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Metabolomics affirms traditional alcoholic beverage *raksi* as a remedy for high-altitude sickness

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Abstract

Raksi, a fermented distilled alcoholic beverage, is an ethnic drink consumed in high altitude regions of Singalila Ridge of the Himalayas and in adjoining high altitude places in Nepal, northern and north eastern part of India and Tibetan plateau. Like *jaanr*, *tongba*, *nigar*, *chhyang*, and other fermented ethnic beverages, *raksi* is considered as an element of ethnopharmacology of high altitude with claims of medicinal properties. An ethnobiological survey was done in the study area prior to collection and identification of *raksi* samples. In this research, two *raksi* samples (*khokim raksi* and *chimphing raksi*) of Singalila Ridge of the Himalayas were investigated by metabolite profiling using gas chromatography-mass spectrometry analysis. Results of the experiment showed presence of several respiratory protective, cardioprotective, neuroprotective, anti-inflammatory, and antioxidant components which have properties to prevent various high altitude illnesses. Moreover, large quantities of bioactive terpenoids, fatty acid derivatives, coumarins, and peptides were detected whose chemotaxonomy and biosynthesis pathways were further studied. This metabolomics investigation not only affirmed *Raksi* as a remedy for high-altitude sickness but also helped in understanding the importance of this type of ethnic foods in high altitude ethnoecology. This research on *raksi* in the fields of ethnobiology and metabolomics is a cumulative approach which has opened the door for in-depth investigations on similar ethnic foods. However, further experiments on *raksi* are surely needed in ethnobiology, microbiology, biochemistry, and food technology.

Keywords: High altitude, *Raksi*, Ethnoecology, Ethnic beverage, GC-MS, Metabolomics

Introduction

Elements of human lifestyle, i.e., habitation, clothing, and food habits, are the components that represent ethnic identity of a society. This ethnic identity may differ depending on the region one lives, its geography, weather, and other environmental conditions. The altitude of a region also plays a great role by influencing human activities because altitude controls the environment affecting human lifestyle, precisely, the food that is grown and consumed by the inhabitants of that region. These habits may also differ depending on the age and ability

to process the components of daily needs for consumption. Moreover, ethnicity and food is more dependent over average digestion capability and physical ability of the group of individuals to process the food available in that particular region. An important portion of the world's population lives at high altitude where daily lifestyle, food production, food availability, food requirements, and food habits are seen to be completely different and assiduous, unlike the rest of the world. In a review article, Picon-Reategui [1] provided information on the food and nutrition of high-altitude populations. According to the study, both food production and availability are definitely tied up to altitude, weather condition, soil, and other environmental factors. Relations between food habits and high altitude were further

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described by many scientists and ethnobiologists. Ethnoecology is the scientific study of how different groups of people living in different regions understand the ecosystems around them. This study also helps us to understand how a society or group of people accepts their surrounding environment or conceptualizes and gets acclimatized with their ecological system [2]. So, according to this principle of ethnoecology, high altitude food habits are distinct just to help locals, tourists, or visitors of high altitudes get acclimatized with the surrounding environment, to stay well and get recovered from high-altitudinal sicknesses like hypoxia, fatigue, dizziness, loss of appetite, sleep problems, headache, weakness, acute mountain sickness, vertigo, peripheral edema (swelling of hands, feet, and face), nose bleeding, and cardiovascular diseases. Kayser [3] described the effect of high altitude on nutrition where high altitudinal ailments like hypoxia, gastroenterological (digestion) problem, weight loss, and anorexia nervosa and symptoms like nausea, vomiting, and loss of appetite were mentioned. Anand et al. [4] described this altitude specific health issues with main focus on gastrointestinal problems. Not only ethnic foods or beverages but also local medicinal herbs and plant organs of a region are also considered as elements of that region's ethnoecology. Ethnoecology is cross-cultural where interrelation between people and their environments are studied [5] with ethnic foods, beverages, and ethno-medicines as key components. Like every other region of the world, high altitude ethnoecology exhibits a rational relationship between its people and their ethnic food habits (food/beverage or other ethno-medicines) which directly or indirectly help a group of people to endure the weather conditions.

Ethnoecology of Singalila Ridge of the Himalayas (the high altitude place in between Darjeeling, Sikkim, and Nepal, i.e., our study area) is very distinctive and enriched with plenty of ethnic food habits where *jand* or *jaanr*, *raksi* or *rakshi*, *nigar*, *chhyang* or *chhaang*, etc. are mentioned as Nepalese and Tibetan ethnic fermented cereal-based alcoholic beverages [6]. Settlements of human ethnic groups (mainly Nepalese) are found in Singalila National Park and adjoining hills. High altitude places like Tumling (2949 m), Phatak (2992 m), Jaubari (2900 m), Gairibans (2625 m), Kaiyakatta (2879 m), Batasay (2971 m), Kalipokhari (3100 m), Bikheybhanjyang (3200 m), Sandakphu (3626 m), and Phalut (3600 m) are the main villages of Singalila Ridge. Other settlements are also found in the buffer zone of Singalila (at approx. 2300 m altitude), i.e., Rammam, Siri, Gurdung, Samanden, and Gorkhey [7]. Interestingly, fermentation knowledge and practice have been considered as an intimate part of food and beverage management in high altitude regions since ancient ages which are actually considered as a part of ethnicity of high altitude regions

in the world. Fermentation is a process which is followed to preserve food, especially where availability of important nutritious food is a matter of stress. Fermentation helps increase shelf-life of processed or harvested food material where beneficial microorganisms are used. Major fermented foods of high altitude regions of the Himalayas (Darjeeling, Sikkim, and Nepal) are fermented legume products (*kinema*, *masauyra*), fermented dairy products (*chhurpi*, *chhu/sheden*, *philu*, *somar*, etc.), fermented vegetables (*gundruk*, *sinki*, *Khalpi*, *Mesu*, etc.), fermented cereal products (*selroti*), fermented meat/fish products and alcoholic beverages like *chhaang* and *raksi* [6]. These homemade fermented foods and beverages are considered as ethnoecological components of the mentioned region for being acclaimed as medicinally beneficial to native people or visitors against a lot of high-altitude sickness.

Raksi or *rakshi* (also pronounced as *roxy*) is one of those locally fermented traditional food, a distilled alcoholic beverage, which have been used as a medicinal and regular beverage by Tibetan, Nepali, and other ethnic group of people living in high altitudes of the Himalayas (parts of Nepal, northern and north eastern part of India and Tibetan plateau). It is a home-made beverage produced from different grains where millet or rice is used as the main substrate. *Raksi* is strong and highly astringent in flavor and taste that gives a burning sensation in the digestive tract during consumption [8]. Microbes involved in fermentation of *raksi* are mainly fermentative yeasts, saccharifying mold, and lactic acid bacteria [9]. Drop of pH or increase of acidity during fermentation is reported in *raksi* which is expected due to occurrence of organic acids as fermentation metabolites. Alcohol concentration of *raksi* is reported as 11 to 13% [9] which is quite acceptable as a regular beverage in high altitudes. *Raksi* is a very popular homemade liquor in Tibet and in high altitudes of Nepal, Darjeeling (West Bengal), and Sikkim, and is also brewed at almost every household in villages of Singalila, not only for their own consumption but also for selling as it is also very popular among the tourists [7]. The liquor is required in various religious rituals and social events performed by Nepalese and Tibetan Society. *Raksi* is also ranked as 41st in the CNN's list of World's 50 most delicious drinks [8].

Raksi, *chyang*, *jand*, etc. are well documented in the field of ethnoecology along with some health claims, but there is a lack of proper scientific research on it. Ray et al. [10] prepared *chyang* in laboratory and performed biochemical analysis of it but *raksi* is scientifically undiscovered as it has not been significantly studied. The ethnomedicinal claim of this high-altitude medicinal drink should be properly evaluated. Therefore, the main aim of the study was to analyze the biochemical composition and biological activities of two different types of *raksi* by

using metabolomics as a tool. In this research, we have analyzed the metabolites (by GC-MS analysis) of two different types of *raksis*, i.e., *khokim raksi* and *chimphing raksi* sipped in Singalila Ridge (the highest altitude region of Darjeeling Himalaya).

Materials and methods

Study area and ethnobiological survey

A fieldwork was conducted in the trekking routes (orthodox trekking route of Singalila Ridge, i.e., Dhوترey-Tonglu-Tumling-Gairibans-Kalipokhari-Bikheybhanjyang-Sandakphu-Phalut) of Singalila National Park located in Darjeeling, India, and Indo-Nepal border area (Fig. 1) to collect samples of *raksi* and identify them by local inhabitants. The local inhabitants or participants of this ethnobiological survey were 55 males and 45 females (24 to 91 years of age groups). Among participants, 68% were regular *raksi* consumers and all of the participants were ethnic Nepalese (belong to Lepcha and Nepali communities) who lived in villages of Singalila ridge, i.e., Tonglu (5%), Tumling (14%), Gairibans (20%), Kalipokhari (17%), Bikheybhanjyang (29%), Sandakphu (11%), and Phalut (4%).

Sample collection and preparation

Two different fresh samples of locally brewed *raksi* (*khokim raksi* and *chimphing raksi*) were collected from a very old (50 years) and famous traditional *raksi* selling shop at Bikheybhanjyang (27° 05' 32" N 88° 00' 47" E, Fig. 2). In between Kalipokhari village (3100 m) and Sandakphu (3626 m, the highest peak of the ridge as well as

of the state of West Bengal, India), Bikheybhanjyang (3200 m) is located (Fig. 3) and reported as one of the oldest human settlements in Singalila National Park [7].

Samples of *raksi* were collected in sterilized laboratory glass bottles (Fig. 4) to avoid microbial contamination and kept inside the ice box and taken to laboratory. Sample preparation for GC-MS analysis was done following the method of Majumder et al. [11] with slight modification. One milliliter of each sample was dissolved in 1 ml of methanol (Merck) overnight to prepare methanolic (50%) extracts of *khokim raksi* (KR) and *chimphing raksi* (CR). Methanol was taken as solvent depending on some facts, like, it is the widely used and ideal most solvent (compared to other organic solvent) for extraction of biochemicals, and polarity of methanol as an organic solvent shows closeness with that of water or ethanol which are primary solvents of any edible beverage [11].

GC-MS analysis

Following the research protocol of Majumder et al. [11, 12], metabolite profiles and metabolomics of compounds from different biosynthesis pathways of KR and CR samples were analyzed using GC-MS based metabolomics. GCMS-QP2010 Plus (Shimadzu Co., Japan) with DB-5 fused-silica capillary column (0.25 µm film thickness, 0.25 mm internal diameter, and 30 m of length) was used in this research. Analysis was performed by split injecting (with a ratio of 20:1) 1 µl of each sample (KR and CR). Injection temperature was 260 °C and interface temperature was 270 °C. Ion source temperature was

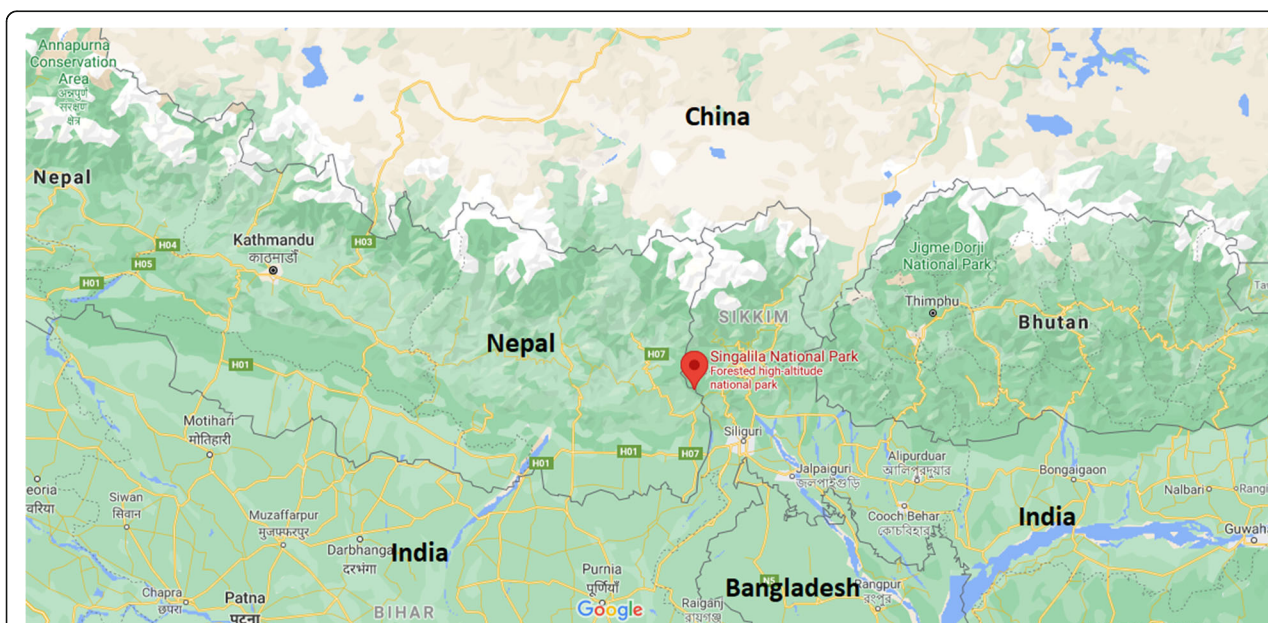


Fig. 1 Location of Singalila National Park at Indo-Nepal border in the Himalayan region



Fig. 2 *Raksi* selling shop at Bikheybhanjyang, from where samples of freshly prepared *rakis* were collected

adjusted to 230 °C. Helium gas (99.9%) was used as carrier gas. Total flow rate and column flow rate were 16.3 ml min⁻¹ and 1.21 ml min⁻¹ respectively. Mass spectra were recorded at 5 scan/s with a scanning rate of 40–650 m/z. The compounds were identified after comparing the spectral configurations obtained with that of the available mass spectral database. The compounds were detected using library databases like NIST08s.LIB and WILEY8.LIB. The chromatogram (TIC or total ion chromatogram) is based on the intensity of fragments produced by the ionization. Quantification of the amount (area %) of each compound was done on the basis of peak areas. The data obtained from GCMS analysis were further studied from available literature.

Results and discussion

Ethnobiological survey

In this study, ethnobiological survey helped to collect proper traditional *raksi* samples from one of the oldest *raksi* brewer of Bikheybhanjyang (one of the oldest

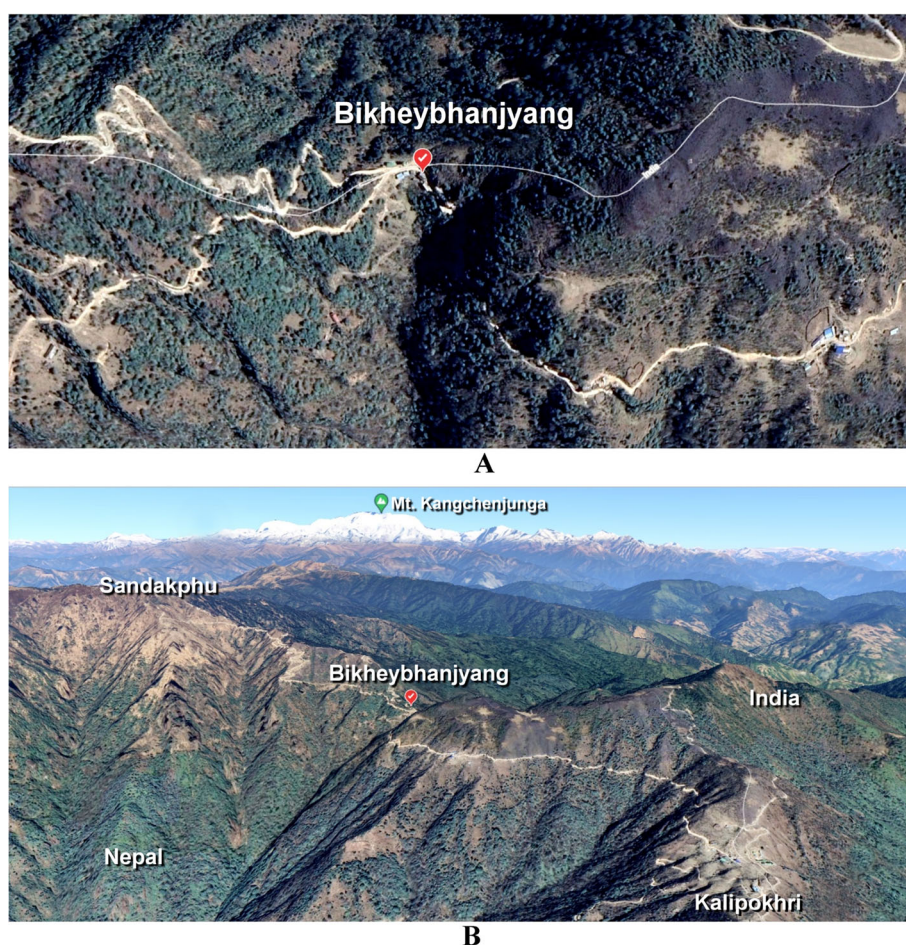


Fig. 3 Bikheybhanjyang extracted from Google Earth (<https://earth.google.com/web/>). **A**—A close view of the village Bikheybhanjyang, the sample collection site. **B**—Location of Bikheybhanjyang in Singalila Ridge (between Kalipokhri and Sandakphu)



Fig. 4 Samples of *raksi* KR (left) and CR (right) kept in sterilized laboratorial glass bottles

human settlements in Singalila) and identify two distinct *raksi* samples: *khokim raksi* and *chimphing raksi*. Moreover, all the participants shared their knowledge on ethnomedicinal claims of *raksi* as cardio-protective, neuroprotective, pain reliever or anti-inflammatory, respiratory illness preventive, and gastro-protective. Furthermore, in this research, scientifically successful tool of metabolomics, i.e., GC-MS metabolite profiling was used to analyze both *raksi* samples to evaluate the unexplored reports of bioactivity or ethno-medicinal claims in a biochemical way.

GC-MS analysis

Gas chromatography-mass spectrometry analysis, a successful tool for metabolomics [12], was used in this research to analyze bioactive components of *raksi* samples and metabolites of the microorganisms involved in *raksi* fermentation. GC-MS analysis exhibited nineteen peaks for different compounds for the sample KR including bioactive terpenoids, derivatives of bioactive fatty acids (methyl esters of linoleic acid and petroselinic acid), and coumarins (meranzin and auraptene) while CR came out with a total of thirteen compounds which is rich in different amino acid derivatives (amines and peptides) including antibiotic and anti-inflammatory drug compound cyclo(gly-pro) as the major compound. Peak reports for both KR and CR have been included in Table 1.

Bioactivities of *raksi* compounds against altitude sickness

Interestingly, after studying previous literatures on these GC-MS detected compounds, it was found that most of

the bioactive components of *raksi*s (Table 1) were reported to prevent diseases and disorders that are cumulatively linked with ailments occurring in high altitude regions, simply known as altitude sickness. This includes a huge number of diseases and disorders, i.e., respiratory disorders like chronic obstructive pulmonary disease (COPD), hypoxia, fatigue, dizziness, loss of energy, and shortness of breath; neurological and psychiatric disorders and diseases like loss of appetite, sleep problems, headache, loss of coordination, cyanosis, confusion and irrational behavior, tightness in the chest, weakness, and vertigo; gastrointestinal problems; pain and inflammation in the body muscle and joints, peripheral edema (swelling of hands, feet, and face); nose bleeding; cardiovascular diseases like high blood pressure, high pulse rate, heart attacks and, at last but not least, acute mountain sickness [13]. Regular high altitude ailments and components of *raksi* reported to prevent them are described below which favorably represent the acceptance of this beverage as a potent high altitude ethnic medicine (on the basis of metabolomics only).

Respiratory illness and *raksi* components

Respiratory disorders and more precisely chronic obstructive pulmonary disease (COPD) are considered as the main illness in altitude sickness [13]. Disorders and symptoms like hypoxia, lung injury, bronchitis, asthma, fatigue, dizziness, loss of energy, shortness of breath, and chest pain as a whole are considered as respiratory illnesses. A total of six different *raksi* components (derivatives of linoleic acid; nootkatone; derivatives of lidocaine; auraptene, calamendiol, etc.) are found to be reported as respiratory illness preventive compounds. Major KR compounds, linoleates [14] and auraptene [15], are reported to prevent hypoxia. According to Takao et al. [16] and Nemmar et al. [17], nootkatone and lidocaine are two lung protective compounds. These two compounds were mainly detected in KR. Moreover, another derivative of lidocaine, lidocaine benzyl benzoate, detected in both KR and CR, is reported as potent bronchodilator [18]. Calamendiol is the only exclusive respiratory protective compound found in CR which helps to prevent bronchitis (infamous high altitude diseases) and sinusitis [19].

Cardiovascular diseases and *raksi* components

It is already described earlier that there are some major cardiovascular diseases reported as high altitude ailments. Sometimes, cardiovascular illness causes life threatening problems to the visitors or tourists after exposure in high altitude environment unlike high altitude residents because, according to principles of ethnoecology described earlier, it is a fact that living at high altitude reduces risk of dying from heart disease [20].

Table 1 Components of KR (*Khokim raksi* extract) and CR (*Chimphing raksi* extract) detected by GC-MS analysis detected by GC-MS analysis

Compound index	Name of compound	Area% in KR	Area% in CR	Type of compound
1	Nootkatone	0.92	-	Sesquiterpenoid
2	Copalol	2.1	-	Diterpenoid
3	Squalene	2.56	-	Triterpenoid
4	Geranylgeraniol formate	1.46	-	Diterpenoid derivative
5	Driman-8,11-diol	2.13	-	Sesquiterpenoid
6	Geranylinalool	-	4.62	Diterpenoid
7	Calamendiol	-	2.53	Sesquiterpenoid
8	Ethyl linoleate	2.58	-	Polyunsaturated omega-6 fatty acid derivative
9	Sandacanol	0.93	-	Organic oil
10	Linoleyl alcohol	0.26	-	Fatty alcohol
11	Methyl palmitate	2.18	-	Saturated fatty acid derivative
12	Methyl linoleate	20.84	-	Polyunsaturated omega-6 fatty acid derivative
13	Methyl petroselinat	11.96	-	Unsaturated fatty acid derivative
14	Methyl stearate	2.94	-	Saturated fatty acid derivative
15	Caprylic triglyceride	0.44	-	Natural fatty acids with glycerin
16	Phenylacetate	-	6.84	Aromatic fatty acid metabolite of phenylalanine
17	5-Chlorovaleric acid, 2,5-dichlorophenyl ester	-	1.14	Saturated fatty acid derivative
18	Octyl methacrylate	-	4.59	Lipid
19	Lidocaine	8.03	-	Amino amide derivative
20	Lidocaine benzyl benzoate	1.56	2.51	Amino amide derivative
21	pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-or cyclo(gly-pro)	-	26.47	Peptide
22	N,N,N'-Triethylethylenediamine	-	8.2	Amines
23	Cyclopropanecarboxamide, N-cycloheptyl	-	17.11	Amines
24	5-Pyrrolidino-2-pyrrolidone	-	16.77	Amines
25	pGlu-phe-pro	-	5.67	Peptide
26	Meranzin	7.26	-	Coumarin
27	Auraptene	14.44	-	Monoterpene coumarin
28	Etiocolanolone glucuronide	1.17	-	Steroid
29	(7R)-cis-anti-cis-Tricyclo[7.3.0.0(2,6)]dodecan-7-ol	-	2.05	
30	Phthalic acid	16.24	-	Dicarboxylic acid
31	Isobutyl tridec-2-yn-1-yl phthalate	-	1.51	Dicarboxylic acid

Results of this research suggested acceptance of health claims of *raksi* as a cardio-protective ethnic medicine in the form of a fermented beverage [21]. Reports of fourteen components of both KR and CR revealed *raksi*'s cardiovascular protective activity. Among fatty acid derivatives detected in KR, linoleates [22], methyl palmitate [23], methyl petroselinat [24], methyl stearate [25], etc. are reported as cardio-protective agents. Linoleic acid can lower blood cholesterol and prevents coronary heart disease [22]. Cardio-protective agent lidocaine and its

derivative lidocaine benzyl benzoate (detected in both CR and KR) prevent hypertension and release high blood pressure [26] which is very important during altitude sickness. Coumarins, the major group of molecules detected in KR (Table 1), are reported to exhibit cardio-protective activities. Monoterpene coumarin auraptene is reported as cardio-protective agent and antihypertensive [27] while coumarin meranzin is reported as anti-atherosclerosis [28]. Bioactive terpenoids like nootkatone (antiplatelet) and squalene (controls cholesterol),

detected in KR, are also reported as cardio-protective compounds [29, 30]. Exclusive CR components pGlu-phe-pro, being thyrotropin releasing hormone, and calamendiol [19] also prevent cardiovascular diseases. Vasodilation is an important cardiovascular activity that has to occur during hypoxic conditions (lack of oxygen) to allow more blood flow in tissues. Vasodilation decreases blood pressure by increasing blood flow rate through our blood vessels which is very important during physical works (i.e., trekking) in high altitudes. This process also prevents some respiratory ailments in high altitudes. Interestingly, *raksi* components are rich in potential vasodilators like lidocaine [31], lidocaine benzyl benzoate [26], methyl palmitate [23], and auraptene [27].

Inflammation, pain, and *raksi* components

Pain/inflammation and high altitude are closely associated. Pain, nociception, and inflammation in the body muscle and joints, peripheral edema (swelling of hands, feet, and face), etc. are common high altitude problems for high altitude tourists or visitors. Not only *raksi*, but several other ethnic medicines served as foods or beverages (*chhyang*, *tongba*, Rhododendron flower juice or wine, last but not least tea) in many places of Singalila Ridge and neighboring hills to get rid of pains. Interestingly, most of the *raksi* components of this study, i.e., nootkatone [32], lidocaine [33], methyl palmitate [23], auraptene [34], squalene [12], geranylgeraniol [35], driman-8-11-diol [36], phenylacetate [37], cyclo(gly-pro) [38], and calamendiol [19] are reported as potential anti-inflammatory, pain reliever, and anti-nociceptive.

Neurological and psychiatric ailments preventive *raksi* components

High altitude neurological and psychiatric ailments may be linked to symptoms like dizziness, mental fatigue, shortness of breath, loss of appetite, sleep problems, headache, loss of coordination, cyanosis, confusion and irrational behavior, tightness in the chest, weakness, and vertigo [13]. KR compounds, i.e., nootkatone [39], auraptene [34], geranylgeraniol [35], and caprylic triglyceride [40], and major CR compound cyclo(gly-pro) [41] are reported as potential neuroprotective components. Major fatty acid components of KR (linoleates and petroselinates) are reported to prevent altitude sickness symptoms like fatigue, dizziness, and vertigo [42–44]. Regarding other KR compounds, lidocaine is reported to prevent intracranial hypertension [45]; major coumarin compound auraptene exhibits all kind of bioactivities against high altitude neurological and psychiatric illness like anxiety, depression, dizziness, and Alzheimer's disease [27]; coumarin meranzin is also an antidepressant [46]; squalene can decrease headache, weakness, and mental fatigue [30]; and caprylic triglyceride [47] is

anticonvulsant, bioactive against Alzheimer's disease and can prevent weakness [40]. Jain et al. [19] reported that sesquiterpenoid compound calamendiol (detected in CR) can prevent headache, fatigue, anxiety, moreover, it is an anti-hallucinogen.

Gastroenterological problems and *raksi* components

Some of the GC-MS detected components described earlier are known to prevent gastroenterological issues occurring due to exposure of high altitude. Lidocaine (present in KR) and lidocaine benzyl benzoate (detected in both KR and CR) are two bioactive components that can reduce food intake by reducing hunger (loss of appetite) and increasing satiety rating in human [48, 49]. Moreover, lidocaine has hepatoprotective activity [50] and lidocaine benzyl benzoate is an antidiabetic agent [51]. Major KR compound auraptene can reduce the loss of appetite potentially [27] which is good for physical activities in high altitude places and it can prevent some digestive problems too [27].

Other bioactivities

pGlu-phe-pro or pyroglutamyl-phenylalanyl-proline (major CR compound) is a thyrotropin-releasing hormone that increases plasma triiodothyronine levels and triiodothyronine (T3 thyroid hormone) then affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate [52] which is surely useful in high altitude condition. Interestingly, KR compound nootkatone, is a signature and major molecule of *Cyperus rotundus* L. plant which exhibits more other potential bioactivities against ailments that are linked with altitude sickness, i.e., laryngitis, tracheitis, pneumonopathy, cough, swelling of throat, nerves weak, headache, dizziness, indigestion, thirst, fever, hypoxia, asthma, and inflammation [53]. Besides high-altitude sickness-related issues described above, these GC-MS detected *raksi* components can exhibit antioxidant, antibiotic, anticancer, or antitumor activities. Antioxidants are generally compounds produced in the human body and found in foods that help defend cells from damage caused by potentially harmful molecules or free radicals during oxidative stress. Free radicals are harmful when their levels become too high in our body. Multiple illnesses, including diabetes, heart disease, and cancer can be caused by these free radicals. Nootkatone [32], methyl palmitate [23], auraptene [34], squalene [30], geranylgeraniol [54], cyclo(gly-pro) [55], etc. are potential antioxidants molecules of *raksi*. Some of the compounds are also reported as antibiotic, such as cyclo(gly-pro) [55], lidocaine [56], methyl palmitate [57], nootkatone [58], squalene [30], and driman-8,11-diol [36]. Compounds with anticancer properties are linoleates [59], nootkatone [60], methyl

palmitate [61], auraptene [34], squalene [30], geranylgeraniol [35], and phenylacetate [37].

Biosynthesis of metabolites present in *raksi* samples

Study of metabolite biosynthesis on the basis of GC-MS analysis is a very basic tool and early concept in metabolomics [12]. Types of metabolites detected in *raksi* and origin of those compounds reveals a number of biosynthesis pathway involved in *raksi* which either originated from microorganisms involved in *raksi* fermentation or from the fermentation substrates (millet or rice) and other organic materials used as the starter culture. Biological classification of a compound or study of its chemotaxonomy actually helps reveal the biosynthesis route of that compound [12]. Chemotaxonomy study confirmed the majority of terpenoids, fatty acid derivatives, and coumarins in KR while CR is rich in amines, peptides fatty acid derivatives, and terpenoids as described earlier. Sesquiterpenes (nootkatone, driman-8,11-diol, and calamendiol), diterpenes (copalol, geranylgeraniol formate, and geranyllinalool), a monoterpene (auraptene) and a triterpene (squalene) were detected in *raksi* (1-7 in Table 1) which are probably derived from either plants (substrate and starter of *raksi*) or microbial fermentation process, because terpenoid biosynthesis and metabolism is reported in microbial fermentation [62]. Moreover, geranylgeraniol (detected as geranylgeraniol formate) of KR or geranyllinalool of CR plays a role as a common yeast metabolite or fermentation component [63]. Similarly, plant derived or microbial fermentation induced metabolomes revealed fatty acid and lipid biosynthesis and degradation pathways behind occurrence of lipids and fatty acid derivatives (8-18 in Table 1) in *raksi*, i.e., linoleyl alcohol, sandacanol, ethyl linoleate, methyl palmitate, methyl linoleate, methyl petroselinate, methyl stearate, phenylacetate, octyl methacrylate and 5-chlorovaleric acid, and 2,5-dichlorophenyl ester; coumarin biosynthesis pathway behind synthesis of auraptene and meranzin; peptide and amino acid biosynthesis pathway behind synthesis of major CR metabolites (19-25 in Table 1) which are chemo-taxonomically peptides or amines, i.e., cyclo(gly-pro); n,n,n'-triethylethylenediamine, cyclopropanecarboxamide n-cycloheptyl; 5-pyrrolidino-2-pyrrolidone; and pGlu-phe-pro. Major CR compound and reported *Saccharomyces cerevisiae* (baker's yeast) metabolite, phenylacetate is also reported as a product of phenylalanine fermentation [64]. Regarding major peptides of CR, pglu-phe-pro is a microbial fermentation derived compound which is also found in different bioactive ethnic fermented foods like Japanese rice wine (sake), different cheese, and soya sauce [65] while 5-pyrrolidino-2-pyrrolidone is reported as a fermentation derived secondary metabolite of *Streptomyces* [66]. Furthermore, aminobenzoates like lidocaines

(lidocaine and lidocaine benzyl benzoate) and steroid like etiocholanolone glucuronide may play role as plant or microbial metabolites if chemotaxonomy is concerned.

Except *raksi*, the decoction or tea of *khokim* root (ingredient of *khokim raksi* or KR) is also used as an ethnomedicine in high altitude which is consumed to treat fever, body ache, muscular pain, etc. [67] and, impressively, a number of compounds have been detected in *khokim raksi* extract (KR) which are potential anti-inflammatory agents or pain relievers. So, reasons behind addition of *khokim*'s root in *raksi* and pain reliever or anti-inflammatory health claims on *khokim raksi* are completely valid. Similarly, exclusive *chimphing raksi* (CR) component, calamendiol [19] and ingredient, i.e., dried fruits of *chimphing* [67], both have been reported to exhibit bioactivities against sinusitis; thus, the use of *chimphing* as an ethnomedicine is also justified.

Conclusion

This is the first study to be reported on ethnobiology and biochemical components of *raksi* beverage. This article described the ethnomedicinal values of *raksi* which have been validated by metabolomics. This research also helped to add more scientific value in Himalaya's ethnoecology. Singalila Ridge and adjoining high altitude places of Darjeeling, Sikkim, and Nepal are hubs of ethnobiology which still remain to be studied and explored. Herbal ethno medicines, ethnic foods and ethnic groups of people, and their way of life need to be subjected for future research which will help researchers to discover more and more unveiled biodiversity, anthropology and life science of the Himalayas. Regarding *raksi*, large peaks of highly bioactive coumarin compound auraptene in *khokim raksi* and antioxidant and antibiotic agent, peptide compound cyclo(gly-pro) in *chimphing raksi*, are key outcomes of this research. Studies on bioactivities of metabolites of *raksi*, and biosynthesis of those components, together helped evaluate the health claims of *raksi* which conclusively discovered that this ethnic beverage is a potential element of Singalila's ethnoecology. This high altitude fermented beverage is actually a medicine for every possible disorder or disease referred to as high-altitude sickness.

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Authors' contributions

SM and MB conceived the idea and designed the protocol. SM, AG, SC, and SS performed collection and laboratory work. SM and MB compiled data and wrote the draft manuscript. All the authors read and approved the manuscript.

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Availability of data and materials

All data analyzed during this study are included in this article.

Declarations**Competing interests**

The authors declare that they have no competing interests.

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