

Chapter 1

Introduction

“Speculation and the exploration of ideas beyond what we know with certainty are what lead to progress.”

-Lisa Randall

From prehistoric civilization to modern humans, every culture throughout the world has been practicing the knowledge of plants as a source of food fodder and medicine for their survival and their day-to-day requirements. This habit took the native people living in synchronisation with nature to evolve an exceptional system of using medicinal plant practices (Teron & Borthakur, 2014). Except for the medicinal uses, plants are the most accepted food for every culture. To increase the shelf life of any plant-based product, fermentation

was one of the old-age techniques. Consumption of fermented beverages prepared from several plant products started way back in the pre-Vedic period (Prakash, 1961). India is the home of several tribal communities and is widespread in diverse geographical regions. Alcoholic beverages are hugely popular throughout the World along with Indian tribal people. Natural fermentation is one of the old-age safeguarding techniques for food and crop preservation. This ancient knowledge has been carried forward

by the indigenous acquaintance of the ancestors among tribes. Across the globe, diverse socio-linguistic groups have rich historical traditions of utilizing various plant materials in the age-old art of fermentation, which extends to crafting a wide array of distinctive beverages. This cultural tapestry weaves together a fascinating narrative of how humanity's linguistic and social diversity has converged with the natural world to create a spectrum of libations that reflect the unique tastes and traditions of each group. Interestingly, almost every tribe has its indigenous alcoholic beverages and the preparation as well as preservation techniques. Cereal-based food and beverages are always in high demand and have a high impact on the human diet. In India and Africa, more than 80% of the inhabitants consume cereal-based food products for their everyday lives (Das et al., 2017). Traditional fermented drinks are primarily prepared from this

cereal-based product such as rice, wheat, millet, maize, etc. although sometimes particular kinds of fruit, vegetables, and different plant parts are also used as a part of the fermentation. In the development of human civilisation, food fermentation plays a crucial role in using indigenous knowledge. For fermentation local grain cereal is used, the variation comes with the starter culture that is locally groomed with different locally grown herb plants. The starter culture of any fermented drink works as an inoculation which starts the process of fermentation. The starter culture contains plenty of microorganisms that change the biochemical and organoleptic features of the substrate or the raw material. The starter culture modifies the edible products by enhancing their taste and nutritional uptake, which makes them socially and culturally accepted by all consumers. Fermented drinks are not only used for consumption purposes

but also have a valuable impact on the religious practices of many communities. In India, many tribal communities are widespread in several geographical areas. Every tribe has its ethnic beverages. However, the preparation technique, raw material, and cultural purposes differ in each group. The tropo-geographical region of each community is responsible for the raw material selection and the herb selection for the final brew. The synergistic effect of the microorganism and the herb plays a crucial role in the production of the end product. Many traditional practices have been neglected, and no proper scientific evaluation has been done in this field. There is a firm belief of all the tribes that despite the serving purposes of the alcoholic beverages, these brews also play various roles in the curing the several acute and chronic diseases. Consequently, they are still trying to preserve their native culture and carry

their inherited gift from generation to generation (Das et al. 2017). The term ethno-microbiology is a fundamental concept in current food microbiology as the aforementioned traditional old-age fermentation techniques encompass the process of conservation and crude sub culturing of essential microbiota using back-sloping techniques by the indigenous community for centuries.

Currently, people are becoming more worried about obesity and prophylaxis chronic diseases, and probiotics and symbiotics play a significant sector in the functional food market. Maximum probiotic beverages are from milk-based products. The propensity to veganism oblique the consumption of non-dairy products known as non-dairy probiotics, which converted an indispensable health-promoting food category (Vasudha & Mishra, 2013). Therefore, kombucha, fermented herb drinks, cereal-based drinks, etc., may replace dairy products for lactose

intolerant people (Gupta & Abu-Ghannam, 2012). Probiotic fermented products are formed from complex microbial communities that remain in open atmospheres categorised by nutritional food sources and growth conditions. The health-enlightening effects of kombucha and other herbal and cereals-based probiotic beverages have been explored in several publications (Bhattacharya et al. 2011, 2013). These fermented drinks with their microbial community are an example of mutualistic metabolic teamwork of prokaryotic and eukaryotic microorganisms such as bacteria and yeast.

Medicinal herbal plants have been used since ancient times as these plants fight several diseases due to their antioxidative properties (Goyal et al., 2011). The starter culture used in making the fermented drink consists of several medicinally formulated herbal plant parts. Several previous reports clearly stated that the herbal

formulation and the microbiota have a synergistic effect that releases phenolic compounds and organic acids into the end product (Dutta et al. 2018). The polyphenols present in different drinks have antioxidant and scavenging properties that act against reactive nitrogen species (RNS) and reactive oxygen species (ROS) that include hydroxyl radical ($\text{OH}\cdot^-$), superoxide anions ($\text{O}_2\cdot^-$), singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxy nitrite radicals (ONOO^-), etc. Reactive oxygen and nitrogen species are formed from mitochondrial respiratory reaction which produces unstable superoxide radicals. A low amount of ROS is necessary for cellular growth, several gene expressions, and defence mechanisms. However, if the body cannot detoxify the excess ROS, it will damage the cell by oxidative stress. Oxidative stress is the reason for several diseases, such as cancer, diabetes, etc.

The oxidative stress also increases cellular inflammation and cell damage (Dutta et al., 2018).

Accumulation of excess ROS activates several signal transduction genes such as activating several signal transduction cascades including changes in transcription factors such as NF- κ B, AP-1, Nrf2, p53, Foxo, etc. The activation of these genes by ROS may lead prolonged inflammatory state and can cause cancer. ROS can also lead to gastrointestinal cancer. Herbal drugs, probiotic food, and drinks produce various phenols, flavonoids, and tannins, which reduces the excess amount of ROS production in the body. During the literal survey, it was found that probiotic drinks and the plants used in the starter cultures play a crucial role in increasing scavenging activity against ROS and RNS. Therefore, an attempt has been made to explore the medicinal values of the different fermented drinks and the plants used to make them.

During the time of fermentation, a group of several metabolites are produced, including lactic acid, enzymes, antimicrobial substances, and aromatic and organic compounds. The aforesaid byproducts enrich the quality of the fermented drinks and also increase the availability of the nutritional uptake and therapeutic potentials (Das et 2017). The global interest in fermented products is increasing due to their low caloric value, unique quality, and high acceptability. Cereals such as rice, maize, millet, and maize are good sources of carbohydrates and moderate amounts of proteins. Although cereals may lack some essential amino acid fermentation of the cereals make them more nutritious as they contain the probiotic microflora. Traditional alcoholic drinks have ritualistic importance among several communities in Asia and Africa where communal activities need provision and intake of

appreciable quantities of fermented drinks. In contrast, the Europeans, as well as Mediterranean ethnic people, have a deep-rooted cultural history for wine. Although several ethnic groups have a variety of fermented drinks, a general scheme of preparation can be presented as a) selection of raw material as a substrate, b) processing of the raw material including sun-drying, washing, and boiling, c) inoculation of starter culture made up of rice and locally grown several herbs d) Distillation of the fermented product f) post distillation processing (Watson, 1993). Several kinds of alcoholic beverages are available, such as:

- Non-distilled or non-filtered alcoholic drinks using amyolytic starter culture such as fermented millet-based alcohol locally known as *kodo ko jhaar* (Thapa and Tamang 1996), fermented rice-based drink locally known as *bhaati jhaar* (Thapa and Tamang 1996), another

fermented rice-based drink known as *Haria/Handia* (Das et al) in India, similarly like this fermented rice-based drink *makgeolli*(fermented rice) is available in Korea (Jung et al., 2012).

- Non-distilled or filtered alcoholic drinks using amyolytic starter culture such as, e.g., *saké* of Japan (Kotaka et al., 2008).
- Distilled alcoholic beverages using amyolytic starter cultures, such as *Joua* rice-based distilled drink used by the Bodo tribe in Assam and West Bengal.
- Alcoholic drinks produced from honey, e.g. *Tej* is an Ethiopian wine that uses honey as a substrate (Fentie et al., 2020).
- Alcoholic beverages produced from different plant parts such as *Toddy* is a traditional slightly alcoholic drink of India, produced from fresh palm saps by natural fermentation.
- Alcoholic beverages are produced from the germination of cereals such

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- as sorghum, maize, etc. Some examples are the “*Bantu*” beer of South Africa and the *pito* of Nigeria (Kutyauripo et al., 2009; Kolawole et al. 2013).
- Wines and Ciders are alcoholic drinks prepared from fruits without distillation, and the distilled part of fermented fruit and cereals are known as whisky and bandy.
 - The malting process of Cereals, that is, alcohol production, is rare in Asian countries. India is home to several cultures and traditions. It is impossible to study all the ethnic foods and beverages at a time. Preparation of amylolytic starter culture is an innovative and back slopping unique technique, cultivating the native core microbiota from several generations in the form of round flat rice cake with a variation of locally grown herbs. The rice cakes are the source of alcohol production in cereal-based ethnic drinks. These rice cakes are locally known as *marcha* in some parts of India, Bhutan, and Nepal; in Vietnam, it is known as *benh men*, *bubod* in the Philippines, in China and Taiwan rice cakes are known as *chiu/chu/daque*, *loogpang* in Thailand (Steinkraus, 1996; Tamang & Fleet, 2006; Tamang2016). Traditional preparation of the starter culture has a considerable variation starting from the type of substrate, herbs, incubation period, and their traditional practices. The amylolytic starter culture has three primary functions and these are the conversion of complex sugar into its simple sugar forms, simple saccharides to alcohol, and finally, alcohol to organic acids (Hesseltine et al., 1988). The microbiota associated with the starter cultures are mainly the lactic acid bacteria, acetic acid bacteria, fermenting Yeast, and moulds (Das et al., 2017, Tamang et al., 2015). These

microorganisms produce different hydrolytic enzymes, and the enzymes can degrade the cereals used for fermented drink production and renovate them into simple sugars, amine derivatives, and different bioactive compounds (Tamang et al., 2016). The metabolites produced by this microflora play a crucial role in enhancing the nutrition uptake of the food (Das et al.). Milk-based probiotic food and beverages are very popular, but recently, for the lactose intolerant population, cereal-based fermented probiotic food and beverages have gained attention for their health benefits.

The microbial culture used in the fermentation of cereals regulates the final taste, essence, and quality of any fermented drink. The wide-ranging set of microbiota present in the amyolytic starter culture or the rice cake entails both culturable and unculturable microbes. Using the conventional

culture-dependent technique to identify all microbiota is nearly impossible. On the other hand metagenomic method is a relatively new technique to identify all the microbes present in any environmental sample that includes the starter culture.

Metagenomic methods are culture-independent replacements for the straight depiction of any microbiota from environmental samples, food, water, faecal and soil, etc (genes99). Two different high-throughput sequencing (HTS) approaches are used in metagenomics equencing, such as amplicon sequencing and Whole Metagenomic Sequencing (WMS) (Walshet al.2017). In HTS amplicon sequencing, 16S rRNA or 18S rRNA markers are amplified by PCR from DNA extracted from any microbial complex, imperilled to direct sequencing, and aligned against a standard database to identify the taxonomic composition of the sample.

In amplicon sequencing, microbial identification is usually done up to the genus level. In contrast, WMS includes the fragmentation and subsequent sequencing, assembly, and annotation of isolated total genomic DNA of the sample, and maximum information is obtained that the identification is possible up to the species level even in some cases, strain-level identification is also possible. WMS offers insights into the metabolic pathway of the given microbial community, whereas amplicon sequencing mainly provides the basic microbial structure (Walsh et al., 2017). Recently, in the field of blooming of microbial ecology, WMS has aimed to gain a deeper understanding of complex microbial structures in various environmental substances. Studying the HTS approach using amplicon sequencing of food samples is very common but the study of food samples with the WMS approach is still limited.

Metabarcoding DNA or metagenomics of multifaceted bacterial and fungal groups by outlining 16S rDNA sequences and internal transcribed regions (ITS) exposed novel scenarios for learning and scheming new competent probiotics based on fermentation methods. Culture-independent techniques of microbial identification include PCR-denaturing gradient gel electrophoresis(DGGE), pyrosequencing, and High-throughput metagenomic amplicon sequencing. These techniques contribute to an understanding of the microbial ecology of natural food fermentation with enlarged precision within a short period (Shangpliang et al., 2018). Among these, recently developed 16S rRNA-based next-generation sequencing (NGS), such as High throughput metagenomic amplicon sequencing, is widely used and accepted. In 16S rRNA-based next-generation sequencing (NGS) such as High throughput metagenomic

amplicon sequencing identifies almost all the bacteria that contain both culture-dependent and culture-independent bacteria present in the starter culture.

Not only bacteria, Yeast also plays a crucial role in the fermentation process. Internal Transcribed Spacer (ITS) region analysis has been extensively valuable for the search for the diversity of yeasts subordinated with countless traditional fermented foods and beverages. (Caggia et al. 2001).

Furthermore, several bioinformatic tools are handy for recovering the microbial genome directly from metagenomic analysis (Scholz et al., 2016). Other techniques of computational biology give immense knowledge regarding the competition and complementation of the microbes present together in the starter culture and also with humans.

The “Adivasi” or “vanyajati” (castes of

the forest) or “adimjati” (primitive people) are the tribal communities that have progressed throughout the topographical ranges they live in the isolated land parts and forests. They are recognised by present-day society for their etymological representation, untouched religious practices, and grassroots culture that form their uniqueness. The native knowledge of the communities is for their attached concord with nature. This accepting nature not only aids in protecting their food habit but also offer appropriate nutrition and rudimentary remedy for diseases. Starting from the name of the drink, the preparation of starter culture, raw substrate, and processing methodology is remarkably exclusive and thereby forms the basis of their culture, mystical rituals, and myths. Almost every tribal community consumes their ethnic brewed liquor during their childbirth, even at funerals. The methodology virtually transmits from generation to

generation and hinges on geographical location and the region they belong (Das et al. 2017). The starter culture preparation, which inductees the fermentation procedure, is an exceptional piece of art and knowledge to be expanded from every tribal community. Though modern food processing and microbiological techniques try to impersonate the process using definite pure starter cultures, they are still unable to develop the indistinguishable tastes and aromas of the beverages. The tribal community improves the quality of the fermented drink liquor by accumulating additives where mostly diverse medicinal or “ayurvedic” plant parts are used. These plant parts are responsible for the foundation of microbes and also as a source of bioactive compounds. Another characteristic of these brews is the belief of the communities that the fermented liquors act as a remedy for several ailments. Scientific analysis of

the practices is a prerequisite to justify the chemical composition and medicinal benefits of the liquors.

North Bengal is the north-western part of Bangladesh and the northern part of West Bengal. North Bengal includes different regions such as Alipurduar, Cooch Behar, Darjeeling, and Jalpaiguri. Starting from Coochbehar to the foothills of the Himalayas, every tribal inhabitant of different tribal communities has its own ethnic brew. Fermented beverages are an essential part of the socio-economic culture of every community. Fermentation stimulates the texture of the food, nutrition uptake, and aroma. Several ethnic communities from north Bengal have their native brew.

Eu is a fermented millet-based drink consumed by one of the tribal inhabitants, the Toto tribe, in the Alipurduar district, West Bengal, India (Dwan, 2014). This tribe is one of the endangered, most nascent Indo-Bhutanese Mongoloid detached

groups in West Bengal, and they are mainly known as “Forest dwellers.” “Totopara” is a small village neighbourhood in Madarihat (gateway to Jaldapara National Park). EU preparation is an integral part of their traditional practices. Both males and females of these communities consume the brew regularly, and it is mandatory on their special occasions such as festivals, marriages, religious ceremonies, etc. As a primitive resident of the sub-Himalayan Region, the Toto tribe has gained a vast knowledge regarding wild medicinal plants. The geographical region is the main driving force for the raw material selection and also the selection of herbs for the starter culture. Eu is prepared from locally grown finger millet (*Eleusinecoracana*). This plant grows in the semi-arid sub-Himalayan region. The traditionally prepared rice cake, locally known as “moi”, groomed with several locally grown

herbs, is the inoculation of microflora that starts the fermentation of the millet seeds. The starter culture used in this brew is a heterogeneous mixture of culturable and non-culturable microbiota.

Rice beer, or ‘Haria’, is a traditional ethnic, alcoholic, refreshing, stable drink prepared from low-grade rice by the native experts of rural areas in India. Like Haria, an alternative rice-based beer used by the Rabha tribe in India, it is recognised as ‘Choko’/‘chokot’. The starter culture used for Chokot preparation is known as ‘Bakhor or phap’. Rabha tribe is limited to the Gopaldhara district in Assam and the Coochbehar district in West Bengal (Chowdhury et al., 2012; Deka et al., 2010). While Choko is a fermented and instinctive ethnic brew of the Rabha community, only a limited number of families are left with the traditional knowledge of preparing this drink. Choko consumption has a high medicinal

impact, the Rabha community believes it. It diminishes headaches, inflammation of body parts, and diarrhoea (Chowdhury et al., 2012). Although the tribal people in this community of every age group consume 'Choko' or 'chokot', this beverage has not gained any scientific attention.

Like *Chokot*, another rice-based drink, *Haria* is consumed by the Orao and Santal tribes of the Jalpaiguri district in West Bengal. The starter culture of these communities is known as *Ranu dabai* (Ghosh et al., 2004).

Different constituents of human lifestyle, i.e. habitat and food habits, are the different elements that define the identity and ethnicity of each community. The environmental region is the chief driving force that differentiates the ethnic groups from each other. The elevation of any geographical region plays a dynamic role in altering the complete everyday life of any community as altitude

shifts the productivity of raw food materials in that area. Many of India's population lives at high altitudes where food making and obtainability are diverse from the rest of the low-elevation and plain areas. The food habits of various communities in the high-altitude region benefit the dwellers, and visitors get acclimatised to the environment and combat high-altitude sickness diseases (Tracy, 2012). Darjeeling is one of the greatest biologically diverse districts in the Eastern Himalayas. The inhabitants of Darjeeling district consume about 70% of the traditionally fermented food and beverages, and the ethnic group includes Tamang, Sherpa, Lepcha, and Limbo (Tamang et al., 1988). One of the popular household liquors consumed in Nepal, Sikkim, and Darjeeling Hill is *Raksi*. The fermented loads of cereals, potatoes, cassava roots, *Rhododendron* flowers, fruits of *zanthoxylum* locally known as *timboor*, ginger rhizome, and leaves of

Viscum articulatum Burm. f. (Harchur) are refined to obtain raksi (Ray et al., 2016; Tamang et al., 1996). The *timboor raksi*, *Harchur raksi* is rarely available in the Darjeeling district. This fermented distilled brew is only available in the high-elevation villages in the Darjeeling Himalayas, such as Sukhiapokhri (2194 m.), Chitrey bazaar (2542 m.), Tumling (2949 m.), Sandakfu (3626 m.) and Phalut (3600 m.) above mean sea level. These high-altitude liquors reduce altitude sickness (Merrey et al., 2018). Despite the ethnomedicinal value, there are only a few pieces of literature on these drinks.

There are very few reports available on the microbial and biochemical profiles of the above-listed amylolytic starters and beverages in North Bengal. Therefore, retaining all of these in mind, an innovative initiative was taken to explore the varied medicinal properties and diversity of microflora of the selected ethnic

beverages with the following objectives:

- Extended survey and proper documentation of the traditional fermented beverages of selected tribal communities and their medicinal applications.
- Proper identification and germplasm maintenance of the plants used in the starter culture.
- Qualitative and quantitative estimation of the phytochemicals present in selected fermented beverages.
- Assessment of Antioxidant profiling of the fermented beverages used by different ethnic groups.
- Nutritional profiling of the selected beverages.
- Determination of active constituent by GC- MS and *in silico* characterisation of the selected fermented beverages.
- Network pharmacological study of the selected fermented beverages.

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- Assessment of antioxidant and antimicrobial activity of the selected plants used in starter culture preparation of the fermented beverages.
- Assessment of the diversity of Yeast in the starter culture of the selected fermented drink.
- Metagenomics of the starter culture to analyse the presence of bacteria in the selected fermented beverages.
- Whole metagenomic sequencing (WMS) of the starter culture of the selected fermented beverages.
- Assessment of Acute and Sub-Acute Toxicity of the selected fermented beverages.
- Metagenomic analysis of the rat gut microbiome following acute oral administration of a fermented beverage.