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Authors:
Jobila Myrphet
Anshuman Khuttiya Deori
Mhajan Patton
Jubin Nath
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Dr. Sudarshana Borah Khanikor

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Mhajan Patton

Jubin Nath

Debojit Mudoi

Rashidul Islam

Dr. Sudarshana Borah Khanikor

School of Pharmaceutical Sciences,

University of Science and Technology,

Techno City, Kiling Road, Baridua, Ri-Bhoi, Meghalaya-793101

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PREFACE

Secondary metabolites are those substances that belong to numerous categories of metabolites as well as can be substantially induced in response to stressful circumstances. Primary metabolites take part in nourishment and reproduction in order to perform important metabolic functions, where an organism interacts with its environment but are not necessary for an organism to live. Metabolites are very important for the human health of our society that include antibiotics, antitumor agents, cholesterol-lowering drugs, immunosuppressants, antihelmintic agents and other antiparasitics, herbicides, ruminant growth stimulators, agricultural fungicides, bio-insecticides, and others. The Northeast India consists of many ethnic tribes and communities having diverse customs and lifestyles with difference in their preparation and consumption of fermented rice beer. Traditional beers play a significant role in the cultural and social fabric of Northeast India. Since it is probiotic-enriched, rice beer is regarded as Mother Nature's elixir. Overall, traditional beer in Northeast India holds a multifaceted role, encompassing cultural, social, and economic aspects. Traditional beers have a number of therapeutic uses that have been proven, and their metabolites may also be beneficial to one's health. To fully grasp the health advantages of these traditionally made rice beers, it is advised that researchers conduct more study on the therapeutic benefits as well as any contaminants. The detection of different phytochemicals in each rice beer sample indicates the presence of unique compounds and varying levels of these compounds. It would involve establishing quality control measures, setting regulatory guidelines and develop standardized protocols to ensure consistent and safe rice beer production. Exploration of therapeutic compounds through chemical profile study would give the researchers a future prospect of understanding the potential therapeutic benefits of these different traditional beverages with minimum toxicity level and meet the consumer demands at a cost effective and cost benefit manner.

- **Authors**

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- **Authors**

Dedicated to
our family members

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Abstract:

Traditional Rice Beer samples: *Sadhier* (Meghalaya), *Xaj-pani* (Assam), *Apong* (Arunachal-Pradesh), *Zutho*, *Khe* (Nagaland) were collected. Various tests to identify phytochemicals in each sample have been carried out, where in *Sadhier*, the presence of carbohydrates, saponins, amino acids, and tannins have been detected. For *Xaj-Pani*, carbohydrates, amino acids, and saponins have been detected. *Apong* showed the presence of carbohydrates, alkaloids, sterols, tannins, and saponins. *Zutho* and *Khe* contained carbohydrates, sterols, amino acids, tannins, and saponins. Thin Layer Chromatography for all the beers sample was performed with the solvent systems: Ethyl acetate: Methanol:Water(14:3:3); 10% of Phenol water; Hexane:Acetone (18:2); Chloroform:methanol:Water (14:6:2) and chloroform:ethy acetate:methanol (2:2:16). The TLC of *'Apong'* revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Saponins and Tannins; of '*Xaj pani*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols, and Saponins; of '*Sadhier*' revealed the presence of 03 classes of compounds that include Carbohydrates, Amino Acids, and Saponins; of '*Zutho*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins; of '*Khe*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins. Liquid Chromatography Mass Spectrometric analysis revealed in *apong* carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine, and tannins; in *Xaj pani* carbohydrate derivatives, histamine, tyramine, and tannins; in *Sadhier* carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine; in *Zutho* carbohydrate derivatives, histamine, tyramine, 2-phenylethylamine and tannins; in *Khe* carbohydrate derivatives, methylamine, tyramine, 2-phenylethylamine and tannins.

Abbreviations:

MD- Doctor of Medicine

FIM- Functional Independent measure

rDNA- Recombinant Deoxyribonucleic acid

LAB- Lactic Acid Bacteria

EDI- Estimated Dietary Intake

RDA- Recommended Dietary Allowance

HPLC – High Performance Liquid Chromatography

TRB- Technology Related Barrier

BC- Before Christ

ROS- Reactive Oxygen Species

AIDS- Aquired Immunodeficiency Syndrome

LCMS- Liquid Chromatography Mass Spectrometry

Rf- Retardation Factor

SM- Secondary Metabolites

DSHEA- Dietary Supplement Health and Education Act

CVDs- Cardiovascular Diseases

Introduction:

Metabolites are the by-products of metabolic reactions that are catalysed by a number of enzymes discovered inside cells. Although it is frequently used to describe larger entities, this phrase is mostly used to describe tiny molecular components. The cell produces primary metabolites because they are necessary for their growth. Amino acids, alcohols, vitamins (B2 and B12), polyols, organic acids, and nucleotides (such as inosine-5'-monophosphate and guanosine-5'-monophosphate) are significant examples.

Compounds that an organism generates referred to as secondary metabolites supply essential environmental and other functions however are not necessary for primary metabolic activities. They encompass pharmaceuticals, flavourings, scents, pigments, pigments, insecticides, and food additives bringing applications in pharmacy, industry, and agriculture (1).

Secondary metabolites (SM) are substances that have a function in the manner that an organism interacts with its environment but are not necessary for an organism to live. These substances frequently play an essential part in plants' defence against biotic or abiotic pressures. Secondary metabolites belong to numerous categories of metabolites as well as can be substantially induced in response to stressful circumstances. Primary metabolites take part in nourishment and reproduction in order to perform important metabolic functions (2).

Metabolites serve diverse survival functions in nature. They are very important for the human health and economics of our society. They include antibiotics, antitumor agents, cholesterol-lowering drugs, immunosuppressants, antihelmintic agents and other antiparasitics, herbicides, ruminant growth stimulators, agricultural fungicides, bio-insecticides, and others. The most important secondary metabolites have been the anti-infective drugs and, among these, the β-lactams are the most important class. Other important classes include the aminoglycosides, tetracyclines, macrolides, lipopeptides, polyenes, and the echinocandins.

Many successful microbial secondary metabolites, including the anthracycline doxorubicin and bleomycin, are used for the treatment of cancer. Taxol and camptothecin are two different plant-based antitumor medications that have proven to be quite effective. If modern medicine is to continue in its present form, novel families of antibiotics and other secondary metabolites must continue to be discovered and enter the marketplace at

regular intervals (3). Metabolites are essential for the normal operation of cells and fulfill various physiological functions.

a) Competitive weapons against other livings such as animals, plants, insects, and
b) Microorganisms
c) Metal transporting agents
d) Agents for symbiotic relation with other organisms
e) Reproductive agent and
f) Differentiation sectors
g) Agents of communication between organisms (4)

Germination and interference with spore production are two other activities. Biological actions such as antibacterial and antiparasitic drugs, enzyme inhibitors and anticancer agents, immunosuppressive agents, etc. are mostly carried out by secondary metabolites. Secondary metabolites have a significant impact on how plants adapt to their changing environment and how they overcome stress-related obstacles. Brown pigments produced by the condensation of chlorogenoquinone with proteins in tobacco limit the spread of stress-induced tissue damage. Beans subjected to heat shock and water stress, respectively, accumulate polyamines and form phenylamides, which perform ROS scavenging (5).

Role of Traditinal Rice Beer in North-East India

The Brahmaputra and Barak River plains, the North-east hills, and the Eastern Himalayas are the three physiographic divisions of the North-east region of India. This region is having very remarkable climate with mostly humid, sub-tropical with hot summer. Moreover, it also has severe monsoons and mild winter (6). The Northeast India is consisting of many ethnic tribes and communities having diverse customs and lifestyles with difference in their preparation and consumption of fermented rice beer (7).

Traditional beer plays a significant role in the cultural and social fabric of Northeast India. The region is known for its diverse indigenous communities, each with its unique traditions, including the production and consumption of traditional beers. These traditional beers hold immense cultural, social, and economic significance in the region. Here are some aspects of the role of traditional beer in Northeast India:

Cultural Significance:

Traditional beer is deeply rooted in the cultural practices of various tribes and communities in Northeast India. Rice beer has played an important role in religion, and

closely associated with their worship. It is apparent that alcohol drinking is influenced by factors such as genetic, socio-environmental, culture, age, gender, accessibility, exposure and personality (8). Brewing beer is often seen as a communal activity, where women from the community come together to prepare it using locally available ingredients and traditional brewing techniques. These beers are associated with cultural identity and heritage, preserving and passing down traditional knowledge from one generation to another.

Social Bonding:

Traditional beer serves as a social glue, bringing people together for celebrations, gatherings, and social interactions. It is often consumed during community feasts, weddings, harvest festivals, and other communal events. Sharing and drinking traditional beer fosters a sense of togetherness, strengthens social bonds, and promotes a spirit of camaraderie among community members.

Economic Importance:

Traditional beer production contributes to the local economy in Northeast India. It creates livelihood opportunities for a family who are involved in brewing and selling beer. Additionally, the production and consumption of traditional beer can also support the local agricultural sector as it requires the use of locally grown grains, herbs, and fruits. In addition to its cultural significance, traditional rice beer also has economic importance in the region. Many households in rural areas engage in small-scale production of rice beer, which serves as a source of income. It has also gained commercial value, with some entrepreneurs.

Traditional Knowledge and Practise:

Different climatic circumstances and the use of locally accessible natural resources may have contributed to the emergence of ethnic rice beer preparation and its variants in consumption (9). Traditional beer-making techniques in Northeast India involve unique recipes and methods passed down through generations. The brewing process often incorporates locally Because it is probiotic-enriched, rice beer is regarded as Mother Nature's elixir. It is a natural antioxidant since it is a rich source of nutrients. The extensive range of therapeutic advantages and medicinal qualities of rice beer are widely recognised. It works wonders to heal infections, prevent gastrointestinal diseases, reduce pain, increase strength, and more. After delivery, newborns in indigenous cultures are given a few drops of the traditionally prepared rice beer. available ingredients like rice, millets,

maize, and herbs, which are specific to the region. By continuing the practice of brewing traditional beer, communities in Northeast India preserve their indigenous knowledge, culinary traditions, and cultural heritage. Each community has its unique method of preparation, using locally available ingredients, such as specific strains of yeast or herbs, which are believed to enhance the flavor and quality of the brew. Elders in the community play a crucial role in teaching younger generations the techniques and rituals associated with rice beer brewing, thereby preserving cultural heritage. The ethnic tribes consume traditional rice beer not only for social, cultural, ritual activity but they also consume it for nutritive value and are also believed to improve their health. Traditional rice beers from many tribes can help people stay healthy and avoid a wide range of ailments (10).

Tourism and Cultural Exchange:

Traditional beer serves as a significant attraction for tourists visiting Northeast India. Travelers are often interested in experiencing the local culture, including trying traditional food and beverages. The availability of traditional beers in local markets, festivals, and cultural events provides tourists with an opportunity to engage in cultural exchange and learn about the rich traditions of the region.

Symbol of Identity:

Rice beer serves as a symbol of identity for the indigenous communities of North East India. It reflects their unique cultural heritage, customs, and traditions. The brewing and consumption of rice beer are deeply rooted in the history and folklore of these communities. It symbolizes their close connection with nature, agricultural practices, and ancestral wisdom. Rice beer acts as a marker of their distinct cultural identity, setting them apart from other regions and contributing to the richness and diversity of India's cultural tapestry.

Because it is probiotic-enriched, rice beer is regarded as Mother Nature's elixir. It is a natural antioxidant since it is a rich source of nutrients. The extensive range of therapeutic advantages and medicinal qualities of rice beer are widely recognised. It works wonders to heal infections, prevent gastrointestinal diseases, reduce pain, increase strength, and more. After delivery, newborns in indigenous cultures are given a few drops of the traditionally prepared rice beer. Overall, traditional beer in Northeast India holds a multifaceted role, encompassing cultural, social, and economic aspects. It not only connects people to their cultural roots but also contributes to community bonding, economic sustainability, and the preservation of traditional knowledge.

Natural Metabolites Role in Traditional Beer:

Metabolites serve diverse purposes, encompassing roles as energy sources, structural components, messengers for signaling, regulators of enzyme activity through both activation and inhibition, and even possess catalytic capabilities. The natural metabolites present in traditional rice beer of North East India play important roles in its flavor, aroma, and potential health benefits.

Metabolites that are found to be traditional beer samples are organic acids, carbohydrates, amino acids, alkaloids, sterols, volatile aromatic compounds, different mineral elements.

Organic Acids:

A variety of organic acids were present in the samples and most of them are natural products of microorganisms or intermediates in their major metabolic pathways (11). These organic acids contribute to the unique and distinctive tartness and flavours of beers and are also responsible for the organoleptic properties apart from aiding in preservation process. The presence of these organic acids helps in increasing the shelf life of the products by inhibiting the growth of spoilage bacteria like *Escherichia coli* and *Salmonella spp.* (12,13). Such inhibition of microbial growth by organic acids is affected by several factors such as inhibition of essential metabolic reactions (14), membrane disruption (15), stress on intracellular pH homeostasis (16) and the accumulation of toxic anions (17).

Carbohydrates:

Carbohydrate provides energy body. The carbohydrates present in beer samples, found in varying amounts, are likely the primary providers of energy. Among these carbohydrates, monosaccharides specifically contribute to the sweetness of the beverage. The presence of simple sugars like glucose represents the easily metabolized carbohydrate, whereas other complex forms like raffinose can act as dietary fibres which have several health benefit effects such as prevention of heart diseases, diabetes, obesity and certain gastrointestinal diseases (18).

Amino Acids:

Amino acids serve as the fundamental components or building blocks that make up proteins. They are essential for the growth and development of yeast. They also contribute to various aspects of the brewing process, including flavor development and the formation of aroma compounds. The relatively high levels of amino acids found in rice beer indicate that it can be a valuable source of essential nutrients and energy for the body's metabolic processes. Moreover, the presence of certain amino acids suggests the existence of low

molecular weight peptides in rice beer, which possess both bioactive and sensory properties (19).

Volatile Aromatic Compounds:

A variety of volatile compounds have been identified as odor-active components in rice beer, contributing to its distinct aroma profile. These compounds are known to impart alcohol-like, sweet, fruity, buttery, and pungent aromas to the beverage (20). Many volatile and non-volatile components contribute to the distinctive flavour of beer (21) and a diverse group of volatile and semi-volatile aromatic compounds were detected in the samples. The impact of these volatile compounds on the overall aroma profile of rice beer is contingent upon their respective threshold values. The perception and contribution of these compounds to the beer's aroma will vary based on their concentration levels and individual sensory thresholds. Phenylethyl alcohol is the most abundant compound in the samples studied and is an important constituent in many essential oils, flavours, and perfumery and moreover it has antimicrobial properties (22).

Different Mineral Elements:

Humans require more than 22 mineral elements, all of which can be supplied by an appropriate diet. However, however improper diet plans very often lead to a deficiency of minerals such as iron, zinc, calcium, magnesium, copper, or selenium. Besides other trace elements such as copper and zinc are essential for and human and animal nutrition (23). The presence of minerals in alcoholic beverages can be attributed to several factors. These include natural sources such as raw materials, the composition of the soil in which the ingredients are grown, the water used in the production process, and even the yeast involved in fermentation. Additionally, the presence of minerals can also result from potential contamination that may occur during various stages of production, transportation, and storage of the beverages. Their levels in beer can be a significant parameter affecting its consumption. These minerals have various beneficial effects on the human body. For example, iron (Fe) is an essential component of hemoglobin, myoglobin, and other enzymes. Zinc (Zn), magnesium (Mg), and copper (Cu) are also crucial for numerous enzyme functions. Copper additionally contributes to the structure of hair, bones, and other organs in the body. These minerals play vital roles in supporting essential physiological functions and overall human health (24).

Alkaloids:

Alkaloids are a class of nitrogen-containing compounds that are found in plants. They are often bitter and have a variety of pharmacological effects. Alkaloids can have a variety of effects on the flavor of rice beer. Some alkaloids can add a bitter or astringent taste to the beer. Others can contribute to the beer's aroma. Some alkaloids have also been shown to have psychoactive effects. For example, the alkaloid caffeine is found in some traditional rice beers. Caffeine is a stimulant that can increase alertness and energy levels (25). In addition to the above, alkaloids can also contribute to the nutritional value of traditional rice beer. Some alkaloids are essential nutrients for humans, and they can help to improve the body's ability to absorb other nutrients. For example, the alkaloid thiamine is essential for the metabolism of carbohydrates. It is also involved in the production of energy and the function of the nervous system.

Sterols:

Sterols are metabolites that help to protect nerves and makes cell tissues and certain hormones. They are important for a variety of functions, including cell membrane structure, hormone production, and cholesterol metabolism. Sterols can have a variety of effects on the flavor of rice beer. Some sterols can add a nutty or oily taste to the beer. Others can contribute to the beer's aroma (26).

In addition to the above, sterols can also contribute to the nutritional value of traditional rice beer. Some sterols are essential nutrients for humans, and they can help to improve the body's ability to absorb other nutrients.

Role of Herbal Nutraceutical:

In 1989, Stephen Defelice, MD, the founder and chairman of the foundation for innovation in medicine (FIM) Cranford, New Jersey, combined the words "nutrition" and "pharmaceutical" to create the term "nutraceuticals (27). Nutraceuticals, according to Defelice, are foods or components of foods that have health advantages, such as the ability to prevent or treat disease" (28). Let food be your medicine, said Hippocrates, a Greek physician regarded as the founder of medicine. "Focus on prevention" is the guiding principle. Dietary supplements, functional foods, multi-functional foods, etc. are other terms used in this area. Functional foods are regular foods with added components or substances that provide them with additional health benefits beyond their nutritional value (29).

A dietary supplement is defined as a product intended to supplement the diet that bears or contains one or more of the dietary ingredients listed below: a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients. Dietary supplements are also defined as products that contain one or more of these ingredients. It can be consumed in the form of a tablet, capsule, or liquid. It is not intended to be consumed as a typical food or as the only component of a meal or diet. It has the designation "dietary supplement"(30). According to the Dietary Supplement Health and Education Act (DSHEA) of 1994, it is the responsibility of the dietary supplement manufacturer to ensure the safety of the product prior to its introduction into the market (31).

The global nutraceutical market is expanding primarily due to current demographic and health trends. Nutraceuticals, which can be broadly defined as foods or food components that have a significant impact on maintaining and enhancing normal physiological functions for overall health, play a key role in this growth. Dietary fibre, prebiotics, probiotics, polyunsaturated fatty acids, antioxidants, and other diverse kinds of herbal/natural foods are some of the food products used as nutraceuticals (32). Nutraceuticals have emerged as valuable allies in combating several major health issues of the century. Conditions such as obesity, cardiovascular disease, cancer, osteoporosis, arthritis, diabetes, and high cholesterol are being addressed with the assistance of nutraceuticals. These products offer potential benefits and play a supportive role in managing and mitigating the impact of these health concerns. Overall, the term "nutraceutical" has ushered in a new era of medicine and health, one in which the food industry has evolved into a field focused on research (33).

In many pathological problems, such as diabetes, recent investigations have revealed these substances to have promising outcomes and also diseases like athrosclerosis, cardiovascular diseases (CVDs), cancer, and neurological disorders These conditions encompass various physiological changes, including modifications in the redox state (34,35). The majority of neutraceuticals possess antioxidant activity, which allows them to counteract the imbalanced redox state associated with various health conditions. Therefore, they are regarded as nutritious options of health promotion, especially for prevention of life-threatening diseases such as diabetes, infection, renal, and gastrointestinal (36,37) disorders.

Herbal nutraceuticals are nutraceutical that are obtained from natural sources with no added chemicals or toxins. These products demonstrate superior efficacy, safety, and tolerability compared to conventional products currently available in the market. It helps in maintaining health and acts against nutritionally induced acute and chronic diseases, which promotes optimal health, longevity, and quality of life. Some common roles and benefits associated with herbal nutraceuticals:

1. Nutritional Support

Herbal nutraceuticals can provide essential nutrients, including vitamins, minerals, and antioxidants, which are important for overall health and well-being. They can complement a balanced diet and help fulfill nutritional gaps.

2. Health Promotion and Disease Prevention

Many herbal nutraceuticals possess bioactive compounds that have been studied for their potential health benefits. They may support immune function, promote cardiovascular health, aid in digestion, and provide anti-inflammatory and antioxidant effects. These properties can help prevent certain diseases and promote overall wellness.

3. Traditional Medicine

Herbal nutraceuticals often draw from traditional medicine systems, such as Ayurveda. Ayurveda is recognized as a holistic system that prioritizes disease prevention and health promotion. Ayurvedic plants possess a more potent influence on the body compared to regular food or spices. These plants have the ability to actively counteract and reverse pathophysiological processes, contributing to the restoration of overall well-being.

Traditional belief of Beer and different types of Beer in Northeast India

An ethnic representation of the rich and varied culture of North-East India is rice beer. The indigenous of North-eastern tribal communities who live in the mountains, particularly the foothills of the Himalayas, drink rice beer, which is brewed using ancient techniques. In many tribal societies, these fermented rice beverages go by different names. For instance, the Garo tribe of Meghalaya calls them Sadhier, the Mishing tribe of Assam calls them *Apong*, and the Angamis tribe of Nagaland calls them Zutho. Additionally, the components used to make the rice beverages are indigenous to the tribes, depending on the geographical habitats (38).

Because it is fortified with probiotics, rice beer is regarded as Mother Nature's elixir. It is a natural antioxidant because it is a rich source of nutrients. The extensive range of therapeutic advantages and medicinal qualities of rice beer are widely known. It works wonders to treat infections, prevent gastrointestinal diseases, relieve pain, increase

strength, and more. Following delivery, newborns in indigenous tribes are given a few drops of the rice beer that is customarily brewed (39).

Rice beer brewing and consumption in India's north-eastern states have long been supported by their sociocultural legacy. Idioms, folktales, ballads, and numerous folk dance forms all stress the need of making rice beer to establish a close connection between nature and humanity. Making rice beer is an essential component of the many traditional events held in the north-eastern States. Bihu, an Assamese harvest festival that honours the changing of the seasons, is a well-known illustration of this type of celebration. The agricultural community celebrates with traditional rice beer brewing and drinking while taking pleasure in or taking part in the celebrations, in addition to prayers, caring for their animals, song and dance performances, and several other sporting events.

Table 1: List of different types of beers in Northeast India

State	**Tribe**	**Rice beer**
Assam	Ahom	*Xaj-pani & Laopani*
	Boro	*Jou bishi*
	Deori	*Sujen*
	Karbi	*Hor*
	Mishing	*Apong*
	Rabha	*Junga Mod*
	Sonowal	*Rohi*
Aruncahal Pradesh	Adi, Nysshing & Mishmi	*Opo*
	Monpa	
	Deori and Khampuri	*Poka*
	Miji	*Rakshi*
	Hill miri	*Mingri*
Manipur	Meiti	*Sekmai*
Mehalaya	Pnar	*Sadhier*
	Garo	*Bitchi*
	Khasi	*U-Phandieng*
Nagaland	Angami	*Zutho*
Sikkim	Gorkha	*Baati jhar*
Tripura	Kalai	*Chuwak*

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Literature Review on '*Apong*'

Borah T *et al.* (2019) stated that the most potential isolate was identified as *Bacillus velezensis* strain on the basis of rDNA gene sequencing which was found to be fulfilling all the basic criteria to be considered as a potential Probiotic candidate. The non-haemolytic and non-cytotoxic nature of the isolate further advocates its potential application for commercial exploitation after further research (1).

Handique P *et al.* (2020) stated that it appears that total phenolic content is a major contributing factor toward antioxidant capability of these beverages. Catechin, caffeic acid p-coumaric acid, ferulic acid and salicylic acid were detected in *Apong* and rice-based alcoholic beverages of mishing tribe of Assam (India) exhibit good antioxidant activity, and this may be attributed to the presence of different phenolic compounds. This study indicates that the traditional rice-based alcoholic beverages are potent sources of antioxidants, consumption of which in moderate quantity can contribute toward the well-being of human health (2).

Das S *et al.* (2019) stated that the presence of LAB (Lactic Acid Bacteria), Acetobacter as well as yeast and moulds play an important role in the final characteristics of rice beer. The synergistic actions of the microbial consortia converted the starchy materials present in rice into potentially beneficial compounds which included mannobiose, sugar alcohol, organic acids and amino acids. The presence of LAB and nutraceuticals in the rice beer may provide health benefts to the consumers which needs to be studied further (3).

Handique P *et al.* (2017) stated that the concentrations of some metals, viz. Na, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb, have been estimated in alcoholic beverages traditionally prepared and consumed by ethnic communities of Assam (India), viz. Deori (sample- *sujen*), Mishing(sample-*Apong*) and Ahom(sample-*Xaj pani*). The EDI(Estimated daily intake) owing to consumption of the traditional beverages was also evaluated and compared with the recommended dietary allowances (RDA). The EDI is well within the limits of the RDA. All of the samples examined can be considered safe for consumption and can be used as supplements of the essential metal nutrients such as K, Mn, Fe and Zn (4).

Yein J *et al.* (2022) stated that alcoholic beverages, particularly Mishings' *Apong*, are often thought to have medicinal properties by them and are primarily consumed as a relaxant. It's also used as an offering in religious ceremonies, crisis rites, and other celebrations, so it's a drink with cultural significance in Mising society. However, the drink

is only brewed in households, and no attempt has been made to commercialise it. The recipe for the drink is a gendered affair, and it is usually only known by the women in the community. There is variation in the brewing process in different regions due to the lack of a standard procedure for the knowledge of the recipe being passed down orally for generations. As a result, a thorough scientific examination of the ingredients and the brewing process will standardise the process. The scientific method will ensure that the right amounts of ingredients are used and that the medicinal properties of the ingredients are preserved. The beverage can then be used for commercial production once the procedure has been formally documented in a scientific manner. Commercialization of the drink could result in revenue that could be used for Mishing society's overall development as well as provides employment for the community's youth (5).

Literature Review on '*Xaj-Pani*'

Keot j *et al.* (2020) stated that this study reports the microbial quality of ethnic starter culture *Xaj-pitha* used for rice wine fermentation in Assam. Here, we collected 60 *Xaj-pitha* samples belonging to Ahom community of the state and enumerated the microorganisms using spread plate technique. Illumina-based whole genome shotgun sequencing detected the presence of microbial contaminants like *Acidovorax*, *Herbaspirillum*, *Methylobacterium, Pantoea, Pseudomonas, Stenotrophomonas, Staphylococcus, Micrococcus, Acinetobacter*, etc. Presence of major health hazards associated with spontaneous rice wine fermentation necessitated method optimization through the development of a defined mixed starter culture. For this, functionally important α-amylase producers viz., Penicillium*, Rhizopus oryzae*, *Mucor guilliermondii* and *Amylomyces rouxii* and eight yeasts viz., *Saccharomyces cerevisiae, Wickerhamomyces anomalus, Saccharomycopsis malanga, Saccharomycopsis fibuligera and Saccharomycopsis malanga* were retrieved using appropriate media. All the mould cultures tested negative for aflotoxins production. Among the yeasts, *Saccharomyces cerevisiae* and decarboxylated lysine HCl and tyramine HCl, respectively, indicating their biogenic amine production ability. For defined mixed starter culture, *Amylomyces rouxii* with α-amylase (5.92 U/ml) and glucoamylase (7.50 U/ml) activities was selected as fungal partner; while *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae* with high ethanol production (up to 10.11% and 9.88% v/v, respectively) were selected as yeast partners. The mixed culture was able to produce high amount of glucose, ethanol and liquid (glucose 10.91% w/v; ethanol 7.5% w/v; liquid 51.0% w/v. Microbial diversity and dynamics of *Xaj-pitha*

was assessed through the culture-dependent approach. The starters contained high counts of fungi, lactic acid bacteria (LAB) and aerobic bacteria (6).

Das AJ *et al.* (2014) stated that the results of the HPLC analysis for the content of different organic acids present in the *Xaj-Pani*. Lactic acid was found to be predominant amongst all of the other acids and its concentration varied significantly. It was recorded as the highest (9119.42 mg/L) in *Xaj-Pani*. Organic acid like- Lactic acid, Oxalic acid, Pyruvic acid, Formic acid are found in *Xaj-Pani*. Trehalose was also detected in *Xaj-Pani.* Carbohydrates like - Glucose, Trehalose, Arabinose, Galactose. Methionine also present in *Xaj-Pani* (7).

Kalita P *et al.* (2021) stated that the tribal people follow fermentation technique unknowingly and have been using microbes for thousands of years to prepare rice beer. Factors that induce beer production primarily include the amylolytic and alcohol producing yeasts (*Saccharomyces cerevisae, Debaryomyces hansenii*), lactic acid bacteria (*Lactobacillus sp., Pediococcus sp. Leuconostoc, Enterococcusand Lactococcus*) and starch degrading moulds *(Mucorand Rhizopussp*) . In the fermentation technique, these microorganisms act as a source of enzyme, Zymaseto converting raw materials of the TRB into fermented forms. The herbal components of different parts of leaves, barks and other parts used in the preparation of the fermented beverage contain a variety of phytochemicals viz., phytoestrogens, terpernoids, carotenoids, limnoids, phytosterols, glucosinolates, polyphenols, flavonoides, iso-flavonoids and anthocyanidins. The *Xaj pani* in Ahom community is used as energy booster during physical labour. It is naturally loaded with probiotics and used as therapeutics for tackling anxiety, depression, stress and to improve overall mood (8).

Bhuyan B *et al.* (2013) stated that *Xaj-Pani* is prepared by using many medicinal plants like- *Centella asiatica, Cinnamomum bejolghota, Cissampelos pareira, Clerodendrum viscosum, Croton caudatus* and many more. All these plants have ethno medicinal properties. These plants are used in chronic dysentery, spermatorrhoea, liver disorder, carbuncle, cuts and wounds, nervous deability, and hermicrania, roots are used in stomach ache, leaves are used in dyspepsia and gastric trouble. Leaves are used as anti-diabetic agent and also used in urinary trouble (9).

Fuloria S *et al.* (2022) stated that the Ahom communities routinely consume this traditionally prepared alcoholic drink with no understanding of its quality and shelf life.

Additionally, a finally produced dried cake, known as *vekur pitha* act as a source of *Saccharomyces cerevisiae* and can be stored for future use. Despite the rampant use in this community, the relationship between *Xaj-pani* consumption, immunological response, infectious and inflammatory processes remain unknown in the presence of factors unrelated or indirectly connected to immune function. Overall, this review provides the guidelines to promote the development of prebiotic- and probiotic-containing functional fermented rice that could significantly have an impact on the health of the consumers. the rice beer preparation done by the Ahom Community of Assam which is deemed as among the favorite. The drink is prepared by rice fermentation with a mix of rare herbs and plays an important role in the socio-cultural lives of the people. Its mild consumption seems to bring relaxation and impart good therapeutic values as it helps in tackling anxiety, depression, stress which uplifts the overall mood of the hardworking population without any reported major side effects (10).

Literature Review on '*Sadhier*'

Jaiswal (2010) states that alcoholic beverages have been used since many centuries BC. They were and are still one of the important parts of many human activities and endeavours including research. It is also being commonly consumed by various individual groups of the society. In the Indian context, wine and alcoholic beverages are important to many societies especially during festivities. These are usually produced and used locally. Hence the types and quality differ greatly with varying traditions. Scientific studies of such process are very important to highlight the various aspects related to the production process and quality of the product. The consumption of rice beer prepared from rice is a common practice among many tribal communities residing in the North-Eastern states of India and many of them have been preparing it since time immemorial. Rice beer (*Kiad*) is also commonly used by the people of Khasi (Khasi Hills) and *Pnar* (Jaintia hills) in Meghalaya during various religious festivals and ceremonies (11).

Samati H *et al.* (2007) stated that in their studies that kiad plays a vital role in the various socio-cultural lives of *Pnar* people associated with each and all religious festivals and ceremonies. Minimum consumption is considered to be good for health and acts as a remedy for various ailments/diseases. *Sadhier* is use to cure urinary trouble, *Kiad-thnam* is used to cure dysentery. A minimum consumption daily, before or after meal is advisable as a health tonic, but maximum or excess consumption may be intoxicated and harmful. *Kiad* production serves as a good source of income for livelihood of the *Pnar* people and a source

of revenue to the state. The nutritional and medicinal potentials of *Kiad* need further detailed study. Many of the tribal people have not carried out modern experiment as they still have an indigenous method of local liquor preparation coupled with a clear understanding of nature and how it works. Therefore, there is an urgent need to take every effort to document, preserve and encourage the practice of the traditional method of the tribal people and scientifically validate before it is too late, for the prosperity of mankind (12).

Literature Review on '*Zutho*'

Teramoto *et al.* (2002) stated that *Zutho* had a fruity aroma and sour taste and its unique aroma had characteristics similar to those of Japanese sake and sprouted rice sake.After biochemical and microbial characterization they reported *Saccharomyces cerevisiae* as main yeast in *Zutho* (13).

Mao A. A. (1998) stated that *Zutho* or *Zhuchu* is an ethnic alcoholic beverage of the Mao Naga prepared from rice (14).

Das*et* J. *et al.* (2012) stated that *Zutho* is known to boost the immune system, lower the blood insulin level, prevent loss of appetite, lower bad cholesterol, assist in wound healing, and prevent infection (15).

Anupma *et al.* (2018) *Khrie/khekhrii* an amylolytic starter culture prepared by germinated sprouted rice grains in Nagaland. This is the only amylolytic starter in North-East India which is not prepared by using the old starter through back-sloping method, rather it is prepared by fermenting germinated sprouted-rice grains and then sun-dried to use as dry starters to prepare the local alcoholic drink called *Zutho* (16).

Literature Review on '*Khe*'

Das A. J. *et al.* (2012) discuss that it plays an important role in the sociocultural life of the tribal people as it is found to be associated with many occasions like merry making, ritual ceremonies, festivals, marriages and even death ceremonies (17).

Miyaji T. *et al.* (2015) reported that this study broadened the prospects of the particular bacterial and yeast strains to be utilized in the preparation of fermented alcoholic beverages. Presently the production of beer in northeast India is limited to households and the manufacturers sell their produce at a domestic level or in the local markets. This optimized methodology for the production of a good quality rice beer has techno-economic feasibility and the potential to promote entrepreneurship (18).

Bhuyan D. J. *et al.* (2014) In their work stated that the rice beer produced in North East India is nutritionally rich and have high therapeutic values. The presence of antiradical activity and other earlier evidences also suggest the possible medicinal properties of this traditional drink (19).

Khawas P. *et al.* (2014) stated that Rice from the North eastern states of India are a potential source of nutrition, owing to their various biochemical compositions such as carbohydrates, amino acids, organic acids and aromatic compounds in appreciable amounts. The average consumption of rice beer by tribal communities in northeast India is around two glasses (~400 mL) in the evening, 3–4 days in the week and no health complications related to the consumption of rice beer have been reported to date (20).

Handique P. (2019) In his work claimed that in traditional rice beers the microflora present in dried starter tablets converts starchy materials to fermentable sugars and subsequently to alcohol and organic acids. The antioxidant activity of rice beers may be derived from the different plant ingredients used for preparation of starter cultures. Analysis of microbial population and antioxidant activity of starter cultures are essential as quality of rice beer is dependent largely on the raw materials used (21).

No literature has been reported on *'Khe'* rice beer.

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Aim and Objectives:

Folklores of Northeast India, prepare rice beers traditionally and with different methods as it is a common socio-culture of several tribes of Northeast India. These traditional beverages may contain many different levels of contaminants and therefore it is essential to understand chemical profile of these traditional formulations, so as to standardise these samples by means of its belief to act as preventive, prognostic, and curative medicine for several ailments. With this aim, the following objectives have been planned:

1. To collect different Traditional beer samples from different parts of N.E., India.
2. To assess phytoconstituents present in the samples qualitatively.
3. To assess metabolites quantitatively by Thin Layer Chromatography and Liquid Chromatography Mass Spectrometry.

Materials and Methods:

Materials

The traditional rice beers sample (1) *'Apong'* was collected from Itanagar (Arunachal Pradesh), sample (2) '*Xaj pani*' was collected from Rani, Kamrup metro (Assam), sample (3) '*Sadhier*' was collected from Jowai, West Jaintia Hills (Meghalaya), sample (4) '*Zutho*' was collected from Kohima (Nagaland) and sample (5) '*Khe*' was collected from Kohima (Nagaland). The solvents and other chemicals were acquired commercially and were used as such without testing and purification. In the Table 2 the following chemicals and solvents have been listed respectively.

Table 2: The list of chemicals needed was procured from the following companies

Chemical Name	Company Name
Silica gel G	Sisco Research Laboratories Pvt.Ltd.
Methanol	Loba Chemie Pvt.Ltd.
Ethyl acetate	Thermo Fisher Scientific India Pvt.Ltd.
Hexane	Thermo Fisher Scientific India Pvt.Ltd.
Chloroform	Finar Limited
Acetone	Pallav Chemicals & Solvents Pvt.Ltd.
Phenol Crystalline	Pallav Chemicals & Solvents Pvt.Ltd.
Iodine	Thermo Fisher Scientific India Pvt.Ltd.
Molisch solution	Nice Chemical Pvt.Ltd.
Sulphuric Acid concentrate	Pallav Chemicals & Solvents Pvt.Ltd.
Dragendorff reagent	Pallav Chemicals & Solvents Pvt.Ltd.
Ferric chloride	Thermo Fisher Scientific India Pvt.Ltd.
Methanol	Avantor Performance Materials India Pvt.Ltd
Hydrochloric Acid	Thermo Fisher Scientific India Pvt.Ltd.
Sulphuric Acid	Pallav Chemicals & Solvents Pvt.Ltd.

Methods

Authentication and Preparation of the plant material

The fresh traditional rice beer sample (1) *'Apong'* was collected from Itanagar (Arunachal Pradesh), sample (2) '*Xaj pani*' was collected from Rani, Kamrup metro (Assam), sample (3) '*Sadhier*' was collected from Jowai, West Jaintia Hills (Meghalaya), sample (4) '*Zutho*' was collected from Kohima (Nagaland) and sample (5) '*Khe*' was

collected from Kohima (Nagaland) as authenticated and claimed by traditional tribes of the community of mishing. Fresh samples were collected of approximately 100 mL, then stored in airtight containers free from moisture at 4^0C until further use.

Phytochemical Investigation

Tests for Alkaloids Dragendroff's Reagent:

To 3 mL of beer sample, few drops of Dragendroff's reagent (potassium bismuth iodide solution), was added and observed for the formation of an orange-brown precipitate. Hager's Reagent: To 3 mL of beer sample, few drops of Hager's reagent (saturated aqueous solution of picric acid) were added and observed for the formation of yellow precipitate. Mayer's Reagent: To 3 mL of beer sample, a few drops of Mayer's reagent (potassium mercuric iodide solution), was added and experiential for the development of cream or white-colored precipitation. Wagner's Reagent: Wagner's reagent (iodine in potassium iodide), of 2 drops was added to 3 mL of beer sample, and observed for the formation of a red-brown precipitate. (b) Tests for Amino acids Ninhydrin Test (General test): To 3 mL of the test solution, 3 drops of 5% Ninhydrin solution were added and heated in a boiling water bath for 10 min and observed for the formation of purple or bluish color.

Tests for Carbohydrates:

In 3 mL of beer sample, the following tests were performed to detect the presence of carbohydrates. Molisch's Test (General test): To 3 mL of beer sample, few drops of α–naphthol (20% in ethyl alcohol) were added. The mixture was shaken well. Then conc. H2SO4 acid of 1 mL volume was poured beside the wall of the test tube. A reddish-purple colored ring at the intersection of the two layers has appeared in the presence of carbohydrates. Benedict's test (Test for reducing sugars): Equal volume of Benedict's reagent and test solution were mixed. The blend was warmed and allowed to boil in a water bath for a period of 5 min and observed for the formation of a characteristic-colored precipitate (green, yellow, red depending on the amount of reducing sugar present in the filtrate). Fehling's Reagent (Test for reducing sugars): To 1 mL of Fehling's solution A, 1 mL of Fehling's solution B were mixed and boiled for 1 min. To this equal volume of test, solution was added and heated in a boiling water bath for 5-10 min and observed for the formation of a brick-red colored precipitate. Tests for Pentose Sugars: Equal amount of test solution and conc. Hydrochloric acid (HCL) was mixed and a crystal of phloroglucinol was added and observed for the formation of red color. Cobalt-chloride test (Test for Hexose

sugars): To 3 mL test solution, 2 mL cobalt chloride were mixed, boiled, and cooled. Few drops of sodium hydroxide solution (NaOH) were added. The solution appeared greenish-blue (glucose), purplish (fructose) or upper layer greenish-blue, and lower layer purplish (a mixture of glucose and fructose). Tollen's phloroglucinol test for galactose: To 2.5 mL conc. Hydrochloric acid and 4 ml 0.5% phloroglucinol were mixed and a 2 mL test solution was added to it and heated, observed for the formation of yellow to red color.

Tests for Flavonoids Shinoda Test:

To 1 mL of beer sample was mixed in 5 mL of alcohol (95% ethanol), was treated with few drops of conc. Hydrochloric acid and 0.5 g of magnesium turnings were added and observed for the formation of pink color.

Tests for Proteins Biuret test:

To a 3 mL test solution, 1 mL of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added and observed for the formation of pinkish or purple violet color. Million's test: To 1 mL of test solution acidified with sulphuric acid Million reagent was added and boiled. It was observed for the formation of white precipitate. After warming the precipitate turned brick red or the precipitate dissolved giving red colored solution. Protein containing sulphur: Test solution of 5 mL was mixed with 2 mL, 40% sodium hydroxide and two drops 10% lead sulfide solution. The solution was boiled and observed for the formation of a black or brownish color solution due to lead sulfide formation.

Tests for Steroids Liebermann-Burchard's Test:

A solution of 3 mL beer sample was dissolved in chloroform ($CHCl_3$) and 2 mL of acetic anhydride ($C_4H_6O_3$) was added along with few drops of conc. Sulphuric acid (H_2SO_4) from the side of the test tube and observed for the formation of first red, then blue and finally green color. Salkowski Test: To the sample, 2 mL of chloroform ($CHCl_3$) and 2 mL of conc. H_2SO_4 acid was added. Shaken well and observed for the formation of red color in the chloroform layer and greenish-yellow fluorescence in the acid layer.

Tests for Terpenoids Noller's test:

Two or three granules of tin metal were dissolved in 2 mL thionyl chloride ($SOCl_2$) solution. 1 mL rice beer sample was added into the test tube and warmed, the formation of pink color indicates the presence of triterpenoids.

Tests for Tannins and Phenolic Compounds:

To 3 mL of beer sample, few drops of the following reagents were added (1, 2).

Thin Layer Chromatography Preparation of plates:

Thin layer chromatographic analysis has been carried out as per the method of Stahl Glass plates of 3 cm x 15 cm size were coated with silica gel G, first air-dried and then activated at 110^0C for 30 min. The concentration of the beer solution was kept at 2 µL of 1% solution and spotted using a capillary tube. The spot was placed equidistant from each other, 1.5 cm above the base of the plate, and kept unimmersed in the mobile phase in the development chamber.

Development of chromatogram:

The solution was spotted on the coated and activated TLC plates, with the help of fine bore capillaries and chromatograms were developed in the chromatographic chamber with the plates kept at an angle of 75° using different solvent systems at room temperature. The plates were developed using the following solvent systems: For the analysis of presence of carbohydrate, we have used Ethyl acetate : Methanol : Water (14:3:3) and for amino acids, we have used 10% of Phenol Water; for sterols, used Hexane : Acetone (18:2); for Saponins used Chloroform : Methanol: Water (14:6:2) and for tannins Chloroform : Ethyl acetate : Methanol (2:2:16).

After the completion of the run of the solvent system, the plates were removed from the chamber and allowed to dry in the air. These plates were visualized for the spots and the Retention factor (Rf) values of the spots were calculated and recorded as given in Equation [1].

Rf = Distance travelled by the solute/ Distance travelled by the solvent … [Equation 1]

The Rf value ranges from 0-1 (3).

Liquid Chromatography Mass Spectrometry

Initially, 1 mL of respective beer sample was mixed with 1 mL of methanol and vortexed well. This mixture was centrifuged for 10 min at 9000 rpm, after which 500 µL of the supernatant was used as an underivatized sample that was analysed for LCMS-8060, Shimadzu using different multiplexed analytical columns.

Results:

Identification and Preparation of Rice Beers samples

The collected rice beers samples from indigenous regions of North east India have been identified from the traditional folklores of the respective regions.

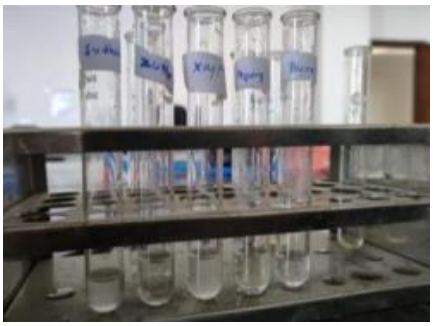
Phytochemical Investigation of Rice Beers samples

The different classes of phytoconstituents present in each beer sample, was analyzed for its phytochemical constituents as depicted in Table 3 and Figure 1.

Table 3: Results of the Preliminary phytochemical screening of rice beer samples based on the polarity of solvent

Metabolites	**Beer Samples**				
	Apong	***Xaj Pani***	***Sadhier***	***Zutho***	***Khe***
Carbohydrates	Present	Present	Present	Present	Present
Alkaloids	Present	Not Present	Not Present	Not Present	Not Present
Sterols	Present	Not Present	Not Present	Present	Present
Tannins	Present	Not Present	Very Less	Very Less	Very Less
Saponins	Very Less	Very Less	Present	Very Less	Very Less
Flavonoids	Not Present	Not Present	Not Present	Not Present	Not Present
Amino Acids	Present	Present	Present	Present	Present

(a)



(b)

(c)

(d)

Figure 1: (a, b, c, d) depicts Phytochemical tests of traditional beer samples carried at laboratory

Thin Layer Chromatographic analysis of Traditional Rice Beer Samples

The rice beer samples *'Apong'*; '*Xaj pani*'; '*Sadhier*'; '*Zutho*'; '*Khe*' were analyzed for TLC profiles. The TLC of *'Apong'* revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Saponins and Tannins with Rf values and spots as depicted in Table 4 and Figure 2. The TLC of '*Xaj pani*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols, and Saponins, with Rf values and spots as depicted in Table 5 and Figure 3. The TLC of '*Sadhier*' revealed the presence of 03 classes of compounds that include Carbohydrates, Amino Acids, and Saponins, with Rf values and spots as depicted in Table 6 and Figure 4. The TLC of '*Zutho*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins, with Rf values and spots as depicted in Table 7 and Figure 5. The TLC of '*Khe*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins, with Rf values and spots as depicted in Table 8 and Figure 6.

Table 4: Thin Layer Chromatographic analysis and Rf values of the following metabolites *Apong*

Carbohydrates	0.94
Amino Acids	0.18
Saponin	0.12
Tannins	0.42

Figure 2: TLC analysis of *Apong*

Table 5: Thin Layer Chromatographic analysis and Rf values of the following metabolites *Xaj Pani*

Carbohydrates	0.49
Amino Acids	0.33
Sterols	0.65
Saponin	0.24

Figure 3: TLC analysis of *Xaj pani*

Table 6: Thin Layer Chromatographic analysis and Rf values of the following metabolites *Sadhier*

Carbohydrates	0.93
Amino Acids	0.15
Saponin	0.16

Figure 4: TLC analysis of *Sadhier*

Table 7: Thin Layer Chromatographic analysis and Rf values of the following metabolites *Zutho*

Carbohydrates	0.36
Amino Acids	0.44
Sterols	0.24
Tannins	0.33

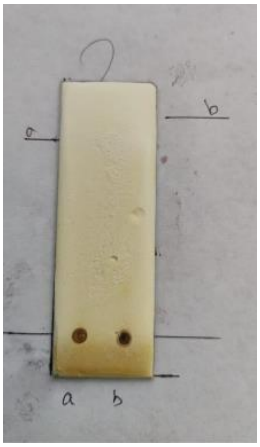


Figure 5: TLC analysis of *Zutho*

Table 8: Thin Layer Chromatographic analysis and Rf values of the following metabolites *Khe*

Carbohydrates	0.9
Amino Acids	0.58
Sterols	0.65
Tannins	0.36

Figure 6: TLC analysis of *Khe*

Liquid Chromatography Mass Spectrometric Analysis of Traditional Rice Beer Samples:

Liquid Chromatography Mass Spectrometric analysis of Traditional Rice Beer Samples was carried out to separate the derivative compounds depending on their derivatization time, temperature, volumes of samples and reagents. The compounds detected in *apong* are carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine, and tannins as depicted in the spectra Figure 7. The compounds detected in *Xaj pani* are carbohydrate derivatives, histamine, tyramine, and tannins as depicted in the spectra Figure 8. The compounds detected in *Sadhier* are carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine as depicted in the spectra Figure 9. The compounds detected in *Zutho* are carbohydrate derivatives, histamine, tyramine, 2-Phenylethylamine and tannins as depicted in the spectra Figure 10. The compounds detected in *Khe* are carbohydrate derivatives, methylamine, tyramine, 2-Phenylethylamine and tannins as depicted in the spectra Figure 11.

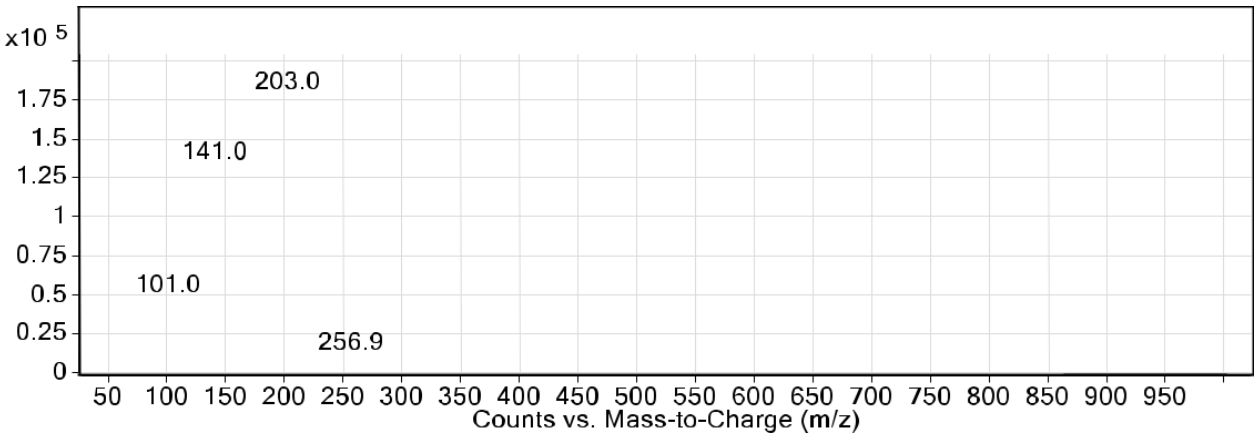


Figure 7: Liquid Chromatography Mass Spectrometric analysis spectra of *Apong*

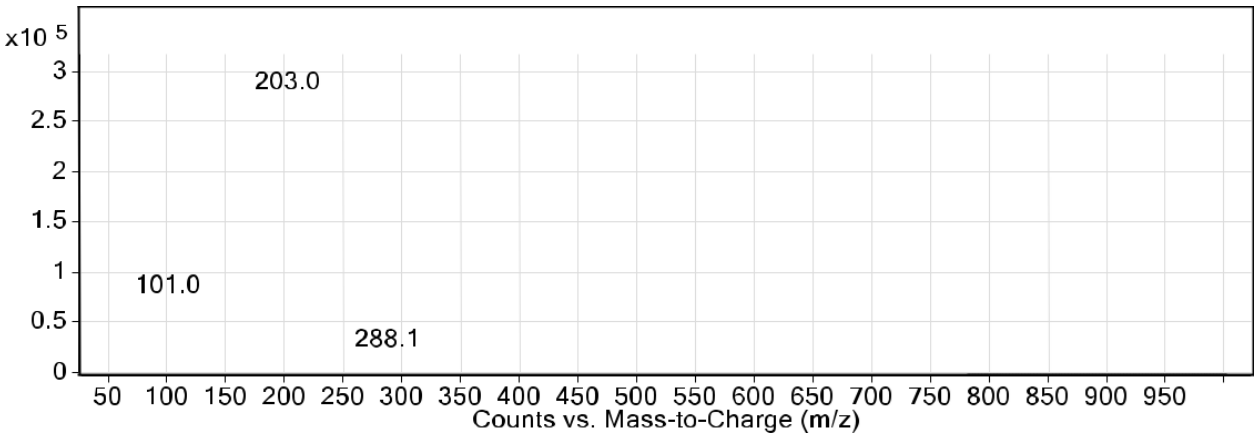


Figure 8: Liquid Chromatography Mass Spectrometric analysis spectra of *Xaj Pani*

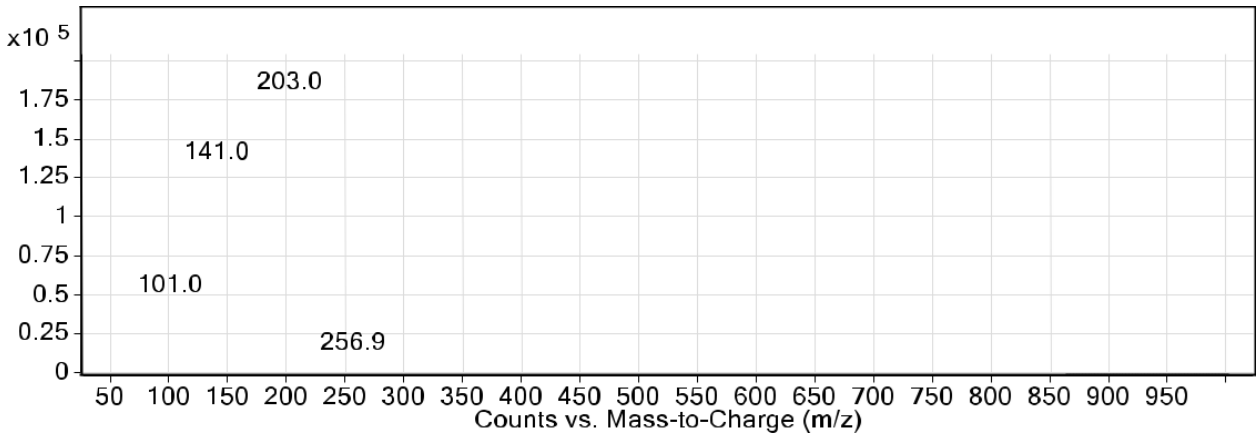


Figure 9: Liquid Chromatography Mass Spectrometric analysis spectra of *Sadhier*

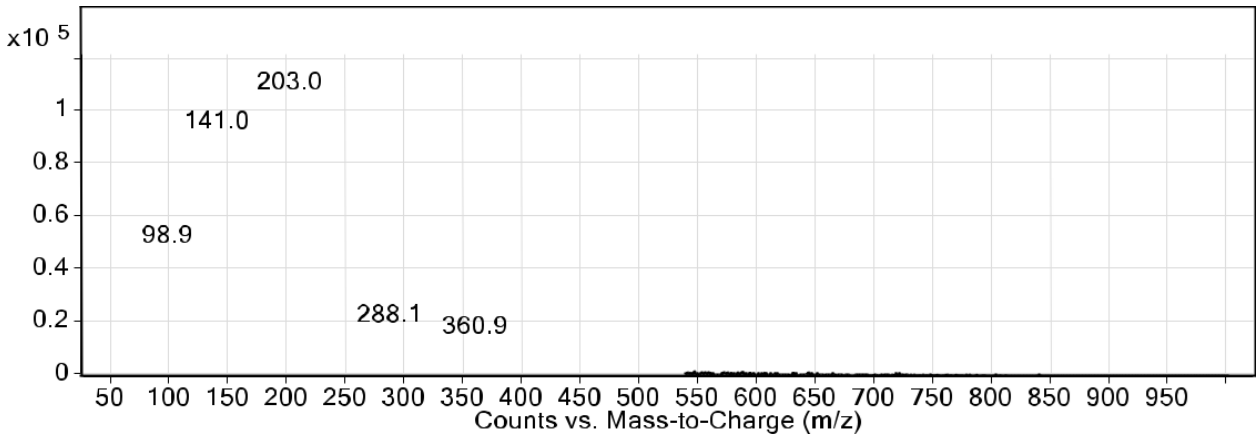


Figure 10: Liquid Chromatography Mass Spectrometric analysis spectra of *Zutho*

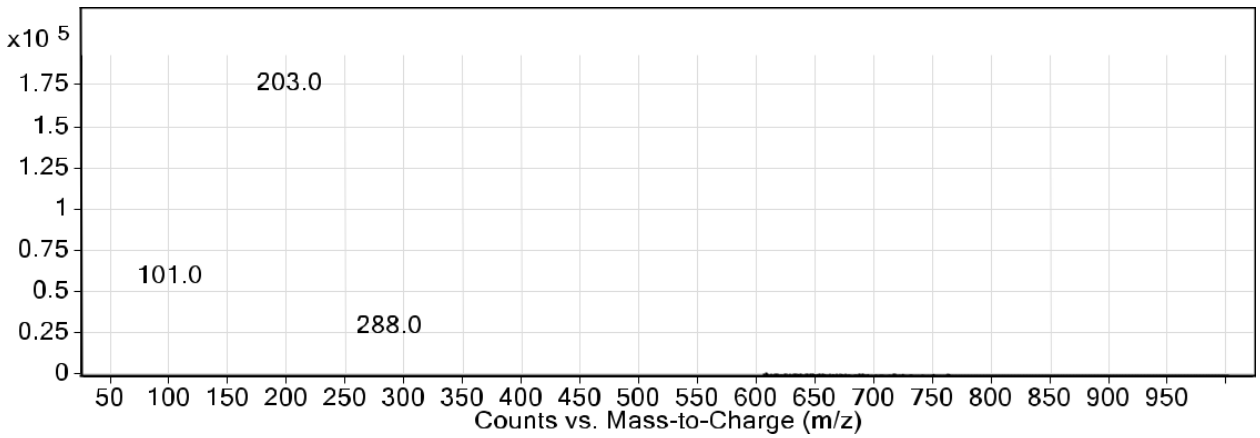


Figure 11: Liquid Chromatography Mass Spectrometric analysis spectra of *Khe*

Discussion:

The ethnic tribes consume traditional rice beer not only for social, cultural, ritual activity but they also consume it for nutritive value and are also believed to improve their health (1). The traditional rice beers of diverse folks are supportive to preservenoble health and for anticipation of various ailments. The phytoconstituents of diverse parts of herbs viz., barks, leaves, roots, rhizomes and different parts used in the research of the fermented brews thatcomprises a diversity of phytochemicals terpernoids, carotenoids, phytosterols, phytoestrogens, polyphenols, flavonoidal compounds, anthocyanidins, etc. These phytoconstituents have been reported to exhibit remarkable impact in the prevention or treatment of different diseases in the traditional healthcare system (2). In addition to human health, the traditional rice beer of Rabha community is used to improve the body strength of cattle. Remarkably, during cholera epidemic in 1960s and 1970s, the local Karbi people used their traditional rice beer to counter such communicable diseases (3).

The antioxidant activity in the rice beer samples was most likely caused by the presence of phenolic acids, polyphenols and flavonoids from various indigenous herbs used in the preparation of the starter culture cake (4). These complexesconstrain the oxidative mechanism that are liable for various diseases and syndromes in people that includediabetes, arthritis, infections, cardiovascular issues, AIDS, cancer, neurodegenerative diseases etc. Rice beer is enriched with probiotics and is a rich cause of nutritional quantities, and a natural antioxidant. Rice beer is acknowledged for its extensive assortment of medicinal properties. It is one accurate corrective measure to boost immunity, pain and inflammation, infections, develop stamina, gastrointestinal infections etc.

Preparation of traditional rice beer in the house holds of several tribes of the North East India has been widely practiced since ancient times (1). Although it is a common tradition to consume the beverage as a part of their socio-cultural development, the mode of preparation and their formulation vary from tribe to tribe and each of them is exceptional from another. Traditional rice beer till today invariably remains an inevitable beverage that is served for all the social gathering of these tribes (3). While the primary ingredient of the traditional beverage is rice from which rice cake is prepared, yet subtle differences exist among the preparations of various tribes. North East India being a rich hot spot of biodiversity, significant number of the tribal populations in these regions gained

invaluable knowledge on the folk and traditional methods of health care practices. Such knowledge that is attained is passed on from one generation to the other only through their local practices (5). However, majority of such knowledge is not documented. Since these tribes gathered a great deal of knowledge on the medicinal and therapeutic properties of various plants and their products, they made sure that extracts of such plants are mixed in the formulations of the starter cake preparation (6).

However, these formulations do vary with the tribe and also with the locality of tribe. Further the method of fermentation and the time of incubation vary. The phytochemical formulations in the preparation of the traditional rice beer, is believed to keep up the good health, and also act as prognostic, preventive and curative medicine for several ailments (3). With rapid developments in the science and technology, there is a decline in the conservation of the invaluable traditional knowledge and practices and hence there is danger of completely losing such knowledge over a period of time if not documented properly (7). Therefore, preserving the indigenous traditional knowledge, the local ethnic practices, culture and health care practices that are of great benefit to the upcoming generations becomes the priority of the current generation (8).

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Summary and Conclusion:

Secondary metabolites are those substances that belong to numerous categories of metabolites as well as can be substantially induced in response to stressful circumstances. Primary metabolites take part in nourishment and reproduction in order to perform important metabolic functions, where an organism interacts with its environment but are not necessary for an organism to live.

Metabolites are very important for the human health of our society that include antibiotics, antitumor agents, cholesterol-lowering drugs, immunosuppressants, antihelmintic agents and other antiparasitics, herbicides, ruminant growth stimulators, agricultural fungicides, bio-insecticides, and others.

The Northeast India consists of many ethnic tribes and communities having diverse customs and lifestyles with difference in their preparation and consumption of fermented rice beer. Traditional beers play a significant role in the cultural and social fabric of Northeast India. Since it is probiotic-enriched, rice beer is regarded as Mother Nature's elixir. Overall, traditional beer in Northeast India holds a multifaceted role, encompassing cultural, social, and economic aspects.

Rice beer brewing and consumption in India's north-eastern states have long been supported by their sociocultural legacy. Idioms, folktales, ballads, and numerous folk-dance forms all stress the need of making rice beer to establish a close connection between nature and humanity. Making rice beer is an essential component of the many traditional events held in the north-eastern States. The agricultural community celebrates with traditional rice beer brewing and drinking while taking pleasure in or taking part in the celebrations, in addition to prayers, caring for their animals, song and dance performances, and several other sporting events.

The traditional rice beers sample (1) *'Apong'* was collected from Itanagar (Arunachal Pradesh), sample (2) '*Xaj pani*' was collected from Rani, Kamrup metro (Assam), sample (3) '*Sadhier*' was collected from Jowai, West Jaintia Hills (Meghalaya), sample (4) '*Zutho*' was collected from Kohima (Nagaland) and sample (5) '*Khe*' was collected from Kohima (Nagaland). Fresh samples were collected of approximately 100 mL, then stored in airtight containers free from moisture at 4°C until further use. Preliminary phytochemical tests were performed for the presence of Alkaloids, Carbohydrates, Flavonoids, Proteins, Steroids, Terpenoids, Tannins. Thin layer chromatographic analysis has been carried, where **t**he plates were developed using the following solvent systems:

For the analysis of presence of carbohydrate, we have used Ethyl acetate : Methanol : Water (14:3:3) and for amino acids, we have used 10% of Phenol Water; for sterols, used Hexane : Acetone (18:2); for Saponins used Chloroform : Methanol: Water (14:6:2) and for tannins Chloroform : Ethyl acetate : Methanol (2:2:16). After the TLC profile check of the of each sample, Liquid Chromatography Mass Spectrometry was carried out, where **i**nitially, 1 mL of respective beer sample was mixed with 1 mL of methanol and vortexed well. This mixture was centrifuged for 10 min at 9000 rpm, after which 500 µL of the supernatant was used as an underivatized sample that was analysed for LCMS-8060, Shimadzu using different multiplexed analytical columns (Hypercarb of 100 mm x 2.1 mm, 5 µm; Raptor C18 of 100 mm x 2.1 mm, 2.7 µm) at 35° C Column temperature and keeping the injection volume at 5 µL.

Results reveal the different classes of phytoconstituents present in each beer sample, that was analyzed for its phytochemical constituents: Carbohydrates are present in *Apong, Xaj Pani, Sadhier, Zutho, Khe*. Alkaloids are present only in *Apong*. Sterols are present only in *Apong* and *Khe.* Sterols are present in *Apong, Zutho* and Khe. Tannins were highly detected qualitatively in *Apong,* however very little qualitative detection in *Sadhier, Zutho* and *Khe*. Saponins were highly detected qualitatively in Sadhier, however very little qualitative detection in *Apong, Xaj Pani, Zutho* and *Khe*. Amino acids were detected in all the four traditional beer samples. The rice beer samples *'Apong'*; '*Xaj pani'*; '*Sadhier*'; '*Zutho*'; '*Khe*' were analyzed for TLC profiles. The TLC of *'Apong'* revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Saponins and Tannins; of '*Xaj pani*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols, and Saponins; of '*Sadhier*' revealed the presence of 03 classes of compounds that include Carbohydrates, Amino Acids, and Saponins; of '*Zutho*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins; of '*Khe*' revealed the presence of 04 classes of compounds that include Carbohydrates, Amino Acids, Sterols and Tannins. Liquid Chromatography Mass Spectrometric analysis of Traditional Rice Beer Samples were carried out to separate the derivative compounds depending on their derivatization time, temperature, volumes of samples and reagents. The compounds detected in *apong* are carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine, and tannins; in *Xaj pani* are carbohydrate derivatives, histamine, tyramine, and tannins; in *Sadhier* are carbohydrate derivatives, histamine, tyramine, methylamine, cadaverine; in *Zutho* are carbohydrate derivatives, histamine, tyramine, 2-

Phenylethylamine and tannins; in *Khe* are carbohydrate derivatives, methylamine, tyramine, 2-Phenylethylamine and tannins.

Traditional Rice Beers of Northeast India, is a common socio-culture of several tribes, with different methods of preparation. These traditional beverages may contain many different levels of contaminants and therefore it is essential to find out the chemical profile of these traditional formulations, as it is believed to keep up the good health, and also act as preventive, prognostic, and curative medicine for several ailments. The association of the natural compounds could be explored for future prospect that would support the hypothesis, preserve the indigenous traditional knowledge and the local ethnic practices. Further it can also be concluded that the proposed scientific report to be novel, and contributory.

Future Prospective:

Traditional beers have a number of therapeutic uses that have been proven, and their metabolites may also be beneficial to one's health. To fully grasp the health advantages of these traditionally made rice beers, it is advised that researchers conduct more study on the therapeutic benefits as well as any contaminants.

The detection of different phytochemicals in each rice beer sample indicates the presence of unique compounds and varying levels of these compounds. It would involve establishing quality control measures, setting regulatory guidelines and develop standardized protocols to ensure consistent and safe rice beer production.

Exploration of therapeutic compounds through chemical profile study would give the researchers a future prospect of understanding the potential therapeutic benefits of these different traditional beverages with minimum toxicity level and meet the consumer demands at a cost effective and cost benefit manner.

References:

1. Harborne, A. J. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* Springer Netherlands, 538-547.
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**** Notes ****

Qualitative Analysis Report

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Comment			

Sample Group **Info.**

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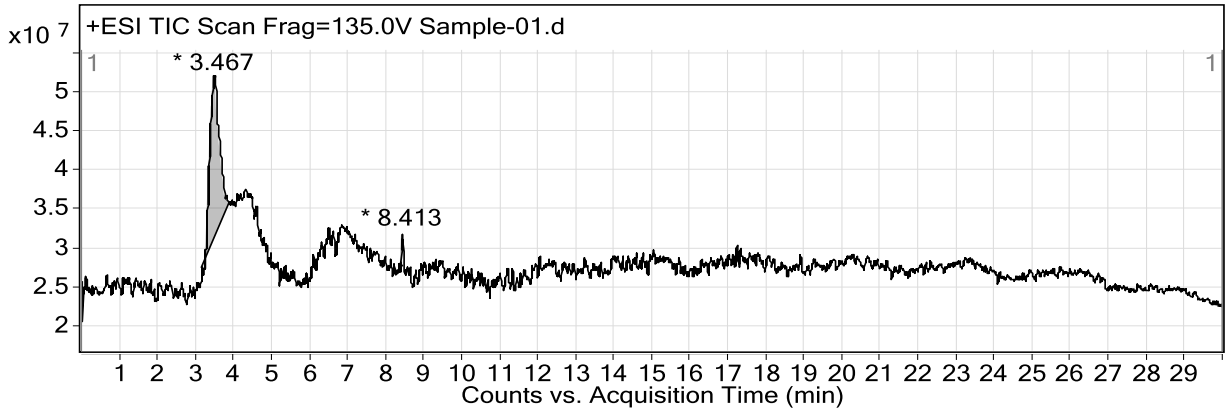

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User Spectra

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
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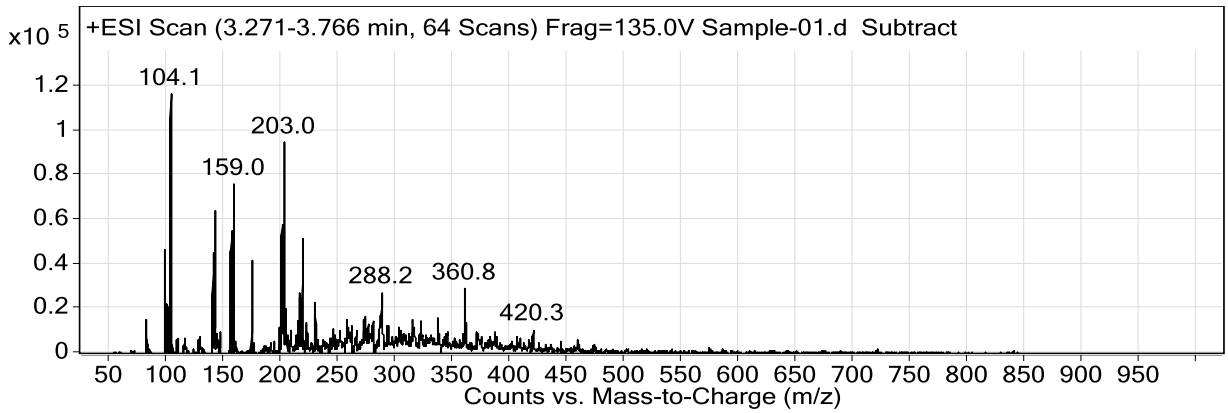

Peak List

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159		76236.2
175.1		41964.6
201.1		57722.6
203	1	94946.7

Qualitative Analysis Report

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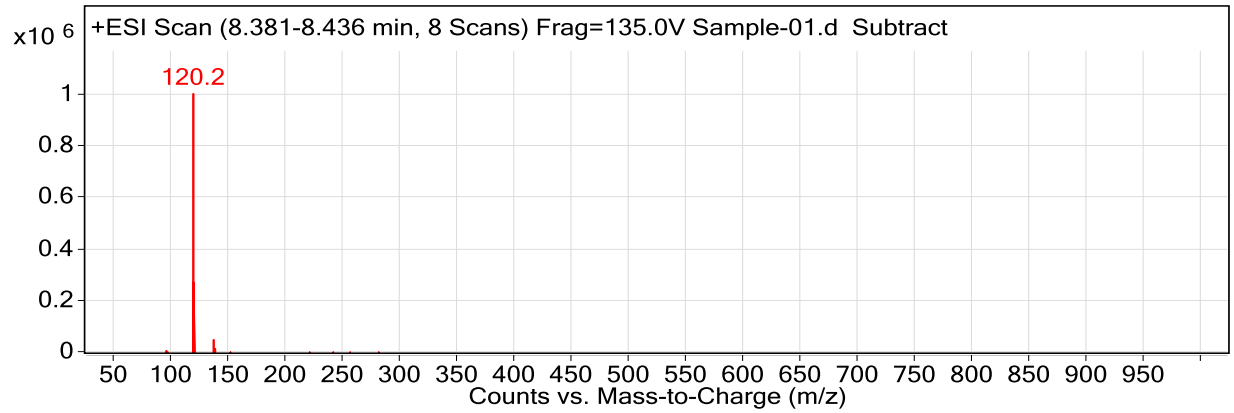

Peak List

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Qualitative Analysis Report

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Comment			

Sample Group **Info.**

User Chromatograms

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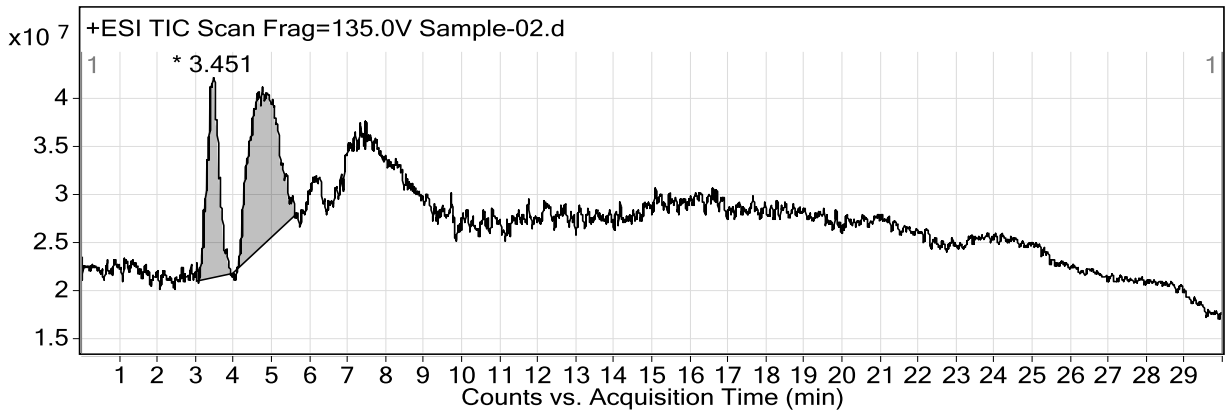

Integration Peak List

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User Spectra

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
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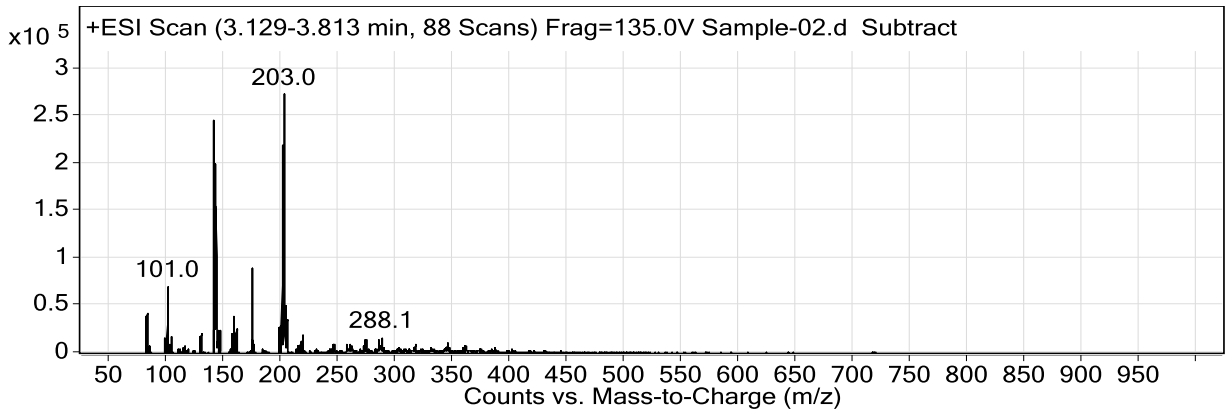

Peak List

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142	1	55512.8
143	1	199873
175.1		90077.1
201	1	219201.2
202	1	43829.2
203	1	273666.3

Qualitative Analysis Report

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Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
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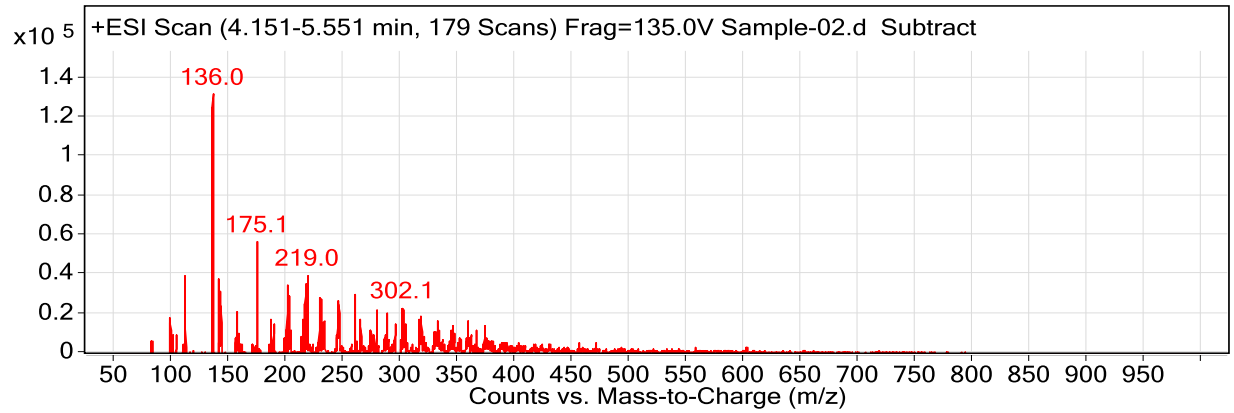

Peak List

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141	1	37828.8
143	1	31411.7
175.1		56826.2
201		34791.5
203		29103.7
217.1	1	35242.7
219	1	39842.5
260.1		29831.9

Qualitative Analysis Report

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Comment			

Sample Group **Info.**

User Chromatograms

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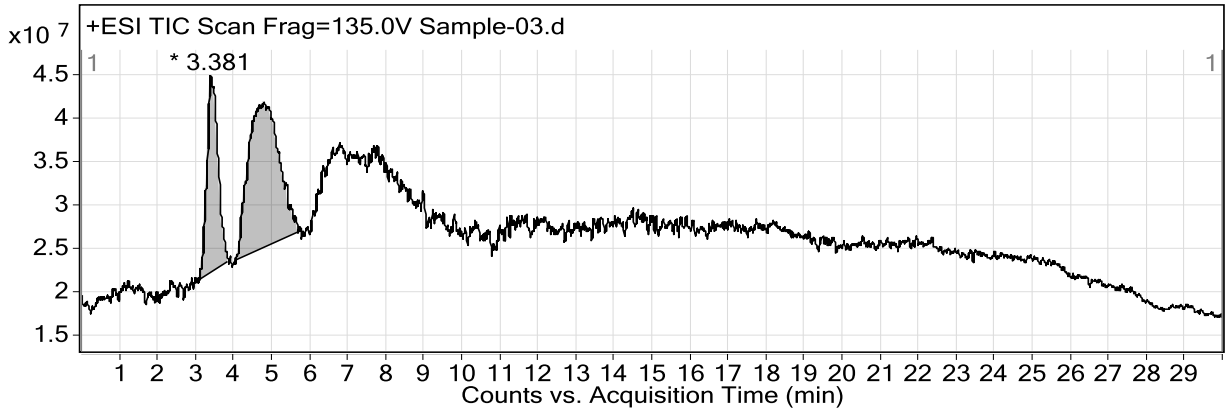

Integration Peak List

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User Spectra

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
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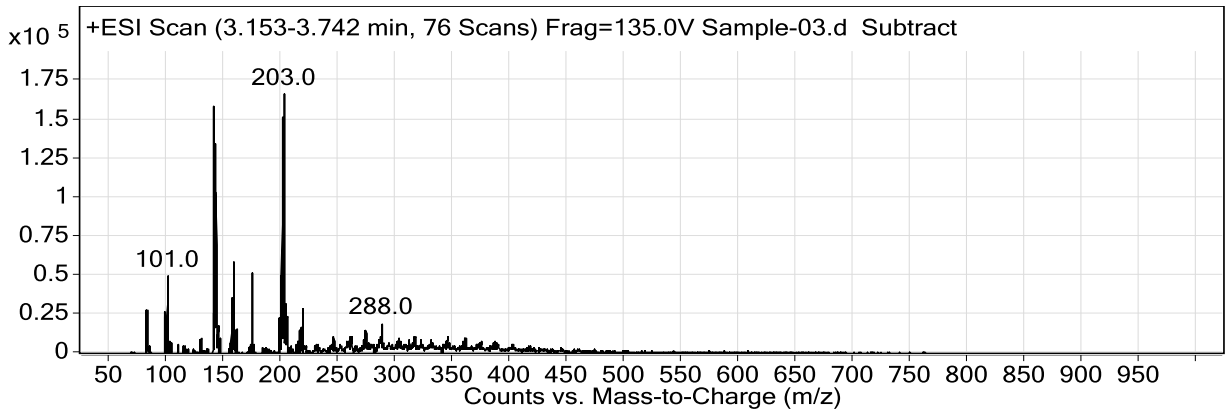

Peak List

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156.9		35613.3
159		58894.8
175.1		51854.4
201	1	151351.4
203	1	166664.6

Qualitative Analysis Report

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Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
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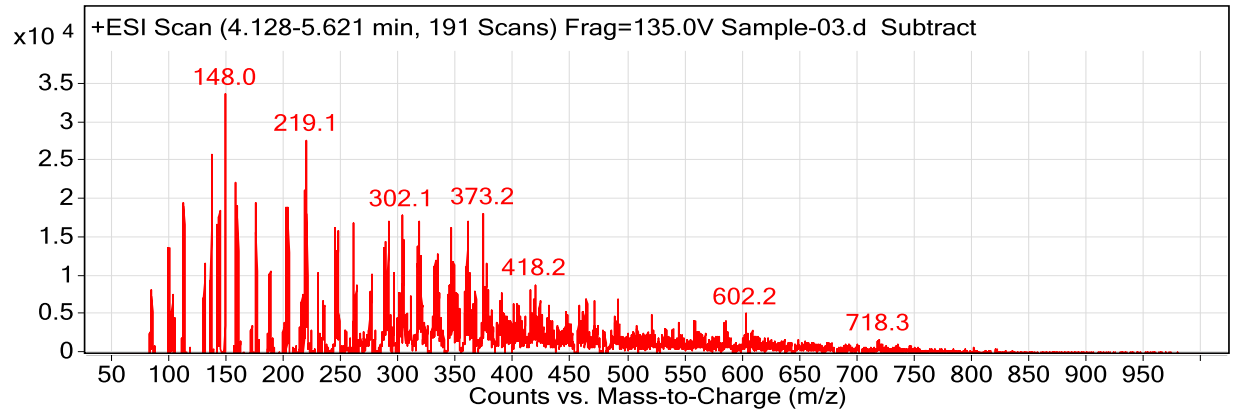

Peak List

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159		19102.6
175.1		19647.9
201	1	18910.3
203	1	19015.3
217.1	1	21175.7
219.1	1	27778.7

Qualitative Analysis Report

Data Filename	Sample-04.d	**Sample Name**	Sample-04
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Comment			

Sample Group **Info.**

User Chromatograms

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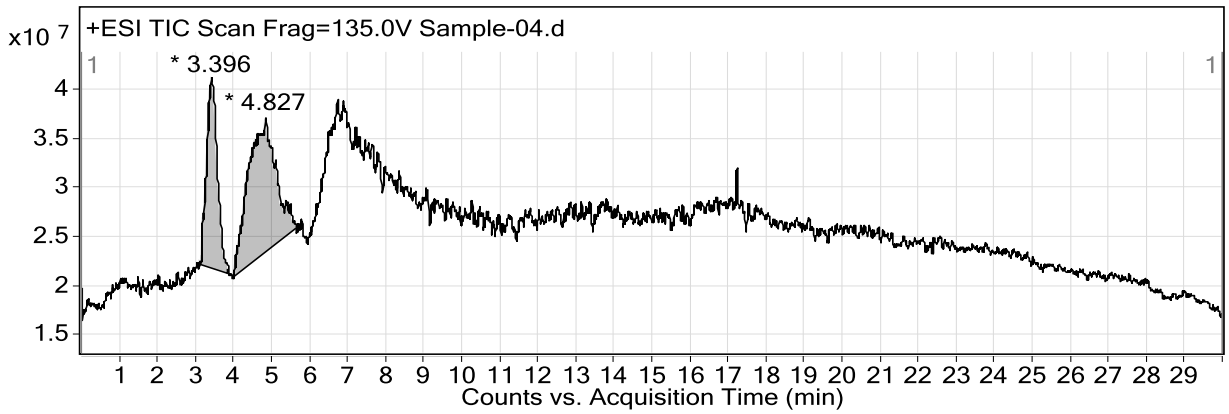

Integration Peak List

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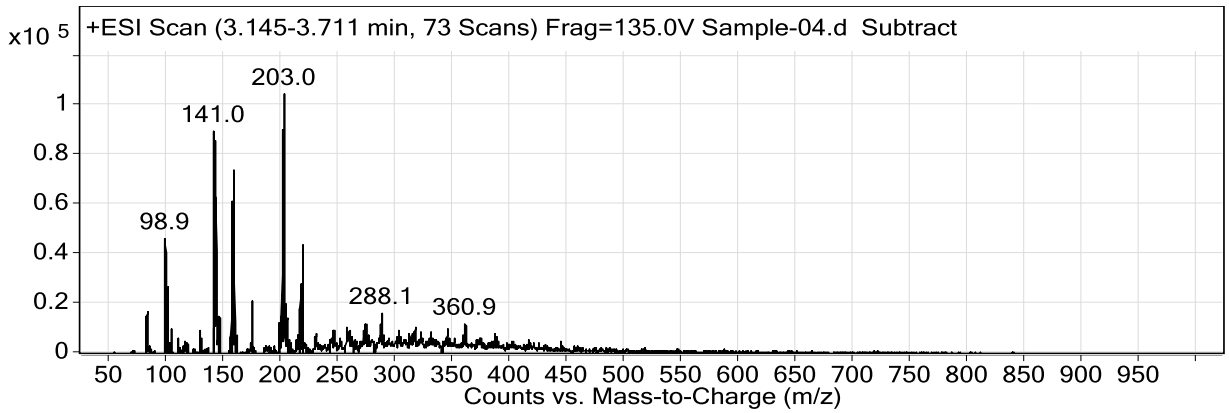
3.978	4.827	5.645	13734787	722771003

User Spectra

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
Peak (1) in "+ TIC Scan"	135	0	ESI



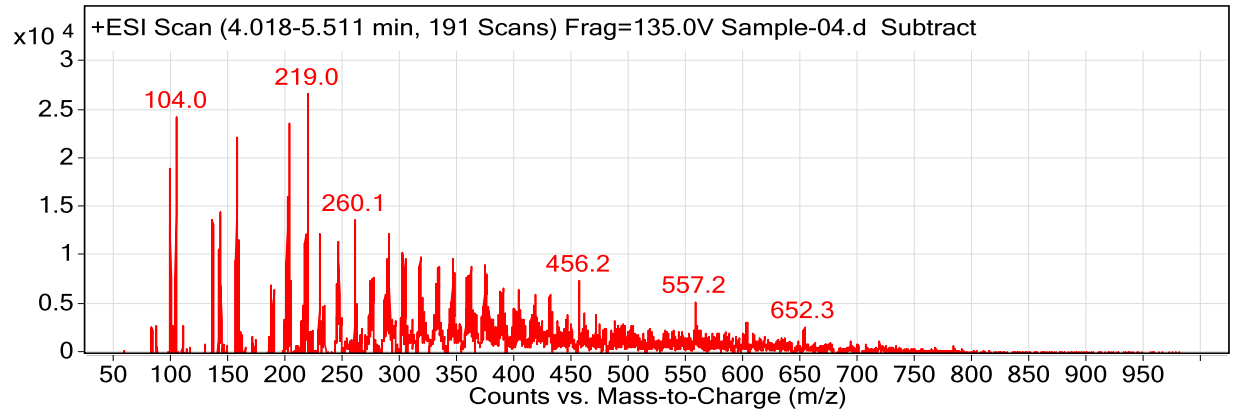
Peak List

m/z	z	Abund
98.9		46421.2
101		27136.8
141	1	89768.9
143	1	85565.1
157	1	61398.8
159	1	73769.1
201.1	1	90023.9
203	1	104762.4
217	1	28310.4

Qualitative Analysis Report

219		43663.9

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
Peak (2) in "+ TIC Scan"	135	0	ESI



Peak List

m/z	z	Abund
98.9		19054.1
104		24364.6
136		13815.8
143	1	14610.3
157	1	22202.1
201		16148.4
203	1	23722.5
219	1	26738.3
260.1		13717.4
290.1	1	12281.6

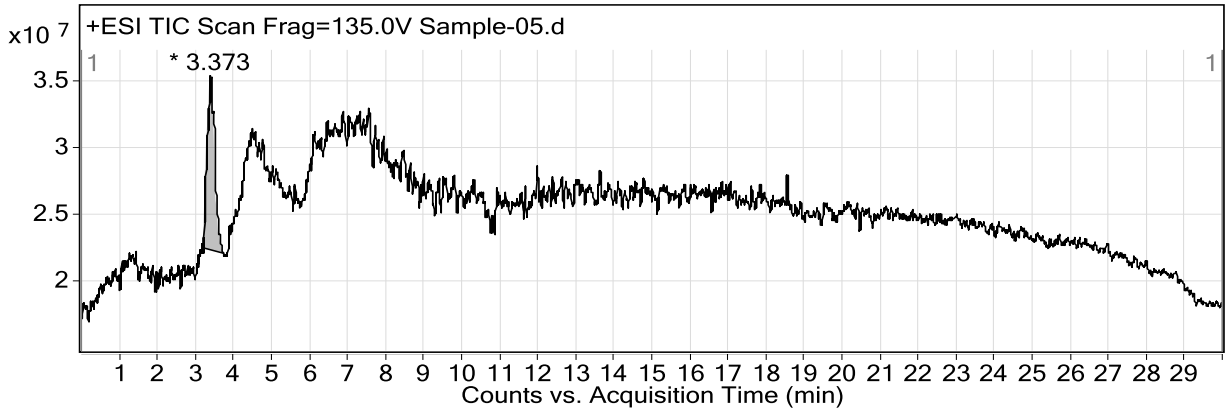
Qualitative Analysis Report

Data Filename	Sample-05.d	**Sample Name**	Sample-05
Sample Type	Sample	**Position**	Vial 5
Instrument Name	Instrument 1	**User Name**	
Acq Method	default_ESI.m	**Acquired Time**	12/3/2022 2:13:01 PM
IRM Calibration Status	Not Applicable	**DA Method**	NDMA SCAN.m
Comment			

Sample Group **Info.**

User Chromatograms

Fragmentor Voltage 135 **Collision Energy** 0 **Ionization Mode** ESI

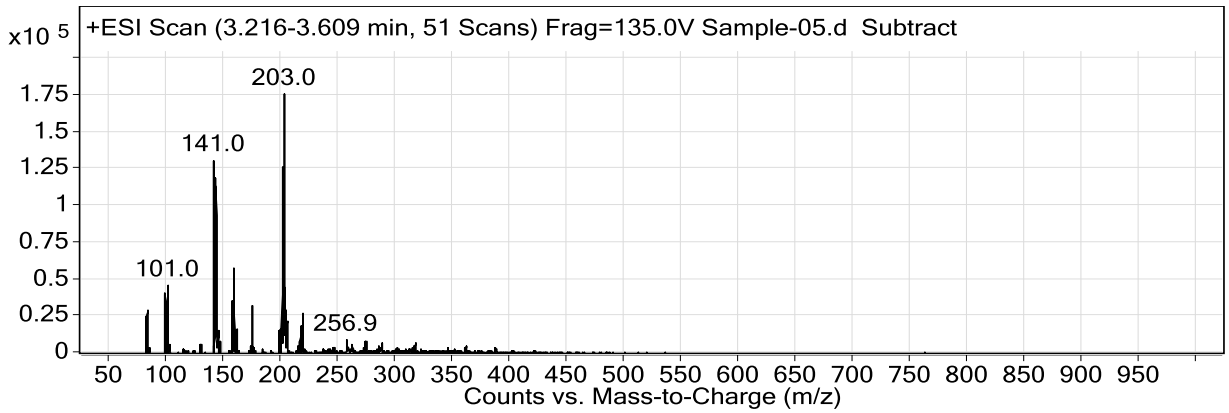


Integration Peak List

Start	RT	End	Height	Area
3.192	3.373	3.734	13147375	183658541

User Spectra

Spectrum Source	Fragmentor Voltage	Collision Energy	Ionization Mode
Peak (1) in "+ TIC Scan"	135	0	ESI



Peak List

m/z	z	Abund
98.9		41467.8
101		46092.6
141	1	131246.5
143	1	119244.5
157		36016
159		57585.6
175.1		32665.3
201	1	126023
203	1	176255.8
204	1	29982.9

Metabolite Analysis of Traditional Rice Beers of Northeast India (ISBN: 978-93-95847-88-9)

About Authors

Ms. Jobila Myrphet is a B.Pharm from the University of Science and Technology, Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200) and a Ladthadlaboh Mookyrdup Jowai West Jaintia Hills Meghalaya resident. She is the finalist of the FAME Biotech 2.0 Hackathon (Prototype Stage), which was organized by BioNEST IASST, Guwahati, India. She has presented papers in International Seminars and she is enthusiastic to work on traditional medicines.

Mr. Anshuman Khuttiya Deori is a B.Pharm from the University of Science and Technology, Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200) and a Bandardewa, Bali Gaon, Pathali pahar, Dist-Lakhimpur, Assam resident. He is the finalist of the FAME Biotech 2.0 Hackathon (Prototype Stage), which was organized by BioNEST IASST, Guwahati, India. He has presented papers in International Seminars and he is enthusiastic to work on traditional medicines.

Mr. Mhajan Patton is a B.Pharm from the University of Science and Technology, Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200) and a Porterlane Colony Kohima, Nagaland resident. He is the finalist of the FAME Biotech 2.0 Hackathon (Prototype Stage), which was organized by BioNEST IASST, Guwahati, India. He has presented papers in International Seminars and he is enthusiastic to work on traditional medicines.

Mr. Jubin Nath is a B.Pharm from the University of Science and Technology, Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200) and a Chinabari, Baguan, Dist-Goalpara, Assam resident. He is the finalist of the FAME Biotech 2.0 Hackathon (Prototype Stage), which was organized by BioNEST IASST, Guwahati, India. He has presented papers in International Seminars and he is enthusiastic to work on traditional medicines.

Mr. Debojit Mudoi is a B.Pharm from the University of Science and Technology, Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200) and a Ketla T.E, Behali, Dist- Biswanath, Assam resident. He is the finalist of the FAME Biotech 2.0 Hackathon (Prototype Stage), which was organized by BioNEST IASST, Guwahati, India. He has presented papers in International Seminars and he is enthusiastic to work on traditional medicines.

Mr. Rashidul Islam, is a D.Pharm and B.Sc. qualifications holder and working as a the store in-charge cum laboratory technician at the School of Pharmaceutical Sciences at the University of Science and Technology Meghalaya, accredited with NAAC Grade A and NIRF India Ranking 2023 (151-200). He completed his Bachelor of Science degree from IGNOU (accredited with NAAC Grade A++) and his Diploma in Pharmacy from the Institute of Pharmacy, Guwahati Medical College. He is a highly experienced and knowledgeable person and looks forward to carry out extensive research on Indian Traditional Medicines.

Dr. Sudarshana Borah, Associate Professor, School of Pharmaceutical Sciences, University of Science and Technology Meghalaya (USTM) accredited with NAAC Grade 'A' and NIRF India Ranking 2023 (151-200) has completed her Ph.D. in Pharmaceutical Sciences, from Department of Pharmaceutical Sciences, Dibrugarh University under the supervision of Dr. Hemanta Kumar Sharma. She has GPAT qualification and also received National Doctoral Fellowship Award as CSIR- Senior Research Fellowship during her Ph.D. research. She has received the INTERNATIONAL YOUNG SCIENTIST AWARD, 2019 on the 5th International Young Scientist Congress, organized by International Science Community Association, at Mid-Western University, Surkhet, Nepal. Dr. Borah is a Lifetime member of International Science Community Association and Association of Pharmaceutical Teachers' of India, and Convenor of Women's Forum, Assam State Branch, APTI. She has also guided students in their research activities in different projects. Dr. Borah has 30 paper publications in journals both National and International,4 Books, 5 Book Chapters, 3 National awards, 2 International awards and 2 Fellowship awards, 4 patents and she looks ahead to further carry forward her research activities on ethnomedicinal plants of Northeast India.

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