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BACTERIOLOGICAL CONTAMINATION OF LOCALLY MANUFACTURED WINES FROM SELECTED REGIONS OF MEGHALAYA.

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ABSTRACT:

This study aims to assess the bacteriological contamination of locally manufactured wines from selected regions of Meghalaya, namely, the Khasi Hills and the Jaintia Hills region respectively. Our research was carried out at the State Food Testing Laboratory, under the Department of Microbiology, Pasteur Hills, Shillong, Commissionerate of Food Safety, Government of Meghalaya. Around 11 wine samples were collected on random sampling from selected regions of Khasi Hills and Jaintia Hills and further analyzed for microbiological parameters as per the Food Safety and Standard Act 2006, for the presence of contaminants. It was observed that out of the 6 (six) wines samples analyzed from the Khasi Hills region, all 6 (six) wines shows absence of bacteriological contaminants indicating that the wine samples collected and analyzed from Khasi Hills regions were found to be safe and fit for consumption. However, 5 (five) wine samples received from Jaintia Hills region showed variable result. It was observed that, from the 5 (five) wine samples collected and analyzed, 2 (two) wine samples showed presence of E.coli and Staphylococcus aureus indicating that wine samples were unsafe and unfit for consumption, whereas another 3 (three) wine samples collected and analyzed from the same region i.e., Jaintia Hills region shows absence of bacteriological contaminants indicating that the wine samples collected and analyzed from these selected region of Jaintia Hills were safe and fit for consumption. Inorder to ensure authenticity and reliability of all analysis performed during the study, all testing protocols were in accordance with the Food Safety and Standards Act 2006, Regulations 2011. Also, 'Negative and Positive controls' for all samples were prepared and kept throughout all stages of analysis for maintaining the quality control of the test. From this study it was found that, from the 11 wine samples collected on random sampling from selected regions of Meghalaya, 2 (two) wine samples shows bacteriological contamination and hence render them unsafe for consumption due to the lack of an effective bottling management system.

Keywords: *Khasi Hills, Jaintia Hills, Wines, Food Safety, Pathogenic bacteria, E.coli, Staphylococcus aureus, Cross-contamination*

1. INTRODUCTION

Wine is an alcoholic beverage which has been around for thousands of years and can be found all around the world from one culture to another. Winemaking is a simple process which only involves the fermentation of natural sugars found in fruits such as grapes by microbes like yeast which converts the sugars into alcohol and carbon-dioxide. Although, the process of making wine may sound simple and easy to understand, however, it takes skill and years of experience and knowledge about the different factors that are involved in all the stages of winemaking in-order to ultimately produce a product which has flavour and one that maintains its original texture and quality during long storage periods (Bartowsky, *et al.*, 2009).

In the ancient Greek and Roman cultures, wine seems to have been the norm for a food beverage. In the Near East and Egypt, wine was almost exclusively the domain of the elite, both secular and religious. The Bible, which mentions the first vineyard ever planted by Noah after the Great Flood, is one of the many religious texts that make reference to wine (Soleas, *et al.*, 1997). Despite the fact that many different religions encourage its consumption, weddings and ceremonial occasions are the main occasions where wine is used. Only in areas where wild grapes naturally grew have vines been introduced to produce a popular beverage (Jackson, 2020). One of the primary methods of identifying wine was the calcium salt of tartaric acid, which is only present in significant amounts in grapes. An old technique that was widely used in antiquity to stop the growth of bacteria in wine was the addition of terebinth tree resin (Soleas, *et al.*, 1997).

Little was known about the causes of spoilage or the fermentation process before the 19th century. Some civilizations probably consumed almost all of their wines within a year of harvest and covered up spoilage by adding flavorings like honey, herbs, cheese, and salt water. The Romans improved oaken cooperage, while the Greeks stored wine in earthenware amphorae. Up until the 17th century, the main aging vessels were wooden barrels, but the mass production of glass bottles and the development of the cork stopper made it possible to age wines in bottles for years (Amerine, 2023).

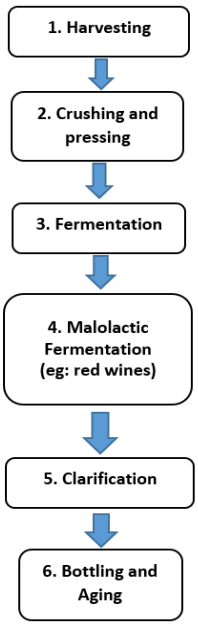

Figure 1: Steps involved in wine making

The presence of certain species of bacteria such as Lactic Acid Bacteria (LAB) (for eg; *Lactobacillus brevis*, *Lactobacillus lindneri*, *Pediococcus damnosus*, *etc)*, *Staphylocccus aureus*, *Salmonella*, *E.coli*, Acetic Acid Bacteria (AAB) (for eg; *Acetobacter cerevisiae*, *Acetobacter pasteurianus)* etc., which are commonly found in

grapes and in non-sterile conditions and equipments like fermentation tanks, barrels, bottles, corks, filters and crushers respectively, may lead to deterioration and will lower the overall quality and safety of wine. Spoilage bacteria can survive in fermented products such as wine and other alcoholic beverages for long periods of time (Jeon, *et al.*, 2015). When these bacteria are introduced through cross contamination in fermentation tanks or in bottles they begin to multiply and release certain secondary metabolites which are detrimental and cause the wine to lose its flavour and texture. The development of certain strains of Lactic Acid Bacteria in the late stages of fermentation lead to a metabolic process known as malolactic fermentation which involves the fermentation of malic acid into lactic acid and CO_2 (Henick-Kling, 1993), which when goes unchecked and if the optimum temperature is not maintained it can lead to the overproduction of volatile compound that promotes the growth of spoilage bacteria and the wine will develop an unpleasant odour. The ample availability of sugars and nutrients in wine promotes the growth of these detrimental and harmful bacteria that lead to the spoilage of wine which can however be prevented by hygienic and sterile pre and post processing practices (Bartowsky, 2009). The formation of biofilms on the wine surfaces is also yet another physical indication of microbial contamination in which wine makers should be hypervigilant about in-order to ensure that their products are safe and acceptable (Tristezza, *et al.*, 2010). The use of chemical agents and preservatives such as sulphur dioxide (SO_2) in the wine making process to control the growth and development of unwanted spoilage bacteria have been a great advantage and a solution for wine makers to produce better quality wine. However, in the present day there is a great increase in demand for more green products which are free of any chemical preservatives (du Toit, *et al.*, 2000). Hence, other alternatives for the preservation of wine are being explored.

There are various factors which should always be kept as a priority in the wine-making industry. These factors are crucial as they are the driving forces which will ultimately influence the quality of wine. Firstly, the raw materials that are required in every wine-making manufacturing industry are grapes or any fruit that will further undergo various steps and fermentation stages before being transformed into a savory and flavorful wine. High quality grapes or fruit are a direct link to the quality of wine produced and hence the growing method and the agricultural aspects should be of high quality in-order for the raw material to be able to be transformed into a top-notch product (Charters, *et al.*, 2007). The use of pesticides in vineyards and on grapes before harvesting have proven to be beneficial against pests, however, prolonged use of these chemicals in excess amount above the permitted levels poses a threat to human health (Dumitriu, *et al.*, 2021). The climate and temperature are also another important factor which directly influences the quality of raw materials used for making wine. The properties of wine such as acidity, total sugars, alcohol potential are directly influenced by the climate in which the variety of raw materials are grown. Hotter climates facilitate the proper ripening of fruits leading to a product with high quality components that are higher in sugar, alcohol potential and acidity. However, this in itself can be a disadvantage that may lead to over-ripening of fruits and retaining of excess acidity by the fruits, hence, the proper knowledge about the various climatic factors such as light, temperature, water quality and even soil quality are essential for wine makers in-order to produce high quality raw materials for a higher quality wine (Yan, *et al.*, 2002).

The aim of this study is to assess the bacteriological quality of locally manufactured wine of selected regions in the state of Meghalaya, India. Over the past 18 years, the production of home-made wine in the state was deemed illegal, but however, in September 2022, the State Government took notice of the potential of local wine makers and their products and decided to legalize the production of locally manufactured fruit wines and to provide a license to the wine makers and entrepreneurs of the state. Therefore, in-order to ensure that the locally manufactured wines are safe and adhere to all requirements as per the Food Safety and Standards Act 2006, Regulations 2011, this present study, hence, caters to the local manufacturers and concurrently sensitize them about the problems of unhygienic and improper techniques of the wine-making process which leads to the possibilities of contamination by various pathogens that degrade the overall quality of wine and can cause serious health complications when consumed.

2. MATERIAL AND METHODS

2.1. Sampling of Wines

a) Random Sampling:

For this study, the bacteriological analysis of wine was carried out by procuring wine samples from different regions of Meghalaya, namely, Khasi Hills region and Jaintia Hills region *(Figure 2)*.

b) Collection of wine samples:

Bottles of wine samples were procured from local vendors and manufacturers from various regions of Khasi Hills and Jaintia Hill in Meghalaya *(Figure 2)*. After procuring the samples from all regions, they were transferred in sterile conditions and were safely delivered and received at the Laboratory for analysis. To ensure that all test results are authentic and undisputed, all analysis performed was in accordance with the Food Safety and Standards Act, 2006.
All equipment and sterilized bottles were allocated by the State Food Testing Laboratory, Pasteur Hills, Shillong.

Figure 2: Different types of wine samples collected from different parts of Meghalaya

c) Preparation of media used for isolation of bacterial contaminants.

The preparation of all media used in this study is as per the manufacturer's instructions.

i. ***Eosine Methylene Blue (EMB) Agar***:

Total No of Sample: 11
Total No of control: 2

For 13 number of plates the total amount of EMB agar required was 14.02 gm in 1000ml of ultra-pure water.

ii. ***Xylose Lysine Deoxycholate (XLD) Agar***:

Total No of Sample: 11
Total No of control: 2

For 13 number of plates the total amount of XLD agar required was 22.20 gm in 1000ml of ultra-pure water.

iii. ***Mannitol Salt Agar (MSA)***:

Total No of Samples: 11
Total No of control: 2

For 13 number of plates the total amount of MSA required was 43.29 gm in 1000ml of ultra-pure water.

d) Bacterial isolation from wine samples

i. *Pour Plate Method*:

1 ml of the sample is pipetted out using a 1000 ml pipette with sterile plastic tips onto a sterile petri plate and is evenly distributed by gently shaking the plate so as to achieve equal and even distribution of the sample. The sterilized media was kept aside for cooling and is then poured into the plates containing the samples. The plates were then sealed properly using parafilm and are then incubated at 37℃ for 24hrs.

ii. *Streak Plate Method*:

- The media was poured into the plate and left to solidify for 10-15 minutes.
- After the media solidifies, an inoculation loop was taken and was burnt until red hot and was allowed to cool. The loop was then dipped in the sample and quadrant streaking was performed.
- The plates were then sealed properly using parafilm and are then incubated at 37℃ for 24hrs.

The methods of isolation were performed for all three media, namely, EMB Agar, XLD Agar and MSA and all methods performed in this study are in compliance with the Food Safety and Standards Act, 2006.

3. RESULTS AND DISCUSSION

3.1.1. Wine samples from Khasi Hills region

After 24hrs of incubation it was observed that almost all the wine samples collected from various regions of the Khasi Hills were found to be safe and free of any bacteriological contamination. There was also no growth observed in any of the control plates for all wine samples *(Figure 6)*. *(Table 1)*

Table 1: Parameters and results of analysis of different wines from various regions of Khasi Hills in Meghalaya

Sl. No	Type of wine	Region	Bacteriological Parameter			Observation
			Eosine Methylene Blue Agar *(E.coli)*	***Xylose Lysine Deoxycholate Agar*** *(Salmonella)*	**Mannitol Salt Agar** *(Staphylococcus aureus)*	
1.	Sohiong (Black cherry) Wine *(KH-1)*	Khasi Hills	*Absent*	*Absent*	*Absent*	*It was observed that all 6 wine samples were safe and free from bacterial contaminants*
2.	Strawberry Wine (*KH-2)*		*Absent*	*Absent*	*Absent*	
3.	*Elaeocarpus Lanceifolius* (Sohkhyllam) Wine *(KH-3)*		*Absent*	*Absent*	*Absent*	
4.	Mulberry (Sohlyngdkhur) Wine *(KH-4)*		*Absent*	*Absent*	*Absent*	
5.	Ginger (Sying) Wine *(KH-5)*		*Absent*	*Absent*	*Absent*	
6.	*Prunus Nepalensis* (Sohiong) wine (*KH-6)*		*Absent*	*Absent*	*Absent*	

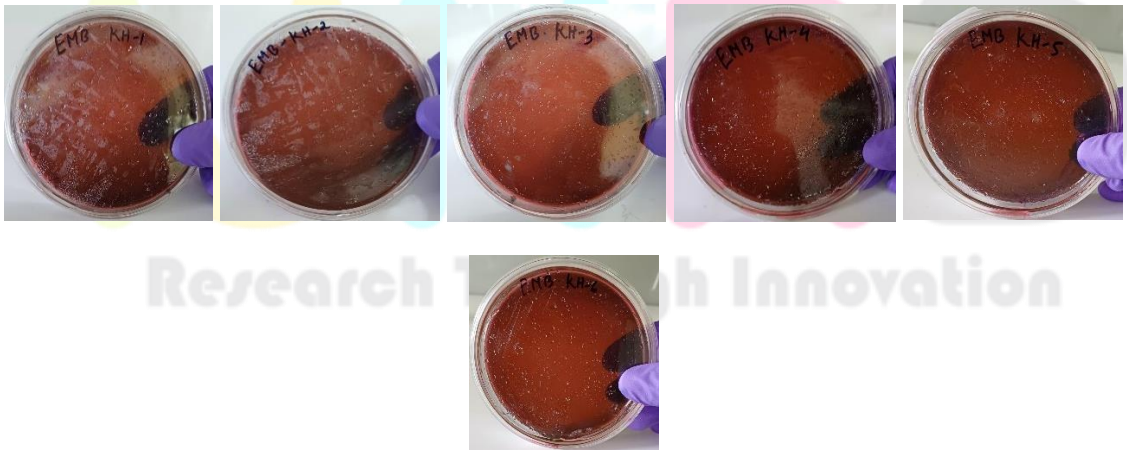

Figure 3: Eosine Methylene Blue Agar (EMB) plates showing negative results for *E.coli* in wines samples from the Khasi Hills region

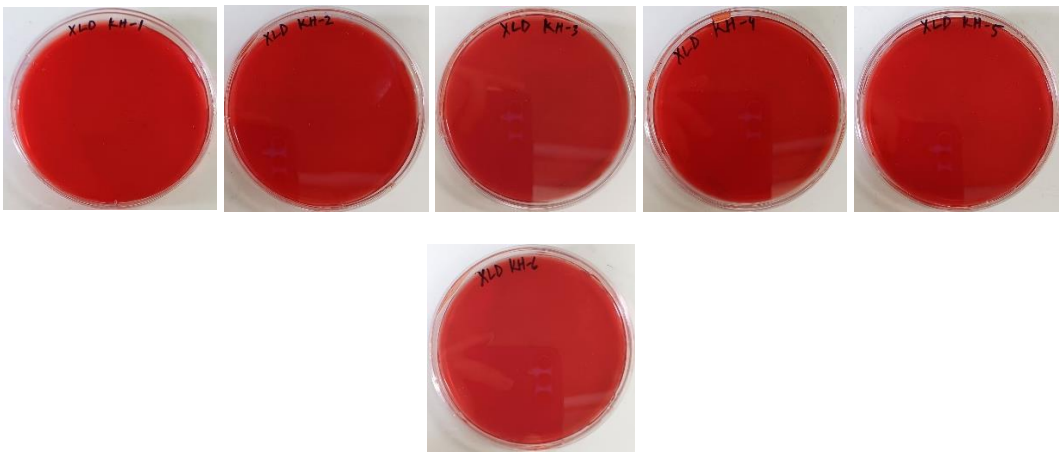

Figure 4: Xylose Lysine Deoxycholate Agar (XLD) plates showing negative results for *Salmonella* in wine samples from the Khasi Hills region

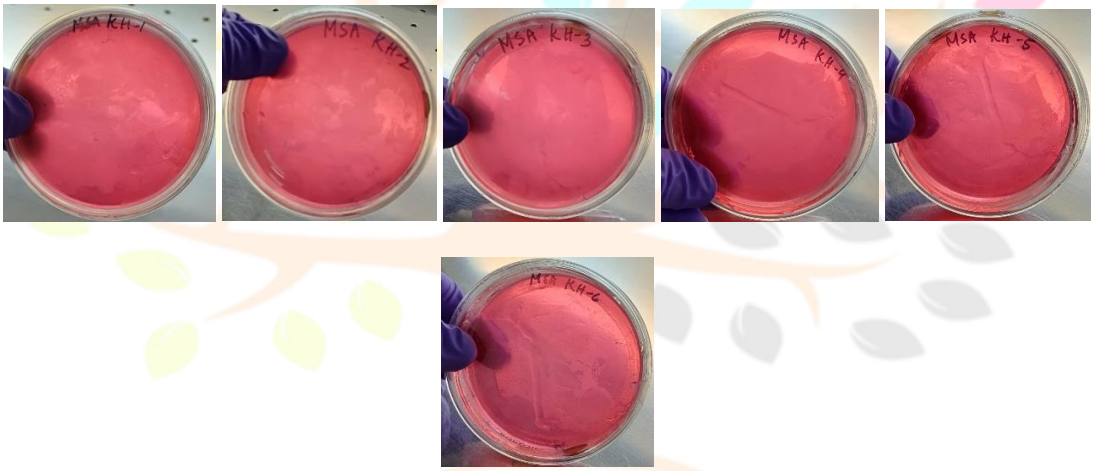

Figure 5: Mannitol Salt Agar (MSA) plates showing negative results for *Staphylococcus aureus* in wine samples from the Khasi Hills region

Figure 6: Controls of EMB, XLD and MSA showing no growth of microorganism

3.1.2. Wine samples from Jaintia Hills region

The observations and results for the wine samples collected from the various regions of Jaintia Hills in Meghalaya showed that out of 5 samples analysed 2 wine samples, i.e., Finger Millet Sidhiar (*JH-1*) and Rose Wine *(JH-3)* were found to be contaminated with *E.coli* and *Staphylococcus aureus (Table 2)*.

i. *Streak Plate Method:* The development of green metallic sheen colonies in Eosine Methylene Blue Agar confirms the presence of *E.coli (Figure 7).* The presence of *Staphylococcus aureus* colonies in Mannitol Salt Agar for the Finger Millet Sidhiar (*JH-1*) and Rose Wine (JH-3) sample was also observed by the change in colour of the media from red to yellow due to mannitol fermentation

(*Figure 10)*. No growth was observed in any of the control plates for all the wine samples *(Figure 13)*.

ii. *Pour Plate Method:* The change in colour of Mannitol Salt Agar (MSA) plates from red to yellow indicates the presence of *Staphylococcus aureus (Figure 11)*.

Table 2: Parameters and results of analysis of different wines from various regions of Jaintia Hills in Meghalaya

Sl. No	Type of wine	Region	Bacteriological Parameter			Observation
			Eosine Methylene Blue Agar (EMB) *(E.coli)*	***Xylose Lysine Deoxycholate Agar (XLD)*** *(Salmonella)*	**Mannitol Salt Agar (MSA)** *(Stapylococcus aureus)*	
1.	Finger Millet Sidhiar (*JH-1)*	Jaintia Hills	*Present*	*Absent*	*Present*	*It was observed that out of 5 wine samples that were analysed 2 wine samples showed the presence of E.coli and Staphylococcus aureus*
2.	Pineapple Wine (*JH-2)*		*Absent*	*Absent*	*Absent*	
3.	Rose Wine (*JH-3)*		*Present*	*Absent*	*Present*	
4.	*Meyna Laxiflora* (Sohmon) Wine (*JH-5)*		*Absent*	*Absent*	*Absent*	
5.	Mulberry Wine (*JH-6)*		*Absent*	*Absent*	*Absent*	

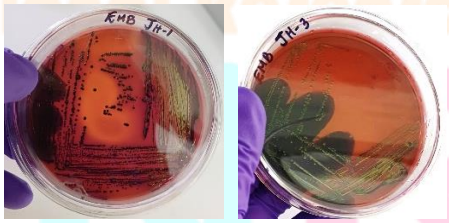

Figure 7: EMB plates showing positive results for *E.coli* (Green metallic sheen colonies) in JH-1 and JH-3 samples from Jaintia Hills region

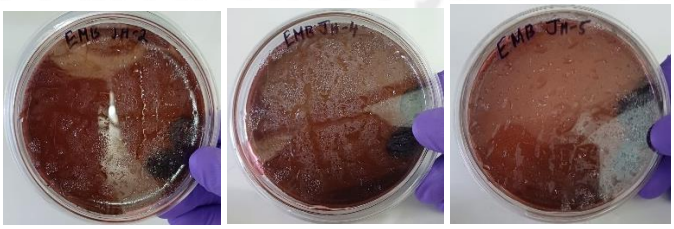

Figure 8: EMB plates showing negative results for *E.coli* in JH-2, JH-4 and JH-5 samples from Jaintia Hills region

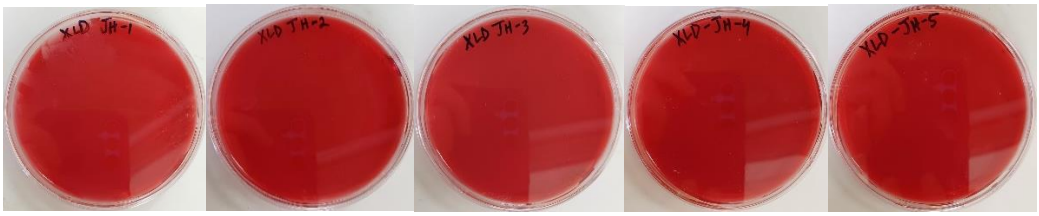

Figure 9: XLD plates showing negative results for *Salmonella* in all wine samples from the Jaintia Hills region

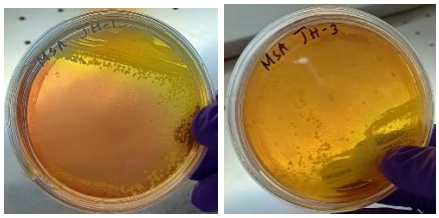

Figure 10: *Streak Plate Method:* MSA plates showing positive results for *Staphylococcus aureus* in JH-1 and JH-3 samples from Jaintia Hills region

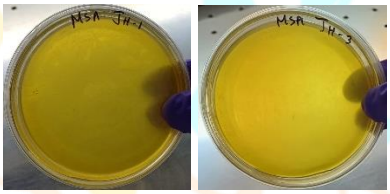

Figure 11: *Pour Plate Method:* MSA plates showing positive results for *Staphylococcus aureus* in JH1 and JH3 samples from Jaintia Hills region

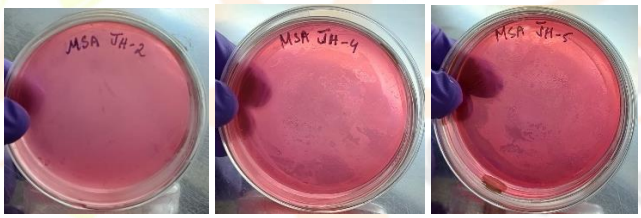

Figure 12: MSA plates showing negative results for *Staphylococcus aureus* in JH-2, JH-4 and JH-5 samples from Jaintia Hills region

Figure 13: Controls of EMB, XLD and MSA showing no growth of microorganism

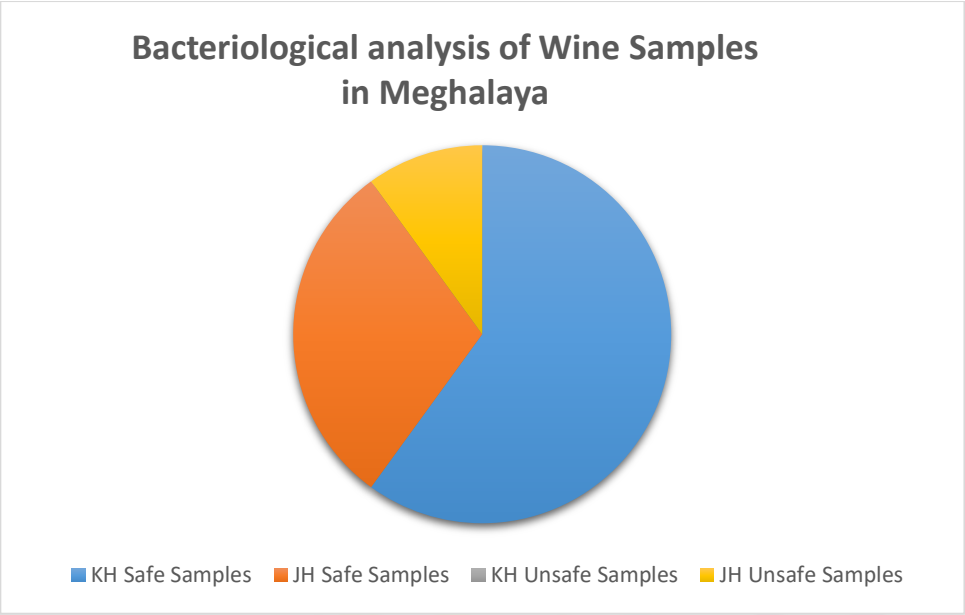

Figure 14: Chart representing the total percentage of all safe and unsafe wine samples collected from the Khasi Hills and Jaintia Hills region of Meghalaya

Table 3: A cumulative result of analysis of all the wine samples from various regions of Meghalaya

Sl. No	Type of wine	Region	Bacteriological Parameter			Observation
			Eosine Methylene Blue Agar *(E.coli)*	***Xylose Lysine Deoxycholate Agar*** *(Salmonella)*	**Mannitol Salt Agar** *(Stapylococcus aureus)*	
1.	Sohiong (Black cherry) Wine *(KH-1)*	Khasi Hills	*Absent*	*Absent*	*Absent*	*It was observed that out of 11 (eleven) wine samples, 8 (eight) wine samples were safe and free from bacteriological contamination. However, 2 (two) wine samples were found to be*
2.	Strawberry Wine (*KH-2)*		*Absent*	*Absent*	*Absent*	
3.	*Elaeocarpus Lanceifolius* (Sohkhyllam) Wine *(KH-3)*		*Absent*	*Absent*	*Absent*	
4.	Mulberry (Sohlyngdkhur) Wine *(KH-4)*		*Absent*	*Absent*	*Absent*	
5.	Ginger (Sying) Wine *(KH-5)*		*Absent*	*Absent*	*Absent*	
6.	*Prunus Nepalensis* (Sohiong) wine (*KH-6)*		*Absent*	*Absent*	*Absent*	
7.	Finger Millet Sidhiar (*JH-1)*		*Present*	*Absent*	*Present*	

8.	Pineapple Wine (*JH-2)*	Jaintia Hills	*Absent*	*Absent*	*Absent*	*contaminated with E.coli and S.aureus*
9.	Rose Wine (*JH-3)*		*Present*	*Absent*	*Present*	
10.	*Meyna Laxiflora* (Sohmon) Wine (*JH-5)*		*Absent*	*Absent*	*Absent*	
11.	Mulberry Wine (*JH-6)*		*Absent*	*Absent*	*Absent*	

3.2. Discussion

From the study, it was observed that most of the wine samples that were procured and analyzed were safe for consumption and free from bacterial contaminants and in compliance with the Food Safety and Standards Act 2006, Regulations 2011. However, two samples namely; Finger Millet: Sidhiar (*JH-1*) and Rose Wine (*JH-3*) depict presence of pathogens namely; *E.coli* and *S.aureus* and are said to be unsafe and unfit for human consumption. Presence of any bacterial contaminants is not acceptable as per the norms under the FSS Act 2006. The 11 (eleven) wine samples that were collected from Khasi Hills and Jaintia Hills regions were analyzed for the microbiological parameters as per the Food Safety and Standard Act 2006 to check for the presence of bacterial contamination. It was observed that, out of the total wine samples collected and analyzed from both regions of Meghalaya, majority of the samples that were analyzed were safe and fit for consumption and shows absence of bacterial growth for organisms viz; *E.coli*, *Salmonella* and *Staphylococcus aureus* respectively. However, out of the 11 (eleven) wine samples received and analyzed, 2 (two) wines, namely, Finger Millet: Sidhiar (*JH-1*) and Rose Wine (*JH-3*) from Jaintia Hills does not comply to the prescribed standard on the basis of the tested parameters of '*E coli*' depicting presence of green metallic sheen colonies in Eosine Methylene Blue (EMB) Agar which confirmed the presence of *E.coli* organism and the change in Colour from red to yellow in Mannitol Salt Agar (MSA) confirmed the presence of *Staphylococcus aureus* in both the wine samples respectively. A 'Positive and Negative controls' were prepared and kept in-order to maintain the quality control of the entire test throughout the procedures. *(Table 3)*

4. CONCLUSION

The study were mainly to evaluate the prevalence of bacteriological contamination in locally manufactured wine from various regions within the state of Meghalaya. The results of this study showed that the wine samples collected from the Khasi Hills region of Meghalaya were safe from any bacteriological contamination and are safe for consumption since they adhere to the Food Safety and Standards norms. However, some wine samples collected and received from the Jaintia Hills region did not pass the bacteriological test and were contaminated with pathogenic bacteria such as *E.coli* and *S.aureus*. The wines did not have any proper bottling or safety management plan and were sold to the public loosely, which therefore poses a great risk for cross-contamination by pathogenic bacteria and overall lowers the quality of the wine. The findings of this research suggested that there is a need for the local winemakers to be sensitized and conscientiously be motived to have a proper quality and safety management plan in-order to ensure that the wine that are manufactured by them are safe and free from any contamination and are safe for consumption. There are many reasons for why bacteriological contamination can be detected in wine mainly due to the unhygienic pre/post processing and storage conditions, irregular maintenance of the correct temperature and moisture which may cause the cork to dry and lead to premature oxidation. (Christaki, *et al.,* 2002) discussed all the possible quality and safety hazards which can be encountered during various different stages of wine making. Their study also indicated that the bottling stage could give rise to certain hazards such as the introduction of spoilage microorganism in wine, presence of microbial pathogens in unclean bottles, contamination of wine from non-sterile equipment's, etc. The presence of *E.coli* and *S.aureus* in the wine samples analyzed in this

study could be due to cross contamination during the post processing stages prior to bottling or could happen during the bottling process itself. Both the failed wine samples that were collected from Jaintia Hills did not have a proper bottling management system and the wines were sold loosely rather than in pre-packed sealed wine bottles. The chance for cross contamination by *E.coli* and *S.aureus* is highly probable in the case of wines sold loosely without a proper bottling and hazard management system. Hence, care should be taken in bottling of all locally manufactured wines inorder to avoid any cross- contaminations.

5. ACKNOWLEDGEMENT

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