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The Himalayan ethnic beverage tongba with therapeutic properties in high-altitude illnesses and metabolomic similarities to Japanese sake

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Abstract. Tongba, a millet-based fermented ethnic drink of the Limbu and other Nepalese-Tibetan communities, is consumed in the highlands of Singalila Ridge of the Himalayas and the adjoining high-altitude places of Nepal, the northern and north-eastern parts of India, and the Tibetan Plateau and is valued for its ethnomedicinal properties. In this research, the GC-MS-based metabolite profiling of an authentic sample of tongba was carried out, identifying various bioactive metabolites. Several biologically active components, such as glycoside, amino acids, fatty acids, and other long-chain hydrocarbon derivatives, terpenoids and phenol, were detected in tongba, which have therapeutic properties against various high-altitude illnesses. Probable biosynthesis routes of those compounds in tongba's broth were also studied, where many similarities were noticed with the Japanese beverage sake. The key finding of this metabolomic investigation was the detection of bioactive ethyl-α-D-glucopyranoside and cyclo(L-Leu-L-Pro) with abundant peak areas, which confirmed tongba's therapeutic importance in high-altitude illnesses and its metabolomic similarities with sake.

Keywords and phrases: traditional foods, ethnomedicine, fermented beverage, metabolomics

1. Introduction

An important portion of the world's population lives at high altitudes where daily lifestyle, food production, food availability, food requirements, and dietary habits are different and traditional, unlike in the rest of the world. According to biologist *Picón-Reátegui* (1978), food production and availability are definitely tied up to the altitude, weather conditions, soil, and other environmental factors of a region. A series of research has revealed how high altitude can influence human nutrition, dietary habits, and other activities of people (*Majumder et al.*, 2021). High-altitude ethnoecology shows a rational relationship between its people and their ethnic dietary habits, which directly or indirectly helps a group of people to endure the altitudinal stresses, including weather conditions (*Picón-Reátegui*, 1978). High-altitude dietary habits are distinct from the rest of the world, as these have been developed to help locals as well as tourists or visitors to get acclimatized to the surrounding environment, to stay well and recover from various high-altitude illnesses. Traditional knowledge on fermentation technology is considered an important part of food and beverage management in high-altitude regions where obtaining of important functional foods is very difficult and the preservation of food is required to increase the shelf life of processed or harvested foodstuff (*Majumder et al.*, 2021). The ethnoecology of Singalila Ridge of the Himalayas (the high-altitude place between Darjeeling, Sikkim, and Nepal) is very distinctive and enriched with plenty of traditional foods prepared by Nepalese and Tibetan ethnic communities, which include a lot of cereal-based fermented beverages such as: jand, or jaanr; raksi, or rakshi; nigar; chyang, or chhaang; tongba or tumba (*Tamang & Kailasapathy*, 2010; *Ray et al.*, 2016; *Majumder et al.*, 2021).

In several regions, finger millet grains are malted and fermented to obtain different traditional fermented drinks such as finger millet sake, one of the traditional millet-based fermented beverages that is consumed mainly in Japan and China. Tongba, or often called tumba, is one of the locally fermented millet-based traditional alcoholic beverages of the Limbu community of eastern Nepal, which is regarded a regular beverage by Tibetan, Nepalese, and other ethnic groups of people living in high-altitude regions in the Himalayas (parts of Nepal, northern and north-eastern parts of India, and the Tibetan Plateau) and is praised for its many ethnomedicinal properties, mainly for its anti-inflammatory or pain-relieving effects (*Tamang & Kailasapathy*, 2010). Tongba is prepared from brown finger millet (*Eleusine coracana*, also known as ragi in India or kodo in Nepal) grown in hilly regions, and it is cooked and combined with traditionally cultured khesung, which is a microbial colony or starter culture (*Tamang & Kailasapathy*, 2010). 'Khesung' is the Limbu version of the Nepali term 'murcha'; the Lepcha call it 'thamik', and Bhutias refer to it as 'phab'. Tongba is traditionally prepared in a round container and served in glass-shaped wooden or bamboo vessels after pouring hot water over the fermented grains and sipped through a special bamboo straw with a perforated bottom that also functions as a filter (*Dangal et al.*, 2021). The resulting whitish liquor is thick and astringent, with a pleasant, mild flavour and distinctive taste. The vessel is refilled three to four times with hot water until the grains lose their potency (flavour, astringency, and taste) and the alcohol is exhausted. The term "tongba" actually means the bamboo vessel that holds the fermented millet beverage, which is traditionally referred to as 'mandokpenaa thee' (*Dangal et al.*, 2021). Traditionally, it is stored or aged for about six months, when the fermentation culture matures and flavours intensify and become mellower (*Harmayani et al.*, 2019).

Tongba, raksi, chyang, and jand, are all well documented in the field of ethnoecology along with some health claims, but only few of them (mainly raksi and chyang) have been subjected to foodomics to be studied scientifically at molecular level (*Majumder et al.*, 2021). So, the objective of this research was to study the volatile profile of tongba and metabolomics to understand the ethnomedicinal properties linked with the beverage. Analytical platforms like gas chromatography-mass spectrometry, liquid chromatography, capillary electrophoresis, nuclear magnetic resonance spectroscopy, etc. are commonly used in metabolomics research, among which GC-MS is believed to be the most used technique to date. GC-MS has also been tightly linked to foodomics since ages, where the technique is used to identify and quantify various substances of foods belonging to various metabolic pathways, including sugars, sugar alcohols, amino acids, fatty acids, and other organic acids, polyamines, terpenoids, alkaloids, etc. This research comprehensively provides a framework to facilitate the metabolomic evaluation of a traditional alcoholic beverage, and the outcomes would help to confirm the ethnomedicinal properties associated with the beverage.

2. Materials and methods

Sample collection

Six-month-old culture of traditionally brewed tongba (*Figure 1*) was collected from the oldest tavern of Kalipokhri village, one of the highest points of Singalila Ridge, situated at an altitude of 3,100 m near the Indo-Nepal border in the Darjeeling District of West Bengal. The sample collection site, Kalipokhri (27°04'45" N, 88°01'03" E), has been described as one of the oldest human settlements in Singalila National Park (*Majumder et al.*, 2021). An ethnobiological fieldwork was conducted in the traditional trekking routes of Singalila National Park (Dhotrey – Tonglu – Tumling – Gairibans – Kalipokhri – Bikheybhanjyang – Sandakphu – Gurdung – Srikhola) located in Darjeeling, India, and the Indo-Nepal border area to collect the authentic sample of tongba.

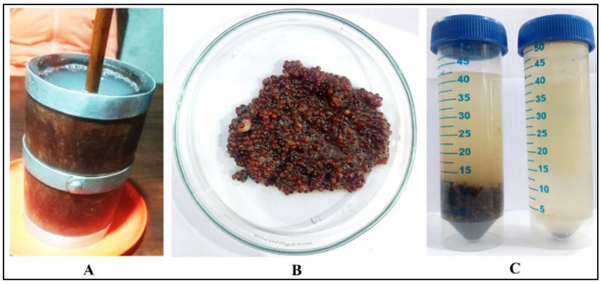

Figure 1. A – traditionally served tongba after pouring hot water over fermented culture, B – six-month-old culture of tongba, C – methanolic solution of the sample

Sample preparation for GC-MS analysis

The sample was collected in a sterilized container to avoid microbial contamination and kept inside the icebox before transferring it to the laboratory. Sample preparation for GC-MS analysis was done following the method standardized for alcoholic beverage analysis (*Majumder et al.*, 2021). A liquid portion (1 ml) from the sample of tongba was diluted in methanol (Merck) overnight to prepare the methanolic (50%) solution of tongba or TM (*Figure 1C*). Being a widely used and ideal solvent for the extraction of biochemicals, methanol was chosen. The polarity of methanol as an organic solvent also shows closeness with that of water or ethanol, which are primary components of alcoholic beverages (*Majumder et al.*, 2021).

GC-MS-based volatile profiling and metabolomics analysis

TM was subjected to GC-MS-based metabolomics following the standardized research protocol developed for the analysis of fermented beverages (*Majumder et al.*, 2021). GCMS-QP2010 Plus (Shimadzu Co., Japan) equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm) was used in this research. The analysis was performed by split-injecting (with a ratio of 20:1) 1 μl of TM. Injection temperature was 260°C, and interface temperature was 270°C. Ion source temperature was adjusted to 230°C. As carrier gas, helium (99.9%) was used. The total and column flow rate were 16.3 ml/min and 1.21 ml/min respectively. Mass spectra were recorded at the scanning rate of 5 scan/s. The compounds were identified after comparing the spectral configurations obtained with that of the available mass spectral databases, i.e. Wiley Registry of Mass Spectral Data, 8th Edition and The NIST 14 Mass Spectral Library (*Majumder et al.*, 2020). The chromatogram (TIC or Total Ion Chromatogram) was based on the intensity of fragments produced by the ionization. Quantification of the amount (area %) of each compound was done based on peak areas. The data obtained from the GC-MS analysis were further studied based on available literature (*Majumder et al.*, 2022).

3. Results and discussions

The positive effect of tongba was reinforced by local inhabitants and brewers, who shared their knowledge on the ethnomedicinal properties of tongba as cardio-protective, pain reliever or anti-inflammatory, respiratory illness preventive, gastro-protective, etc. They also praised the drink for exhibiting potential moisture retention properties in the human body. Furthermore, the GC-MS metabolite profiling of this ethnic beverage was performed to determine its volatile composition and evaluate the claims of being therapeutic against high-altitude illnesses through studying the bioactivities of the metabolites that are described below.

The volatile profile of tongba and GC-MS-based metabolomics

A total of thirty-three peaks were found in the GC-MS chromatogram showing twenty-six different compounds (*Table 1*), where ethyl-α-D-glucopyranoside (α-EG) and cyclo(L-Leu-L-Pro) or (3S,8aS)-hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-a]pyrazine-1,4-dione were found as major components occupying 53.95% and 16.96% of the total peak areas respectively. *Figure 2* includes a pie chart showing the percentage share of the types of metabolites based on chemotaxonomy and biosynthesis pathways involved in the broth of tongba (TM), where most of the glucoside has been found through the peak of α-EG. The sample also contained derivatives of terpenoids, fatty acids, and long chains of similar hydrocarbons, amino acids, phenols, sugar alcohol, antibiotic (actinomycin), alkaloid (dihydroergotamine), etc. (*Table 1*). The chromatograph is shown in *Figure 3*. Moreover, studying metabolomics has revealed various routes involved in the biosynthesis of those components whether they were directly derived from the substrate (finger millet) or due to fermentation, as metabolites of the khesung (traditional starter of tongba), which are described below.

Table 1. GC-MS peak report showing volatiles in TM

Name of compound	**Area (%)**	**Type of compound**
Tyrosol	2.72	Phenol
Ethyl-α-D-glucopyranoside	53.95	Glucoside
5-Methylcyclohexane-1,3-diol	0.37	Fatty alcohol
1,3-Methylene-D-arabitol	1.25	Sugar alcohol
Neophytadiene	0.42	Diterpenoid

Name of compound	Area (%)	Type of compound
Cyclo(L-Leu-L-Pro)	16.96	Cyclic dipeptide
Actinomycin C2	1.43	Cyclic peptide
14-Methyl pentadecanoate	0.36	Fatty acid
Ethyl palmitate	1.01	Fatty acid
Ethyl linoleate	1.47	Fatty acid
Methyl oleate	0.67	Fatty acid
Phytol	0.26	Diterpenoid
D,L-Pyroglutamic acid	0.32	Amino acid derivative
Ethyl linolenate	2.64	Fatty acid
Ethyl pentadecanoate	0.29	Fatty acid
3-Cyclopentyl-propionic acid, 2-dimethylaminoethyl ester	0.46	Fatty acid derived carboxylic acid
2,5-Di(trifluoromethyl) benzoic acid, 3-hexadecyl ester	1.05	Fatty acid derivative
Dihydroergotamine	2.7	Alkaloid
Phthalic acid	0.37	Chemical pollutant
Ethyl(dimethyl)(pentadecyloxy)silane	1.29	Fatty acid derivative
Arachidyl alcohol	1.96	Fatty alcohol
4-(2-Methoxyhexadecoxymethyl)-2,2-dimethyl-1,3-dioxolane	1.48	Fatty acid derivative
Farnesol	0.37	Sesquiterpenoid
β-sitosterol-methyl ether	0.85	Phytosterol
1-(2,3-Dimethoxypropoxy)-2-methoxyhexadecane	4.5	Long-chain alkane
β-Sitosterol	0.85	Phytosterol

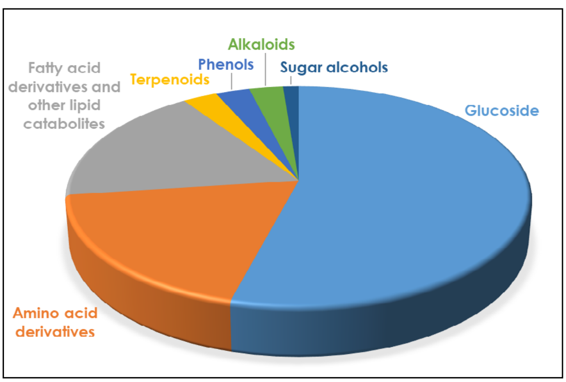

Figure 2. The metabolite types of tongba based on the GC-MS peak area

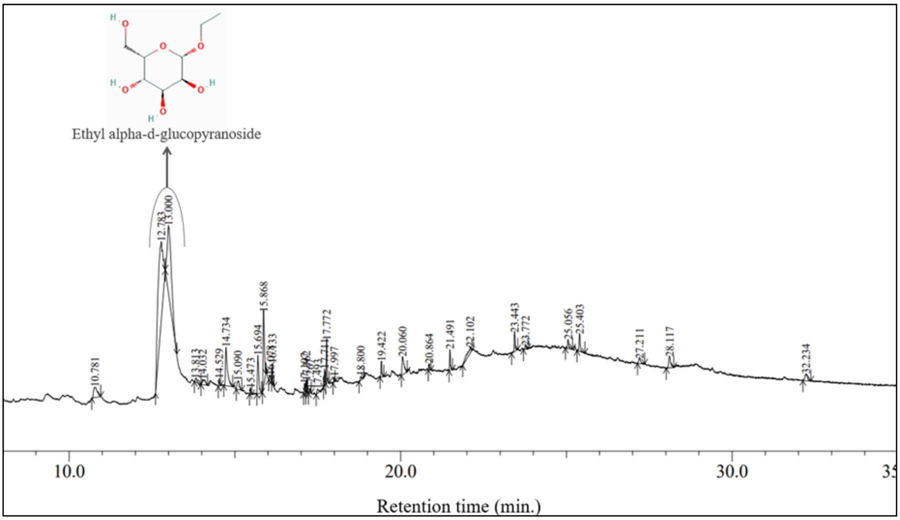

Figure 3. The GC-MS total ion chromatogram of the methanolic extract of tongba revealing the major compounds as α-EG (the compounds corresponding to peaks are listed in *Table 1*)

Glucoside

In tongba, the major component α-EG was detected with a total chromatographic peak area of 53.95%. This umami-flavoured compound is an abundant and typical component of sake, a Japanese traditional fermented beverage (*Bogaki et al.*, 2017). α-EG was later recognized as a safe and acceptable food component and also as one with beneficial biological activities on human health (*Mishima et al.*, 2005). Reports on the production of α-EG in sake by *Aspergillus oryzae* (*Imanari & Tamura*, 1971; *Bogaki et al.*, 2017) shed light on the probable biosynthesis pathway of α-EG in fermented broth, where α-EG is produced via starter-derived α-glucosidase activity due to the trans-glycosylation of substrate-derived maltose or any maltooligosaccharides. The α-EG's precursor maltose is a disaccharide derived from starch due to the amylase activity of amylolytic microbes present in tongba. Interestingly, the total starch content of finger millet/kodo (tongba's substrate) is about 59% (*Devi et al.*, 2014), which has been reflected in tongba's volatile profile, as the overall peak area occupied by starch catabolites (glucoside, sugar alcohol, and terpenoids) was 57.95%.

Amino acid derivatives

Three amino-acid-derived compounds detected in TM occupied a total of 18.71% of the area including peaks of major compound cyclo(L-Leu-L-Pro) (of 16.96% of total peak area), actinomycin C2, and DL-pyroglutamic acid. Cyclo(L-Leu-L-Pro), a homodetic cyclic dipeptide composed of leucyl and prolyl residues, functions as a fermented metabolite (bacterial). The Japanese traditional fermented beverage sake, the distilled liquor awamori (*Yamamoto et al.*, 2016), and Himalaya's millet-based distilled liquor raksi (*Majumder et al.*, 2021) are reported to contain this major compound of tongba. It is a taste-modulating compound responsible for the characteristic taste and aroma of various fermented beverages such as beer, wine, and sake (*Yamamoto et al.*, 2016; *Acharyya et al.*, 2021). Pyroglutamic acid was also reported in different fermented foods such as sake and soya sauce (*Gazme et al.*, 2019), the Tanzanian traditional millet-based liquor togwa (*Mugula et al.*, 2003), and the Korean ethnic rice wine makgeolli (*Kim et al.*, 2008). *Gazme et al.* (2019) also demonstrated the biosynthesis of pyroglutamic acid in fermented sake due to the protein digestion by the protease enzyme of koji or *Aspergillus oryzae* (starter culture of sake). However, pyroglutamic acid is reported as a metabolite of finger millet (*Kim et al.*, 2013), the substrate of tongba. The pyroglutamic acid in the sample may be derived either from substrate or after fermentation as the breakdown products of proteins and peptides if metabolomics is considered. Another cyclic peptide, the actinomycin C2, is an antibiotic of bacterial (*Streptomyces* sp.) origin (*Saravana Kumar et al.*, 2014); so, the actinomycin in tongba-fermented broth is definitely a microbial metabolite.

Fatty acid derivatives and other lipid catabolites

Including the derivatives of long-chain hydrocarbons such as fatty acids, fatty alcohols, alkanes, and carboxylic acid derived from lipid breakdown, a total of thirteen such components of TM (*Table 1*) have been grouped together based on biosynthesis (lipid metabolism) and for possessing chemotaxonomic similarities. The occurrence of these components in TM causes no confusion, as the substrate kodo or finger millet contains lipids of about 1.3% (*Devi et al.*, 2014), and fermenting microbes also metabolize lipids to produce fatty acids and alcohols. Fatty acids such as linoleic acid and linolenic acid were previously reported as finger millet metabolites (*Kumar et al.*, 2016). Moreover, the fermented millet-based distilled alcohol raksi was also reported to contain palmitic acid and linoleic acid (*Majumder et al.*, 2021). *Majumder et al.* (2021) referred to those fatty acids of raksi as fermentation metabolome existing in various fermented beverages. The Japanese ethnic liquor sake also contains fatty acids, such as palmitic acid, linoleic acid, and linolenic acid (*Ishikawa & Yoshizawa*, 1979), which shows a metabolomic relationship between tongba and sake, in the same way as discussed above in relation to glycoside and amino acid derivatives. Arachidyl alcohol, palmitic or hexadecanoic acid, and pentadecanoic acid were already reported in finger millet sake (*Liu et al.*, 2015), which helped to validate this metabolomic study. The ethyl and methyl esterification of fatty acids is a common phenomenon during alcoholic fermentation, which reportedly contributes to the formation of distinct wine-like flavour (*Yin et al.*, 2019). Earlier, *Ishikawa & Yoshizawa* (1979) described this same event in the fermentation of sake.

Terpenoids

Diterpenoid neophytadiene, its precursor phytol, sesquiterpene alcohol farnesol, and the phytosterol compound beta-sitosterol are components of the terpenoid biosynthesis pathway of TM (*Table 1*). Farnesol is reported as an important component of the Korean ethnic rice wine makgeolli (*Ha et al.*, 2014), which is also the precursor of various bioactive terpenoids (*Majumder et al.*, 2020), which metabolomically validates its presence as a fermented product of tongba. Phytosterol, beta-sitosterol, chlorophyll-derived phytol and its derivative neophytadiene are typical plant metabolites, where beta-sitosterol is specifically reported as a millet metabolite (*Islam et al.*, 2018). Thus, metabolomics suggested that these terpenoids in tongba were obtained directly from substrate.

Other components

Three other bioactive components with notable peaks were detected in TM, which are sole representatives of their chemotaxonomical categories; these are: tyrosol of the phenol, sugar alcohol – 1,3-methylene-D-arabitol, and dihydroergotamine of the alkaloid. The naturally occurring phenol tyrosol is a millet metabolite (*Sun,* 2017) and is documented as a millet fermentation product as well (*Ren et al.*, 2021). The production of tyrosol by *Saccharomyces cerevisiae* is reported in sake and the glutinous millet-based wine huanhjing (*Soejima et al.*, 2012; *Ren et al.*, 2021), which collectively validated tongba's similarity with sake and the occurrence of this compound in this millet-based beverage. The sweetener compound arabitol is a yeast metabolite and can be found in various fermented foods (*Kordowska-Wiater*, 2015). *Saha et al.* (2007) reported this compound as the main product of glucose fermentation by *Zygosaccharomyces* sp., starter of various fermented beverages, including kombucha. A series of research has revealed sugar alcohol arabitol as an important and typical component of sake besides amino acid derivatives and major glucoside compound α-EG (*Imanari & Tamura*, 1971; *Takenaka et al.*, 2000). Alkaloid dihydroergotamine is actually a secondary metabolite of ergot fungi, and, additionally, millet (substrate of tongba) is an important host of ergots (*Haarmann et al.*, 2009); therefore, metabolomics has confirmed this component as a substrate-derived contamination or microbial output.

The glycoside compound α-EG; amino acid derivatives, i.e. cyclo-D-Leu-L-Pro and pyroglutamic acid; fatty acid derivates; other compounds, i.e. phenolic tyrosol and sugar alcohol arabitol, etc. revealed a metabolomic similarity between tongba and sake. The biosynthesis of these fermented metabolites are dependent on both substrate and starter material. It is known that millet is the substrate for both tongba and sake. On the other hand, starter cultures are also similar, comprising mixed cultures of fungi and bacteria with starchy cereals as the base. These starter cultures are usually found in the form of dried powder, flattened cakes, or hard balls of various sizes (*Koay et al.*, 2022). Both sake and tongba are fermented using amylolytic starter cultures usually containing amylolytic fungi for starch hydrolysis and yeast for alcohol production (*Koay et al.*, 2022). The spores of the *Aspergillus oryzae* (koji-kin) are added to the steamed rice, which is then incubated to produce koji, starter of sake. *Aspergillus oryzae* is a filamentous mould grown on rice (also known as koji mould), which is responsible for the production of most of the sake's metabolites but mainly of the major compound α-EG. Tongba's starter khesung or murcha is also prepared by pounding overnight-soaked glutinous rice (*Tamang & Kailasapathy*, 2010) that contains a microflora rich in the rice-grown amylolytic filamentous mould *Rhizopus oryzae* yeast species such as *Saccharomycopsis fibuligera*, *Saccharomyces bayanus*, *Candida glabrata*, *Pichia anomala*, *Saccharomycopsis capsularis*, and *Pichia burtonii*, and probiotic-candidate amylolytic lactic acid bacteria (*Tamang & Kailasapathy*, 2010; *Koay et al.*, 2022; *Olee et al.*, 2022). Therefore, the presence of rice-grown fungi in the rice-based starter khesung or murcha is natural, these fungi being responsible for the production of metabolites similar to those of sake. Just like *Aspergillus oryzae*, both *Rhizopus oryzae* and *Saccharomycopsis fibuligera* show glucoamylase, alpha-amylase, and protease activity (*Olee et al.*, 2022). Hence, the production of the starch breakdown product α-EG and the protein-derived cyclo(L-Leu-L-Pro) and pyroglutamic acid during the fermentation of tongba is acceptable. However, the microbiological assessment of tongba is yet to be performed, as besides this metabolomic interpretation, it is required to evaluate the biosynthesis pathways of α-EG and other compounds in tongba.

The bioactive compounds of tongba and their therapeutic effects in high-altitude illnesses

In highlands, moisture availability in the atmosphere decreases with elevation, where the human body, mainly the skin, must retain moisture on its own or by using supplementations. Reportedly, TM's major compound α-EG can have skin moisturizing and moisture retention effects and can decrease skin irritation also (*Bogaki et al.*, 2017). *Bogaki et al.* (2017) discovered α-EG's potential proliferation-activating effect on the fibroblasts of human dermis, which consequently increases the production of collagen, the most abundant protein of the human body (main structural protein in the extracellular matrix of connective tissues) to exhibit effective damage-repairing activities or speedy healing properties on various body parts, including the skin, tendons, ligaments, bones, and muscles. A formulation enriched with α-EG would definitely be beneficial for humans in highlands, where a speedy healing of the skin, muscles, ligaments, and bones is required. Therefore, a traditional beverage like tongba, offering the same, should always be welcomed in high-altitude conditions. Earlier, *Mishima et al.* (2005) described α-EG as a diuretic component that increases urine production without affecting renal cells or functions, the blood glucose level, and insulin production. This biological activity can be cardioprotective, as frequent urination lowers high blood pressure.

Cyclo(L-Leu-L-Pro) is a potential antifungal metabolite that can also possess antiviral and antibacterial (*Zhao et al.*, 2021), antimutagenic, antifouling, antiprotozoal, and various similar properties of pharmacological importance (*Acharyya et al.*, 2021). Antibiotic actinomycin C2 is an antioxidant and displays cytotoxic activity against various human pathogens (*Saravana Kumar et al.*, 2014). Pyroglutamic acid possesses hepatoprotective, antidepressant, and anti-inflammatory properties (*Gazme et al.*, 2019). Similarly, the millet-based fermented beverage raksi is reported to exhibit biological activities against different diseases and disorders associated with altitude sickness due to the presence of such fatty acids (*Majumder et al.*, 2021). Fatty acid palmitate and linoleate are cardio-protective agents that can exhibit properties as vasodilators, antihypertensive and coronary heart disease preventives, which seems to be helpful in highlands (*Majumder et al.*, 2021). Palmitic acid and pentadecanoic acid have many other biological activities such as anti-inflammatory, antioxidant, and antibiotic (*Anyasor et al.*, 2015; *Venn-Watson et al.*, 2020; *Majumder et al.*, 2021). Linoleate is reported to prevent hypoxia and related respiratory illnesses and altitude-sickness-related symptoms such as fatigue, dizziness, vertigo, etc. (*Majumder et al.*, 2021). Moreover, even-chain fatty acid palmitate [C:16] and odd-chain pentadecanoate [C:15] are reported as beneficial for human health, and both have been detected in the sample TM including three derivatives of each (*Table 1*). Recently, *Venn-Watson et al.* (2020) have reported the efficacy of pentadecanoate in reducing cardiometabolic diseases, inflammation, anaemia, breathing problems, and chest pains causing disorders, pulmonary fibrosis. As major compound α-EG, straight-chain fatty alcohol and arachidyl alcohol also help to prevent skin damages by retaining moisture (*Lukic et al.*, 2021). Arachidyl alcohol is typically produced from arachidonic acid, which also exhibits a wide range of bioactivities, but mainly anti-inflammatory, cardio-protective, antidiabetic, antiviral, and antibacterial activity, and it can inactivate the severe acute respiratory syndrome (SARS-CoV-2) (*Das*, 2020). Beta-sitosterol, phytol, and neophytadiene are reported for their anti-inflammatory, antioxidant, and antimicrobial activities (*Bhandari & Lee*, 2013; *Islam et al.*, 2018). Phytol can exhibit neuroprotective properties such as anxiolytic and anticonvulsant effects (*Islam et al.*, 2018). Farnesol is a potential cardio-protective, antimicrobial, antitumor, antioxidant, antidiabetic, and neuroprotective compound (*Delmondes et al.*, 2020). This compound acts like a vasodilator and natural calcium channel blocker to reduce hypertension and exhibits bioactivities against disorders directly linked to high-altitude illnesses such as asthma, edema, and inflammation (*Delmondes et al.*, 2020; *Chen et al.*, 2019). Tyrosol has a wide range of medicinal properties, i.e. antioxidant, anti-inflammatory, antiulcer, antimicrobial, antitumor, antiviral, anticancer, antidiabetic, and antiallergic activities (*Sun*, 2017). Having pharmacological effects, a considerable amount of dihydroergotamine can be acceptable in food (*Beuerle et al.*, 2012), especially in a high-altitude beverage, as it can prevent migraine disorders and is reported as effective pain reliever for people with cluster headache and very frequent migraine attacks (*Tfelt-Hansen & Koehler*, 2008).

4. Conclusions

The ethnobiology of Singalila Ridge and the adjoining high-altitude places of Darjeeling, Sikkim, and Nepal are rich in various traditional elements, which remains to be studied and explored. This metabolomic study revealed that tongba, containing the collagen-producing and moisture-retaining compound ethyl-α-EG, could heal damages occurred to the skin and other connective tissues due to exposure to high altitude. Inflammation and high altitude are closely associated, as body muscle inflammation, joint pains, peripheral edema (swelling of hands, feet, and face), etc. are common high-altitude problems. Interestingly, in Singalila and the neighbouring hills, drinks like tongba are served as healing beverages to get rid of pains. Here, metabolomics validated this property by revealing the presence of many anti-inflammatory compounds such as pyroglutamic acid, palmitic acid, pentadecanoic acid, phytol, neophytadiene, β-sitosterol, and tyrosol. Studies on the bioactivities of the metabolites of tongba and on the biosynthesis of those components have conclusively discovered that this ethnic beverage is metabolomically similar to sake and is a potential element of Himalaya’s ethnoecology.

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