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Chemical Analysis and Documentation of an Ethnic Indigenous Beverage, *Tej* and *Areki* of Ethiopia

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ABSTRACT

Alcoholic beverages have been a part of human dietary culture for a long time. In Africa, people consume fermented alcoholic beverages on different occasions, and the production of these beverages depends on the type of food, the fermentation process duration, and the microbes used for fermentation. Ethiopia is one of the countries where many traditional fermented beverages, such as *Tej, Areki, Tella, kribo, borde*, and *korfe*, are prepared and consumed. However, there is limited information available regarding these indigenous products. Most of the recipes and preparation methods are not documented or studied. To address this, a study was conducted to analyze the chemical characteristics and documentation of indigenous knowledge of *Tej* and *Araki*. The results showed that the mean pH of *Tej* was 3.63, the mean pH of Araki was 4.26, and the mean electrical conductivity value of the Tej sample was 535.1µS/cm. The average electrical conductivity of the Areki sample was 18.89µS/cm. In Tej, the mean ethanol content at 15.56°C was 5.12 % (v/v), ranging from 1% to 12%. For *Areki*, the mean ethanol content at a temperature of 15.56°C was found to be 34.9% (v/v), with a range of 14% (v/v) to 51% (v/v). *Tej* exhibited an acidic nature with a low pH, which is essential for microbial inhibition and product preservation. However, paying attention to sensory properties is important to avoid undesirable effects. As for Areki, participants reported using diverse ingredients, with maize, wheat, and Gesho being the most common. *Areki* had a lower acidity and higher pH than Tej, influencing its flavor and shelf life.

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Keywords: Tej, Areki, Physical and Chemical Properties, Ingredient, Fermentation, Indigenous Knowledge, Cultural Values

Abbreviations

AOAC: Official Method of Analysis of The Association of Official Analytical Chemist
EPHI: Ethiopian Public Health Institute
ES: Ethiopian Standard
ISO: International Standard Organization

Introduction

Globally, in nearly all countries, some alcoholic beverage native to its region is prepared and consumed. Alcoholic beverages are a part of the human dietary culture and have an inseparable relationship with the life of humanity in history. The making and drinking of alcoholic beverages enhance the nutritional significance and social relationships for human beings. In Africa, fermented alcoholic beverages are consumed on different occasions, such as marriage, naming, and rain-making ceremonies. Beverage production depends on the type of food, fermentation process duration, and application of microbes utilized for fermentation. Fermented beverages can be classified based on the sources, mainly into four categories: grain, fruit juice, vegetables, and other sources [1-4].

Ethiopia is one of the countries where a wide variety of traditional fermented beverages are prepared and consumed. Tej, Areki, Tella, Kribo, Borde, and Korfe are the most common. The fermentation method for traditional alcohols is inexpensive and adaptable at the household level in conventional communities. Fermentation of Tej, like other traditionally fermented alcoholic beverages, is spontaneous and depends on the microflora naturally present in the substrates, utensils, and equipment used. The different metabolic products of these randomized microflorae at various stages, the physical and chemical environments, and the duration of fermentation and concoction practices result in physicochemical variations in the final product [5-7].

Fermented beverages produced from cereals are usually referred to as beers; the recipes are handed down from generation to generation and are still widely used in many developing countries. Traditionally, fermented beverages are low-cost products in all aspects, as they are usually manufactured using only simple utensils. Because of their cheapness, low socioeconomic resource groups primarily consume them. Nearly half of the Ethiopian population reported drinking alcohol at some point in their life, and one in five men reported heavy episodic drinking [1,8-10].

Areki is a distilled beverage made of ground Gesho (Rhamnus prenoides) leaves, malt, and water that is kept for three to four days, and after that, a "kita" (flat bread) made of cereals such as teff, sorghum, or maize and germinated barley or wheat are added. The mixture is allowed to ferment for five to six days and then distilled. In the villages, distillation is carried out with

a traditional clay pot. The local beer Tella can also be refined to produce Areki [6,11].

However, information regarding this indigenous product is limited. Most of the recipes and the preparation methods are not documented and studied. In contrast, the product doesn't have national standards. The research aimed to investigate the chemical composition and functional properties of Tej and Areki collected in different parts of Ethiopia and recipe documentation for developing the national standard.

History, culture, and Ethnic aspects of Tej

The history of Tej in Ethiopia dates back 2000 years; evidence from the excavation site of Axum has found accounts of the consumption of honey wine and its use in rituals [12]. Tej is a home-processed but also commercially available honey wine. It is prepared from honey, water, and leaves of Gesho (Rhamnus prenoides). Perhaps Ethiopia owns about 10 million bee colonies, the largest bee population in Africa, and the ninth-largest honey producer worldwide. The total honey production is estimated at 43,000 tons [13,14].

During the preparation of Tej, the fermentation pot is seasoned by smoking over smoldering Rhamnus prenoides stems and olive wood. One part of honey mixed with 2 to 5 (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment, after which wax and top scum is removed. Some must be boiled with washed Rhamnus prenoides and returned to the fermenting must. The pot is covered and fermented continuously for another five days in warmer weather or 15-20 days in colder cases. The mixture is stirred daily and filtered through cloth to remove sediment and Rhamnus prenoides. Good quality Tej is yellow, sweet, effervescent, and cloudy. Tej's flavor depends on the part of the country where the bees have collected the nectar and the climate [15].

History, culture, and Ethnic aspects of Areki

Ancient archeological evidence in many countries confirms alcohol use in human history dating back as early as 7000 BC. Traditionally, people used fermented alcoholic beverages as medicines for illnesses such as fever and other ailments by adding barks or stems of certain plants [3,16].

Ethiopia is rich in cultural diversity, and the variety of processed foods, beverages, and consumption indicates this diversity. The various traditional fermented beverages are household art on a reasonably small scale and usually for local consumption either within the household or for local markets. The Ethiopian fermented beverages are varieties of Tella, Tej, Borde, Areki, Keribo, and Korefe, which rely on the microorganisms in the substrates, fermentation containers, or utensils. These indigenous fermented beverages are products of the acid-alcohol type of fermentation. So, with the variable microflora of such spontaneous fermentation, variability of the product is imminent [5].

Material and Methods

Study area

Samples of Tej were collected from various regions such as Adigrat, Tenben, Raya Kobo, Woreillu, Lalibela, Woreta, Gondar, Bahir Dar, Debere Markos, Ankober, Nekemte, Gimbi, Bonga, Bula Hora, Masha, Sululta and Addis Ababa. These regions are known for their consumption and production of Tej. Similarly, samples of Areki were collected from Bahir Dar, Dembecha, Merawi, Dejene, Deberetsige, Debere Birhan, Mahalmeda, Aresinegele, Assela, Balegoba and Addis Ababa. The samples were collected from households, manufacturers, shops, and retailers. Collection and processing of samples were done by the Ethiopian Standard ES ISO ES 829:2002 sampling protocols.

Study Design and Sampling Techniques

Several consultative workshops were organized with experts from various organizations such as the Ministry of Agriculture, Ministry of Trade and Industry, Ministry of Health, Food and Drug Control Authority, Ethiopian Standard Agency, Universities (Hawwasa, Bahir Dar, Gondar, Harramaya, and Addis Ababa), Producers, Consumers Protection Authority, Private companies, and NGOs at the EPHI training facility. The main objective of these workshops was to gain knowledge about the areas where Tej and Areki production is superior and to gather opinions from experts on critical Tej and Areki characterization parameters. Based on the experts' recommendations, producers, retailers, and households were identified as sample collection points from areas where potential production and consumption occur nationally.

Before conducting the interviews and collecting data, we followed a cross-sectional study design and obtained informed consent from the participants. Trained data collectors used semi-structured questionnaires, interviews, and focus group discussions to gather qualitative data on indigenous knowledge, skills, practices, ingredients used, and modes of preparation. The questionnaires were written in English and translated into the local language by a senior researcher at EPHI, who is also one of the authors of this manuscript. The informants were questioned about the ingredients used, methods of preparation, indigenous practice beliefs, and mode of preparation. During the interview and sample collection processes, regional tourism bureaus, woreda-level administrators, regional public health institutes, and local guides actively participated. 104 Tej and 87 Areki samples were collected from the selected sampling areas.

Method Validation, Sample Preparation, and Transportation

Samples of Tej and Areki were collected from 11 regions and two city administrators across 17 and 11 sampling areas, respectively. Each sample was contained in a single litter, placed in a glass bottle, labeled with a permanent marker, and stored under suitable conditions (4Co) until transportation to the Ethiopian Public Health Institute (EPHI); upon arrival at EPHI, a designated individual inspected all the samples according to the prepared protocols. Before sample analysis, analytical methods were validated, and reference material (99% Ethanol analytical grade) was prepared.
Inclusion Criteria

The inclusion criteria were knowledge, practice, skills, and preparation mode for Tej and Areki. Participants over 20 were considered to possess indigenous knowledge, experience, and skills in preparing Tej and Areki.

Chemical composition

The ethyl alcohol content of the Tej and Areki samples was determined according to the method described by AOAC official method number 942.06. The methyl alcohol content of the Tej sample was determined according to the method described by AOAC official method number 905.01. Volatile acidity, fixed acidity, and total acidity of the Tej and Areki samples were determined according to the method described by East African Standard. Esters and aldehydes of the Tej and Areki samples were determined according to the method described, method number 950.05 [17,18].

physical properties

The specific gravity of the Areki sample was determined according to the pycnometer method described by method number 945.06; the pH, conductivity, and total dissolved solid of the Areki samples were measured by dipping the electrode of a digital pH, conductivity, and total dissolved solid meter (model: BANTE 902 pH/Conductivity/TDS) into the samples after proper calibration of the meter with the standard solution. The degree of brix of the Areki sample was determined by a Handheld Refractometer (model: TC Hand Refractometer American Optical) [17].

Result and Discussion

Tej

Documentation of Recipes

Ninety-one respondents have participated in Tej recipe documentation from different sample collection sites for commercial purposes and household consumption. Most participants reported honey, Gesho (Rhamnus prenoides), and water are used to produce Tej. These results are in agreement with the written list of ingredients used to prepare Tej [7,12,15].

Functional properties and chemical composition

pH scale is logarithmic and inversely indicates the concentration of hydrogen ions in the solution (a lower pH indicates a higher concentration of hydrogen ions). The mean pH of Tej was 3.63. This result was in agreement with the reported values of previous studies. This study revealed that Tej is an acidic beverage with a low pH that inhibits the growth of microorganisms and preserves the product. However, a very low pH can also affect the sensory properties of Tej, such as its color, flavor, and aroma. Therefore, monitoring and controlling Tej production and storage pH is essential [6,19-22].

Foods that are predominantly liquid conduct electricity. Unlike metals, the charge carriers in foods are ions instead of electrons. The concentration and mobility of ions determine the electrical conductivity of food; the higher the concentration and mobility of ions, the higher the electrical conductivity. The mean electrical conductivity value of the Tej sample was 535.1µS/cm. This result was supported by Tadesse et al.'s reported values. This finding indicates a variation in conductivity throughout the sample due to unregulated fermentation and the proportion of honey to water used in production. Conductivity has an inverse relationship with product sugar content. When conductivity decreases, sugar content will increase. This probably indicates the availability of free sugar and the extent of fermentation [20,23,24].

For a given liquid, the specific gravity may be defined as the ratio of the density of the liquid to the density of water. The mean specific gravity of Tej obtained is 1.015, ranging from 0.99 to 1.18. The gravity decreases with increasing alcohol content, while increasing sugar content leads to higher specific gravity. Furthermore, the mean degree of Brix @20oc of the Tej sample was 10.17, ranging from 1.5 to 29. This result agreed with the reported values of Demewez et al. This implies that the sugar content of Tej varies across the sample due to differences in the source and quantity of honey used to produce it [25-27].

In any food product, methanol is considered an undesirable content. In humans, it can cause central nervous system depression, blindness, coma, and death. According to (table 1) the mean methanol content of Tej at 15.56oc is 0.46 %(v/v). The result agreed with Teshome et al.'s reported values. According to the East African Standard, fermented alcoholic beverages such as wine cannot exceed a methanol content of 0.1% (v/v). This study revealed that Tej exceeded the maximum limit [21,27,28].

Ethanol is a substance naturally produced through the fermentation of sugars by yeasts. It can also be made via petrochemical processes. Ethanol is commonly consumed as a recreational drug. In Tej, the mean ethanol content at 15.56°C was 5.12 % (v/v), ranging from 1% to 12%. Ethanol is the primary product in Tej's making. However, the alcoholic content of the final product varies across different samples due to the extent of the fermentation period. This finding is supported by the reported values in references [6-29].

The total acidity results from the contribution of non-volatile or fixed acids, such as malic and tartaric. Tej's mean entire acidity content was 14.89 g/L, ranging from 9.5 to 22 g/L. The result was supported by the reported values of Seferu et al. Also, fixed acidity ranges from 0.84 g/L to 0.15g/L. In contrast, the volatile acidity content of the Tej sample ranges from 1.53 g/L to 0.672g/L. The process of fermenting Tej is typically uncontrolled and spontaneous, resulting in the formation of acidity as a default. As a result, the product has a lower pH scale and is more acidic. However, this research has found that the level of acid content in Tej is lower than the threshold acidic level, which can cause tooth enamel demineralization [20,30,31].

In nature, esters are well-known contributors to the aroma of flowers and ripe fruits. Most esters are secondary or tertiary flavor compounds largely absent from the raw material but formed during fermentation and storage. The mean esters content of Tej was 0.75 g/L. This finding indicates that Tej contains higher ester levels than beer and wine, which are also common fermented alcoholic beverages. Acetaldehyde is a quantitatively minor end product of carbohydrate metabolism by lactic acid bacteria. The average aldehydes content of Tej was 0.1 g/L [28,32,33].

Table 1: *Tej* Functional Properties and Chemical Composition (N= 104)

Parameters		Mean ± SD	Medin	Range		Q1	Q3
				Min	Max		
Physical properties	pH	3.63 ± 0.21	3.63	3.26	4.15	3.5	3.76
	Conductivity µS/cm	535.1±195	510	183.7	1174	401	634
	Specific Gravity @20°c	1.01±0.02	1.007	0.993	1.18	0.999	1.022
	Degree of Brix @20oc	10.17±4.85	9.5	1.5	29	6.65	12
	Suspended Solid (%)	.06±0.005	0.04	0	0.1	0.0027	0.069
Chemical composition	Methyl alcohol @15.56oc (%v/v)	0.46±0.1	0.484	0.116	0.656	0.45	0.5
	Ethyl alcohol @15.56oc (%v/v)	5.12±2.03	5.01	0.93	12.16	3.91	6.03
	Total Acidity (g/L)	14.89±3	14.4	9.45	22	12.89	17.15
	Volatile Acidity (g/L)	9.7±2.34	9.27	6.65	15.3	7.71	11.23
	Fixed Acidity (g/L)	5.01±1.58	4.96	1.5	9.63	4.02	6.02
	Aldehydes (g/L)	0.11±0.01	0.09	0.009	0.704	0.022	0.172
	Ester (g/L)	0.75±0.44	0.63	0.14	2.43	0.42	0.95

Areki

Recipe documentation

According to the results presented in Figure 1, during Areki recipe documentation from different sample collection sites both for commercial purposes as well as household consumption, participants have reported Hop 'Gesho' (Rhamnus provides), maize, wheat, barley, sorghum, millet, and lupine were main ingredients for Areki preparation. Most respondents said maize and wheat with hop 'Gesho' were the most popular ingredients. Whereas millet and lupine with 'Gesho' was the least reported ingredient used for Areki preparation across sample collection sites. This list of ingredients agrees with the documented list of ingredients used to prepare Areki [15].

Physical Properties and Chemical Composition

pH scale is logarithmic and inversely indicates the concentration of hydrogen ions in the solution (a lower pH indicates a higher concentration of hydrogen ions). The mean pH of Areki was 4.26, with an interquartile range (Q1 and Q3) of 4.12 and 4.42. This result was supported by the reported values of Tadesse et al. and Yohannes et al. [19,20,34].

The electrical conductivity of foods, especially those mostly liquid, depends on the presence and movement of ions, which differ from the electrons that carry the charge in metals. The average electrical conductivity of the Areki sample was 18.89µS/cm with a minimum of 8.86 µS/cm and a maximum of 86.1 µS/cm. This result was consistent with the previous study by Tadesse et al. This research also showed an inverse relationship between electrical conductivity and alcohol content. In other words, the higher the alcohol content, the lower the electrical conductivity. Compared to Tej, Areki had a much lower electrical conductivity. This was in line with the finding by Chongwei et al. that electrical conductivity and alcohol concentration were negatively correlated [20,23,35].

The specific gravity of a liquid is determined by measuring the density of the liquid compared to that of water. This method called a pycnometer, involves measuring the density of an alcohol-water solution. The mean specific gravity of Areki was 0.945, ranging from 0.91 to 0.97. This finding indicates that the specific gravity of the samples varied across the board. This is due to differences in the levels of fermentation and distillation efficiency. The alcohol content level has an inverse correlation with specific gravity, meaning that as the alcohol content increases, the specific gravity decreases. The mean degree of Brix at 20oc of the Areki sample was 15.77 [25,36].

Methanol is a harmful substance that can be found in various food products. When consumed, it can cause serious harm to the human body, leading to central nervous system depression, blindness, coma, and even death. To ensure safety, the East African Community has set a regulatory standard that states the maximum level of methanol should be no more than 0.005% (v/v)(. According to the data presented in Table 2, the mean methanol content of Areki was 15.56oc 0.49% (v/v). This finding is consistent with the reported values of Teshome, which suggests that Areki products contain a high level of methanol. However, this result does not agree with the reported values of Tadesse et al. Given the potential risks associated with high methanol levels in food products, it is crucial to closely monitor and regulate the methanol content of Areki and other similar products. The findings presented in this study can serve as a basis for further research and regulatory action to ensure the safety of consumers [20,21,27,37].

Ethanol, also known as ethyl alcohol, is a clear, colorless liquid with a slightly sweet taste and a distinct odor. It is a chemical compound produced naturally by the fermentation of sugars by yeasts or artificially through the petrochemical process. Ethanol is commonly consumed as a popular recreational drug due to its psychoactive effects on the human body. In the specific case of Areki, the mean ethanol content at a temperature of 15.56°C was found to be 34.9% (v/v), with a range of 14% (v/v) to 51% (v/v). These results are consistent with those reported in other studies, which suggest that the ethanol content of Areki can vary widely [6,16,20,21].

Organic acids such as malic and tartaric in Areki contribute to acidity. The total acidity in Areki is a combination of non-volatile or fixed acids, which remain in the solution after distillation or heating, and volatile acids, which evaporate during the process. The mean total acidity content of Areki was 0.9 g/L, while the mean fixed acidity was 0.1 g/L. In contrast, the mean volatile acidity of the Areki sample was 0.67 g/L. These findings were obtained through the analysis of several samples of Areki and are supported by the values reported by Seferu et al. The acidity of Areki is an important parameter that affects its flavor, aroma, and shelf-life [20].

Esters are organic compounds formed by the reaction of an alcohol and a carboxylic acid. These compounds are commonly found in the aroma of flowers and ripe fruits, and can contribute to the flavor and fragrance of many foods and beverages. Most esters are secondary or tertiary flavor compounds, meaning they are largely absent from the raw material but formed during fermentation and storage. In the case of Areki, the mean ester content was found to be 0.26 g/L. The presence of esters in Areki is likely due to the fermentation process used to produce the beverage, which involves the conversion of sugars into alcohol and other compounds. These esters can be essential to the beverage's overall flavor and aroma profile, contributing to its unique taste and sensory characteristics [32].

During carbohydrate metabolism by lactic acid bacteria, acetaldehyde is produced in small amounts. This organic compound is a minor end product of the metabolic pathway . Areki has been found to have an average aldehyde content of 0.297, which may be attributed to the metabolic activity of lactic acid bacteria during the fermentation process [33].

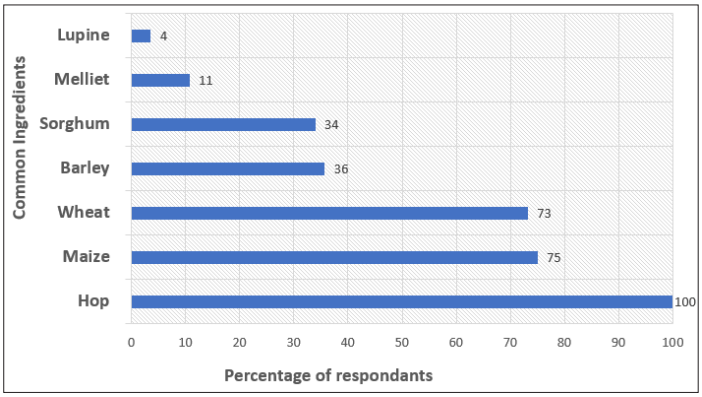

Figure 1: provides a comprehensive overview of the fundamental ingredients in the production of Areki; it is crafted by combining a variety of essential components, each contributing district characteristics to the final product. The primary grains utilized in Areki production are cereals, which include maize, wheat, barley, sorghum, and mellite. In certain regions, lupine, a dominant crop, is incorporated into the Areki production process. Furthermore, one common thread observed across various Areki-making traditions is the incorporation of hops. Hops are introduced during the Areki fermentation process to modify and enhance the beverage's taste and aroma profile.

Table 2: Functional Properties and Chemical Composition of Areki (N=87)

Parameters		Mean ± SD	Median	Range		Q1	Q3
				Min	Max		
Physical properties	pH	4.26 ± 0.23	4.26	3.67	4.98	4.12	4.42
	Conductivity µS/cm	18.89±10.8	16.15	8.86	86.1	12.75	6.36
	Specific Gravity @20°c	0.94±0.01	0.94	0.91	0.97	0.93	0.95
	Degree of Brix @20°c	15.77±2.36	16.5	7	24	14.6	17
	TDS µS/cm	9.42±5.45	8.05	4.3	43.1	6.36	10.36
Chemical composition	Methyl alcohol @15.56oc (%v/v)	0.49±0.019	0.49	0.42	0.52	0.48	0.49
	Ethyl alcohol @15.56oc (%v/v)	34.9±6.88	38.74	14.0	51.0	35.4	41.55
	Total Acidity (g/L)	0.90±0.36	0.89	0.19	1.85	0.67	1.14
	Volatile Acidity (g/L)	0.67±0.27	0.66	0.19	1.37	0.49	0.82
	Fixed Acidity (g/L)	0.11±0.09	0.08	0.03	0.68	0.06	0.12
	Aldehydes (g/L)	0.29±0.1	0.29	0.044	0.52	0.23	0.36
	Ester (g/L)	0.26±0.06	0.27	0.02	0.42	0.25	0.28

Conclusions and Recommendations

In Ethiopia, people celebrate national and religious holidays as well as personal events like weddings, birthdays, festivals, and funerals. Alcoholic beverages are often consumed during these occasions, with Tej and Areki being popular choices. However, there has been limited research on these drinks. The study examined the recipe documentation, functional properties, and chemical composition of *Tej* and *Areki*. The preparation of *Tej* involved honey, Gesho, and water, which aligns with documented ingredient lists. *Tej* exhibited an acidic nature with a low pH, which is essential for microbial inhibition and product preservation. However, it is important to pay attention to sensory properties to avoid undesirable effects. The electrical conductivity indicated variations in fermentation and sugar content, emphasizing the need for controlled production. *Tej* exceeded the recommended methanol limit, which poses potential health risks. For *Areki*, participants reported using diverse ingredients, with maize, wheat, and Gesho being the most common. *Areki* had a lower acidity and higher pH compared to *Tej*, which influenced its flavor and shelf life. The electrical conductivity showed an inverse relationship with alcohol content. Methanol levels in Areki exceeded regulatory limits, highlighting the need for stringent monitoring and regulatory measures. Ethanol, the primary product in both beverages, exhibited variability in content due to the duration of fermentation and distillation efficiency. The study suggests that these products could be used for income generation through exports to

the international market and value addition. This information could also benefit governmental and non-governmental institutions, product standardization agencies, universities, research centers, product exporters, product developers, enterprises, academicians, students, and regulatory bodies. However, the study did not address the microbial and chemical safety analysis or the shelf life of the products.

Declaration

Ethical approval

The Institutional Review Board (IRB) of the Ethiopian Public Health Institute approved the study protocol. Written informed consent was obtained from study participants.

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Author Contribution

Temesgen Awoke contributed to investigation, conceptualization, data collection tool development, training, data collection, interpretation, and manuscript drafting. Tesfaye Zeru contributed to data collection tool development, data collection, and method validation. The remaining coauthor contributed field work, data collection, method validation, and laboratory analysis. All authors read and approved the manuscript.

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Availability of Data and Materials

This study includes all data collected.

Consent for Publication

We confirm that this article and the entire study route were efficiently completed. Any academic material included in the manuscript has been appropriately cited. We certify that this work is unique and has not been previously published. Furthermore, it is not currently being considered for publication elsewhere.

Competing Interests

The authors state that they do not have any conflict of interest.

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