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Evaluation of antioxidant and cytotoxic activity of herbal teas from Western Himalayan region: a comparison with green tea (*Camellia sinensis*) and black tea

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Abstract

Background: Herbal tea, known as health-promoting due to its therapeutic potential for several ailments and consumption increased over decades. The Western Himalayan region of India affluent with herbs has therapeutic values. However, these herbs have not been used in the tea and are still untouched by mankind. Therefore, the present study aimed to pioneer and manufacture herbal teas from Western Himalayan region plants.

Results: Our findings showed that total polyphenol content was ranged from 4.42 ± 0.53 to 13.37 ± 0.50% compared to green tea (GT) and black tea (BT) (13.37 ± 0.50 and 10.05 ± 0.11%) of *C. sinensis*, total flavonoid and total catechin content was ranged from 1.81 ± 0.67–4.68 ± 0.26% to 4.43 ± 0.28–15.17 ± 0.53% in all the herbal tea samples. Moreover, antioxidant activity was ranged in DPPH from (27.58–226.28 µg/mL) and in ABTS (14.17–117.62 µg/mL). Highest antioxidant activity was observed in GT and lowest was observed in rose tea (RT). Heatmap was made for catechin visualization in green herbal teas (GHT). Principal Component Analysis (PCA) showed the variation of amino acids in all the herbal tea samples which was found in the range from 0.82 to 2.86%. Taxus green tea (TGT) exhibited remarkable cytotoxic activity against SW480 (50.9 ± 0.7 at 200 µg/mL). Whereas, sea-buckthorn green tea (SGT) exhibited the highest activities on A549 cells (87.01 ± 1.1 at 200 µg/mL). Maximum volatile organic compounds (VOCs) were identified in lemongrass black tea (LBT) (96.23%), namely, geranial, levoverbenone, pulegone, L-linalool and cineol. In addition, the sensory analysis revealed that herbal tea shows sweet and better taste with high sensory attributes.

Conclusions: Current study revealed that the Western Himalayan region plants could be used as herbal tea with additional health benefits. The prepared herbal teas can be used in nutraceuticals as a beverage and a new dietary source for bioactive compounds.

Keywords: Herbal teas, *Camellia sinensis*, Antioxidant activity, Cytotoxic activity, Catechins

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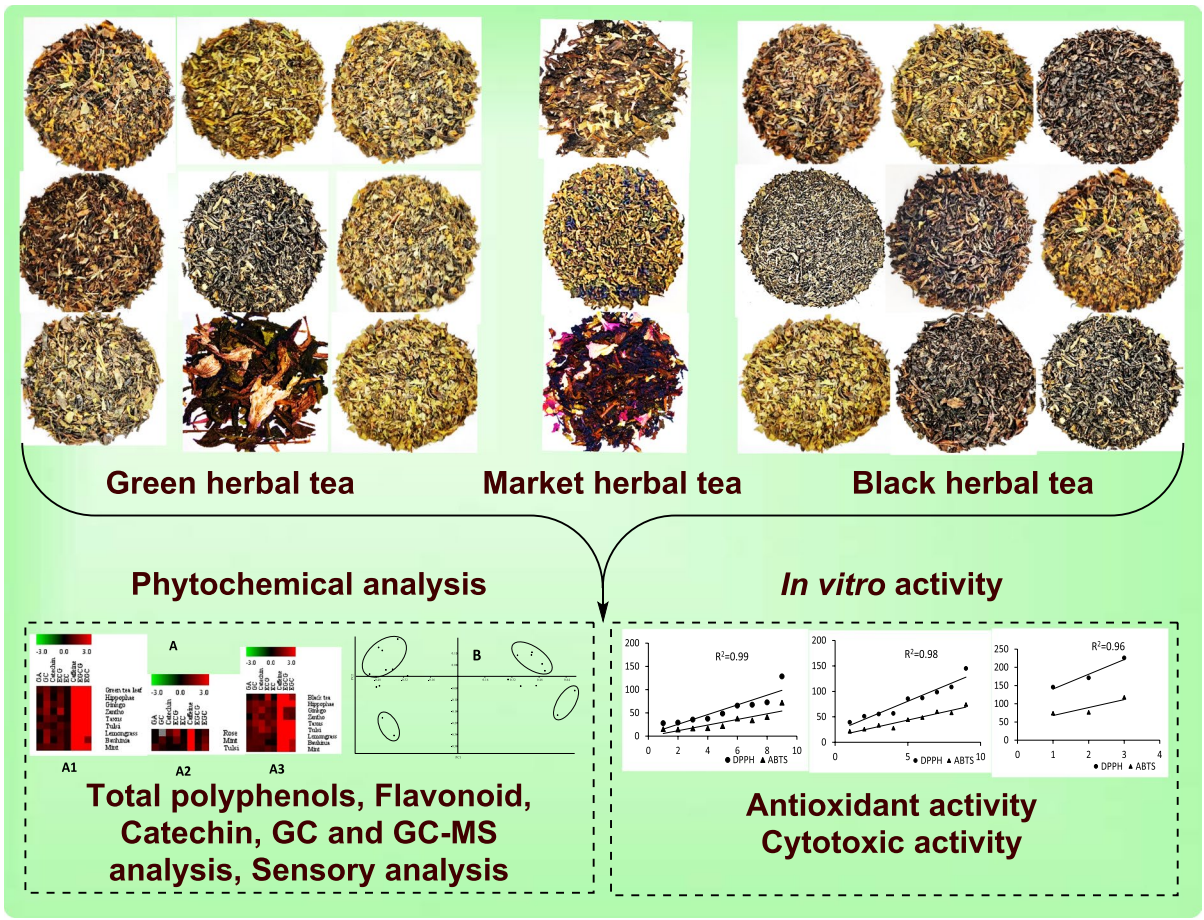
Graphical Abstract



Background

Tea (*Camellia sinensis*) is the most widely consumed non-alcoholic beverage, next to water [1]. There are different types of teas manufactured from the *C. sinensis,* such as green tea, black tea, oolong tea and white tea [2]. These are used as a healthy beverage in traditional Chinese medicine for the last many years [3] contains a large amount of polyphenolic compounds with the health beneficial properties [4]. These teas are manufactured in different geographical regions, such as India, Kenya, Sri Lanka, Taiwan and Bolivia [5].

Nowadays, people have started consuming more processed foods and beverages, as a result, health problems have been increasing [6]. Therefore, the interest of people towards herbal tea is increasing day by day which is an alternative to conventional beverages [7]. In this context, herbal plants are rapidly being explored to promote health beneficial effects with attractive flavor and taste [8]. Herbal tea is highly consumed as a nutritional and traditional beverage for the numerous diseases [9] and have a long history of use as complementary therapy of various disorders in human and still, play an important role in traditional medicine. Herbal tea consumption is mainly based on the local plants, which is influence by the local culture, tradition and regions [10]. In several countries, tea drinking is very common; therefore, people choose to take several types of teas or herbal tea (e.g., chamomile and linden) [11].

Till today, herbal teas and green teas are considered as a popular beverage in the global due to their aroma, taste and beneficial health effects [12]. A large numbers of herbal teas are sold in the market in the blended form [13]. Herbal tea is the infusions or decoctions made from the leaves, flowers and fruit of the plant material other than the *C. sinensis* leaves [7]. The plants used for making herbal tea are *Cinnamon, Cloves, Peppermint, Chamomile, Ginger, Jasmine, Hibiscus* and *Fennel* [14]. Some

researchers introduced the *Cyclopia genistoides* and *C. subternata* for the preparation of herbal teas [15]. The most commonly present compounds in herbal tea are the polyphenolic compounds mainly phenolic acids and flavonoids show high antioxidant activity [16]. Furthermore, herbal tea also showed various biological activities, such as anticancer, antidiabetic, anti-inflammatory, antitumor, antibacterial and antiviral activities [17].

Herbs are widely used in pharmaceutical and culinary purposes due to their flavour and taste quality [18]. Flavour is the most important element in tea which describe the taste and aroma of tea quality. Herbal tea of *Rooibos* and *Honeybush* are known for their better aroma, flavour and taste [11]. The herbal tea of honeybush (*Cyclopia* spp.) is not accepted by the consumers, because it shows a vegetal aroma; therefore, the aroma of honeybush is improved by the steam treatment [19]. The high-quality herbal tea of *Cyclopia genistoides, C. subternata* and *C. maculata* were manufactured with sensory attributes and aroma characteristics [15].

Herbal plants play an important role in human life; approximate 31,000 species of plants are used all over the world for the medicinal purpose [20]. International market of foods and beverages is predicted in the range of 356.3 billion USD in the upcoming years [21]. Approximately 80% of the people around worldwide are used herbal plants as a traditional medicine with a market price of USD 72 billion [22].

In India, about 80% of rural populations use medicinal herbs which is estimated that about 960 plant species are used by the Indian industries, approximating about 80 billion [23]. Herb includes medicine of AYUSH (Ayurveda, Unani, Siddha and homeopathy) products that occupy approximately 3% of total Indian pharmaceutical export. About 70% of the herbal sector consists of raw material estimated at 10 billion per annum and 30% of export consists of the final product [23]. India shares nearly 1% of global herbal exports [23]. According to FSSAI in India, the food product follow the following requirement, such as total soluble solid not less than 10%, fruit content and ready to serve beverage not less than 5% and all other beverage/drink not less than 10% [24]. FSSAI provides the regulation for fruit beverages, fruit drinks, green tea, and black tea, but did not mention the proper regulation for herbal tea [24]. In EU, the regulation has been given for flavoured tea or flavoured tea with other food ingredients are provided [25]. It lacks the regulation for herbal tea, such as safety, effective use, good quality before reaching the public [26]. In UK regulation regarding food, it show the nutritional properties of food, such as low fat content with the potential health benefits for the consumption of food [27]. Further studies will be required in this area, such as manufacturing conditions of herbal tea, types of herbs used, shelf life, stability study and safety.

Also, the manufacturing of herbal teas from Western Himalayan region plants is much less investigated and the plants are used for making herbal teas by the local people are not well explored.

Therefore, the aim of present study is to utilize the Western Himalayan region plants for making herbal teas with health beneficial properties. A total of 16 different herbal teas were manufactured and compared with green and black teas of *C. sinensis*. Total polyphenols, total flavonoids and total catechins content were estimated using a spectrophotometer. Antioxidant activity was carried out using DPPH and ABTS free radicals. Cytotoxic activity was performed in three different cell lines viz, SW480, A549 and SiHa. Analysis of individual catechin content was also performed using HPLC. Amino acids profile was performed using UPLC. Identification and characterization of VOCs were performed using GC and GC–MS followed by sensory evaluation studies.

Materials and methods

Chemicals

HPLC grade acetonitrile, methanol and dichloromethane were procured from Merck, Mumbai, India. Potassium carbonate (≥ 99.9%) was of analytical grade procured from Sigma Aldrich, Bangalore. Anhydrous sodium sulphate (≥ 99.0%) was taken from Merck, Mumbai, India. Authentic catechins standards (≥ 98%) were purchased from Sigma Aldrich, Bangalore.

Collection of plant materials

Fresh leaves of *Camellia sinensis, Ginkgo biloba, Bauhinia variegate, Ocimum basilicum, Cymbopogon flexuosus* and *Mentha piperita* were collected from CSIR–IHBT, Palampur. *Taxus baccata* was taken from institute experimental farm Bandla, Palampur. *Zanthoxylum armatum* was taken from Bandla Palampur and *Hippophae rhamnoides* was taken from the Lahoul Spiti. The plant material was authenticated by the Taxonomist of the institute and voucher specimens were submitted to the herbarium of CSIR–Institute of Himalayan Bioresource Technology, Palampur, India. The voucher number of *Bauhinia variegate* L. is PLP-16495, *Zanthoxylum armatum* is PLP-16496, *Taxus baccata* is PLP-16497, *Ginkgo biloba* is PLP-16498, *Mentha piperita* is PLP-16499, *Ocimum basilicum* is PLP-16500, *Cymbopogon flexuosus*is PLP-18561 and *Hippophae rhamnoides* is PLP-4400. Rose tea, mint tea and tulsi tea were acquired from the local market of Palampur, India. The market herbal tea samples were kept at 4 °C until further analysis.

Herbal teas preparation

Fresh shoots of *C. sinensis* cultivars of "Kangra Jat" were obtained from tea experimental farm Banuri, Palampur.

Each sample was collected and manufactured with herbal plant material on a w/w basis—i.e., 93 weight % of *Camellia sinensis* shoots and 7 weight % of herbal plant material. Based on manufacturing processes, two different types of herbal tea; green (unfermented) and black (fully fermented) herbal tea were manufactured.

Infusion preparation

Each 2 g of tea infusion was prepared with 100 mL of hot distilled water and stirred for 3 min, which is maintained for 4–6 min then allowed to cool for 20 s [28]. Furthermore, tea infusions were filtered using a Whatman filter paper and stored at 4 °C used within 24 h. The experiment was performed in triplicate.

Total polyphenols content

Total polyphenols content were measured in all the herbal tea samples using Folin Ciocalteu reagent, followed the method of Joshi et al. [29]. 40 µL of herbal tea infusion was added into the 25 mL volumetric flasks followed by the addition of 500 µL Folin Ciocalteu reagent (1 N) after that 100 µL of Na_2CO_3 solution was added and then made up to 25 mL using distilled water. Further incubation given for 30 min at room temperature and measured at 730 nm using Shimadzu UV–Vis spectrophotometer. Gallic acid was used as a standard, and a calibration curve was plotted in a concentration of 50–300 µg/mL. Experiment was performed in triplicate set.

Total flavonoids content

The total flavonoid content of all the herbal tea samples was analysed followed Joshi et al. method [29]. One millilitre of herbal tea samples was added into volumetric flask which is made up to 5 mL using distilled water followed by the addition of 0.3 mL of 5% $NaNO_2$ then incubated for 5 min. After that 10% $AlCl_3$ was added followed by the addition of 1 M NaOH (2 mL), and then made up to 10 mL using distilled water. The absorbance was measured at 510 nm on spectrophotometer. Quercetin was used as standard and total flavonoid contents were expressed as quercetin equivalent (QE).

Total catechin content

Total catechin content was measured followed the method of Joshi et al. with slight modification [29]. 40 µL of each sample was dispensed into a 25 mL volumetric flasks. 1 mL of diazotized sulphanilamide reagent was added, followed by 1 mL of HCl (30%). The flasks was incubated for 1 h at room temperature, and absorbance was taken at 425 nm on spectrophotometer after making the volume to 25 mL with distilled water.

Antioxidant activity

DPPH free radical-scavenging activity

Antioxidant activity of all the herbal tea samples was performed using DPPH free radical followed the method of Joshi et al. [29]. Concentration of the samples ranged from (25–200 µg/mL) with 70% methanol (v/v). For the determination, each sample was diluted to a serial dilution of 25–200 µg/mL with 70% (v/v) aqueous methanol. Separately, 5 ml of DPPH (0.06 mM) solution was prepared in the 70% (v/v) methanol. Aliquot (0.5 mL) of each dilution of each sample was taken, followed by the addition of 3 mL of DPPH solution. The mixture was vortexed vigorously followed by incubation for 30 min at ambient temperature in the dark. The absorbance was measured at 517 nm on spectrophotometer against methanol as a blank.

ABTS free radical-scavenging activity

Antioxidant activity of all the herbal tea samples were carried out using ABTS free radical followed the method of Joshi et al. [29]. Concentration of samples was ranged from (25–200 µg/mL) with 70% methanol (v/v) which is followed by the addition of 3 mL diluted ABTS solution. The absorbance was taken 734 nm.

HPLC analysis of catechins and caffeine

For the analysis of catechins in all the samples followed the method of Sharma et al. with some modifications [30]. The LiChrospher RP-18 column (250 × 4 mm, 5 µm) was used and the column temperature was set at 35 °C. Injection volume was used as 10 µL. The mobile phase (A) consisted 0.1% ortho-phosphoric acid in water (w/v) and (B) acetonitrile with a flow rate of 1 mL/min used. The gradient method was used as follows A:B; 90:10 (0–10 min), 70:30 (10–15 min), 65–35 (15–18 min), 80–20 (18–20 min) then again back to 90:10 in 20 min. The absorbance was measured at 254 nm. All the samples were performed in triplicate.

Amino acids analysis

Amino acid analysis of tea samples was performed using UPLC followed the method of Joshi et al. with slight modification [29]. Amino acid analysis was performed on a Waters UPLC system, with a binary solvent manager. Column was used as Waters AccQ Tag (2.1 mm i.d. 100 mm, 1.7 µm) and temperature was set at 35 °C with the flow rate of 0.1 mL/min. Mobile phase A and B were used as AccQ Tag ultra-pure solvent. The gradient method are as follows A:B; 100:0 (0–3 min), 90:10 (3–4 min), 85:15 (4–5 min), 82:18 (5–7 min), 80:20 (7–9 min), 79:21 (9–10 min), 75:25 (10–11 min), 50:50 (11–12 min), 0:100 (12–13 min) then again back to 100%

in 15 min. Injection volume was used as 0.5 µL. The PDA detector was set at 254 nm. All the experiment were performed in triplicate.

Cell lines and cell culture

SW480 (human colon adenocarcinoma), A549 (human lung carcinoma), and SiHa (human cervical cancer) cells were taken from National Centre for Cell Sciences, Pune (India), following the method reported by Kumar et al. [31].

SRB assay

Cytotoxicity potential of herbal tea samples was performed using Sulphorhodamine B (SRB) assay followed the method reported by Kumar et al. [31]. Briefly, all the cells (SW480, A549 and SiHa) distributed at a density of 20,000 cells/well into 96-well flat bottom plates. The concentrations of cells was set as (20, 50, 100 and 200 µg/mL). Vinblastine (1 µM) was used as a positive control. 100 µL/well of each concentration was added and incubated in CO_2 incubator for 48 h. After incubation, 50 µL trichloroacetic acid (50%) were added to the wells and incubated for 1 h at 4 °C. Plates were washed using water and dried at room temperature. Which is followed by the addition of 100 µL of SRB (0.4% W/V prepared in 1% glacial acetic acid), and then placed in a dark for 30 min at room temperature. Subsequently, the plates were washed with 1% glacial acetic acid, air-dried, and the dye was dissolved using 100 µL of Trisbase (Sigma Aldrich, India). The absorbance was taken at 540 nm using microplate reader (BioTek Synergy H1 hybrid reader, Winooski, VT, USA).

Extraction of volatile organic compounds using simultaneous distillation extraction (SDE)

Volatile organic compounds were extracted using 100 g dried herbal tea samples, following the method as described by Rawat et al. [32]. Likens–Nickerson apparatus was used which is attached to an extended condenser. The pressure was maintained as 0.267 bar at the air vent which is connected to a vacuum pump. The process was continued for 20 min for the extraction of VOCs. The extracted volatile compounds from 5 different batches of SDE, for each herbal tea samples, were pooled together, followed by concentrating it to 5 mL at 35 °C in the Vigreux column (Perfit India, Ambala, India). The obtained volatile extracts of herbal teas were concentrated to 5 mL in an inert atmosphere for the removal of moisture and other impurities and then passed through anhydrous Na_2SO_4.

Aroma extract dilution analysis (AEDA)

The obtained volatile extracts of herbal teas were diluted in a serial dilution of the ratio of (1:2, 1:4, and so on) using dichloromethane then given for analysis.

Gas chromatography analysis

For the analysis of volatile compounds GC was used of An Agilent 7890 Series gas chromatograph equipped with fused silica column (30 m × 0.25 mm i.d., coated with a 0.25 µm film of HP-5). The injection was used in a spitless mode. Column temperature was maintained between 40 and 210 °C at the rate of 5 °C/min. Injector temperature was used as 250 °C and detector temperature was used as 230 °C. The carries gas was used as a hydrogen with the flow rate of 1 mL/min. Flame ionization detector (FID) was used.

Gas chromatography–mass spectrometry (GC/MS) analysis

Volatile organic compounds of herbal tea samples were estimated on a Shimadzu GC/MS–QP2010SE instrument followed the method reported by Joshi and Gulati [33]. A DB-5 column was used (30 m × 0.25 mm × 0.25 µm), the carrier gas was helium, and the injector temperature was 220 °C with 1: 10 split ratio. The oven temp was maintained at 70 °C for 4 min, then increased to 220 °C at 4 °C/min and held at 220 °C for 5 min. The flow rate of the column was maintained at 1.10 mL/min and the ion source temperature was 200 °C. MS were scanned at 70 eV over 40–600 a.m.u. The injection volume of the samples was 1 µL.

Identification of flavour components

The identification of compounds was performed by associating the retention indices (RI) relative to a C_8–C_{23} *n*-alkanes mixture. Compounds were characterized by comparing their mass spectra library with the NIST 02 and Wiley 7 library [34]. Validation of identified compounds was accomplished by comparing the mass spectra already available in our library, which is further followed by matching the compounds to the RI of compounds [35].

Sensory evaluation

Each (2 g) green and black herbal tea were infused with 100 mL of hot distilled water and maintained for 4–6 min then allowed to cool for 20 s. The obtained tea infusions were filtered and used for taste evaluation. The total quality score (TQS) of tea infusion was measured according to the standard procedure by the trained tea-tasting panel [36]. Which was classified into five different tea sensory quality attributes viz, the appearance of leaf,

aroma, infusion colour, taste, and shape of infused leaves. The TQS of the sample was measured by the sum of each taste quality and spearmen linear correlation analysis.

Statistical analysis

Determinations of each sample was carried out in triplicate; statistical analysis was performed using STATISTICA 7 variance. Heatmap visualization was performed using ClustVis variance software for the analysis of individual catechins profile. Principal component analysis (PCA) was performed by PAST variance 3 software for the analysis of amino acid content.

Results

Three different types of herbal tea were categorized. Based on manufacturing condition using herbal plant material with *C. sinensis* shoots viz, green herbal tea (GHT), black herbal tea (BHT) and market herbal tea (MHT) is purchased from the local market Palampur. All these three types of teas were compared with green and black tea of *Camellia sinensis*. A total of 16 green and black herbal teas were manufactured using the Western Himalayan region herbs, such as sea-buckthorn (*Hippophae rhamnoides*), tulsi (*Ocimum basilicum*), lemongrass (*Cymbopogon flexuosus*), mint (*Mentha piperita*), timur (*Zanthoxylum armatum*), taxus (*Taxus baccata*), ginkgo (*Ginkgo biloba*) and kachnar (*Bauhinia variegate*). The manufactured GHT category include the kachnar green tea (KGT), timur green tea (TiGT), ginkgo green tea (GGT), mint green tea (MGT), lemongrass green tea (LGT), tulsi green tea (TuGT), taxus green tea (TGT) and sea-buckthorn green tea (SGT). The BHT manufactured category include the ginkgo black tea (GBT), kachnar black tea (KBT), timur black tea (TiBT), mint black tea (MBT), tulsi black tea (TuBT), taxus black tea (TBT), lemongrass black tea (LBT) and sea-buckthorn black tea (SBT). Rose tea (RT), mint tea (MT) and tulsi tea (TT) was purchased from the local market are in the MHT category.

Total polyphenols content (TPC)

Total polyphenol content was measured using Folin–Ciocalteu method which was ranged from 4.42 ± 0.53 to 13.37 ± 0.50%. Among all the teas, GT is the rich source of TPC (13.37 ± 0.50%) (Table 1). In green herbal tea (GHT), polyphenols content was ranged from 10.75 ± 0.14 to 13.37 ± 0.50%. From GHT, GT showed the highest 13.37 ± 0.50%, whereas KGT contain the lowest 10.75 ± 0.14% polyphenol content. In addition, there are some exceptions were observed in green herbal teas such as SGT (13.23 ± 0.10%) and TuGT (12.47 ± 0.19%) showed an almost similar level of TPC compared to

Table 1 Total polyphenols, total catechin, total flavonoid and antioxidant activity using DPPH and ABTS in herbal teas

	Sample code	TPC (%)	TCC (%)	TFC (%)	DPPH (µg/ml)	ABTS (µg/ml)
Green herbal tea	GT	13.37 ± [a]0.50	15.17 ± 0.53	2.86 ± 0.21	27.58 ± 4.74	14.17 ± 4.09
	SGT	13.23 ± 0.10	12.19 ± 0.21	4.68 ± 0.26	28.86 ± 6.37	15.15 ± 1.46
	GGT	11.37 ± 0.38	12.76 ± 0.47	2.89 ± 0.17	128.36 ± 4.24	71.81 ± 4.00
	TiGT	10.89 ± 0.27	12.45 ± 0.38	2.73 ± 0.25	72.51 ± 3.63	40.88 ± 4.34
	TGT	11.93 ± 0.28	10.18 ± 0.29	2.90 ± 0.28	37.41 ± 2.62	17.03 ± 3.15
	TuGT	12.47 ± 0.19	10.65 ± 0.83	2.96 ± 0.13	35.38 ± 1.24	16.41 ± 3.61
	LGT	11.47 ± 0.25	14.76 ± 0.44	2.24 ± 0.27	65.5 ± 2.64	37.88 ± 7.42
	KGT	10.75 ± 0.14	9.30 ± 0.15	2.26 ± 0.18	67.3 ± 5.59	33.75 ± 2.83
	MGT	11.37 ± 0.47	14.57 ± 0.53	2.80 ± 0.11	48.27 ± 4.04	21.59 ± 2.61
Black herbal tea	BT	10.05 ± 0.11	12.25 ± 0.14	2.53 ± 0.01	39.3 ± 2.74	21.48 ± 1.65
	SBT	8.69 ± 0.33	12.21 ± 0.12	3.10 ± 0.52	50.95 ± 1.84	25.81 ± 2.99
	GBT	5.61 ± 0.37	10.83 ± 0.20	1.81 ± 0.67	108.4 ± 1.61	57.66 ± 0.62
	TiBT	6.16 ± 0.18	7.91 ± 0.76	2.71 ± 0.59	55.35 ± 5.52	33.21 ± 3.51
	TBT	8.28 ± 0.44	8.13 ± 1.02	3.24 ± 0.73	144.8 ± 3.46	74.17 ± 3.96
	TuBT	8.18 ± 0.24	10.74 ± 0.56	1.85 ± 1.16	56.48 ± 7.27	27.61 ± 5.47
	LBT	8.68 ± 0.56	9.51 ± 0.71	2.08 ± 0.97	86.82 ± 3.63	48.55 ± 3.97
	KBT	6.06 ± 0.68	10.12 ± 1.02	1.92 ± 0.96	98.43 ± 5.9	60.23 ± 3.63
	MBT	7.41 ± 0.29	8.62 ± 0.82	2.22 ± 0.65	85.46 ± 4.19	44.53 ± 4.48
Market herbal tea	RT	4.42 ± 0.53	9.59 ± 0.64	2.31 ± 0.21	226.28 ± 2.72	117.62 ± 7.59
	MT	6.78 ± 0.71	4.43 ± 0.28	1.82 ± 0.63	171.28 ± 8.37	76.71 ± 3.32
	TT	7.61 ± 0.36	4.46 ± 0.20	2.67 ± 0.34	145.65 ± 2.85	74.21 ± 5.46

[a] ± standard deviation

GT. In BHT highest polyphenol content was observed in BT (10.05 ± 0.11%) and lowest was observed in GBT (5.61 ± 0.37%). The findings of this study suggested that the highest polyphenol content was observed in GHT followed by BHT and the least was observed in MHT.

Total catechin content

Catechins is the class of flavonoid commonly known as flavan-3-ols. Total catechin content in all the samples was ranged from 4.43 ± 0.28 to 15.17 ± 0.53% (Table 1). In GHT, GT showed the higher catechins content (15.17 ± 0.53%) and KGT show the lowest catechin content (9.30 ± 0.15%). While in BHT, BT showed the higher catechins content (12.25 ± 0.14%) which was almost equal to SBT 12.21 ± 0.12% and lowest was observed in TiBT (7.91 ± 0.76%). In MHT, RT contain the highest (9.59 ± 0.64%) and MT show the lowest catechin content (4.43 ± 0.28%).

Total flavonoid content

The total flavonoid content was ranged from 1.81 ± 0.67 to 4.68 ± 0.26% in all the tea samples. In GHT flavonoid content was ranged from 2.24 ± 0.27 to 3.10 ± 0.52% and in BHT it was ranged from 1.81 ± 0.67 to 4.68 ± 0.26% (Table 1). In MHT flavonoid content was ranged from (1.82 ± 0.63–2.67 ± 0.34%). SGT showed the highest flavonoid content (4.68 ± 0.26%) compared to GT (2.86 ± 0.21%), while GBT showed almost similar and lower amount of flavonoids (1.81 ± 0.67–1.82 ± 0.63%). In MHT, TT contain the highest amount of total flavonoid content (2.67 ± 0.34%), while in MT, it was lowest (1.82 ± 0.63%).

HPLC analysis of catechins

Total catechins content was ranged from 8.10 to 22.69% in all the tea samples. The highest amount of catechins was determined in GT (22.69%) in GHT, while in BHT, TuBT showed the highest (20.84%) and in MHT, MT show the highest catechin content (11.12%). In GHT, EGCG (8.08 ± 0.25%) content was highest in GT and lower in SGT (4.18 ± 0.17%). While in BHT, BT contain high (7.24 ± 0.16%) and GBT (1.21 ± 0.17%) contain low amount of EGCG. In MHT, MT contain high amount 1.45 ± 0.34% and RT contain low EGCG 0.87 ± 0.12% Caffeine content was also found higher in GT (5.92 ± 0.15%) in GHT, while BT (5.81 ± 0.21%) contain the hight caffeine content in BHT. The variation in the catechin content was shown in the heatmap (Fig. 1A). From the heatmap, it was observed that the catechins content was higher in GHT compared to BHT and the least was present in MHT.

Amino acids analysis

Amino acid profile was studied in all the herbal teas using UPLC which shows the variations in their chemical constituents. A total of 14 amino acids were determined ranged from 0.82 to 2.86%. A higher amount of L-theanine was found in GT (1.24%) in GHT samples. Principal component analysis (PCA) was executed for the comparison and classification of amino acids in herbal tea samples (Fig. 1B). Amino acids content showed variations in all the herbal tea samples. By applying PCA, the two components were extracted which explained the variation of amino acid content in all the herbal tea samples. Figure 1B shows the score plot of herbal tea for the component 1 and 2. GT, SGT, GGT, TGT, BT, TuBT, LBT and KBT were observed on the positive side of component 1. Whereas at the upper side KGT, TGT, TuGT, LGT, KGT, MGT, SBT, GBT and TBT appeared on component 2. Amino acids such as L-theanine, histidine, glycine and phenylalanine show the positive side of components 1, which contribute to the tea quality. RT, MT and TT are correlated to the negative side of component 1. In case of component 2, asparagine, serine, histidine and L-theanine were present on the positive side which contributes

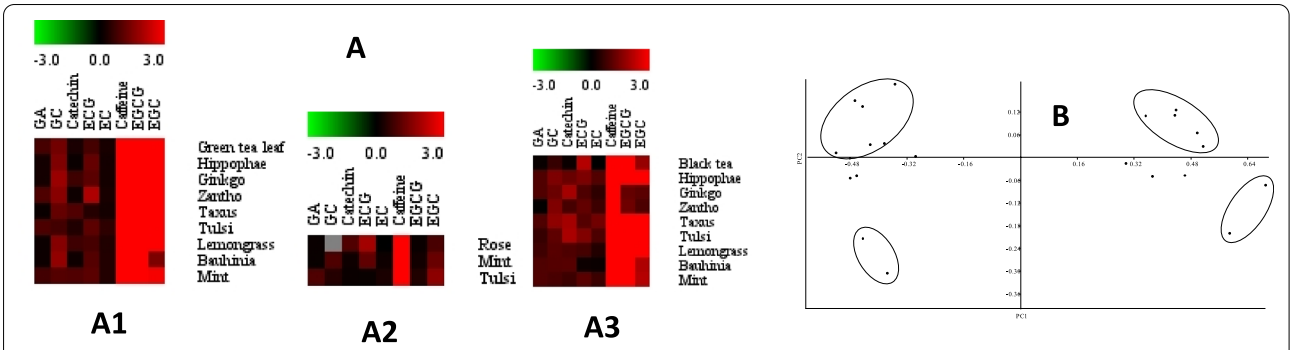


Fig. 1 Analysis of catechins and amino acids in teas and herbal teas. **A** Heat map of individual catechin profile (A1) Green herbal tea (A2) Market herbal tea (A3) Black herbal tea; **B** principal component analysis (PCA) of amino acids in all the tea samples

to the tea quality. Amino acids content shows the variation in a different class of herbal tea, the highest amount was identified in GHT followed by BHT and the least was observed in MHT.

Antioxidant activity

The antioxidant activity of herbal tea samples was studied using DPPH and ABTS assays (Table 1).

DPPH free radical-scavenging activity

The antioxidant activity of 21 teas was studied using DPPH free radical. Which was ranged from 27.58 ± 4.74 to 226.28 ± 2.72 µg/mL GAE. In the studied GHT, GT (27.58 ± 4.74 µg/mL) showed the highest, while GGT (128.36 ± 4.24 µg/mL) showed the lowest antioxidant activity. Some exception was observed in the SGT (28.86 ± 6.37 µg/mL) which shows approximately similar antioxidant activity to GT (Table 1). In BHT, DPPH free radical-scavenging activity was ranged from 39.3 ± 2.74 to 144.8 ± 3.46 µg/mL. While in MHT, TT show the highest 145.65 ± 2.85 µg/mL and RT show the lowest 226.28 ± 2.72 µg/mL antioxidant activity.

ABTS free radical-scavenging activity

Among all the tea samples, antioxidant activity was ranged from 14.17 ± 4.09 to 117.62 ± 7.59 µg/mL using ABTS free radical (Table 1). In GHT, ABTS free radical-scavenging activity was ranged from 14.17 ± 4.09 to 71.81 ± 4.00 µg/mL. While, in BHT it was ranged from 21.48 ± 1.65 to 74.17 ± 3.96 µg/mL and in MHT, it was ranged from 74.21 ± 5.46 to 76.71 ± 3.32 µg/mL. The efficacy of antioxidants mainly depends on the polyphenolic compounds present in tea infusions. The high antioxidant activity in GT is due to the presence of high content of polyphenols.

Correlation analysis

The correlation was observed in GHT, BHT and MHT between the TPC, TFC and antioxidant activity of DPPH and ABTS. The best correlation was observed in GHT between the DPPH and ABTS ($R^2 = 0.99$) (Fig. 2). In addition, correlation observed between TPC and TFC in GHT, BHT and MHT is positive ($R^2 = 0.77$, 0.89, 0.98), respectively. Moreover, the correlation between DPPH and ABTS was positive in GHT ($R^2 = 0.99$), BHT ($R^2 = 0.98$) and MHT ($R^2 = 0.96$).

Cytotoxic activity by SRB assay

Cytotoxic potential of tea samples was tested on three different cell lines, such as SW480, A549 and SiHa. The results revealed that TGT showed promising activity against SW480 cells (50.9 ± 0.7 at 200 µg/mL). Herbal tea samples exhibited remarkable cytotoxic potential against A549 cells in the SGT (87.01 ± 1.1 at 200 µg/mL), respectively. Whereas, TT did not show a considerable effect on A549 cells. However, herbal tea samples exhibited the highest activity against SiHa cells in the LGT (67.1 ± 0.4 at 200 µg/mL), respectively. Therefore, the tested sample of herbal tea showed dose-dependent

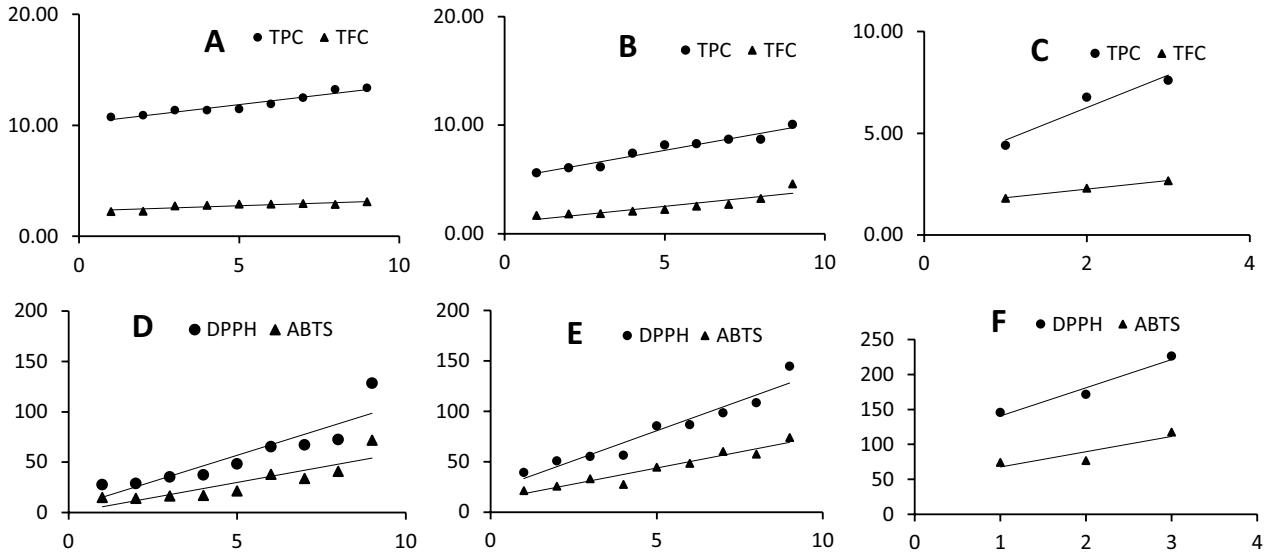


Fig. 2 Correlation analysis of different teas. **A** green herbal tea; **B** black herbal tea; **C** market herbal tea in the total polyphenol content and total flavonoid content; **D** green herbal tea; **E** black herbal tea; **F** market herbal tea in the DPPH and ABTS

activity against all the cells, except RT and TT on SW480 cells.

Volatile organic compound analysis

Herbal tea samples with the total composition ranged from 62.03 to 96.23%. Qualitative and quantitative analysis on VOCs in herbal teas were carried out. The VOCs were extracted using Liken–Nickerson apparatus and analysed by GC and GC–MS. A total of nine different classes of compounds were identified and quantified in herbal tea samples viz, aldehydes, ketones, alcohols, nitrogenous compounds, hydrocarbons, acids, esters and other compounds. Among all the classes of VOCs alcohols and aldehydes were the most important class. A total of 188 VOCs were characterized, the most commonly identified compounds were 2-hexenal, L-linalool, hotrienol and α-terpineol. Some substantial variations in the volatile profile of GHT, BHT and MHT were observed. The BHT contained the commonly present compound viz, 2-hexenal, geraniol, 2-pentanol. The variation in the volatile profile could be due to the processing of tea shoots. The highest number of VOCs were identified in LGT (96.23%) and the lowest was observed in TBT (62.03%). The unique aroma compounds identified in LGT are octenal (0.19), *trans*-chrysanthemal (1.68), levoverbenone (8.57), pulegone (5.03), borneol (0.56), citral (0.92), carane (11.22), *dl*-limonene (2.05), α-terpinolene (0.39), cyclopentene (0.21), cineole (0.56) and 6-methyl-6 heptone-2-one (6.95). It was observed that the BHT contains a large number of VOCs compared to GHT and MHT. In addition, ZGT contains unique aroma compounds, such as cryptone (0.19), β-phellandrene (2.84), limonene (1.01) and β-ocimene (0.18). The least number of VOCs were identified in TGT and TBT. This could be due to the presence of very complex molecules in taxus plant which cannot be detected, contain unique VOCs, such as 2-pentadecanone (0.31), neophytadiene (1.08), heptadecyl trifluoroacetate (1.93), di (2-ethylhexyl) adipate (0.33) and *O*,*O*′-biphenol, 4,4′-difluoro (0.96). MGT comprised of (*E*)-*p*-2,8-menthadien-1-ol (0.29), β-terpineol (0.96), menthol (9.69), thymol (0.36), 1-octadecene (0.23), methyl acetate (0.68), 2,5-dimethyl tetrahydrofuran (0.29), *cis*-2-cyclohexene-1-ol-1-methyl-4 (1-methylethyl) (1.52) and menthacamphor (5.81). The unique VOCs with sweet taste were observed in LBT, MBT, TuBT, LGT, MGT and TuGT. Therefore, these teas can be used as beverages with unique VOCs.

Aroma extract dilution analysis (AEDA)

Volatile organic compounds have a great influence on the flavour quality even when present in small amount which are due to their low threshold value. The odour threshold play an significant role for the determination of VOCs. These VOCs are present in very small amount and show a great influence on the quality.

A total of 90 odour active compounds were identified using AEDA in herbal tea samples (Tables 2, 3, 4). The commonly identified class of compounds are aldehydes and alcohols. AEDA was performed and compared between GHT, BHT and MHT which shows the variations in their volatile profile. *cis*-Linalool oxide, 1-dodecene, methyl salicylate and epoxylinalool show the sweet floral note in GT. Geranial (fresh, lemon-like), geranylacetone (faint woody floral), *cis*-linalool oxide (fruity), *trans*-linalool oxide (fruity and fresh), L-linalool (floral, fruity) and nerolidol (floral green) are the odour active compounds were identified in BT. While, LGT show the neral (lemon-like), geranial (fresh, lemon-like), levoverbenone (spicy, mint, camphor), pulegone (peppermint camphor fresh herbal), *trans*-linalool oxide (fruity and fresh), L-linalool (floral, fruity) and cineole (minty) odour active compounds. The most important aroma contributors present in MBT is the 2,4-heptadienal (green fruity), levoverbenone (spicy, mint, camphor), gernylacetone (faint woody floral), cineole (minty), menthol (minty) and citronella (floral, citrus). It was noticed that herbal tea samples such as LGT, MGT, TuGT, RT, GT and BT produce floral, fruity, green and sweet floral note. Fatty and roasted note were highly observed in TGT.

Sensory analysis

Sensory analysis was performed by the tea tasting panel. Six sensory terms were taken, including leaf appearance, infusion colour, taste, aroma, infused leaves and total score (Table 5). Sensory analysis was performed for all the herbal tea samples. The colour palate of the infusion of GHT showed a light brown colour with a greenish tint. This may be likely due to the presence of catechins which was extracted into tea infusions with a light green–brown colour. TQS for aroma, infusion colour, appearance, taste and infused leaves were observed by the tea-tasting panel were observed from 37 to 87 and averaged 65.85 (Table 5). The highest TQS was found in LGT (87) followed by TuGT (86) then TiGT (85) and least were observed in TBT (37). LGT infusion shows the sensory analysis for leaf appearance (15), infusion colour (8), taste (28), aroma (29) and infused leaves (7). Tasters showed blended flavours, with unique mouthfeel and sweet aftertaste for LGT, MGT and TuGT (Fig. 3). The astringency and bitter taste were observed in a large number of teas, which include GT. This is due to the presence of catechins in green tea and theaflavins in black tea. The bitter and astringent attributes are decreased in the herbal teas; therefore, these herbal teas are accepted by the consumer

Table 2 Potent aroma active compounds (FD ≥ 2) in green herbal teas using AEDA analysis

Compounds[a]	Odour perception[b]	Green herbal teas									
		RRI	GT	SGT	GGT	TiGT	TGT	TuGT	LGT	KGT	MGT
2-Pentenal	Sweet grassy	nd[c]	2	nd	4	nd	4	4	4	2	8
n-Hexanal	Green	nd	nd	4	2	nd	nd	16	8	16	256
3-Hexenal	Freshly cut grass	nd	nd	nd	16	nd	nd	64	nd	nd	16
2-Hexenal	Grassy green	nd	2	16	32	nd	nd	2	32	32	4
Tridecane	Hydrocarbon-like	nd	nd	nd	nd	nd	nd	nd	nd	4	nd
2-Hexenol	Sweet floral	861	32	16	nd	nd	nd	nd	nd	nd	nd
3-Hexenol	Floral	869	64	2	nd	nd	16	4	nd	128	2
n-Heptanal	Fatty green	916	nd	8	nd	nd	nd	nd	nd	nd	32
Ethylpyrazine	Nutty and roasted	920	4	nd	nd	nd	nd	nd	nd	nd	8
α-Pinene	Woody	939	nd	nd	nd	2	nd	256	16	16	nd
Isovaleraldehyde	Chocolate	961	nd	nd	nd	nd	nd	nd	nd	4	nd
Benzaldehyde	Green	967	nd	2	nd	nd	nd	nd	nd	128	128
Hexanoic acid	Sour fatty sweat cheese	971	nd	nd	512	nd	nd	256	nd	1024	32
Sabinene	Warm, oily-peppery	974	nd	nd	nd	4	nd	nd	nd	nd	nd
3-Octenol	Green, meaty	976	nd	nd	nd	nd	nd	nd	nd	nd	8
2,4-Heptadienal	Green fruity	989	4	16	128	nd	8	nd	nd	256	nd
β-Myrcene	Metallic	993	nd	nd	nd	16	nd	16	nd	256	256
6-Methyl-5-hepten-2-one	Citrus, musty, grass, lemon	1002	32	nd	nd	nd	nd	nd	32	nd	256
Octanal	Citrusy	1022	nd	nd	nd	nd	nd	nd	nd	nd	512
Cineole	Minty	1035	nd	nd	nd	nd	nd	nd	64	nd	nd
(*E*)- *β* ocimene	Citrusy green woody	1038	nd	nd	nd	nd	nd	2	128	nd	nd
Benzyl alcohol	Floral balsamic	1039	nd	4	nd	nd	nd	nd	64	256	nd
Benzeneacetaldehyde	Green	1042	nd	32	nd	nd	32	nd	nd	32	512
Limonene	Citrus-like, fresh	1049	nd	nd	nd	nd	nd	16	nd	nd	nd
Phenyl acetaldehyde	Floral Honey	1051	nd	nd	512	nd	nd	nd	nd	nd	1024
cis-Linalool oxide	Fruity	1074	512	32	4	32	nd	16	nd	8	16
4-Thujanol	Woody balsam	1075	nd	nd	nd	2	nd	nd	nd	nd	nd
trans-Linalool oxide	Fruity and fresh	1093	16	128	16	8	nd	8	128	nd	64
Rosefuran	Caramel green minty	1096	nd	nd	nd	nd	nd	nd	8	nd	nd
L-Linalool	Floral, fruity	1098	8	8	32	16	nd	2	512	2	32
Hotrienol	Ginger-like	1108	nd	256	128	2	nd	nd	8	64	2
Phenylethanol	Floral, rose-like	1119	2	nd	256	nd	nd	nd	4	nd	nd
1,6-Octadien-3-ol	Fresh floral woody	1136	nd	nd	512	nd	nd	32	nd	nd	nd
Ocimenol	Fresh citrus, sweet	1158	nd	nd	nd	nd	nd	nd	nd	256	nd
Menthol	Minty	1159	nd	nd	nd	nd	nd	nd	nd	nd	2
Rose furan epoxide	Green earthy citrus	1169	nd	nd	nd	nd	nd	nd	4	nd	nd
Methyl salicylate	Wintergreen-like	1175	128	64	nd	nd	128	16	nd	4	nd
1-Dodecene	Hydrocarbon-like	1186	128	16	nd	nd	nd	nd	nd	32	nd
cis-Hexenyl butyrate	Fresh, green apple, fruity	1192	nd	nd	nd	nd	nd	8	nd	nd	4
α-Terpineol	Floral and sweet	1198	64	8	1024	nd	nd	4	8	8	4
Safranal	Sweet green, floral	1201	6	256	nd	nd	16	nd	nd	nd	2
Levoverbenone	Spicy, mint, camphor note	1208	nd	nd	nd	nd	nd	nd	32	4	nd
trans-Geraniol	Sweet rose odor	1216	nd	32	4	nd	32	nd	nd	nd	64
Nerol	Floral	1226	32	4	8	nd	16	nd	nd	16	8
β-Citronellol + nerol	Clean, rose-like	1229	nd	nd	nd	nd	nd	nd	16	nd	nd
Neral	Lemon-like	1239	nd	nd	nd	nd	nd	nd	512	nd	16
Pulegone	Peppermint camphor fresh herbal	1241	nd	nd	nd	nd	nd	nd	8	nd	32
Geranial	Fresh, lemon-like	1246	nd	nd	nd	nd	nd	nd	256	4	nd

Table 2 (continued)

Compounds[a]	Odour perception[b]	Green herbal teas									
		RRI	GT	SGT	GGT	TiGT	TGT	TuGT	LGT	KGT	MGT
Linalyl acetate	Floral, sweet and citric	1256	nd	nd	nd	nd	nd	nd	nd	16	nd
Thymol	Sweet phenolic–herbal–medicinal	1263	nd	nd	nd	nd	nd	nd	nd	nd	512
Citral (Z)	Strong lemon	1270	nd	512	2	nd	nd	nd	nd	32	4
Geraniol	Sweet floral	1276	2	nd	nd	nd	8		128	64	256
Nerol acetate	Floral	1284	nd	2	nd	nd	nd	nd	nd	4	nd
2-Undecanone	Floral and fatty	1298	nd	nd	4	nd	nd	nd	nd	nd	nd
Menthyl acetate	Peppermint	1305	nd	nd	nd	nd	nd	nd	nd	nd	32
Neryl acetate	Floral	1354	nd	nd	nd	nd	nd	nd	nd	64	nd
cis-Isoeugenol	Spice-clove odor	1366	nd	2	nd	nd	nd	16	nd	nd	nd
α-Ionone	Warm woody	1398	2	2	nd	nd	4	nd	nd	2	64
Epoxylinalool	Sweet woody	1402	512	nd	nd	nd	nd	nd	nd	nd	128
E-Caryophyllene	Spicy, woody and terpenic	1413	nd	8	nd	8	nd	8	nd	nd	nd
β-Ionone	Dry, floral–fruity	1415	4	nd	8	nd	nd	nd	nd	nd	256
β-Caryophyllene	Spicy and peppery	1429	nd	nd	nd	nd	nd	nd	nd	64	nd
Elemene	Herbal	1445	nd	nd	nd	nd	nd	4	nd	nd	nd
α-Humulene	Earthy, woody, and spicy	1454	nd	nd	nd	nd	4	nd	nd	2	nd
(*E*)-*β*-Farnesene	Woody type odor	1456	nd	nd	nd	nd	nd	32	nd	nd	nd
Gernylacetone	Faint woody floral	1458	8	nd	nd	nd	8	nd	nd	16	1024
Caryophyllene oxide	Sweet fruity	1465	nd	nd	8	4	nd	nd	nd	nd	nd
Germacrene *D*	Woody spice	1490	nd	64	nd	16	nd	8	nd	nd	nd
Dihydroactinidiolide	Roasted	1491	16	nd	nd	nd	nd	nd	nd	nd	nd
Bicyclogermacrene	Green woody weedy	1494	nd	nd	nd	64	nd	nd	nd	512	nd
β-Bisabolene	Herbal type	1507	nd	nd	nd	nd	16	nd	nd	nd	nd
Cyclopentene	Spicy	1509	nd	nd	nd	nd	nd	nd	4	256	nd
Geranyl buytrate	Fruity	1547	nd	nd	nd	nd	256	4	nd	nd	64
Nerolidol	Floral green	1562	16	64	16	64	512	nd	nd	256	nd
δ-Cadinene	Thyme herbal woody	1574	nd	nd	nd	256	nd	256	nd	1024	nd
α-Cadinol	Herb wood	1653	nd	nd	nd	nd	nd	nd	nd	512	nd
Heptadecene	Hydrocarbon-like	1800	nd	nd	2	nd	nd	4	8	8	4
Hexadecanal	Green fatty grassy	1832	nd	nd	nd	nd	nd	nd	nd	nd	16
Isopropyl myristate	Faint oily fatty	1847	nd	nd	nd	nd	nd	nd	256	8	nd
Nonadecane	Hydrocarbon-like	1904	nd	nd	32	nd	nd	64	32	nd	nd
Tricosane	Waxy	1920	nd	nd	64	nd	nd	nd	nd	32	nd
Palmitic acid	Waxy	1968	nd	8	256	nd	nd	512	nd	nd	4
Oleic acid	Fresh cut grass-like	2091	4	nd	nd	nd	nd	nd	nd	nd	8
Phytol	Floral green	2105	nd	nd	32	nd	nd	2	nd	nd	4
Linoleic acid	Green	2159	nd	16	nd	nd	nd	nd	8	nd	nd
Eicosane	Hydrocarbon-like	2188	nd	nd	128	nd	4	128	nd	16	8
9-Tricosene	Fatty waxy	2276	nd	nd	nd	nd	nd	nd	nd	nd	16
Hexadecane	Weak waxy	2321	nd	nd	nd	nd	2	nd	nd	128	nd
1-Heneicosanol	Pungent, etherial, fuel oil, fruity and alcoholic, sweet	2345	nd	nd	128	nd	nd	8	nd	nd	nd
2-Tridecanone	Slightly spicy	2546	nd	nd	64	nd	nd	256	nd	nd	nd

[a] The compound was identified by comparing its retention time and mass spectrum with the reference standard

[b] Odour quality perceived through the previously published literature [29]

[c] Not detected

Table 3 Potent aroma active compounds (FD ≥ 2) in black herbal teas using AEDA analysis

Compounds[a]	Odour perception[b]	Black herbal tea									
		RRI	BT	SBT	GBT	TiBT	TBT	TuBT	LBT	KBT	MBT
2-Pentenal	Sweet grassy	nd[c]	4	2	nd	2	nd	16	8	2	2
n-Hexanal	Green	nd	nd	32	16	nd	nd	2	nd	4	8
3-Hexenal	Freshly cut grass	nd	nd	16	nd	nd	nd	nd	nd	nd	16
2-Hexenal	Grassy green	nd	8	8	8	16	nd	4	1024	16	64
Tridecane	Hydrocarbon-like	nd	nd	4	nd	nd	4	1024	nd	128	8
2-Hexenol	Sweet floral	861	32	512	nd	nd	nd	16	16	nd	nd
3-Hexenol	Floral	869	64	2	256	nd	16	32	nd	2	nd
n-Heptanal	Fatty green	916	nd	2	nd	nd	nd	64	16	nd	nd
Ethylpyrazine	Nutty and roasted	920	8	8	nd	nd	nd	512	nd	nd	nd
α-Pinene	Woody	939	nd	nd	nd	nd	nd	4	nd	64	nd
Isovaleraldehyde	Chocolate	961	nd	nd	nd	nd	nd		nd	64	nd
Benzaldehyde	Green	967	32	8	nd	nd	nd	32	64	512	4
Hexanoic acid	Sour fatty sweat cheese	971	nd	2	nd	nd	nd	16	nd	2	16
Sabinene	Warm, oily-peppery	974	nd	nd	nd	4	nd	nd	nd	nd	nd
3-Octenol	Green, meaty	976	nd	nd	nd	nd	2	nd	nd	nd	nd
2,4-Heptadienal	Green fruity	989	nd	nd	nd	64	nd	128	nd	8	128
β-Myrcene	Metallic	993	nd	8	nd	nd	nd	8	nd	512	nd
6-Methyl-5-hepten-2-one	Citrus, musty, grass, lemon	1002	8	nd	nd	nd	nd	2	nd	nd	nd
Octanal	Citrusy	1022	nd	512	nd	nd	nd	nd	8	nd	nd
Cineole	Minty	1035	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*)-*β* ocimene	Citrusy green woody	1038	nd	nd	nd	nd	nd	32	nd	nd	nd
Benzyl alcohol	Floral balsamic	1039	nd	4	1024	nd	nd	64	nd	4	nd
Benzeneacetaldehyde	Green	1042	nd	4	nd	nd	nd	32	nd	32	nd
Limonene	Citrus-like, fresh	1049	16	nd	nd	nd	nd	256	8	nd	nd
Phenyl acetaldehyde	Floral Honey	1051	nd	16	nd	nd	nd	256	nd	nd	8
cis-Dodecene oxide	Fruity	1074	128	32	4	2	nd	128	64	16	16
4-Thujanol	Woody balsam	1075	nd	nd	nd	16	nd	nd	nd	nd	nd
trans-Linalool oxide	Fruity and fresh	1093	256	4	2	8	nd	4	nd	8	8
Rosefuran	Caramel green minty	1096	nd	nd	nd	nd	nd	nd	nd	nd	nd
L-Linalool	Floral, fruity	1098	512	64	8	34	nd	2	nd	512	4
Hotrienol	Ginger-like	1108	nd	8	nd	32	nd	nd	nd	nd	nd
Phenylethanol	Floral, rose-like	1119	16	512	512	nd	nd	nd	nd	nd	nd
1,6-Octadien-3-ol	Fresh floral woody	1136	nd	1024	32	nd	nd	128	nd	nd	nd
Ocimenol	Fresh citrus, sweet	1158	nd	nd	nd	nd	nd	nd	nd	128	nd
Menthol	Minty	1159	nd	nd	nd	nd	nd	nd	nd	nd	128
Rose furan epoxide	Green earthy citrus	1169	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl salicylate	Wintergreen-like	1175	nd	2	nd	nd	4	16	nd	4	nd
1-Dodecene	Hydrocarbon-like	1186	nd	2	64	nd	nd	nd	nd	1024	64
cis-Hexenyl butyrate	Fresh, green apple, fruity	1192	nd	8	nd	nd	nd	4	nd	nd	128
α Terpineol	Floral and sweet	1198	4	4	8	4	nd	nd	nd	512	4
Safranal	Sweet green, floral	1201	16	nd	nd	8	nd	nd	512	nd	nd
Levoverbenone	Spicy, mint, camphor note	1208	nd	nd	nd	nd	nd	nd	nd	16	4
trans-Geraniol	Sweet rose odor	1216	nd	16	nd	nd	2	2	nd	nd	16
Nerol	Floral	1226	nd	2	nd	16	nd	8	nd	1024	nd
β-Citronellol + nerol	Clean, rose-like	1229	nd	nd	nd	nd	nd	nd	nd	nd	nd
Neral	Lemon-like	1239	64	nd	nd	nd	nd	nd	nd	nd	nd
Pulegone	Peppermint camphor fresh herbal	1241	nd	nd	nd	nd	nd	nd	nd	nd	nd
Geranial	Fresh, lemon-like	1246	512	nd	nd	nd	nd	nd	nd	4	nd

Table 3 (continued)

Compounds[a]	Odour perception[b]	Black herbal tea									
		RRI	BT	SBT	GBT	TiBT	TBT	TuBT	LBT	KBT	MBT
Linalyl acetate	Floral, sweet and citric	1256	nd	nd	nd	nd	nd	8	nd	16	nd
Thymol	Sweet phenolic–herbal–medicinal	1263	nd	nd	nd	nd	nd	nd	nd	nd	nd
Citral (*Z*)	Strong lemon	1270	nd	34	nd	nd	nd	512	nd	32	nd
Geraniol	Sweet floral	1276	8	32	4	nd	nd	nd	nd	nd	256
Nerol acetate	Floral	1284	nd	nd	256	nd	nd	nd	nd	nd	nd
2-Undecanone	Floral and fatty	1298	nd	4	8	nd	4	nd	nd	128	2
Menthyl acetate	Peppermint	1305	nd	nd	nd	nd	nd	nd	nd	8	nd
Neryl acetate	Floral	1354	nd	16	nd	nd	nd	64	nd	16	nd
cis-isoeugenol	Spice-clove odor	1366	nd	nd	nd	nd	nd	16	nd	nd	nd
α Ionone	Warm woody	1398	nd	32	nd	2	nd	nd	2	nd	nd
Epoxylinalool	Sweet woody	1402	64	8	nd	nd	nd	nd	1024	nd	nd
E-caryophyllene	Spicy, woody and terpenic	1413	nd	nd	nd	nd	nd	2	nd	nd	nd
β Ionone	Dry, floral–fruity	1415	8	16	16	nd	nd	4	nd	nd	64
β-Caryophyllene	Spicy and peppery	1429	nd	nd	nd	16	256	nd	nd	8	nd
Elemene	Herbal	1445	nd	nd	nd	nd	nd	128	nd	32	nd
α-Humulene	Earthy, woody, and spicy	1454	nd	nd	nd	64	16	nd	nd	64	nd
(*E*)-*β*-Farnesene	Woody type odor	1456	nd	nd	nd	256	nd	512	nd	nd	nd
Gernylacetone	Faint woody floral	1458	128	128	32	128	nd	8	32	512	128
Caryophyllene oxide	Sweet fruity	1465	nd	nd	16	64	nd	nd	nd	nd	2
Germacrene *D*	Woody spice	1490	nd	nd	nd	nd	nd	1024	nd	2	nd
Dihydroactinidiolide	Roasted	1491	nd	256	nd	nd	nd	nd	8	nd	32
Bicyclogermacrene	Green woody weedy	1494	nd	nd	nd	nd	nd	nd	nd	nd	nd
β-Bisabolene	Herbal type	1507	nd	nd	nd	nd	nd	nd	nd	128	nd
Cyclopentene	Spicy	1509	nd	2	nd	nd	nd	nd	nd	nd	nd
Geranyl buytrate	Fruity	1547	nd	nd	nd	nd	nd	256	nd	nd	nd
Nerolidol	Floral green	1562	512	32	256	nd	4	128	nd	2	512
δ-Cadinene	Thyme herbal woody	1574	nd	nd	nd	nd	nd	4	nd	512	nd
α-Cadinol	Herb wood	1653	nd	nd	nd	nd	nd	nd	nd	nd	nd
Heptadecene	Hydrocarbon-like	1800	nd	3	8	nd	nd	2	nd	256	nd
Hexadecanal	Green fatty grassy	1832	nd	2	nd	nd	nd	nd	nd	nd	16
Isopropyl myristate	Faint oily fatty	1847	2	nd	nd	nd	nd	nd	128	nd	nd
Nonadecane	Hydrocarbon-like	1904	nd	nd	nd	nd	nd	32	nd	nd	nd
Tricosane	Waxy	1920	nd	32	16	nd	64	nd	nd	4	nd
Palmitic acid	Waxy	1968	nd	8	nd	nd	nd	256	nd	16	8
Oleic acid	Fresh cut grass-like	2091	16	64	nd	nd	nd	1024	256	nd	64
Phytol	Floral green	2105	4	4	nd	nd	8	nd	nd	256	nd
Linoleic acid	Green	2159	4	4	nd	nd	nd	nd	nd	nd	nd
Eicosane	Hydrocarbon-like	2188	nd	nd	nd	nd	8	nd	nd	8	nd
9-Tricosene	Fatty waxy	2276	nd	nd	64	nd	nd	nd	nd	nd	nd
Hexadecane	Weak waxy	2321	nd	16	128	nd	nd	nd	nd	nd	2
1-Heneicosanol	Pungent, etherial, fuel oil, fruity and alcoholic, sweet	2345	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Tridecanone	Slightly spicy	2546	nd	1024	128	nd	nd	nd	nd	nd	256

[a] The compound was identified by comparing its retention time and mass spectrum with the reference standard

[b] Odour quality perceived through the previously published literature [29]

[c] not detected

Table 4 Potent aroma active compounds (FD ≥ 2) in market herbal teas using AEDA analysis

Compounds[a]	Odour perception[b]	Market herbal teas			
		RRI	RT	MT	TT
2-Pentenal	Sweet grassy	nd[c]	4	4	4
n-Hexanal	Green	nd	2	2	2
3-Hexenal	Freshly cut grass	nd	nd	64	256
2-Hexenal	Grassy green	nd	64	16	32
Tridecane	Hydrocarbon-like	nd	nd	32	64
2-Hexenol	Sweet floral	861	nd	256	8
3-Hexenol	Floral	869	16	512	16
n-Heptanal	Fatty green	916	nd	32	8
Ethylpyrazine	Nutty and roasted	920	nd	2	4
α-Pinene	Woody	939	64	16	256
Isovaleraldehyde	Chocolate	961	nd	nd	nd
Benzaldehyde	Green	967	nd	128	8
Hexanoic acid	Sour fatty sweat cheese	971	nd	16	256
Sabinene	Warm, oily-peppery	974	nd	nd	nd
3-Octenol	Green, meaty	976	nd	2	128
2,4-Heptadienal	Green fruity	989	nd	256	32
β-Myrcene	Metallic	993	16	64	128
6-Methyl-5-hepten-2-one	Citrus, musty, grass, Lemon	1002	nd	32	nd
Octanal	Citrusy	1022	nd	nd	32
Cineole	Minty	1035	nd	nd	nd
(E)-*β* ocimene	Citrusy green woody	1038	nd	128	512
Benzyl alcohol	Floral balsamic	1039	nd	32	nd
Benzeneacetaldehyde	Green	1042	nd	512	16
Limonene	Citrus-like, fresh	1049	nd	1024	nd
Phenyl acetaldehyde	Floral Honey	1051	nd	1024	2
cis-Linalool oxide	Fruity	1074	8	1024	2
4-Thujanol	Woody balsam	1075	nd	nd	nd
trans-Linalool oxide	Fruity and fresh	1093	4	16	8
Rosefuran	Caramel green minty	1096	nd	nd	nd
L-Linalool	Floral, fruity	1098	2	2	16
Hotrienol	Ginger-like	1108	nd	8	32
Phenylethanol	Floral, rose-like	1119	nd	16	256
1,6-Octadien-3-ol	Fresh floral woody	1136	nd	nd	nd
Ocimenol	Fresh citrus, sweet	1158	nd	nd	nd
Menthol	Minty	1159	nd	1024	nd
Rose furan epoxide	Green earthy citrus	1169	nd	nd	nd
Methyl salicylate	Wintergreen-like	1175	512	nd	nd
1-Dodecene	Hydrocarbon-like	1186	nd	2	1024
cis-Hexenyl butyrate	Fresh, green apple, fruity	1192	1024	16	4
α Terpineol	Floral and sweet	1198	nd	4	4
Safranal	Sweet green, floral	1201	nd	nd	4
Levoverbenone	Spicy, mint, camphor note	1208	nd	nd	nd
trans-Geraniol	Sweet rose odor	1216	nd	nd	2
Nerol	Floral	1226	nd	2	64
β-Citronellol + nerol	Clean, rose-like	1229	256	nd	nd
Neral	Lemon-like	1239	nd	nd	nd
Pulegone	Peppermint camphor fresh herbal	1241	nd	nd	nd
Geranial	Fresh, lemon-like	1246	nd	nd	nd

Table 4 (continued)

Compounds[a]	Odour perception[b]	Market herbal teas			
		RRI	RT	MT	TT
Linalyl acetate	Floral, sweet and citric	1256	nd	64	16
Thymol	Sweet phenolic–herbal–medicinal	1263	nd	16	nd
Citral (*Z*)	Strong lemon	1270	256	4	128
Geraniol	Sweet floral	1276	nd	4	512
Nerol acetate	Floral	1284	nd	2	8
2-Undecanone	Floral and fatty	1298	nd	4	4
Menthyl acetate	Peppermint	1305	nd	8	nd
Neryl acetate	Floral	1354	nd	128	256
cis-isoeugenol	Spice-clove odor	1366	nd	2	1024
α Ionone	Warm woody	1398	nd	8	2
Epoxylinalool	Sweet woody	1402	nd	8	nd
E-caryophyllene	Spicy, woody and terpenic	1413	nd	nd	nd
β Ionone	Dry, floral–fruity	1415	32	2	16
β-Caryophyllene	Spicy and peppery	1429	nd	nd	nd
Elemene	Herbal	1445	nd	2	4
α-Humulene	Earthy, woody, and spicy	1454	8	nd	nd
(*E*)-*β*-Farnesene	Woody type odor	1456	nd	8	256
Gernylacetone	Faint woody floral	1458	nd	64	64
Caryophyllene oxide	Sweet fruity	1465	nd	nd	nd
Germacrene *D*	Woody spice	1490	4	32	2
Dihydroactinidiolide	Roasted	1491	nd	16	32
Bicyclogermacrene	Green woody weedy	1494	nd	nd	nd
β-Bisabolene	Herbal type	1507	nd	nd	nd
Cyclopentene	Spicy	1509	nd	nd	nd
Geranyl buytrate	Fruity	1547	nd	512	nd
Nerolidol	Floral green	1562	nd	16	16
δ-Cadinene	Thyme herbal woody	1574	nd	64	16
α-Cadinol	Herb wood	1653	nd	nd	nd
Heptadecene	Hydrocarbon-like	1800	nd	nd	64
Hexadecanal	Green fatty grassy	1832	nd	nd	256
Isopropyl myristate	Faint oily fatty	1847	256	256	256
Nonadecane	Hydrocarbon-like	1904	8	nd	32
Tricosane	Waxy	1920	16	256	nd
Palmitic acid	Waxy	1968	nd	2	4
Oleic acid	Fresh cut grass-like	2091	nd	nd	nd
Phytol	Floral green	2105	nd	4	8
Linoleic acid	Green	2159	nd	4	1024
Eicosane	Hydrocarbon-like	2188	4	512	8
9-Tricosene	Fatty waxy	2276	nd	nd	2
Hexadecane	Weak waxy	2321	64	4	16
1-Heneicosanol	Pungent, etherial, fuel oil, fruity and alcoholic, sweet	2345	nd	nd	16
2-Tridecanone	Slightly spicy	2546	64	nd	2

[a] The compound was identified by comparing its retention time and mass spectrum with the reference standard

[b] Odour quality perceived through the previously published literature [29]

[c] not detected

Table 5 Sensory analysis of green herbal teas, black herbal teas and market herbal teas

S. no.	Sample type	Leaf appearance[a] (0–20)	Infusion colour (0–10)	Taste (0–30)	Aroma (0–30)	Infused leaves[b] (0–10)	Total score (0–100)
Green herbal tea	GT	16	7	24	24	8	79
	SGT	14	7	26	23	6	76
	GGT	18	9	24	26	7	84
	TiGT	17	8	27	25	8	85
	TGT	13	7	27	24	7	78
	TuGT	17	8	26	26	9	86
	LGT	15	8	28	29	7	87
	KGT	15	3	25	29	8	80
	MGT	14	4	24	27	7	76
Black herbal tea	BT	12	5	24	17	6	64
	SBT	9	4	16	16	5	50
	GBT	11	4	21	15	5	56
	TiBT	12	3	19	13	6	53
	TBT	7	2	11	13	4	37
	TuBT	8	3	21	21	6	59
	LBT	7	2	18	18	5	50
	KBT	13	6	13	18	6	56
	MBT	12	5	26	18	6	67
Market herbal tea	RT	8	2	15	12	4	41
	MT	14	6	18	21	7	66
	TT	9	4	16	17	7	53

[a] Including uniformity of colour, shape, cleanliness in twist of leaf tea

[b] Features of post-infused leaf based mainly on bud and leaves proportions and appearance and cleanliness

with better aroma and taste. The aromatic compounds of LGT, MGT, TuGT and TiGT dominate the characteristic compounds of GT and BT. The mild flavour was observed in the KGT, GGT, SBT, GBT and KBT. Herbal tea is accepted by all the tea tasting panels showing a unique and sweet taste.

Discussion

Polyphenols are the major class of chemical compounds having one or more hydroxyl groups attached to an aromatic ring contribute to its antioxidant activity [37]. Herbal tea contains a wide variety of polyphenol content. Therefore, it is essential to determine the TPC in all the herbal tea samples. The highest amount of polyphenols was observed in GT which is due to the presence of flavan-3-ols, such as C, EGCG, ECG, EGC and EC. The findings of the study are consistent with the previous study which showed the highest amount of polyphenols was observed in GT among the various herbal tea samples [38]. The differences in the polyphenol content are due to the various factor, such as species, plant material, soil and climate conditions that affect the phytochemical constituents (Additional file 1) [39].

Flavonoids are the major class of phenolic compounds with the basic moiety of C_6–C_3–C_6 carbon skeleton bearing the hydroxyl group [40]. The flavonoids present in tea and herbal tea play a significant role in health beneficial properties. The findings of this study showed a significant difference in the total flavonoid content among all herbal teas. The highest quantity of flavonoids was observed in SGT that is due to the presence of its major chemical constituents, such as isorhamnetin, quercetin and kaempferol glycosides [41]. In the previous literature, *Hippophae rhamnoides* showed the various biological activities, such as anticancer, coronary heart disease, and abdominal pain [42] which suggest that SGT show a beneficial therapeutic potential in the health care and nutraceutical industries.

Catechins belongs to the flavonoid family are comprised of EGC, EC, EGCG and C [43]. The individual catechin profile was previously determined on HPLC revealed that the higher content of EGCG and ECG in the green tea samples [44]. The high amount of catechins was determined in GT which is due to the presence of catechins, such as EC, EGCG, ECG, EGC, C and GC. The low catechin content in BHT is due to the manufacturing

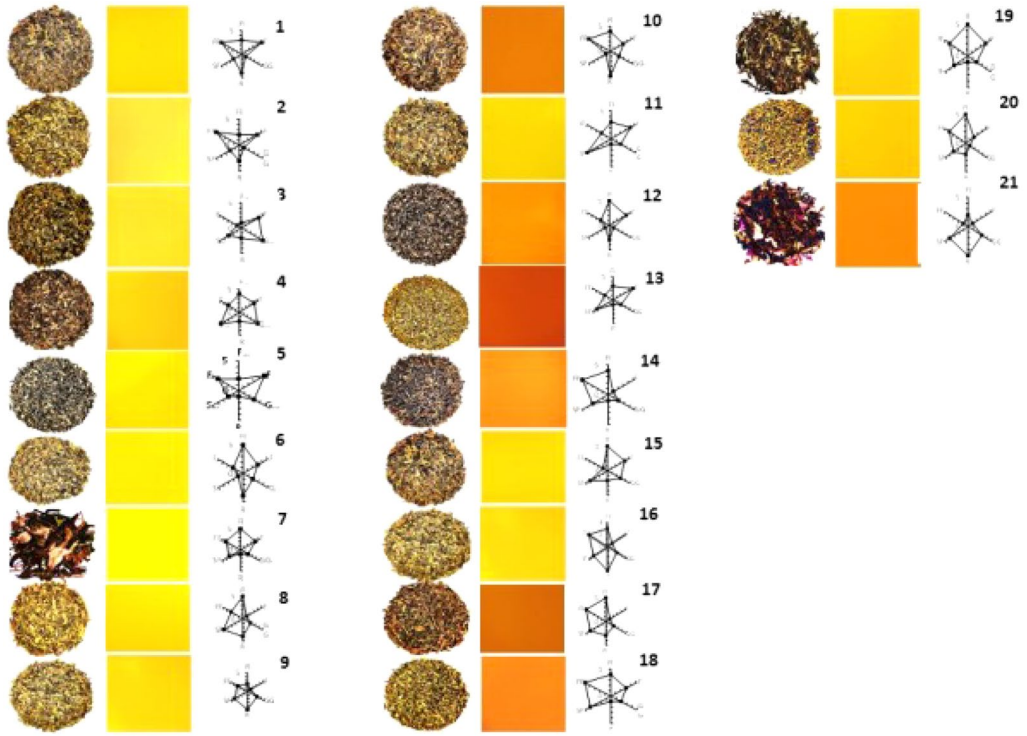


Fig. 3 Sensory analysis profile of teas and herbal teas—1. green tea (GT) 2. taxus green tea (TGT) 3. timur green tea (TiGT) 4. lemongrass green tea (LGT) 5. mint green tea (MGT) 6. ginkgo green tea (GGT) 7. kachnar green tea (KGT) 8. sea-buckthorn green tea (SGT) 9. tulsi green tea (TuGT) 10. black tea (BT) 11. taxus black tea (TBT) 12. timur black tea (TiBT) 13. lemongrass black tea (LBT) 14. mint black tea (MBT) 15. ginkgo black tea (GBT) 16. kachnar black tea (KBT) 17. sea-buckthorn black tea (SBT) 18. tulsi black tea (TuBT) 19. mint tea (MT) 20. tulsi tea (TT) 21. rose tea (RT)

of black tea from green tea in the presence of enzyme PPO during the enzymatic oxidation reaction [45]. After this process, catechins were converted into complex molecules, such as theaflavins and thearubigins [45]; therefore, catechin content was decreased in BT. The catechins present in tea show the various biological activities, such as antioxidant, anticancer and anti-inflammatory activity [46] which revealed that the prepared herbal tea show the biological activity.

The highest amount of amino acids was observed in GT. The major amino acid present in herbal tea is theanine which contributes to the quality and umami taste of tea infusion [47]. L-theanine is a free amino acid that is present in tea [48] and showed a large number of biological activities, such as increased alpha brain waves, relaxed the mind, reduces stress and blood pressure [48]. Therefore, we conclude that the amino acids present in tea contribute to the taste quality [49].

The highest antioxidant activity was observed in GHT compared to BHT. The results are in line with the previous findings which showed the highest antioxidant activity of GT followed by BT [37]. The high antioxidant activity in GT is due to the presence of compounds, such as EC, ECG, EGC and EGCG [50]. In the previous study, it was reported that the GT shows the higher antioxidant activity in terms of DPPH and ABTS free radical [38].

The correlation observed between TPC, TFC, DPPH and ABTS in GHT, BHT and MHT were positive. Previously in the literature, a correlation between TPC and TFC is correlated with antioxidant activity in the leaf of *Olea europaea* L. and *Argania spinosa* (L.) Skeels [51]. Therefore, in our study, we revealed that the correlation between TPC and TFC was directly related to antioxidant activity. Therefore, we hypothesize that there are some synergistic interactions between *C. sinensis* shoots and herbal plant material with unique taste and quality. In addition, in the previous studies correlation was observed between TPC/TFC and antioxidant activity show the synergistic interactions in the extracts [51].

The results reported that the cytotoxic activity was observed higher in LGT in SiHa cells. Our results are in consistent with the previous literature which showed that the lemongrass extract is used as traditional medicine for the treatment of diabetes, cancer, fever and inflammation [52]. In addition, SGT show the remarkable cytotoxic potential against A549 cells. Previously researcher reported that the sea-buckthorn leaves containing the

high amount of flavanol glycosides, such as isorhamnetin and quercetin derivatives [53]. These reported chemical constituents show the various biological activity, such as antioxidant, antitumor, and anti-inflammatory activity with astringent taste [53]. Therefore, we can conclude these herbal teas could be used for the health beneficial properties.

A large number of different types of VOCs was observed in all the herbal tea samples. The differences in the volatile profile of herbal tea-based kombuchas is due to the different manufacturing conditions of teas [54]. LGT show the highest content of VOCs containing geranial, L-Linalool, 6-Methyl-5-hepten-2-one, *trans*-chrysanthemal, levoverbenone, borneol and carane with a unique flavour. The results are consistent with the previous literature which reported the major aroma compounds, such as geranial, neral, linalool and geraniol [55]. AEDA is used for the determination of VOCs with high odour active values which is expressed as the flavour dilution (FD) factor [56]. High odour active compounds were observed in LGT showed the fresh, lemon-like, spicy, mint, floral and fruity odour. In addition, in the previous research high FD factors were identified in the Chinese green tea infusions showing the sweet, seasoning-like, floral, green, and spicy odour [57]. LGT showed the highest TQS with the sweet taste and aroma of the infused leaves. Results are consistent with the previous literature, in which sensory analysis was performed in lemongrass herbal infusion which shows the citric odour and taste with high perception [55]. However, ferulic acid, quercitrin, and chlorogenic acid are accompanied by the taste, odour and after-taste in the infusions of lemongrass leaves [55]. In addition, previously herb-based kombuchas are accepted higher scores compared to tea-based kombuchas because of the differences in their sweetness and pleasant sensory analysis [54].

Conclusions

In the present study, first-time herbal tea was manufactured using western Himalayan region plants. TGT, SGT and LGT herbal tea show better antioxidants and cytotoxic activities compared to GT and BT. Herbal tea also shows a unique taste and quality with high sensory attributes. Altogether, findings of the study implicate that the prepared herbal teas could be used as a new dietary source having therapeutic potential in the high-paced era.

Abbreviations

HPLC: High-performance liquid chromatography; SDE: Simultaneous distillation extraction; UPLC: Ultra-performance liquid chromatography; GHT: Green herbal tea; BHT: Black herbal tea; MHT: Market herbal tea.

Supplementary Information

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Additional file 1. Additional Tables S1–S6 and Figures S1–S44.

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Author contributions

Conceptualization, methodology, data analysis and manuscript writing are performed by KA. Biological activity, experimental, data analysis and manuscript writing are accomplished by KD. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interest.

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