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Phenolic metabolites, vitamins, minerals and antioxidant profiling of medicinal teas of Ladakh

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Abstract
The traditional pharmacopoeia of Ladakh comprises of a wide arsenal of wild plants having medicinal properties used in folk medicine and in foods as beverages and spices. Many of these plants are near to extinction and at the same time very less scientific studies have been done so far in this response. This paper explores and discusses the biochemical constituents of some wild plant parts traditionally used for therapeutic purposes in Ladakh. Bark of wild rose/*sia*, leaves of willow/*chashing* and seeds of iris/*tesma* used in the form of tea were evaluated while trying to relate with their nutritional and health promoting benefits. Extracts from these plant parts were reported to contain remarkable amount of nutrients (vitamins and minerals) and secondary metabolites (polyphenols and flavonoids) that exhibited important biological functions. *Sia* detected maximum quantity of total vitamins studied and *londo* (iris seed) detected maximum amount of total minerals analyzed while *chashing* was shown to have the best antioxidant activity with comparatively more phenolic acids and flavonoids. It would be beneficial to consume such types of natural plants having potent activity in limited doses as substitutes for chemical therapeutics.

Keywords: *Chashing*, *sia*, *londo*, medicinal tea, willow, wild rose, iris, Ladakh

Introduction
Ladakh is a reservoir of herbal plants growing in wild conditions having health promoting benefits. Since ancient times, these wild plants are being used in traditional *Amchi* medicinal system (*Sowa rigpa*) which is amongst the well-known medical practices of the world (Dawa *et al*., 2013) [1]. The wild plants are also utilized by the natives of this tribal region as food (vegetable) and beverage (tea) (Hussain *et al*., 2022; Akbar *et al*., 2013) [2, 3]. Extractions of some locally available wild plants were used by the locals for the treatment of various ailments, which at this time, are under under-utilization as the locals rely more on allopathic system of medicine. Among these, the extractions of bark of wild rose/*sia*, leaves of willow/*chashing* and seeds of iris/*tesma* have been traditionally used in the form of tea under the supervision of *amchis* (local practitioners).
Wild rose (*Rosa webbiana*), spread almost in the whole world has been and still is a source of international investigation due to its various health-related aspects. Flowers are used as stomachic and fruit decoction is used for asthma treatment. Petals are used to counter chest infection, nasal swelling and nasal bleeding, hepatitis, jaundice and liver disorders (Kumar *et al*., 2015) [4]. The decoction of root bark has the ability of purification of blood and mental relaxation and the stem bark can treat skin and blood pressure issues (Khan *et al*., 2015) [5]. Tea made from the stem bark is useful for fever, sore throat and cough. The leaves of willow (*Salix sp.*) have been mentioned in ancient texts from Egypt, Sumer and Assyria and the physician Hippocrates, in ancient Greece wrote about medicinal properties in 5th century B.C. Willow exhibits anti-rheumatic, anti-inflammatory, antipyretic, antigesic, antiseptic, and antidotic properties (Banes *et al*., 2002) [6]. It is very much believed that the leaf of willow is the primary source of salicylic acid (the precursor of aspirin). White willow and other closely associated species are being used to manage fevers and relieve joint pain for thousand years (Vane and Botting, 2003) [7]. Branches and leaves are externally used for allergy and itching. Extract of fresh leaves is drunk thrice a day to treat malaria.

Herbal tea and poultices made from willow leaves were used to relieve toothaches, arthritis, gout, digestive problems, minor body pains, and rashes. Tea made from leaves is soothing and used against headache. Infusion of the leaves can treat nervous insomnia due to its calming effect (Kumar and Namgyal, 2019) [8] and post-birth bleeding (Chang *et al*., 2017) [9]. Willow leaves are also prescribed to be chewed to ease the pain of childbirth. Iris (*Iris lactea* P.) in local language is known as *tesma* and the seed is known as *londo*. The decoction made from dried plant powder is consumed orally to cure stomach cramps, intestinal obstruction, food poisoning and boost appetite. It also gives remedy for cold, cough and liver diseases (Vane and Botting, 2003) [7]. The species of Iris were traditionally used to treat inflammation, cancer, viral and bacterial infections, etc. (Wang *et al*., 2010) [10]. Cherokee, the American Indians used to take tea brewed from iris rhizomes for renal, bladder and gastrointestinal complications (Crisan and Cantor, 2011) [11]. Keeping in mind the health benefits, the primary focus of this research trial is to quantify the essential components present in these plants. This will be a promising step towards the revival of forgotten medicinal teas of Ladakh.

Material and Methods

Collection and preparation of samples

The herbal plants used for the current investigation were collected manually from different regions of Leh district of Ladakh as described in Table 1. Plants were identified or authenticated with the help of scientific literature, subject matter specialists, *amchis*, elderly people and internet sources. Since the plants are growing naturally, so no specified permissions were required for the activities carried over in the said locations. No other species and protected area were disturbed during the fieldwork. The plant parts used for the study were cleaned, washed and shade dried. For analytical purpose, the samples were ground into very fine powder. Another lot of the dried parts were coarsely powdered for making tea. The powders were then packed in air tight glass containers and kept at cool and dry place for further use. The images of the herbal plants, plant parts and developed teas studied in this study are shown in Fig. 1.

Table 1: Details of the samples and collection

Plant	Scientific name	Local name	Part used	Collection site	Collection time	Altitude (ft)
Wild rose	*Rosa webbiana*	*Sia*	Bark	Mahe, Changthang	August, 2022	13770
Iris	*Iris lactea*	*Tesma*	Seeds (*londo*)	Chuchot, Leh	July, 2022	10718
Willow	*Salix eleagnos angustifolia*	*Chashing*	Leaves	Turtuk, Nubra	July, 2022	9846

Fig 1: Herbal plants, plant parts used and developed teas (a) *Sia* plant; (b) *Sia* bark; (c) *Sia* tea; (d) *Chashing* plant; (e) *Chashing* leaf; (f) *Chashing* tea; (g) *Tesma* plant; (h) *Londo*; (i) *Londo* tea

Reagent and standard solutions
All reagents/chemicals engaged in the present study were of analytical grade. The reagents and standards were ordered from Sigma-Aldrich Chemicals Co.

Analytical methods
Determination of Water Soluble Vitamins
Standard preparation
For the preparation of stock standard solutions of thiamine, niacin, pantothenic acid, pyridoxin and biotin, 25 mg of each standard was dissolved in 1 ml 0.1 M hydrochloric acid in 25 ml standard volumetric flask. The standard stock solutions of riboflavin, folic acid and cyano-cobalamine were prepared by dissolving 25 mg of each standard in 1 ml 0.1 M sodium hydroxide in a 25 ml standard volumetric flask. The standard solutions were stored in refrigerator at 4 °C kept in amber-glass bottles. Phosphate buffer (1M, pH 5.5) was diluted for the preparation of the working standards.

Extraction
1 g each of dried sample was soaked in 10 ml water followed by extraction with 1 ml 0.1M NaOH. 10 ml phosphate buffer (1M, pH 5.5) was added to it and kept in a dark place for 24 hours. The solution so obtained was filtered through a filter paper (Whatman No. 1) and the recovered filtrate was taken in a 25 ml volumetric flask. The solution was topped with HPLC grade water up to the mark. Before injection into LC system, the sample solution was filtered through membrane filter (0.45 mm). The stock solutions of sample were refrigerated for further use.

Procedure
The procedure described by Seal *et al.* [12] with insignificant modifications was followed for the chromatographic analysis. The mobile phase carries acetonitrile as Solvent A and aqueous trifluoro acetic acid (0.01% v/v) as Solvent B. The column was thermostatically controlled at 220 °C while the injection volume was kept at 20 ml. A gradient elution was done by varying the proportion of solvent A to solvent B. Each sample was analyzed for 35 minutes. A photo diode array UV/detector at four different wavelengths (210, 245, 275 and 290 nm) was used to detect HPLC chromatograms of all vitamins according to absorption maxima of analyzed compounds. The compounds were detected in same manner as that of flavonoids and phenolic acids.

Determination of Fat Soluble Vitamins
Standard preparation
The preparation of standard solutions of vitamins was established through serial dilutions up to 1 mg/ml of vitamin D_1 and D_2, 5 mg/ml of vitamin E, 1.4 mg/ml of vitamin K_1 and 1 mg/ml of vitamin K_2 on daily basis from a stock solution kept in a dark place at -20 °C. 20 µl was injected and to generate the standard curve data, peak areas were determined. Slope of standard curves (6 concentrations levels) was get by linear regression. All quantization was by peak area using Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Based on the established chromatographic conditions, repeated injections of different concentrations of the standard FSV were made 5 times onto the HPLC system.

Extraction of oils
The extraction of oils from the powdered sample was made with two different solvents [n-hexane (H) and chloroform/methanol (CM) (2:1 v/v)] in a Soxhlet apparatus as per the protocol given by Folch *et al.* [13]. During the extraction with CM, the addition of 0.75% aqueous sodium chloride solution (0.2 v) with the extracted lipid was required. The layers were allowed to separate after thorough mixing (without shaking), and the chloroform layer was obtained. The lipid so extracted was collected in a flask was dried with sodium sulfate, filtered, and the solvent was removed on a rotary evaporator at 40 °C. The lipids were then stored at 4 °C under nitrogen for further analyses.

Procedure
A Solvent Delivery Module LC-9A, Shimadzu HPLC (Kyoto, Japan) was employed for liquid chromatography analysis. The chromatographic system consisted of a Model 87.00 Variable Wavelength Monitor Detector (Knauer, Berlin, Germany) with stainless steel column (25 cm × 4 mm i.d.), packed with Zorbax-Sil (5 µm). Isocratic elution was based for the separation of all vitamins and the flow-rate of solvent was kept at 1 ml/minute with a column back-pressure of 65 to 70 bar. The solvent system, retention time and UV detection of vitamin E are Isooctane/ethyl acetate 96:4 v/v, 9 mins and 295 nm, respectively. The solvent system for D_1, D_2, K_1 and K_2 are Isooctane/isopropanol 99:1 v/v. The retention time for D_1 and D_2 are 17 mins and for K_1 and K_2 are 10 mins and 5.5 mins, respectively. The UV detection of D_1 and D_2 and K_1 and K_2 are 254 nm and 244 nm, respectively. The sample oil (20 µl) so extracted or its diluted solution in the selected mobile phase was directly injected onto the HPLC column. Fat soluble vitamins were determined following the comparison between their retention times and authentic standards. All tasks were carried out precisely under weak light conditions.

Determination of phenolic acids and flavonoids
Acetonic extracts (100 µg/ml) of samples were subjected to filtration through a membrane filter (0.45 µm) prior to injection into the HPLC system. An LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source (ESI) and operated in negative ionization mode was used to perform LC-ESI-MS analysis. The quadrupole temperature was DL: 275 °C and that of heat block was 450 °C. Mass spectrometer was paired online with an ultra-fast liquid chromatography system that consisted of a LC-20AD XR binary pump system, SIL- 20AC XR auto-sampler, CTO-20AC column oven and DGU-20A 3R degasser (Shimadzu, Kyoto, Japan). A DiscoVery BIO Wide Pore C18-5 (Thermo Electron, Dreieich, Germany) (15 cm x 4.6 mm, 5 µm) was used for analysis. The mobile phase consists of A and B with 0.1% formic acid in H_2O, v/v and 0.1% formic acid in methanol, v/v, respectively, with a linear gradient elution of 0 to 14 min, 10% B; 14 to 24, 20% B; 27 to 37 min, 55% B; 37 to 45 min, 100% B; 45-50 min, 10% B. A 5 minutes re-equilibration duration between individual runs was retained. The mobile phase has the flow rate of 0.4 ml/ min, the column temperature of 40 °C and the injection volume of 5 µl. Selected Ion Monitoring (SIM) mode was employed to monitor spectra followed by processing through Shimadzu LabSolutions LC-MS software (Tlili *et al*., 2019) [14].

Determination of minerals
Plant samples kept in pre-weighed clean silica crucibles were burned in a muffle furnace set at 400 °C till the smoke

get ceased. The crucibles were kept in a desiccator for cooling. Sulphuric acid (conc.) was added to the ash (carbon free) to moisten it and continuously heated on a heating mantle till the evolution fumes of sulphuric acid stopped. Continuous heating of the sulphated ash at 600 °C till constant weight, was followed (approx. 2-3 h). 1 g of the ash was dissolved in 100 ml of 5% hydrochloric acid (HCl). Determination of required minerals was done through atomic absorption spectroscopy (AAS) (AA 800, Perkin-Elmer Germany). Standard solutions of the elements studied were prepared separately and respective calibration curves were outlined accordingly (Indrayan *et al*., 2005) [15].

Assessment of antioxidant activities

DPPH Radical Scavenging Activity Assay

The DPPH (1,1-diphenyl-2-picrylhydrazil) radical scavenging capacity of the plant extracts was determined according to the method previously described by Murthy *et al.* [16] with minor modifications. Plant extracts of 0.5 ml at various concentrations namely 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 500 µg/ml in methanol or the standard i.e. ascorbic acid solution was added to 2.5 ml of a 0.1 mM methanolic solution of DPPH. The mixtures underwent dark incubation at 30 °C for 30 min. after proper vortex. By taking methanol as the blank, the absorbance was recorded at 517 nm in a spectrophotometer. The extract ability to scavenge DPPH was expressed in terms of percent inhibition of DPPH free radicals which was calculated using Eq. (1).

$$\text{Inhibition (\%)} = \frac{A_{control}-A_{sample}}{A_{control}} \times 100 \quad (1)$$

Where $A_{control}$ corresponds to the absorbance of the control (which exclude the test extract) and A_{sample} to the absorbance of the test extract. The percent inhibition against the extract (µg/ml) was plotted for the calculation of IC_{50} value of each sample.

Hydrogen Peroxide Scavenging Assay

The present study follows the protocol given by Ruch *et al.* [17] with slight modifications. The preparation of H_2O_2 solution (40 mM) was done in 0.1 M phosphate buffer (pH 7.4). The plant extract (2 ml) was mixed with 2 ml of 40 mM H_2O_2 solution at 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml concentrations in distilled water. Incubation of the reaction mixture was ensured for a period of 10 min. The absorbance was then measured at 230 nm by taking phosphate buffer as the blank. The standard used was ascorbic acid (50 to 500 µg/ml of distilled water). The calculation of hydrogen peroxide scavenging capacity was done using Eq. (1). Regression analysis of a plot of the percent inhibition against the extract concentration (µg/ml) was utilized to express IC_{50} values.

Ferric Reducing Antioxidant Power Assay

The method given by Benzie and Strain [18] with requisite modifications was followed for the determination of ferric reducing antioxidant power (FRAP). This assay works on the basis of the fact that, antioxidants reduce the ferric tripyridyltriazine which is colorless to ferrous-tripyridyltriazine which is blue in color, at low pH. First, 0.031 g TPTZ was dissolved in 10 ml 40 mM hydrochloric acid for the preparation of TPTZ (2,4,6-tripyridyl-s-triazine) solution. This was followed by the preparation of fresh working FRAP reagent by dissolving 25 ml of 300 mM acetate buffer (pH 3.6) in 2.5 ml TPTZ solution and 2.5 ml of 20 mM ferric chloride solution. Each plant extract or ascorbic acid were prepared using concentrations of 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 500 µg/ml and 2 ml of FRAP reagent was added to 1 ml of each (extract or ascorbic acid). The mixture underwent incubation at 37 °C for 30 min after thorough mixing. The absorbance with acetate buffer as the blank was read at 593 nm in a spectrophotometer. Standard curve of ferrous sulfate with concentration levels of 5 µM, 10 µM, 20 µM, 40 µM, and 60 µM was plotted. The antioxidant capacity was calculated and expressed as μMFe^{2+} equivalent and the determination of EC50 value of the FRAP capacity of each extract took place.

Statistical analysis

Detailed statistical analysis was not carried out in case of vitamins, phenolic acids and flavonoids but the average figure with their standard derivation has been worked out with the help of MS Office 2021, Excel Worksheet. As per the biochemical analysis of minerals and antioxidant activities is concerned, analysis of variance (ANOVA) was carried out followed by Duncan test using SPSS v.20 software. Significance level was considered at $p<0.05$. A minimum of three observations were pooled down for each of the samples to calculate the average values.

Results and Discussion

The present experiment is based upon the estimation of biological constituents of extracts from three wild plants traditionally used as tea in folk medicines. The data in the literature about these plants is scarce and especially the parts that were studied, thus, comparison of the results obtained in the current study was difficult. However, a few papers reported some biological constituents and associated activities of other parts of the selected plants or plants from same families.

Vitamins

Vitamin profiling of the samples reveal significant concentrations of fat as well as water soluble vitamins as depicted in Table 2. The water soluble vitamin content in *sia* can be graded in ascending order of their mean concentrations as riboflavin < thiamine < folic acid < biotin < pyridoxin < cyano-cobalamin < pantothenic acid < niacin, that of *chashing* as riboflavin < folic acid < biotin < thiamine < cyano-cobalamine < niacin < pyridoxin < pantothenic acid and of *londo* as folic acid < riboflavin < biotin < cyano-cobalamine < thiamine < niacin < pyridoxin < pantothenic acid. Fat soluble vitamin content in *sia* ascended in their mean concentrations as Vitamin K_2 < Vitamin D_1 < Vitamin K_1 < Vitamin D_2 < Vitamin E, that of *chashing* leaf Vitamin D_1 < Vitamin D_2 < Vitamin K_2 < Vitamin K_1 < Vitamin E and of *londo* as Vitamin D_1, < Vitamin K_2, < Vitamin D_2 < Vitamin E < Vitamin K_1. There are no published studies on vitamin concentrations in the selected plants that could be compared with the current results. So, the outputs presented here are the first indication of the nutrients level. However, Golubtsova, (2017) [19], reported thiamin (0.05 mg 100 g^{-1}), riboflavin (0.33 to 0.88 mg 100 g^{-1}), folic acid (0.1 to 0.25 mg 100 g^{-1}), niacin (0.6 mg 100 g^{-1}), Vitamin K_1 (0.6 to 1.2 mg 100 g^{-1}) and Vitamin

E (1.0 to 8.8 mg 100 g^{-1}) in wild rose (*Rosa majalis Herrm*) fruits.

Table 2: Vitamins (mg g^{-1} of dw) of traditional teas

Component	*Sia* tea	*Chashing* tea	*Londo* tea
Water Soluble Vitamins			
Niacin	12.14±1.47	12.09±1.61	5.59±0.74
Pyridoxin	6.14±0.58	30.82±0.50	36.30±1.20
Pantothenic acid	11.50±1.19	109.53±7.87	211.33±5.90
Biotin	0.50±0.04	0.71±0.04	0.32±0.01
Thiamine	0.12±0.11	1.73±0.13	3.03±0.30
Riboflavin	0.02±0.10	0.07±0.10	0.14±0.28
Folic acid	0.14±0.01	0.28±0.03	0.11±0.01
Cyano-cobalamine	9.12±0.22	3.68±0.21	0.36±0.05
Fat Soluble Vitamins			
Vitamin D_1	12.11±2.64	0.52±0.05	1.32±0.01
Vitamin D_2	42.53±3.01	0.84±0.03	3.22±0.09
Vitamin E	301.44±3.84	55.42±4.26	18.48±3.51
Vitamin K_1	23.90±3.46	4.13±0.43	54.09±2.21
Vitamin K_2	6.04±0.74	2.09±0.27	2.64±0.38

Values are presented as mean±standard deviation and n = 3. N.D. stands for Not Detected

Phenolic acids

Mass spectrometry of the phenolic profile of extracts of *sia*, *chashing* and *londo* led to the quantification of 18 phenolic acids (Table 3). The mean values of the various phenolic acids reveal that elagic acid with a value of 259.29 µg g^{-1} was the most abundant compounds in *sia* among the extracts studied. Similarly, salicylic acid and gallic acid with values 207.72 µg g^{-1} and 107.82 µg g^{-1} topped the phenoilc acids in *chashing* and *londo*, respectively. Phenolics exhibit multiple beneficial effects, especially the antioxidant activity, which give protective properties to the foods against chronic diseases. Earlier Nowak, 2006 [20] observed significant levels of ellagic acid in rose hips of several species. Berries that belong to Rosaceae family such as cloudberry, red raspberry, strawberry, and arctic bramble are considered as rich sources of ellagic acid (Hakkinen *et al*., 2000) [21]. A few papers reported presence of some phenolics such as gallic acid, p-hydroxy benzoic acid, vanillic acid, p-coumaric acid, ferulic acid and t-cinnamic acid in *Iris lactea* (Hoang *et al*., 2020) [22]. Gasecka *et al.* (2017) [23] while analyzing eight leaves of species and hybrids of Salix namely *Salix* × *rubra* Huds., *Salix* × *smithiana* Willd., *Salix purpurea* L. × *triandra* L. × *viminalis* L., *Salix purpurea* L., *Salix cinerea* L., *Salix* × *smithiana* Willd., *Salix alba* L. and *Salix eriocephala* Michx. found benzoic acid (13.86 to 42.81 µg g^{-1} d.m.), chlorogenic acid (12.25 to 32.94 µg g^{-1} d.m.), ferulic acid (8.90 to 24.21 µg g^{-1} d.m), p-coumaric acid (13.65 to 34.86 µg g^{-1} d.m), sinapic acid (13.81 to 36.38 µg g^{-1} d.m), t-cinnamic acid (9.10 to 423.20 µg g^{-1} d.m.), vanillic acid (8.87 to 75.31 µg g^{-1} d.m.). These results thus confirm the presence of the studied phenolics in the selected plants. p-Hydroxy benzoic acid and protocatechuic acid have antioxidant, antitumor and antimicrobial properties, salicylic acid have anti-inflammatory; gentisic acid have antioxidant and antitumor properties; p-coumaric acid have antioxidant, antitumor, anti-inflammatory, antimicrobial, anticholesterolemic and antihypertensive properties; vanillic acid have antioxidant and antimicrobial properties; gallic acid have antioxidant, antitumor, anti-inflammatory and antimutagenic properties; caffeic acid have antioxidant, anti-inflammatory, antitumor, antitumorantimicrobial and anticholesterolemic properties; ferulic acid have antioxidant, antitumor, anti-inflammatory, and antimicrobial properties; syringic acid have antioxidant, antitumor, antimicrobial and antidiabetic properties; sinapic acid have antioxidant, antitumor, anti-inflammatory, antimicrobial and antidiabetic properties; ellagic acid have antioxidant, anti-inflammatory, antitumor, antimicrobial antidiabetic and antihypertensive properties; chlorogenic acid have antioxidant, antitumor, antidiabetic, anti-inflammatory, antimicrobial, anticholesterolemic and antimutagenic properties (Al-Jitan *et al*., 2018) [24].

Table 3: Phenolic acids (µg g^{-1} of dw) of traditional teas

Component	*Sia* tea	*Chashing* tea	*Londo* tea
Benzoic acid	0.29±0.01	0.79±0.06	7.45±0.04
p-Hydroxy benzoic acid	1.97±0.02	3.05±0.11	0.17±0.01
Salicylic acid	41.91±2.74	207.72±2.58	3.19±0.21
3-Hydroxy benzoic acid	1.74±0.02	2.79±0.01	0.15±0.06
t-Cinnamic acid	0.26±0.02	1.12±0.09	0.56±0.08
2,4-dihydroxybenzoic acid	12.98±1.42	9.27±0.63	3.48±0.22
Gentisic acid	90.98±4.08	30.77±0.55	6.01±0.20
Protocatechuic acid	15.70±0.14	2.40±0.17	11.32±0.45
p-Coumaric acid	230.77±0.23	31.54±2.8	70.81±1.29
o-Coumaric acid	30.93±1.74	200.02±1.23	4.17±0.22
Vanillic acid	6.64±0.17	5.04±0.39	2.70±0.04
Gallic acid	60.10±2.66	15.28±3.67	107.82±0.18
Caffeic acid	10.86±1.00	121.98±2.05	23.88±2.48
Ferulic acid	0.07±5.50	44.83±1.15	9.23±5.21
Syringic acid	0.52±0.02	1.46±0.01	91.67±0.48
Sinapic acid	4.13±0.15	2.42±0.12	0.17±0.20
Ellagic acid	259.29±1.99	9.67±0.48	0.64±0.04
Chlorogenic acid	0.16±0.29	2.33±0.02	0.51±0.78

Values are presented as mean±standard deviation and n = 3. N.D. stands for Not Detected

Flavonoids

The results from the determinations of the flavonoid contents in the extracts of *sia*, *chashing* and *londo* are presented in Table 4. The flavonoids quantified were umbelliferone, apigenin, galangin, naringenin, kaempferol, luteolin, fisetin, eriodictyol, catechin, epicatechin, hesperetin, quercetin, epigallocatechin, myricetin and rutin. However, fisetin and eriodictyol were not detected in all the

three samples and in addition to that, galangin was also not detected in *sia*. In *sia*, quercetin (83.60 µg g^{-1}) was the most abundant flavonoid followed by hesperetin (38.61 µg g^{-1}) and naringenin (7.61 µg g^{-1}). Similarly, quercetin (138.87 µg g^{-1}) ranked top amongst the flavonoids followed by epigallocatechin (50.45 µg g^{-1}) and myricetin (33.51 µg g^{-1}) in case of *chashing*. *Londo* exhibited highest content of quercetin (50.64 µg g^{-1}) followed by hesperetin (13.20 µg g^{-1}) and epicatechin (4.59 µg g^{-1}). Flavonoids of iris have been reviewed by Wong *et al.* (2006) [25]. Apigenin was detected from the leaves of *I. reichenbachii*, *I. gracilipes* and *I. rossii*. Naringenin was reported from the rhizomes of *I. domestica.* Luteolin was reported in the rhizomes of *I. dichotoma* and aerial parts of *I. sisyrinchium* and *I. germenica* and *I. setosa*. Kaempferol was reported from *I. bracteata*, *I. douglasiana*, *I. domestica* and *I. germanica*. Hesperetin was reported in rhizomes of *I. milesii*, I. *crocea* and I. *tectorum*. Quercetin was isolated from the rhizomes of *I. bulleyana*, *I. bracteata*, *I. delavayi*, *I. chrysographes* and *I. domestica*. Myricetin was found in *I. tenax*, *I. douglasiana*, *I. domestica*, and *I. pseudacorus.* Rutin was found in *I. dichotoma*, *I. domestica*, *I. ensata*, *I. japonica and I. laevigata*. Earlier studies reported the presence of luteolin, apigenin, myricetin and quercetin in leaves of six willow species *viz*. *S. borealis* (Fr.), *S. phylicifolia* L., *S. myrsinites* L., *S. lapponum* L., *S. glauca* L. and *S. reticulate* (Nyman and Julkunen-Tiitto, 2005) [26]. Several species of *Cotoneaster* (Rosaceae) are reported to contain some flavonoids such as bark of *C. integerrimus* contains myricetin, quercetin, catechin and epicatechin; twigs contain hesperidin; fruits contain kaempferol. Leaves of *C. thymaefolia* contain apigenin, leafy twigs of *C. orbicularis* contain naringenin; leafy twigs of *C. horizontalis* contain luteolin (Kicel *et al.*, 2019) [27]. Flavonoids are reported to have considerable scavenging activities against an array of oxidants. In particular, *chashing* recorded a richer flavonoid profile as compared to other plants analyzed, with a high quantity of apigenin and luteolin, which are documented in the prevention of cancer (Sung *et al.*, 2016) [28]. *Londo* recorded 9 times higher rutin, a glycoside of quercetin with potent anti-inflammatory and anti-carcinogenic activities (Perk *et al.*, 2014) [29] than the average value observed in other herbal plants. *Chashing* reported to have higher quantities of rutin, epicatechin and catechin, that are reported to have prominent role in the management of inflammatory diseases. Gasecka *et al.* (2017) [23] while analyzing eight leaves of species and hybrids of Salix found that myricetin ranges between 116.20 and 938.16 µg g^{-1} (d.m.) and quercetin between 74.85 and 1222.54 µg g^{-1} (d.m.). Quercetin, present in foods such as green tea, berries, and grains has been most effective in colorectal carcinogenesis prevention. Luteolin has neuroprotective capability and also has a protective capacity against neuro-disorders that prevails with aging (Fan *et al.*, 2017) [30]. Quercetin, in its conjugated form improves vascular health, and slow down cardiovascular disease risks. Quercetin and its derivatives prevent chances of thrombosis (blood clotting) and stroke (Terao, 2017) [31]. Luteolin, having pro-apoptotic activity in hepatocellular carcinoma (HCC) cells, scavenge the cancer cell cycle at the G2/M stage. Kaempferol can reduce the risk of several types of cancer. It acts as antioxidant stimulant against cancer causing free radicals (Nabavi *et al.*, 2015) [32]. Myricetin has anticancer and anti-inflammatory potentials. It shows antimitotic effects in liver cancer and kill cancer cell by targeting different metabolic pathways in mitochondria (Devi *et al.*, 2015) [33]. Hesperidin and hesperetin showed antioxidant, anticancer effects, antimicrobial and anti-inflammatory (Roohbaksh *et al.*, 2014) [34]. These flavonoids are reported to have pharmacological effects on nervous system, including antidepressant, neuroprotective, and memory (Roohbaksh *et al.*, 2015) [35]. Rutin showed several biological activities like cytoprotective, anticancer, antioxidant, etc. (Ganeshpurkar and Saluja, 2017) [36]. Epicatechin, an antioxidant flavonoid has an analog 3-O-methyl epicatechin which has neurotoxicity inhibiting capacity *in vitro* (Lamuela-Raventos *et al*., 2016) [37].

Table 4: Flavonoids (µg g^{-1} of dw) of traditional teas

Component	*Sia* tea	*Chashing* tea	*Londo* tea
Umbelliferone	N.D.	0.60±1.35	0.06±0.82
Apigenin	0.18±0.09	3.22±0.84	0.31±1.02
Galangin	N.D.	0.35±0.37	0.78±2.98
Naringenin	7.61±0.22	9.80±0.26	0.79±0.09
Kaempferol	2.19±0.84	0.13±0.91	0.12±4.06
Luteolin	1.95±0.11	5.59±0.26	3.56±0.28
Fisetin	N.D.	N.D.	N.D.
Eriodictyol	N.D.	N.D.	N.D.
Catechin	5.62±0.77	21.10±0.20	2.54±0.84
Epicatechin	1.18±0.06	1.81±0.09	4.59±0.13
Hesperetin	38.61±1.32	24.11±1.22	13.20±0.10
Quercetin	83.60±2.52	138.87±2.14	50.64±0.72
Epigallocatechin	2.12±0.26	50.45±3.08	1.09±0.11
Myricetin	2.95±0.09	33.51±4.85	3.44±0.64
Rutin	6.49±0.61	4.87±0.25	0.89±0.09

Values are presented as mean±standard deviation and n = 3. N.D. stands for Not Detected

Minerals

The concentrations of mineral elements are illustrated in Fig. 2 and the results present the mean concentrations of calcium, iron, magnesium and potassium. In the present experimental trail, the highest calcium content of 14.66 g Kg^{-1} was reported in *chashing* followed by 11.47 g Kg^{-1} in *sia* and 14.28 g Kg^{-1} in *londo*. *Londo* exhibited highest iron (9.24 g Kg^{-1}) amongst the herbs followed by *chashing* (2.16 g Kg^{-1}) and *sia* (1.05 g Kg^{-1}). The magnesium content was 4.01 g Kg^{-1} in *chashing*, 1.38 g Kg^{-1} in *sia* and 12.46 g Kg^{-1} in *londo*. Potassium content was highest in *sia* (45.76 g Kg^{-1}) and lowest in *chashing* (2.16 g Kg^{-1}) with *londo* (14.85 g Kg^{-1}) in between these. Calcium gives rigidity and strength to teeth and bones. Calcium ions are also helpful in transmission in neuro-muscles, promoting muscular contraction and clotting of blood. It also acts as enzyme activators such as arginine kinase, phospholipase, adenyl kinase and adenosine triphosphatase. Calcium ions act as a mental depressant when exceed in the extracellular fluids and in low levels lead to spontaneous discharge of nerve fibers which results in tetany. Iron is the most important mineral in biological system as it performs a number of essential activities. Many of these functions are related to the redox reactions and energy conserving processes in the body. It is an intrinsic part of haemoglobin, metalloflavoproteins, myoglobin and cytochromes and some enzymes such as peroxidases and catalase. Iron is thus very necessary for oxygen transportation and for functioning of oxidation systems within the cells. Iron deficiency causes anemia. Magnesium is considered to be one of the most important cations in human body. Most of the magnesium is

contained in bones associated with phosphate and calcium and rest in the body fluids and soft tissues. In muscles and other tissues, many of the enzymes involved in carbohydrate metabolism and DNA and RNA synthesis are activated by intracellular magnesium ions. Magnesium also plays an important role in binding of ribosomal particles. Increase in extracellular magnesium content reduces the skeletal muscles contraction whereas lees quantity of magnesium concentration results in nervous system irritability, peripheral vasodilation and cardia arrhythmias. Potassium is the main intracellular cation and also considered as a very prominent component of the extracellular fluids. Potassium ions help in the electrical impulse transmission in the nervous system as well as maintenance of balance of body fluid. Venkataraman and Gopal Krishnan (2002) [38] found remarkable contents of Fe, Ca and K in nine wild plants and established that higher potassium concentrations could be because of the diuretic action of these plants. We found high concentration of potassium in *sia* thus was in conformity with the above study. Fruits of *Rosa rubiginosa* contain 11.38 to 15.43 g Kg^{-1} and 19.93 to 27.0 g Kg^{-1} of calcium and potassium, respectively (Mabellinia *et al.*, 2011) [39]. Golubtsova (2017) [19], reported calcium (16 to 28 g 100 g^{-1}), iron (24 to 115 g 100 g^{-1}), magnesium (6 to 8 g 100 g^{-1}) and potassium (23 to 51 g 100 g^{-1}) in wild rose (*Rosa majalis Herrm*) fruits. *Iris lactea* contain calcium 2600 mg g^{-1} (d.w.) and 2000 mg g^{-1} (d.w.), iron 130 mg g^{-1} (d.w.) and 810 mg g^{-1} (d.w.), magnesium 2000 mg g^{-1} (d.w.) and 2100 mg g^{-1} (d.w.) and potassium 22 mg g^{-1} (d.w.) and 48 mg g^{-1} (d.w.) in its shoot and root, respectively (Guo *et al.* 2017) [40]. The variations so observed in the mineral contents of the plants studied are due to several factors, such as the plant age, the botanical structures, the climatic conditions, the soil properties, and in between biological differences.

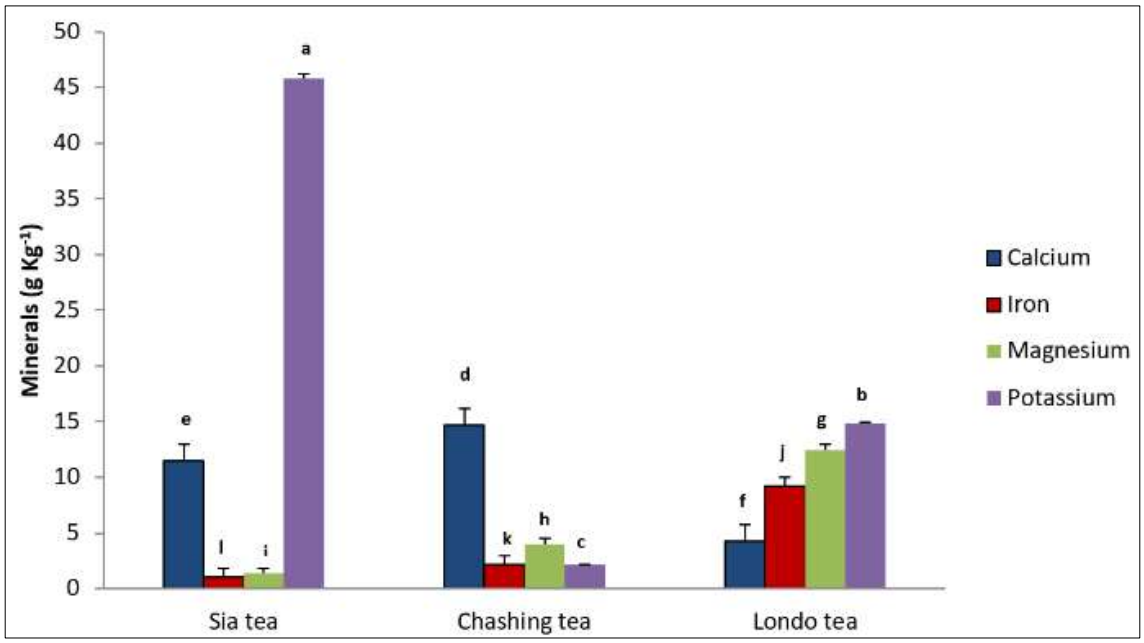

Fig 2: Mineral contents (g/Kg) of extracts of *sia* tea, *chashing* tea and *londo* tea. Values are presented as mean±standard deviation and n = 3. N.D. stands for Not Detected. Different letters in the graph represent significant differences (p<0.05)

Antioxidant activity

The DPPH, H_2O_2 and FRAP assays, were employed to determine the antioxidant capacity of plant extracts while comparing with the activity of the ascorbic acid (standard) (Fig. 3). AEEs (Aqueous Ethanolic Extracts) and AMEs (Aqueous Methanolic Extracts) of *sia*, *chashing* and *londo* depicted significant (p<0.05) scavenging effects on the described assays. The IC_{50} (µg ml^{-1}), corresponds to extract/standard concentration that can inhibit DPPH or H_2O_2 scavenging activities up to 50%, and the EC_{50} (µg ml^{-1}) corresponds to extract/standard concentration that can inhibit FRAP capacity up to 50%. These values were compared with the IC_{50} and EC_{50} of the ascorbic acid, standard antioxidant taken. Lower the IC_{50} and EC_{50} values, higher the antioxidant activity. As per the outputs of the current research, the AME of *sia* had the hydrogen peroxide neutralizing capacity with an IC_{50} value of 140.84±1.73 µg/ml, which is lower than that of IC_{50} value of AEE (280.49±6.85 µg/ml) but comparable to that of ascorbic acid (135.65±0.45 µg/ml). But in the DPPH assay, the IC_{50} values of the AEE (78.35±1.47 µg/ml) and AME (69.14±1.46 µg/ml) of *sia*, were notably higher than that of IC_{50} value of ascorbic acid (18.05±0.55 µg/ml), which specified a lower antioxidant activity. Moreover, the EC_{50} value (68.15±1.49 µg/ml) observed for AEE of *sia* in the FRAP assay was notably ($p < 0.05$) higher than that of AME (57.36±5.82 µg ml^{-1}), which shows that the AME exhibits a higher reducing power than the AEE. Here, it is interesting to describe that the extracts of *chashing* showed promising free radical scavenging activities similar to ascorbic acid in both the DPPH and H_2O_2 assays. The IC_{50} values of AEE (20.53±1.40 µg/ml) and AME (18.87±1.28 µg/ml) of *chashing* in the DPPH assay, exhibited a non significant difference between their scavenging activities which indicate their encouraging free radical scavenging potentialities. These findings correspond to those observed previously in extracts from of *Adenium obesum* (Hossain *et al.*, 2014) [41]. The results obtained in the H_2O_2 assay with both extracts of *chashing* (IC_{50} values of 150.34±5.04 µg/ml and 143.11±3.87 µg/ml) were also comparable to the IC_{50} value of ascorbic acid (IC_{50} 135.65±0.45 µg/ml) and further established the potential antioxidant activity of *chashing* extracts. Interestingly, the AME of *chashing* recorded better reducing potential in the FRAP assay than the AEE, which

is true with the antioxidant capacities of *sia* extracts in the FRAP assay. While comparing the extracts i.e. AME and AEE, *londo* showed similar trends as that of *sia* and *chashing* and the antioxidant capacities of the former lied between the latter two. The slight antioxidant capabilities recorded by the AEE of *sia*, especially in the H_2O_2 assay, could be justified partially by the relatively low flavonoid content in this extract. The current findings corroborate to data previously documented by Abdel-Farid *et al*. (2014) [42]. The noticeable radical scavenging activity exhibited by *chashing* extracts on the DPPH and H_2O_2 assays could be because of higher concentrations of phenolic compounds in general and flavonoids in particular. AME of *chashing* and *sia* extracts displayed better reducing capacity in the FRAP assay than the AEE. This result indicates that the ferric reducing capacity of the former can be linked to the phenolic content, which is in line with the previous findings of Wong *et al.* (2006) [25]. There is a noteworthy relationship between the chemical composition particularly the polyphenols and flavonoids with the antioxidant activity. Polyphenolic compounds act as reducing agents, antiradicals, singlet oxygen quenchers, etc., thus promote the natural antioxidative defense mechanisms and protect the enzyme activity (Huwaitat *et al.*, 2018) [43].

The discussed herbal plants used in traditional medicare can have side effects and interactions with certain medications. Iris seeds contain a number of compounds that can be toxic in high doses including irisin, iridin and iridinol. These compounds can cause nausea, vomiting, diarrhea and other symptoms if consumed in large quantities. Willow leaves contain compound called as tannin, which cause gastrointestinal upset if consumed in excess. Ingestion of another compound salicin in large amounts can be toxic. If one is considering using these plants for consumption, it is important to consult with a food technologist, healthcare provider or licensed herbalist first to determine the appropriate dosage to ensure the safety. It is also important to authenticate these plants from genuine sources to ensure their purity and quality. In general, the dosage can range from 1-6 g per day, divided into several doses throughout the day and can be consumed as tea.

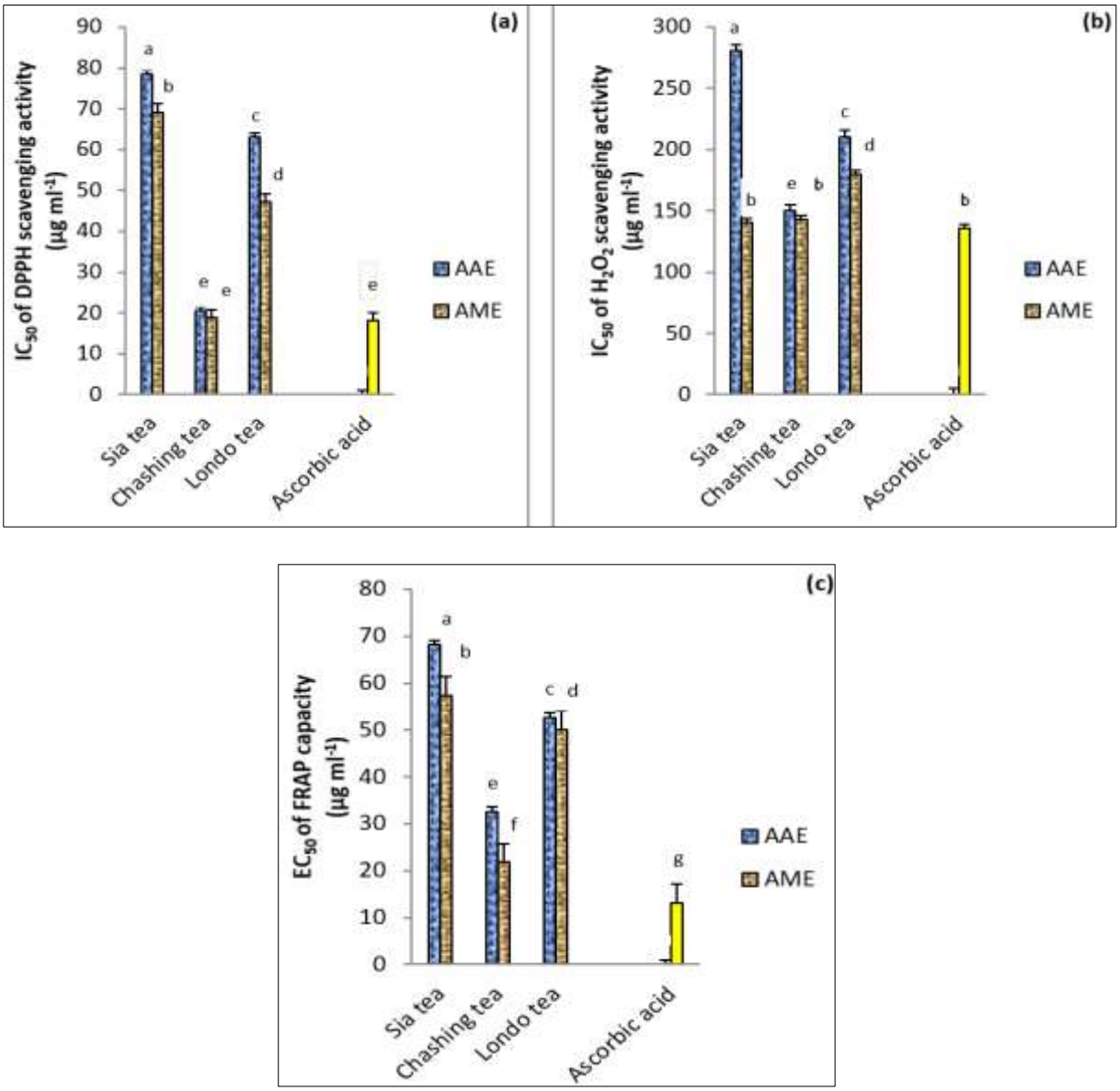

Fig 3: Antioxidant activities of extracts of *sia* tea, *chashing* tea *and londo* tea. Values are presented as mean±standard deviation and n = 3. Different letters in the graph represent significant differences ($p<0.05$)

Conclusion

The current investigation offers an overview of both nutritional and health promoting properties of the herbal plants, traditionally used in Ladakh region. The plants studied exhibited a noticeable presence of essential nutrients and bioactive metabolites, involved in several important biological activities responsible for prevention and cure of many diseases. In particular, *chashing* had a high content of polyphenols. However, these herbal plants may contain toxic compounds thus its consumption should be done under the guidance of qualified professionals to determine the appropriate dosing information.

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