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Tracing early life on Mars: lessons from organics produced in high-altitude hotsprings of Ladakh

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Abstract

Hotspring waters have traditionally been recognized for their therapeutic benefits. More recently, high-altitude hotsprings have received attention for astrobiological studies because they replicate several parameters of extreme conditions that once occurred on early Mars. Investigation of such hotspring environments can provide us with more relevant biomarker tools to study the search for possible extinct life on Mars that existed in similar environments. This study presents the first qualitative data (semiquantitative) on organic compounds' origin and distribution in Ladakh's high-altitude hotspring waters. The study was conducted on the Chumathang, Panamik, Changlung, and Puga hotspring sites with thermal water temperatures and pH ranging from 50.4° to 84.9 °C and 7.01 to 8.08, respectively. These sites were dominated by bacterially produced organic compounds, mainly n-alkanes, esters, alcohols, carboxylic acids, and alkenes. Non-distinguishable thermogenic abiotic organic compounds may represent a minor fraction of low molecular weight n-alkanes (C12 and C14). A semiquantitative understanding of organic compounds based on peak area percentage exhibits that larger proportions of organic compounds in the thermal waters of these sites were dominated by a diverse range of bioactive compounds in response to various extreme environmental factors. Compared to the low-altitude hotspring waters, the high-altitude hotspring waters contain a significantly higher number of bioactive compounds. These compounds are stable both chemically and physically in the extreme environments commonly found in high-altitude hydrothermal environments, which makes them promising candidates as biomarkers for the search for early life in Mars' hydrothermal deposits.

Keywords High altitude, Hotspring, Thermogenic, Organic compounds, Biomarker, Ladakh

1 Introduction

Since their discovery in the late 1970s (Corliss et al. 1979), mid-oceanic ridge hydrothermal systems have been considered favorable locations for the abiotic synthesis of organic compounds (Ingmanson and Dowler 1977) with the potential to understand the abiogenic steps in the origin of life (Corliss et al. 1981; Baross and Hoffman 1985; Macleod et al. 1994). This idea of a hydrothermal system as a "cradle of earliest life on the Earth" was initially developed for the alkaline vents where the serpentinization reaction also yields hydrogen gas, and the water temperature is well within the range that microbiological life can withstand (Martin and Russell 2003, 2007; Lane and Martin 2012; Barge et al. 2017). A range of ultramafic-hosted mid-oceanic ridge hydrothermal systems have been found to contain traces of what appear to be abiogenic methane and straight-chain hydrocarbons (Charlou et al. 2000, 2002; Proskurowski et al. 2008). These systems were recorded to contain various organic compounds,

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such as aliphatic, mono-, and polyaromatic hydrocarbons (HC), carboxylic acids, and amino acids (Konn et al. 2009, 2011, 2015; Shulga and Peresypkin 2012; Aubrey et al. 2009; Holm and Charlou 2001; Lang et al. 2010; Klevenz et al. 2010; McCollom et al. 2015; Reeves et al. 2014; Raznitsin et al. 2018; Sorokhtin et al. 2018; Simoneit 2004).

However, more recently, especially after Damer and Deamer (2020) hypothesized that terrestrial hotsprings offer a better physicochemical environment [wet–dry cycles, K^+/Na^+ ratio close to cytoplasm composition (seawater K^+/Na^+ ratio is orders of magnitude higher than the cell cytoplasm), i.e., freshwater] for the synthesis and polymerization of life essential organic molecules than the subseafloor hydrothermal system, terrestrial hotsprings have become a renewed target for research to understand the prebiotic soup and the origin of life. The information on the organic composition of hotspring waters or vapor condensate is available from only a few numbers of studies conducted in Spain (González-Barreiro et al. 2009), Hungary (Kárpáti et al. 1999), Italy (Gioia et al. 2006), Japan (Suda et al. 2022, 2014), Mexico (Sánchez-Avila et al. 2021), Russia (Poturay and Kompanichenko 2019; Kompanichenko et al. 2016; Kompanichenko and Poturay 2022), USA (Clifton et al. 1990), and New Zealand (Czochanska et al. 1986).

A study of hydrothermal systems in Kamchatka (Russia) revealed the presence of glycine of abiogenic origin in its vapor–water mixture's condensates (Mukhin et al. 1979). Low molecular weight alkanes with a composition dominated by n-C10–C14 in the same hydrothermal system were suggested to have a thermogenic origin (Poturay and Kompanichenko 2019). Furthermore, Kompanichenko et al. (2015) found a homologous series of biologically essential compounds: carboxylic acids, alcohols, ethers, aldehydes, and ketones from the Uzon caldera of Kamchatka. A larger fraction of these organic compounds belongs to the carboxylic acid series, i.e., octanoic acid (C8) nonanoic (C9), decanoic acid (C10), dodecanoic (C12), tridecanoic acid (C13), tetradecanoic (C14), hexadecanoic (C16), and octadecanoic (C18) (Kompanichenko 2020). Carboxylic acids are the vital hydrophobic groups of phospholipids that play a crucial role in building biological membranes. Apel et al. (2002) suggested that if carboxylic acids have nine or more carbons in their hydrocarbon chains, they can organize into membranous vesicles. Therefore, the presence of long-chain monocarboxylic acids in hydrothermal water is particularly interesting due to their biological significance.

High-altitude hotsprings serve as unique hydrothermal environments that harbor diverse thermophilic microorganisms capable of producing a wide range of secondary metabolites also known as bioactive organic compounds (Al-Dhabi et al. 2016; Prihantini et al. 2018; Deamer et al. 2019; Tyagi et al. 2024, 2021; Yan et al. 2017; Aissaoui et al. 2021; Al-Daghistani et al. 2021). These thermophiles, thriving at temperatures above 45 °C, generate secondary metabolites such as amines, alkaloids, fatty acids, glycoproteins, and phenols, many of which exhibit significant medicinal properties, including antifungal, antibacterial, anticancer, and anti-inflammatory effects (Prihantini et al. 2018; Al-Daghistani et al. 2021). While these compounds have been extensively studied in laboratory cultures, their presence and role in natural hydrothermal ecosystems remain less explored (Al-Dhabi et al. 2016; Prihantini et al. 2018; Deamer et al. 2019; Tyagi et al. 2024, 2021; Yan et al. 2017; Aissaoui et al. 2021; Al-Daghistani et al. 2021). Investigating these biomolecules in extreme terrestrial environments can provide valuable insights into potential biosignatures for astrobiological studies, particularly in the search for life on Mars, where hydrothermal systems may have once supported microbial activity. This study aims to analyze organic biomarkers from high-altitude hotsprings to enhance our understanding of life's adaptability in extreme conditions and its implications for extraterrestrial habitability.

Ladakh is a high-altitude cold desertic region located in the Trans-Himalaya that presents geomorphological features and several extreme environmental parameters, such as a thin atmosphere, subzero temperatures for around six months, the presence of glaciers and permafrost, and high UV exposure (Pandey et al. 2020; Ansari et al. 2020). That makes it one of the most suitable regions for Martian analog studies. Hotsprings in this region are one such analog site that can help not only to understand prebiotic (abiotic) organic synthesis but also to get an idea of possible biogenic organic compounds that might have been preserved in the hydrothermal silica and carbonate deposits widely present on the Martian surface (Cady et al. 2018; Brown et al. 2010; Niles et al. 2013; Michalski and Niles 2010; Michalski et al. 2018; Parnell et al. 2002). To our knowledge, this study is the first to detail the origin and distribution of organic compounds in the thermal waters of high-altitude Trans-Himalayan Ladakh hotspring sites.

2 Geology of Ladakh

Ladakh is situated in the Trans-Himalayan India amidst the Kohistan–Ladakh batholith at an average altitude of 11,482 feet. This cold and arid desert region lies on the Ladakh batholith and is surrounded by the Karakoram batholith in the north. The Shyok suture zone runs between these two batholith units (Fig. 1), while the Indus suture zone borders the Ladakh batholith along the southwestern margins. The Tethyan Himalayas

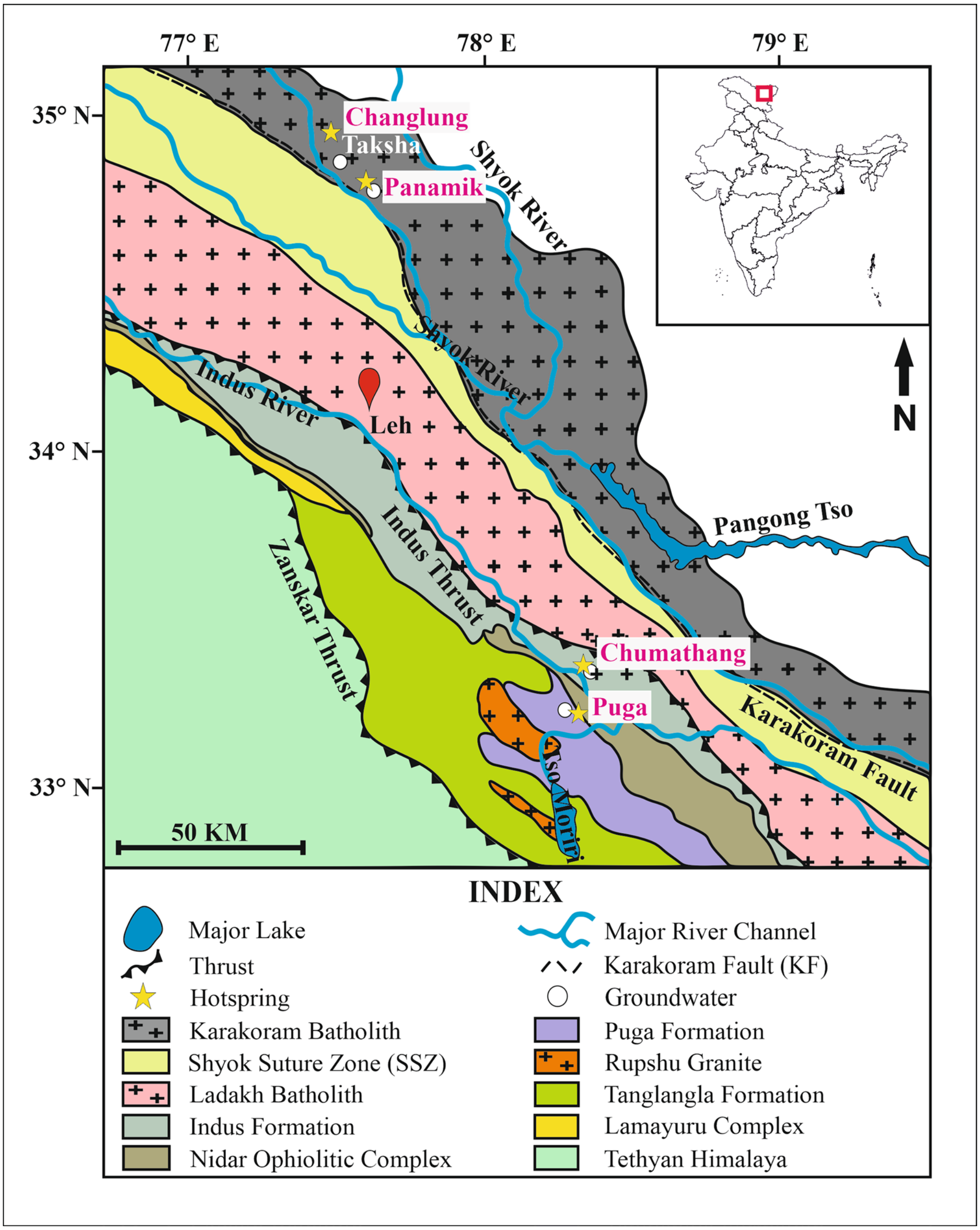

Fig. 1 Geological map of Ladakh, India, showing sampling locations

are further south of the Indus suture zone (Thakur and Rawat 1992; Bandyopadhyay 1990).

Extensive bodies of dominantly granites and high-grade metamorphic rocks form the Karakoram batholith. These granites of the Karakoram batholith (Fig. 1) intrude south Eurasian Palaeozoic to Triassic sedimentary sequences (Crawford and Searle 1992; Debon et al. 1987; Srimal 1986; Weinberg and Searle 1998; Sinha et al. 1997). The dextral Karakoram fault on the northern side separates the Shyok suture zone (SSZ) from the Karakoram batholith (Thanh et al. 2010). A sedimentary unit consisting mainly of sandstone, conglomerate, and minor Albian limestone makes the upper member of the Shyok Formation, while its Lower Member is composed of volcaniclastic rocks (Matsumaru et al. 2006; Ehiro et al. 2007). Terrigenous mudstone with thin sandstone beds and intercalations of sandstone and mudstone are the characteristic lithology of the Callovian Tsoltak Formation (Ehiro et al. 2007).

The Ladakh batholith is a plutonic complex in the Trans-Himalaya zone that has been emplaced between 100 and 40 Ma (Honegger et al. 1982; Ahmad et al. 1998; Kumar et al. 2007). It is wider in the northwestern parts, narrower in the southeastern parts, and runs about 600 km long. It comprises I-type granitoid and minor amounts of noritic gabbro and diorite (Thakur and Rawat 1992; Searle et al. 1999). Minor amounts of rhyolitic to andesitic rocks of the Early Cretaceous to Late Eocene are also formed during the subduction of the Neo-Tethys oceanic plate under the Eurasian continental plate (Sharma 1983; Jain et al. 2002). Cretaceous to Tertiary ophiolite and ophiolitic mélanges constitute the NW–SE-trending Indus suture zone (Ahmad et al. 1996, 2008; Robertson 2000; Kojima et al. 2001; Upadhyay 1998). The molasse sequence varies from coarse conglomerates to shales and onlaps unconformably onto the Ladakh–Kohistan batholith.

The Tethyan Himalayas, in the northern part of the Indian subcontinent, are dominated by Precambrian to Cretaceous fossiliferous sediments (Rao et al. 2006). Quaternary deposits of river, glacier, lake, and eolian sediments constitute the composite physiographical setup of the Upper Indus River Basin (URIB), with a large number of substantial alluvial fans, moraines, talus and scree cones, bajadas, dunes, and loose sediments creating a rough topography of the region (Lone et al. 2020). Dras, Suru, Zanskar, and Nubra sub-basins of UIRB, Ladakh, have the most significant glacial and eolian deposits in the region. Deep gorges, moraines, river cliffs, narrow V-shaped and U-shaped valleys, outwash plains, and waterfalls are the standard features in UIRB, Ladakh (Lone et al. 2021).

3 Methodology

3.1 In situ physicochemical measurements and water sample collections

Temperature, pH, TDS (total dissolved solids), salinity, and conductivity in all these sites were measured by Thermo Scientific Orion Star (A329) Multi-parameter Water Quality Field Instrument. The thermal water samples were collected from high-altitude hotsprings of Changlung (Fig. 2a) and Panamik (Fig. 2b) (in the Nubra Valley, the northern part of Ladakh) and Chumathang (Fig. 2c) and Puga (Fig. 2d) (in the Indus Valley, the southern part of Ladakh) which are among the few active high-altitude hotspring sites across the globe (Fig. 3). The hotspring water sample was collected in a 60-mL sterile polypropylene syringe, prewashed thrice with the same water. The collected water in the syringe was then passed into a Tarson 60-mL sterile polypropylene centrifuge tube through a fitted Merck 0.22 µm filter. The initial filtered water was used to wash the centrifuge tube three times before the final sample collection. Similarly, nonthermal groundwater samples were collected from the hand pumps nearest each hotspring site. The filtered water samples in the centrifuge tubes were stored in an ice box and transported to Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow, for further analysis.

3.2 GC–MS–MS analysis

Analysis was performed using a gas chromatograph coupled to a quadrupole mass spectrometer (TSQ8000 EVO, Thermo Fischer Scientific Private Limited, USA). Separation on Gas chromatography was achieved using a DB-5MS capillary column (30 m × 250 µm × 0.25 µm, Agilent Technologies) and a split/splitless injection port of 250 °C with a split ratio of 20:1. The column oven temperature program was initiated at 65 °C for 4 min, increased by 4 °C/min to 210 °C, and held for 5 min with a run time of 56 min. High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The auto-injection volume was 10 µL. The GC–MS interface temperature was 300 °C with the electron ionization at 70 eV. The ionization source and the quadrupole mass analyzer temperatures were set at 230 °C and 150 °C, respectively. The mass spectrometer was operated in full-scan mode, and the mass range was 20–450 AMU. The nonpolar organics were identified using the NIST11 library, and quantitative results were processed with the Data Analysis Software (Thermo Fischer Scientific Private Limited, USA). Retention time and the characteristic ions were used to confirm the target compounds, and the base peak (the most abundant ion) was used as the quantitative ion. The screening was conducted within an m/z range of 50 to 800, with 50 as the lowest detectable m/z limit, using 2 µl of the sample over a run time of approximately 50.60 min. The equipment has a detection sensitivity of up to 0.5 fg for octafluoronaphthalene (OFN).

3.3 Liquid–liquid extraction (LLE)

The nontarget extraction of organics in the water sample was using sequential extraction. A 50 mL water sample was extracted using 25 mL of n-hexane in a 250-mL separatory funnel by shaking vigorously for 5 min till the milky emulsion. The phase was allowed to separate, and the solvent layer was collected, pooled, and dried using anhydrous sodium sulfate. The extracted solvent fraction was then concentrated using a rotary evaporator to 1 mL, filtered through a 0.22 µm filter, and transferred to the amber color vial for GC–MS–MS analysis.

Fig. 2 Photographs of the hotspring sampling sites: **a** Changlung, **b** Panamik, **c** Chumathang, and **d** Puga. Photographs of the thermophilic microbial mats of various colors such as green, brown, black, yellow, and white at the **e** Changlung, **f** Panamik, **g** Chumathang, and **h** Puga

4 Results

4.1 Physicochemical characterization of hotspring waters

Physicochemical parameters such as temperature, pH, TDS, salinity, and conductivity of the hotspring waters are provided in Table 1. Temperature variability among thermal waters of the investigated sites ranged from

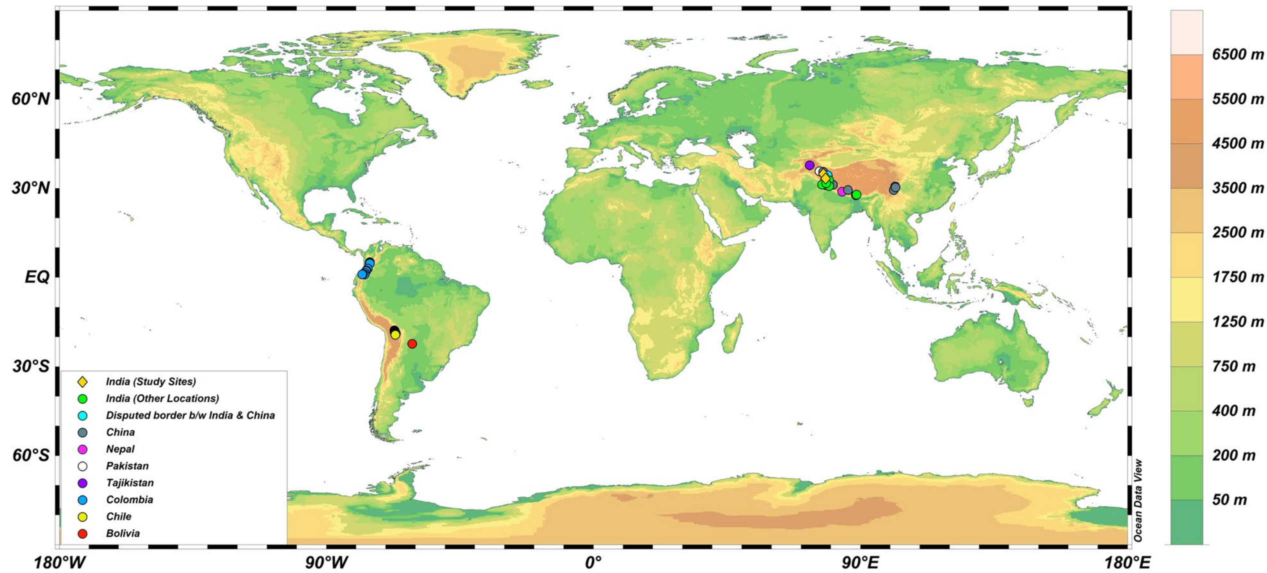

Fig. 3 Global distribution of active high-altitude hotspring sites (Waring et al. 1965; Barbieri et al. 2014; Garzón et al. 2004; Munoz et al. 2015; Tang et al. 2018; Kidov et al. 2023). The scale is given in meters above mean sea level (a.s.l.)

Table 1 Altitude of the sampling sites (a.s.l.) and the physicochemical parameters of respective hotspring waters

Parameters	Chumathang HS	Panamik HS	Changlung HS	Puga HS
Elevation (m)	3988	3232	3376	4375
Temperature	84.9 °C	73.8 °C	73.6 °C	50.4 °C
pH	7.12	7.31	7.01	8.08
TDS	1530 ppm	387 ppm	2880 ppm	0.5 ppm
Salinity	1632 ppm	429 ppm	2630 ppm	690 ppm
Conductivity	3130 μS/cm	789 μS/cm	5370 μS/cm	0 μS/cm

50.4 to 84.9 °C. The pH value shows that the Chumathang, Panamik, and Changlung hotspring waters were almost neutral (~7), whereas Puga hotspring waters were slightly alkaline. The TDS and salinity values were higher in the Changlung (TDS = 2880 ppm, salinity = 2630 ppm) and Chumathang (TDS = 1530 ppm, salinity = 1632 ppm) and lower in the Puga (TDS = 0.5 ppm, salinity = 69 ppm) and Panamik (TDS = 387 ppm, salinity = 429 ppm).

4.2 Organic compounds in the hotspring waters

GC–MS–MS qualitative analysis of dissolved organic content in the hot spring waters of Chumathang, Panamik, Changlung, and Puga reveals the presence of 60, 56, 68, and 60 compounds, respectively (Table 2). These compounds belong to alkanes, alkenes, alkynes, ketones, alcohols, carboxylic acids, esters, aldehydes, benzenes, amides, azoles, phosphates, and phosphites (Fig. 4 and Table 2). The majority of the dissolved organic pool consisted of alkanes, esters, alcohols, and carboxylic acids (Fig. 4 and Table 2). Several common and abundant organic compounds within this pool are classified as bioactive compounds including decanedioic acid, bis(2-ethylhexyl)ester, phenol,2,5-bis(1,1-dimethylethyl)-, hexadecane, E-15-heptadecenal, n-hexadecanoic acid, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, dibutyl phthalate, 1-heneicosanol, phthalic acid, di(2-propylpentyl)ester, 13-docosenamide, (Z)-, 1,4-benzenedicarboxylic acid, and bis(2-ethylhexyl)ester (Table 2 and Supplementary Material 1). Nonthermal groundwater samples from nearby areas of the hotspring sites contained a few silylated alkanes, i.e., traces of hexasiloxane, tetradecamethyl-, pentasiloxane, dodecamethyl-, silane, and [(1-methoxy-1,3-propanediyl)bis(oxy)]bis[trimethyl- (Table 3 and Supplementary Material 2).

The SI value of identified compounds was used to divide the compounds into four groups. The compounds showing SI > 900, 900–800, 800–700, and 700–600 were attributed as excellent match, good match, fair match, and below fair match (Table 2). Among the investigated sites, alkanes (6.6–7.6%), esters (23.9–54.1%), alcohols (6.5–13.5%), carboxylic acids (6.2–17.3%), aldehyde (2–2.7%), nitrogen-bearing compounds (2.1–32.3%), phosphorus–bearing compounds (1–4.6%), and alkenes (1.3–2.7%) formed the major groups of compounds in the thermal waters (Fig. 4b). Alkynes were detected only in the Chumathang hotspring, and aldehydes were detected only in the Chumathang and Puga hotsprings (Fig. 4a–b). The n-alkanes were dominated by low molecular weight C12 to C20 compounds. Among the few nitrogen-containing compounds, 13-docosenamide, (Z)- was common

Table 2 List of the different groups of organic compounds detected in the Changlung, Chumathang, Panamik, and Puga hot spring water samples

Compounds	Retention time (RT)	Chumathang HS	Panamik HS	Changlung HS	Puga HS
Esters					
Oxalic acid, cyclohexyl propyl ester	6.44				+ + +
Oxalic acid, cyclohexyl ethyl ester	6.44			+ +	
2-Propenoic acid, tridecyl ester	25.66			+ +	
3-Chloropropionic acid, heptadecyl ester	25.66	+			
Phthalic acid, hept-3-yl isobutyl ester	29.17		+ + +		
Phthalic acid, isobutyl 2,4,4-trimethylpentyl ester	29.18			+ +	
Dibutyl phthalate	31.08/31.09	+ + +	+ + +	+ + +	
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	31.08				+
l-(+)-Ascorbic acid 2,6-dihexadecanoate	31.24	+			
Docosyl pentafluoropropionate	36.77/36.78	+ +			+ +
3-Hydroxypropyl palmitate, TMS derivative	41.64	+		+	
Phthalic acid, di(2-propylpentyl) ester	42.21/42.22	+ + +	+ + +	+ + +	+ + + +
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	44.04	+ + +	+ +	+ + +	+ +
Decanedioic acid, bis(2-ethylhexyl) ester	44.46	+ + +	+ + + +	+ + + +	+ + + +
Alkanes					
Dodecane	13.11		+ +	+ +	+ +
Cyclopropane, 1-butyl-2-pentyl-, cis-	18.42				+
Tetradecane	18.63				+ +
Heneicosane, 11-(1-ethylpropyl)-	18.98/20.81		+		+
Heptadecane	20.71				+ +
Dodecane, 2,6,11-trimethyl-	20.8				+ + +
Tetradecane, 2,6,10-trimethyl-	21			+	+ +
Hexadecane, 2,6,11,15-tetramethyl-	21.93/26.73	+ +	+ + +	+	
Hexadecane	23.54/23.55	+ + +	+ + +	+ +	+ + +
Heptadecane, 2,6,10,15-tetramethyl-	25.77	+ +		+ +	
Tridecane, 6-propyl-	25.81		+ +		
Eicosane, 10-methyl-	26.73	+	+		+ + +
Octadecane	27.98		+ + +	+	+ + +
Heptadecane, 2,6,10,14-tetramethyl-	27.99			+ +	
Eicosane	27.99/32.02	+ + +	+	+	
Heptadecane, 9-hexyl-	30.20/34.61	+ +	+ +	+ +	+
Heptadecane, 9-octyl-	30.2	+		+	
Octadecane, 5,14-dibutyl-	30.2		+		+ +
Heneicosane	34.17		+ +	+	
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	35.74/39.72	+		+ +	
Cyclotetracosane	40.71				+ +
Eicosane, 7-hexyl-	40.78			+ + +	
Heptadecane, 2,3-dimethyl-	43.74			+ +	
CPI		**0.00**	**0.43**	**0.25**	**0.06**
AVL		**18.74**	**18.76**	**19.08**	**16.41**
Alkenes					
1,3-Cyclopentadiene, 5-(1-methylpropylidene)-	6.31		+		
Mesitylene	7.2			+	
5-Octadecene, (E)-	23.36				+ + +
7-Hexadecene, (Z)-	23.37			+ + +	
1-Octadecene	27.84		+ + +		
3-Eicosene, (E)-	31.9		+ + +		

Table 2 (continued)

Compounds	Retention time (RT)	Chumathang HS	Panamik HS	Changlung HS	Puga HS
Heptacos-1-ene	40.71	+ +			
Nonacos-1-ene	40.72			+ + +	
10-Heneicosene (c,t)	42.86	+ + +	+		
Squalene	44.58				+ +
Alkyne					
1-Hepten-5-yne, 2-methyl-3-methylene-	6.32				+
Alcohols and phenols					
2,4-Di-tert-butylphenol	21.26		+ + +	+ + +	+ +
Phenol, 2,5-bis(1,1-dimethylethyl)-	21.26	+ + +			
1-Hexadecanol	23.36		+ + +		
Hexadecen-1-ol, trans-9-	23.37	+ + +			
n-Nonadecanol-1	27.84			+ +	
1-Heneicosanol	31.9	+ + +		+ + +	
2,6-Dimethyl-1-nonen-3-yn –5-ol, TMS derivative	32.19			+	
n-Tetracosanol-1	36.77/36.78		+ +	+ + +	
1-Dodecanol, 2-octyl-	36.77		+ + +		
[1,1′-Biphenyl]−2,3′-diol, 3,4′,5,6′-tetrakis(1,1-dimethylethyl)-	40.03			+	
1-Heptacosanol	42.86				+ +
Carboxylic acids					
n-Hexadecanoic acid	31.23/31.24	+ +	+ +	+ +	+ +
Palmitic acid, TMS derivative	32.9	+ +		+	
Octadecanoic acid	35.73/35.74	+ +	+		+
Aldehyde					
E-15-Heptadecenal	27.84	+ + +			+ + +
Ketones					
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	20.18		+		
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	30.08/30.09/30.10/30.11	+ +	+	+ +	+ +
Ether					
Hexadecane, 1,1-bis(dodecyloxy)-	18.88				+
Benzenes					
Benzene, 1-ethyl-3-methyl-	6.31/7.99	+	+ +		
Benzene, 1,2,4-trimethyl-	7.2				+
1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	39.57			+	
Phosphorus-bearing compounds					
Triethyl phosphate	10.75/10.77	+			+ + +
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	50.62	+	+	+ +	
Tris(2,4-di-tert-butylphenyl) phosphate	53.96	+	+		+
Nitrogen-bearing compounds					
Cyclohexanespiro-5′-(2′,4′, 4′-trimethyl-2′-oxazoline)	6.44	+ +	+ +		
Benzothiazole, 2-(2-hydroxyethylthio)-	32.19	+			
13-Docosenamide, (Z)-	44.33/44.36	+ + +	+	+ +	+ + +

In the table, + + + +, + + +, + +, and + denote excellent match, good match, fair match, and below fair match, respectively. Bioactive compounds are highlighted by the bold text. The CPI and ACL denote the carbon preference index and average chain length, respectively

(based on the area %) and made the largest fraction of the total dissolved organic carbon pool in the Panamik hotspring waters (Fig. 4b and Table 2). The carbon preference index (CPI = Relative abundance of odd carbon number n-alkane/relative abundance of even carbon number n-alkane) and average chain length [(ACL) average number of carbons] for n-alkane ranged from 0 to 0.43 and from 16.41 to 19.08, respectively. Four silylated

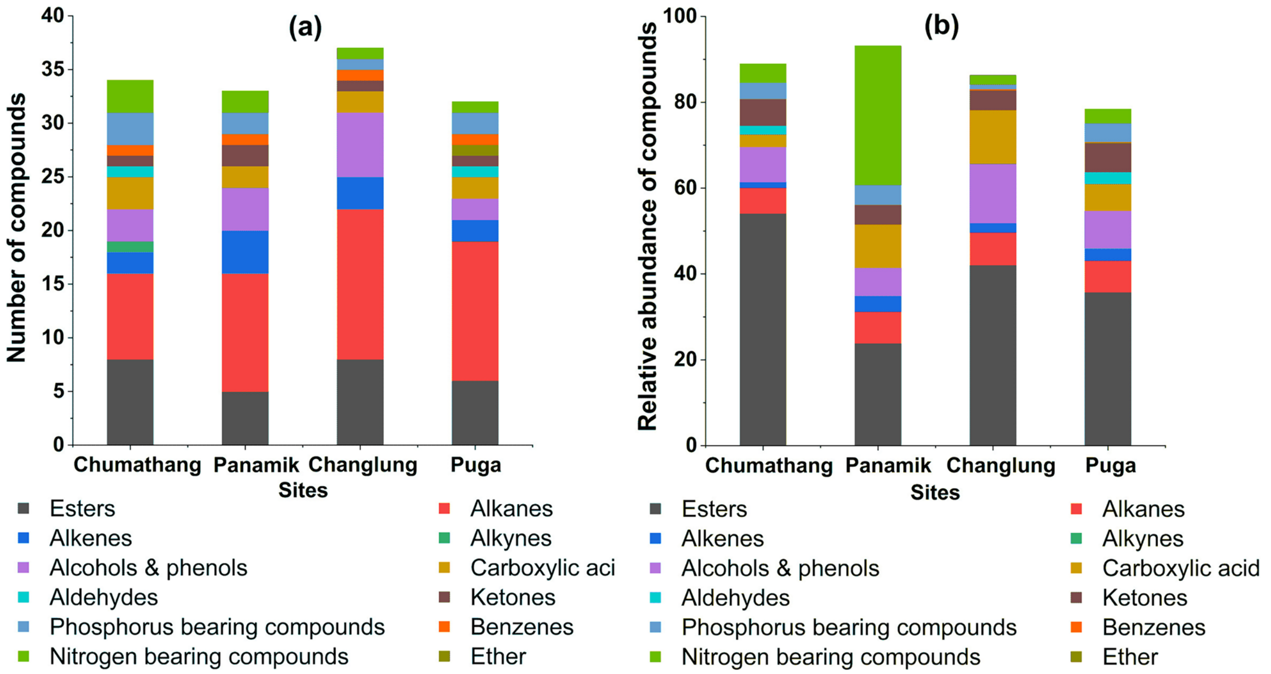

Fig. 4 Bar plot for the **a** number and **b** relative abundance of organic compounds of different groups in the Changlung, Chumathang, Panamik, and Puga hotspring water samples

alkanes detected in the nonthermal groundwaters were identified under the fair match category, and the rest were identified under the below fair match category. Thermal waters exhibited some variations in the composition of the organic compound groups despite the overall similarities in the dissolved organic pools of these waters.

5 Discussion

The organic compound composition of high-altitude hotspring waters (> 3200 m a.s.l.) in Ladakh reveals a distinct pattern when compared to the organic profiles observed in low-altitude hotspring waters (< 3000 m a.s.l.) across various global regions (Table 4). In the Ladakh hotspring waters, esters, carboxylic acids, alcohols, amides, and alkanes make up the majority of the dissolved organic pool (Fig. 4a, b), with esters comprising up to 54.1% and amides comprising up to 32.3% of the total dissolved organic compounds. Among the 27 compounds are recognized as bioactive compounds or bacterial secondary metabolites (Tables 3 & 4). This finding does not align with the major dissolved organic composition of low-altitude hotspring waters (Table 4).

The organic compound profiles available from low-altitude terrestrial hotsprings in regions such as Spain (González-Barreiro et al. 2009), Hungary (Kárpáti et al. 1999), Italy (Gioia et al. 2006), Russia (Poturay and Kompanichenko 2019; Kompanichenko et al. 2016), and the USA (Clifton et al. 1990) exhibit a different composition, with notably lower proportions of esters and the absence of phosphorus-bearing organic compounds. For instance, the proportion of esters in low-altitude hotspring waters does not exceed 20.7% and the number of bioactive compounds identified in these waters is up to 12 (Table 4). These differences can be attributed to variations in environmental conditions, microbial activity, and the chemical composition of the surrounding geology.

In Ladakh, the high-altitude hotspring waters not only contain a higher proportion of esters but also feature phosphorus-bearing organic compounds (up to 4.6%) (Table 4). Phosphorus-bearing compounds are key indicators of biogeochemical cycling in microbial ecosystems, particularly communities in geothermal environments (Soo et al. 2017; Gupta et al. 2013). The relatively higher abundance of esters and phosphorus-bearing compounds suggests a unique microbial influence or distinct biochemical pathways in these extreme environments, possibly linked to the adaptation of thermophilic organisms to the harsh high-altitude conditions.

The presence of 27 bioactive compounds that compose 38 to 98% of the total dissolved organic content in the high-altitude hotspring waters of Ladakh is another significant observation. Bioactive compounds, including antimicrobial agents, are frequently associated with thermophilic bacteria and archaea found in hot springs (Giddings et al. 2015; Al-Daghistani et al. 2021; Banerjee et al. 2023). These compounds may serve ecological functions,

Table 3 List of the organic compounds observed in the nonthermal groundwater samples from the hand pumps near the Changlung, Chumathang, Panamik, and Puga hot spring sites

Compounds	Retention time (RT)	Chumathang HS	Panamik HS	Changlung HS	Puga HS	Chumathang GW	Panamik GW	Taksha GW	Puga GW
Silane, [(1-methoxy-1,3-propanediyl)bis(oxy)]bis[trimethyl-	6.41					+ +		+ +	+ +
Cyclotetrasiloxane, octamethyl-	7.3					+			
Methyltris(trimethylsiloxy)silane	9.14/9.81					+	+		
4-Tert-octylphenol, TMS derivative	9.81								+
Tetrasiloxane, decamethyl-	9.81							+ +	
Pentasiloxane, dodecamethyl-	13.91/16						+ +	+ +	+
Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	16					+			
Hexasiloxane, tetradecamethyl-	21.56/21.57					+ +	+	+ +	+ +
Cycloheptasiloxane, tetradecamethyl-	24.02						+		
Heptasiloxane, hexadecamethyl-	26.49/26.50	+			+	+	+	+	+
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	26.5								+
Cyclooctasiloxane, hexadecamethyl-	28.82					+			
Cyclononasiloxane, octadecamethyl-	32.99								+
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	39.93		+		+		+		+
Cyclodecasiloxane, eicosamethyl-	45.35/46.31	+				+	+	+	

In the table, + + + +, + + +, + +, and + denote excellent match, good match, fair match, and below fair match, respectively

such as defense mechanisms or chemical signaling (Prihantini et al. 2018; Al-Daghistani et al. 2021), and their high concentration in Ladakh's hotspring waters suggests a unique microbial synthesis or metabolic process at the high-altitude hotspring sites.

While the composition of dissolved organic compounds in Ladakh hotsprings is distinct, it bears some similarities to other high-temperature environments where organic compounds of biogenic origin—such as alcohols, ketones, aldehydes, carboxylic acids, and esters—are commonly found (Table 4). Thermophilic bacteria, which thrive in the extreme conditions of geothermal springs, are known to produce a wide variety of organic compounds through fermentation, respiration, and other metabolic processes (Zeikus 1979; Takai et al. 2005; Tang et al. 2009; Kristjansson and Stetter 2021; Amend and Shock 2001). In the case of Ladakh, the predominance of carboxylic acids, esters, and n-alkanes in the dissolved organic pool (Table 2) strongly supports the idea that thermophilic bacteria and their degradation products are major contributors to the organic composition of these waters.

The presence of thermophilic biofilms at the study sites (Fig. 4e–h) further reinforces the hypothesis that microbial activity plays a crucial role in shaping the organic compound profile of the Ladakh hotspring waters. Biofilms in geothermal systems are known to be rich in microbial communities that contribute significantly to the local chemical environment by producing or modifying organic compounds (Sand 2003; Álvaro et al. 2021; Lerm et al. 2013). These biofilms can alter the composition of the dissolved organic pool by secreting extracellular polymeric substances (EPS) and releasing metabolites that interact with the surrounding environment.

Furthermore, the detection of a small number of silylated organic compounds in local nonthermal groundwaters provides an intriguing contrast to the hot spring waters. Silylated organic compounds are typically associated with certain chemical reactions, such as silanization. Their presence in nonthermal groundwaters suggests that

Table 4 Comparative tables for elevation, physicochemical, and dissolved organic characteristics for the high-altitude (> 3000 m a.s.l.) and low-altitude hot springs (< 3000 m a.s.l.)

Parameters	Low-altitude hotsprings					High-altitude hotsprings
	Yellowstone National Park, USA (Clifton et al. 1990)	Urup Island and Uzon Caldera, Russia (Kompanichenko et al. 2016; Poturay and Kompanichenko 2019; Kompanichenko and Poturay 2022)	Calabria region, Italy (Di Gioia 2006)	Pannonian Basin, Hungary (Kárpáti et al. 1999)	Ourense, Spain (González-Barreiro et al. 2009)	Ladakh, India (this study)
Elevation	2220 m	< 998 m	< 370 m	400 m	90–115 m	3232–4375 m
pH	6–8	3–9	NA	NA	7.5–8.5	7–8
Temperature	40°–80 °C	50°–90 °C	NA	50°–60 °C	45°–66 °C	50°–85 °C
Total number of compounds	NA	95	7	23	70	88
Major groups	Alkanes, aromatics, alcohols, and carboxylic acids	Alkanes, aromatics, ketones, alcohols, carboxylic acids	Alcohols	Aromatics	Aldehydes, esters, ketones	Alkane, esters, carboxylic acids, alcohols, alkenes
Alkanes (%)	< 83	< 41.8	NA	< 0.4	< 8.2	< 7.6
Alcohols (%)	NA	< 29.4	NA	< 18.3	< 15.3	< 13.8
Aldehydes (%)	NA	< 4.1	NA	ND	< 3.2	< 2.7
Carboxylic acids (%)	NA	< 18.9	NA	< 21.9	< 16.3	< 10.1
Esters (%)	NA	ND	NA	ND	< 20.7	< 54.2
Nitrogen-bearing compounds (%)	NA	ND	NA	ND	< 40.8	< 32.3
Phosphorus-bearing compounds (%)	NA	ND	ND	ND	ND	< 4.6
C_{max} (n-Alkane)	C23	C11	NA	NA	NA	C19
CPI (n-Alkane)	1	> 1	> 1	NA	NA	< 0.43
Number of bioactive compounds	Absent	12	Absent	Absent	8	27
Proportion of bioactive compounds (%)	NA	NA	NA	NA	NA	38.5–98.1

In this table, NA denotes that data were not available, and ND denotes that the compound was not present or below the detection limit

different geochemical processes might be at play in non-thermal aquifers compared to the more dynamic and biologically active hotspring environments.

5.1 Alkanes

A comparative analysis of the dissolved organic parameters, particularly the distribution of n-alkanes, between high-altitude hotsprings (e.g., those in Ladakh) and low-altitude hotsprings reveals significant variations in the composition and structural characteristics of carbon compounds. One critical parameter in this comparison is the CPI, which provides insights into the sources and transformation processes of organic matter. In low-altitude hotsprings, CPI values are typically ≥ 1, indicating either a lack of odd–even preference (CPI ≈ 1) or a distinct preference for odd carbon number n-alkanes (CPI > 1). This pattern is often associated with biogenic sources, particularly terrestrial organic matter such as vascular plant waxes, which contribute significantly to the organic inputs in these environments (Bray and Evans 1965).

In contrast, the CPI values of n-alkanes in high-altitude hotsprings, such as those in Ladakh, are consistently ≤ 0.43, reflecting a strong preference for even carbon number n-alkanes. This suggests the dominance of thermogenic processes in these high-altitude systems, where hydrocarbons are significantly altered by extreme geothermal activity, pressure, and temperature. Such conditions favor the synthesis of even carbon number n-alkanes, potentially originating from inorganic carbon sources or microbial activity adapted to these harsh environments (Peters et al. 2005).

Several factors may contribute to these differences. The distinct geothermal dynamics of high-altitude hotsprings, characterized by steeper thermal gradients and more extreme pressure conditions, significantly influence organic compound formation pathways (Tissot and Welte 2013). Additionally, the source of organic matter plays a pivotal role. Low-altitude hotsprings are typically surrounded by vegetation, which introduces terrestrial organic matter, enriching these waters with odd carbon number n-alkanes (Seki et al. 2010). In contrast, high-altitude regions like Ladakh are sparsely vegetated, and organic matter inputs primarily derive from microbial activity, geological processes, or atmospheric deposition.

Microbial and biogeochemical processes further shape these differences. High-altitude microbial communities adapted to extreme conditions may preferentially degrade odd carbon number n-alkanes or synthesize even carbon number ones, contributing to the lower CPI values observed (Pu et al. 2017; Finkel et al. 2023). Thermal alteration processes, facilitated by high temperatures and pressures, also play a significant role in modifying organic matter in these systems. Environmental conditions at high altitudes, including intense UV radiation and lower oxygen levels, can influence the chemical structure and stability of dissolved organic matter, contrasting with the milder conditions of low-altitude springs that preserve more biogenic signatures (Brocks and Pearson 2005).

These findings highlight how high-altitude and low-altitude hotsprings reflect fundamentally different sources and transformation pathways of dissolved organics. While low-altitude hot springs exhibit signatures of biogenic influence with odd carbon number n-alkane dominance, high-altitude hotsprings like those in Ladakh show evidence of thermogenic processes, marked by even carbon number n-alkane preference. These differences provide crucial insights into carbon cycling, thermal alteration processes, and the role of extreme environments in shaping organic matter characteristics.

5.2 Major bioactive compounds: high-altitude vs low-altitude hotspring waters

An essential source of organic compounds in hotspring waters could be bioactive molecules produced by thermophiles thriving at a temperature > 45 °C (Al-Dhabi et al. 2016; Prihantini et al. 2018; Deamer et al. 2019; Tyagi et al. 2024, 2021; Yan et al. 2017; Aissaoui et al. 2021; Al-Daghistani et al. 2021). Secondary metabolites include amines, alkaloids, fatty acids, glycoproteins, phenols, etc. These secondary metabolites possess medicinal values such as antifungal, antibacterial, anti-HIV, anticancer, anti-inflammatory, and antiulcer properties (Prihantini et al. 2018; Al-Daghistani et al. 2021). However, most of these secondary metabolites have so far been reported from the pure laboratory-based cultures of thermophilic bacteria (Prihantini et al. 2018; Al-Dhabi et al. 2016; Deamer et al. 2019; Tyagi et al. 2021, 2024; Yan et al. 2017; Aissaoui et al. 2021; Al-Daghistani et al. 2021).

Extremophiles exhibit a range of physiological and molecular adaptations, such as the production of extrapolates, ice-nucleating proteins, pigments, extremozymes, and exopolysaccharides (Rawat et al. 2024). Key limiting factors include osmotic and hydrostatic pressure, solar, terrestrial, and cosmic radiation, oxidative stress, and nutrient availability (D'Amico et al. 2006). As a result, some of the significant adaptations involve strategies for surviving extreme conditions through the use of specialized proteins and lipid membranes that maintain cellular integrity (Chauhan et al. 2023; De Maayer et al. 2014; Musilova et al. 2015; Ghosh et al. 2023). The chemicals and enzymes produced under such extreme conditions are chemically and physically more stable (Mashakhetri et al. 2024; Barzkar et al. 2024).

When compared to bioactive compounds reported in low-altitude hotsprings from regions like Spain (González-Barreiro et al. 2009), Hungary (Kárpáti et al. 1999), Italy (Gioia et al. 2006), Russia (Poturay and Kompanichenko 2019; Kompanichenko et al. 2016), and the USA (Clifton et al. 1990), Ladakh's high-altitude hotsprings were found to contain a higher number and greater variety of bioactive compounds (Tables 3 & 4). The high-altitude hotsprings of Ladakh present a unique environment that could explain the increased diversity of dissolved bioactive compounds found in their waters. These extreme conditions, including intense ultraviolet (UV) radiation, arid climates, low atmospheric pressure, low oxygen levels, and significant seasonal temperature fluctuations, create a harsh habitat that pushes the limits of biological life. In such an environment, organisms must adapt to these stressors, potentially leading to the production of a wider array of bioactive compounds as survival mechanisms. In contrast, low-altitude hotsprings typically experience milder environmental conditions, such as more stable temperatures, lower UV radiation, and a more temperate climate. These conditions impose fewer selective pressures on the organisms that live there, resulting in a less diverse array of bioactive compounds (Kompanichenko et al. 2016; González-Barreiro et al. 2009). The organisms in such environments do not need to produce the same level of biochemical diversity to cope with stress, as the environment offers fewer challenges to survival. Thus, the unique combination of high-altitude stressors in Ladakh's hot springs fosters a greater diversity of bioactive compounds. The organisms in these hotsprings have adapted in ways that might provide valuable insights into how life can thrive in extreme environments such as the hotsprings of Mars, which were once active. These adaptations not only highlight the resilience of life but also open up possibilities for discovering novel compounds that could have applications in medicine, agriculture, and biotechnology, as well as could serve as a biomarker for the search for life on Mars. Some of the major bioactive compounds detected in the hotspring waters of Chumathang, Panamik, Changlung, and Puga are given below.

5.2.1 Decanedioic acid, bis(2-ethylhexyl)ester

This compound was detected in the thermal waters of all the four hotspring sites. It is commonly known for its antimicrobial and antifungal properties. Pure laboratory culture study of bacteria *Bacillus thermotolerance* (DHT 26), *Bacillus cereus* (Tambekar et al. 2017, 2014) isolated from Lonar Lake, and endophytic bacteria *Bacillus atrophaeus* isolated from medicinal plant *G. uralensis* (Li et al. 2018) have demonstrated the release of this compound as a secondary metabolite. In addition, this compound has been detected in the extract of freshwater filamentous green alga *Spirogyra elongate* (Abdel-Aal et al. 2015) and shrubs such as *Alhagi mannifera* (Fabaceae family) (Jaradat et al. 2022) and *Carthamus oxycantha* (Asteraceae family) (Rafiq et al. 2017).

5.2.2 Phthalic acid, di(2-Propylpentyl)ester

This compound was found in the thermal waters of all the four hotspring sites. Recently, this compound was discovered in the pure laboratory culture of the marine bacteria *Streptomyces* sp. (Al-Dhabi et al. 2020; Chakraborty et al. 2022). The extract of aquatic cyanobacteria *Leptolyngbya* sp. and *Desertifilum* sp. (Shawer et al. 2022) and endosymbiotic bacteria *Bacillus atrophaeus* (Mohamad et al. 2018) has also been discovered to synthesize this compound. This compound has demonstrated antibacterial, antifungal, anti-inflammatory, and anticancer properties (Oludare and Gamberini 2019; Chakraborty et al. 2022).

5.2.3 4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester

This compound was detected in the thermal waters of all the four hotspring sites. It has been reported in the extract of fungi *Arthrobotrys oligospora* (Bahena-Nuñez et al. 2024), *Aspergillus flavipes* strain (Verma et al. 2014), *Aspergillus unguis* (Sajna et al. 2020), in the extract of cyanobacteria (Li et al. 2021), marine algae *Padina boergesenii* and *Polycladia Myrica* (Ramezanpour et al. 2021), and hydrothermally treated sludge. It is known for antibacterial, antifungal, nematocidal activity, and anticancer properties (Verma et al. 2014).

5.2.4 13-Docosenamide, (Z)

This compound was detected in the thermal waters of all the four hotspring sites. It has been detected in halophilic *Bacillus* sp. (Donio et al. 2013; Nas et al. 2021) and in marine algae *T. suecica* (Abu-Hussien et al. 2022). It is also found associated with the succession of *Flavobacterium* and the inhibition of nitrifying bacteria (*Nitrosomonas* and *Nitrospira*) (Fan et al. 2024). According to Fan et al. (2024), this bioactive compound is essential for bacterial and algal cell contact and the development of synergy between the two, while cell contact lessens the antagonistic effects. 13-Docosenamide, (Z) is well known for its potent antiviral, antifungal, and anticancer activities (Donio et al. 2013; Nas et al. 2021; Chen et al. 2018).

5.2.5 n-Hexadecanoic acid

This compound was detected in the thermal waters of all the four hotspring sites. N-hexadecanoic acid is commonly reported in thermophilic bacteria (Merkel and Perry 1977), bacterial cells of the microbial mat from the marine intertidal region (Scherf and Rullkötter 2009), marine algae (Zakaria et al. 2011; Thirunavukkarasu et al. 2014), and freshwater algae (Shawer et al. 2022). Many cyanobacteria and photosynthetic algae have high concentrations of n-octadec-9(Z)-enoic acid in their fatty acid composition, which is dominated by n-hexadecanoic acid (Chuecas and Riley 1969; Grimalt et al. 1992). It shows antibacterial and antifungal (Zakaria et al. 2011; Shobier et al. 2016; Karthikeyan et al. 2014).

5.2.6 9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione

This compound was detected in the thermal waters of all the four hotspring sites. It has been reported in thermophilic cyanobacteria *Leptolyngbya* sp. (Tyagi et al. 2024), cave-dwelling bacteria *Streptomyces* sp. (Fatima et al. 2021), halophilic bacteria *Brevibacillus borstelensis* (Hamedo et al. 2023), etc. This compound has also been reported in crude oils (Goma-Tchimbakala et al. 2022) and marine sediments (Chakraborty et al. 2022). It shows antiplatelet and antioxidant properties (Kumar et al. 2023) and is also reported to be beneficial in cancer treatment (Hamedo et al. 2023).

5.2.7 4-Di-tert-butylphenol

This compound is found in the thermal waters of Panamik, Changlung, and Puga hotspring sites. The compound has been reported from the thermophilic bacteria *Bacillus licheniformis* (Aissaoui et al. 2019), other gram-positive and gram-negative bacteria (Viszwapriya et al. 2016; Dharni et al. 2014; Varsha et al. 2015; Belghit et al. 2016; Sang and Kim 2012), and cyanobacteria *Leptolyngbya sp.* (Tyagi et al. 2021; Zhang 2018). This phenolic compound possesses various biological properties, including antioxidant, antifungal, antibacterial, and anticancer properties (Dharni et al. 2014; Varsha et al. 2015). Additionally, there are mentions of antioxidant and anticancer effects (Varsha et al. 2015; Choi et al. 2013).

5.2.8 5-Bis(1,1-dimethylethyl)phenol

This compound was detected only in the thermal waters of the Chumathang hotspring site. It has been reported from thermophilic cyanobacteria *Leptolyngbya* sp. (Tyagi and Singh 2020) and in the extract of *Streptomyces* sp. It is known for antibacterial activity.

5.3 Implications for the search for life on Mars

A review by Ansari (2023) demonstrated that organic geochemical analysis of Martian surface rocks has so far been random and requires a more focused approach, i.e., which rocks should be targeted? What kind of organic compounds should be expected in those rocks? This information will be crucial in preparing our next mission to Mars and collecting suitable rock samples, which are expected to reach Earth with the Mars sample return mission. Hence, ideas about the ideal target for searching for life-associated organic molecules (biomarkers) on the Martian surface have been proposed. Widespread hydrous opaline silica and carbonate deposits around prehistoric hydrothermal sites on the Martian surface (Farmer et al. 1996), such as Nili Patera Caldera, Nili Fossae, Leighton Crator, and Gusev Crator of Mars, are identified as one of the best targets (Cady et al. 2018; Brown et al. 2010; Parnell et al. 2002; Niles et al. 2013; Michalski and Niles 2010; Michalski et al. 2018).

These sites are likely to contain entrapped fluid inclusions, remnants of hydrothermal fluid discharge from the time when the respective hydrothermal system was active (Parnell et al. 2002). These inclusions are generally expected to be < 5 µm in size, but the ability to extract information from the fluids—such as biosignatures and actual remains of living organisms—highlights the benefits of technical advancements in studying fluid inclusions. Assume that a similar microbial life would have inhabited the hydrothermal sites on the Martian surface in the past. We expect to find the compounds that we see in hotspring waters in similar environments on the Earth such as diverse bioactive compounds that we detected in our study of high-altitude hotspring waters of Ladakh. Accordingly, the instruments should be standardized, and methods should be refined for the on-site detection of these organic compounds.

Crushing large samples may release inclusion fluids for on-site analysis on Mars. Existing methodology for the in-situ study of the Martian surface has already applied mass spectrometry, including its application for the detection of particular organic molecules (Biemann et al. 1977; Lauer et al. 2009; Leshin et al. 2013; Ming et al. 2014; Eigenbrode et al. 2018; Freissinet et al. 2015; Millan et al. 2022a, 2022b; Szopa et al. 2020; Glavin et al. 2013). The development of microfluidic devices, which can be linked to mass spectrometers, will enable the administration of chemicals to assist in cleaning up the module and extraction of particular types of compounds (specially compounds commonly detected in hotspring waters and deposits) through online microdialysis (Parnell et al. 2002).

6 Conclusion

The study revealed the presence of diverse organic compounds in the high-altitude hotspring waters of Ladakh. They are predominantly biogenic in origin, either directly released by living bacteria or recycled products of dead organic matter via thermogenic processes. This study shows a diverse range of bioactive compounds with high medicinal properties, such as antibiotic, antifungal, antiviral, and anticancer. The high diversity of the bioactive compounds was most likely associated with the multiple extreme environmental factors that are unique to the high-altitude hotsprings of Ladakh. Assuming that the relict hydrothermal sites with widespread deposition of opaline silica and carbonates had once hosted a similar microbial life, this suit of bioactive compounds can be used as a standard biomarker for searching for evidence of extinct life in that area on Mars. Hence, for future missions dealing with the in situ biomarker exploration in hydrothermal opaline silica and carbonate deposits on Mars, this suit of organic compounds may be used to prepare the respective methodology.

Supplementary Information

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Supplementary material 1

Supplementary material 2

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Author contributions

A.H.A. was involved in conceptualization; A.H.A., A.D., N.G.A., and A.S. were responsible for methodology, formal analysis, investigation, data curation, writing-original draft preparation, and writing-review and editing; A.H.A., A.D., and N.G.A. helped with resources; and A.H.A. and A.D. assisted with visualization. A.D. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s)).

Declarations

Competing interests
The authors declare that they have no competing interests.

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