

# Abstract

The widespread adoption of preserving food through natural fermentation has ancient origins, primarily stemming from the ancestral wisdom of Tribal communities. Ethnic foods and beverages are described as culinary offerings that have their roots in the heritage and culture of a particular ethnic group, drawing upon their understanding of locally sourced plant and animal ingredients. India houses a multitude of tribal communities spread across diverse geographical regions. Among these communities, alcoholic beverages hold considerable popularity among the tribal population. As a preservation technique, the ancestral practice of natural fermentation has endured through the indigenous

knowledge transmitted by ancestors within these tribes. The mildly alcoholic, sweet-flavoured beverages are regarded more as food than alcoholic drinks due to their high-calorie content, vitamins, and beneficial lactic acid bacteria and yeast. Traditionally prepared alcoholic beverages are cereals such as rice, wheat, maize, or ragi, which have been the substrate of choice for their preparation, depending on the habitat of the tribal population. For generations, alcoholic beverages have been consumed at diverse cultural, social, and religious events. Most fermented drinks predominantly use cereals as a base, incorporating many plants and fruits as the primary raw materials. This combination

contributes to a distinct and unique taste in the beverage. Numerous traditional ethnic practices have been ignored, resulting in insufficient documentation for scientific analysis. Therefore, the present investigation was conducted to evaluate the appropriate scientific examination of select tribal beverages in North Bengal, India. Initially, various areas inhabited by tribal communities were selected to gather their respective traditional beverages, used plant parts and the starter culture. The initial screening was based on the alcohol content of the beverages. Eu, a millet-based drink utilized by the Toto tribe in North Bengal, Chokot, a rice-based drink consumed by the Rabha tribe in North Bengal, Harchur raksi, and Timboor raksi, traditional medicinal plant-based drinks used by the tribal community in the high-altitude region in Darjeeling Himalaya, and Haria, a rice-based drink consumed by the Orao and

Santal communities in North Bengal, were selected for further study. The injection of starter cultures, serving as the source of ethnic microbiota to induce the fermentation process, was also included in the present study. In vitro, the antioxidant study was also conducted to evaluate the most potent among those, as mentioned earlier, low-alcoholic ethnic drinks. The study results show that Eu is the most potent beverage among the other tribal drinks.

The Harchur raksi (HR) is a lesser-known beverage consumed by the indigenous people residing in the high-altitude regions of the entire Himalayan range. HR is unique in its traditional processing method, an indigenous knowledge held by the Darjeeling-Himalayan tribal community. This knowledge should be meticulously documented to preserve its uniqueness. Although HR is considered an anti-inflammatory brew with various ethnomedicinal claims by

the local inhabitants, there has been no scientific evaluation thus far. A comprehensive survey was conducted across the Darjeeling Himalayan region to unearth the ethnobotanical knowledge related to HR. HR was identified and collected in collaboration with a local expert. This work aims to assess the physical, biochemical, and metabolite profiling of HR using different biochemical tests and Gas Chromatography–Mass Spectrometry (GC–MS) experiments. To get a deeper understanding of the medicinal properties of HR, in silico molecular docking studies were conducted. The analysis revealed that HR, with a notably low alcohol content (around 6–8%), exhibits high antioxidant activity. GC–MS analysis of the brew identified 27 bioactive compounds, which were further subjected to bioinformatics analysis. Molecular docking analysis demonstrated the inhibitory activity of most of these compounds against

oxidative stress, inflammation, and specific potent viral proteins related to their pathogenicity. Timbur raksi is a distilled fermented alcoholic beverage from the high-altitude region of Darjeeling Himalaya. The methanolic extract of the sample exhibited 60% inhibition at a higher concentration (200µg/ml). The GC-MS evaluation of the sample revealed the presence of 43 bioactive compounds, which have potential applications as scavengers for reactive oxygen species, anti-inflammatory agents, and anticancer compounds. 'Choko'/'Chokot' is a rice-based beverage consumed by the ethnic tribal community 'Rabha' in North Bengal and Assam, India. While rice is the main ingredient in this fermented product, introducing the starter culture, popularly known as 'Bakhor or phap,' brings variation to its composition. 'Choko'/'Chokot' constitutes a significant portion of the daily caloric intake for the Rabha

tribe. Metabolite profiling was examined through GC-MS analysis. Additionally, the Metagenomic analysis was employed to assess the total microbial load of the starter culture (Bakhor). Reverse ecology analysis delved into the interaction between the microbes present in the brew and humans. The results indicated that the compounds in this beverage mostly exhibit antimicrobial and antifungal effects despite its relatively higher alcohol content than other tribal ethnic drinks. The metagenomic analysis identified several dominant bacterial genera, including *Bacillus*, *Arthrobacter*, *Lactobacillus*, *Ilyobacter*, *Clostridium*, and *Lactococcus*. Haria is a fermented rice beverage crafted using a starter culture (Ranu dabai) to boiled rice grains. It is a casual drink in daily life and is also utilized to alleviate ailments such as fever, headache, and nausea. Including herbal plants in the starter culture

enhances the beverage, providing it with beneficial organoleptic and therapeutic properties. While extensive research has been conducted on Haria, we have not included Haria in our analysis. However, our study does encompass an examination of the plants used in the starter culture of Haria, an area where comprehensive research still needs to be done. The plant-derived phytochemicals demonstrated significant antioxidant activity, suggesting their capacity to reduce reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during oxidative stress. Consuming the beverage may help overcome the growth of cancerous cells and slow the ageing process. Additionally, the herbal plants exhibit active antimicrobial properties, indicating a therapeutic role in inhibiting the growth of human-pathogenic bacteria and protecting the body from severe infections. "Eu" is a traditional mild alcoholic

beverage made from millet and consumed by the Toto tribe in West Bengal. The preparation of Eu involves millet fermentation with the locally cultivated starter culture known as "Moaa." Ethnobotanical data and samples were collected through semi-structured interviews. Qualitative assays, such as acute toxicity, antioxidant analysis, gas chromatography-mass spectrometry (GC-MS), in vitro anti-inflammatory assay, molecular docking, and physiochemical assessments (pH and alcohol content), were conducted. The findings indicate that Eu has a low alcohol content (approximately 1-3%) and a high antioxidant capacity. GC-MS analysis identified thirteen bioactive compounds. The starter culture employed in this brew comprises a diverse microflora. Identifying these microbes will enhance our comprehension of the brew and allow us to quantify its effects on the human system post-

consumption. Leveraging advancements in Illumina sequencing technology, meta-profiling of the 16S rRNA (V3–V4 region) has become a valuable resource for scrutinizing the microbiome of various samples. We have utilized this tool to investigate the microbiome population of Eu. The microbial diversity found in the brew primarily consisted of probiotic and fermenting bacteria. Nutrient profiling of Eu assessed the levels of protein, carbohydrates, free amino acids, and free fatty acids. The nutritional content in the brew likely supports the growth of probiotic microflora. Reverse ecology-based network analysis unveiled substantial complementary interactions between humans and the microflora in the Eu. GC–MS analysis identified thirteen potent compounds in Eu, and an extensive network pharmacological study on these compounds revealed the anticancer properties of Eu, particularly against gastrointestinal

malignancies. This was achieved by reducing the proliferation of malignant cells and forming pro-inflammatory cytokines. The probiotic microflora and the Eukaryotic Yeast also play a crucial role in fermentation. Several yeast cells were isolated from the starter culture, and from the isolated cultures based on biochemical screening, three strains were selected for identification. Molecular phylogenetic analysis based on ITS region (ITS1 and ITS4) sequencing of these three yeast isolates showing similarity with *Saccharomyces cerevisiae*.

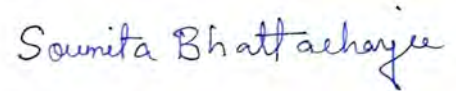
The MTT assay against the ACHN human renal adenocarcinoma cell line determined the cytotoxicity of Eu. The brew showed no cytotoxic effect. Whole metagenomic sequencing, or WMS, is particularly valuable in studying complex microbial ecosystems, where diverse microbial communities coexist. Whole metagenomic sequencing was done on

the starter culture of Eu; the result showed .42% of Archaea, 60.80% of Bacteria, 22.84% of fungi, 11.95% of protozoa and 2.95% of Viruses within the sample.

In vivo, acute and sub-acute oral administration toxicity study was conducted to show the efficacy of the drink Eu on a rat model. In the acute toxicity study, female and male Wistar rats received oral administrations of Eu of doses 250 and 500 mg/kg over 28 days following OECD Guidelines 425. In the sub-acute study, no significant reduction or gain of weight was observed when compared with the control group. The haematological and histopathological study also showed no significant alteration of the parameters when compared with the control. The spleen weight of both males and females was changed slightly compared to the standard, but this change is not dose-dependent. The result of sub-acute toxicity showed that an adequate amount of the drink

didn't have a cytotoxic effect. Moreover, the gut microflora was compared with the treated rat with the non-treated rat. Culture-independent 16s rRNA (V3-V4) regions were analyzed for both samples.

Results revealed that consumption of Eu can regulate the gut microflora.



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