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PRESERVATIVE ACTIVITY OF *Xylopia aethiopica* FRUITS BIO-ACTIVE FRACTIONS ON FRESH MEAT

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ABSTRACT

The availability of food and its accessibility to people has been an important concern in most developing countries where food preservation techniques have been very inadequate. In comparison with synthetic additives, natural preservatives may be more acceptable to consumers and regulatory agencies and also potentially of benefit to human health. Fractionation of crude ethanolic extract, phytochemical screenings of fractions, evaluation of antimicrobial and preservative activity were carried out. GC-MS on the most active fraction was also carried out. Phytochemical screening uncovered the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antimicrobial activity results showed S. aureus with 18.33±0.33mm zone of inhibition and Aspergillus niger had least activity with 15.33±0.33mm at 4000µg/ml. The activity of the ethyl acetate fraction at highest concentration (4000µg/ml) against S. aureus was found to be not significant (p>0.05). Results of GC-MS analysis revealed the identity of the bio active compounds in the fraction such as phenols and terpinen-4-ol. The meat treated with 5% ethyl acetate fraction, recorded pH of 6.75 compared with negative control 7.74 while 5% vinegar (positive control) showed pH of 6.58 at 96 hours of storage. At 48 hours of storage, there were significant differences (P<0.05) in the pH of the treated meat with fractions, vinegar and untreated were observed. The sensory evaluation of the preserved meat by the judges showed meat treated with 5% vinegar had 50% likeness while meat treated with 5% ethyl acetate fraction recorded 30% likeness at 96 hours of storage.

Key words: Preservative, Phytochemical, bio active, fraction, Antimicrobial

INTRODUCTION

Meat is highly perishable, being with typically high levels of amino acids, which microorganisms metabolize, producing ammonia, organic acids, ketones and sulphur compounds (Mohammed *et al*. 2008). Meat begins to spoil as soon as they are slaughtered, the spoilage is caused by microorganisms, other spoilage results from chemical changes within the food itself due to natural process such as action of enzymes or oxidation (Olunike, 2014). The high temperatures of the tropics, lack of basic infrastructures and the unsanitary production conditions prevailing in most developing countries predispose meat to spoilage (Doulgeraki, *et al.* 2012). It has been estimated that 25% of all food produced globally is wasted post-harvest or post slaughter due to the microbial spoilage (Gram *et al*., 2002). Preservatives from natural sources can be useful in extending the shelf life of meat by reducing or inhibiting survival of spoilage microorganisms and increasing the quality of meat. *Xylopia aethiopica* contains a variety of active ingredients that are extracted in various forms as crude organic or aqueous extracts. A major advantage of *X. aethiopica* in preservation of meat is strong aroma which serve as a limitation in preservation of juice (Ogbonna, 2013). Several studies carried out by scientists on spices were concentrated on their medicinal properties. However, this study was carried to determine the preservative activities of *X. aethiopica* fruits bio-active fractions on fresh meat.

MATERIALS AND METHODS

Sample Collection and Processing

The fruits of *X. aethiopica* were purchased as dried fruits from famous Kurmi market in Kano city and authenticated by a botanist from Department of Plant Biology, Bayero University, Kano. Voucher specimen with reference number BUKHAN 0510 was deposited.

Processing and extraction of *X. aethiopica* fruits

The plant was air dried at room temperature for two weeks (28+2^{0}C) and ground into powdered form using mortar and pestle (Mukhtar and Tukur, 1999). The powdered spices (100g) was percolated in 1000ml ethanol in 2L conical flasks, stoppered and kept for two weeks with an intermittent shaking. The percolate was filtered with Whatman's No. 1 filter paper and resulting ethanol (BP 78.370C) extract was concentrated at 40^{0}C by complete evaporation of solvent using rotary evaporator. The crude extract was weighed and kept into airtight container under refrigeration at 4^{0}C prior to use (Fatope *et al.* 1993).

Fractionation of the crude extract

Crude extract was undertaken for liquid-liquid partitioning using the method of Kupchan *et al. (*1973) and modified version of Wagenen *et al*. (1993). The crude ethanolic extract (5g) was partitioned by dissolving in 10% aqueous ethanol (ethanol: water; 9:1 v/v) to make homogeneous solution which was successively partitioned by three solvents chloroform, petroleum ether and ethyl acetate in order of increasing polarity by using separating funnel and concentrated by evaporation using rotary evaporator at 40^{0}C.

Phytochemical Analysis of the fractions

The fractions were subjected to phytochemical analysis for identification of bioactive chemical constituents (AOAC, 2002).

Preparation of Different Concentrations of the fraction

Stock solution (400mg/ml) was prepared by dissolving 0.8g in 2ml of DMSO. Serial doubling dilution was carried out by adding 1ml of DMSO at each serial dilution. Concentrations (500µg, 1000µg, 2000µg and 4000µg) were prepared and kept at 4^{0}C prior to use (Bukar *et al*. 2010).

Test Organisms

Spoilage microorganisms were isolated from fresh meat sample. The meat sample (10g) was homogenized in 90ml of sterile distilled water and streaked on appropriate media for isolation, cultural, morphological and biochemical characterization of isolates was carried out using procedures described by FAO (1997).

Preservation of Fresh Meat Sample

Different treatments

A – Untreated fresh meat (Negative control)

B – Treatment with 1% XAFEA w/v

C – Treatment with 5% XAFEA w/v

D - Treatment with 5% (v/v) vinegar (Positive control)

Fresh meat was aseptically cut into pieces (10g) and were dipped in each treatment as above for 30 minutes and gently swirled with a sterile glass rod, removed and allowed to drain on a stainless wire mesh screen for 10 minutes. Subsequently, the treated samples were placed in plastic containers with a lid, labeled and stored at ambient temperature (Eniolorunda, *et al.* 2014). A 10g of meat sample from the different treatments was homogenized for bacterial count, pH and sensory analysis was carried out at 0 hour, 24, 48, 72 and 96 hours (Bukar, 2012).

Determination of pH

Determination of pH was carried out according to Terefe (2016). Ten grams of meat from each treatment were homogenized in 100 ml distilled water. The homogenate was filtered and the pH of the filtrate was taken by inserting the glass electrode of the pH meter into the filtrate using a portable Jenway digital pH meter.

Sensory Analysis

Organoleptic properties were assessed by judges through sensory evaluation (Idise, 2011). Scores were graded on a Hedonic scale, the treatment most preferred and the number of hours for each treatment to deteriorate were recorded and compared with the control (Bukar, 2010).

Statistical Analysis

Results were analyzed by one way analysis of variance (ANOVA) to determine the differences at 5% probability level of significant using Microsoft Excel 2010 software.

RESULTS

The physical characteristics of the *Xylopia aethiopica* crude ethanolic extract recovered 33.7%, dark blue with a sticky texture on Table 1. Phytochemical properties of the bioactive fractions revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, phenols and terpenoids in ethyl acetate fraction while flavonoids and saponins were absence in chloroform fraction as shown in Table 2. From the results of GC-MS analysis on Table 3, twenty nine (29) compounds including phenolic compound (phenols, d6, terpinen-4-ol) and monoterpine hydrocarbon (B-pinene, a-sapinene, B-phellandrene) were recovered from ethyl acetate fraction. The results of antimicrobial activity, revealed ethyl acetate fraction was the most active (18.33$\pm$0.33 mm) than other fractions but the differences were not statistically significant (P>0.05) on Table 4. At 0 hour the mean aerobic bacterial count (APC) of meat was 5.86 log cfu/g as presented on (Figure 1), the reduction in APC observed in ethyl acetate fraction (treatment C) ranged from 1.06 to 0.54 log cfu/g at 72 hours while in vinegar (treatment D) decreased in APC ranged from 1.12 to 0.12 log cfu/g at 92 hours.

Significant increases (P<0.05) in APC were observed at 72 hours of storage. Figure 2, at 0 hour (before treatment) fresh meat had pH of 5.86, while treatment C recorded pH 6.75. However treatment D had pH of 6.58 at 96 hours and significant differences (P<0.05) were observed in the pH of treated and untreated meat. From the result of general acceptability of preservative meat (figure 3) showed the judges rejected the untreated meat at 48 hours while treatment C (5% *Xylopia aethiopica*) and treatment D (5% vinegar) were rejected at 72 and 48 hours respectively.

TABLE 1: Physical characteristics of the *Xylopia aethiopica* ethanolic extract

Properties	**XAFEA**
Weight of Powder (g)	100
Volume of Solvent(ml)	1000
Weight Recovered(g)	33.70
Percentage Recovered (%)	33.7
Colour	Dark Blue
Texture	Sticky
Odour	Pungent

KEY: XAFEA= *Xylopia aethiopica* fruit ethanolic extract

TABLE 2: Phytochemical properties of the *X. aethiopica* Fruit

Fractions	**Alkaloids**	**Flavonoids**	**Saponins**	**Steroids**	**Tannins**	**Phenols**	**Terpenoids**
Ethyl- acetate	+	+	+	+	+	+	+
Pet. Ether	+	+	—	+	--	+	+
Chloroform	+	--	--	+	+	+	+

KEY: + = Presence --- = Absence

Table 3: GC-MS analysis of *X. aethiopica* most active fraction (ethyl acetate)

PEAK	Retention Time	Composition By Area (%)	Compound Name	Molecular Formula	Molecular weight
1.	6.775	0.11	Octanoic acid, methyl ester Heptanoic acid methyl ester	$C_9H_{18}O_2$	58.24
2.	14.944	0.70	2,4-Decadienal (E,E)	$C_{10}H_{16}O$	152.23
3.	15.746	0.36	2(1H)-Pyrimidinone,4-amino-5-methyl	$C_{10}H_{14}N_4O_4$	212.30
4.	19.449	0.06	Terpinen-4-ol	$C_{10}H_{18}O$	154.25
5.	28.110	0.35	Hexane,3-ethyl-3-methyl	C_9H_{20}	128.25
6.	31.319	0.19	Nonanoic acid,9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186.25
7.	50.552	2.24	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
8.	52.897	0.13	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48
9.	53.666	0.11	12-methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O_2$	280.49
10.	86.637	49.10	3-quinolinearboxylic acid 6,8-difluoro-4-hydroxy-ethyl ester	$C_{12}H_9F_2NO_3$	253.205

Table 4: Zone of inhibition (mm) of *Xylopia aethiopica* fractions

Fractions	Ethyl-acetate				Chloroform				Petroleum ether			
Conc. (µg/ml)	SA	EC	AN	MC	SA	EC	AN	MC	SA	EC	AN	MC
500	8.66 ±0.3	9.66 ±0.3	8.33 ±0.3	8.00 ±0.0	7.66 ±0.3	8.66 ±0.3	7.33 ±0.6	7.66 ±0.3	7.66 ±0.6	7.33 ±0.3	7.00 ±0.0	7.75 ±0.5
1000	11.33 ±0.3	12.00 ±0.0	10.33 ±0.3	9.66 ±0.3	9.66 ±0.3	11.33 ±0.3	9.66 ±0.3	10.33 ±0.6	9.00 ±0.0	8.66 ±0.3	7.66 ±0.5	9.33 ±0.8
2000	13.66 ±0.6	13.33 ±0.3	13.33 ±0.3	12.33 ±0.3	12.33 ±0.33	13.66 ±0.33	12.00 ±0.0	11.66 ±0.0	10.33 ±0.3	9.66 ±0.6	10.00 ±0.0	10.66 ±0.0
4000	18.33 ±0.3	16.66 ±0.6	15.33 ±0.3	17.00 ±0.0	15.00 ±0.33	15.33 ±0.33	13.33 ±0.3	14.00 ±0.0	12.33 ±0.6	13.00 ±0.0	11.66 ±0.3	12.33 ±0.3

Values are mean ± SE and each value is mean of three determinations $p>0.05$

KEY: SA= *Staph. aureus* , EC= *E. coli,* AN= *Aspergillus niger,* MC= *Mucor*

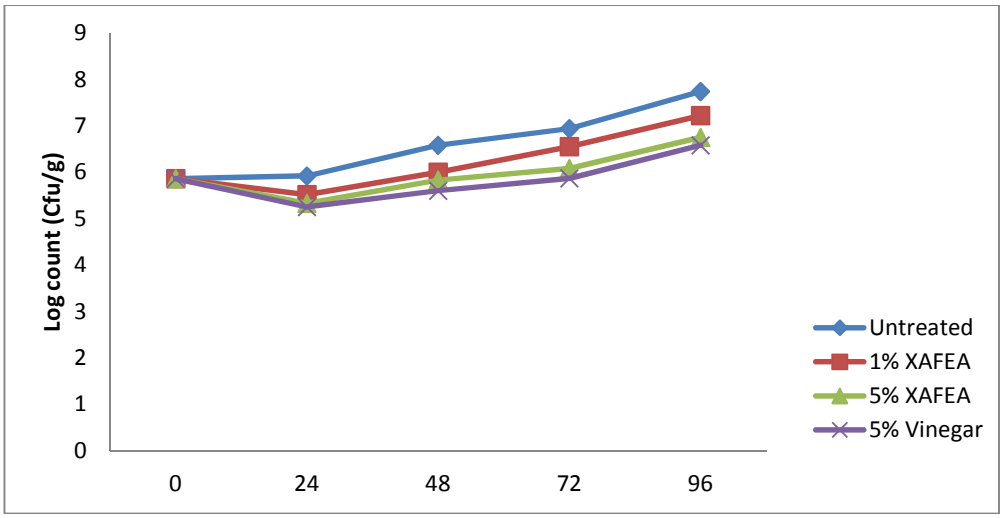

Figure 1: Aerobic bacterial count of preserved meat (CFU/g)

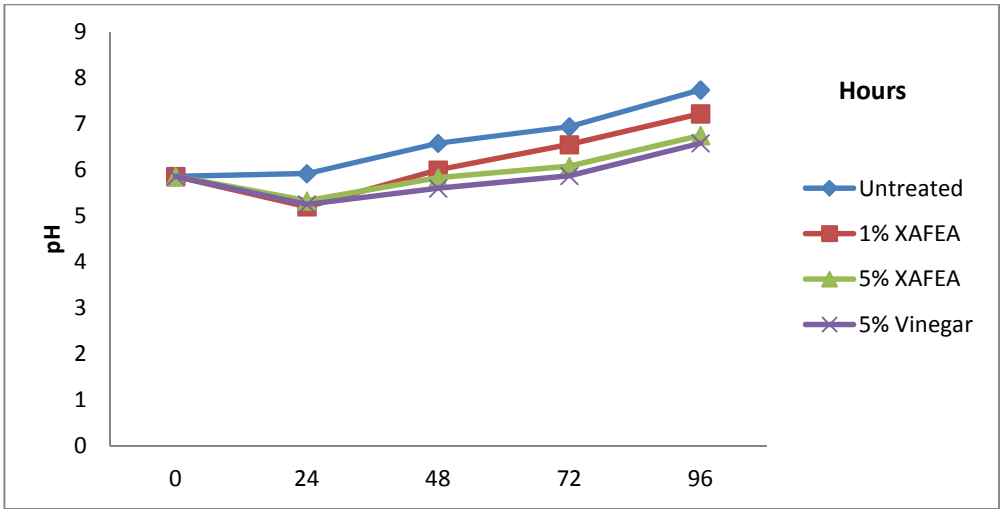

Figure 2: pH Changes of preserved Meat

Key: XAFEA= *Xylopia aethiopica* fruit ethanolic extract

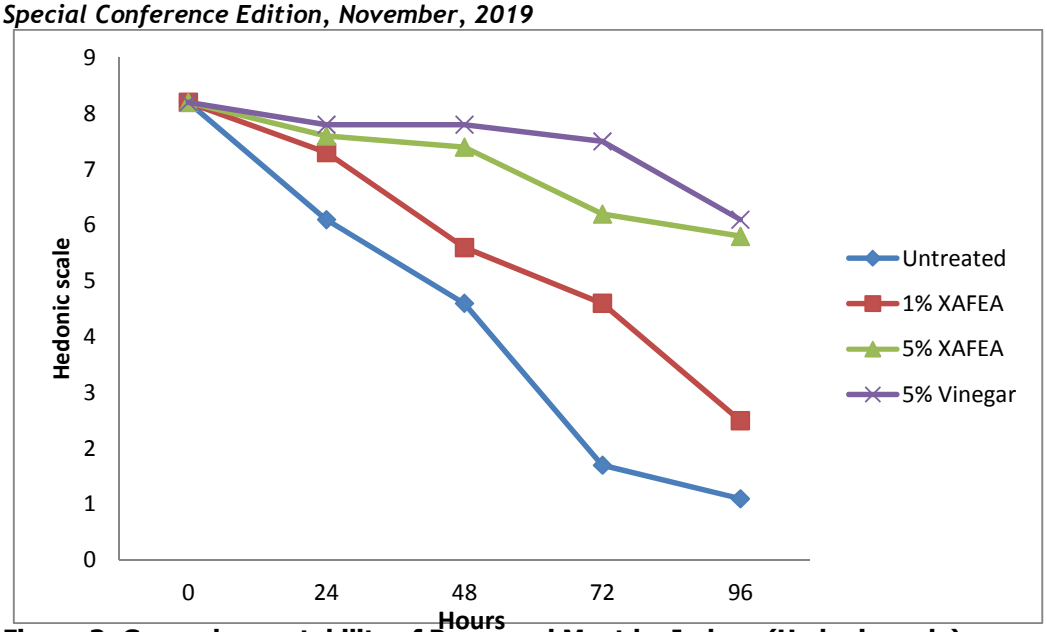

Figure 3: General acceptability of Preserved Meat by Judges (Hedonic scale)

DISCUSSION

Microorganisms isolated in the study were similar to the microorganisms reported by Omorodion and Odu (2014) and also in conformity with report by Fasanmi and Sanusi (2008). Storage of meat at ambient temperature for a long time which is commonly done by meat retailers allow multiplication of spoilage bacteria (Chukwu and Imodiboh 2009). The result is in agreement with the findings of Aguoro *et al*. (2016) and John-Dewole *et al*. (2012) where alkaloids, flavonoids, tannins, phenols and saponins were recovered from *X. aethiopica* fruit. The relative solubility of phytochemicals compound in different solvents depends on the nature of polarity of the solvents and their chemical bond. Polar solvents such as ethyl acetate recovered more phytochemical than less polar solvents (Udobi and Onolapo, 2009). Ethyl acetate fraction has the highest sensitivity among the other fractions, the sensitivity might be as a result of numerous bioactive compounds in the fraction. Fractionation across different solvent phases may have caused the breakdown of active compounds due to different solubility in the solvents (Adebayo *et al.* 2013). Fractions contain pure forms of bioactive compounds than the crude extracts which may contain inactive compounds that may resist the antimicrobial properties (Ebi and Ofoefule, 1997). The phenolic compounds and monoterpine hydrocarbon discovered from the GCMS analysis might have contributed towards preservative properties. GCMS analysis of *Xylopia aethiopica* fruit by Noujou *et al*. (2007) and Tatsadjieu *et al*. (2003) identified compounds mainly B-pinene and B-phellandrene. The reduction of APC observed in treatment C was probably due to antibacterial activity of *Xylopia aethiopica* on some spoilage microorganisms, this could be due to ability of the ethyl acetate fraction to recovered more phytochemicals than other solvents used for the fractionation

This corroborates with the findings of Bukar (2012) who reported treatment of meat with 5% *Parkia bigloposa* pod extract and 5% vinegar prevented the meat from spoilage for a period of 4 days.

CONCLUSION

The study has shown *X. aethiopica* fruits was found to contain active compounds that are responsible for effective preservative activity. Ethyl acetate fraction applied to fresh meat could help decrease pH, reduce microbial load and preserve fresh meat at ambient temperature for up to 96 hours. Hence the fraction of the *X. aethiopica* fruits represent a potential source of natural preservative in food as an alternative to synthetic chemical preservatives.

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