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The Effects of Herbal Plants on Quality and Microbial Safety of Cow Milk Produced by Smallholder Dairy Farmers in Northern Ethiopia

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Abstract
Cow milk is a perishable commodity that spoils easily. Its low acidity and high nutrient content makes to be a medium for bacteria growth. The study was conducted to evaluate the effect of five herbal plants (*Olea europaea L, Vernonia amygdalina, Solanum schimperianum hochst, Acacia etbaica schweinf, Aoe elegans*) on improving the quality and microbial safety of cow milk. Milk samples were collected and poured in to 6 calabash and 6 plastic containers each with 200 ml. Non-fumigated plastic and calabash containers were used as control group. Bacterial load was estimated using standard plate and coliform counts. Effects of herbal plants were assessed on the basis of selected organoleptic (odor and taste) and physical attributes (temperature and pH) parameters. The lowest standard plate ($7.8x10^5$) and coliform ($4.5x10^5$) bacterial count was obtained from milk samples fumigated by *Vernonia* amygdalina. The highest bacterial count was recorded from milk samples of non-fumigated containers. *Vernonia amygdalina* was identified as the best herbal plant in improving the quality of milk. Milk stored in plastic and calabash containers had an excellent taste and odour but did not significantly decreased bacterial load. There is a need to investigate the active ingredients contained in the herbal plants.
Keywords: Calabash, Coliform count, Plastic, Fumigation, Standard plat count

1. Introduction

Cow milk is a perishable commodity that spoils easily and quickly. Its low acidity and high nutrient content makes it an ideal medium for the growth of bacteria and poses serious human health threats. Smallholder livestock keepers in rural areas rely on traditional plants to preserve the quality of raw cow milk. Such indigenous knowledge is unevenly distributed among each community members. Location, religion, linguistic and cultural backgrounds are among the factors that influence the use of herbal plants for preserving raw milk. The knowledge on medicinal plants is mostly transferred from generation to generation through oral as well as written documentation as inscribed in Parchments which partly characterize the traditional medical system described as medico religious written in ‘’Geez’’ manuscripts of the 15th century (Gelahun, 1989; Dawit and Ahadu, 1993). Other ancient written sources include the book of remedy (*Metsehafe Fews*) of the 17th century which contains a wide range of medicinal plants prescription (Fekadu, 2001). These are the medical traditions of the followers of Coptic Christianity. In addition cultural groups in the country have their own written or oral traditions that could be associated with individual clans or groups (Gelahun, 1976; Abbink, 1995).

Ethiopia’s more than 6500 higher order flora is diverse and makes the country one of the six plant biodiversity rich regions (Edwards, 2001). Some of these plants have been traditionally used to preserve raw and fermented milk for example Lippia javanica, Olkingiri and Olea europaea used by the Maasai community in Kajiado district to process and preserve milk were collected from the field (onyango *et al*., 2014). There is considerable potential for utilization of natural antimicrobials in food, especially in fresh milk (Burt, 2004; Davidson, 2006; Gaysinsky and Weiss, 2007). Timely cooling ensures that the quality of the milk remains good for processing and consumption (O’Connor, 1994). However, in rural places where there is no refrigerator facility people use traditional approaches to maintain the quality and safety of milk. In Tigray use of herbal plants as milk preservation or as milk container fumigation is a common practice for long period of time. However, there was no documented study on the role of these herbal plants and all herbal plants are considered as equally effective in improving the quality and microbial safety of milk. So the objective of this paper is to identify the effect of herbal plants on quality and microbial safety of milk in Eastern zone of Tigray.

2. Materials and methods

2.1. Study Area

The study was conducted in eastern zone of Tigray, Northern Ethiopia. The zone covers about 6050 km^2 with a total population of 830,503. With 13,977 ha of irrigated area largely used for vegetable and fruit production and with 414,408 cattle population. The elevation in the zone ranges from 946 to 3,298 masl. Annual rainfall is variable within 420-689mm. Temperature ranges from 8 to 26°C (BoARD, 2014). The zone consists of seven districts i.e. two districts were selected for the study these are Kilte-Awlaelo and Ganta-Afeshum, based on the

history on use of herbal plants as fumigators of milk containers (Fig. 1).

2.2. Collection and identification of herbal plants

Five herbal plants frequently used by smallholder dairy farmers as milk container fumigators to maintain the quality of milk were identified through discussion with knowledgeable farmers. These plants were *Olea europaea L,Vernonia amygdalina, Solanum schimperianum hochst, Acacia etbaica* schweinf*, Aoe elegans.* The collection of herbal plants from the study areas was according to traditional practices. For all herbal plants the stem or branch parts are used for smoking except Olea europaea L where the stem and leaf parts are used together for fumigation of milk containers.

2.3. Milk sampling

The milk sample was taken from five dairy farms that own more than three lactating cows of apparently healthy udder and free of any clinical mastitis. All farms did not fumigate their milk containers, and this was done to ensure the impact of the study plants. The milk samples were poured in to 6 calabash and 6 plastic containers with 200 ml milk. Milk with non-fumigated plastic and calabash containers was used as control groups and five plastic and five calabash containers fumigated with each of the herbal plants were used as the experimental groups. Each herbal plant was tested in five batches of milk samples, and this served as a replication. The test was run at 24 hours interval two times.

2.4. Milk Quality Parameters

Organoleptic tests (odor and taste) were done by three experienced taste panelists who scored the odor results excellent, very good, good, bad and normal. The three taste and odor panelists were test for the odor and taste of the treated and untreated milks for five batches (6 treatment x 5 batches =30 times) of each person's. Physical attributes (temperature and pH) were carried using pH meter (PH-870Model).

2.5. Fumigation method

A smoke free fire was lit using charcoal to which air dried herbal plant was added with sufficient amount to produce enough smoke and burned by covering the top part with aluminum foil, which had a small opening that served as a smoke outlet. The dried milk containers were held upside down for 3-5 minutes, and 200 ml milk poured and tightly clothed (Fig. 2).

2.6. Bacterial Load Determination

Standard plate count (SPC) and coliform count methods were used for determining the number of total viable bacteria and coliforms in the control and treatment milk samples. Serial tenfold dilution up to 10^6dilutions was prepared for each sample using 0.85% sterile saline solution. Pour on plate method was used to make viable count. After incubation of 24 and 48 hours, plates from the different dilutions having bacterial colonies ranging from 30–300 were counted using the illuminated colony counter. The counts for each plate were expressed as colony forming unit of the suspension (cfu/mL) (Quinn *et al.,* 2002).

2.7. Data analysis

Bacterial load counts were subject to analysis of variance using a repeated measures model. Container type and herbal plant used to fumigate the container were considered as the main treatments and the time between the first and second measurement taken at 24 hours interval was the repeated measure. Temperature and pH measures were considered as covariates. Analysis was carried out using SPSS Statistical software version 17.0 (SPSS, 2008).

3. Results

Milk samples from fumigated plastic containers showed statistically significant variation for both standard plate count and coliform counts (P = 0.000). The lowest bacterial count was obtained from milk samples fumigated by *V. amygdalina* for both standard plate count ($7.8x10^5$) and coliform count ($4.5x10^5$). The highest bacterial count was obtained from the untreated milk. Temperature and pH has not statistically significant variation. (Table 1). Similarly, milk samples from fumigated *calabash* containers had statistically significant variation for both standard plate count and coliform counts (P = 0.000). The lowest bacterial count was obtained from milk samples fumigated by *V. amygdalina* for both standard plate count ($8.4x10^5$) and coliform count ($4.7x10^5$). The highest bacterial count was measured in the untreated milk samples. Considering temperature and pH doesn't have statistically significant (Table 2). At day 0, temperature, pH, SPC and coliform counts were 25.4^{o}C, 5.3, $20.4x10^5$/cfu/ml and 15.6 $x10^5$/cfu/ml, respectively. There is no significant variation of bacterial load in Standard plate and coliform count in day one and day two. Temperature and pH showed statistically significant association with the type of container (Table 3). Milks samples stored in calabash containers had excellent taste

and odor in day one and day two compared to milk samples stored in plastic containers from accordingly to the respond of the taste and odor panelists 90% of the organoleptic result was shown excellent for the odor and taste for the milk in calabash (Table 4).

The highest mean difference was measured in milk samples treated by *Vernonia amygdalina* and the lowest was measured in milk samples treated with *Solanum schimperianum* hochst (Table 5). According to the mean difference, ranking of the herbal plants on their ability to decrease the bacterial count in milk was performed and the plants are listed from first to the least as: *Vernonia amygdalina*, *Olea europaea* L, *Acacia etbaica* schweinf, *Aoe elegans*, *Solanum schimperianum* hochst.

5. Discussion

Milk samples from fumigated and non-fumigated on plastic and calabash containers showed statistically significant variation for both standard plate count and coliform counts ($p < 0.05$). This indicates that there is a difference on bacterial load because of the fumigation. The lowest bacterial count was obtained from milk samples fumigated by *Vernonia amygdalina* for both standard plate count and coliform count. The highest bacterial count was recorded from the sample taken from non-fumigated. This result has similarity from a research result which is previously done by (Mogessie, 2002) revealed that the total count of bacteria in milk in non-smocked container is higher than the smocked the coliform bacteria too. Considering temperature and pH as factors for the treatments, no statistically significant variation was observed between the different treatment groups for both milk samples stored in plastic and calabash containers. So PH and temperature will not be influenced due to fumigation and type of herbal plant. Regarding the PH the previous research result by (Onyango, 2014) was opposite revealed by there was significant difference in pH in the five fermented milk samples stored with different treatments for 14days. This is due to the type of herbal plant of the previous research is different than the present study

Comparisons of bacterial counts between the treatments were conducted in different storage of days. Mean comparison of day one and day two of standard plate count and coliform count from milk sample stored in plastic and calabash containers didn't show statistically significant association. This result has a difference with a research result revealed by (Onyango, 2014) says total plate counts increased constantly in all the milk samples up to the 14th day. However, temperature showed a statistically significant relation with storage times in plastic and calabash ($P<0.05$) stored milk samples. So a milk which is treated with herbal plant stored for one day or for two day doesn't have visible and meaningful change on bacterial load of the milk. In addition, there is no difference on improving the quality and microbial safety of the milk due to container difference. This is revealed by the mean comparisons of bacterial counts were made in standard plate count and coliform count considering the storage containers. However, the association in both cases was insignificant. Were as Temperature ($P < 0.05$) and pH ($P<0.05$) showed statistically significant association with the type of container. In calabash due to its nature which can resist by being influenced by the external air condition the milk in calabash was slightly with low temperature and high PH. Milks samples stored in calabash containers had excellent taste and odor in day one and day two in compared to milk samples stored in plastic containers. But all plants had very nice aroma with their exclusive natural taste. In relation to the days, at day one the smell and taste was very strong than day two. This is because the smoke in calabash can strongly intermingle with the calabash itself so this can help on maintain the aroma for a long time in comparative to plastic in which can't hold the smoke for a long period.

The comparative mean analysis of the fumigated and non-fumigated group were revealed strong statistically significant difference in their bacterial load count. The highest mean difference was encountered from milk samples treated by *Vernonia amygdalina* and the lowest mean difference was seen in milk samples treated *Solanum schimperianum* hochst. Accordingly ranking of the herbal plants on their ability to decrease the bacterial count in milk was performed so from first to the least were *Vernonia amygdalina*, *Olea europaea* L, *Acacia etbaica* schweinf, *Aoe elegans*, *Solanum schimperianum* hochst. This is due to the nature of *Vernoni*a that a previous research result indicates that, *Vernonia amygdalina* is used as an antibacterial, laxative and acts as an antioxidant (Georgewill, *et al*., 2009).

6. Conclusion

Vernonia amygdalina was identified as the best in improving the quality and microbial safety of milk. Herbal plants used for fumigation of plastic and calabash milk containers did not differ in improving the milk quality as measured by bacterial loads. Milk stored in calabash had an excellent taste and odor compared to milk stored plastic containers.

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8. Conflict of Interest Statement

The Author Haftom Yemane, Author Habtamu Taddele, Author Yayneshet Tesfay declare that they have no conflict of interest.

9. Statement of Animal Rights

The study animals were treated and physically examined on the way the welfare of the animals couldn't be damaged.

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Table 1. Average bacterial load count in treated and untreated milk samples stored in plastic containers

Treatment groups	Standard Plate Count		Coliform Count	
	Mean ± SD	*P Value*	Mean ± SD	*P Value*
Control	$16.4x10^5$±4.95	0.0000	$13.2x10^5$±9.19	0.0000
O. europaea L	$9.1x10^5$±5.65		$7.3x10^5$±7.07	
V. amygdalina	$7.8x10^5$±7.07		$4.5x10^5$±7.78	
S. schimperianum hochst	$11.4x10^5$±1.41		$9.8x10^5$±2.12	
A. etbaica schweinf	$10.2x10^5$±11.31		$9.2x10^5$±4.24	
A. elegans	$10.6x10^5$±7.78		$10x10^5$±4.42	

Table 2. Average bacterial load count in treated and untreated milk samples stored in calabash container

Treatment groups	Mean comparison with Standard Plate Count		Mean comparison with Coliform Count	
	Mean ± SD	*P Value*	Mean ± SD	*P Value*
Control	$16.8x10^5$±2.83	0.0000	$12.7x10^5$±0.78	0.0000
O. europaea L	$9.4x10^5$±5.66		$7.3x10^5$±9.19	
V. amygdalina	$8.4x10^5$±3.54		$4.7x10^5$±7.07	
S. schimperianum hochst	$11.9x10^5$±7.07		$9.9x10^5$±9.19	
A. etbaica schweinf	$10.8x10^5$±2.83		$10.4x10^5$±2.82	
A. elegans	$11.3x10^5$±5.66		$10x10^5$±5.66	

Table 3. Mean comparison of milk samples in calabash and plastic containers

Container type	Mean comparison on Standard Plate Count		Mean comparison on Coliform Count		Mean comparison in T°		Mean comparison in pH	
	Mean ± SD	*P Value*	Mean ± SD	*P Value*	Mean ± SD	*P Value*	Mean ± SD	*P Value*
Calabash	$11.4x10^5$±28.21		$9.1x10^5$±26.86		21.11±0.48		2.99±0.20	
Plastic	$10.9x10^5$±28.57	0.6551	$9x10^5$±28.15	0.8834	21.10±0.60	0.0147	2.78±0.21	0.0167
Total	$11.1x10^5$±27.90		$9.1x10^5$±26.92		21.41±0.61		2.89±0.21	

Table 4. Organoleptic test

Container type	Days		Organoleptic	
	Day one	Day two		
Control group (Non fumigated)	x	x	Taste	Odor
Calabash group (Fumigated)	xxxx	xxx	Taste	Odor
Plastic group (Fumigated)	xxx	xx	Taste	Odor

N.B. xxxx- excellent, xxx- very good, xx-good, x- normal milk odor and taste

Table 5. Mean difference comparison of control group with the different treatments

Untreated	Treated	Mean difference	P value	S.E
Control	*Olea europaea L*	65.50	0.001	6.279
	Vernonia amygdalina	86.15	0.000	
	Solanum schimperianum hochst	41.85	0.008	
	Acacia etbaica schweinf	50.65	0.003	
	Aoe elegans	44.80	0.006	

The mean difference is $x10^4$

Figure 1.Map of the study districts

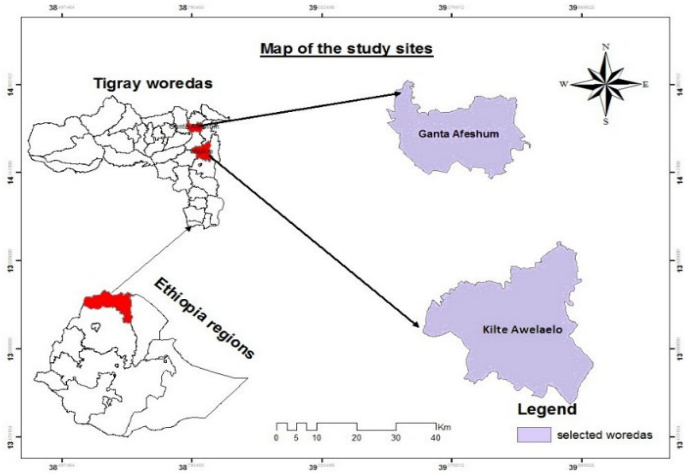

Figure 2. Fumigation of milk containers with herbal plants