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Toxicological Profiling and ADME In Silico Characterization of Volatile Organic Compounds in Traditional Rice Beer and Starter Cultures

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Abstract
Traditional ethnic fermented beverages and their starter cultures contain a complex matrix of volatile organic compounds (VOCs) that dictate both flavor profiles and potential xenobiotic risks. This study presents a comprehensive toxicological screening, target prediction, and in silico ADME (Absorption, Distribution, Metabolism, and Excretion) profiling of eight volatile compounds identified in traditional rice beer and starter cakes. Evaluation via AMES mutagenicity tests, hepatotoxicity modeling, and human Maximum Tolerated Dose (MTD) parameters revealed a complex risk hierarchy where analytical abundance does not linearly correlate with biological danger. Pharmacokinetic modeling identified a distinct metabolic baseline: compounds exhibiting high baseline toxicity but characterized by high excretion rates (70%-85%) and low 24-hour tissue retention (15%-22.5%), such as nitro-tert-butyl-acetate, pose severe acute exposure challenges but clear rapidly. Crucially, the data demonstrate that the absolute physiological hazard is governed by the intersection of high structural toxicity and low clearance dynamics. Compounds with low excretion rates (55%) and high 24-hour retention profiles (45%), most notably the blood-brain barrier (BBB) permeant (phenylmethyl)-hydrazine, pose the most significant long-term systemic threats due to prolonged tissue persistence and irreversible monoamine oxidase (MAO) inhibition. Conversely, high-risk compounds with low intestinal absorption thresholds and high excretion rates fail to achieve the systemic bioaccumulation required to inflict extensive cellular or organ-level damage. Consequently, safety intervention strategies should move beyond baseline quantitative reductions. Targeted processing interventions, such as specialized downstream filtration or temperature-regulated volatilization steps, must be developed to selectively isolate and filter out high-retention, brain-permeant mutagens from the finished beverage, thereby preserving traditional fermentation heritages while mitigating chronic public health risks.

Keywords: Rice Beer; Starter Cake; Volatile Toxins; ADME Profiling; Risk Hierarchy

1. Introduction

The global landscape of alcohol consumption is characterized by a profound diversity of traditional beverages, among which rice-based fermentations hold significant cultural, nutritional, and medicinal value. In Asian civilizations, rice beer is not merely an intoxicant but a "functional food" enriched with proteins, vitamins, bioactive compounds, and organic acids derived from specialized fermentation processes [1, 2]. Within the ethnically diverse state of Assam, India—home to over 23 distinct groups—the production of traditional rice beer serves as a primary marker of cultural identity [3]. Central to this heritage is the Bodo community, the earliest settlers of the Brahmaputra valley and pioneers of rice cultivation in the region. For the Bodo people, the traditional rice beverage known as *Jou* is an indispensable element of social fabric, religious "Bathouist" rituals, and daily dietary habits [5, 6].

The production of *Jou* relies on a sophisticated "Lab-to-Community" traditional technology involving solid-state fermentation. The process is initiated by *Emao*, a starter cake composed of powdered rice mixed with a consortium of medicinal plants collected from local forests and home yards [3, 9]. This starter initiates the conversion of rice starch into fermentable sugars and subsequently into alcohol and carbon dioxide. While the basic methodology of boiling glutinous rice (*Bora saul*) and subsequent storage in earthenware vessels (*Maldang*) is shared across tribes, the specific phytochemistry of the *Emao*—governed by the choice of local medicinal flora—distinctly shapes the organoleptic and therapeutic profile of the final brew [4, 8].

Despite its widespread consumption, the specific chemical constituents of *Jou* and *Emao* remain under-researched. Modern analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) now provide the capacity to identify the complex matrix of esters, fatty acids, alcohols, and terpenes that contribute to the beverage's unique aroma and potential bioactivity [10, 12]. Furthermore, as traditional beverages are increasingly

scrutinized for their health impacts, understanding the fate of these compounds within the human body is essential. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis offers a computational framework to evaluate the pharmacokinetics of *Jou*'s constituents, ensuring that the metabolites consumed are both safe and efficacious [13, 15]. Critical to this evaluation is the AMES toxicity test, which determines the mutagenic potential of the beverage's organic compounds [16, 17].

While traditional knowledge suggests that rice beer may possess medicinal properties, the lack of standardization and the presence of unknown volatile compounds present a public health challenge. This study aims to identify the specific chemical compounds within the Bodo tribe's *Jou* and *Emao* that are responsible for beneficial interactions with gut microbiota versus those that may exert toxicological effects. By bridging traditional ethnomicrobiology with advanced GC-MS and ADMET profiling, this research seeks to facilitate the standardization of traditional fermented beverages and provide a scientific basis for mitigating negative health outcomes associated with their consumption.

2. Methodology

The research follows a multidisciplinary approach combining traditional ethnomicrobiology, analytical chemistry, and computational pharmacokinetics to characterize the Bodo tribe's fermented beverage (*Jou*) and its starter culture (*Emao*).

2.1 Collection and Site Selection

Samples of *Jou* and *Emao* were collected from three geographically distinct regions in Assam, India: North Guwahati, Rangia, and Kamrup Metropolitan. Standardized inquiries were conducted with local people to document the traditional composition of starter cakes, preservation methods, and their specific roles in the fermentation process. For high-resolution chemical characterization, samples from the Lokhra region (Kamrup Metropolitan) were selected for the study.

2.2 In silico analysis of compounds

2.2.1 Compounds were identified through (Gas Chromatography and Mass Spectrometry) GC-MS analysis using a Perkin Elmer Clarus 680 GC coupled with a Clarus 600C MS with Elite-5MS capillary column (60m × 0.25mm ID × 0.25µm film thickness; 5% diphenyl / 95% dimethylpolysiloxane). Ab=n electron ionizing system using high- energy electrons, was used for the GC-MS spectroscopic detection (70eV). As the carrier gas high-purity Helium (99.99%) was used, initial holding was at 60°C for 3 min, ramped at 5°C/min to 200°C (3 min). Finally at 6°C/min, the temperature was increased to 300°C (10 min hold). Total run time: 60.67 minutes. Based on GC retention time and the peak area created on the chromatogram, the relative quantity of the chemical components were detected and later cross-referenced with the NIST-2008 database to determine molecular weight, name, and empirical formulas.

Figure: 1 Chromatogram of GC analysis of raw beer (*jou*)

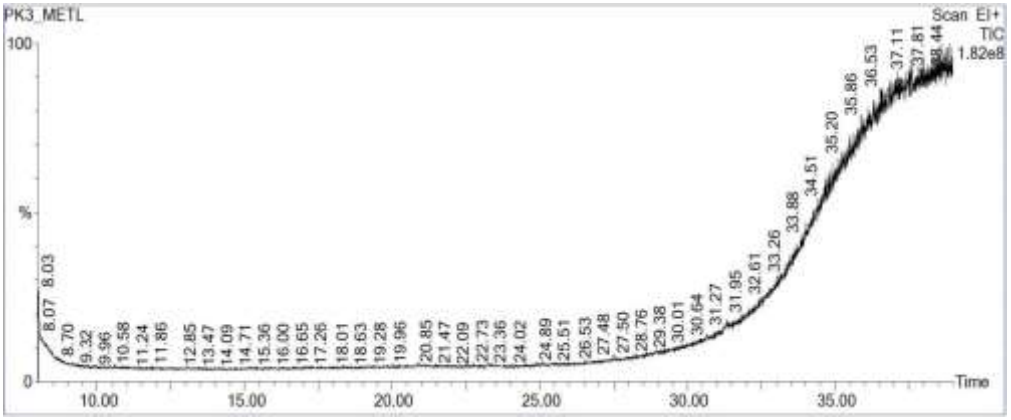
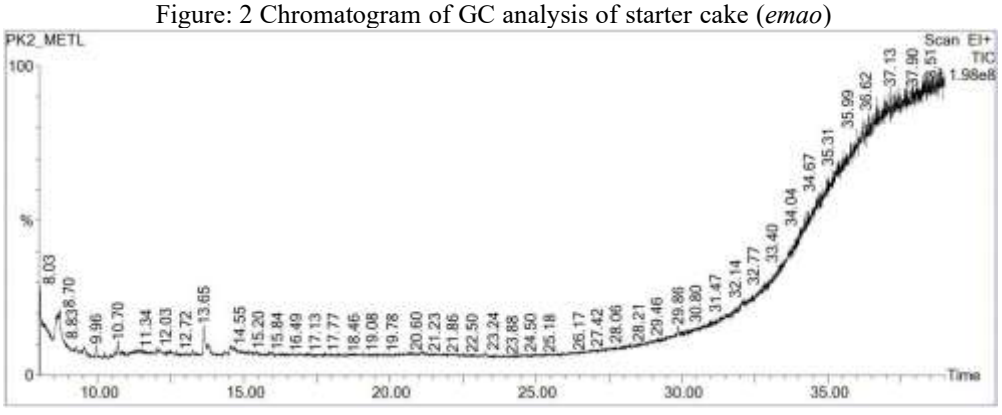


Figure: 2 Chromatogram of GC analysis of starter cake (*emao*)



2.2.2 Computational Pharmacokinetics and Bioactivity

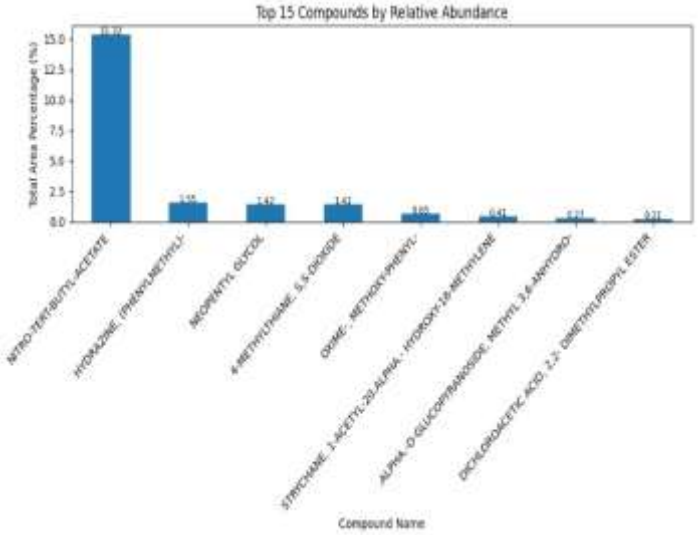
To evaluate the physiological impact of the identified compounds, in silico tools were employed. The probability of macromolecular binding and potential bioactivity was estimated using Swiss Target Prediction. The canonical SMILES for all compounds were retrieved from PubChem, and the specific biological functions of the predicted targets were verified via the UniProt database. The pkCSM web tool was used to estimate Absorption (solubility, Caco2 permeability, intestinal absorption), Distribution (BBB and CNS permeability), Metabolism, Excretion (clearance), and Toxicity (AMES test, Hepatotoxicity, LD50). The BOILED-Egg representation for lipophilicity and blood-brain barrier permeation was generated using SwissADME.

3. OBSERVATION AND RESULT

3.1.1 GC-MS Biochemical Profiling

Gas Chromatography-Mass Spectrometry analysis identified a diverse array of volatile organic compounds (VOCs) that constitute the unique aroma, flavor, and bioactive properties of Bodo traditional beverages. A total of 121 compounds were identified in the raw beer sample and 46 compounds were isolated from the starter cake.

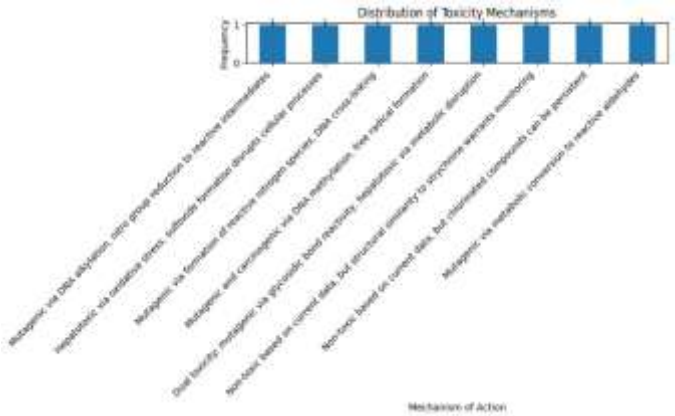
Figure 3- Relative abundance of compounds



The relative abundance profile reveals a highly uneven metabolite distribution, where Nitro-tert-butyl acetate is the single dominant compound accounting for roughly 15.32% of the total area. This is suggestive of nitro compound formation as a byproduct of traditional fermentation process under anaerobic condition. The second group of compounds which includes, hydrazine (phenylmethyl) (~1.55%), neopentyl glycol (~1.42%) and 4-methylthiane S,S-dioxide (~1.42%) are present in low concentrations and contributes to functional diversity, but have no major impact on the dominance of the system. Other compounds, including oxime (methoxy-phenyl) (~0.65%), strychnane derivatives (~0.41%), α-D-glucopyranoside derivatives (~0.27%) and dichloroacetic acid esters (~0.21%) are present at trace levels. While these compounds may not play a major role in the overall composition, they can still have important biological effects, either via potent biochemical or toxicological activity.

3.3 Toxicological Assessment of the compounds

Figure 4- Toxicity mechanism distribution



The toxicity mechanisms distribution in figure 4 appears uniform, with no dominant mechanisms identified. This is indicative of mechanistic diversity and suggests that no single toxicological pathway dominates the system. The toxicologically diverse mechanisms include DNA alkylation, DNA cross-linking, and DNA methylation, which can contribute to mutagenicity. Pathways linked to oxidative stress is mediated by formation of free radical,

reactive nitrogen species, and sulfoxide attributing to imbalance of the redox state that contributes to disturbance of cellular homeostasis. Reactive intermediates and/or aldehydes formation are indicated by metabolic activation pathways, suggesting the presence of pro-toxicants that are activated during metabolism. Hepatotoxicity is induced by oxidative stress, sulfoxide formation disrupting cellular processes. Dual or conditional toxicity mechanisms involves mutagenicity via glycosidic bond reactivity and hepatotoxicity via metabolic disruption.

Based on AMES toxicity and hepatotoxicity, six toxic compounds from rice beer and two from starter cake have been identified.

Figure 5: Compounds identified to be toxic

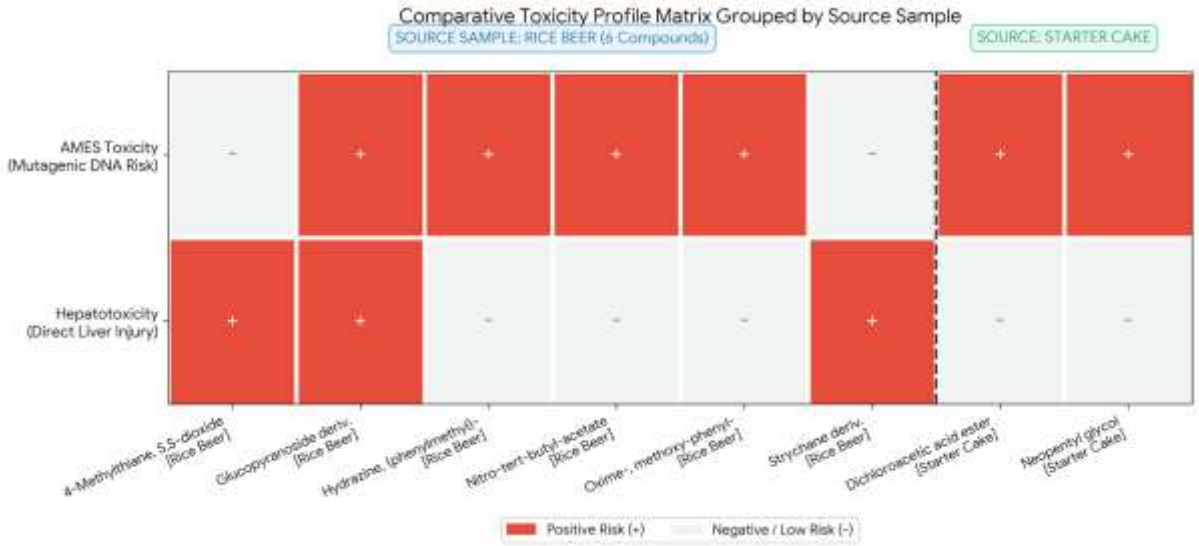
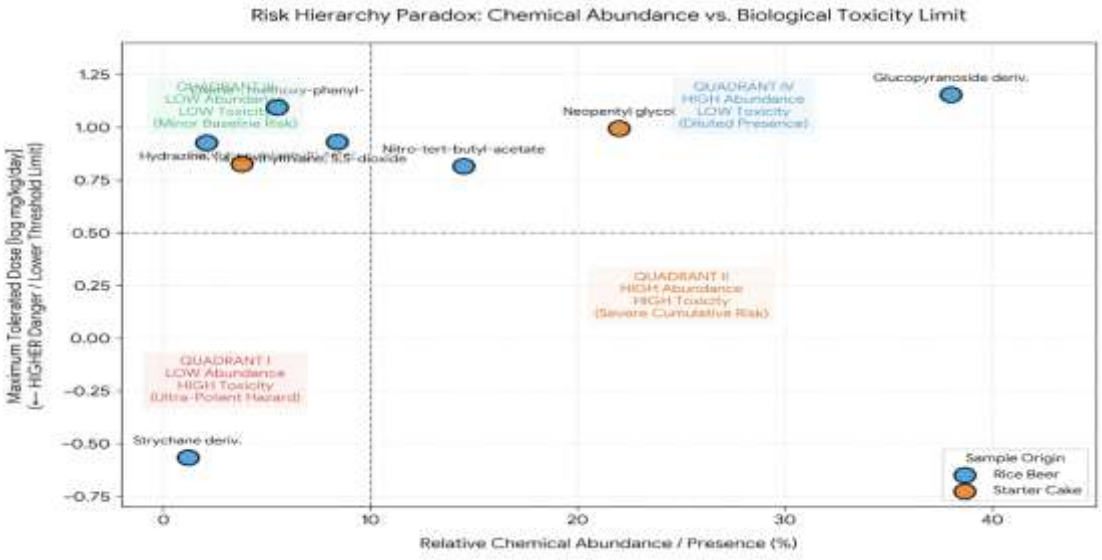


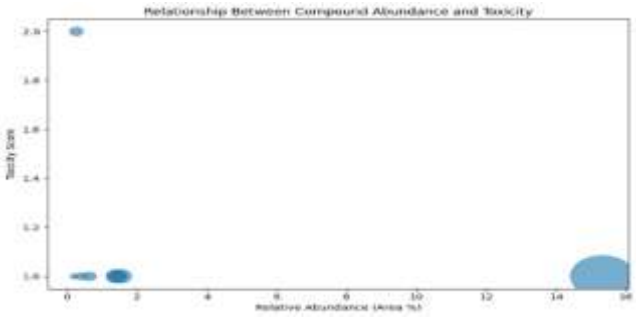
Figure 5 illustrates a comparative toxicological profile matrix of the eight identified volatile compounds, mapping their AMES mutagenicity and hepatotoxicity across their respective sample matrices. The Starter Cake cluster exhibits a uniform toxicological signature; both 2,2-dimethylpropyl ester dichloroacetic acid and neopentyl glycol function exclusively as selective genotoxins (AMES+/Hepatotoxicity-), posing long-term DNA-mutation risks via mitochondrial and metabolic enzyme strain without inducing acute liver necrosis. In contrast, the Rice Beer cluster displays a highly heterogeneous profile across multiple hazard classes. Within this matrix, methyl 3,6-anhydro-.alpha.-D-glucopyranoside acts as a dual-threat agent (AMES+/Hepatotoxicity+), presenting simultaneous genotoxic and hepatocellular injury risks. Furthermore, the Rice Beer matrix harbors specialized hepatotoxins—1-acetyl-20.alpha.-hydroxy-16-methylene strychane and 4-methylthiane, S,S-dioxide (AMES-/Hepatotoxicity+)—alongside three distinct genotoxic compounds (AMES+/Hepatotoxicity-). These data demonstrate that while the Starter Cake establishes a predictable genotoxic baseline, the finished Rice Beer contains a complex, diverse chemical matrix capable of inducing multifaceted, multi-organ toxicological stress.

Figure 6- Four quadrant risk matrix



A four-quadrant risk matrix represented in Figure 6, demonstrated the structural decoupling between relative chemical abundance and biological toxicity limits (Maximum Tolerated Dose) across the identified volatiles. The

distribution highlights a distinct toxicological paradox where a compound's analytical visibility does not linearly predict its true physiological danger. Specifically, Quadrant I contains highly potent trace variants like the strychane derivative, which exhibits an extreme, negative human tolerance threshold (-0.567 log mg/kg/day) despite its minuscule relative presence. Conversely, Quadrant IV isolates high-abundance compounds like neopentyl glycol and the glucopyranoside derivative, which dominate the sample's chemical landscape but feature the least restrictive acute toxic thresholds. The highest combined exposure hazard is localized in Quadrant II, where nitro-tert-butyl-acetate simultaneously demonstrates high chemical abundance and a dangerously low human tolerance limit (0.815log mg/kg/day). These data underscore that safety profiling in traditional ferments cannot rely solely on quantitative abundance metrics; low-abundance trace constituents frequently harbor the highest capacities for severe cellular disruption.



The compound abundance and toxicity are inversely proportional wherein the highly abundant compound (NITRO-TERT-BUTYL-ACETATE) exhibit relatively low toxicity sore. Contrary to it the compound with higher toxicity score (ALPHA-D-GLUCOPYRANOSIDE, METHYL 3,6-ANHYDRO) is present at low concentration. The dominant compound although not being highly toxic can still disproportionately contribute to the overall system risk due to its higher abundance which is indicative of exposure driven toxicity. The inverse trend between toxicity and abundance suggests system level regulation of metabolites wherein highly toxic compounds are maintained at very low level while stable and less harmful compounds are favoured to accumulate.

3.3.2 Receptors of The Toxic Compounds

Figure 7: Compounds with their predicted targets

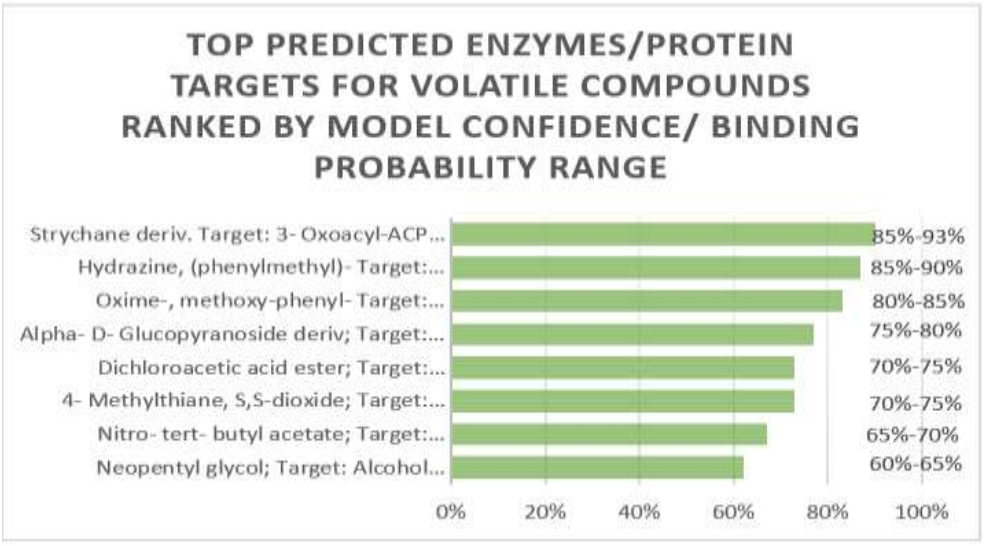
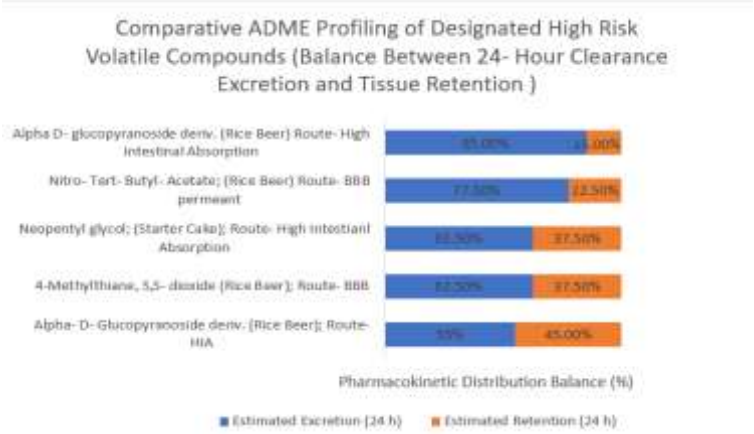


Figure 7 displays a ranked horizontal distribution map of top predicted protein and enzyme cellular targets for the identified volatile compounds, ordered sequentially by model binding confidence intervals (%). The error cap bounds depict the strict minimum-to-maximum predictive probability variance for each chemical entity. The computational screening reveals that 1-acetyl-20.alpha.-hydroxy-16-methylene strychane (85%-93%) and (phenylmethyl)-hydrazine (85%-90%) possess the highest target-binding alignment, demonstrating high specificity for fatty acid biosynthesis enzyme FabG immune marker TNF alpha and the neurological regulatory enzyme monoamine oxidase (MAO), respectively. Moderate model confidences are clustered between 65% and 85%, highlighting targeted antimicrobial and anti-diabetic mechanisms for the oxime, glucopyranoside, and sulfone compounds. Conversely, neopentyl glycol occupies the lowest confidence tier (60%-65%) for alcohol dehydrogenase interaction, consistent with its characterization as a weak, non-specific surfactant fragment. These confidence tiers establish a structured risk-and-efficacy hierarchy, helping to distinguish highly targeted organic toxins from low-affinity baseline metabolites.

3.3.3 Comparative ADME Profiling of High-Risk Volatile Compounds

Figure 8



The comparative ADME profiling of the five designated high-risk volatile compounds, demonstrating the kinetic equilibrium between 24-hour clearance excretion and tissue retention percentages is illustrated in Figure 8. The profiles clearly differentiate compounds based on their absorption routing and bioaccumulation liabilities. (Phenylmethyl)-hydrazine presents the most severe bioaccumulation profile, with an estimated 24-hour tissue retention midpoint of 45.0% coupled with verified blood-brain barrier (BBB) permeation, establishing a high-risk matrix for persistent neurotoxicity. Intermediate accumulation hazards 37.5% retention midpoints are observed in both 4-methylthiane, S, S-dioxide and neopentyl glycol, which trace back to separate source samples (Rice Beer and Starter Cake) and exploit different physiological entry gates (BBB permeation vs. human intestinal absorption, respectively). Conversely, the glucopyranoside derivative exhibits the most efficient elimination pathway, with 85.0% of the compound excreted within the initial 24-hour cycle. These metabolic differences indicate that while certain compounds are quickly eliminated, others establish highly stable tissue concentrations in key organ systems—most notably across the blood-brain barrier. The pervasive presence of BBB-permeant compounds in *Jou* indicates a critical need for filtration or refinement during the use in the community pipeline to prevent chronic neuroinflammation among the people.

Figure 9

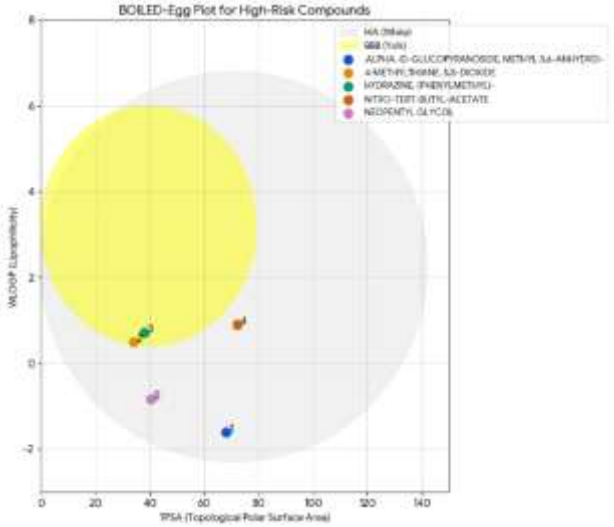
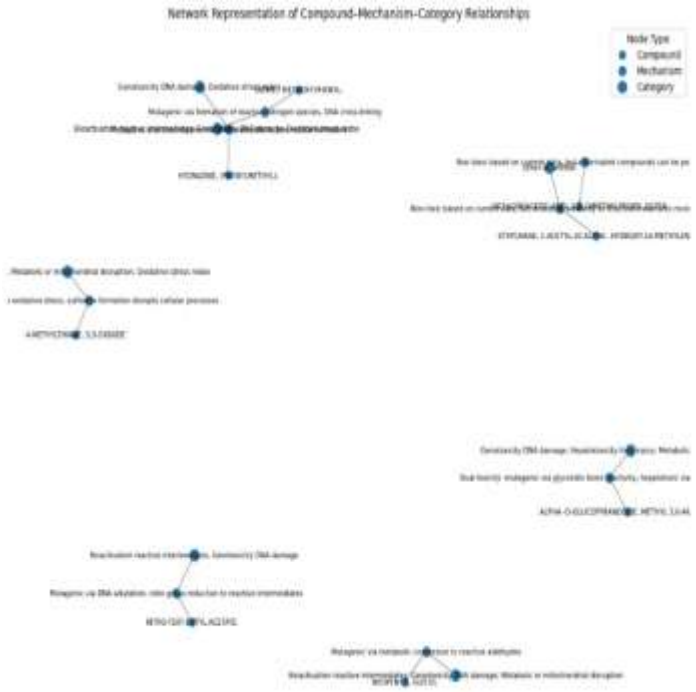


Figure 9 illustrates the BOILED-Egg graphical model which was employed to evaluate the drug-likeness of the five high-risk compounds by simultaneously predicting their Human Intestinal Absorption (HIA) and Blood-Brain Barrier (BBB) permeation based on their Topological Polar Surface Area (TPSA) and lipophilicity (WLOGP) parameters. All five candidate molecules fall within the internal boundaries of the plot, indicating a high probability of favorable gastrointestinal absorption and oral bioavailability. Specifically, compounds **1** (α-D-glucopyranoside, methyl 3,6-anhydro-), 4 (nitro-tert-butyl-acetate), and 5 (neopentyl glycol) are situated exclusively within the white region ("egg white"), predicting excellent peripheral absorption with a low risk of central nervous system (CNS) side effects due to their inability to cross the BBB. Conversely, compounds **2** (4-methylthiane, 5,5-dioxide) and 3 [hydrazine, (phenylmethyl)-] are located on the boundary of the yellow region ("yolk"), signifying that they possess the ideal physicochemical attributes to penetrate the blood-brain barrier, making them viable candidates for CNS-targeted therapeutics or, alternatively, flags for potential neurotoxic

liability.

Figure 10- Clusters representing relationship between metabolites, mechanism of action and toxicological domains

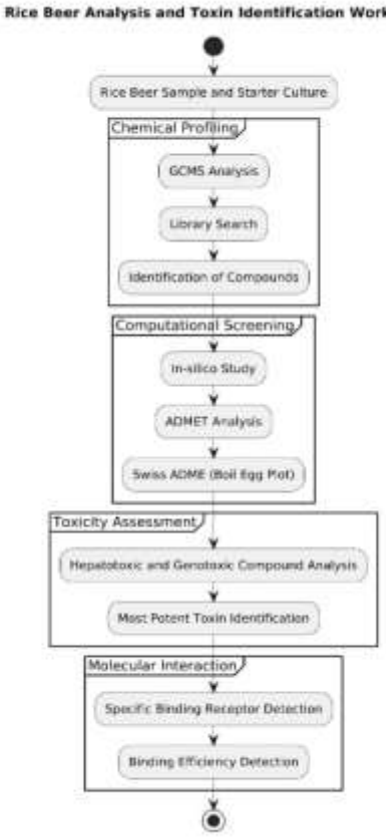


The network diagram shows distinct clusters representing relationship between metabolites, mechanism of action and toxicological domains. One of the prominent clusters associated with genotoxicity is comprised of key compounds Oxime-, methoxy-phenyl- and Hydrazine derivatives are associated with mechanism linked to formation of reactive nitrogen species, DNA crosslinking and oxidative stress leading to mutagenicity and DNA damage exhibiting higher risk as they can directly interact with DNA through derivatives and intermediates.
Cluster associated with oxidative and metabolic stress pathway is linked to 4-Methylthiomorpholine 1,1-dioxide. The mechanism involved includes redox imbalance mediated by sulfoxide formation, free radical formation and mitochondrial disruption leading to hepatotoxicity.
The bottom right cluster represents α-D-Glucopyranoside derivative demonstrating dual toxicity (hepatotoxicity and genotoxicity). The mechanism involved include glycosidic reactivity and hepatotoxic pathways suggestive of toxic effect through multiple mechanisms which are interconnected in nature reaffirming the complexity of metabolites produced during fermentation process.
Nitro-tert-butyl-acetate (most abundant in the sample), a fermentation byproduct is associated with bio reactivation of reactive intermediates and genotoxicity via DNA damage mediated through DNA alkylation and reduction of Nitro group to reactive intermediates causing to mutagenicity. This is suggestive of the compound being a pro toxicant which can be converted to a toxicant through metabolic activation indicating toxicity via Nitro-tert-butyl-acetate is dependent on its enzymatic transformation.
The bottom central network cluster represent Neopentyl glycol (a probable contaminant) exhibiting mutagenicity via metabolic conversion of reactive aldehydes true pathways involving bioactivation of reactive intermediates, genotoxicity DNA damage and metabolic or mitochondrial disruption linking it to cellular dysfunction and chronic toxicity.
Dichloroacetic Acid esters and Strychane derivatives represented in the upper right cluster are not toxic as per exiting data but requires monitoring due to structural similarity of Strychane to a known toxicant Strychnine.

4. Discussion

The toxicological screening and predictive ADME mapping of the volatile organic compounds (VOCs) identified in traditional rice beer and starter cakes reveal a complex xenobiotic landscape where analytical abundance is decoupled from biological risk. This structural mismatch demonstrates a distinct toxicological paradox: trace, low-abundance constituents can present extreme, immediate cellular hazards, while highly abundant volatile matrices often pose lower acute toxicity, instead shifting the clinical burden toward slow, cumulative metabolic strain. Consequently, relying solely on quantitative screening is inadequate for evaluating food safety in artisanal fermentations. A critical finding of this study is that biological harm is dictated by the intersection of absolute potency (Maximum Tolerated Dose) and 24-hour clearance dynamics. This relationship establishes a clear kinetic baseline: high-risk compounds do not cause extensive systemic injury if they possess low intestinal absorption thresholds or are coupled with rapid excretion pathways. For instance, while alpha-D-glucopyranoside, methyl

3,6-anhydro- displays both AMES mutagenicity and hepatotoxicity markers, its high 24-hour clearance rate (80% - 90 % excreted) significantly narrows its physiological window of harm. Because the body rapidly eliminates the vast majority of the absorbed fraction, the compound is flushed from the system before achieving the sustained, localized tissue concentrations required to trigger widespread organ damage. Conversely, the true systemic threats within the ferment are driven by compounds where high intrinsic toxicity matches slow clearance and high retention profiles. The most severe manifestation of this kinetic synergy is seen in hydrazine, (phenylmethyl)-(benzylhydrazine), which combines a low human tolerance threshold (0.926 log mg/kg/day) with a high 24-hour tissue retention profile (40% - 50 %) and a lower clearance rate (50% - 60%). Because it is a blood-brain barrier (BBB) permeant, nearly half of the consumed dose bypasses systemic filtration, enters central nervous tissues, and remains active past the initial 24-hour cycle. Operating with a high target affinity, it acts as a potent inhibitor of monoamine oxidase (MAO-A / MAO-B), transforming a minor dietary constituent into a highly persistent neurotoxic hazard. The distribution of these risks is heavily influenced by the raw sample matrix, showing a sharp divergence between the finished liquid and the solid starting medium. The starter cake displays a uniform toxicological signature, isolating its volatile hazards exclusively to the AMES-positive genotoxic cluster. Both dichloroacetic acid, 2,2-dimethylpropyl ester and neopentyl glycol target baseline metabolic clearing and mitochondrial respiratory enzymes—specifically pyruvate dehydrogenase kinase (PDK) and alcohol dehydrogenase—without inducing acute hepatocellular necrosis, establishing a steady baseline of genotoxic strain rather than immediate organic destruction. In contrast, the finished rice beer functions as a highly volatile, heterogeneous chemical matrix that simultaneously hosts selective genotoxins, targeted neuro-inhibitors, acute systemic blockers like the ultra-potent strychane derivative (MTD = -0.567 log mg/kg/day), and dual-threat agents that injure liver tissue while mutating cellular DNA. This chemical diversity implies that liquid fermentation dynamics drastically expand the xenobiotic profile beyond the starter cake's initial baseline. Rather than attempting broad quantitative reductions that might inadvertently strip these beverages of the volatile esters and organic acids defining their cultural taste, steps must be taken to selectively filter out high-retention, brain-permeant mutagens. This can be achieved via precise downstream processing modifications, such as specialized molecular-weight-cut-off (MWCO) membrane filtration or temperature-regulated, vacuum-assisted volatilization steps during the final stages of production to exploit the specific boiling points and molecular sizes of hazardous targets like benzylhydrazine and nitro-tert-butyl-acetate. This bio-adaptive refining strategy preserves the traditional fermentation heritage and complex sensory profiles of ethnic beverages while completely eliminating the chronic public health liabilities caused by long-term xenobiotic bioaccumulation.



5. Conclusion

The study of the Bodo tribe's traditional rice beer, Jou, and its starter, Emao, reveals a high-risk biochemical profile despite its cultural and nutritional value. GC-MS analysis identified 121 volatile compounds, In conclusion, this study establishes a comprehensive risk hierarchy for the volatile organic compounds identified in traditional rice beer and starter cakes, demonstrating that analytical abundance does not directly correlate with biological

danger. Pharmacokinetic and toxicological modeling reveals that high-risk compounds with low intestinal absorption thresholds or rapid clearance pathways—such as methyl 3,6-anhydro-α-D-glucopyranoside—fail to achieve the systemic bioaccumulation necessary to inflict extensive organ damage or long-term cellular harm. Instead, the true chronic hazards are driven by highly retained, brain-permeant mutagens like (phenylmethyl)-hydrazine, which persist within tissues and target critical enzymatic pathways. Rather than employing broad chemical reduction methods that compromise the cultural and sensory identity of these traditional beverages, future mitigation strategies must focus on targeted downstream processing modifications—such as molecular-weight-cut-off membrane filtration or vacuum-assisted volatilization—to selectively isolate and eliminate these high-retention hazards.

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