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Antimicrobial Evaluation of Vinegar Produced from Pineapple and Pawpaw Fruits with their Peels

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Abstract : Thevinegar produced from locally grown pineapple and pawpaw (with and without their peels) were evaluated to determine their antimicrobial properties on some clinical isolates. Agar well diffusion method was used for this analysis. The zones of inhibitions were measured in millimeters. The results of the antimicrobial analysis showed that the vinegar exhibit antimicrobial activities on the clinical isolates.On Escherichia colithere was 8 ± 1.5mm diameters for the aqueous extract of the pineapple juice vinegar and 11 ± 0.44mm for the methanol extract of the pineapple peel vinegar, 10 ±1.23mm and 12 ±0.19 for the aqueous and methanol extract of pawpaw juice vinegar and 6 ±0.36mm and 7 ±0.77mm for the aqueous and methanol extract of pawpaw peel vinegar respectively. On Staphylococcus aureus, there was 9 ± 0.50mm and 10 ±1.00mm for the aqueous and methanol extract of the pineapple juice vinegar respectively while the pineapple peel vinegar extract showed 10 ± 1.50mm and 7 ±0.50mm for the aqueous and methanol extract, 7 ±0.29mm and 8 ±0.77 for aqueous and the methanol extract of the pawpaw juice vinegar, 10±0.55mm and 7 ±0.86mm for aqueous and methanol extract of pawpaw peel vinegar. On Bacillus sp there was 9 ± 0.99mm diameter for the aqueous extract of the pineapple juice vinegar and 8 ±1.30mm for the methanol extract, 9 ±0.98mm and 7 ± 0.99mm for the aqueous and the methanol extract of the pineapple peel vinegar, 9 ±0.55mm aqueousextract of pawpaw juice vinegar and 13±0.76mm for aqueous extract of pineapple peel vinegar. On Candida albicans there 8 ± 0.12mm diameter for the aqueous extract of the pineapple juice vinegar and 9 ± 0.99mm for the methanol extract10 ±1.00mm and 7 ± 0.76mm for the aqueous and the methanol extract of the pineapple peel vinegar, 8 ±0.65mm and 7 ±0.9mm for aqueous and methanol extract of pawpaw juice vinegar and 9±1.22mm and 10 ±0.22mm for aqueous and methanol extract of pineapple peel vinegar. This shows that the produced vinegars exhibited antimicrobial characteristics and their use should be encouraged

Introduction

Vinegar is a solution of acetic acid produced by a two-step bioprocess. In the first step, fermentable sugars are transformed into ethanol by the action of yeast. In the second step, AAB oxidize the ethanol into acetic acid in an aerobic process. Vinegar can be produced by different methods and from various raw materials.

Acetic acid is a carboxylic acid with antibacterial and antifungal properties found in Vinegar. Not only this, it acts as a preservative because of its acetic acid content and consequently, lowers the pH of food and hence, help in preservation (Joshi and Thakur, 2000). Vinegar has been reported to retard microbial growth and improve the sensory properties of foods.

Vinegar may be produced from a variety of raw materials, the main requirement being satisfactory economic source of ethanol (Ezemba*et al*., 2021). The basic requirement for vinegar production is a raw material that will undergo an alcoholic fermentation such as apples, pears, grapes, honey, syrups, cereals, hydrolyzed starches, beer and wine (Kadere*et al.,* 2008) or any other sugary food (Bamforth, 2005).
The antimicrobial properties of vinegar have made it useful for a number of applications. Vinegar has served cleaning purposes, treating nail fungus, head lice, warts, and ear infections (Rutala*et al.,* 2000; Dohar, 2003). Consumers usually prefer the use of natural preservatives for inhibiting the growth of food pathogens in the foods (Rauha *et al.,*2000).The organic acids in vinegar and mainly acetic acid usually penetrate into the cell membranes of microorganisms which causes bacterial cell death (Booth and Kroll, 1989; Brul and Coote, 1999; Blackburn and McClure 2002; Bjornsdottir*et al.,* 2006; Chang and Fang, 2007). The bacterial characteristics like strains, temperature, pH, acid concentration and ionic strength has a direct effect on the antimicrobial activity of organic acids (Buchanan and Edelson, 1996; Entani*et al.,* 1998; Cheng *et al.,* 2003). Naturally, many organic acids like acetic, lactic, ascorbic, citric, malic, propionic, succinic, and tartaric acids are found in many fruits and fermented foods and in non-excessive levels, they ardangerous to human health (Escudero*et al.,* 1999; Brennan *et al.,* 2000; Fang and Hsueh, 2000; Sengun and Karapinar, 2004).On comparing of the effect of organic acids on foodborne pathogenic bacteria, it was reported that most lethal acid to *Escherichia coli* O157:H7 was acetic followed by lactic, citric, and malic acids (Entani*et al.,* 1998; Ryu*et al.,* 1999). Different studies have shown that inhibition pathogenic bacteria on fresh fruits and vegetables could be achieved using vinegar (Wu *et al.,* 2000; Rhee *et al.,* 2003; Sengun and Karapinar, 2004; Chang and Fang, 2007).The trend in consuming vinegar in Nigeria is on the increase, and in as much as so many benefits have been observed in the use of vinegar, little attention has been given to the locally produced vinegar from locally grown fruits (Ezemba*et al*., 2021). Hence the aim of this work is to determine the antimicrobial properties of the locally produced vinegar from locally grown pawpaw and pineapple fruits and peels.

Materials and Methods

Procurement of materials

Pineapples and pawpaw bunches were purchase from a village market called eke market in Aguata Local Government area, Anambra state. It was taken to Chukwuemeka OdumegwuOjukwu University, Microbiology Departmental laboratory for analysis. Samples of 50 g and 20g of peel and core respectively, were stored in freezer (-18 °C) prior to use. Laboratory works were carried out both at MCB Dept. COOU and Chychy Gilgal research Laboratories Anambra Nigeria. All reagents used were of analytical grades.

Methods

Production of Vinegar

Fruits to be used were washed with distilled water. Twenty grams (20 g) of different fruits were weighed, peeled and soaked in distilled water and allowed to ferment naturally at room temperature in 500 mL of conical flask. The distilled water was poured to about three-quarters capacity of the flask, corked with cotton wool for 28 days and stirred daily. During this period, the mixture ferments into alcohol, the mixture was decanted and poured into a bottle. The mixture was allowed to open at room temperature for several weeks, blended, inoculated with acetic acid bacteria and allowed to ferment. The mixture was then

transferred into a larger glass container (5L) and covered with cheese cloth. The bottles were placed in the dark at 28°C.The fermentation was allowed for 28days and then the products was filtered using a tea strainer to remove the produced slime before chemical analysis and sensory evaluation. During this period of fermentation, physical observations like pH, Specific gravity and alcohol analysis were conducted and proper changes noted on the samples daily, until the desired strength is reached.

Measurement of Antimicrobial Activity of the Vinegar

The methanol extract was got by measuring two millilitres of the sample into a test-tube with lid. Four millilitres of methanol was used for extraction procedure. The covered test-tube was then sonicated in an ultrasonic bath at 70 °C for 30 min. Organic layer was syphoned into a clean beaker/round-bottom flask, dried with sodium sulfate. The sample extract was then concentrated to ~2 mL using a rotary evaporator. Cultures of *E. coli*, *B. Specie* and *S. aureus* were grown in nutrient media whereas *C. albican*s was grown in Sabourand Dextrose media. All cultures were cultivated in a shaking incubator at 37 °C for 24 h overnight prior to use. Each microbe was swabbed evenly onto plates containing MHA. For sample addition, 100 µL of the vinegar at varying concentrations was added to the wells made on the agar using a sterile cork borer. Incubation will be at 37 °C for 24 h. Zones of inhibition surrounding samples was determined using a micro ruler and measured in mm (Ostrovsky, 2008).

Data Analysis

The experiment was conducted in triplicates and the results were expresesd a mean±standard deviation (SD). Data was analysed using one-way analysis of variance (ANOVA) to determine the statistical significance within alpha value of 0.05 using Statitistical Package for Social Science (SPSS) verision 20.

Results

Table 1 shows the antibiotic susceptibility of the organisms to the aqueous and methanol extracts of the vinegars. The organisms evaluated or their susceptibility to the vinegars includes *Staphylococcus aureus, Escherichia coli, Bacillus sp,* and *Candida albicans*. The zones of inhibitions were measured in millimeters. On *E.coli*, the zones of inhibition of the aqueous extract of the pineapple juice vinegar gave 8 ± 1.5mm and the methanol extract gave no activity. The aqueous extract of the pineapple peel vinegar extract didn't show any activity but the methanol extract showed 11 ± 0.44mm zone of inhibition. The aqueous extract of the pawpaw juice vinegar showed an inhibition diameter of 10 ±1.23mm and 12 ±0.19 for the methanol extract. The aqueous extract of the pawpaw peel vinegar gave the inhibition diameter of 6 ±0.36mm while the methanol extract showed 7 ±0.77mm as the inhibition diameter. On *S. aureus*, the zones of inhibition of the aqueous extract of the pineapple juice vinegar gave 9 ± 0.50mm and the methanol extract showed a diameter of 10 ±1.00mm. The aqueous extract of the pineapple peel vinegar extract showed a diameter of 7 ±0.50mm but the methanol extract showed 10 ± 1.50mm zone of inhibition. The aqueous extract of the pawpaw juice vinegar showed an inhibition diameter of 7 ±0.29mm and 8 ±0.77 for the methanol extract. The aqueous extract of the pawpaw peel vinegar gave the inhibition diameter of 10±0.55mm while the methanol extract showed 7 ±0.86mm as the inhibition diameter. On *Bacillus sp,* the zones of inhibition of the aqueous extract of the pineapple juice vinegar gave 9 ± 0.99mm and the methanol extract showed a diameter of 8 ±1.30mm. The aqueous extract of the pineapple peel vinegar extract showed a diameter of 9 ±0.98mm but the methanol extract showed 7 ± 0.99mm zone of inhibition. The aqueous extract of the pawpaw juice vinegar showed an inhibition diameter of 9 ±0.55mm but the methanol extract showed no activity on the organisms. The aqueous extract of the pawpaw peel vinegar gave the inhibition diameter of 13±0.76mm while but the methanol extract showed no activity on the organisms. On *Candida albicans*, the zones of inhibition of the aqueous extract of the pineapple juice

vinegar gave 8 ± 0.12mm and the methanol extract showed a diameter of 9 ± 0.99mm. The aqueous extract of the pineapple peel vinegar extract showed a diameter of 10 ±1.00mm but the methanol extract showed 7 ± 0.76mm zone of inhibition. The aqueous extract of the pawpaw juice vinegar showed an inhibition diameter of 8 ±0.65mm and 7 ±0.96 for the methanol extract. The aqueous extract of the pawpaw peel vinegar gave the inhibition diameter of 9±1.22mm while the methanol extract showed 10 ±0.22mm as the inhibition diameter. The positive control which is Amoxil tablet showed diameters of 16 ±0.01mm, 13 ±0.01mm, ±21 ±0.02mm, 11 ±0.20mm for *Staphylococcus aureus, Escherichia coli, Bacillus sp,* and *Candida albicans* respectively. The negative control used was distilled water and it showed no activity on the organisms tested.

Table 1: Antimicrobial activities of the produced vinegar on some clinicalisolates

Vinegar sample	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Bacillus sp* (mm)	*Candida sp* (mm)
PJA (Aqueous extract)	8±1.5	9±0.50	9±0.99	8±0.12
PJA(Methanol extract)	-	10±1.00	8±1.30	9±0.99
PJB (Aqueous extract)	-	7±0.50	9±0.98	10±1.00
PJB(Methanol extract)	11±0.44	10±1.50	7±0.99	7±0.76
PPC (Aqueous extract)	10±1.23	7±0.29	9±0.55	8±0.65
PPC(Methanol extract)	12±0.19	8±0.77	-	7±0.96
PPD (Aqueous extract)	6±0.36	10±0.55	13±0.76	9±1.22
PPD(Methanol extract)	7±0.77	7±0.86	-	10±0.22
Amoxil (positive control)	16±0.01	13±0.10	21±0.02	11±0.20

Distilled water (Negative control)	-	-	-	-

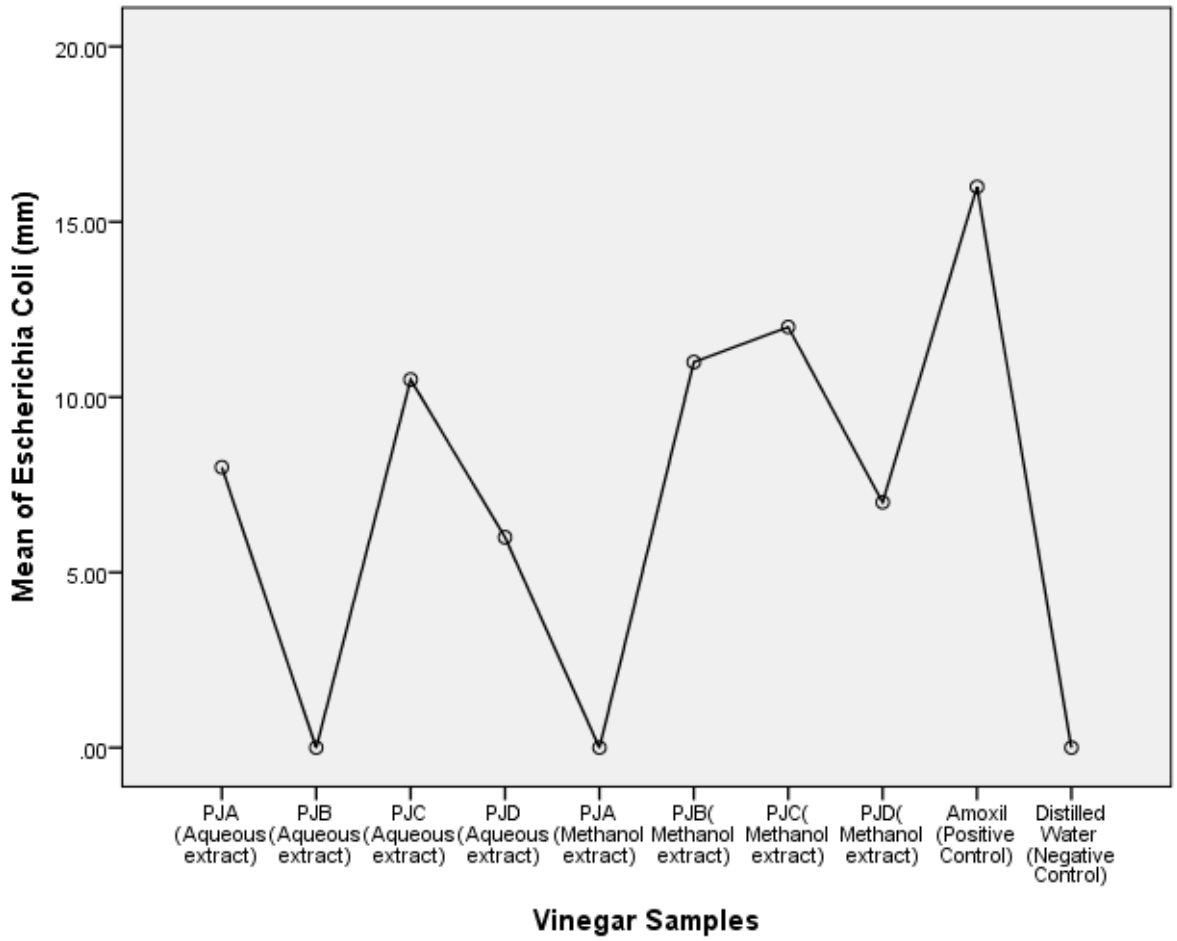

Figure 1: Plot showing the effects of the vinegar extracts on *Escherichia coli*

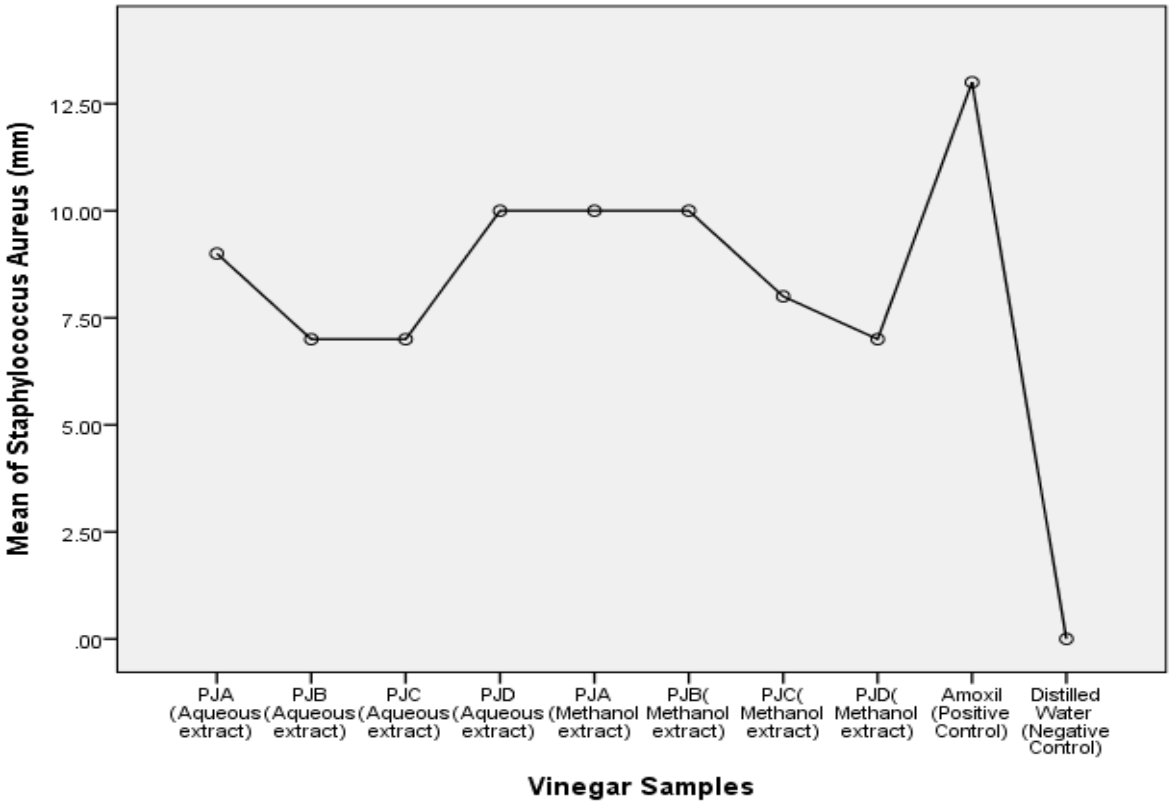

Figure 2: Plot showing the effects of the vinegar extracts on *Staphylococcus aureus*

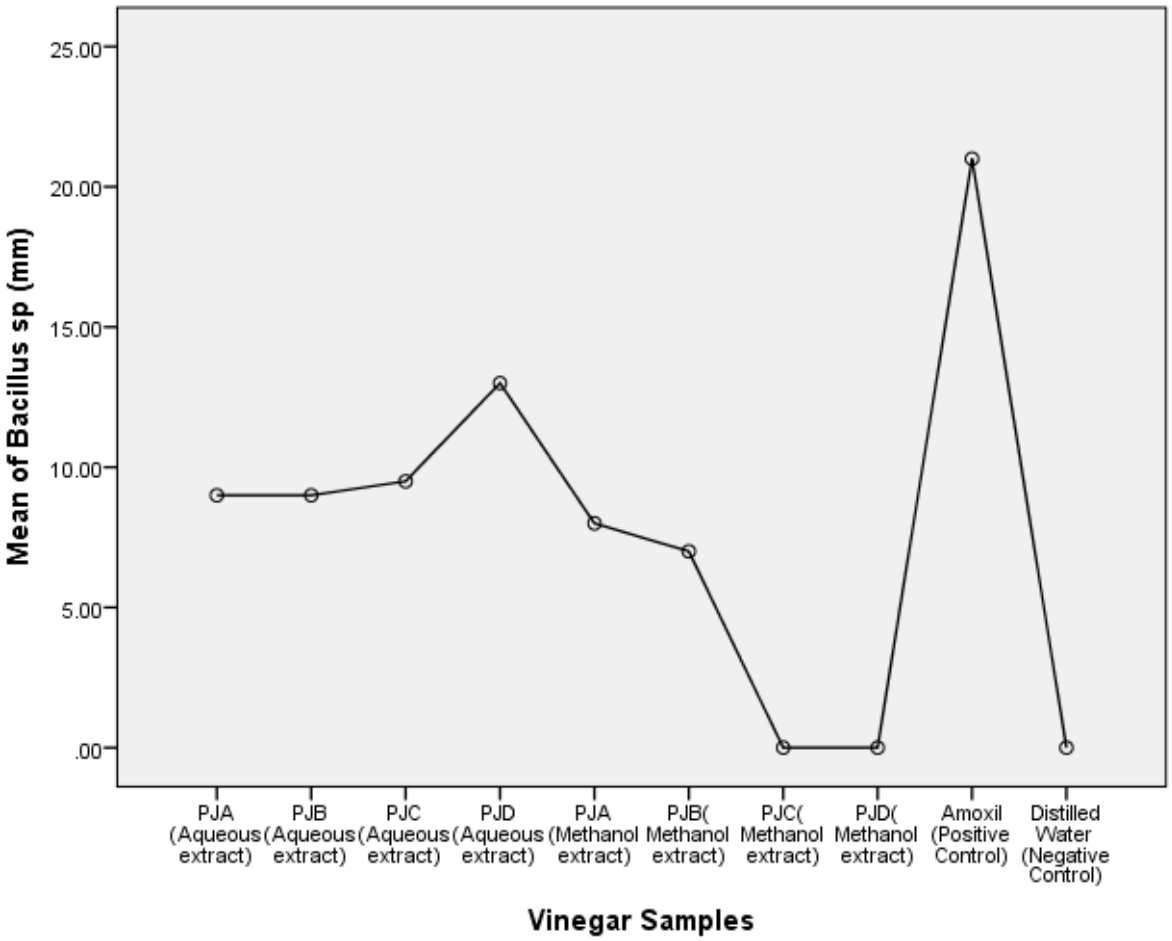

Figure 3: Plot showing the effects of the vinegar extracts on *Bacillus sp*

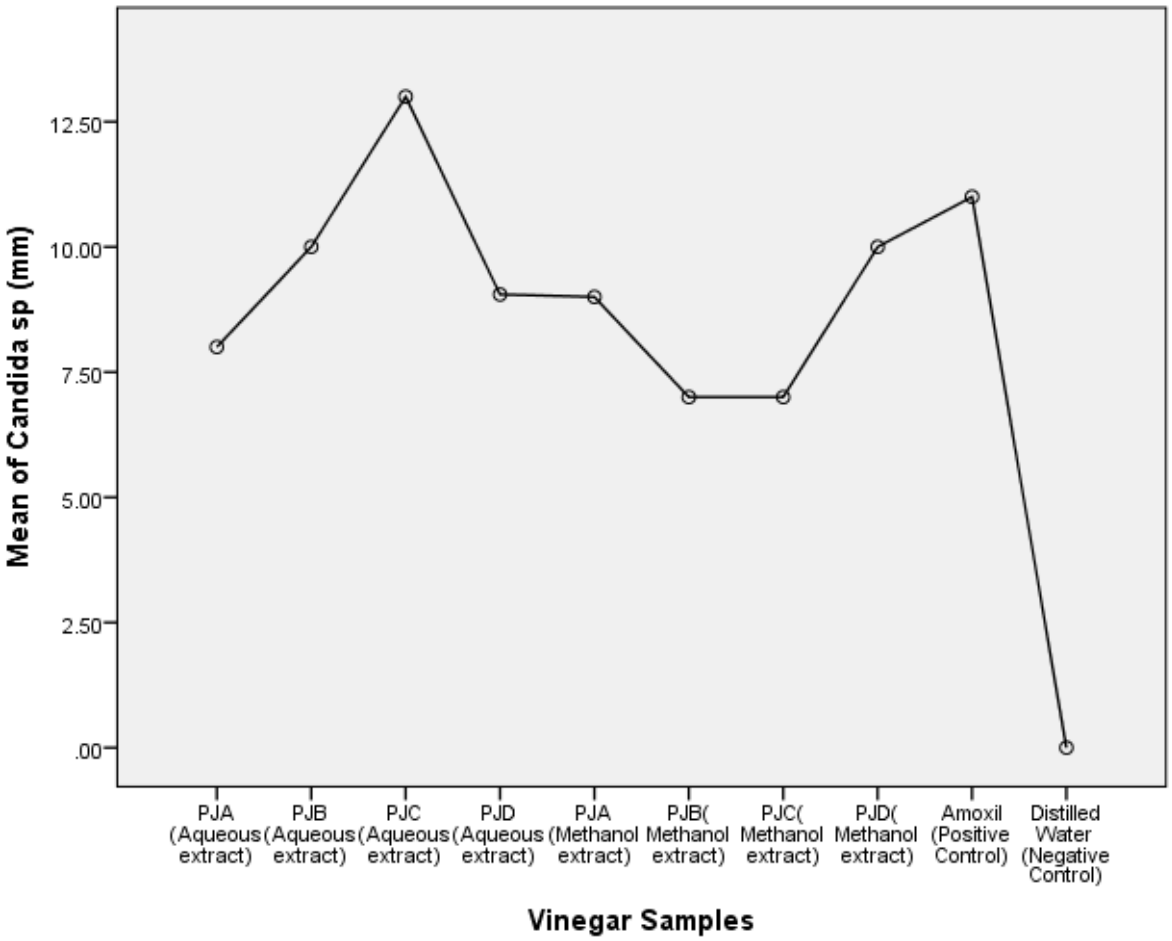

Figure 4: Plot showing the effects of the vinegar extracts on *Candida sp*

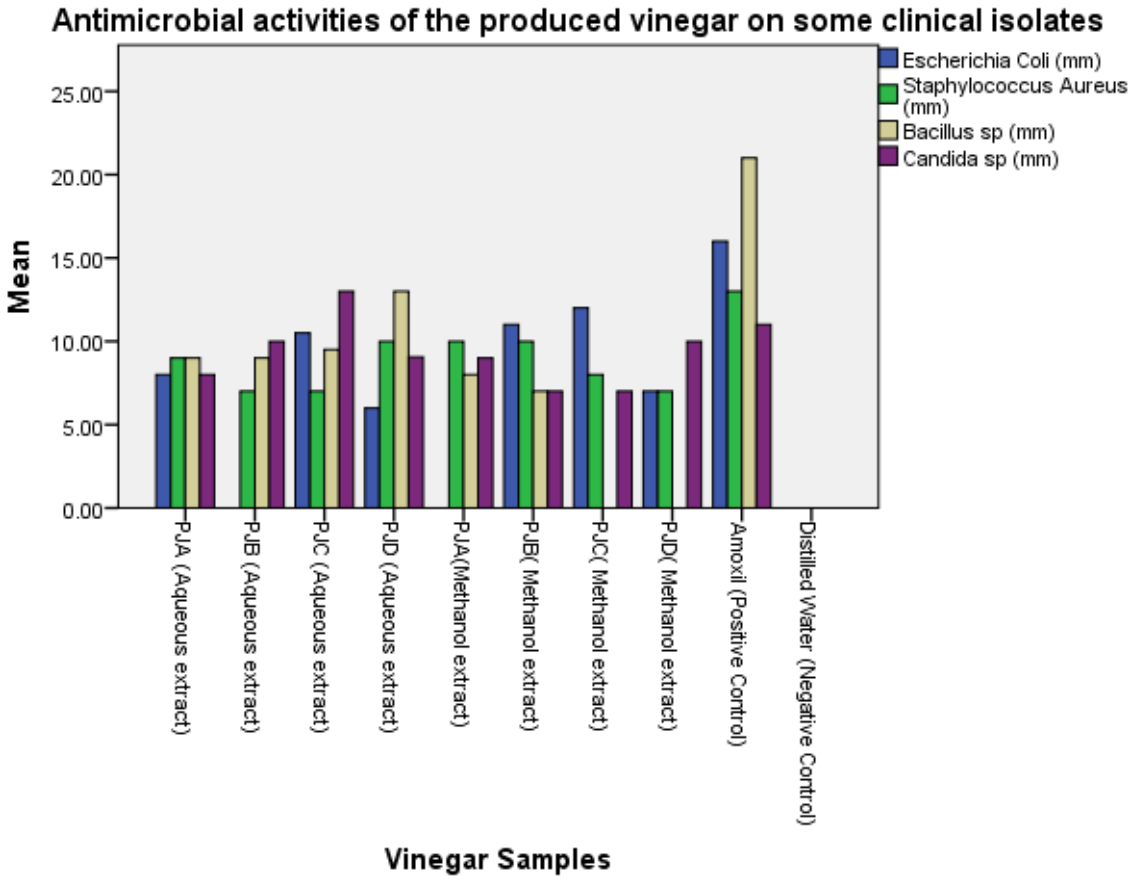

Figure 5: Chart showing the antimicrobial activities of the produced vinegar extracts on clinical isolates

Discussion and Conclusion

The antimicrobial properties of vinegar have made it useful for a number of applications. Vinegar has served cleaning purposes, treating nail fungus, head lice, warts, and ear infections (Rutala*et al.,* 2000; Dohar, 2003). The vinegars were tested using the aqueous and methanol extract to check their effect on clinical isolates which includes *Escherichia coli, Staphylococcus aureus , Bacillus sp* and *Candida sp*.From the result, the aqueous extract of the pineapple juice vinegar was effective on the *E.coli, S. aureus , Bacillus sp* and *Candida sp*. the methanol extract of the pineapple juice vinegar was effective on *S. aureus , Bacillus sp* and *Candida sp* but not on *E. coli*. This result is in keeping with the work of Tumane*et al* (2008) who reported that apple cider vinegar has antibacterial activity against gram positive and gram negative bacteria strain. The aqueous extract of the pineapple vinegar was effective on *S. aureus , Bacillus sp* and *Candida sp* but not on *E. coli*. The methanol extract was effective on *E. coli, S. aureus , Bacillus sp* and *Candida sp*. The aqueous extract of pawpaw juice vinegar and pawpaw peel vinegar was effective on *E.coli, S.aureus*, *Bacillus sp* and *Candida sp* while the methanol extract was effective on all the clinical isolates except *Bacillus sp*. It could be seen that the methanol extracts of both pawpaw juice

vinegar and pawpaw peel vinegar had no effect on *Bacillus sp*. This could be substrate or solvent related because it could be said that the methanol extract of pawpaw has no antimicrobial effect on *Bacillus sp*.
On comparing of the effect of organic acids on foodborne pathogenic bacteria, it was reported that most lethal acid to *Escherichia coli* O157:H7 was acetic followed by lactic, citric, and malic acids (Entani*et al.,* 1998; Ryu*et al.,* 1999) which is similar to the result from this work as the vinegar produced showed a positive activity on the *E.coli* isolate. Different studies have shown that inhibition pathogenic bacteria on fresh fruits and vegetables could be achieved using vinegar (Wu *et al.,* 2000; Rhee *et al.,* 2003; Sengun and Karapinar, 2004; Chang and Fang, 2007).This work is in keeping with the work of Chang and Fang. (2007) who evaluated the antimicrobial effect of rice vinegar on lettuce inoculated with *E. coli* O157:H7.This result agrees with the work of Chan *et al*. (2012) who reported that Matang wood vinegar displayed potent antibacterial activity against the strains of Gram-positive *Bacillus cereus, Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative *Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginos*

The inhibition of microbial growth increases by lowering pH of the media, and most microorganisms are susceptible to antimicrobial effects in the presence of organic acids. This phenomenon is due to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process takes place spontaneously due to pH and osmolarity gradients that exist between the inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. To counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics (Hassan *et al*., 2015). From this result, it shows that the vinegar have antimicrobial activities on the microorganisms which it was tested on. Hence there is need to encourage the use of vinegar as an antimicrobial agent.

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