Shonbo" (*C. frutescens* var. fingerh); W, "Ata Wewe" (*C. frutescens* var. baccatum).

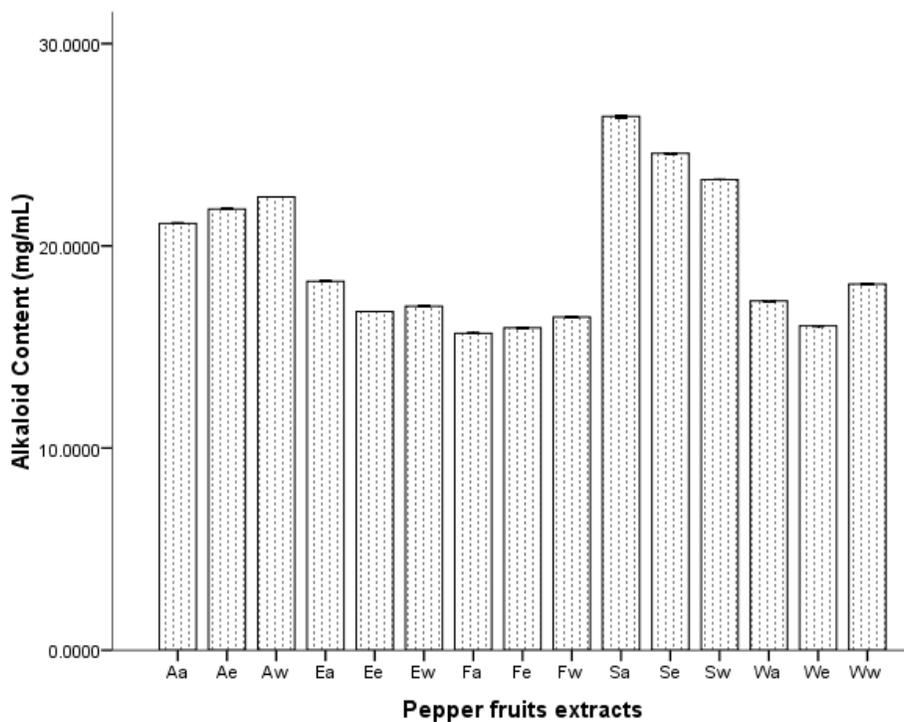


Figure 3. Alkaloid contents of the pepper fruit extracts. a, Acetone extract; e, ethanolic extract; w, water extract. A, "Ata Ekanna Asa" (*C. frutescens* var. chacoense); E, "Ata Eye" (*C. frutescens* var. minima); F, "Ata Funfun" (*C. frutescens* var. maxima); S, "Ata Shonbo" (*C. frutescens* var. fingerh); W, "Ata Wewe" (*C. frutescens* var. baccatum).

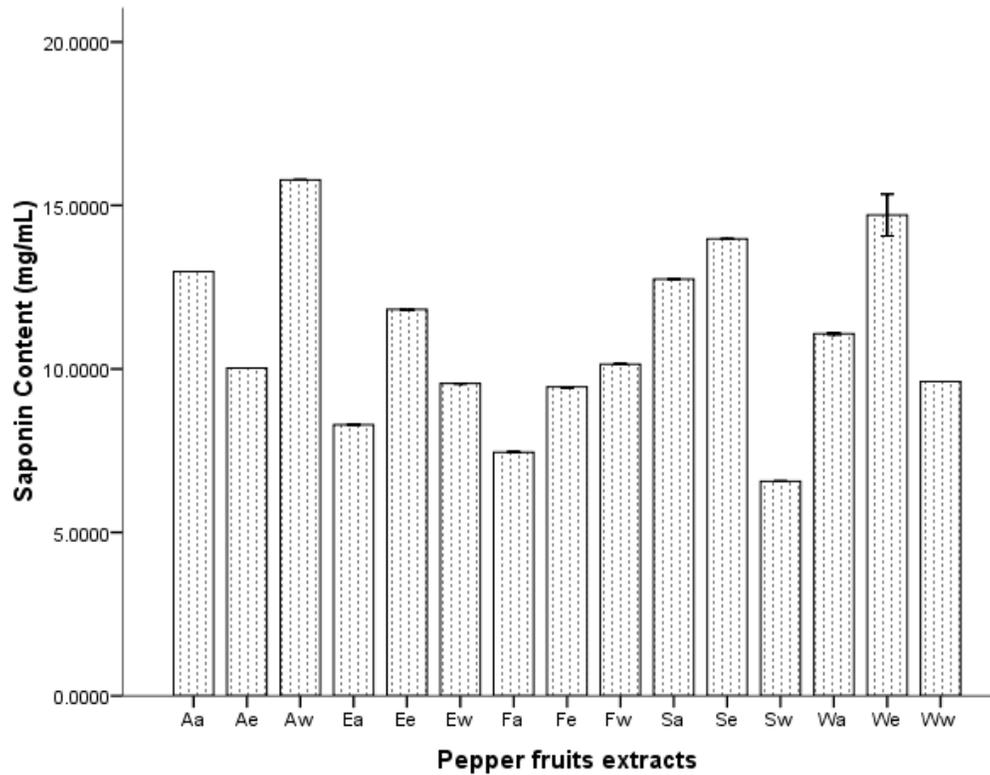


Figure 4. Saponin contents of the pepper fruit extracts. a, Acetone extract; e, ethanolic extract; w, water extract. A, “Ata Ekanna Asa” (*C. frutescens* var. chacoense); E, “Ata Eye” (*C. frutescens* var. minima); F, “Ata Funfun” (*C. frutescens* var. maxima); S, “Ata Shonbo” (*C. frutescens* var. fingerh); W, “Ata Wewe” (*C. frutescens* var. baccatum).

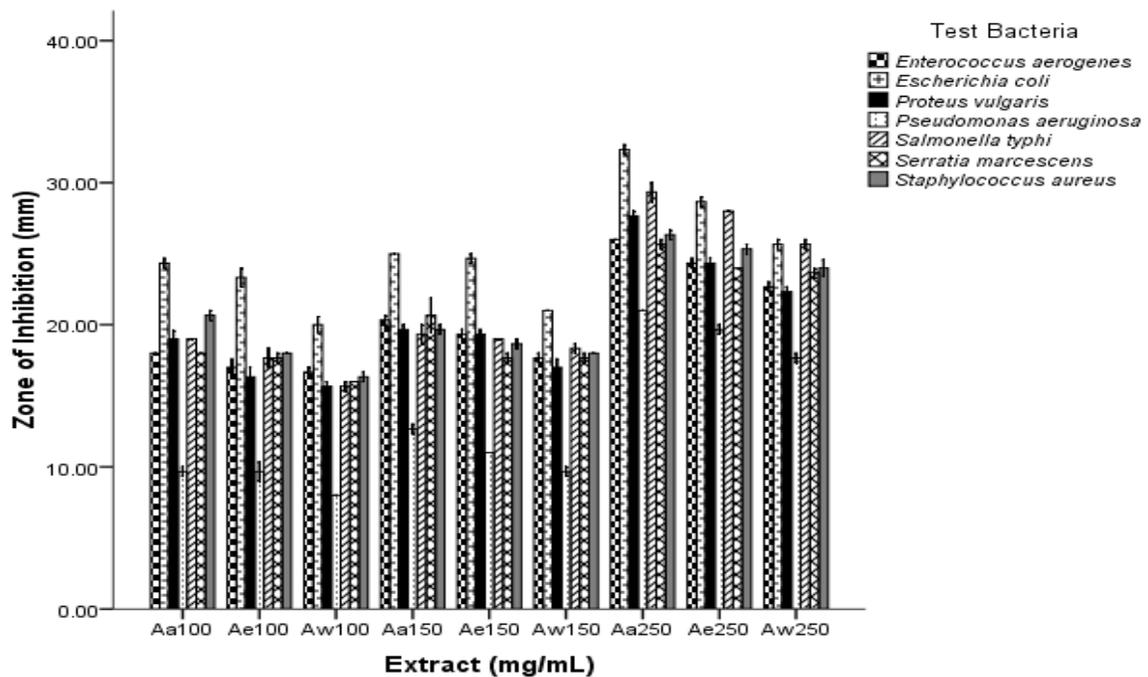


Figure 5. Effect of different extraction solvents and extract concentrations on the antibacterial activity of *Capsicum frutescens* var. fingerh (“Ata Shonbo”). Aa, Acetone extract; Ae, ethanolic extract; Aw, water extract. 100, 150 and 250: Concentrations of extracts at 100, 150 and 250 mg/ml, respectively.

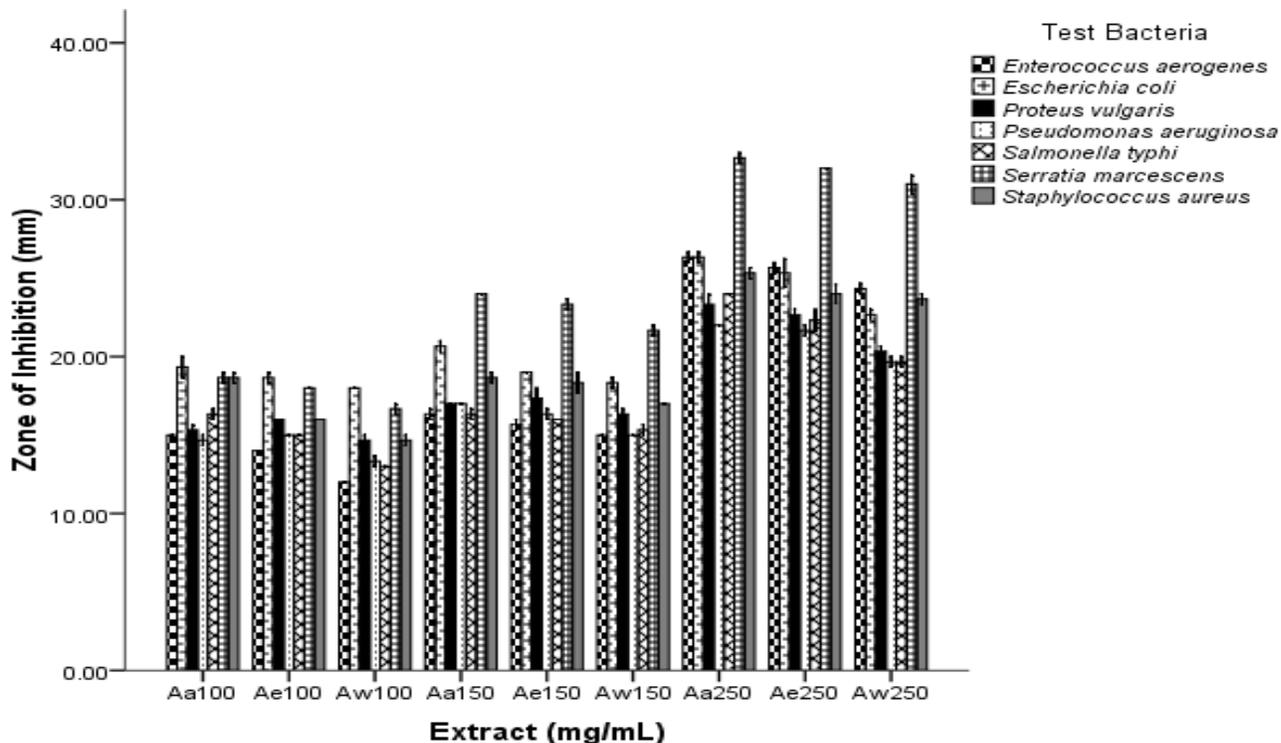


Figure 6. Effect of different extraction solvents and extract concentrations on the antibacterial activity of *Capsicum frutescens* var. *chacoense* ("Ata Ekanna Asa"). Aa, Acetone extract; Ae, ethanolic extract; Aw, water extract. 100, 150 and 250: Concentrations of extracts at 100, 150 and 250 mg/ml, respectively.

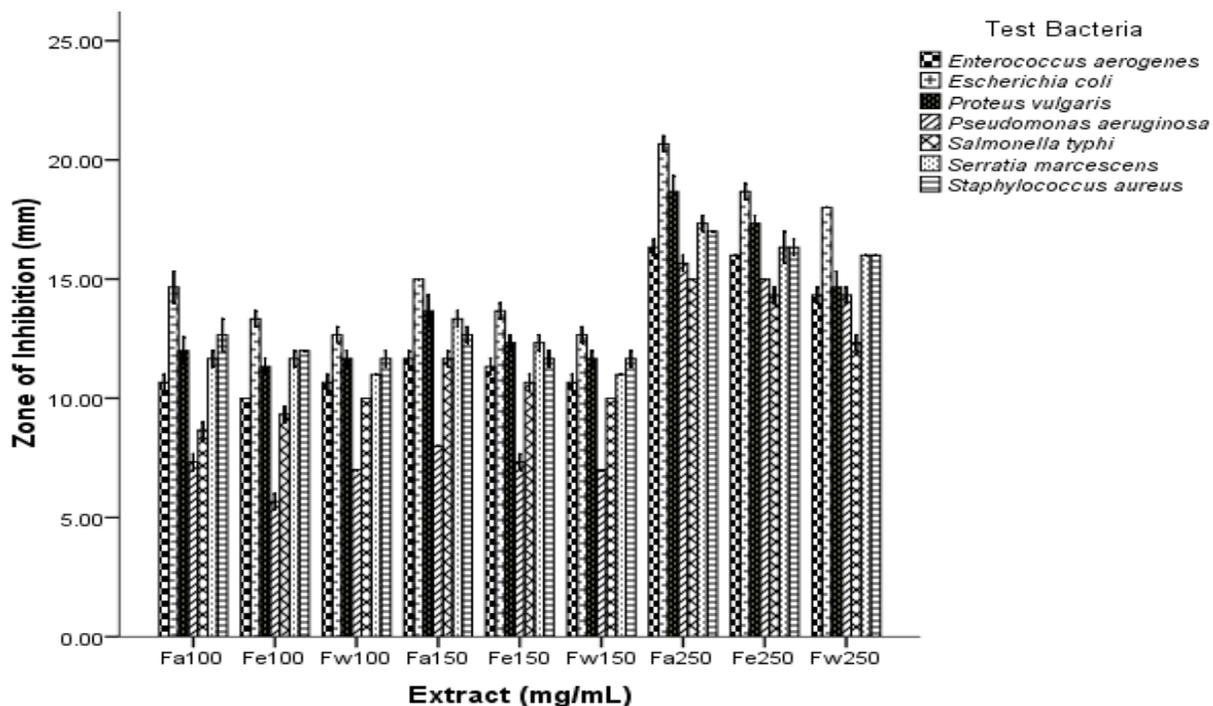


Figure 7. Effect of different extraction solvents and extract concentrations on the antibacterial activity of *Capsicum frutescens* var. *maxima* ("Ata Funfun"). Aa, Acetone extract; Ae, ethanolic extract; Aw, water extract. 100, 150 and 250: Concentrations of extracts at 100, 150 and 250 mg/ml, respectively.

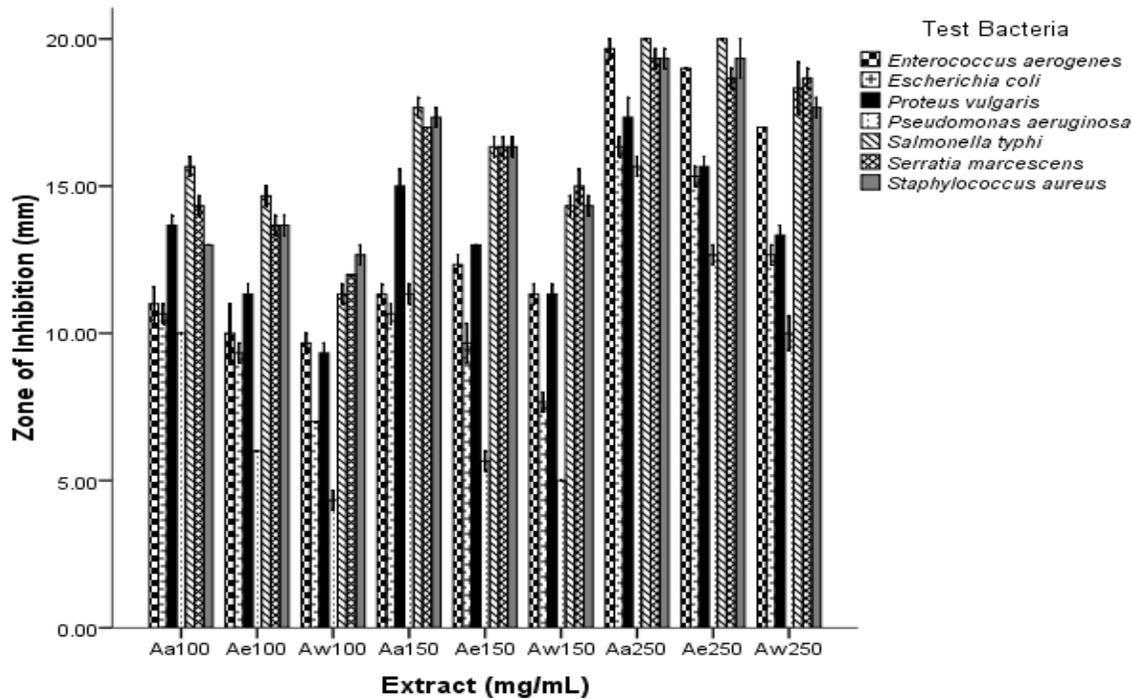


Figure 8. Effect of different extraction solvents and extract concentrations on the antibacterial activity of *Capsicum frutescens* var. *baccatum* ("Ata Wewe"). Aa, Acetone extract; Ae, ethanolic extract; Aw, water extract. 100, 150 and 250: Concentrations of extracts at 100, 150 and 250 mg/ml, respectively.

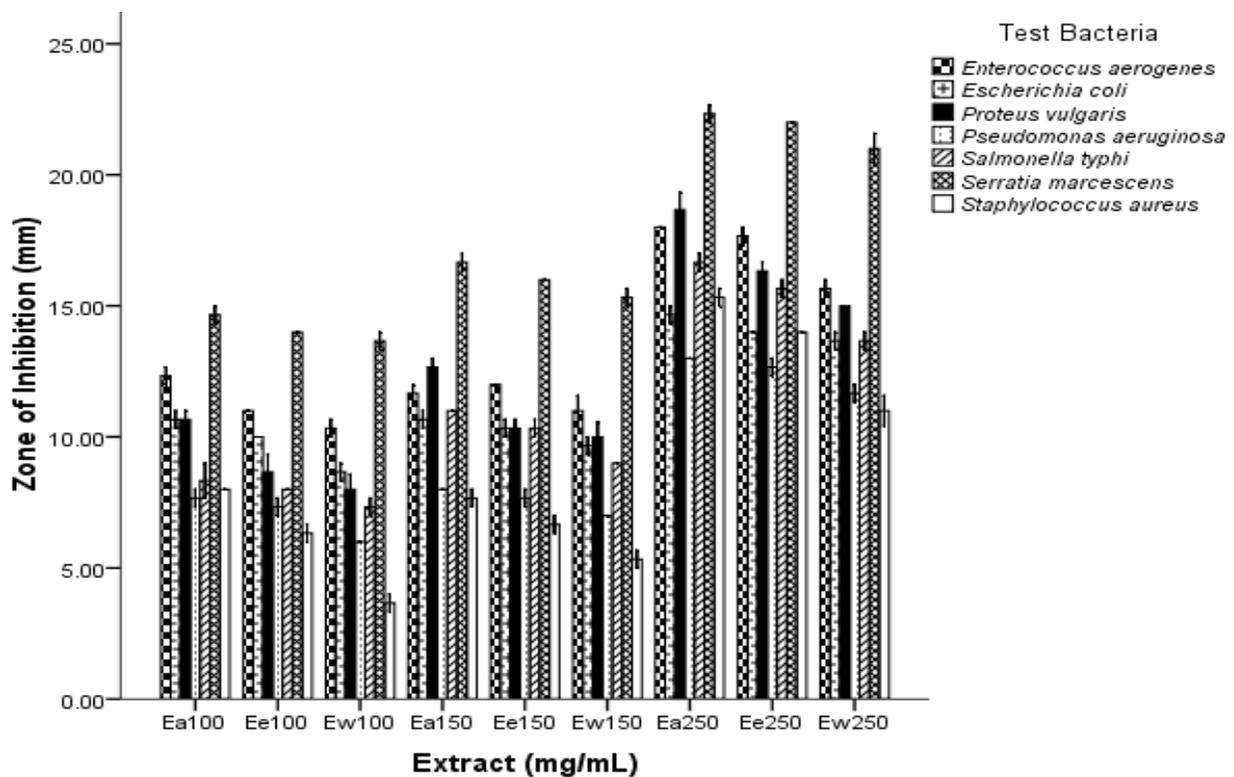


Figure 9. Effect of different extraction solvents and extract concentrations on the antibacterial activity of *Capsicum frutescens* var. *minima* ("Ata Eye"). Aa, Acetone extract; Ae, ethanolic extract; Aw, water Extract. 100, 150 and 250: Concentrations of extracts at 100, 150 and 250 mg/ml, respectively.

Table 2. Minimum inhibitory concentration (mg/mL) for the pepper fruits extracts.

Solvent	Pepper Variety	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. Marcescens</i>	<i>E. aerogenes</i>	<i>S. typhi</i>	<i>P. vulgaris</i>
Water	A	11.54±0.12 ^a	7.8±0.9 ^a	11.54±1.3 ^{ab}	15 ±1.2 ^b	11.54±0.52 ^{ab}	7.8±0.5 ^a	7.8±0.0 ^a
	E	37.5±0.3 ^e	27.3±0.56 ^c	32.6±0.37 ^d	32.6±0.78 ^d	37.5±0.21 ^{ef}	37.5±2.0 ^d	15±0.4 ^b
	W	27.3±0.45 ^c	37.5±1.23 ^d	27.4±0.53 ^c	27.3±0.87 ^c	27.3±0.11 ^c	15±0.1 ^b	11.54±0.3 ^{ab}
	S	11.54±0.16 ^a	7.8 ± 0.26 ^a	7.8 ± 1.5 ^a	7.8 ± 0.22 ^a	7.8 ± 0.19 ^a	7.8±0.2 ^a	7.8±0.3 ^a
	F	32.6± 0.28 ^d	15 ± 0.65 ^b	37.5±2.76 ^e	15±0.37 ^b	15 ± 1.12 ^b	27.3±3.0 ^c	27.3±3.2 ^c
Ethanol	A	15 ± 0.22 ^b	7.8± 0.45 ^a	15 ± 1.5 ^b	15±0.44 ^b	15 ± 1.12 ^b	7.8±0.3 ^a	7.8±0.2 ^a
	E	27.3± 0.50 ^c	27.3±1.35 ^c	27.3± 1.6 ^c	27.3±0.58 ^c	37.5±3.45 ^{ef}	37.5±1.2 ^d	15±0.1 ^b
	W	27.3± 0.32 ^c	37.5 ± 4.5 ^d	27.4±1.35 ^c	27.3±1.50 ^c	27.4±1.30 ^d	15±0.0 ^b	15±0.1 ^b
	S	15 ± 0.20 ^b	11.54±0.47 ^{ab}	7.8 ± 0.3 ^a	11.54±1.0 ^{ab}	7.8 ± 1.10 ^a	7.8±0.19 ^a	7.8±0.4 ^a
	F	27.3 ± 0.5 ^c	15 ± 0.61 ^b	37.5±2.35 ^e	15 ± 0.23 ^b	27.4 ± 1.6 ^d	27.3±0.4 ^c	27.3±2.4 ^c
Acetone	A	15 ± 0.34 ^b	7.8 ± 0.5 ^a	15 ± 0.15 ^b	15 ± 1.4 ^b	15±0.0 ^b	7.8±0.2 ^a	7.8±1.4 ^a
	E	27.3± 0.40 ^c	27.3 ± 1.1 ^d	27.3 ± 1.2 ^c	27.3± 2.14 ^c	32.6±0.1 ^e	37.5±3.2 ^d	15±0.3 ^b
	W	27.3± 0.34 ^c	37.5 ± 2.5 ^d	15 ± 0.4 ^b	27.3±0.13 ^c	15±0.0 ^b	15±2.3 ^b	15±0.4 ^b
	S	15 ± 0.45 ^b	7.8 ± 0.5 ^a	7.8±0.53 ^a	7.8 ± 1.10 ^a	11.54±1.1 ^{ab}	7.8±0.19 ^a	7.8±0.1 ^a
	F	27.3± 0.28 ^c	15 ± 0.71 ^b	37.5±1.21 ^e	15±1.10 ^b	15±0.0 ^b	27.3±0.2 ^c	27.3±2.3 ^c

Values are mean ± STD of replicates. Values with the same alphabet across column are not significantly different at P = 0.05. A, *C. frutescens* var. chacoense ("Ata Ekanna Asa"); E, *C. frutescens* var. minima ("Ata Eye"); W, *C. frutescens* var. baccatum ("Ata Wewe"); S, *C. frutescens* var. fingerh ("Ata Shonbo"); F, *C. frutescens* var. maxima ("Ata Funfun").

Capsicum frutescens var. maxima (Figure 7) and *C. frutescens* var. baccatum (Figure 8) appeared to be less active than the other three varieties (Figures 5, 6, 8) tested in this research. The two varieties are similar in their inhibitory activities except that var. maxima created largest halo which, was a little larger (20.66 mm) than the widest zone of inhibition formed by var. baccatum (20.00 mm). The highest inhibitory activity exhibited by *C. frutescens* var. maxima extracted with acetone, ethanol and water at all concentrations was against *Escherichia coli* (Figure 7). At 100 and 150 mg/ml of acetone and ethanolic extracts of the "Ata funfun", *P. aeruginosa* was least inhibited. In the case of *C. frutescens* var. baccatum (Figure 8), the highest inhibitory activity was observed against *S. typhi*. Acetone and ethanolic extracts at 100 and 150mg/ml acted best against *Serratia marcescens* and *S. aureus*. Whereas at 250 mg/ml, the acetone extract was more inhibitory against *Enterococcus aerogenes* and *S. marcescens* with *S. aureus* except that ethanolic extract was more inhibitory against *S. aureus* than *E. aerogenes* and *S. marcescens*.

The highest inhibitory activity of *C. frutescens* var. minima in all solvents was observed against *S. marcescens* with zones of inhibition that ranged from 13.66 to 22.33 mm, followed by *E. aerogenes* with zones of inhibition of 10.33-18 mm (Figure 9) except for acetone extracts at 150 and 250 mg/ml.

All the pepper fruits showed inhibitory activity against all the human pathogens tested. Generally, at varying

concentrations, the *C. frutescens* varieties in different solvents displayed similar patterns of antibacterial activity. The largest zones of inhibition for each pepper variety were obtained at 250 mg/ml in acetone and ethanol followed by water. The least inhibition was observed at 100 mg/ml for each solvent irrespective of the solvent type used (Figures 5, 6 and 8) except for variety maxima and baccatum (Figures 7 and 9). The smallest zones of inhibition for varieties maxima and baccatum, was obtained at 100 mg/ml with acetone and ethanol. The water solvent extracts of the two peppers gave the least zones of inhibition at 100 and 150 mg/ml.

Table 2 shows the MIC of the pepper fruit extracts. All the pepper fruits, independent of the extracting solvents had a MIC range of 7.8 - 37.5 mg/ml. The water extract of variety fingerh inhibited all the test bacteria at a MIC of 7.8 mg/ml except *P. aeruginosa* that were inhibited at 11.54 mg/ml. A MIC range of 7.8 - 15 mg/ml was observed for the water extract of variety chacoense.

The water extract of variety minima, had a MIC of 37.5 mg/ml (against *P. aeruginosa*, *Enterococcus aerogenes* and *Salmonella typhi*), 32.6 mg/ml (against *S. aureus* and *S. marcescens*) and 27.3 mg/ml (against *E. coli*). The MIC range for variety maxima was 15 mg/ml (against *E. coli*, *S. marcescens* and *E. aerogenes*) to 37.5 mg/ml (*S. aureus*).

The extracts of *C. frutescens* var. chacoense and fingerh showed the highest activity against the tested pathogens while var. minima and maxima showed the

least activity. The higher alkaloid and saponin content of these two pepper varieties (chacoense and fingerh) could have accounted for this. The amount of alkaloids and saponins higher than those of tannins and flavonoids determined quantitatively in the fruits of the *Capsicum frutescens* varieties, could be responsible for their antibacterial properties. This is in agreement with the work of Otunola et al. (2010) who reported high alkaloid and saponin content in pepper. Saponins are produced by plants as a defense mechanism to stop attacks by foreign pathogens, which makes them natural antibiotics (Otunola et al., 2010). Alkaloids are heterocyclic indole compounds, which have been proven to have pharmacological properties such as hypotensive activity, anticonvulsion activity, antiprotozoal and antimicrobial activities (Mallikharjuna et al., 2007). Purified alkaloids as well as their synthetic agents are used as analgesic, antimalarial, antiseptic and bactericidal (Otunola et al., 2010).

Although the mechanism of action of these extracts was not studied, the phytochemical compounds such as tannins are known to coagulate the wall proteins, while saponins facilitate the entry of toxic material or leakage of vital constituents from the cell (Onwuliri and Wonang, 2005). Flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low concentrations (Olajuyigbe and Afolayan, 2012).

All the pepper varieties' extracts tested showed antibacterial properties on both Gram- positive and Gram-negative bacteria used in this investigation. This is in conformity with previous studies carried out by Soetarno et al. (1997). This means that all the varieties of *C. frutescens* used in this study are useful as potential antibacterial agents. It was also observed from this study that acetone and ethanol extracts exhibited higher inhibitory activity on the test organisms. This can be attributed to the ability of ethanol and acetone to extract more of the secondary metabolites, which are believed to exert antibacterial activity on the test organisms.

The variations in the antibacterial activities of the *C. frutescens* fruits may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients in the pepper varieties (Nwachukwu and Uzoeto, 2010). This variation could also be ascribed to the morphological differences between the tested pathogens. Vital and Rivera (2009), stated that cell wall of Gram positive bacteria is more complex and lacks the natural sieve effect against large molecules due to the small pores in their cell envelop. Gram-negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porin constitute a selective barrier to the hydrophilic solute with exclusion limit of about 600Da (Prescott et al., 2009; Vital and

Rivera, 2009). It can be concluded that the pepper fruits varieties possessed a broad spectrum of antibacterial activity.

Extracts with known antimicrobial properties can be of great importance in therapeutic treatments and their active principles have been considered alternative cheap and effective herbal drugs against common microbial infections (Rojas et al., 2006). The significant antibacterial activities of *C. frutescens* indicated its medicinal and preservative potential that could be used against infectious and food borne pathogens.

Reports showed that the chemical constituents responsible for the pungency in *Capsicum* are capsaicinoids. The capsaicinoids are composed of 12 different compounds, of which the two major compounds are capsaicin and dihydrocapsaicin (80 - 95%) (Soumya and Nair, 2012). Zhang et al. (2003) found the potential of capsaicin in inhibiting the growth of adult T – cell leukemia cells. There are different opinions regarding the antimicrobial properties of capsaicin. Kurita et al. (2002) suggested that capsaicin is a major antimicrobial factor. Cichewicz and Thorpe (1996) observed that the pure capsaicin and dihydrocapsaicin had no antimicrobial properties. Other components like the lectins from *C. annum* and *C. frutescens* are reported to exhibit antifungal and sugar binding characteristics (Soumya and Nair, 2012).

However, further research has to be carried out in order to determine the bioactive components in the long pepper fruits (*C. frutescens*) used in this research.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- AOAC (2002). Official Methods of analysis. 17th edition, Washington D.C, Association of Official Analytical Chemists.
- Cichewicz RH, Thorpe PA (1996). The antimicrobial properties of chile peppers (*Capsicum* spp) and their uses in Mayan medicine. *J. Ethnopharmacol.* 52(2):61-70.
- Dong MW (2000). How hot is that pepper? *Today's Chemist at Work* 9(5):17-20.
- Dorantes L, Colmenero R, Hernandez H, Mota L, Jaramillo ME, Fernandez E and Solano C (2000). Inhibition of growth of some food borne pathogenic bacteria by *Capsicum annum* extracts. *Int. J. Food Microbiol.* 57:125-128.
- Dutta AC (2001). *Botany for Degree Students*. Sixth Edn. Oxford University Press. pp. 321-329.
- Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical Constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 4(7):685-688.
- George FOA, Ephraim RN, Obasa SO, Bankole MO (2010). Antimicrobial properties of some plant extracts on organisms associated with fish spoilage. *Afr. J. Biotechnol.* 9 (6): 202-206.
- Kurita S, Kitagawa E, Kim CH, Momose Y, Iwashashi H (2002). *Biosci. Biotechnol. Biochem.* 66(3):532-536.
- Mallikharjuna PB, Rajana LN, Sharananbasappa GK (2007). Phytochemical studies of *Strychnos potatorum* L. f – A medicinal plant. *Elect. J. Chem.* 4(4):510-518.

- Nwachukwu E, Uzoeto HO (2010). Antimicrobial activity of some local mushrooms on pathogenic isolates. *J. Med. Plants Res.* 4(23):2460-2465.
- Nwokem CO, Agbaji EB, Kagbu JA, Ekanem EJ (2010). Determination of Capsaicin content and pungency level of five different peppers grown in Nigeria. *New York Sci. J.* 3(9):17-21.
- Oboh G, Rocha JBT (2006). Water extractable phytochemicals from *Capsicum pubescens* (Tree pepper) inhibit lipid peroxidation induced by different pro – oxidant agents in brain. UNESCO and International Atomic Energy Agency. Available at: http://www.ictp.it/pub_off
- Olajuyigbe OO, Afolayan AT (2012). Antimicrobial potency of the ethanolic crude bark extract of *Ziziphus mucronata* Subsp. *mucronata* Willd. *Afr. J. Pharm. Pharmacol.* 6(10):724-730.
- Oni MO (2011). Evaluation of seed and fruit Powders of *Capsicum annum* and *Capsicum frutescens* for control of *Callosobruchus maculatus* (F.). *Int. J. Biol.* 3(2):185-188.
- Onwuliri FC, Wonang DL (2005). Studies on the combined antibacterial action of ginger (*Zingiber officinale* L.) and garlic (*Allium sativum* L.) on some bacteria. *Nig. J. Bot.* 18: 224-228.
- Otunola GA, Oloyede OB, Oladiji AT, Afolayan JA (2010). Comparative analysis of the chemical composition of three Spices- *Allium sativum* L., *Zingiber officinale*, Rosc. and *Capsicum frutescens* L. commonly consumed in Nigeria. *Afr. J. Biotechnol.* 9(41): 6927-6931.
- Prescott LM, Flarley JP, Klevin OA (2009). *Microbiology*. 6th edition. McGraw-Hill education. New York.
- Rojas R, Bustamanate B, Bauer J, Fernandez L, Albaro J, Lock O (2006). Antimicrobial activity of selected Peruvian Medicinal plants. *J. Ethnopharmacol.* 88:199-204.
- Showemimo SJ, Olarenwaju JD (2004). The chemical composition of some pepper varieties commonly grown in the Northern guinea Savannah of Nigeria. *J. Food Technol.* 2(2):100-102.
- Soetarno S, Sukrasno E, Sylvia Y (1997). Antimicrobial Activities of the Ethanol Extracts of *Capsicum* fruits with different levels of pungency. *Jurnal Matematika & Sains* 2(2):57-63.
- Soumya SL, Nair BR (2012). Antifungal efficacy of *Capsicum frutescens* L. extracts against some prevalent fungal strains associated with groundnut storage. *J. Agric. Technol.* 8(2): 739-750.