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Evaluation of antioxidant, phytochemicals and antibacterial potential of *Mormordica charantia* (Linn) against pathogenic bacteria isolated from ready to eat food sold in Akure Metropolis, Nigeria

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Abstract

Background: Food hygiene and safety is of utmost importance for public health, as it helps to protect the health of consumers from foodborne illnesses. Although, hundreds of plant species have been screened and tested for antimicrobial properties, the vast majority of these plants have not been adequately screened and evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aimed to evaluate antioxidant, phytochemicals and determine the antibacterial activity of *M. charantia* (Linn) *on* pathogenic organism from Ready-to-eat food sold in Akure metropolis.

Results: Bacteria isolated from RTE food studied belong to the following genera; *Staphylococcus, Streptococcus, Citrobacter, Pectobacterium, Klebsiella, Bacillus, Kocuria, Kluyveria, Serratia, Pantoea, Enterobacter and Salmonella*. The antibiotic susceptibility pattern revealed that the Gram negative organisms were more susceptible to Gentamycin (30 µg) while Streptomycin (30 µg) was found to be more potent on Gram positive organisms. Water and ethanol extracts showed significant to moderate antibacterial activity toward all tested isolates except; *Escherichia vulneris* and *Kluyveria intermedia* which are both resistant to the aqueous extracts of *M. charantia* and the latter which was resistant to the ethanoic extract. The highest inhibitory activity was observed for *Bacillus cereus* with zone of inhibition of diameters 28 ± 0.29^{l} (mm), followed by *Escherichia vulneris* 25 ± 0.29^{l} (mm). DDPH% Inhibition has higher value in the water extract (69.21 ± 0.882^{a}) while lower value was recorded in the ethanol extract (67.89 ± 0.155^{b}). % Iron chelation has higher value in the ethanol extract (121.87 ± 0.882^{a}) while lower value was recorded in the water extract (97.28 ± 0.155^{b}). In all the concentration, vitamin C was higher in the ethanol extract than in the water extract.

Conclusions: The study revealed that all the Ready-to-eat food had a total bacterial count that are below potentially hazardous count although the presence of some microorganisms that are of health significance were observed. The study also confirmed that *M. charantia* (Linn) extracts had antibacterial effect against tested isolates from Ready-to-eat food sold in Akure metropolis. Based on the findings of this great research work, *Mormordica charantia* (Linn) possesses antioxidant activity that could prevent oxidative stress and degenerative diseases.

Keywords: Antibacterial, *Mormordica charantia* (Linn), Pathogenic bacterial, Read-to-eat food

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Background

Man's life and survival would be impossible without 'symbiosis' with, and extensive use of plants and plant products. Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Boklage and Lehmkuhl 2018). According to the World Health Organization "a medicinal plant" is any plant which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs (Awuchi 2019).

Medicinal plants have become the subject of very intense pharmacological study in recent years (Ahn 2017). The continuous emergence of multidrug resistances microorganisms has drastically reduces the efficacy of our antibiotic armory, and consequently, increases the frequency of therapeutic failure (Awuchi 2019). Although, hundreds of plant species have been screened and tested for antimicrobial properties, the vast majority of the plants have not been adequately screened and evaluated. Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aimed to carryout phytochemical screening and evaluates *Mormordica charantia* (Linn) for antimicrobial activity on pathogenic bacteria from RTE food sold in Akure metropolis.

Mormordica charantia (Linn) is an annual to perennial monoecious climbing or sprawling herb. It may be either hairless or slightly hairy. There is a central taproot, from the apex of which the stems spread to climb over any available support (Deepti et al. 2017). *M. charantia* (Linn) is a medically important vine (Khalid et al. 2021). It has also been utilized in different traditional medicines for the treatment of bronchitis, cholera, anemia, ulcer, blood diseases, diarrhea, gonorrhea rheumatism, gout, colic, worms, dysentery, disease of liver and spleen, cancer and diabetes etc. (Jandari et al. 2020). The main constituents of this plant include protein, triterpene, alkaloid, steroid, inorganic, lipid, and phenolic compounds, which are responsible for biological and pharmacological activities such as anti-diabetic, anti-cancerous and anti-tumorous, anti-microbial, anti-helmintic, antimalarial, anti-ulcerative and immunomodulatory (Guarniz et al. 2019)).

The food eaten has a direct influence on health, it is therefore an important task for food inspectors, food manufactures and food handlers to keep food safe from pathogenic microorganisms, especially when such food are to be consumed without further processing (Banik et al. 2019), some ready-to-eat foods also are regarded as 'potentially hazardous'. Such foods can support the growth of pathogenic (food poisoning) bacteria and must be kept at certain temperatures to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food (NSW 2019) Foodborne diseases due to bacteria occur with the consumption of food contaminated by pathogen microorganisms (Mboto et al. 2012). At present, the spectrum of foodborne diseases is increasing and these diseases create a major health problem. Different foodborne pathogens have been associated with outbreaks of foodborne disease.

The continuous emergence of multidrug resistances microorganisms have drastically reduced the efficacy of our antibiotic armory, and consequently, increases the frequency of therapeutic failure. The food eaten has a direct influence on health, it is therefore an important task for food inspectors, food manufactures and food handlers to keep food safe from pathogenic microorganisms, especially when such food are to be consumed without further processing. At present, the spectrum of foodborne diseases is increasing and these diseases create a major health problem. Hence this study would determine the antibacterial activity of *M. charantia* (Linn) *on* pathogenic organism from Ready-to-eat food sold in Akure metropolis.

Hence, this study was carried out to evaluate antioxidant, phytochemicals and determine the antibacterial activity of *M. charantia* (Linn) *on* pathogenic organism from Ready-to-eat food sold in Akure metropolis.

Methods

Plant sample

The plant *M. charantia* (Linn) selected for this study was collected along the school farm of Federal University of Technology, Akure, Ondo state, Nigeria (7.2571° N, 5.2058° E). The plant material used was identified at the herbarium of Crop, Soil and Pest Management, Akure with voucher specimen number FUH 3622.

Food samples

Ready to eat food samples were collected for 30 days. A total of 103 samples were transferred from different vendor to the microbiology laboratory aseptically. The samples consisted of white rice, moimoi, fried rice and suya.

Other materials

test tubes, conical flasks, sterile syringes, wire loops, sterile disposable Petri dishes, beaker, autoclave, incubator, cover slip, microscope slide, plain bottles, weighing balance, cotton wool, ethanol, paper tape, measuring cylinder, hand gloves, distilled water, Bunsen burner, refrigerator, test tube rack, retort stand, thermometer, burette, pH meter, bioreactor and anaerobic jar.

Sterilization of materials

All glassware were washed with detergent and rinsed thoroughly. They were placed in a rack to dry and then autoclaved at 121 °C for 15 min to kill microorganisms.

Preparation of plant materials for extraction

The plant materials were dried (in a ventilated room away from direct sun light) for 28 days. The dried pieces (whole plant) were milled into powdery form using a mortar and pestle, and put in a container. The weights of the powdered form of leaves were recorded.

Preparation of ethanol extract

Two hundred and fifty grams (250 g) of the powdered plant materials were weighed and soaked in one thousand two hundred and fifty (1250 ml) of Ethanol and kept for 72 h in a Gallenkamp shaker at 65 revolutions per minute. The contents were sieved using muslin cloth, the liquid sieved was then filtered by pouring the contents sieved into a beaker containing a funnel and Whatman filter paper no. 1 inserted into it. The filtrate was poured into a round bottom flask and kept away from direct sunlight undisturbed, thereafter it was subjected to further extraction using soxhlet apparatus for subsequent sterile filtration and filtrate oven dried at 40 °C till it became paste-like in appearance. The paste-like filtrate was weighed and stored in the refrigerator at 4 °C until used. The crude extract was thereafter weighed and dissolved in a known volume of dimethyl sulphoxide.

Preparation of water extract

Two hundred and fifty grams (250 g) of the powdered plant materials were weighed and soaked in one thousand two hundred and fifty (1250 ml) of distilled water and kept for 72 h in a Gallenkamp shaker at 65 revolutions per minute. The contents were sieved using muslin cloth, the liquid sieved was then filtered by pouring the contents sieved into a beaker containing a funnel and Whatman filter paper inserted into it. The filtrate was poured into a round bottom flask and kept away from direct sunlight undisturbed, thereafter it was subjected to further extraction using soxhlet apparatus for subsequent filtration and filtrate oven dried at 40 °C till it becomes paste-like in appearance. The paste-like filtrate was weighed and stored in the refrigerator at 4 °C until used. The crude extract was thereafter weighed and dissolved in a known volume (10 ml) of dimethyl sulphoxide.

Isolation of bacteria

Bacteria isolation was carried out using the pour plate technique. 0.1 ml of serial diluents 10^{-2} and 10^{-4} was measured and poured into different sterile Petri dishes respectively and it was then overlaid with the molten sterilized Nutrient agar (NA) media, this was also done for MacCokay agar, Salmonella-Shigella agar, Mannitol salt agar and Eosine methylene blue agar. The plates were rocked gently to ensure homogeneity on the media in the plates and were then allowed to solidify. After solidification, the plates were then incubated aerobically in the incubator at 37 °C for 24 h. Pure cultures of the microorganisms were obtained using the streak plate method. Distinct colonies were examined and enumerated. Identification of bacterial isolates was performed according to standard bacteriological techniques previously established.

Antibacterial assay

The effect of the plant extracts on the bacterial strain was assayed by Agar well diffusion method and further confirmed by Disc diffusion method. Agar well diffusion method was used to test the antibacterial activity of the extracts. The bacteria inoculi were prepared by transferring the isolates from already incubated Nutrient Agar into a freshly prepared broth using inoculating loop and incubated for 24 h at 38 °C. After incubation, standardized inoculum was streaked on petri dishes containing freshly prepared Muller Hinton Agar using a sterile swab sticks. five 6 mm wells were bored on the already inoculated MHA plates using a sterile cork borer, the susceptibility test was carried out in triplicate. Each of the wells were filled up with 100 mg/ml, 50 mg/ml and 25 mg/ml of the reconstituted extracts respectively. The fourth well, being the positive control was filled up with 25 mg/ml Chloramphenicol while 2% DMSO was used as the negative control. The plates were incubated at 38 °C for 18 to 28 h and zones of inhibition were measured with milliliter ruler.

Evaluation of minimum inhibitory concentration and minimum bactericidal concentration

For minimum inhibitory concentration, 0.2 ml of extract (100 mg/ml, 50 mg/ml and 25 mg/ml) was dispense into different bottles containing standardized organism suspension. The bottles were incubated at 38 °C for 24 h after which turbidity was measure using spectrophotometer. For minimum bactericidal concentration, 1 ml of aliquots of test organism suspension from the minimiun inhibitory concentration test bottles was pour plate into a fresh culture media after, incubated at 38 °C for 24 h.

Phytochemical analysis

Test for the screening and identification of chemical bioactive chemical constituent *Mormordica charantia*

(Linn) were carried out with the extract using the standard procedure as described.

Some in-vitrol antioxidant analyses of the plants samples

Determination of DPPH free radical scavenging ability

The 1, 1-diphenyl-2-picryhydrazyl (DDPH) free radical scavenging ability of the extract was determined using standard method. Briefly, 1.0 mL of different concentrations (20, 40 and 80 mg/mL) of the extracts was placed in respective test tubes. 1.0 ML of 0.1 Mm methanolic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 min before absorbance measured at 516 cm. Decreased absorbance of the sample indicates DDPH free radical scavenging capability.

Distilled water was replaced for the extract in the control.

Percentage radical scavenging ability was calculated using the following expression.

Abs sample

$$\%\text{DDPH radical scavenging ability} = 1 \rightarrow \times 100$$

Abs control

Determination of ferric reducing antioxidant power

The reducing property of the extract was determined using standard method. This method is based on the reduction of (Fe3+) ferricyanide in stoichiometric excess relative to the antioxidants. Different concentrations of the methanolic extract of the sample and its various fractions (10–50 g/Ml) was added to 1.0 mL of 200 Mm of sodium phosphate buffer PH 6.6 and 1.0 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min, thereafter 1.0 mL of freshly prepared 10% TCA was quickly added and centrifuged at 2000 rpm for 10 min, 1.0 mL of the supernatant was mixed with 1.0 mL of distilled water and 0.25 ml 0f 0.1% of $FeCl_3$ solution was added. Distilled water was used for blank without the test sample while control solution contained all other reagents except the 0.1% potassium ferricyanide. Absorbances of these mixtures were measured at 700 nm using a spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample.

$$\text{The percentage Ferric reducing antioxidant power (\%) was subsequently calculated} = [(\text{Abscontrol} - \text{Abssample})/\text{Abscontrol}] \times 100$$

Determination of Fe2 + chelation

The ability of the extract to chelate Fe^{2+} was determined using standard method. Freshly prepared 500 M $FeSO_4$ (150 L) was added to a reaction mixture containing 168 L of 0.1 M Tris-HCL (PH 7.4), 218 L saline and the different concentrations of extracts (0–25 L). The reaction mixture was incubated for 5 min before the addition of 13 L of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer.

The percentage of Fe (II) chelating ability was subsequently calculated with respect to the control (which contains all the reagents without the test sample).

$$\text{The } Fe_2 + \text{chelation ability} + [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100$$

Determination of vitamin C (ascorbic acid) contents in various fruit and vegetable by UV—spectrophotometry and titration methods

The aim of the work is to determine the amount of Ascorbic acid in the samples.

Extraction from plant source

Mormordica charantia (Linn) was blended, 1 g of the sample was transferred into a 100 ml volumetric flask. 10 ml of water was added to the sample. 5 ml of Acetic acid was added to the sample using a pipette for homogenization and it was shaken appropriately.

The extraction was left for 3 h. The solution was filtered and a clear solution was obtained.

Estimation

To the filtered solution, Few drops of Bromine water was added until the solution became coloured. This is to confirm the complete oxidation of Ascorbic acid to dehydroascorbic acid. Few drops of thiourea solution was added to it to remove excess bromine and thus a clear solution was obtained. Then, 2–4 Dinitrophenyl hydrazine solution was added thoroughly with all standards and also with the oxidized Ascorbic acid and make up to 10 ml mark with Acetic acid. The absorbance of the sample was examined at 520 nm using UV spectrophotometer to determine the concentration of Ascorbic acid under testing.

Statistical analysis

All the data are presented as standard error of the mean ± (SEM) from triplicate experiments and were analysed using Statistically Package for Social Sciences

(SPSS) software version 22.0. A one-way analysis of variance (ANOVA) was used for multiple comparison. The correlation between result properties was described by the Pearson product-movement correlation.

Results

Extract yield

After the extraction process, the 70% ethanol yielded the highest value of the extract The ethanol extracts of *Mormordica charantia* Linn showed yields of 22.45% while the yields from water extracts is 18.60% (Fig. 1).

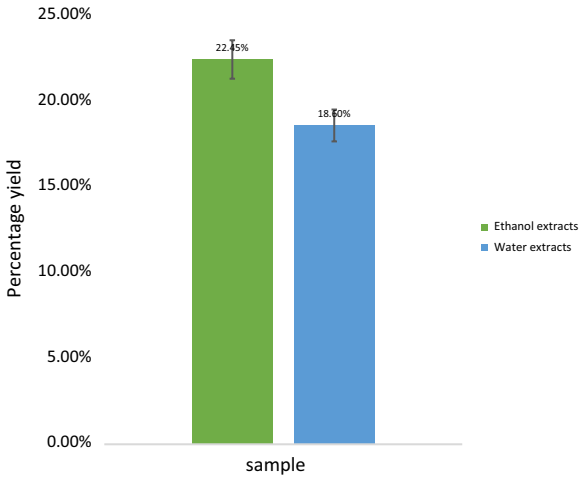

Fig. 1 Percentage yields of ethanol and water extracts of *M. charantia* (Linn) per 250 g. Error bar +/-2 SE

Bacterial load of ready-to-eat food samples sold in Akure metropolis

Total bacterial loads of ready-to-eat food samples sold in Akure metropolis

The total bacterial counts of Ready-to-eat food samples sold in Akure metropolis is shown in Figs. 2, 3, 4, 5. Moimoi purchased from vendor A had a significantly high bacterial loads with a value of $1.38 \pm 0.012 \times 10^4$ cfu/g whereas Moimoi from vendor C had the lowest total bacterial loads $1 \times 10^3 \pm 0.012$ cfu/g. Total bacterial counts of Fried rice from different vendors varied with no significant difference ($P \leq 0.05$), sample from vender A had the highest bacterial loads with value of $2.5 \pm 0.01 \times 10^3$ cfu/g whereas sample from vendor E had the lowest bacterial loads with value of $1.2 \times 10^3 \pm 0.01$ cfu/g. Suya Sample purchased from vendor A had the highest bacterial loads ($1.33 \pm 0.02 \times 10^4$ cfu/g), whereas sample from vendor S had the lowest bacterial loads ($1.25 \pm 0.02 \times 10^4$ cfu/g). White rice purchased from vendor C had the highest bacterial loads with value of $2.7 \pm 0.03 \times 10^3$ cfu/g compare to sample from other vendors (Figs. 2, 3, 4, 5).

Among all the samples collected, Moimoi purchased from vendor A had the highest bacterial loads with value of $1.38 \pm 0.02 \times 10^4$ cfu/g, followed by Suya

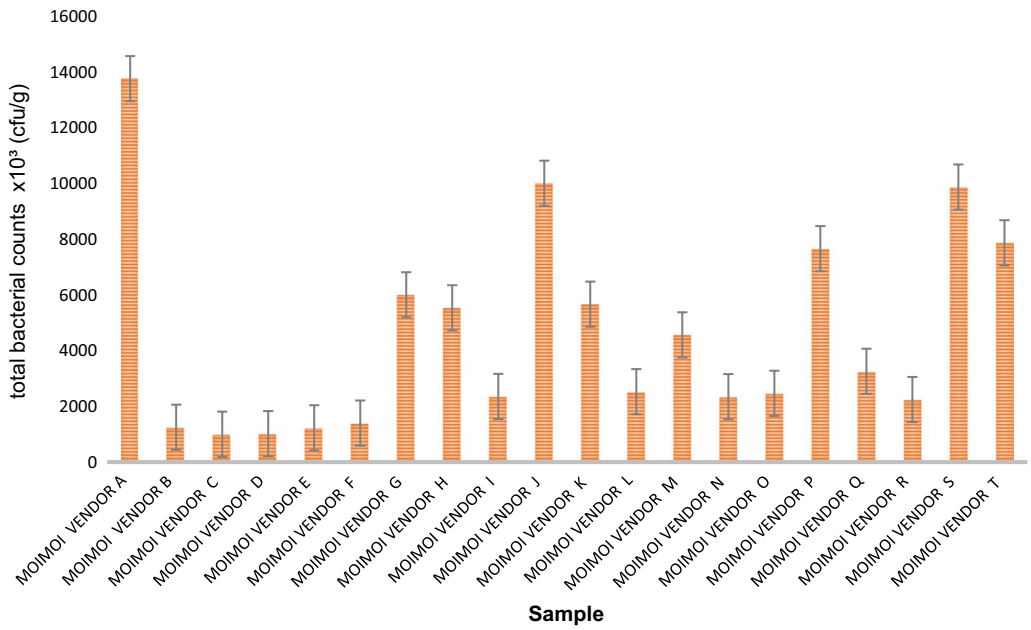

Fig. 2 The total bacterial counts of Ready-to-eat Moimoi sold in Akure metropolis. Error bar +/-2 SE

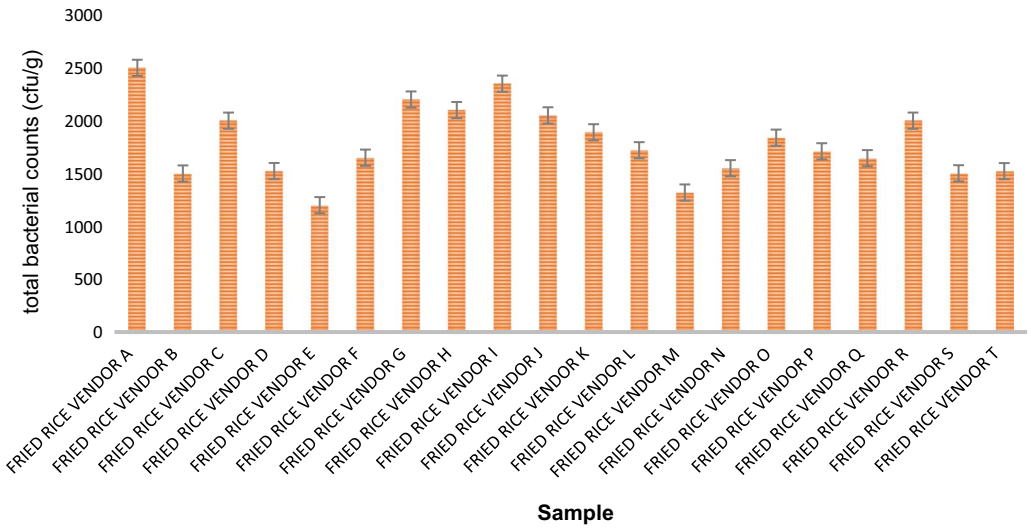

Fig. 3 The total bacterial counts of Ready-to-eat Fried rice sold in Akure metropolis. Error bar +/-2 SE

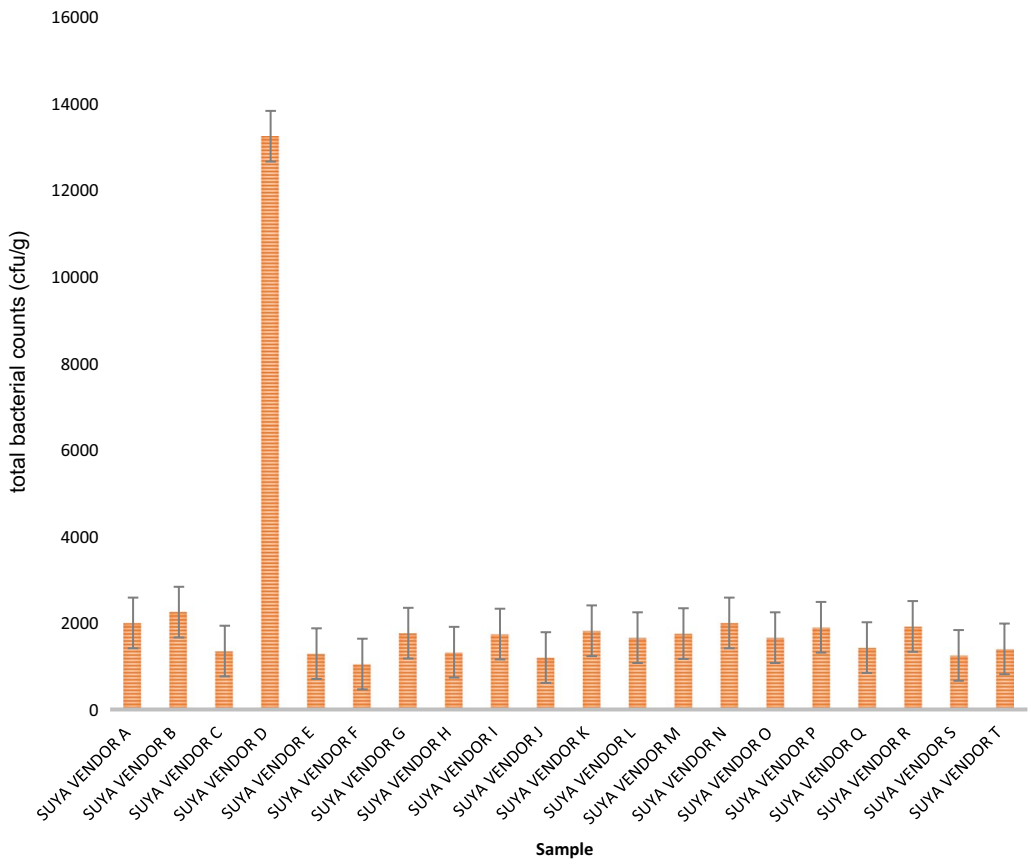

Fig. 4 The total bacterial counts of Ready-to-eat Suya sold in Akure metropolis. Error bar +/-2 SE

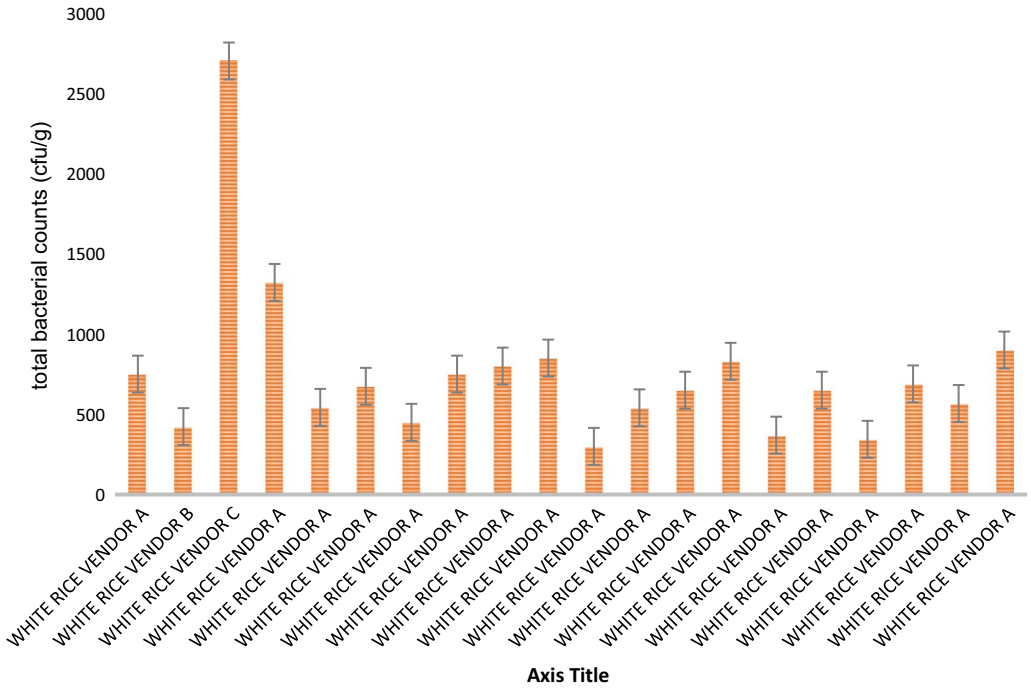

Fig. 5 The total bacterial counts of Ready-to-eat White rice sold in Akure metropolis. Error bar +/-2 SE

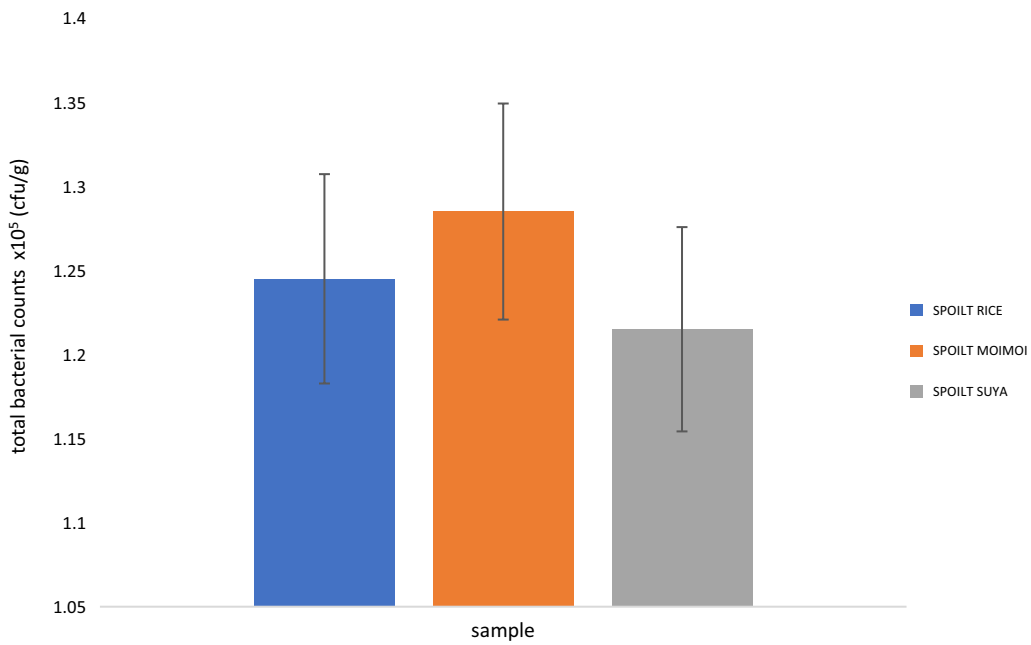

Fig. 6 The total bacterial counts of isolates from spoilt form of Ready-to-eat food sold in Akure metropolis. Error bar +/-2 SE. Error bar +/-2 SE

sample purchased from vendor D whereas white rice purchased from vendor B had the lowest bacterial loads. The total bacterial load of spoilt food sample is higher than Ready-to-eat food sample. Among the spoilt sample analyzed spoilt Moimoi had the highest bacterial loads with value of $1.29 \pm 0.04 \times 10^3$ cfu/g (Fig. 6).

Morphological and biochemical characteristics of bacteria species isolated from ready-to-eat food sample sold in Akure metropolis

The morphological and Biochemical results of bacteria isolate from Ready-to-eat food is shown in Table 1. The percentage of occurrence of different bacterial genera in Ready-to-eat food sample analyzed is shown in Fig. 7. The bacteria isolated from the samples belong to the genera of *Staphylococcus, Citrobacter, Enterobacter, Salmonella, Enterococcus, Klebsiella, Bacillus, Escherichia, Kocuria, Streptococcus, Kluyveria, Shinwellia, Pseudomonas, Serratia, Pectobacterium* and *Pantoea. Enterobacter* had highest frequency of occurrence of 23.1% among all the bacterial genera isolates from Ready-to-eat food samples, Followed by *Staphylococcus* and *Salmonella* with uniform frequency of occurrence of 17.9%.

Distribution of bacteria species isolated from ready-to-eat foods sold in Akure metropolis

Distribution of Bacteria species isolated from Ready-to-eat foods sold in Akure metropolis and their spoilt form with their percentage of occurrence in bracket is shown in Table 2. *Enterobacter (Cronobacter) sakzaki* was isolated from almost all types of food samples used whereas S*taphylococcus heamolyticus, Klebsiella pnuemoniae, Enteric group 69, Salmonella typhi* and *Pantoea agglomerans* were present in only one type of RTE food samples. Figure 7 showed the percentage of occurrence of different bacterial genera in Ready-to-eat food sold in Akure metropolis.

Antibiotic susceptibility pattern result for pathogenic organism isolated from RTE food sold in Akure metropolis

The Antibiotic susceptibility pattern for Gram negative and Gram positive bacterial isolated from RTE food sold in Akure metropolis is shown in Tables 3 and 4. *Kluyveria intermedia* resist the effect all the antibiotics used. Gentamycin (30 µg) is the most effective antibiotics on Gram negative bacteria isolates whereas Pefloxacin (30 µg) had the lowest effect on Gram negative bacteria isolates. Streptomycin (30 µg) was effective for all the Gram positive bacteria isolates whereas Ampiclox (30 µg) and Pefloxacin (30 µg) were effective on one of the gram positive bacteria isolates.

Phytochemical composition of the extracts

Tannin, saponin, phlobatannin, steroids and alkaloid were observed to be present in the water extract of *Momordica charantia* (Linn) with no traces of flavonoids, cardiac glycosides, anthraquinones and phenols. phlobatannin, steroids, cardiac glycosides alkaloids and phenols where present in ethanolic extracts sample of *Momordica charantia* (Linn). Table 5 and Table 6 showed the components present.

Antibacterial activity of ethanolic and water extracts on bacterial isolates from RTE foods sold in Akure metropolis

The Zone of inhibition (mm) of *Momordica charantia* (Linn) extracts against bacterial isolates from RTE foods sold in Akure metropolis is shown in Tables 7 and 8. The results from the agar well-diffusion method revealed that the ethanol and water extracts showed significant to moderate antibacterial activity toward all tested isolates except; *Escherichia vulneris* and *Kluyveria intermedia* resist the effect of water extracts. Only *Kluyveria intermedia* resist the effect of ethanol extracts. The highest inhibitory activity of the water extracts was observed for *Bacillus cereus* with wide inhibition zone diameters 28 ± 0.29^{l} (mm), followed by *Staphylococcus aureus* 24 ± 0.29^{k} (mm) whereas *Escherichia vulneris* 25 ± 0.29^{l} (mm) S*taphylococcus heamolyticus* 24 ± 0.29^{k} (mm), and *Staphylococcus aureus* 24 ± 0.29^{k} (mm) had the highest inhibitory activity for ethanol extracts.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of ethanolic and water extracts on bacterial isolates from RTE foods sold in Akure metropolis

Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of Ethanolic and Water Extracts on bacterial isolates from RTE foods sold in Akure metropolis is shsown in Table 9. The MIC and MBC values indicated that *Momordica charantia* Linn specie extracts inhibited the growth of pathogenic bacterial from on bacterial isolates from RTE foods sold in Akure metropolis is shown in Table 9. The minimum inhibitory concentrations (MICs) of the extracts for the test bacteria were relatively lower in water extract compared to the ethanolic extract The concentration ranges from 25 to 100 mg/ml.

Quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and %Iron chelation of water and ethanol extract of *Mormordica charantia* (Linn) at 100 mg/ml

Table 10 shows the quantitative composition of DDPH% Inhibition and Iron chelation analysis in the sun dried water and ethanol extracts of *Mormordica charantia* (Linn). DDPH% Inhibition has higher value in the water extract (69.21 ± 0.882^{a}) while lower value was recorded in the ethanol extract (67.89 ± 0.155^{b}). % Iron chelation has higher value in the ethanol extract

Table 1 Result of morphological and biochemical results of bacteria isolates from Ready-to-eat food sold in Akure metropolis

Biochemical tests																											Suspected bacteria
Tsia																						Gram reaction					
Sucrose	Glucose	Lactose	Gas	H2s	Galactssose	Rhamnose	mannitol	Maltose	raffinose	xylose	Fructose	arabinose	Citrate	Motility	Indole	Nitrate	Mr	vp	Starch	Shape	Gram	Catalase	Coagulse	Pigment	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	Nd	Rod	−	+	−	+	*Citrobacter freundii*
+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	Nd	Rod	−	+	−		*Enterobacter sakzakii*
−	+	−	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	Rod	−	+	−	+	*Salmonella enterica subsp enterica*
−	+	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	Cocci	+	+	−	+	*Enterococcus casseliflavus*
−	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	Nd	Rod	−	+	−	+	*Enterobacter nimipressuralis*
+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	−	+	Nd	Rod	−	+	−	+	*klebsiella pnuemoniae*
+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−	+	Nd	Rod	−	+	−	+	*Cronobacter sakazakii*
−	+	−	−	+	−	−	−	+	+	+	+	−	+	+	−	+	−	+	+	Rod	+	+	−	+	*Bacillus cereus*
−	+	+	+	−	+	+	+	+	−	+	−	+	−	+	+	+	+	−	Nd	Rod	−	+	−	+	*Escherichia coli*
−	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	+	−	−	+	Cocci	+	+	−	+	*Kocuria varians*
+	+	+	−	−	+	−	+	+	−	−	−	−	+	−	−	+	+	+	−	Cocci	+	+	+	+	*Staphylococcus aureus*
+	+	+	−	−	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	Cocci	+	+	−	+	*Streptococcus salivarius subp:t*
+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	+	+	+	Nd	Rod	−	+	−	Nd	*Kluyveria intermedia*
+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	−	Nd	Cocci	+	+	−	Nd	*Staphylococcus gallinarum*
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	Rod	−	+	−	+	*Shinwellia pseudoproteus*

Table 1 (continued)

Biochemical tests																										Suspected bacteria
Tsia																					Gram reaction					
Sucrose	Glucose	Lactose	Gas	H2s	Galactssose	Rhamnose	mannitol	Maltose	raffinose	xylose	Fructose	arabinose	Citrate	Motility	Indole	Nitrate	Mr	vp	Starch	Shape	Gram	Catalase	Coagulse	Pigment	
–	–	–	+	–	–	–	+	–	–	–	–	–	+	+	–	+	–	–	–	Rod	–	+	–	+	*Pseudomonas aeruginosa*
+	+	–	+	–	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	Rod	–	+	–	+	*Serratia liquefaciens*
–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	Rod	–	+	–	+	*Enteric group 69*
–	+	–	+	–	+	–	+	+	+	+	+	+	–	+	–	+	+	+	–	Rod	–	+	–	+	*Escherichia vulneris*
+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	Nd	Rod	–	+	–	+	*Pectobacterium carotovorum*
–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	–	Nd	Rod	–	+	–	+	*Salmonella typhi*
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	Rod	–	+	–	Nd	*Pectobacterium carotovorum*
–	+	–	–	–	+	+	+	+	+	+	+	+	+	+	–	Nd	+	+	Nd	Rod	–	+	–	+	*Pantoea agglomerans*

Nd: not define

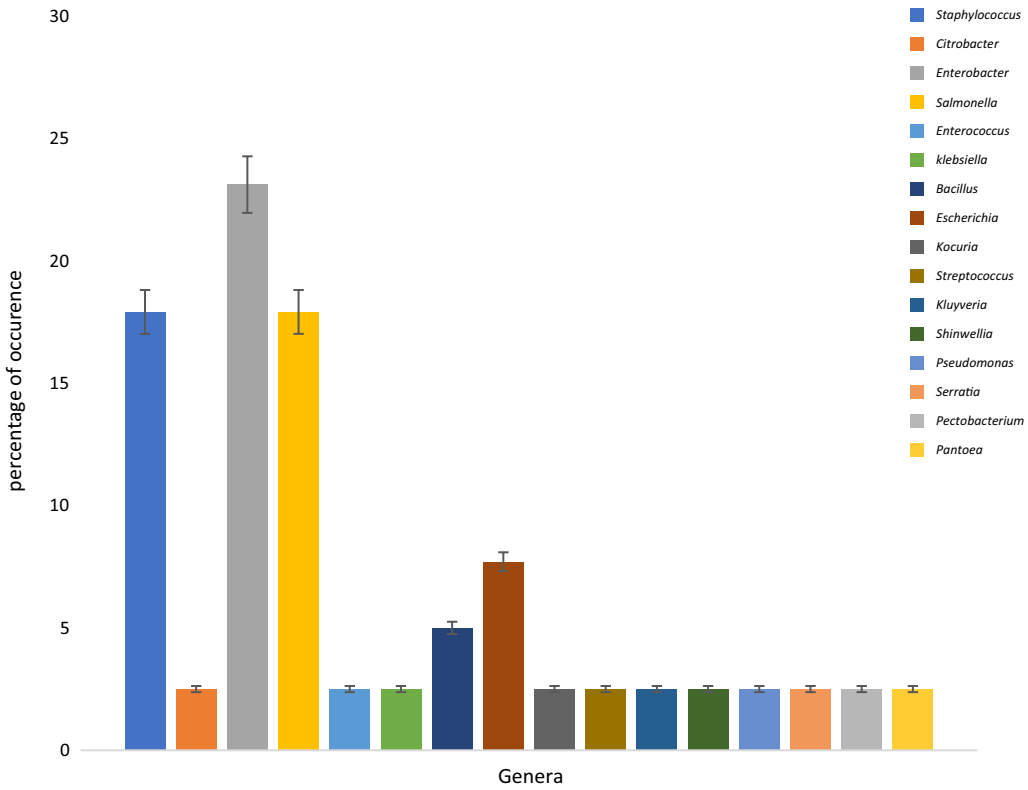

Fig. 7 Percentage of occurrence of different bacterial genera in Ready-to-eat food sold in Akure metropolis

(121.87 ± 0.882^{a}) while lower value was recorded in the water extract (97.28 ± 0.155^{b}).

Analysis of vitamin C in *Mormordica charantia* (Linn)

Table 11 presents the concentration of vitamin C in *Mormordica charantia (Linn).* In all the concentration, vitamin C was higher in the ethanol extract than in the water extract (Fig. 8).

Discussion

The findings in this research indicate that the percentage yields of the extract using different solvents varied. The results corroborate with those obtained by Zaini et al. (2018) in which the percentage recovery of *M. charantia* (Linn) varied with different extraction solvents. The 70% ethanol yielded the highest value of the extract because ethanol is complex chemical structures compared with water extracts. Thus, there was a significant increase in the bioactive substances ($P \leq 0.05$) from ethanolic extract than water extract respectively and this may be due to the polar nature of ethanol.

The study revealed that all the "ready-to-eat" food had a total bacterial counts that are below potentially hazardous counts according to the International microbiological standards recommended limits of bacteria contaminated for foods which is less than 10^5 cfu/g of food for total bacteria plate counts (Owhe-Ureghe et al. 2017).

Bacterial counts obtained and the presence of different species of bacteria in Ready-to-eat food in this study may be attributed to several factors which include the initial contamination on the raw materials to the handling of the finished products. The utensils used in the preparation of the food and sanitary condition of the processing environment especially air and dust-in-air may have contributed to increase in extent of contaminants. The contaminants used up the nutrient in the food, grew and multiplied in their numbers. According to Devi et al. (2016), the total effects of such contaminating factors determines the quality of the food, its probable shelf-life and the potential health risks specially if the contaminants are pathogenic in nature. The result is in harmony with Adewale et al. (2015) that reported different genera of bacteria present in RTE food. Most of the isolates are soil and water microflora, this is however not surprising since soil and water are included among the sources of microbial contaminants in food (Devi et al. 2016). Some of the bacteria isolated are normal flora of man, which are likely to have been introduced by food handlers through

Table 2 Percentage of occurrence of bacteria species isolated from Ready-to-eat foods sold in Akure metropolis

Bacterial isolates	Ready to	Eat food	Sample		Spoilt food	Sample	
	Moimoi N1 = 20	Fried Rice N2 = 20	Suya N3 = 20	White rice N4 = 20	Spoilt rice N5 = 1	Spoilt Moimoi N6 = 1	Spoilt Suya N6 = 1
Staphylococcus gallinarum	66%	–	–	33%	100%	–	
Citrobacter freundii	–	–	–	–	–	100%	100%
Enterobacter (Cronobacter) sakzaki	33%	50%	25%	–	100%	100%	100%
Salmonella enterica subsp enterica	33%	–		66%	–	100%	
Enterococcus casseliflavus	–	–	25%	–	–	–	
Enterobacter nimipressuralis	–	–	50%		100%		
Staphylococcus heamolyticus	33%	–	–	–	–	–	
Klebsiella pnuemoniae	–	50%	–	–	–	–	
Bacillus cereus	–	–	25%	33%	–	–	100%
Escherichia coli	33%	–	25%	–	–	–	
Kocuria varians	–	50%	–	–	–	–	
Staphylococcus aureus	–	–	20%	–	100%	–	100%
Streptococcus salivarius subsp: thermophilus	–	–	–	–	100%	–	
Kluyveria intermedia	–	–	–	33%	–	–	
Escherichia vulneris	33%	–	–	–	–	100%	100%
Shinwellia pseudoproteus	–	50%	–	–	–	–	
Pseudomonas aeruginosa	–	–	–	–	–	100%	100%
Serratia liquefaciens	–	–	25%	–	–	–	
Enteric group 69	33%	–	–	–	–	–	
Pectobacterium carotovorum	–	–	–	–	–	100%	
Salmonella typhi	–	–	50%	–	–	–	
Pantoea agglomerans	–	–	–	–	100%	–	

ND—Not detected, N1… N7 = Number of vendor for food sample collected

sneezing, talking and coughing. The presence of enteric bacteria in the RTE food was not unexpected, however it can be attributed to the water used in preparing the rice by the sellers is most of the time untreated. This points to the fact that not everybody has access to potable water in Nigeria. This study showed that the group of organisms in RTE food in FUTA South gate were prepared under a low standard of hygiene. Some of the microorganisms apart from being able to cause spoilage of the food through their metabolic activities can also pathogenic to the consumers.

The present of some microorganism likes *Salmonella enterica subsp enterica, Klebsiella pnuemoniae, Salmonella typhi, Staphylococcus heamolyticus, Bacillus cereus, Staphylococcus aureus* e.t.c. occurring in high numbers is of health significance as some of them are known to be associated with food poisoning, infection and intoxication capable of causing various ailments in adults and children which may be fatal.

Commercial antibiotics (standard) were tested against Gram negative and Gram positive isolates respectively. The findings in this research work indicate that the effect of commercial antibiotics (standard) on those isolates varied. Gentamycin (30 µg) had the highest inhibition zone for Gram negative bacteria isolates whereas Pefloxacin (30 µg) had the lowest effect on Gram negative bacteria isolates. Similar results was obtained by Sharif et al. (2014) that reported gentamycin to be effective against aerobic gram negative bacteria due to the fact that gentamycin irreversibly binds to the 30S ribosomal subunits. Streptomycin (30 µg) was effective on all the Gram positive bacteria isolates whereas Ampiclox (30 µg) and Pefloxacin (30 µg) were effective on one of the gram positive bacteria isolates. This result may be due to the fact that streptomycin is a broad spectrum antibiotics and had two mechanism of action, it can bind to the small 16S of 30 subunits. Moreover, resistance of *Kluyveria intermedia* to all commercial antibiotics used is worrisome. Unfortunately, resistance of these bacteria to some of this antibiotics is becoming more common globally. As such, appropriate treatment varies with geographic distribution of resistant strains (Mogasale et al. 2021).

Table 3 Antibiotic susceptibility pattern result of Gram negative bacterial isolates from RTE food sold in Akure metropolis

Isolate	Antibiotics (MM)									
	CPX(mm) S ≥ 21 I = 16–20 R ≤ 15	AM(mm) S ≥ 23 I = 14–22 R ≤ 13	AU(mm) S ≥ 18 I = 14–17 R ≤ 13	CN(mm) S ≥ 15 I = 13–14 R ≤ 12	PEF (mm) S ≥ 24 I = 16–23 R ≤ 15	OFX(mm) S ≥ 21 I = 16–20 R ≤ 15	S (mm) S ≥ 15 I = 13–14 R ≤ 12	SXT(mm) S ≥ 17 I = 11–16 R ≤ 10	CH(mm) S ≥ 18 I = 13–17 R ≤ 12	SP (mm) S ≥ 25 I = 16–23 R ≤ 15
Salmonella enterica subsp enterica	22 ± 0.29^{g}	5 ± 0.33^{a}	19 ± 0.29^{e}	18 ± 0.17^{h}	24 ± 0.17^{d}	22 ± 0.29^{e}	14 ± 0.29^{c}	11 ± 0.29^{b}	17 ± 0.29^{e}	22 ± 0.29^{e}
Enterobacter sakzakii	21 ± 0.29^{f}	21 ± 0.29^{e}	18 ± 0.29^{d}	17 ± 0.29^{g}	23 ± 0.17^{c}	4 ± 0.33^{a}	15 ± 0.17^{d}	12 ± 0.17^{b}	13 ± 0.29^{b}	24 ± 0.29^{g}
Escherichia vulneris	17 ± 0.17^{c}	21 ± 0.17^{e}	16 ± 0.29^{c}	15 ± 0.17^{e}	23 ± 0.29^{c}	21 ± 0.17^{d}	17 ± 0.29^{f}	20 ± 0.29^{g}	17 ± 0.29^{e}	20 ± 0.17^{c}
Pectobacterium carotovorum	14 ± 0.29^{b}	15 ± 0.17^{b}	15 ± 0.17^{b}	13 ± 0.17^{c}	24 ± 0.29^{d}	22 ± 0.29^{e}	13 ± 0.29^{b}	15 ± 0.29^{f}	18 ± 0.29^{f}	24 ± 0.17^{g}
Escherichia coli	0 ± 0^{a}	22 ± 0.29^{c}	7 ± 0.28^{a}	14 ± 0.17^{d}	23 ± 0.17^{c}	19 ± 0.17^{c}	16 ± 0.17^{e}	17 ± 0.17^{g}	15 ± 0.29^{c}	19 ± 0.17^{b}
Serratia liquefaciens	14 ± 0.29^{b}	18 ± 0.29^{d}	20 ± 0.29^{f}	16 ± 0.29^{f}	22 ± 0.44^{bc}	22 ± 0.29^{e}	13 ± 0.29^{b}	19 ± 0.17^{h}	17 ± 0.29^{e}	20 ± 0.29^{c}
Enteric group 69	18 ± 0.17^{d}	3 ± 0.17^{a}	16 ± 0.29^{c}	16 ± 0.29^{f}	22 ± 0.29^{b}	19 ± 0.29^{c}	13 ± 0.29^{b}	19 ± 0.29^{h}	18 ± 0.17^{ef}	21 ± 0.29^{d}
Enterobacter nimipressuralis	22 ± 0.17^{g}	23 ± 0.17^{e}	19 ± 0.29^{e}	15 ± 0.17^{e}	25 ± 0.29^{e}	25 ± 0.29	19 ± 0.17^{g}	21 ± 0.17^{i}	22 ± 0.17^{h}	24 ± 0.29^{f}
klebsiella pneumoniae	21 ± 0.17^{f}	22 ± 0.17^{d}	21 ± 0.17^{g}	17 ± 0.17^{g}	23 ± 0.17^{c}	25 ± 0.29^{f}	19 ± 0.17^{g}	20 ± 0.29	20 ± 0.17^{g}	23 ± 0.17^{f}
Citrobacter freundii	20 ± 0.17^{e}	24 ± 0.29^{h}	21 ± 0.29^{g}	17 ± 0.29^{g}	23 ± 0.17^{c}	21 ± 0.29^{d}	20 ± 0.29^{h}	13 ± 0.29^{c}	16 ± 0.29^{d}	20 ± 0.17^{c}
Salmonella typhi	22 ± 0.29^{g}	23 ± 0.29^{g}	23 ± 0.17^{h}	13 ± 0.17^{b}	23 ± 0.29^{c}	16 ± 0.17^{b}	21 ± 0.29^{i}	14 ± 0.29^{d}	19 ± 0.29^{g}	23 ± 0.29^{f}
Kluyveria intermedia	0 ± 0^{a}	1 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	2 ± 0^{a}	0 ± 0^{a}	4 ± 0^{a}	2 ± 0^{a}	5 ± 0^{a}

OFX = Ofloxacin (10 µg), PEF = Pefloxacin (30 µg), CPX = Ciprofloxacin (30 µg), AU = Augmentin (10 µg), CN = Gentamycin (30 µg), S = Streptomycin (30 µg), SXT = Septrin (30 µg), AM = Amoxicillin (30 µg), CH = Chloramphenicol (30 µg), SP = Sparfloxacin (30 µg). R = resistant, I = intermediate and S = susceptible according to the Clinical and Laboratory Standards Institute (CLSI, 2018). Values are expressed in mean ± SEM. Means of antibiotic without a common superscript letters differ $P < 0.05$ was considered to be statistically significant

Table 4 Antibiotic susceptibility pattern of Gram positive bacterial isolates from RTE food sold in Akure metropolis

Isolate	Antibiotics									
	AM(mm) S ≥ 18 I = 14–17 R ≤ 13	**Z (mm) S ≥ 18 I = 15–17 R ≤ 14**	**APX(mm) S ≥ 23 I = 14–23 R ≤ 13**	**CN(mm) S ≥ 15 I = 13–14 R ≤ 12**	**PEF (mm) S ≥ 24 I = 16–23 R ≤ 15**	**E (mm) S ≥ 23 I = 14–22 R ≤ 13**	**S (mm) S ≥ 15 I = 13–14 R ≤ 12**	**SXT(mm) S ≥ 17 I = 11–16 R ≤ 10**	**CPX(mm) S ≥ 21 I = 16–20 R ≤ 15**	**R (mm) S ≥ 20 I = 13–19 R ≤ 12**
Enterococcus casseliflavus	19 ± 0.17^{d}	19 ± 0.29^{b}	21 ± 0.17^{d}	16 ± 0.17^{d}	21 ± 0.17^{c}	24 ± 0.29^{d}	17 ± 0.29^{a}	19 ± 0.29^{d}	22 ± 0.29^{c}	22 ± 0.29^{d}
Staphylococcus heamolyticus	17 ± 0.29^{b}	17 ± 0.29^{a}	15 ± 0.29^{b}	14 ± 0.29^{b}	24 ± 0.17^{e}	18 ± 0.29^{b}	17 ± 0.29^{a}	20 ± 0.29^{e}	24 ± 0.29^{d}	15 ± 0.17^{a}
Kocuria varians	17 ± 0.17^{b}	21 ± 0.17^{d}	23 ± 0.17^{e}	15 ± 0.17^{c}	0 ± 0^{a}	16 ± 0.17^{a}	19 ± 0.17^{b}	13 ± 0.17^{b}	22 ± 0.17^{bc}	16 ± 0.17^{b}
Bacillus cereus	0 ± 0^{a}	20 ± 0.29^{c}	0 ± 0^{a}	16 ± 0.29^{d}	17 ± 0.29^{b}	15 ± 0.29^{a}	17 ± 0.29^{a}	0 ± 0^{a}	19 ± 0.29^{a}	20 ± 0.29^{c}
Staphylococcus aureus	0 ± 0^{a}	22 ± 0.17^{e}	19 ± 0.29^{c}	13 ± 0.29^{a}	23 ± 0.29^{d}	23 ± 0.29^{c}	17 ± 0.29^{a}	18 ± 0.17^{c}	21 ± 0.29^{b}	22 ± 0.29^{e}

OFX = Ofloxacin (30 µg), PEF = Pefloxacin (30 µg), CPX = Ciprofloxacin (30 µg), AU = Augmentin (10 µg), CN = Gentamycin (30 µg), S = Streptomycin (30 µg), SXT = Septrin (30 µg), AM = Amoxicillin (30 µg), Z = zinnacel (30 µg), APX = Ampiclox (30 µg). R = resistant. I = intermediate, S = susceptible according to the Clinical and Laboratory Standards Institute (CLSI, 2018). Values are expressed in mean ± SEM. Means of antibiotic without a common superscript letters differ $P < 0.05$ was considered to be statistically significant

Table 5 Qualitative phytochemical composition of Ethanol and aqueous Extracts of *Mormordica charantia* Linn

Bioactive compound	Ethanol	Water
Saponins	+	–
Tannins	+	–
Phlobatannins	+	+
Flavonoids	–	–
Steroids	+	–
Terpenoids	–	+
Cardiac glycosides	–	+
Alkaloids	+	+
Anthraquinones	–	–
Phenols	–	+

+ = Detected, – = Not detected

Table 6 Quantitative phytochemical composition of Ethanol and aqueous extracts of *Mormordica charantia* Linn

Bioactive compound	Ethanol	Water
Saponins	6.787 ± 0.0075^{d}	2.291 ± 0.054^{a}
Tannins	5.775 ± 0.0110^{c}	2.834 ± 0.0100^{b}
Phlobatannins	3.109 ± 0.0040^{a}	4.732 ± 0.011^{de}
Flavonoids	–	–
Steroids	7.088 ± 0.0130^{e}	
Terpenoids	–	4.899 ± 0.00145^{e}
Cardiac glycosides		4.583 ± 0.016^{d}
Alkaloids	3.873 ± 0.006^{ab}	3.393 ± 0.0055^{c}
Anthraquinones	–	–
Phenols	–	9.131 ± 0.012^{f}

Values are expressed in mean ± SEM. Means without a common superscript letters differ $P < 0.05$ was considered to be statistically significant

It was observed that phytochemical constituents of the ethanolic extract and water extract of *M. charantia* (Linn) varied. Water extract contained more bioactive compounds than that of ethanolic extracts as indicated by colour change. This result corroborates with the report of Oliveira et al. (2018) that the relative amount of phytochemical substances from plant extraction depends on their solubility in the solvent used for extraction. In this study, Flavonoids and Anthraquinones were absent in both extracts, which could be traced to the choice of solvent, extraction technique, age and specie type (Naqab et al. 2017; Olayinka 2018). These bioactive compounds that are known to protect the plant against bacterial and fungi in combating bacteria were confirmed in the extracts of sample after phytochemical screening (Amit and Ranjeeta 2018).

This investigation had shown that the extracts exhibited potent inhibitory potential against the studied bacterial isolate from RTE food. The results revealed that the ethanol and water extracts showed significant to moderate antibacterial activity toward all tested isolates except; *Escherichia vulneris* and *Kluyveria intermedia* that are less susceptible to water extracts. Only *Kluyveria intermedia* is less susceptible to ethanol extracts. The highest inhibitory activity was observed for *Bacillus cereus* with wide inhibition zone diameters, followed by *Escherichia vulneris*, *Staphylococcus heamolyticus*, and *Staphylococcus aureus*. Since microorganisms differ markedly in their susceptibility, the presence of the active components in plants is influenced by several factors such as age of the plants, method of extraction and extracting solvent.

The minimum inhibitory concentrations (MICs) of the extracts for the test bacteria were relatively lower in water extract compared to the ethanolic extract. This could probably be due to susceptibility of the bacteria to the extracts after which the extract damages that microbe which is not tolerate to the extracts (Adegbola et al. 2016). Thus water extracts used was more effective against the isolates than ethanolic extracts in this study since a lower MIC value is indicative of the fact that antimicrobial activity against the test pathogenic organism could be achieved at lower concentrations. Results obtained from this study, indicated that, the water extracts showed the strongest antimicrobial activity than the ethanolic extracts. Similar result was was reported by Gonelimali et al. (2018) in which the effectiveness of antimicrobial agent varies with the solvent used and nature of organisms being treated.

At the same concentration, commercial antibiotics were more effective against the bacterial isolates when compared to *M. charantia* (Linn) extracts. This may be as a result of refined materials used in the production of antibiotics. Oladunmoye (2007) reported that antibiotics have high degree of purity; conventional antibiotics and other pharmaceutical products are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures expressing purity and high fractionation which certainly will enhance antimicrobial effect than crude extracts.

DPPH% Inhibition (2,2-diphenyl-1-Picryl-hydrazyl-hydrate) has an antioxidant properties, DPPH% Inhibition has higher value in the water extract which implies that, The higher the percentage value, the higher the

Table 7 Zone of inhibition (mm) of water extracts of *Mormordica charantia* Linn on bacterial isolates from RTE foods sold in Akure metropolis

Isolate	Zone of inhibition (mm)			
	Water extracts			
	200 mg/ml (mm)	100 mg/ml (mm)	50 mg/ml (mm)	25 mg/ml (mm)
Salmonella enterica subsp *enterica*	20 ± 0.29^{i}	18 ± 0.17^{i}	12 ± 0.29^{d}	0 ± 0^{a}
Enterobacter sakzakii	16 ± 0.29^{f}	14 ± 0.29^{f}	12.5 ± 0.29^{d}	11 ± 0.17^{c}
Escherichia vulneris	10 ± 0.17^{b}	7 ± 0.29^{b}	0 ± 0^{a}	0 ± 0^{a}
Pectobacterium carotovorum	18 ± 0.29^{h}	16.5 ± 0.33^{gh}	15 ± 0.29^{f}	13 ± 0.17^{e}
Escherichia coli	17 ± 0.29^{g}	16 ± 0.29^{g}	13.5 ± 0.17^{e}	12 ± 0.29^{d}
Serratia liquefaciens	13 ± 0.29^{c}	11 ± 0.29^{c}	0 ± 0^{a}	0 ± 0^{a}
Enteric group 69	16 ± 0.17^{f}	12 ± 0.29^{d}	10 ± 0.29b	0 ± 0^{a}
E*nterobacter nimipressuralis*	22 ± 0.17^{j}	17 ± 0.29^{h}	14 ± 0.29^{e}	11 ± 0.29^{c}
klebsiella pnuemoniae	20 ± 0.29^{i}	18 ± 0.17^{i}	16 ± 0.17^{g}	14 ± 0.17^{f}
Citrobacter freundii	15 ± 0.17^{e}	13 ± 0.17^{e}	12 ± 0.29^{d}	7 ± 0.29^{b}
Salmonella typhi	17 ± 0.29^{g}	14 ± 0.29^{f}	12 ± 0.17^{d}	0 ± 0^{a}
Kluyveria intermedia	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Enterococcus casseliflavus	14 ± 0.17^{d}	13 ± 0.29^{e}	11 ± 0.17^{c}	7 ± 0.29^{b}
Staphylococcus heamolyticus	23 ± 0.17^{j}	17 ± 0.29^{h}	14 ± 0.29^{e}	12 ± 0.17^{e}
Kocuria varians	18 ± 0.29^{h}	16.5 ± 0.33^{gh}	14 ± 0.29^{3}	12.5 ± 0.33^{d}
Bacillus cereus	28 ± 0.29^{l}	20 ± 0.29^{j}	15 ± 0.29^{f}	14 ± 0.17^{f}
Staphylococcus aureus	24 ± 0.29^{k}	18 ± 0.29^{i}	16 ± 0.29^{g}	13 ± 0.29^{e}
Pseudomonas aeruginosa	17 ± 0.17^{g}	14 ± 0.17^{f}	11 ± 0.17^{c}	7 ± 0.17^{b}

0–10 = resistant, 11–13 = intermediate, ≥ 14 = susceptible Values are expressed in mean ± SEM. Means of an extract without a common superscript letters differ $P < 0.05$ was considered to be statistically significant

antioxidant scavenging ability while lower value was obtained in the etthanol ectract which also implies that the lower the percentage value, the lower the antioxidant scavenging ability. % Iron chelation has higher value in the ethanol extract which is a therapy that prevents the accumulation of iron reaching harmful levels by matching iron intake from blood transfusion with iron excreted by iron chelation. The higher, the better. Therefore ethanol extract is highly recommended for iron chelation. The analysis of vitamin C in *Mormordica charantia (Linn)* helps in building immunity against diseases. According to the result obtained *Mormordica charantia (Linn)* is a good source of Vitamin C. The absorbance which is the FRAP (Ferric reducing antiodant power) was plotted against concentration. FRAP is a reducing agent which is acceptable, it has antioxidant power, it changes Fe^{3+} to Fe^{2+}.

This outcome constitutes an encouraging boost for the crude extract, thus paving way for further studies on the plant, as this study of medicinal plants serve as a source of new types of drugs with greater therapeutic activity, lower toxicity, better biocompatibility and more accessibility to the population, which, due to cultural aspects (Awuchi 2019; Anjamma et al. 2018), has a good acceptance, reflecting good prospects in the market of therapeutic products made from active natural Ingredients.

Conclusions

The study revealed that all the Ready-to-eat food sold in Akure metropolis had total bacterial counts that are below potentially hazardous count although the presence of some microorganisms that are of health significance were observed, as some of them are known to be associated with food poisoning, infection and intoxication capable of causing various ailments in adults and children which may be fatal.

The study also confirmed that *M. charantia* (Linn) extracts had antibacterial effect against isolated pathogenic bacterial from Ready-to-eat food sold in Akure metropolis, water extracts were found to be more potent being capable of exerting significant inhibitory activities against the majority of the isolates. At the same concentration, commercial antibiotics were more effective

Table 8 Zone of inhibition (mm) of ethanolic extracts of *Mormordica charantia* Linn on bacterial isolates isolates from RTE foods sold in Akure metropolis

Isolate	Zone of inhibition			
	Ethanol extracts			
	200 mg/ml (mm)	100 mg/ml (mm)	50 mg/ml (mm)	25 mg/ml (mm)
Salmonella enterica subsp *enterica*	21 ± 0.29^{fi}	15 ± 0.29^{f}	11 ± 0.29^{c}	7 ± 0.29^{b}
Enterobacter sakzakii	23 ± 0.29^{j}	19 ± 0.29	13 ± 0.29^{e}	7 ± 0.29^{b}
Escherichia vulneris	25 ± 0.29^{l}	16 ± 0.29^{g}	12 ± 0.44^{de}	9 ± 0.29^{d}
Pectobacterium carotovorum	17.5 ± 0.17^{e}	16 ± 0.29^{g}	14.5 ± 0.33^{i}	12 ± 0.29^{e}
Escherichia coli	17 ± 0.29^{e}	14.5 ± 0.33^{de}	13 ± 0.29^{e}	7 ± 0.29^{b}
Serratia liquefaciens	22.5 ± 0.33	18 ± 0.28^{h}	15.5 ± 0.33^{g}	8.5 ± 0.17^{c}
Enteric group 69	16.7 ± 0.29^{de}	13.5 ± 0.17^{c}	11 ± 0.29^{c}	0 ± 0^{a}
E*nterobacter nimipressuralis*	16 ± 0.29^{d}	14 ± 0.29^{cd}	12 ± 0.29^{d}	8 ± 0.29^{b}
klebsiella pnuemoniae	22.5 ± 0.17^{j}	19 ± 0.29^{j}	17 ± 0.29^{h}	15 ± 0.29^{h}
Citrobacter freundii	17.5 ± 0.29^{e}	15 ± 0.17^{ef}	12 ± 0.29^{d}	0 ± 0^{a}
Salmonella typhi	21 ± 0.1^{i}	17 ± 0.29	13 ± 0.17^{e}	7 ± 0.29^{b}
Kluyveria intermedia	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Enterococcus casseliflavus	13 ± 0.29^{b}	11 ± 0.29^{b}	8.5 ± 0.17^{b}	0 ± 0^{a}
Staphylococcus heamolyticus	24 ± 0.29^{k}	19 ± 0.29^{j}	16 ± 0.17^{g}	14 ± 0.29^{g}
Kocuria varians	20 ± 0.29^{f}	18 ± 0.29^{h}	14 ± 0.29^{f}	12 ± 0.29^{e}
Bacillus cereus	15 ± 0.17^{c}	14 ± 0.29^{cd}	12 ± 0.29^{d}	0 ± 0^{a}
Staphylococcus aureus	23 ± 0.29^{i}	19 ± 0.29^{j}	15.5 ± 0.17^{g}	13 ± 0.29^{f}
Pseudomonas aeruginosa	20 ± 0.29^{f}	15 ± 0.17^{def}	13 ± 0.17^{e}	8 ± 0.29^{c}

0–10 = resistant, 11–13 = intermediate, ≥ 14 = susceptible. Values are expressed in mean ± SEM. Means of an extract without a common superscript letters differ $P < 0.05$ was considered to be statistically significant

Table 9 Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of extracts on bacterial isolates from RTE foods sold in Akure metropolis

Isolate	Ethanol extracts		Water extracts	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Salmonella enterica subsp *enterica*	50	50	50	50
Enterobacter sakzakii	25	50	25	25
Escherichia vulneris	25	25	-	-
Pectobacterium carotovorum	25	25	25	25
Escherichia coli	50	50	25	25
Serratia liquefaciens	50	50	100	100
Enteric group 69	50	50	50	100
E*nterobacter nimipressuralis*	25	50	25	25
klebsiella pnuemoniae	25	25	25	25
Citrobacter freundii	50	50	50	100
Salmonella typhi	50	50	25	50
Kluyveria intermedia	-	-	-	-
Enterococcus casseliflavus	50	100	50	50
Staphylococcus heamolyticus	25	25	25	25
Kocuria varians	25	25	25	25
Bacillus cereus	50	50	25	25
Staphylococcus aureus	25	25	25	25
Pseudomonas aeruginosa	50	50	50	100

Table 10 Quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and %Iron chelation of sun dried water and ethanol extract of *Mormordica charantia (Linn)* at 100 mg/ml

Phytochemicals	Water	Ethanol	
DPPH% Inhibition	69.21 ± 0.882^{a}	67.89 ± 0.155^{b}	
% Iron chelation		97.28 ± 0.155^{b}	121.87 ± 0.882^{a}

Means with the same letter within rows are not significantly different at $P \leq 0.05$

DPPH—Diphenyl Picryl Hydrazyl Radical

Table 11 Analysis of Vitamin C in *Mormordica charantia (Linn)*

Absorbance Conc	Vit. C std	Water	Ethanol
20 mg/ml	0.424 ± 2.646^{a}	0.414 ± 2.646^{a}	0.422 ± 2.646^{a}
40 mg/ml	0.572 ± 1.155^{c}	0.433 ± 1.155^{b}	0.467 ± 1.155^{a}
80 mg/ml	0.644 ± 0.882^{c}	0.476 ± 1.155^{ab}	0.480 ± 0.882^{b}
100 mg/ml	0.730 ± 0.882^{c}	0.498 ± 1.155^{b}	0.492 ± 2.646^{a}

FRAP—Ferric Reducing Antioxidant Power. Means with the same letter within rows are not significantly different at $P \leq 0.05$

against the bacterial isolates when compared to *M. charantia* (Linn) extracts.

Based on the findings of this great research work, *Mormordica charantia (Linn)* possesses antioxidant activity that could prevent oxidative stress and degenerative diseases.

The availability and accessibility of *M. charantia* (Linn) being a biological substance, its application would have no or less adverse effect to man and environment, this could make a better alternative to the conventional antibiotics drugs which many pathogens have render less effective due to the development of resistance. This attest that the plant extract could potentiate the development of a new drug against disease caused as a result of food infection or food poisoning.

Recommendation

In order to reduce the incidence of food poisoning and food infection measure such as thorough cooking, the provision of clean food container, using clean wrapping materials and cutleries, serving the food hot to reduce their microbial load as much as practice of basic sanitary rules in preparing foods and good personnel hygiene should be employed. There is need for continuous monitoring of Ready-to- eat food sold in Akure metropolis by the health officers.

More research should be conducted using bacterial of different species to further establish reproducibility of the antibacterial potency of *M. charantia* (Linn) as it can be developed into commercial drugs for conventional use, thereby reducing global antibiotics resistance crisis.

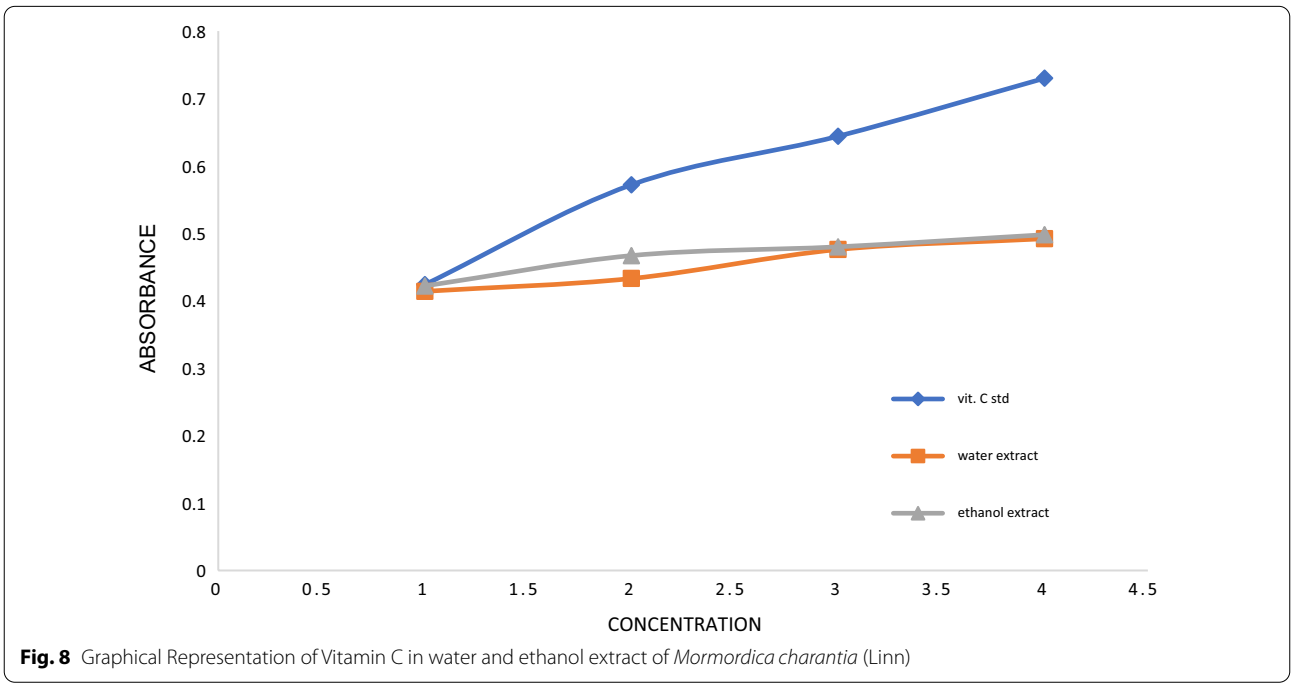

Fig. 8 Graphical Representation of Vitamin C in water and ethanol extract of *Mormordica charantia* (Linn)

Abbreviations
FUTA: Federal University of Technology Akure; RTE: Ready-to-eat; DMSO: Dimethylsulfoxide; NA: Nutrient agar.

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Authors' contributions
TAI: conceived and designed the experiments, contributed to sample preparation, carried out the experiment, processed the experimental data, performed the analysis, wrote the manuscript, drafted the manuscript, designed the figures and contributed to the interpretation of the results. JOA: involved in planning and supervised the work, contributed to the interpretation of the results, other contribution. Both authors discussed the results and commented on the manuscript. Both authors read and approved the final manuscript.

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Declarations

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References

Adegbola A, Akinbile Y, Awotoye J (2016) Mormodica charantia Linn A potential antibiotic, anti-fungal drug. Int J Pharm Sci Invent 5(2):21–27
Adewale O, Olanike MB, Simeon KO, Emmanuel O (2015) Bacteria associated with contamination of ready-to-eat (RTE) cooked rice in Lagos-Nigeria. Int J Biol Chem Sci 9(5):2324–2333
Ahn K (2017) The worldwide trend of using botanical drugs, strategies for developing global drugs. Biochem Mol Biol Rep 50(3):111–116
Amit I, Ranjeeta K (2018) Phytochemical screening, anti-microbial activity of *M charantia* (Linn). Int J Pharmacol Res 8(7):63–65
Anjamma N, Lakshmi B (2018) Comparative Antibacterial, Antioxidant activity from root, fruit extracts of *Momordica charantia (*Linn*)*, *Momordica dioica*. Int J Sci Res Sci Technol 4(5):2395–6011
Awuchi CG (2019) Medicinal plants: the medical, food, nutritional biochemistry, uses. Int J Adv Acad Res 5(11):220–241
Banik A, Maruf A, Suvamoy D, Syeda T (2019) Microbiological quality of ready - to - eat food from Dhaka, Bangladesh. Curr Res Nutr Food Sci 7(1):161–168
Boklage E, Lehmkuhl M (2018) Coverage of antimicrobial resistance in the German Press: 1993–2013. J Community Health 34(9):958–963
Chokki M, Zongo C, Dah-Nouvlessounon D, Cudalbeanu M, Noumavo P, Ghinea LO, Furdui B, Savadogo A, Dinica RM, Baba-Moussa L, Baba-Moussa F (2020) Phytochemical screening, antimicrobial activity of *Momordica charantia* (Linn), *Morinda lucida* Benth extracts from Benin. Afr J Microbiol Res 14(8):426–435
Clinical Laboratory Standard Institute (CLSI) (2018) Performance Standards for antimicrobial susceptibity tests Document M100–527 CLSI, Wagne, PA. Clin Microbiol 45(1):199–205
Deepti, K, Vijender, S, Mohd A (2017) Biochemistry, therapeutic uses of medicinal plants. Discovery Publishing House Pvt Ltd, New Delhi (India) 45(3):978–93
Devi U, Bharani A, Sindhura P, Thejaswi S (2016) Isolation, identification of pathogenic bacteria from Hussain Sagar Lake, hyderabad, their antibiotic sensitivity. Eur J Biomed 3(1):238–241
Gonelimali F, Jiheng L, Wenhua M, Jinghu X, Fedrick C, Hatab S (2018) Antimicrobial properties, mechanism of action of some plant extracts against food pathogens, spoilage microorganisms. Front Microbiol 9:1639
Guarniz W, Canuto K, Ribeiro P, Dodou H, Magalhaes K, Sa K (2019) Momordica charantia L variety from Northeastern Brazil: analysis of antimicrobial activity, phytochemical components. Pharmacogn J 11(6):1312–1324
Jandari S, Abed G, Rahele Z, Nattagh-Eshtivani E, Mohammadi H (2020) Effects of Momordica charantia L on blood pressure: a systematic review, meta-analysis of randomized clinical trials. Int J Food Prop 23(1):1913–1924
José G, Adriana R, Fabíola F, Rodrigues (2015) Antibacterial activity of *Momordica charantia* (Curcubitaceae) extracts. Fractions J Basic Clin Pharm 2(1):23–123
Khalid Z, Hassan S, Shahzad S, Khurram H (2021) A review on biological attributes of Momordica charantia. Adv Biosci Bioeng 9(1):8–12
Madueke SN, Awe S, Jonah A (2014) Microbiological analysis of street foods along Lokoja-Abuja Express Way, Lokoja. Am J Res Commun 2(1):196–211
Mboto CI, Agbo BE, Ikpoh IS, Agbor RB, Udoh DI, Ambo EE, Ekim MA (2012) Bacteriological study of raw meat of Calabar Abattoir with public health, veterinary importance. J Microbiol Biotechnol Res 2(4):529–532
Mogasale V, Saldanha P, Pai V (2021) A descriptive analysis of antimicrobial resistance patterns of WHO priority pathogens isolated in children from a tertiary care hospital in India. Sci Rep 11:5116
Muronga M, Cristina Q, Phumudzo P, Tshikhudo A, Ahmad F (2021) Three selected edible crops of the genus momordica as potential sources of phytochemicals: biochemical, nutritional, medicinal values. J Pharm Pharmacogn Res 9(4):537–548
Naqab K, Abdulhaleem S, Ejaz AK, Muhammad S, Samiullah K, Natasha B (2017) Phytochemical analysis, biological activities of stem bark extract of *Jatropha curcas* L. Int J Pharmacogn 2:155–168
New South Wales (NSW) (2019) Food Authority: www.foodauthoritynsw.gov.au. Retrieved November 15, 2020
Oladunmoye MK (2007) Comarative evaluation of antimicrobial activities of leaf extract of *Mirabilis jalapa*, microbial toxins on some pathogenic bacteria. Trends Med Res 2(2):108–112
Olayinka TO (2018) Phytochmicals—God's endowment of curative power in plants. Phytochem Source Antioxidants Role Disease Prevent 12:1–12
Oliveira M, Wanessa A, Fernanda W, Marilena E, Raul F (2018) Phytochemical profile, biological activities of *Momordica charantia* L (Cucurbitaceae): a review. Afr J Biotechnol 17(26):829–846
Owhe-ureghe UB, Ekundayo AO, Agbonlahor DE, Oboh PA, Orhue P (1993) Bacteriological examination of some Ready-to-eat foods marked in Ekpoma, Edo state of Nigeria. Nigeria Food J 11:45–52
Owusu E, Ahorlu M, Afutu E, Akumwena A, Asare G (2021) Antimicrobial activity of selected medicinal plants from a sub-Saharan African country against bacterial pathogens from post-operative wound infections. J Med Sci 9:23
Palomino JC, Martin A, Portaels F (2007) rapid drug resistance detection in *Mycobacterium tuberculosis*: a review of colorimetric method. Clin Microbiol Infection 13:754–762
Parra A, Roberto M, Piloto F, Pham T, Nguyen P, Addis B, Gilberto L (2021) Antidiabetic, hypolipidemic, antioxidant, anti-inflammatoryeffects of Momordica charantiaLfoliage extract. J Pharm Pharmacogn Res 10(4):647–653
Samuel P, Joan-Miquel B, Amanda H, Robert C (2016) Multicenter assessment of gram stain error rates. J Clin Microbiol 54:6
Sharif A, Mohammad R, Alizargar J (2014) Antibiotic resistance of gram-negative bacteria to ampicillin, gentamicin, imipenem in samples of the patients admitted to Shahidbeheshti Hospital in Kashan. World Appl Sci J 30(6):778–781
Shubha AS, Devaraju SMB, Srinivasa V, Kantharaj Y, Ravi CS, Akshay A, Yallesh Kumar HS, Shanwaz A (2018) Medicinal, nutritional importance of bitter melon (*M charantia* (Linn)). J Pharmacogn Phytochem 3:297–300
Sofowora A (1995) Medicinal, traditional medicine in Africa (Second edition). Spectrum Books Limited, Ibadan, Nigeria, p 130
Şükriye S, Beytullah K (2014) Assessment of microbiological quality of ready-to-eat foods in Istanbul, Turkey. J Food Agricult Environ 12:3–4

Thiyam B, Sharma G (2013) Isolation, identification of fungi associated with local fruits of barak Valley. Curr World Environ Int Res J 8(2):319–322
United Kingdom Standard for Microbiology Investigation (USMI (2018) Methods for antimicrobial dilution, disk susceptibility testing of infrequently isolated or fastidious bacteria methods for antimicrobial dilution, disk susceptibility testing of infrequently isolated or fastidious bacteria. UK Standard Microbiol Invest 15(3):1–32
Villarreal-La T, Sagástegui G, Silva-Correa C, Cruzado-Razco L, Siche R (2020) Antimicrobial activity, chemical composition of momordica charantia: a review. Pharmacogn J 12(1):213–222
Zaini A, Noor A, Nicky R, Syafiza A, Mohd Johari K, Zuhaili I, Mohd A (2018) Comparison of charantin extract from *M charantia (*Linn*)* using modified supercritical carbon dioxide, soxhlet extraction method. MJFAS 4:462–466

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