ANTIBACTERIAL EFFECTS OF PALM WINE (Elaeis guineensis) ON *SALMONELLA TYPHI* ISOLATED FROM DIFFERENT SOURCES

ABSTRACT

This research work is to investigate the antibacterial effects of palm wine (*Elaeis guineensis*) on *Salmonella typhi* isolated from different sources. The samples were collected overnight from palm trees (*E. guineensis*). And it was assayed for antibacterial activity on *S. typhi* isolated from different sources using Agar well diffusion method. The effect of dilution and fermentation duration of the palm wines on isolated *S. typhi* was also determined. Palm wine (*E.guineensis*) inhibited*S. typhi* isolated, with diameter zones of inhibition ranging from 6.33±0.67 to 39.33±0.33 mm respectively. Palm wine from both palm trees was found to be more active against *S. typhi* than the conventional antibiotics (Chloramphenicol, Amoxycillin, Gentamycin and Ciprofloxacin) used, with diameter zones of inhibition ranging from 1.00± 0.33 to 20.67±0.57.The greatest inhibitory effect was on *S. typhi* isolated from well water (6.67± 0.31 to 44.67± 0.67 mm), while the least effect was on *S. typhi* isolated from an apparently healthy individual with inhibition ranging from 7.33±0.33 to 29.67± 0.33 mm. Also, the growth inhibitory effects of both palm wines on all *S. typhi* isolates used increased with increase in period of fermentation with diameter zones of inhibition ranging from 15.67±0.67 to 44.33± 0.33mm for palm wine from *R. vinifera* and 6.33±0.33to 39.33±0.33 mm for palm wine from *E. guineensis*. It is conceivable therefore that palm wine subjected to natural fermentation could be used to treat infections caused by *S. typhi* that is typhoid fever.

Keywords: Palm wine, fermentation, inhibition, typhoid fever, antibacterial

1.0 INTRODUCTION

All over the world, fermented foods and beverages continue to constitute an important part of human diet. Fermentation is a process involving the transformation of simple raw materials into a range of value-added products through the activities/growth of microorganisms on various substrates (William and Dennis, 2011). Food fermentation is one of the oldest known uses of biotechnology.

Palm wine is a traditional alcoholic beverage produce by natural fermentation of the sap of palm trees. It is whitish in colour with different varieties of flavours, ranging from sweet to sour and vinegary (Elijah *et al*., 2010). Palm wine is normally use traditionally for the extraction of active ingredients from leaves, barks and stems of some medicinal trees which is use in the treatment of various diseases like malaria, dental yellow fever, pains and stomach disorders. It is also used to treat cases of skin rashes in children and related diseases like smallpox, chicken pox and measles (Uraih *et al*., 1992).

Antimicrobial agents are substances that inhibit the growth and survival of microorganisms (Mackie and McCartney, 1989). The discovery of new antimicrobial agents from different sources such as microorganisms, animals, plants and plant products has been the major challenge of researchers. The screening may result in the discovery of effective compounds (Tomoko *et al*., 2002). The increase in drug resistant by microorganisms, higher cost commercially produced antimicrobial agent coupled with development of new strains of microbes adds urgency to the search for new antimicrobial agents (Sieradzki *et al*., 1999).

Therefore, there is the need for testing this local beverage for antimicrobial property. This project was carried to evaluate antibacterial effects of palm wine (*E.* guineensis) on *S. typhi* isolated from different sources.

2.0 METHODOLOGY

2.1 Collection of samples

Palm wine from *E. guineensis* (Oil palm) samples were purchased from palm wine tappers at Ijare, Ondo State, Nigeria. The samples were collected in sterile containers and transported immediately to the Microbiology laboratory, Federal University of Technology, Akure for further analyses.

2.3 Isolation of Test organisms

Typed isolate of *S. typhi* was obtained from National Institute of Medical research (NIMER) Yaba Lagos Nigeria, clinical isolates of *S. typhi* were obtained from Don Bosco Hospital, Akure Nigeria. The clinical *S. typhi* were isolated from stool/blood samples of typhoid fever patients. Other *S. typhi* used were isolated from well water, fresh crayfish and apparently healthy individual using standard microbiological methods.

2.4 Preparation of *S. typhi* for antibacterial assay

The approximate number of bacteria used was standardized using 0.5 McFarland turbidity standards. McFarland (0.5) was prepared by adding 9.95 ml of 1% H_2SO_4 to 0.05 ml of 1% $BaCl_2$. The absorbance of the solution was then checked using a spectrophotometer at 623nm (it has to be between 0.08-0.1). The test bacteria were prepared by adding inoculum of 24 hours old culture of the test microorganisms into sterile distilled water in a test tube until it has the same turbidity as the prepared McFarland standard. Which represent 1.5 x 10^8 cfu/ml of the microorganisms (Sutton, 2011).

2.5 Effect of dilution on the antibacterial activity of five-day fermented palm wine on *S. typhi* isolates

The effect of dilution was assayed using agar dilution method. Palm wine was diluted serially (0.1, 0.01, 0.001) using sterile distilled water (volume by volume) into a set of sterile tubes. Each tube was inoculated with 0.1 ml of *S. typhi* containing 1.5x10^8 cfu/ml and the tubes were plated on Salmonella – Shigella agar, using pour plate technique to enumerate the viable count after incubation at 37°C for 24 hours (Adebolu *et al.*, 2011).

2.6 Assessment of the growth inhibitory activity of some antibiotics on *S. typhi* isolates

Four different conventional antibiotics; chloramphenicol, amoxicillin, gentamycin and ciprofloxacin was used for this assay. About 0.1 ml of *S. typhi* containing 1.5x10^8cfu/ml was spread on already prepared and solidified Muller-Hinton agar using sterile glass spreader. Five wells were made on each plate using sterile cork borer, each antibiotic was prepared to the concentration on conventional antibiotic sensitivity disk (30 µg for Chloramphenicol, 25 µg for Amoxycillin, 10µg for Gentamycin and 10 µg for Ciprofloxacin). Each of the antibiotics in solution (0.1 ml) was introduced into separate wells, one type per well, while sterile distilled water was added to the well at the centre. The plates were incubated at 37 ^oC for 24 hours after

which the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured using a transparent ruler. The antibiotics served as positive control (Olorunfemi *et al.,* 2006).

2.7 Determination of antibacterial activity of palm wine on *S. typhi* isolates.

The antibacterial activity of palm wine on *S. typhi* isolates was determined by agar well diffusion method. About 20 ml of already sterilized Muller-Hinton agar was allowed to cool to about 45^{o}C, after which it was aseptically poured into sterile petri dishes and left to solidify, 0.1 ml of *S. typhi* suspension containing 1.5x10^{8} cfu/ml was spread on solidified agar using a sterile glass spreader. Two wells were made on each plate using a 6mm sterile cork borer, 0.1 ml (100 µl) of palm wine was introduced into a well and 0.1 ml (100 µl) of sterile distilled water into the other, this served as control. The plates were incubated at 37^{o}C for 24 hours after which they were observed for zones of inhibition. The diameter of zone of inhibition was measured using a transparent ruler (Adedayo and Ajiboye, 2011).

3.0 RESULTS

3.1 Effect of dilution of the growth inhibitory activity for five-day fermented palm wine (*E. guineensis*) on *S. typhi* isolated from different sources

The effect of dilution of growth inhibitory activity for five-day fermented palm wine (*E. guineensis*) on *S. typhi* isolated from different sources is represented (Table 1). The result shows that palm wine (*E. guineensis*) had the highest effect of dilution growth inhibitory activity (8.40) on *S. typhi* isolated from the stools of patients from Federal Medical Center, Owo, on *S. typhi* isolated from stool of patients from State Specialist Hospital, Akure (7.10), on *S. typhi* isolated from well water (8.10), on *S. typhi* isolated from poultry droppings (8.90), on *S. typhi* isolated from raw beef (6.67), on *S. typhi* isolated from stream water (7.43), on *S. typhi* isolated from apparently healthy individual (6.67) and *Salmonella enterica* serovar *Typhi* ATCC 33458 (7.67) were observed at dilution factor of 10^{3} respectively. While, the highest effect of dilution growth inhibitory activity (5.36) was observed on *S. typhi* isolated from Don Bosco Hospital, Akure with undiluted *E. guineensis* and highest effect of dilution growth inhibitory activity (6.06) on *S. typhi* isolated from fresh crayfish and on *S. typhi* isolated from poultry soil (6.26) with dilution factor of 10^{2} respectively.

Table 1: Effect of dilution on the growth inhibitory activity of five-day fermented palm wine (*E. guineensis*) on *S. typhi*

***S.TYPHI* ISOLATES**	**Undiluted (10^0)**	**1:10 (10^2)**	**1:100 (10^2)**	**1:1000 (10^3)**
		***S. typhi* count**	**(cfu/ml)**	
FMC	4.10±0.06^{c}	1.55±0.03^{a}	3.13±0.09^{b}	8.40±0.06^{d}
DB	5.36±0.18^{d}	1.13±0.03^{b}	4.06±0.06^{c}	1.10±0.05^{a}
SH	4.10±0.06^{c}	1.13±0.03^{a}	3.10±0.10^{b}	7.10±0.06^{d}

AH	2.03±0.03[b]	1.96±0.03[a]	3.40±0.03[c]	6.67±0.03[d]
RB	3.00±0.28[b]	1.96±0.03[a]	5.86±0.13[c]	6.67±0.33[d]
FC	5.00±0.28[c]	1.15±0.03[b]	6.06±0.06[d]	1.06±0.06[a]
WW	8.10±0.03[d]	1.10±0.05[a]	6.33±0.08[c]	1.45±0.02[b]
SW	3.10±0.05[b]	1.13±0.08[a]	5.10±0.05[c]	7.43±0.12[d]
PS	3.50±0.28[b]	1.40±0.10[a]	6.26±0.12[c]	1.13±0.06[a]
PD	7.50±0.28[c]	1.73±0.03[a]	4.73±0.14[b]	8.90±0.10[d]
ATCC 33458	6.67±0.03[c]	1.53±0.26[a]	3.63±0.18[b]	7.67±0.03[d]

Data are represented as mean ± standard error (n=3) with the same superscript down the column are not significantly different ($p \leq 0.05$).

Key words: FMC- Federal Medical Center, Owo. Ondo State, WW- Well water, RB- Raw beef, DB- Don Bosco Hospital, Akure. Ondo State, SW- Stream water, FC- Fresh crayfish, SH- State Specialist Hospital, Akure, PS- Poultry soil, AH- Apparently healthy individual, PD- Poultry dropping

3.3 Fermentation duration inhibitory effect of palm wine (*E. guineensis*) on *S. typhi* isolated from various sources.

Table 2 shows the effect of fermentation period of palm wine (*E. guineensis*) on *S. typhi* isolated from various sources. The results show that palm wine (*E. guineensis*) had the highest inhibitory effect (33.33 mm) at 7 days on *S. typhi* isolated from the stools of patients from Federal Medical Center, Owo, stool of patients from State Specialist Hospital, Akure (26.67 mm) at 5 days, Don Bosco Hospital, Akure (35.30 mm) at 5 days, fresh crayfish (31.67 mm) at 5 days, well water (30.33mm) at 5 days, poultry droppings (31.67 mm) at 6 days, raw beef (31.67 mm) at 5 days, poultry soil (44.33 mm) at 5 days, stream water (43.33 mm) at 5 days, apparently healthy individual (39.63 mm) at 5 days and *Salmonella enterica* serovar *Typhi* ATCC 33458 (26.33.mm) at 6 days.

Table 2: Fermentation duration inhibitory effect of palm wine (*E. guineensis*) on *S. typhi* isolated from various sources

Fermentation duration (day)	FMC	DB	SH	AH	RB	FC	WW	SW	PS	PD	ATCC33458
	Diameter zones of inhibition (mm) of *S. typhi* ± S.E										
1	0.00± 0.00[a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00[a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]
2	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]	0.00± 0.00 [a]
3	10.67±0.33 [b]	8.33± 0.33 [b]	11.67±0.33 [b]	7.33± 0.33 [b]	14.33±0.33[b]	12.67±0.33[b]	6.33± 0.67 [b]	6.67± 0.31 [b]	11.33±0.67 [b]	8.00± 0.57 [b]	8.33± 0.33 [b]
4	20.67±0.33[c]	19.00±0.57 [c]	18.00±0.57 [c]	22.33±0.33 [c]	17.67±0.33 [c]	18.00±0.57[c]	17.67±0.33 [c]	27.67±0.31 [c]	24.33±0.33 [d]	21.33±0.67 [c]	16.67± 0.33 [c]
5	31.67±0.33 [e]	35.30±0.33[f]	26.67±0.57 [e]	39.63±0.33 [f]	31.67±0.57 [e]	31.67±0.57 [f]	30.33±0.33 [f]	43.33±0.67 [e]	44.33±0.33 [f]	26.33±0.33 [f]	26.33± 0.31 [f]
6	32.67±0.67 [d]	31.67±0.67 [e]	24.33±0.33 [e]	37.33±0.33 [e]	30.67±0.33 [e]	29.67±0.33 [e]	28.33±0.33 [e]	36.33±0.33 [d]	36.67±0.31 [e]	31.67±0.33 [e]	27.67± 0.31 [e]
7	33.00±0.57 [d]	23.33±0.33 [d]	25.33±0.67 [de]	25.33±0.33 [d]	20.67±0.67 [d]	19.67±0.67 [d]	19.33±0.33 [d]	27.67±0.67 [c]	22.67±0.33 [d]	22.67±0.33 [d]	24.33± 0.33 [d]

Data are represented as mean ± standard error (n=3) with the same superscript down the column are not significantly different ($p<0.05$).

Key words: FMC- Federal Medical Center, Owo. Ondo State, WW- Well water, AH- Apparently healthy individual DB- Don Bosco Hospital, Akure. Ondo State, SW- Stream water, RB- Raw beef, SH- State Specialist Hospital, Akure, PS- Poultry soil, PD- Poultry droppings FC- Fresh crayfish.

3.3 The growth inhibitory effect of palm wine (*E. guineensis*) and selected antibiotics on *S. typhii* isolated from different sources

The growth inhibitory activities of palm wine (*E. guineensis*) and selected antibiotics are represented (Figures 1-11). The results show that Palm wine (*E. guineensis*) had the highest inhibitory effect (35.33 mm) on *S. typhi* isolated from the stools of patients from Federal Medical Center, Owo, stool of patients from State Specialist Hospital, Akure (26.67 mm), Don Bosco Hospital, Akure (35.30 mm), fresh crayfish (31.67 mm), well water (30.33mm), poultry droppings (26.33 mm), raw beef (31.67 mm), poultry soil (44.33 mm), stream water (43.33 mm), apparently healthy individual (39.63 mm) and *Salmonella enterica* serovar *Typhi* ATCC 33458 (26.33.mm) compared to selected antibiotics.

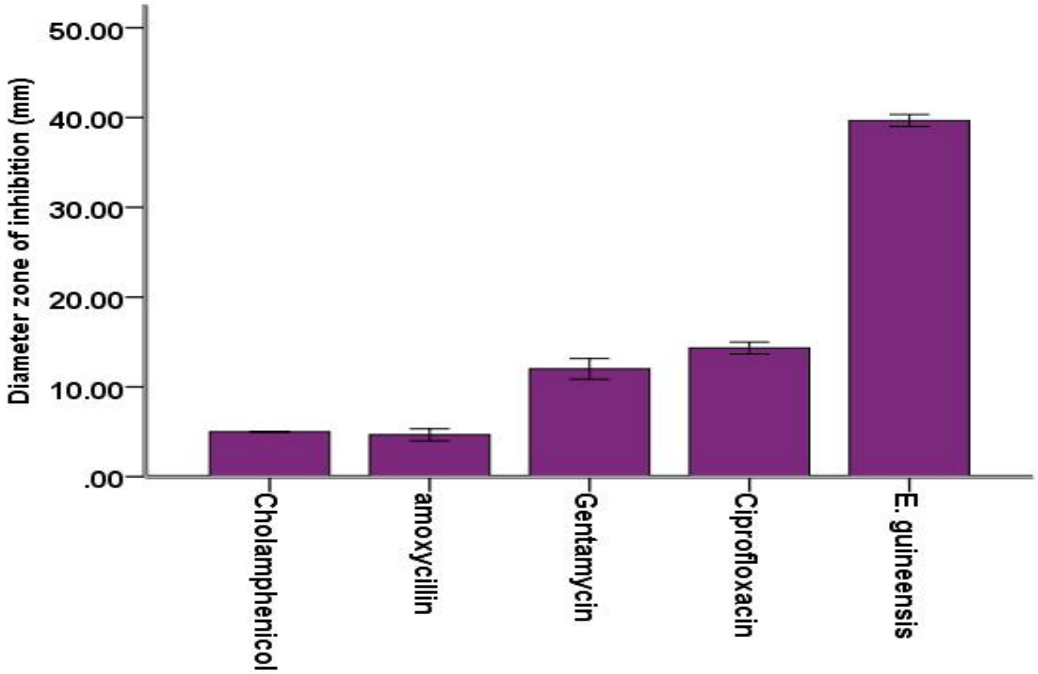

Figure 1: Diameter zone of inhibition of the growth of *S. typhi* isolated from apparently healthy individual by palm wine (*E. guineensis*) and selected antibiotics

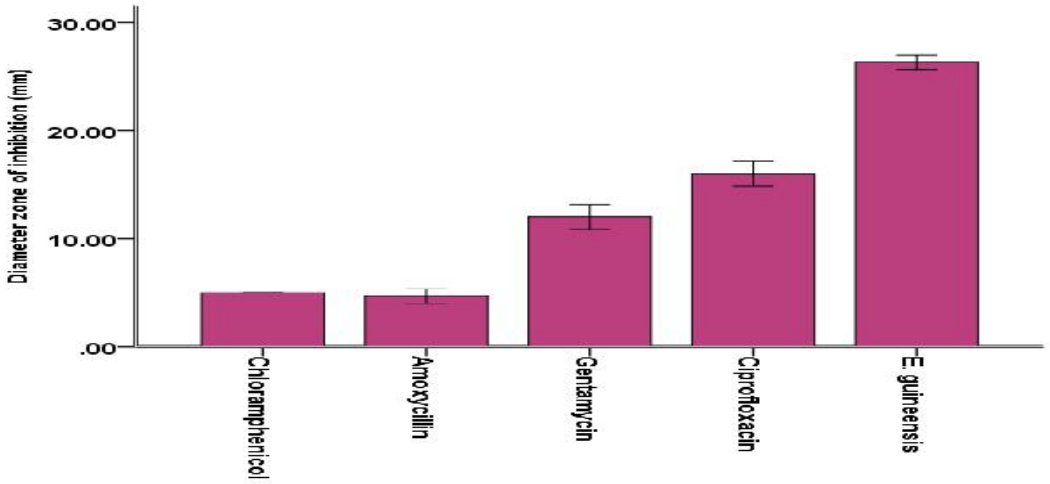

Figure 2: Diameter zone of inhibition of the growth of *Salmonella enterica* serovar *Typhi* ATCC 33458 by palm wine (*E. guineensis*) and selected antibiotics

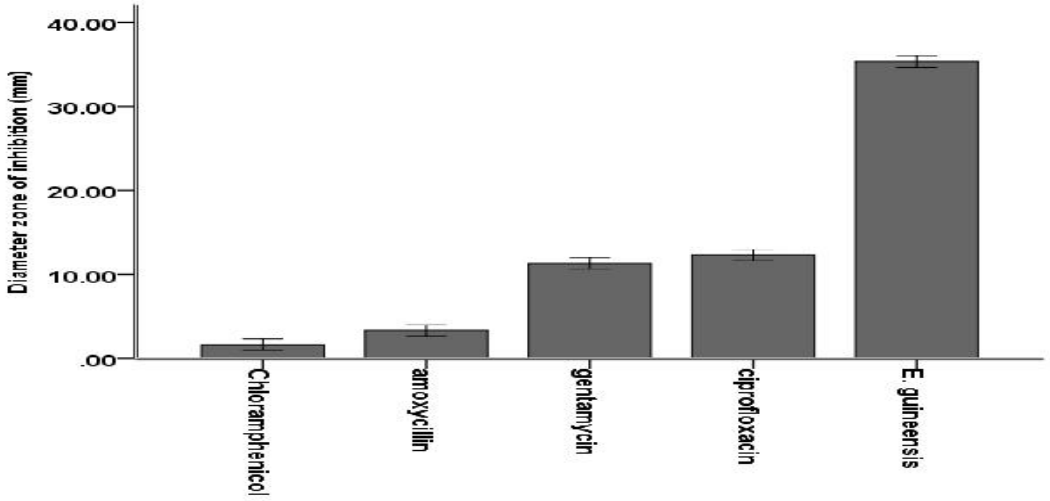

Fig 3: Diameter zone of inhibition of the growth of *S. typhi* isolated from stool sample of patients at Don Bosco Hospital, Akure by palm wine (*E. guineensis*) and selected antibiotics

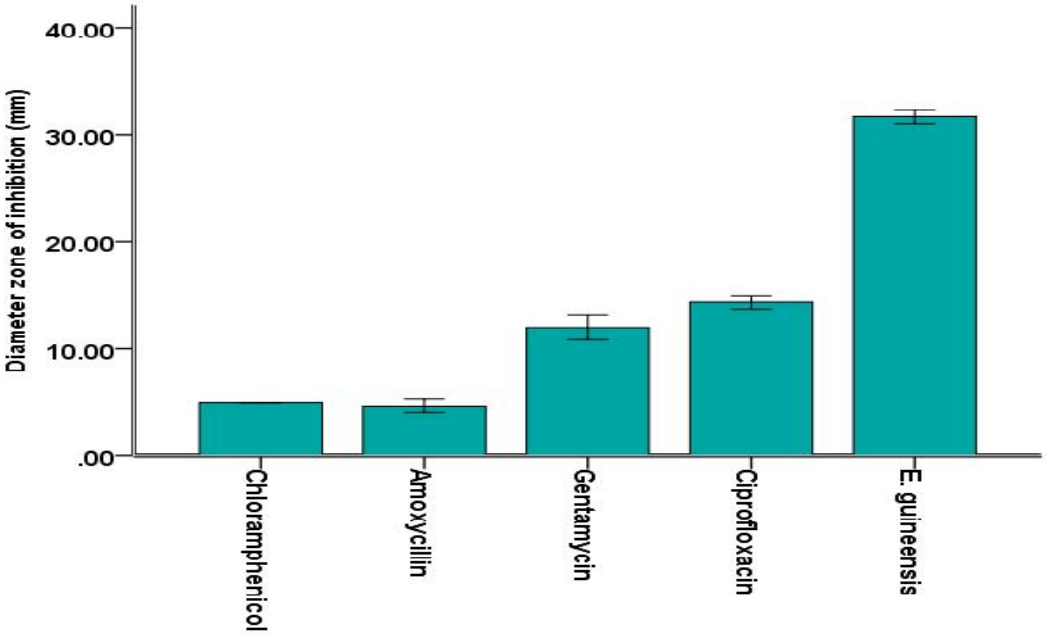

Figure 4: Diameter zone of inhibition of the growth of *S. typhi* isolated from fresh crayfish by palm wine (*E. guineensis*) and selected antibiotics

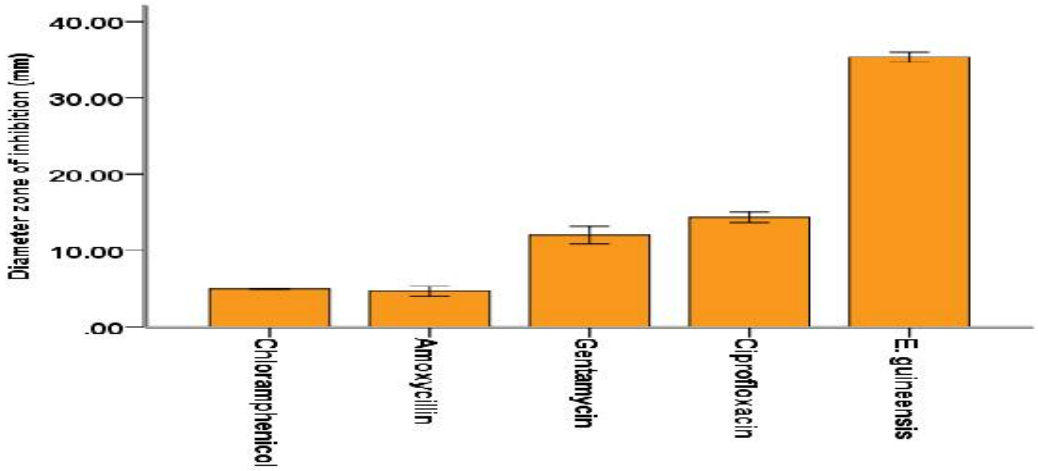

Figure 5: Diameter zone of inhibition of the growth of *S. typhi* isolates from the stool of patient from Federal Medical centre, Owo by palm wine (*E. guineensis*) and selected antibiotics

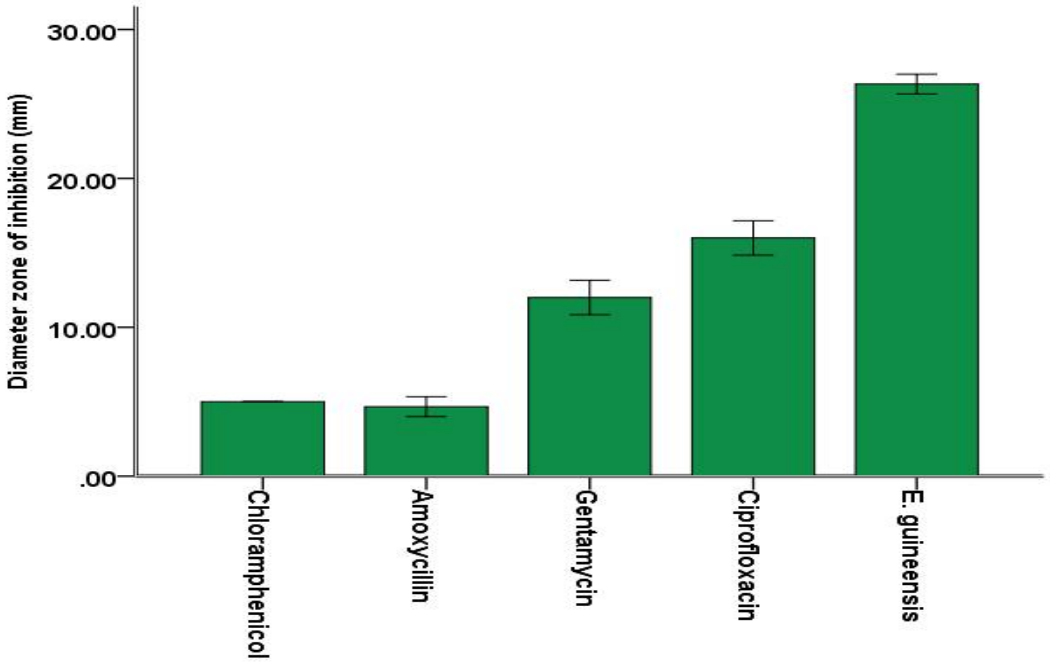

Figure 6: Diameter zone of inhibition of the growth of *S. typhi* isolated from Poultry dropping by palm wine (*E. guineensis*) and selected antibiotics

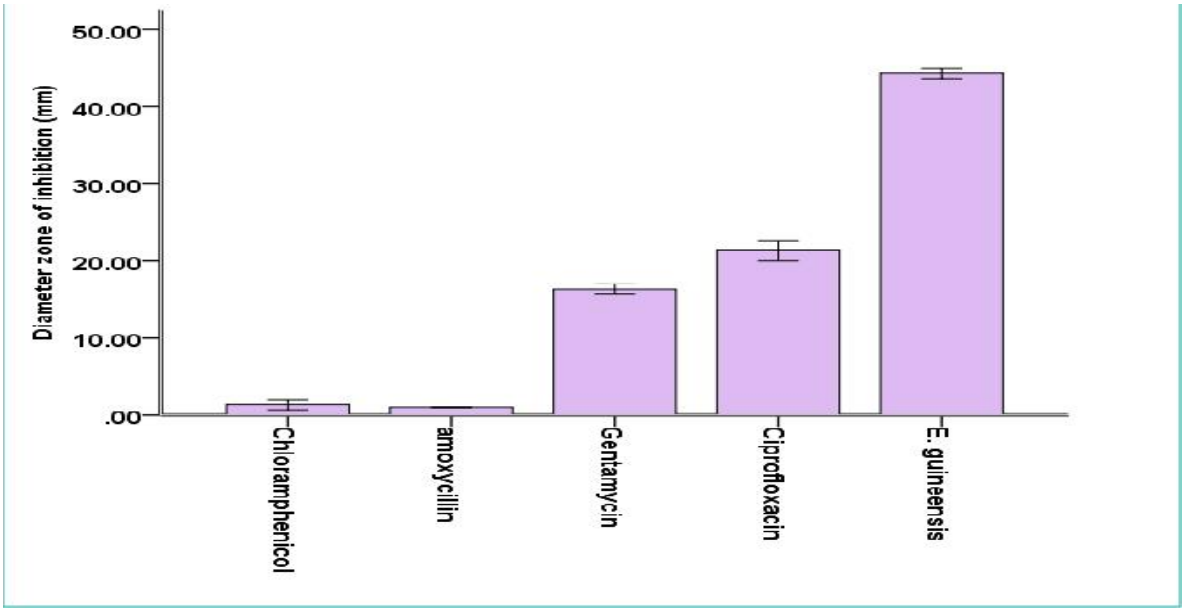

Figure 7: Diameter zone of inhibition of the growth of *S. typhi* isolated from Poultry soil by palm wine (*E. guineensis*) and selected antibiotics

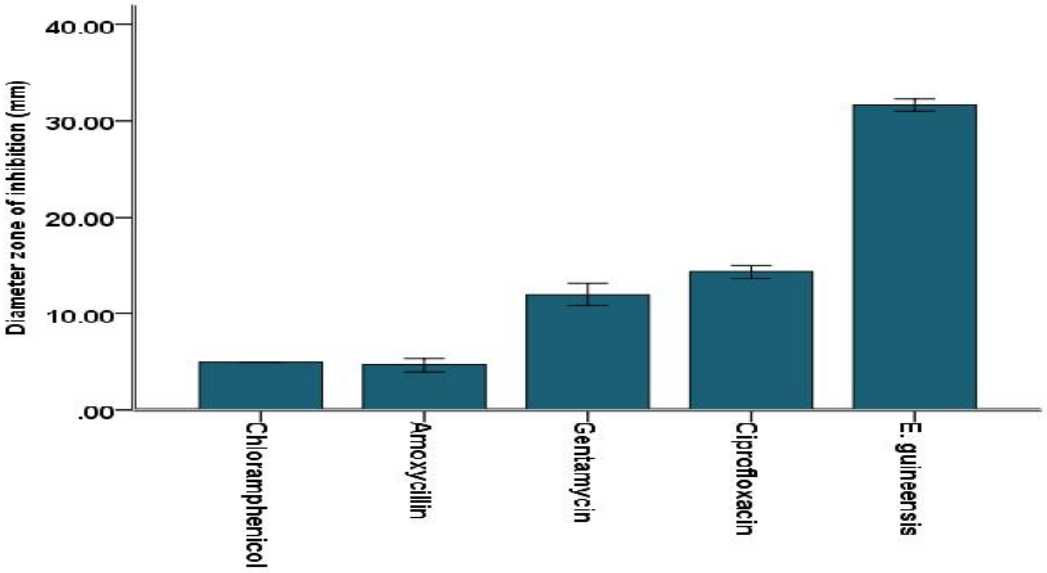

Figure 8: Diameter zone of inhibition of the growth of *S. typhi* isolated from raw beef by palm wine (*E. guineensis*) and selected antibiotics

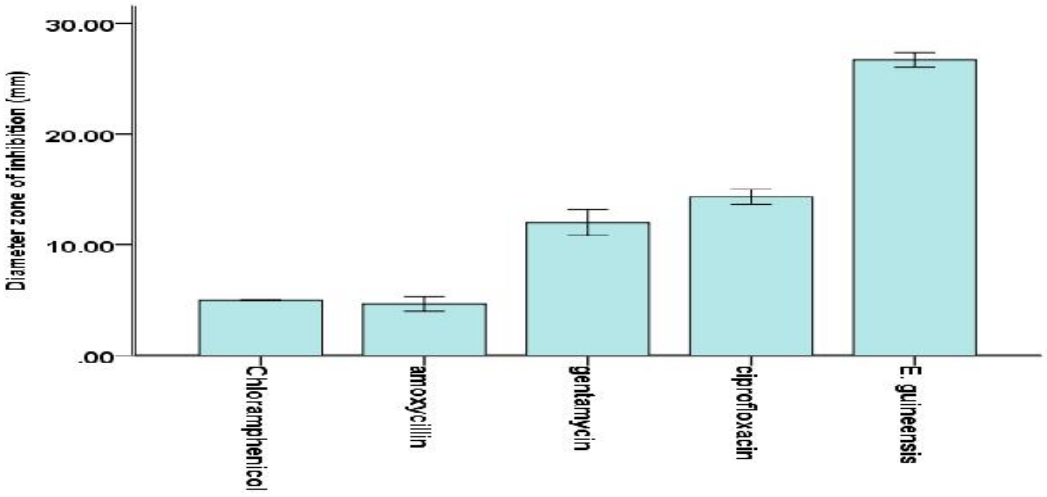

Figure 9: Diameter zone of inhibition of the growth of *S. typhi* isolated from stool sample of patients from State Specialist Hospital, Akure by palm wine (*E. guineensis*) and selected antibiotics

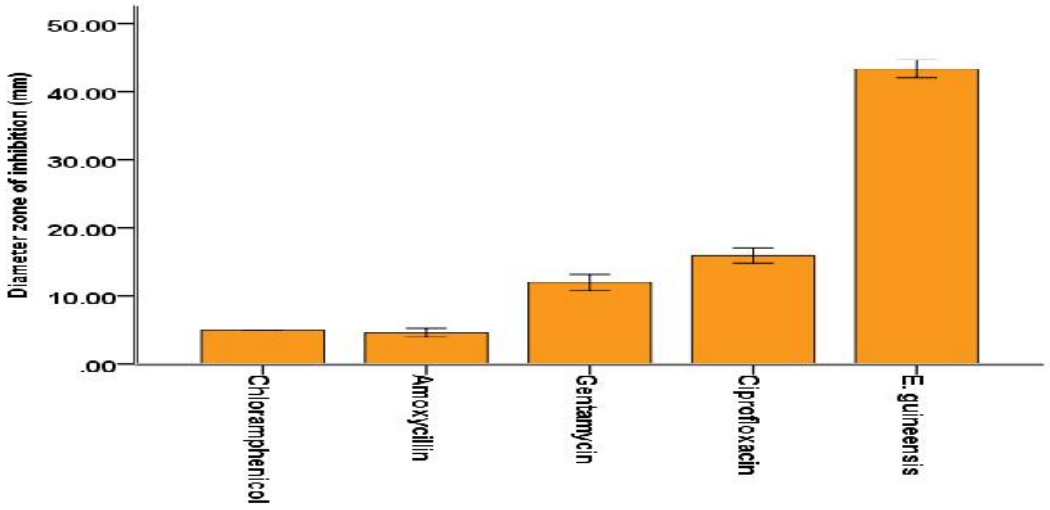

Figure 10: Diameter zone of inhibition of the growth of *S. typhi* isolated from stream water by palm wine (*E. guineensis*) and selected antibiotics

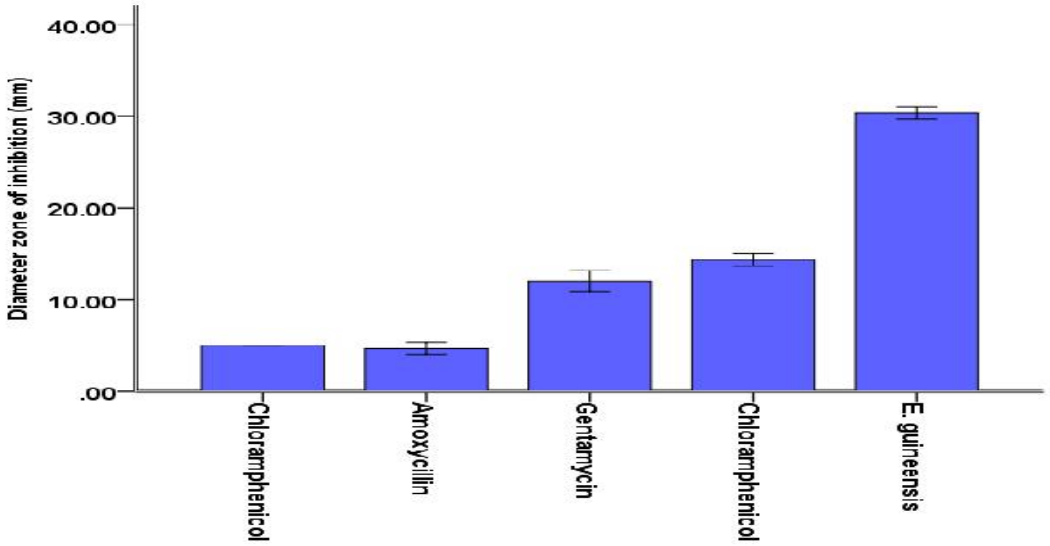

Figure 11: Diameter zone of inhibition of the growth of *S. typhi* isolated from well-water by palm wine (*E. guineensis*) and selected antibiotics

4.0 Discussion

Antibacterial effects of palm wine (*Elaeis guineensis*) on *S. typhi* isolated from different sources were studied. The inhibitory effect of the palm wine used however was discovered to reduce with dilution which shows that higher

concentrations are needed for effective actions (Kigigha *et al*., 2016). Akinsanya (1980) and Kigigha *et al*. (2016) reported that when a bacteristatic agent is excessively diluted, it becomes inactivated and bacteria may even survive in it. Moreover, when a bactericidal agent is excessively diluted it becomes bacteriostatic. This result agrees with the findings of Aibinu *et al*. (2007); Odunayo *et al.* (2007) and Adedayo and Ajiboye(2011) who have used palm wine in various ways as extractant (solvent) for different parts of medicinal plants used in the local treatments of various diseases of microbial origin and antimicrobial activity on various pathogenic microorganisms.

Palm wine was found to exhibit growth inhibitory effect on all the *S. typhi* isolates used this might be as a result of the acidic content of the palm wine (Amoa-Awua *et al*., 2007). The increase in acidity may be responsible for the inhibitory action of the 5-day fermented palm wines on all the isolates of *S. typhi* used (Shanson, 1990).

In this study, the effect of source of *S. typhi* on its susceptibility to the antibacterial effect of palm wine was also carried out. The highest diameter zone of growth inhibition observed in *S. typhi* isolated from poultry soil might be due to the fact that the bacterium has not devised means of resistance against antibiotics (Odunayo *et al*., 2007). *S. typhi* from apparently healthy individual had the lowest diameter zone of inhibition. This might be as a result of developed resistance when evading the human host immune system or due to acquired resistance from previous exposure to antibiotics (Akinsulure *et al*., 2007). This is in agreement with Adedayo and Ajiboye (2011) who reported on antimicrobial property of palm wine.

4.0 CONCLUSIONS

Palm wine used (*E. guineensis*) in this research work has showed to exert antibacterial activity against *S. typhi*. Moreover, palm wine dilution factors coupled with fermentation duration plays an important role in its antibacterial activity. Higher concentration of palm wine and longer fermentation period tends to increase its antibacterial activity of palm wine against *S. typhi*. It is therefore conceivable that palm wine subjected to natural fermentation could be used to treat infections caused by *S. typhi* that is, typhoid fever in the absence of antibiotics.

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