ASSESSMENT OF *olea africana* (CHARCOAL) AND *xylopia aethopion* ('KIMBA') AS PRESERVATIVES ON MICROBIOLOGICAL SAFETY OF MILK

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ABSTRACT
A Comparative study of two traditional methods of milk preservation on microbiological safety was designed and conducted in two stages. The first stage was a survey which identified various methods of preservation of milk as well as the consumption pattern of traditional milk products in the study area. The second stage was a laboratory activity where two traditional preservatives (*Xylopia aethopica* (kimba) and charcoal) were used to preserve milk for 24 hours at six hours interval to investigate the microbiological safety of milk. Data from the survey were expressed in descriptive statistics while data on microbiological analysis was analyzed using ANOVA. The survey result shows that (70%) of the respondents were female and mostly used traditional methods (82.5%) in preserving milk. 77.5% do not add any substance to the milk for preservation, but (22.5%) of respondents add traditional substances to preserve milk. The main reason mentioned by the respondents was to add flavor (70 %) and extend the shelf-life of milk. Data on microbial load shows that except at zero hours, there was significant difference ($P < 0.05$) among all treatments. A trend was observed in that, for control samples, increase in preservation time lead to increase in microbial count, however, for the treated samples, a decrease in microbial count was observed with increase in preservation time, suggesting that the traditional preservatives had antimicrobial effect. *Staphylococcus, E.coli* and *Bacillus spp.* were the bacteria isolated from the milk. It was concluded that a decrease in microbial count was observed with increase in preservation time for treated samples with *xylopia aethopion* ('kimba') having lower bacterial count.
Keywords: *Olea Africana, Xylopia Aethopion,* Preservatives, Microbiological Safety, Milk

INTRODUCTION
Background of the Study
Milk is a nutrient rich, white liquid food produced by the mammary glands of mammals. It is the primary source of nutrient for infant mammals (including humans who are breast fed) before they are able to digest other types of food. Early lactation milk contains colostrum, which carries the mother's antibodies to its young and can reduce the risk of many diseases. It contains many other nutrients including protein and lactose (Pehrson, *et al.*, 2000). As an Agricultural product, milk also called dairy milks, is extracted from farm animals during or soon after pregnancy.

Milk consumption occurs in two distinct overall types, a natural source of nutrition for all infant mammals and a food product obtained from other mammals for consumption by humans of all ages. Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf life. Since milk is often sold in areas of the world for distance from the production sites, the also extended safe shelf life for milk has also expand (Rassoli, 2007). Milk has a complex biochemical composition and its high water activity and nutritional value serves as an excellent medium for growth and multiplication of many kind of micro-organisms when suitable condition exist (Parekh and subhash, 2008). Because of the hand milking system pastoralists, it's not easy to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining it equipment, feed, soil, cow faeces, and grass (Khan *et al.*, 2008).

Thus, although milk and milk products are minor constituent in most diets but contaminated milk is responsible for up to 90% of all dairy related disease of humans (Shirima, *et al.*, 2003). The risk of infection by milk borne zoonotic disease such as tuberculosis and brucellosis among other disease is one of the reasons for public health regulations, which discourage the informal milk markets and consumption of raw or unpasteurized milk (Shirima, et al., 2003).

Traditional milk processing methods, the pastoral are the most important sources of domestic milk in Nigeria. The wives of pastoralist usually process fresh milk into various traditional milk products.

These include nono (sour milk), kindirmo (sour yoghurt), manshanu (local butter), cuku (Fulani cheese) and wara (Yoruba cheese).these product are usually hawked around the local area by women or are sold in a specific location (CBN, 1999).

In rural settings particularly in several pastoralist communities, use of medicinal plants is the commonly used technique for different food preservation including milk. Several plants, herbs and spice extracts have been reported to possess antimicrobial activity against a range of bacteria, yeast and molds (Burt, 2004). Charcoal from *Oleaeuropea, lagenariasiceria* and *Olea Africana* were documented to be used to coat the inside of milk storage gourds to add flavor/ aroma and also to protect the milk against spoilage micro-organisms. Therefore, it is important to evaluate antimicrobial activities of indigenous plant which are commonly used by pastoral communities for milk preservation. Therefore, this study is aimed at assessing two local milk preservation substances for total microbiological load of milk.

MATERIALS AND METHODS

Study Area

The study was carried in Animal Science Laboratory of Kebbi State University of Science and Technology Aliero. Aliero Is Town in Kebbi state, Northern Nigeria.

Experimental Design

The experiment was conducted using a completely randomize design (CRD) consisting of three treatments replicated four times. The treatments were two traditional milk preservation substances (*Olea Africana* (Charcoal) and *xylopia aethopion* ('kimba'). The three (3) litres of milk was divided i to two (1 liter each) for the two treatments respectively. Each treatment was replicated four (4) times i.e. 250ml and one (1g) of *xylopia aethopica* (kimba) was added to first treatment and one (1g) of *Olea Africana* charchol was added to second treatment with the exception of control. Data was taken after an interval of 6 hours. The experiment lasted for 24 hours.

Microbiological Analysis

Nutrient agar medium

The medium was prepared by 7g of nutrient agar in 240mls of distal water. The mixture was dissolved on hot plate and mouth of the conical flask was covered as curved with cotton wool and aluminum. The medium was sterilized using an autoclave at 121^{o}c for 15 minutes. The medium was allowed to cold to 45^{o}c and dispensed into sterile petri-dishes in the manner of (Oyeleke and Manga, 2008). Five folds serial diilution were prepared and labeled as 10^{-1} , 10^{-2}, 10^{-3}, $10^{-4,}$ and 10^{-5} in order to obtain discrete colony. An inoculum (0.1ml) from the last dilution (10^{-5} was be pipette and dispense onto the solidified nutrient agar medium, sterile bent glass rod was used to spread the inoculums for even growth. This was incubated at 37^{o}c for 24hrs. After incubation plate showing between 3 – 11 colonies on agar plates was counted. The colonies are efficiently counted by inverting the plate and each colony counted was marked with marker to avoid recounting. Biochemical tests were carried out as outlined by Cheesebrough *et, al*. (2002).

RESULT AND DISCUSSION

Heterotrophic bacterial count from milk samples preserved using traditional preservatives.

Table 1 shows the result of Heterotrophic bacterial count from milk samples preserved using two traditional preservatives (*Olea Africana* (Charcoal) and *xylopia aethopion* ('kimba'). Results indicate that there was no significant difference ($P > 0.05$) between treatments at 0 hours of preservation. However, there was significant difference ($P < 0.05$) among all hours of preservation across all treatments. The mean total bacterial count is also significantly different indicating that *xylopia aethopica* ('kimba') has lower bacterial count than *Olea Africana* (Charcoal).

Table 1: Heterotrophic bacterial count from milk samples preserved using traditional preservatives.

Treatment (cfu/ml)	Times (hrs)					
	0hr	6hrs	12hrs	18hrs	24hrs	Mean
Control	7.3	6.8^{c}	7.5^{c}	8.0^{c}	8.8^{c}	7.7^{c}
X. aethopica	7.4	7.8^{b}	6.4^{a}	5.0^{a}	4.5^{a}	6.3^{a}
Olea Africana	7.5	7.3^{a}	7.1^{b}	6.5^{b}	5.5^{b}	6.8^{b}
S.E	0.117	0.047	0.061	0.058	0.047	0.047

The significant differences observed at 6, 12, 18 and 24 hours among the treatments with *Xylopia aethopica* recording lower (6.3cfu/ml) than *Olea Africana* (6.8 cfu/ml) and control (7.7 cfu/ml) might be as a result of *xylopia aethopica* having antimicrobial effect as widely reported (Samah *et al.*, 2019; Davide *et al.*, 2016; Kassahun, 2013). A trend was also observed in the bacterial count across the treatments. Bacterial count in control increased with increase in storage time. But, bacterial count decreases with increased in storage time there by suggesting that the preservatives might have exhibited their antimicrobial effect as the storage time is prolonged.

Incidence of some bacteria in milk samples treated with two traditional preservatives

Results in Table 4.3 shows the morphological characteristics of some bacteria isolated from milk samples treated with two traditional preservatives (*Olea Africana* (Charcoal) and *xylopia aethopion* ('kimba'). The result indicated that *Staphylococcus*, *E.coli* and *Bacillus spp.* were the bacteria isolated with *Staphylococcus* and *E.coli* having more incidence of occurrence.

Table 4.3 Incidence of some bacteria in milk samples treated with two traditional preservatives

Treatments	**Incidence**		
	E.coli	*Staphylococcus* spp	*Bacillus*
Control	0	4	0
Olea Africana	3	1	1
xylopia aethopion	1	3	1
Percentage(%)	**33.33(%)**	**66.66(%)**	**16.66(%)**

From the results, it was observed that *E.coli* and *Bacillus spp.* were not found in the control samples but were however observed in treated samples. The presence of these pathogens in the samples could only mean that they might be introduced along with the test preservative which might not be qualitative as reported by Samah *et al*, (2019) that only the highest quality herbs or spices can be added to dairy products to combat contaminating microorganisms.

CONCLUSION

From the research findings, it was concluded that preservation time lead to increase in bacterial count in control samples. However, for the treated samples, a decrease in microbial count was observed with increase in preservation time. The mean total bacterial count among the treatments indicated that *xylopia aethopion* ('kimba') has lower bacterial count than *Olea Africana* (Charcoal).

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