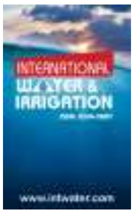

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Production and Characterization of Traditionally Distilled Alcohol (Raksi)

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Abstract Raksi is an ethnic alcoholic drink, distilled from traditional fermented cereal beverages. The objective of this work was to characterize the traditionally fermented alcohol (raksi). We processed six raksi samples at home using traditional methods, and collected four locally from hotels in various districts. During the distillation of raksi, raksi samples were physically analyzed and found to be colorless, strongly concentrated, and alcoholic in nature. While examining the collected alcohol samples, they were found to be light yellow, cloudy, with strong concentration, and unpleasantly alcoholic in nature. All the samples were acidic with a pH range of 3.6-4.5, turbidity range from 3.5-7.8, and TDS less than 14, TSS ranging from 35-850 ppm, and TA ranging from 0.016-0.234%. The alcohol percentage was found in the range 8-46%. Alcohol percentage was highest in sample LAR10, i.e., 45% using a hydrometer, 45.32% using a pycnometer, and 35.95% using HS-GC. Sample LAR4 showed a minimum alcohol % of 10% using a hydrometer, 10.11% using a pycnometer, and 8.68% using HS-GC. There was no detection of methanol and propanol from the HS-GC result; only the ethanol was detected. Almost all the distillates lied in the score panel of "good" to "very good" by using the hedonic scale. The total bacterial count was found in the range of 0-30 CFU/100 ml and 0-7 CFU/ml from the MF technique and pour plate method, respectively. Morphological and biochemical studies confirmed that the isolated organisms were Bacillus spp. We found most of the alcohol samples effective against Pseudomonas aeruginosa. The substrates used for the fermentation of alcohol and their antibacterial efficacy showed insignificant association, i.e., $p = 0.82 > 0.05$. But the association between the alcohol percentage of samples and their antibacterial effectiveness was significant, i.e., $p = 0.01 < 0.05$. These findings highlight the characterization and detection of methanol and propanol in raksi, and they enhance the further findings of any hazards.

Keywords- Raksi, Traditional, Fermentation, Characterization, Antibacterial, GCMS, CFU.

1. INTRODUCTION

Raksi is an ethnic alcoholic drink from the Himalayan regions that has a characteristic aroma and is distilled from traditional fermented cereal beverages. They ferment various substrates such as maize, rice, wheat, and millet to produce a variety of undistilled and distilled alcoholic drinks. These alcohols are primarily consumed by those who cannot afford expensive bottled beverages [1].

The most popular alcoholic drink in the Mongolian community, raksi, is a strong Nepali drink made by distilling a mixture of fermented rice, millet, or fruits. Aeylaa is an essential drink on many different occasions and has religious and cultural significance in the Newari society. A tiny container known as an eylaa, resembling tequila, serves the high concentration of alcohol. Rakshi has several other names in different ethnic communities, such as arak (Tibetans, Bhutia, Drukpa, Sherpa), aarok (Lepcha), aaerak (Tamang), aeylaa (Newar), sijongwaa aara (Limbu), aarakha/hemma (Rai), paa (Gurung), rindho (Sunwar), dhise (Magar), etc. Quality Aeylaa will burn completely with a bluish flame. People also use it as a remedy for indigestion, cough, and colds. They also rub a little aeylaa on sore knees, the back, and the chest to relieve pain [2].

In Nepal, the term "raksi" commonly refers to an alcoholic drink. A large cylindrical metallic vessel measuring 40 cm x 30 cm x 25 cm continuously distills bhaati jaanr, poko, makai ko jaanr, kodo ko jaanr, and gahoon ko jaanr, fermented masses of buckwheat, potato, canna, and cassava roots, over firewood in an earthen oven for 2-3 hours. Bottles capped with a piece of dry corncob typically store raksi. Sometimes, during distillation, people mix petals of Rhododendron spp. to give raksi a distinct aroma. The rhododendron-growing region of the Himalayas commonly prepares this type of raksi. The alcohol content of raksi is 22-27% (v/v). The consumption of traditional fermented alcohol, known as raksi, has been steadily increasing among young men in Nepal in recent times. We can assume that microbial contamination of this type of beverage contributes to outbreaks of foodborne diseases and other diseases such as gastroenteritis, enteric fever, and sore throats. The water and ingredients of traditionally fermented alcohol are the other sources of contamination.[3].

The volatile compounds like ethanol, acetaldehyde, methanol, and acetone are present in the traditionally fermented alcohol whose detection and quantification can be used as biomarkers of several diseases and/or intoxication [4]. Ethanol and methanol have similar physicochemical properties and are present as by-products after distillation [5]. The determination of methanol in traditional fermented alcohol has been a recurrent subject of research because the small amount present makes it very difficult to detect. Thirty milliliters of methanol can be lethal, while as little as ten milliliters can result in irreversible blindness. Alcohol dehydrogenase breaks down methanol to produce formaldehyde, the metabolite responsible for methanol's toxicity. The observed lethal dosage of methanol for humans through oral ingestion ranges from 15 to 250 g [6]. This work focuses on quantifying the concentrations of methanol in traditionally fermented alcohol available in Kathmandu and laboratory-processed alcohol by using gas chromatography mass spectrometry as an analytical method.

In the context of Nepal, consumers are unaware of the harmful effects of alcohol content and are not conscious of its quality. Reports in the past years have documented numerous incidents, including the deaths of individuals consuming local alcohol. However, the production in these local areas has not ceased, and a significant number of people continue to consume these products. The available alcohols (raksi) may have a high risk of consumption due to contamination of different unwanted substrates as well as the organisms. We are undertaking this study to analyze the physio-chemical parameters and their contents in an attempt to reduce the risk of alcohol consumption.

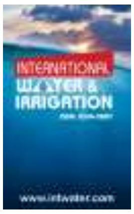
The quality of alcohol is designed for the consumers so that their lives are kept away from threatening. To maintain the quality, the hygiene of personnel and a neat and clean environment are necessary. Besides these, the production, processing, storage, and distribution are drawn as per the public health conscious. Every day, the poor developing countries of Africa and Asia conduct surveillance studies and monitor disease outbreaks.

2. LITERATURE REVIEW

2.1 Historical background

In ancient times, the traditionally fermented alcoholic beverages were made from materials such as maize, millet, rice, and sorghum, and the majority of the fermented alcoholic beverages (binuburan, amba beer, sake, dolo, pito, and tchoukoutou) were produced using spontaneous fermentation and industrial fermentation (use of starter cultures) techniques in South Africa [7].

Oriental countries like Japan used koji, or human saliva, as a source of starch and a saccharifying agent to make alcoholic beverages for celebrations and religious festivals. Some native tribes in the Pan Pacific area made other alcoholic beverages by chewing during the mashing process, using human saliva as the saccharifying agent [8].



Ancient scriptures like "VEDAS," "KIRAT MUNDHUM," and "BIBLE" describe the use of alcohol in various ritual practices such as feasts, festivals, ceremonies, parties, and the worship of gods and goddesses[9]. In some communities, quacks and faith healers continue to offer alcoholic beverages, a remnant of Nepal's ancient traditional culture [10].

People from poorer societies in Asia and Africa consume traditionally fermented alcohol as a cheaper beverage. There was no monitoring of this type of beverage's production, licensing, or sale. Around the world, traditionally fermented alcohols exhibit varying alcohol concentrations, ranging from 22-45% in India, 6-11% in Uganda, and 2-3% in Ghana [11].

2.2 Traditional fermented beverages

Traditional fermented beverages are prepared from cereals such as bhaati jaanr, makai ko jaanr, kodo ko jaanr, gahoon ko jaanr, etc. [12]. Alcoholic beverages are especially beers, wines, and distilled spirits, which are derived from sugars and starches [13].

Microorganisms of various groups appear to be involved in the fermentation of beverages indigenous to different parts of the world. The sources of the microorganisms are usually the ingredients and the traditional utensils used for fermentation processes [14]. Initially, a wide variety of microorganisms were involved, but most gave way to more adaptive genera as the fermentation went on. It may, thus, be said that the initiation of fermentation of most traditional fermented beverages may be undertaken by different groups of microorganisms as far as sufficient fermentable sugars are available in the substrate [15]. As the fermentation proceeds and the environment becomes more and more acidic, yeasts and lactic acid bacteria dominate the fermentation. These two groups of microorganisms usually determine the alcohol content and flavor of the final products [16].

2.3 Methods of preparation of traditionally fermented alcohol (raksi)

2.3.1 Traditional method of preparation of raksi

The local distilled alcohol (raksi) typically relies on the preparation of jaand by various communities. The traditionally made apparatus known as phunga, paini, phasi, and baaata distills the slurry of jaand. The iron tripod known as odhan houses this apparatus. The Phunga, a small metallic collector for distillated alcohol, is located inside the Paini. The Baata, a metallic vessel filled with water, serves as a condenser for the vapor that emerges from the phasie, which contains the slurry of jaand. The 2-4 kg of jaand can distill about 1-2 liters of raksi after replacing the condensing water three times in these traditional distillation apparatus [17].

The preparation of chyang or jaand, which involves the use of barley and millet, can vary from one region to another in the process of making raksi [18]. The strength of the raksi depends upon the duration of fermentation of the substrate; i.e., the higher the extension of fermentation, the higher the alcohol content. The Himalayan region of India, Nepal, Bhutan, and Tibet traditionally distills the jaand/chyang after fermentation [19].

2.3.2 Modern method of preparation of raksi

The distilled alcohol (raksi) is distilled from the distillation plant in which the brewers can ferment high gravity by the addition of high-gravity sugar levels in mashes of fermented substrate. Several methods have been developed to yield higher ethanol concentration by volume that, to a great extent, depend on the preparation of mashes with low viscosity [20].

2.4 Types of raksi found in Nepal

2.4.1 Types of raksi based on replacement of water (condenser)

The raksi has been classified according to the replacement of condensing water. If the condensing water is replaced about three times while preparing the raksi, then the raksi is known as teen pani raksi. Similarly, when we replace the condensing water approximately five times during raksi preparation, we refer to this type of raksi as panch pani raksi.

The teen pani raksi has a higher amount of alcohol content than the panch pani raksi, and traditionally prepared teen pani raksi is usually used for religious purposes [17].

The first lot of distilled alcohol that is obtained from the condensation of cooked fermented substrates is known as ek pani raksi. This type of raksi has higher alcohol strength than other distilled alcohol, and it is used by the choice of people drinking strong alcohol rather than for commercial purposes [21].

2.4.2 Types of raksi based on the substrates used

The raksi is a common word in Nepali meaning alcoholic drink based upon the type of substrate used in jaand making process. These types of jaand include bhaati jaand, poko, makai ko jaand, kodo ko jaand, gahoon ko jaand, and so on. If the jaand is made of millet and then distilled, this type of alcohol is called kodo ko raksi. Similarly, if the jand is made of barley, the alcohol is gahoon ko raksi, and if made using corn, then the alcohol is makai ko raksi, and so on [22].

When the petals of Rhododendron spp. are mixed during the distillation process, these types of raksi emit a distinct aroma. The Himalayan rhododendron-growing regions commonly prepare this type of raksi, known as guras ko raksi [2].

2.5 Microbiology and biochemistry of raksi

Bacteria like *Zymomonas mobilis*, *Aerobacter*, *Bacillus*, *Klebsiella*, *Thermoanerobacter*, and *Aeromonas* are used to ferment ethanol. Yeasts and fungi such as *Saccharomyces cerevisiae*, *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* are equally important. *cherichia coli* and *Bacillus subtilis* have the capacity to produce high amounts of derivatives of fatty acids, isoprenoids, and bio-alcohol. *Thermococcus*, *Thermotoga*, *Caldicellulosiruptor*, and *Pyrococcus* species have the ability to produce ethanol but produce a high amount of hydrogen [23].

In the fermentation of ethanol, mainly two organisms are involved: *Saccharomyces cerevisiae* and *Zymomonas mobilis*. *Saccharomyces cerevisiae*, a species of yeast, facilitates brewer's fermentation, while *Z. mobilis*, an anaerobic, gram-negative bacterium, converts glucose into ethanol. The primary metabolic pathway for ethanol fermentation is glycolysis, also known as the Embden–Meyerhof–Parnas or EMP pathway, where *S. cerevisiae's* metabolic activity metabolizes one glucose molecule and produces two pyruvate molecules. The Enter-Doudoroff (ED) pathway is used by *Z. mobiliz* in the fermentation of ethanol along with the enzymes pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). The EMP pathway of *S. cerevisiae* involves the cleavage of fructose-1, 6-bisphosphate by fructose bisphosphate aldolase, resulting in the production of one molecule each of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Under anaerobic conditions, the release of CO2 further reduces the pyruvate to ethanol. *Z. mobilis* produces less biomass than *S. cerevisiae*, but it feeds more carbon to the ethanol fermentation, resulting in a 3-5% higher ethanol production by *Z. mobilis* compared to *S. cerevisiae* [24].

2.6 Contamination and spoilage of raksi

Traditional alcoholic beverages often contain impurities and contaminants that may pose severe health risks, including carcinogens [25]. Contaminated grains or fruits, unsuitable for commercial nutritional purposes, heighten the likelihood of using rejected grain for alcoholic beverage production [26]. There have been reports of mycotoxins, especially zearalenone and aflatoxin, in traditional alcoholic beverages [27]. Mycotoxins are toxic secondary metabolites produced by filamentous fungi in a wide range of agricultural commodities worldwide, including cereals, nuts, legumes, spices, fruits, and their products [28]. Due to competition, brewers may incorporate various ingredients, such as bark from herbal plants, to attract customers [29].

Traditional fermentation of the murcha starter culture can produce ethanol. The murcha contains the mixed culture of about 10^6 CFU/g of molds, 10^8 cfu/g of yeast, and 10^7 CFU/g of lactic acid bacteria. The moulds present in murcha are *Mucor circinelloids*, *Rhizopus chenesis* and *R. stolonifer*. These organisms cause undesirable changes in the fermented product [30].

According to reports, *Saccharomyces cerevisiae* plays a significant role in the traditional fermentation of ethanol. When wild yeast (*Pichia silvicola* and *P. anomala*) contaminate the yeast, it produces aromatic and flavor compounds, stimulates lactic acid bacteria, and engages in probiotic activities, among other things. If *Saccharomyces cerevisiae* stops the mycotoxin-producing fungi from working, they will make ethanol and methanol by breaking down poisonous cyanogenic glycosides and enzymes that break down tissue, like cellulose and pectinase [31].

2.7 Parameters of alcoholic beverages

In the years 1996 and 1999, researchers analyzed the parameters of black mulberry alcoholic beverages, including pH, titratable acidity, volatile acidity, TSS, specific gravity, potential alcohol, potassium, sodium, tannins, and total phenol index. They found differences in the behavior of the cultivars analyzed in terms of shelf life, firmness, titratable acidity, and anthocyanin compounds [32].

The quality of traditional beverages is determined by physical-chemical and organoleptic attributes such as consistency, color, taste, alcohol content, total soluble solids, titratable, volatile, and fixed acidity, pH, and specific gravity, which undergo changes during storage under ambient conditions [33].

2.7.1 Temperature

The change in temperature affects on the physicochemical parameters like change in pH, acidity and sugars [34].

2.7.2 pH

Local beverages in Osun State, Nigeria, exhibit a pH value ranging from 4.2 to 6.3, indicating a slightly acidic nature [35].

Researchers found that the pH range of the regional state of Ethiopia's population, nations, and local alcoholic beverages was 3.66 to 4.02 [36].

2.7.3 Turbidity

The turbidity meter measured the alcoholic beverages with an accuracy of 5% Nephelo-metric Turbidity Units (NTU). Turbidity is a measure of the cloudiness of alcohol; cloudiness in opaque liquid beverages usually results from the scattering of light by suspended particulates in the continuous phase. There is no actual value for the turbidity of alcoholic beverages; it typically relies on the difference in refractive index between the particle size, the medium, and their colloidal size distribution in the liquid phase [37].

The traditional homemade alcoholic beverages of Tanzania vary in quality depending on preparation techniques, type, and the amount of raw materials used. They are characterized by a high content of soluble solids (turbidity), lack of clarity (opaqueness), varying alcohol concentrations, and short shelf life and are sold and consumed while still in an active state of fermentation [38].

2.7.4 Alcohol percentage

The alcohol percentage of the traditionally fermented beverages has a varying ethanol concentration. The distillated alcoholic beverages had higher ethanol concentration than that of non-distillated alcoholic beverages. Distilled alcohol's ethanol concentration ranges from 3% to 40%, while non-distilled alcoholic beverages fall between 1% and 18.9% [39].

Various reports indicate varying percentages of alcohol in native Ethiopian beverages. Tella and tej have varying alcohol contents, with 3.5 to 6.65% (v/v) and 4 to 11.5% (v/v), respectively, according to a survey on the alcoholic content of various traditional (local) Ethiopian beverages. The study also states that the alcoholic content of beer is between 4.25 and 6% (v/v), and the alcoholic content of arak is between 20.56 and 30.83% (v/v). Different methods of preparation and fermentation account for the variations in alcohol content among the drinks. The Ethiopian people have determined that the local alcoholic beverages, known as tella and areki, have the highest alcohol concentration values, at 10.39% and 24.54% respectively. The local alcoholic beverage, named Areki, had the highest alcohol content among the other alcoholic drinks in Ethiopia. This local drink has been known to cause severe liver and kidney damage in the body [36].

2.7.5 GCMS

Headspace gas chromatography/mass spectrometry is the most effective method for separating and detecting the volatile compound present in alcoholic beverages [40].

Different countries use gas chromatography mass spectrometry to create component profiles of the various traditionally fermented alcohols. The profiles report the components of traditional alcoholic beverages. All types of alcohol contain volatile components. The volatile components are methanol, ethanol, acetone, acetaldehyde, etc. Methanol has emerged as the most popular adulterant in developing countries due to its affordability and easy accessibility. The qualitative and quantitative analysis of methanol in alcoholic beverages has been determined in the report [41].

We use the headspace GC-MS method to determine the concentration and analyze the volatile organic compounds. The method adheres to the universal principle of chromatography, where a carrier gas (mobile phase) transports the gaseous analyte through the column. Ethanol and ethyl hexanoate, butanoic acid, hexanoic acid, and methyl butyrate were the most abundant compounds in the headspace, while the headspace gas chromatography-mass spectrometry detected volatile compounds like aroma and flavor [42].

2.7.6 Sensory evaluation

The organoleptic panelists primarily evaluate the alcoholic products based on their color, taste, and smell, using a nine-point scale that ranges from "dislike extremely(1)" to "like extremely(9)" [43].

Randomly selected and untrained individuals typically use the hedonic scale to analyze alcoholic beverages, assigning a score ranging from 1 (one) to 10 (ten) for the attributes of aroma, aroma intensity, and taste. We evaluate the aroma in three categories and assign scores: excellent/very good (7 to 10), good (4 to 6), and fair/poor (0 to 3). The aroma intensities are as follows: weak (0 to 3), moderate (4 to 6), and strong (7 to 10). The taste is evaluated and classified in disgust (0 to 3), indifferent (4 to 6) and liked (7 to 10). We also ask the evaluator to identify the aroma of the beverages [44].

2.8 Merits and demerits of raksi

2.8.1 Merits of raksi

The traditionally prepared raksi containing available grain, fruit, and herbs acts as medicine. During pregnancy, it encourages childbirth and relieves pain, and it helps breastfeeding mothers to increase milk production [45].

The traditionally fermented alcohol (raksi) is the fermented beverage that strengthens the body and keeps our body away from the coldness. It eliminates intestinal worms, purifies the blood, and relieves urination issues [46].

The varieties of fermented alcoholic drinks are produced and consumed for various reasons, including as a component of a standard diet, to alleviate stress, and for hygienic or medicinal reasons [47].

2.8.2 Demerits of raksi

Traditional fermented alcohols, such as methanol, contain numerous life-threatening compounds. A small amount of methanol can have a highly toxic effect on the human body. It can lead to numerous complications and chronic diseases, including blindness, dizziness, and respiratory diseases, among others [48].

The use of methanol-adulterated ethanol results in severe intoxication due to the formation of formic acid. This methanol has a long half-life intoxication and results in severe acidosis [41].

Traditional fermented alcohol may contain toxic compounds such as methyl alcohol. The production of methanol can lead to the degeneration of the receptor cells in the retina, the optic disk, and the nerve, as the body absorbs the oxidized methanol. Methanol intoxication can also pose a serious problem, leading to symptoms such as headache, nausea, vomiting, and abdominal pain [49].

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3. MATERIALS AND METHODS

We conducted the study both in a homely environment and at the Microbiology Research Laboratory of St. Xavier's College. We processed a total of ten local alcohol raksi (LAR) samples during this period, comprising six home-made, traditionally fermented samples and four randomly collected samples from various hotels and communities in Nepal.

3.2 Methodology

3.2.1 Sampling method

A simple random sampling method was used. We obtained six samples by fermenting alcohol using a locally available apparatus. The remaining four samples were collected from different places in Nepal by asking the details of substrate used, incubation period, fermentation method, and distillation process. These ten samples were taken for the comparative study by comparing the physico-chemical parameters and their antimicrobial efficacy.

3.2.2 Sample collection

We collected the samples without any contamination and handled those using clean apparatus. After collection, we processed six samples using a local traditional fermentation apparatus in a clean, homely environment. After their production, the six prepared samples underwent physical analysis. Finally, we determined the physico-chemical parameters and antimicrobial properties of all the samples through analytical means.

3.2.3 Procedure for making traditionally fermented local wine (jand) and local alcohol (raksi) by using local yeast (murcha)

We took the finger millet (kodo) seed, cleaned it, and washed it properly. We cooked it for about 30-40 minutes until it became glutinous, then drained off the excess water. We spread the cooked millet on a clean mat for cooling. Add slightly warmed local yeast (murcha) onto the cooled, cooked millet and mix gently. We transferred the saccharified mass into an earthen pot or any plastic container, ensuring it was airtight. Depending on the season, we fermented it for 7 days at ambient temperature (longer days in winter). Finally, the local wine (Jand) was prepared. We physically checked the prepared jam for its color, odor, and taste. We kept the fermented starchy local wine (jand) in the vessel. We distilled it using the traditionally distilled alcohol (Raksi), distillation apparatus. We carry out the distillation process for 2-4 hours by repeatedly replacing the condenser, or water, in the upper metallic vessel. The traditionally distilled alcohol (raksi) was collected in a metallic vessel inside the earthen vessel. Finally collected traditionally distilled alcohol (raksi) was characterized.

3.2.4 Analysis of physio-chemical parameters

3.2.4.1 Temperature

The temperature was measured by using the sensitive thermometer. To record measurements, the thermometer was submerged in the sample-containing vessel for three to five minutes.

3.2.4.2 pH

Using a pH meter was the most practical and trustworthy way to measure pH. In order to calibrate the pH meter, a standard buffer solution with a known pH was used, then after the reference electrode was dipped into beaker containing sample and reading was recorded.

3.2.4.3 Turbidity

The sample was kept in the cuvette of the turbidity meter measuring 2-3ml. The reading was taken after 30 seconds and noted.

3.2.4.4 Brix percentage/ Total Dissolved Solids (TDS)

Using a refractometer or the brix technique, the total amount of sugar in the test sample was calculated. Distilled water was used to zero the refractometer, and the temperature was set to 20 degrees Celsius.

3.2.4.5 Total Suspended Solids (TSS)

The filter paper was placed in clean desiccators for an hour after being dried for one hour at 105 degrees Celsius in the oven. The filter paper was then weighed. After that, the filter paper was put in the filter paper holder and soaked in clear, distilled water. After passing 5 milliliters of the sample through filter paper, the used membrane filter paper was dried for an hour at 105 degrees Celsius in a drying oven. After that, it was placed in sanitized desiccators for an hour and weighed again.

TSS was calculated as:

$$\text{TSS (ppm)} = \frac{\text{Wt of final MF (g)} - \text{Wt of original MF(g) X1000000}}{\text{Vol. of sample taken (ml)}}$$

3.2.4.6 Titrable Acidity (TA)

Total acidity of the sample was determined by the titration method using the indicator phenolphthalein. 10 ml of sample was pipetted into a clean beaker, and 2-3 drops (2 ml) of phenolphthalein indicator were added. After that, 0.1N NaOH was used to titrate the test sample until a faint pink color emerged. The burette reading was finally recorded together with the amount of NaOH used.

Total acidity was computed as:

$$\text{Percentage total acidity} = \frac{\text{Titre x Normality of alkali x Eq. wt. of acetic acid}}{\text{Volume of sample taken x 1000x } 1/100}$$

3.2.4.7 Alcohol percentage

The following two techniques were used to calculate the sample's alcohol percentage:

i. Using alcohol meter

Initially, the test sample was transferred into a sterile beaker. After then, the sample was immersed into the alcohol meter. The percentage of alcohol in the sample was finally reported based on the reading.

ii. Using pyknometer

At first, the specific gravity bottle and stopper were cleaned using the cleansing agent. The bottle was washed thoroughly again with distilled water. The bottle was dried, keeping it horizontal to prevent condensation of moisture from the neck in the hot air oven at 120^0C for 1 hour. The bottle was taken out and allowed to cool sufficiently. If water vapor was observed, it was dried in the bottle completely. The weight of the empty, clean, dried pycnometer (specific gravity bottle) was weighed and noted down (We). The specific gravity bottle was filled with distillate (without spilling) slightly above the base of the neck. The capillary stopper was gently inverted to ensure that the capillary is filled to the top. If the alcohol overflowed quickly and completely soaked with a blotting paper, but if the capillary is not filled, the stopper was taken out and a few more drops of distillate were added in the bottle, and the insertion of the stopper until the capillary is completely filled. The entire surface of the bottle and stopper was wiped with dry filter paper. The weight of the bottle with distillate was noted down (Wd). The bottle was emptied, and the capillary was blown out to remove the content. The inner portion of the bottle was repeatedly rinsed (45 times) with 10-20 ml portions of distilled water. The bottle was filled with distilled water, and a stopper was inserted. The surface of the bottle was dried, and weight was noted down (Ww). It was calculated according to the US Standard Bureau Bulletin table.

Calculation:

$$\text{Specific gravity} = \frac{\text{wt. of given vol. of distillate at } T^0\ C}{\text{wt of sample vol. of D/W at } T^0\ C}$$

$$= \frac{Wd - We}{Ww - We}$$

Where,

We = Weight of specific gravity bottle

Wd = Weight of specific gravity bottle + Distillate

Ww = Weight of specific gravity bottle + Distillate water

3.2.4.8 Volatile compound detection

First of all, the samples, calibrators, and controls were allowed to come to room temperature before preparing the aliquots. Each sample was kept in a rocker or inverted for several times. For calibrator solutions, 1 ml of internal standard (ISTD), viz., NIST traceable ethanol standards, were pipetted in five different HS-vial labels with 25 mg/dl, 50 mg/dl, 100 mg/dl, 200 mg/dl, and 400 mg/dl serially. 0.5 ml control alcohols were delivered with corresponding concentration. All headspace vials were sealed by crimping the aluminum rings over the Teflon seals. Headspace vials were loaded in the headspace auto sampler. Finally, 1 ml of internal standard and 0.5 ml of samples were pipetted in an HS vial and sealed immediately. The result was detected on the screen.

3.2.4.9 Sensory evaluation

A group of 10 panelists was chosen for the sensory evaluation of the beverages based on a 5-point hedonic scale in terms of color, aroma, texture, taste, and general acceptability. The panelists were asked to give numbers 1 to 5 for each parameter, which indicates not good (1), fair (2), good (3), very good (4), and excellent (5) [19].

3.2.5 Microbiological analysis

3.2.5.1 Membrane filter (MF) technique

This technique worked well with samples that have few bacteria in them. Each sample was put into a sterile Millipore filter assembly in an amount of 100 ml, and the microorganisms were retained by using an aspirator or vacuum pump to force the sample through a membrane filter. After that, the membrane was put on a Nutrient Agar (HiMedia) plate and let to incubate for 24 hours at 37^0C. After incubation, the isolated colonies were counted and recorded.

3.2.5.2 Pour plating

Nutrient Agar (HiMedia) was prepared and poured over heat- sterilized glass petri dishes with 1 ml raksi sample already placed in it. The plates were rotated in 8 shaped for few times to thoroughly mix the samples and allowed to solidify. The plates were then incubated in inverted position at 37^0 C for 24 hours. After incubation, the plates were observed for bacterial growth as isolated colonies and counted as CFU/ ml [18].

3.2.6 Antimicrobial properties of traditionally fermented alcohol

The susceptibility of the test organisms to the traditionally fermented local alcohol (raksi) with the varying alcohol percentage was investigated using the well variant of the agar diffusion method. ATCC culture plates of *Escherichia coli* (ATCC 25923), *Staphylococcus aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 700603) were taken and kept at room temperature for a few minutes. The ATCC cultures were incubated in nutrient broth tubes for 1-2 hours at 37^0 C. The growth of cultures (turbidity) in the tube was compared with 0.5 McFarland. Using sterile cotton swabs, the cultures were carpet cultured (swabbed) over the whole agar surface in all directions—horizontally, vertically, and around the periphery—on sterile Mueller Hinton agar (MHA) plates. The inoculum plates were left to dry for a few minutes at room temperature with the lid closed. Then a sterile 6mm cork borer

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was taken, and four equally spaced holes were bored in the agar plate with one hole in the center of the plate. Fifty microliters (50 µL) of the samples were then introduced into each of the 4 wells while the central well was filled with an equal volume of sterile water to serve as a control. This was done for all the test organisms and the sample taken. The plates were incubated for 24 hours at 37°C in an upright position. They were then examined for zones of inhibition, which indicate the degree of susceptibility or resistance of the test organism to the antibacterial agent. Inhibition zones were measured with the aid of a ruler (mm) and noted [50].

3.3 Quality control

All the traditional and laboratory equipment were regularly monitored for their performance and immediately corrected if any deviation occurred. Reagents and media were checked for manufacture and expiry date and stored in proper conditions after use.

Data were analyse statistically using Chi-square test and z-test to find p-value by using given formula.

1) Chi-square test

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where,
χ^2 =the test statistic
O =Observed data
E =Expected data

2) Z- test

$$Z = \frac{\hat{P} - P^0}{\sqrt{\frac{P^0(1-P^0)}{N}}}$$

Where,
$\hat{P}$= Sample Proportion
P^o= assumed population proportion in the null hypothesis
N= sample size

To determine the matching P level based on the obtained z value

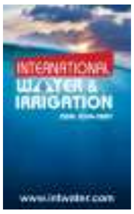
4. RESULTS AND DISCUSSION

4.1 Physical analysis determination of Local wine (jand) prepared

The local wine (jand) was prepared using the substrates barley, rice, and millet with the starter culture: local yeast (murcha). The physical analyses were determined by checking the color, odor, and flavor of the local wine (jand) in 3 different incubation periods: 10, 20, and 30 days. The temperature and pH were also recorded during the jand preparation which is mentioned in **Table 1.**

Table 1: Physical analysis determination of local wine (jand) prepared

Substrate used	Starter culture	Incubation Period	Temp	pH	Colour	Odour	Flavour
Barley	Murcha	10 days	37^0C	4.4	White	Pleasant	Sweet
		20 days	36^0C	4.4	White	Pleasant	Very Sweet
		30 days	32^0C	4.3	Brownish	Pleasant	Sweet sour
Rice	Murcha	10 days	35^0C	4.5	White	Pleasant	Very Sweet
		20 days	34^0C	4.4	White	Pleasant	Sweet



		30 days	34^{0}C	4.2	White	sour	Sweet sour
Millet	Murcha	10 days	37^{0}C	4.5	White	Pleasant	Sweet
		20 days	35^{0}C	4.2	White	Pleasant	Sweet
		30 days	34^{0}C	4.1	Reddish	Pleasant	Sweet

4.2 Physical analysis determination of distilled local alcohol (raksi)

After 30 days of substrate incubation, the distillation of six samples (coding LAR1, LAR2, LAR3, LAR4, LAR9, and LAR10) was carried out. Out of six samples, three were distilled by three replacements of water, followed by four, five, and six replacements of water. The physical analyses of these samples were done by checking their color, odor, and flavor which is shown in **Table 2**.

Table 2: Physical analysis determination of distilled local alcohol (raksi)

Sample code	Distillate substrate	Substrate incubated	Replacement of cooler	Colour	Odour	Flavour
LAR1	Millet	30 days	4	colourless	Alcoholic	Concentrated
LAR2	Barley	30 days	5	colourless	Alcoholic	Mild-concentrated
LAR3	Barley	30 days	3	colourless	Alcoholic	Strong concentrated
LAR4	Rice	30 days	6	colourless	Alcoholic	Concentrated
LAR9	Rice	30 days	3	colourless	Alcoholic	Strong concentrated
LAR10	Millet	30 days	3	colourless	Alcoholic	Strong concentrated

4.3 Physical analysis determination of samples collected

The samples were collected from different location with their detailed information where the sample coded as LAR5 made using millet of 15 days from Kathmandu with 6 times replacement of cooler was colourless, alcoholic and mild-concentrated flavour. The sample coded as LAR6 from Sankhuwa- sabha was processed by 3 times replacement of cooler (tin pani) after thirteen days of incubation of mixture of millet and dates. This sample was light yellow in colour, unpleasant alcoholic but strong concentrated flavour. The sample coded as LAR7 from Makwanpur was processed from mixed fruits by the distillation of 3 times replacement of cooler (tin pani) after incubation of twelve days. This sample was light yellow in colour, unpleasant alcoholic and concentrated flavour. Another sample coded as LAR8 from Taplejung was processed from millet only by the distillation of 3 times replacement of water after the incubation period of thirty days. This sample was colourless, alcoholic and highly concentrated which is mentioned in **Table 3**.

Table 3: Physical analysis determination of sample collected

Sample code	Distillate substrate	Substrate incubated	Replacement of cooler	Colour	Odour	Flavour
LAR5	Rice	15 days	6	Colourless	Alcoholic	Mild-concentrated
LAR6	Millet +	28 days	3	Light yellow	Unpleasant	Strong

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	dates				Alcoholic	concentrated
LAR7	Mixed fruits	31 days	3	Light yellow	Unpleasant Alcoholic	Concentrated
LAR8	Millet	29 days	3	Cloudy	Alcoholic	Strong concentrated

4.4 Physio-chemical parameters

4.4.1 Temperature, pH, turbidity, TDS, TSS and T.A of distillate local alcohol (raksi)

All the samples were acidic with pH range 3.6-4.5, turbidity range from 3.5-7.8 and TDS less than 14, TSS ranging from 35- 850 ppm and titrable acidity ranging from 0.016-0.234% which is shown in **Table 4**.

Table 4: Temperature, pH, turbidity, |TDS,TSS and T.A of distillate local alcohol (raksi)

Sample	Temperature	pH	Turbidity (NTU)	TDS (^{0}Brix)	TSS (ppm)	T. A %
LAR 1	27^{0}C	3.7	5.9	8	660	0.102
LAR2	28^{0}C	3.6	4.9	7	70	0.016
LAR3	27^{0}C	4.4	3.5	14	35	0.036
LAR4	29^{0}C	3.7	7.1	5	60	0.066
LAR5	29^{0}C	3.8	7.8	6	850	0.042
LAR6	28^{0}C	4.4	7.0	12	140	0.024
LAR7	28^{0}C	3.6	6.7	6	320	0.234
LAR8	28^{0}C	3.6	7.3	10	560	0.270
LAR9	26^{0}C	4.5	4.8	14	45	0.054
LAR10	26^{0}C	4.1	4.5	15	40	0.030

4.4.2 Alcohol percentage of distillate local alcohol (raksi)

Alcohol % was highest in sample LAR10, i.e. 45% using hydrometer, 45.32% using pycnometer and 35.95% using HS-GC. Sample LAR4 showed minimum alcohol % of 10% using hydrometer, 10.11% using pycnometer and 8.68% using HS-GC which is given in **Table 5**.

Table 5: Alcohol percentage of distillate local alcohol (raksi)

Sample	Instrument used				
	Hydrometer		Pycnometer		HS-GC
	Temperature Ethanol %	Alcohol %	Sp. Gravity		Alcohol %
LAR1	27^{0}C	28%	0.9643≈ 0.9640	27.59%	16.678%
LAR2	28^{0}C	23%	0.9712≈ 0.9710	22.19%	14.740%
LAR3	27^{0}C	40%	0.9467≈ 0.9465	39.19%	24.886%
LAR4	29^{0}C	10%	0.9868≈ 0.9860	10.11%	8.686%
LAR5	29^{0}C	14%	0.9820	13.29%	10.877%

LAR6	28⁰C	39%	0.9475	38.60%	29.099%
LAR7	28⁰C	29%	0.9638≈ 0.9635	27.96%	23.063%
LAR8	27⁰C	37%	0.9512≈0.9510	36.47%	29.856%
LAR9	26⁰C	44%	≈0.9375	44.25%	32.865%
LAR10	26⁰C	45%	≈0.9355	45.32%	35.954%

4.4.3 Determination of methanol, ethanol and propanol of different distillate local alcohol samples from HS-GC

HS-GC showed no detection of methanol and propanol only the ethanol percentage was observed which is show in **fig. 1.**

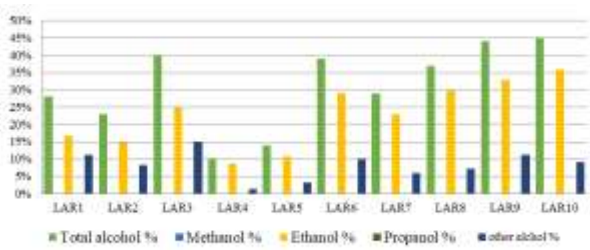

Figure 1: Determination of alcohol percentage in different alcohol sample

4.4.4 Sensory evaluation

Using Hedonic scale, almost all the distillates lied in the score panel of “good” to “very good”, i.e. with good acceptability which is given in **Table 6**.

Table 6: Sensory evaluation

Local distillate	Colour	Aroma	Texture	Taste	General Acceptability
LAR1	5	3.3	3.4	3.7	3.9
LAR2	5	3.5	3.2	3.4	3.6
LAR3	5	4.3	4.5	4.5	4.8
LAR4	5	3.1	2.5	3.1	3.3
LAR5	5	3.1	2.9	3.1	3.4
LAR6	3	3.4	4.3	3.2	3.7
LAR7	3	3.4	3.2	3.3	4.0
LAR8	4	4.1	3.6	3.6	4.4
LAR9	5	4.2	4.6	4.6	4.9
LAR10	5	4.0	4.1	4.2	4.7

Sensory evaluation of the beverages based on 5 point Hedonic scale (average of 10 panellists): 1 = Not good; 2 = Fair; 3 = Good; 4 = Very well; 5 = Excellent.

4.5 Microbiological study

4.5.1 Total bacterial count using membrane filtration technique

Microbial counts of different samples were carried out according to the standard techniques via, membrane filter technique which is given in **Table 7**.

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Table 7: Bacterial count using membrane filtration technique

Technique used	Media	Sample	Volume of sample	Dilution factor	No. of isolated colonies	Total no. of organism (CFU/100ml)
Membrane filtration technique	NA	LAR1	100 ml	1	-	-
		LAR2	100 ml	1	3	3
		LAR3	100 ml	1	-	-
		LAR4	100 ml	1	30	30
		LAR5	100 ml	1	13	13
		LAR6	100 ml	1	-	-
		LAR7	100 ml	1	2	2
		LAR8	100 ml	1	8	8
		LAR9	100 ml	1	-	-
		LAR10	100 ml	1	-	-

The results of the total count using the MF examine are given in CFU/100ml.

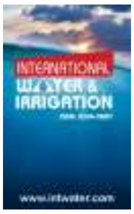
4.5.2 Total bacterial count using pour plate technique

Microbial counts of different samples were carried out according to the standard techniques via, pour plate technique which is given in **Table 8**.

Table 8: Bacterial count using pour plate technique

Technique used	Media	Sample	Volume of sample	Dilution factor	No. of isolated colonies	Total no. of organism (CFU/100ml)
Pour plate technique	NA	LAR1	1ml	1	-	-
		LAR2	1ml	1	2	2
		LAR3	1ml	1	-	-
		LAR4	1ml	1	7	7
		LAR5	1ml	1	4	4
		LAR6	1ml	1	-	-
		LAR7	1ml	1	1	1
		LAR8	1ml	1	2	2
		LAR9	1ml	1	-	-
		LAR10	1ml	1	-	-

In the pour plate technique, the total count findings are given as colony forming units (CFU/ml).



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4.5.3 Morphological/cultural characterization of isolates

Most of the isolates were round in shape, creamy in color, with mucoid consistency, opaque and convex in nature which is mentioned in **Table 9**.

Table 9: Morphological/cultural characterization of isolates

Sample	media	shape	size	colour	margin	consistency	opacity	elevation
LAR2.1	NA	Round	2mm	Whitish	entire	Soft	Opaque	Flat
LAR4.1	NA	Round	3mm	Creamy	irregular	Mucoid	Opaque	Convex
LAR4.2	NA	Round	2mm	Creamy	undulate	Mucoid	Opaque	Convex
LAR4.7	NA	Round	3mm	Creamy	irregular	Mucoid	Opaque	Convex
LAR5.1	NA	Round	3mm	Whitish	Undulate	Mucoid	Opaque	Flat
LAR5.3	NA	Round	3mm	Whitish	Irregular	Mucoid	Opaque	Flat
LAR7.1	NA	Round	1mm	Creamy	Entire	Mucoid	Opaque	Convex
LAR8.2	NA	Round	1mm	Creamy	Entire	Mucoid	Opaque	Convex

4.5.4 Biochemical characterization of isolates

All the isolates were gram positive, rod-shaped, in chains with sub terminal (few terminal) spores, catalase- and oxidase-positive, and fermentative organisms. Hence, the organism was expected to be *Bacillus spp* which is mention in **Table 10**.

Table 10: Biochemical characterization of isolates

Isolate	Gram nature	Arrangement		Catalase	Oxidase	O/F	Inference
		Shape	Spore				
LAR2.1	+ve	Rod in chain	Sub-terminal	+ve	+ve	F	*Bacillus* spp.
LAR4.1	+ve	Rod in chain	Terminal	+ve	+ve	F	*Bacillus* spp.
LAR4.2	+ve	Rod in chain	Sub-terminal	+ve	+ve	F	*Bacillus* spp.
LAR4.7	+ve	Rod in chain	Sub-terminal	+ve	+ve	F	*Bacillus* spp.
LAR5.1	+ve	Rod in chain	Sub-terminal	+ve	+ve	F	*Bacillus* spp.
LAR5.3	+ve	Rod in chain	Sub-terminal	+ve	+ve	F	*Bacillus* spp.
LAR7.1	+ve	Rod in chain	Terminal	+ve	+ve	F	*Bacillus* spp.
LAR8.2	+ve	Rod in chain	Terminal	+ve	+ve	F	*Bacillus* spp.

Note: F-fermentative, O-oxidative

4.6 Antimicrobial properties

4 out of 10 alcohol samples showed no antibacterial effect against test organisms, while 5 samples along with 75% lab alcohol showed an effect against at least 3 test bacteria. Most of the alcohol samples were found effective against *Pseudomonas aeruginosa*, while no samples but only lab alcohol were effective against *Klebsiella pneumoniae* which is show in the **fig. 2.**

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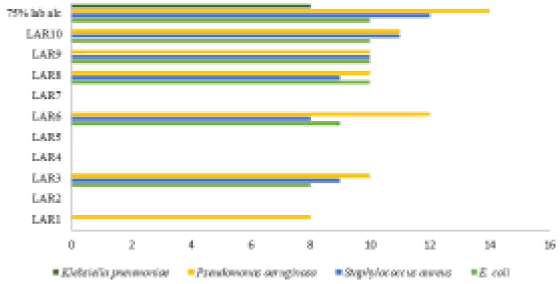


Figure 2: Diameter of zone of inhibition (mm) of alcohol samples against test organisms

4.7 Antibacterial effectiveness of the alcohol samples according to the alcohol percentage

The association between alcohol % of samples and their antibacterial effectiveness was found to be significant (p-value<0.05) which is shown in **Table 11**.

Table 11: Association between alcohol % and antibacterial effectiveness of samples

Alcohol % of raksi	No. of effective samples	No. of ineffective samples	Total	Percentage of effective samples	p-value
<25%	0	3	3	0%	
>25%	6	1	7	85.71%	0.011
Total	6	4	10		

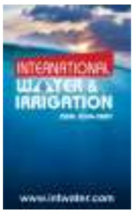
4.8 Antibacterial effectiveness of the alcohol samples according to the substrates used

The association between substrates used for the fermentation of alcohol and the antibacterial effectiveness of alcohol samples was found to be insignificant (p-value > 0.05). Sample LAR10 (substrate: millet) showed antibacterial activity against 3 of the 4 test bacteria which is mentioned in **Table 12**.

Table 12: Association between substrates used for samples and antibacterial effectiveness of samples

Substrate used	No. of effective samples	No. of ineffective samples	Total	Percentage of effective samples	p-value
Millet	2	0	2	100%	
Barley	1	1	2	50%	0.82
Rice	1	1	2	50%	
Total	4	2	6		

The purpose of this work was to investigate the production of locally distilled alcohol (raksi) and to determine its physiochemical parameters and microbial characteristics. The bacteriological and chemical properties of locally distilled alcohols were considered to be relatively hygienic. The purpose of this work was to investigate exogenous contamination of locally distilled alcohol. Six out of 10 samples were processed in local traditional fermentation apparatus in a clean environment using three different substrates. The substrates used in fermentation were barley, rice, and millet, and the starter culture was local yeast (murcha). The fermented product local wine (jand) was prepared by the traditional method of fermentation by cleaning, washing, cooking, cooling, and adding starter culture, kept for saccharification of substrate, and finally left for fermentation [51]. The physical analysis of local wine (jand) was done by checking color, odor, and



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flavor in 3 different incubation periods of 10, 20, and 30 days. The analysis revealed that the local wine (jand) was predominantly white, pleasant, and sweet, with an acidic pH within a temperature range of 32-370C. After 30 days of fermentation, the local wine (jand) was distilled to make locally distilled alchol (raksi) by using the traditional local apparatus. Three replacements of water (tin panie) led to the distillation, followed by four, five, and six replacements (chaar paanie, paach paanie, and chaa paanie). The physical analyses of locally distilled alcohol (raksi) were done by checking the color and flavor. The samples of local alcohol (raksi) were found to be colorless, strong, and concentrated in nature. The remaining 4 samples were collected randomly from hotels and communities in different places in Nepal with their detailed information. And the samples were found to be light yellow, cloudy, strong, concentrated, and unpleasantly alcoholic in nature. The MF technique and the pour plate technique were used to conduct the bacterial study.

The analyzed physiochemical parameters were temperature, pH, turbidity, total dissolved solids (^{0}Brix), total suspended solids (ppm), titrable acidity, and alcohol percentage. If these characteristics are not precise, the product may undergo unfavorable alterations, such as changes in the locally distilled alcohol (raksi).

The physiochemical parameter table yielded a temperature range of 26^0-29^0C. This aspect of the storage environment also influences the microorganisms present in the beverages. Microorganisms are capable of growing in a very broad temperature range. Therefore, it would be prudent to consider the temperature ranges at which important organisms thrive when determining the optimal temperature for storing various types of beverages. From this study, the pH values obtained from the samples were acidic in nature, ranging from 3.6 to 4.5, which was similar to the study of [52], who stated that the standard pH of alcoholic beverages lies between 3.35 and 4.5. The samples (LAR2, LAR7, and LAR8) were more acidic than other samples. LAR9 was less acidic than other samples. It is important to measure the pH of alcohol accurately since an inaccurate pH can encourage the growth of various microorganisms and make alcohol unpleasant to drink. Contamination of organisms, improper storage, or long periods of fermentation may result in an acidic pH value. Hence, pH is an important parameter for maintaining the microbiological as well as chemical qualities of beverages. It is the measurement of the active acidity that determines a product's flavor and palatability as well as the processing needs. In this study, we obtained turbidity within the range of 4-8 NTU. Sample LAR5 yielded the highest turbidity, while sample LAR3 yielded the lowest. That means the suspended particles might be present in the samples [53].

A refractometer was used to determine the beverages' total dissolved solids (Brix %). The given samples yielded total dissolved solids within the range of 5-15^0Brix. The samples (LAR4, LAR5, and LAR6) had lower total dissolved solids (^{0}Brix) due to the low sugar content of the distilled products, while LAR10 had the highest ^{0}Brix due to the higher sugar content of the distilled products [54]. Generally, we use the Brix percentage to assess the quality of locally distilled alcohol (raksi). The measurement of total dissolved solids is a crucial factor in assessing the quality of alcoholic beverages, as sugar promotes the development of various microflora [55]. The study's observed sugar percentage was almost in line with the prescribed quantity; however, variations in handling, processing, and storage conditions could account for variations in the Brix value. However, since sugar contributes to the flavor of beverages, it is crucial to ensure its precise measurement. The quantity of sugar in alcoholic beverages also contributes to maintaining the alcohol's quality [35].

Alcohol samples' total suspended solids ranged from 34 to 850 ppm. Out of 10 samples, LAR5 had the highest total suspended solids (TSS) at 850 ppm, indicating significant mishandling and contamination during the distillation, packaging, and storage processes. Similarly, LAR3 had the lowest TSS with 35 ppm, i.e., this sample was less mishandled and contaminated during distillation, packaging, and storage. Total titratable acidity changes the fermentation products and provides a measure of the acidity and lower pH [36]. This study revealed that the total titratable acid content in local alcohol sample (raksi) ranged from 0.016-0.270%. We measured the acetic acid and tartaric acid of beverages using the titrable acidity method. We measured the titratable acidity of the regional drinks, or raksi, using tartaric acid, which exists in various forms such as free acid, cream of tartar, and small amounts of calcium tartrate. Increased titratable acidity values may result from using conventional methods and from non-standardization of raw material preparation, equipment use, final product quality, and handling. There is an inverse relationship between acidity and pH, whereby higher acidity results in lower pH [35].

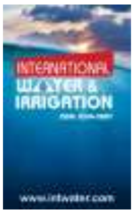
Two methods were used to determine the alcoholic percentage of the local alcohol samples (raksi): volume/weight and volume/volume. The average values of alcoholic percentages of local alcohol samples (raksi) were 30.9%, 30.497%, and 22.6704% from the hydrometer, pycnometer, and headspace gas chromatography, respectively. Although the alcohol content of local alcohol samples (raksi) ranged from 8 to 46% V/V, there was no set standard restriction on the percentage. Variations in fermentation and preparation are the cause of the drinks' varying alcohol contents. According to [36], the use of alcohol with the highest concentration should be avoided as it has the potential to damage the neurological system and enter the bloodstream.

The HS-GC result showed that none of the 10 local alcohol samples contained methanol or propanol, indicating that yeast and bacteria were fermenting alcohol and malolactic on the used substrate [56]. Sometimes, the fermentation of substrate with high pectin leads to ferment methanol [31]. The presence of higher ethanol indicates the higher rate of fermentation occurred by the substrate used [42]. The sample LAR10 showed highest ethanol percentage and the sample LAR4 had showed lowest ethanol percentage. According to [57], acetaldehyde in alcoholic beverages has a pungent, irritating odour and can pose a health risk due to its carcinogenicity. Therefore, it is important to reduce the presence of acetaldehyde in the distillates to protect consumers from potential safety risks. This study only detected ethanol in the control sample.

Sensory properties of products were evaluated in terms of color, aroma, texture, taste, and general acceptability. All samples, with the exception of three, had excellent color. From these three samples, two (LAR6 and LAR7) had a good color, and another (LAR8) had a very good color. The average results for the samples' aroma ranged from good to very good, with LAR3 scoring the highest, and LAR4 and LAR5 scoring the lowest. The average texture of all samples ranged from fair to excellent, with LAR9 having the highest excellent score and LAR4 having the lowest fair score. Similarly, the average taste of all samples ranged from good to excellent, with LAR9 having the highest excellent score and two samples (LAR4 and LAR5) having the lowest good score. Finally, in terms of general acceptability, the average acceptability was from good to excellent, in which LAR9 had the highest excellent score and LAR4 had the lowest good score.

This study showed that the samples LAR1, LAR3, LAR6, LAR9, and LAR10 had nil bacterial growth obtained from both the MF and pour plate techniques. The membrane filtration method yielded a total bacterial count ranging from 0 to 30 CFU/100 ml, while the pour plate method produced a total bacterial count ranging from 0 to 7 CFU/ml. LAR4 had the higher bacterial count: 30 CFU/100 ml and 7 CFU/ml from the membrane filtration technique and pour plate technique, respectively. In the study by [55], the numbers of total viable cells, lactic acid bacteria, and yeast in the control and sample were found to be 2.88×107 and 3.57×107, 1.50×106 and 1.84×106, and 1.90×107 and 2.17×107 CFU/ml, respectively. During fermentation, the numbers of all microorganisms in all samples increased. The test revealed that microorganisms had contaminated the samples. The morphological study revealed that the isolates were round, creamy, and entire, while some were irregular, mucoid, opaque, convex, and flat in nature. In the biochemical study, nearly all the isolates were found to be gram positive, rod-shaped, in chains with sub terminal (few terminal) spores, catalase- and oxidase-positive, and fermentative organisms. Therefore, we confirmed that the isolated bacteria were *Bacillus spp.* Under an oil immersion lens (100X) light microscope, endospores had a vivid green color after staining, while vegetative cells displayed a brownish red to pink color. *Bacillus spp.*, commonly known as food spoilage bacteria, may be present due to alcoholic contamination of water after and before local alcohol (raksi) distillation, as well as utensils and unhygienic handling.

The antimicrobial activity of local alcohol (raksi) relates to the alcoholic compound, which denatures protein and lyses the cell of the bacterial membrane. The traditional fermented beverages, Sopi and Moke, with alcohol percentages of 33% and 39%, have antimicrobial activities against *E. coli* and *Salmonella* spp. according to [58]. According to [59], the ethanol with low water content had antimicrobial activity against *Staphylococcus aureus*. In this study, the local alcoholic beverages (raksi), specifically LAR1, LAR3, LAR6, LAR8, LAR9, and LAR10, with alcohol percentages of 28%, 40%, 39%, 37%, 44%, and 45%, respectively, demonstrated a bactericidal effect on *E. coli*, *Staphylococcus aureus*,



Pseudomonas aeruginosa, and *Klebsiella pneumoniae*. The Food and Drug Administration (FDA)[60] reports that the alcohol (ethanol or isopropanol) concentration range of 60%-95% has the greatest germicidal efficacy.

5. CONCLUSIONS

This study was conducted to analyze the physico-chemical and microbiological parameters with antimicrobial tests of different local alcohol samples (raksi). Temperature, pH, turbidity, total dissolved solids (^{0}Brix), total suspended solids (ppm), titrable acidity, and alcohol % were all analyzed in terms of physio-chemical parameters. The values of all parameters were obtained as the standard recommended range of alcohol. The aim of this study was to detect the methanol and propanol from the locally distilled alcohol (raksi). But there was no detection of methanol and propanol in the samples used. The samples only contained ethanol. The sensory test, using the hedonic scale, predominantly yielded good and very good score panels. Both the membrane filtration technique and the pour plate technique yielded no bacterial isolates in the alcohol above 25%. Microorganisms contaminated the alcohol sample with the minimum alcohol percentage. The morphological and biochemical studies confirmed the presence of isolated *Bacillus spp*. in the samples. The alcohol percentage above 25% demonstrated an antimicrobial effect, primarily against *Pseudomonas aeruginosa*. Therefore, this study concluded that the locally fermented local alcohols (raksi) possess all the necessary characteristics to enhance their quality and compete with commercial alcohols in the market.

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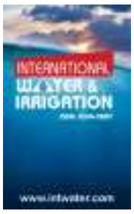

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