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RESEARCH ARTICLES

Altitudinal variation in gallic acid content in fruits of *Phyllanthus emblica* L. and its correlation with antioxidant and antimicrobial activity

Shagun Sheoran[1] · Prakriti Nidhi[1] · Vikas Kumar[1] · Gajender Singh[3] · Uma Ranjan Lal[2] · Anuradha Sourirajan[1] · Kamal Dev[1]

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Abstract

Gallic acid is one of the most important organic compounds in fruits of *Phyllanthus emblica.* Therefore, the objective of the present study is to find out the effect of altitude on gallic acid content in fruits of *P. emblica* and its correlation with antioxidant and antimicrobial activity. Phytochemicals such as phenolics, tannins, flavonoids, carbohydrates, glycosides, phytosteroids, alkaloids and saponins were detected in fruit extracts of *P. emblica* collected from different regions of Himachal Pradesh. Fruits extract from Mandi (239.74 ± 39.28 mg/g gallic acid equivalents, GAE) district showed a higher amount of total phenolic content (TPC), whereas, total flavonoid content (TFC) was higher from Kangra (356 ± 27.63 mg/g rutin equivalents, RE) district. Methanolic extracts showed inhibition to the growth of both Gram-positive (*B. subtilis*, *S. aureus*), and Gram-negative bacteria (*E. coli*, *K. pneumoniae*). The methanolic extract of fruits of Bilaspur district showed the highest antibacterial activity against *B. subtilis* (19.5 ± 0.71 mm), *S. aureus* (21.0 ± 1.41 mm), *E. coli* (17.5 ± 0.71) and *K. pneumoniae* (21.5 ± 2.12) as compared to other regions and amoxyclav. High-performance thin-layer chromatography (HPTLC) method was used for the quantification of gallic acid in the extracts of fruits of *P. emblica*. HPTLC chromatogram showed the highest content of gallic acid in methanolic extracts of fruits from Kangra followed by Bilaspur, Mandi, and Una. However, antioxidant and antibacterial activity was higher in fruits extracts of high altitude (Bilaspur region). In summary, Bilaspur region of Himachal Pradesh could be used for mass cultivation of fruits of *P. emblica* because of their high antioxidant and antimicrobial potential under these geographical conditions.

Keywords Antibacterial · Antioxidant · HPTLC · *Phyllamthus emblica* · Phytochemical variation

Introduction

Medicinal plants act as a reservoir of bioactive compounds, which have been exploited for the preparation of safe and less toxic drugs (Gangwar and Deepali 2010). The bioactive molecules of therapeutic significance include alkaloids, flavonoids, phenols, quinones, tannins, and terpenoids. The quality and therapeutic efficacy of medicinal plants not only depend on the amount of bioactive compounds, but also many environmental factors (Gairola et al. 2010). The plants from temperate habitat possess high amount of UV-B protective compounds such as anthocyanins, ascorbic acid, flavonoids and phenolic acid responsible for their high antioxidative potential (Zidorn 2010). Due to enhancement in the utilization of the green medicines, it has become very crucial to study the effect of altitudinal variation on the production of therapeutically

✉ Anuradha Sourirajan
asourirajan@gmail.com

✉ Kamal Dev
kamaldevbhardwaj1969@gmail.com

[1] Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, PO Sultanpur, District Solan, Bajhol 173229, Himachal Pradesh, India

[2] Department of Natural Products, National Institute of Pharmaceutical Education, Ahmedabad, India

[3] School of Pharmaceutical Sciences, Shoolini University of Biotechnology and Management Sciences, PO Sultanpur, District Solan, Bajhol 173229, Himachal Pradesh, India

important secondary metabolites and related medicinal properties to select effective chemotypes. In India, out of 17,000 species of higher plants, 7500 are known for their medicinal importance (Samal 2016; Shiva 1996). This proportion of medicinal plants is higher than that in any other country of the World. These medicinal plants not only constitute a major reservoir of traditional medicines and herbal industry, but also provide livelihood and health security to a large section of the Indian population (Samal 2016). Around 80% demand for ayurvedic medicines, 46% of Unani drugs and 33% of allopathic drugs have been fulfilled by India (Samant et al. 2007; Sharma et al. 2011). Himachal Pradesh is located between 28°–33°N and 75°–79°E and about 26% of total state area is under forest cover, which is higher than that of the average estimate for India (20.64%). Himachal Pradesh consists of medicinal plants of about 180 families belonging to 1038 genera and about 3400 species (Chaudhary and Wadhwa 1984). A large proportion of the rural population depends on locally available medicinal plants to meet their health care requirements leading to enhancement of demand for the medicinal plant species. Therefore, the increased demand for plant-based drugs has put heavy pressure on some selected high value wild medicinal plant due to over-harvesting.

Phyllanthus emblica Linn (syn. *Emblica officinalis*) commonly known as Indian gooseberry or amla, is an important herbal plant of Unani and Ayurvedic systems of medicine (Charmkar and Singh 2017; Mirunalini and Krishnaveni 2010). The plant is used both as a medicine and as a tonic to build up lost vitality and vigor. *P. emblica* is a highly nutritious and important dietary source of Vitamin C, amino acids and many minerals (Gaire and Subedi 2014; Mirunalini and Krishnaveni 2010). The fruits are rich in ascorbic acid (vitamin C), along with phenols, such as ellagic acid, gallic acid, quercetin, kaempferol, corilagin, geraniin, furosin, gallotanins, emblicanins, flavonoids, glycosides, and proanthocyanidins (Anila and Vijayalakshmi 2002; Bajpai et al. 2005; Bhattacharya et al. 2002; Kumaran and Karunakaran 2006; Nisha et al. 2004; Zhang et al. 2001, 2004). The fruits are mainly attributed to its strong antioxidant activity (Bajpai et al. 2005; Naik et al. 2005a, b). The antioxidant activities of fruits are mainly due to ascorbic acid (45–70%) and has been studied widely (Nisha et al. 2004). However, the compounds such as emblicanins, gallic acid, methyl gallate, corilagin, furosin, and geraniin also contribute to the antioxidant properties of fruits (Poltanov et al. 2009; Scartezzini and Speroni 2000). Several studies have reported about the phytochemistry and biological activities of various parts of *P. emblica.* However, the effect of altitudinal variation on secondary metabolite (such as gallic acid) of *P. emblica* and its correlation with antimicrobial and antioxidant activities has not been adequately explored. Thus, the present study was undertaken to analyze TPC, TFC and gallic acid content in *P. emblica* collected from different regions of Himachal Pradesh and its correlation with antimicrobial and antioxidant activity.

Materials and methods

Collection of plant materials

The present study was conducted in the Indian state of Himachal Pradesh, which is predominantly a mountainous and higher altitudinal variation (450 m to over 6826 m above sea level). It is located in North-west part of India. The fruit samples were collected from four different locations of Himachal Pradesh (Fig. 1a). The details of samples collection sites along with altitudes from sea level are given in Table 1.

Chemicals and solvents

Chemicals and solvents used in the current study were of analytical grade. Ascorbic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ [2, 4, 6-Tri(2-pyridyl)-s-triazine], gallic acid and rutin were purchased from Sigma Chemicals (St. Louis, MO, USA). HPTLC was performed on CAMAG, Switzerland system and TLC silica gel plates purchased from Merck.

Preparation of fruit extract of *P. emblica*

The chopped fruits samples (250 g) were dried at 40 °C after washing with running tap water. After drying, the methanolic extract was prepared using cold maceration method (Kumar et al. 2018). The extract was evaporated at 40 °C in a rotary evaporator and stored in the dark at 4 °C.

Qualitative analysis of phytocompounds

Methanolic extracts of fruit samples of *P. emblica* from different locations of Himachal Pradesh were tested for the presence of various secondary metabolites such as phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, phytosteroids and carbohydrate by using standard protocols (Harbone 1998).

Spectrophotometric quantification of total phenolic and flavonoids

The total phenolic content and flavonoid content of methanolic fruit extracts of *P. emblica* from different locations of Himachal Pradesh were determined by Folin–Ciocalteau reagent method (Singleton et al. 1999) and aluminium chloride ($AlCl_3$) method (Zhishen et al. 1999) respectively.

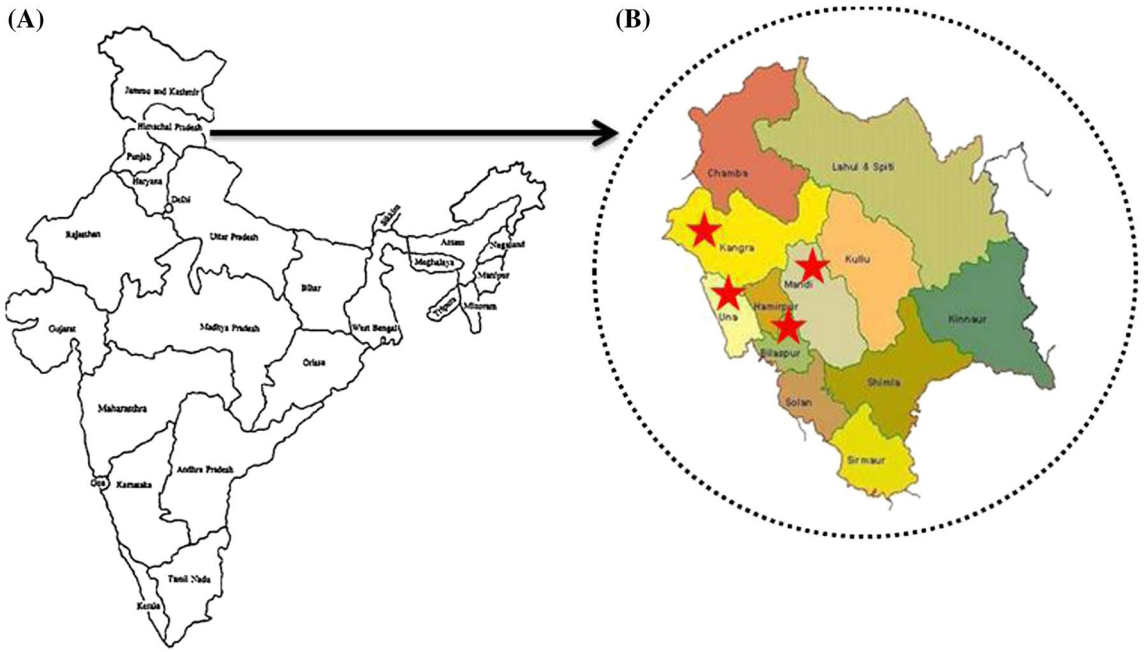

Fig. 1 Maps showing sites of collection (red star) of fuits of *P. emblica*

Table 1 Sample collection sites of *P. emblica* fruits from different locations of Himachal Pradesh

Districts	Location	Altitude (meters above sea level)	Latitude	Longitude
Una	Amb	478	31.6798	76.1175
Kangra	Nagrota bagwan	800	32.1054	76.3789
Bilaspur	Ghumarwin	1500	31.3525	76.69487
Mandi	Baggi	1600	31.7764	77.0765

Analysis of variation in antimicrobial and antioxidant activity

Antimicrobial activity

Agar well diffusion method was used for determination of antimicrobial activity of methanolic fruit extract of *P. emblica* from different regions of Himachal Pradesh (Perez et al. 1990; Kumar et al. 2018; Chandel et al. 2019). The antibacterial activity was evaluated against two Gram's positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram's negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*). All the bacterial strains were available in the Yeast Biology Lab of Shoolini University, Solan, Himachal Pradesh, India. Amoxyclav (25 µg) was used as positive control, while DMSO in which extracts were dissolved was taken as negative control.

In vitro antioxidant activity

Antioxidant activity of methanolic fruit extracts of *P. emblica* from different locations was determined using DPPH radical scavenging and Ferric reducing antioxidant power method (FRAP). For both assays, stocks of methanolic fruit extracts of *P. emblica* were prepared at a concentration of 1 mg/ml and then different dilutions were prepared (5–40 µg/ml). Ascorbic acid was used as standard antioxidant compound in all the assays. The effectiveness of extracts as antioxidants was evaluated in terms of IC_{50}, value. Lower the value of IC_{50}, higher will be the antioxidant potency.

DPPH radical scavenging activity

DPPH radical scavenging activity of the extract was measured by the method described by Barros et al. (2007).The capability of scavenging DPPH radical was calculated using the following equation:

$$\%\ \text{DPPH radical scavenging activity} = \frac{\text{A (control)} - \text{A (sample)}}{\text{A (control)}} \times 100$$

where A (control) is the absorbance of control (DPPH) and A (sample) is the absorbance of plant extracts (ascorbic acid).

Ferric Reducing antioxidant power (FRAP) assay

FRAP activity was evaluated in terms of μM Fe(II) equivalents per gram of the extract according to the method described by Benzie and Strain (1996). The antioxidant capacity of extract and standard was calculated from the linear calibration curve of $FeSO_4$ (2.5–20 μM).

Quantification of gallic acid using high performance thin layer chromatography (HPTLC) method

Ten microliter (μl) of sample solutions and standard (1 mg/ml) were spotted (6 mm width) with a CAMAG microliter syringe on pre-coated silica gel aluminium plate 60 F_{254} (20 cm × 10 cm) with 250 mm thickness. The plates were pre-washed in methanol and activated at 60 °C for 5 min prior to chromatography. The slit dimension was kept at 6 mm × 0.45 mm, and 20 mm/s scanning speed was employed. The slit band width was set at 4 mm, each track was scanned thrice, and baseline correction was used. The mobile phase (10 ml) consisted of toluene–ethyl acetate–acetic acid (5:4:1) (v/v). Linear ascending development was carried out in 20 cm × 10 cm twin-trough glass chamber (CAMAG, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at 25 ± 2 °C at relative humidity of 60 ± 5%. The length of chromatogram run was 8 cm and after the run, TLC was stained with *p*-anisaldehyde solution. Subsequent to the scanning, TLC plates were dried with the help of an air dryer. Densitometric scanning was performed with CAMAG TLC Scanner 3 in the reflectance–absorbance mode at 254 and 366 nm and operated by win CATS software (1.3.0 CAMAG). Concentrations of the compounds were determined from the calibration curve of gallic acid prepared over a concentration range of 1–5 ng/band. The gallic acid was quantified by comparing peak areas with linear regression.

Bioautography assay for gallic acid as antioxidant compounds in fruits of *P. emblica*

TLC bioautography method was performed with gallic acid and extracts were spotted on TLC and run in a solvent system of toluene, ethyl acetate, acetic acid in ratio of 5:4:1, (v/v). After separation on TLC plates, the compounds with free radical scavenging activity were detected in situ with DPPH reagent (0.004%) (Nickavar et al. 2014). After some time, TLC plate was observed under visible light. Samples producing yellowish bands against the purple background indicated the antioxidant potency.

Results

Qualitative analysis of phytochemicals in methanolic extracts of fruits of *P. emblica*

The preliminary analysis of fruits extracts of *P. emblica* from different locations of Himachal Pradesh showed the presence for alkaloids, phenolics, tannins, carbohydrates, glycosides, steroids, saponins and free amino acids, except proteins. A comparative summary of phytochemical analysis is shown in Table 2. These results suggested the presence similar phytochemicals in the extracts from all the locations, irrespective of different geographical conditions.

Quantification of total phenolic and flavonoids

Methanolic extracts of *P. emblica* from different regions of Himachal Pradesh were further subjected to quantification of total phenolics and flavonoids using spectrophotometric method. As shown in Fig. 2a, the amount of total phenolics content was comparable in methanolic extracts of fruit samples from Mandi, Una, Bilaspur and Kangra. In case of total flavonoid content, highest TFC was comparable in fruits extract of Kangra (356 ± 27.63 mg/g, RE), Bilaspur

Table 2 Quantitative analysis of phytocompounds in methanolic extracts of fruits of *P. emblica* collected from different altitude of Himachal Pradesh, India

Phytocompounds	Tests	Places of sample collection			
		Una	Kangra	Bilaspur	Mandi
Alkaloids	Dragendroff test	+	+	+	+
Phenolics and tannins	Ferric chloride test	+	+	+	+
	Gelatin test	+	+	+	+
Flavonoids	Lead acetate test	+	+	+	+
Carbohydrate	Fehling test	+	+	+	+
Glycosides	Borntrager test	+	+	+	+
Proteins	Millon test	–	–	–	–
Saaponins	Foam test	+	+	+	+
Terpenoids	Salkowsky test	+	+	+	+

"+" sign indicated the presence, while "–" sign indicated the absence of phytocompounds

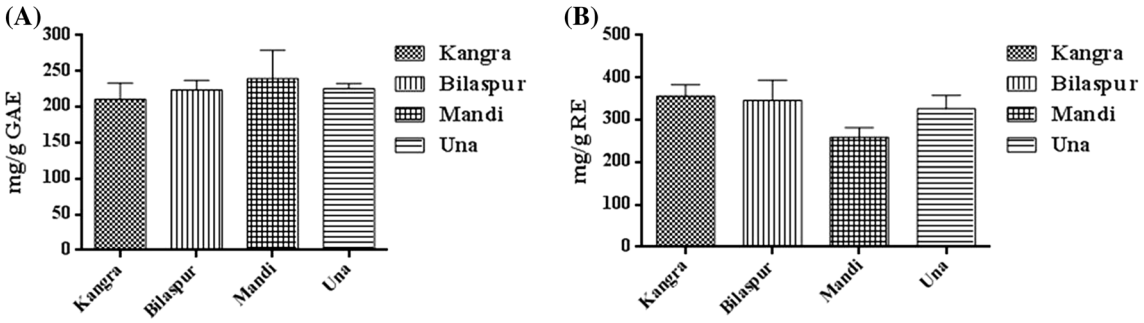

Fig. 2 Variation in total phenolic (**a**) and flavonoid content (**b**) of methanolic extracts of fruits of *P. emblica* from different altitude of Himachal Pradesh. TPC was expressed in terms of mg/g Gallic acid equivalents; however, TFC was expressed as mg/g rutin equivalents. Values are expressed as mean ± SD of three independent experiments

(346.41 ± 47.88 mg/g, RE) and Una (326.27 ± 31.86 mg/g, RE) as shown in Fig. 2b. TFC was lowest in fruits extract of Mandi district (259.28 ± 22.18 mg/g, RE).

Antibacterial activity of methanolic extract of *P. emblica*

Antibacterial activity of methanolic extracts of fruits of *P. emblica* was tested against Gram-positive and Gram-negative bacteria. It was found that the methanolic extract of fruits of Bilaspur showed the highest antibacterial activity against *B. subtilis* (19.5 ± 0.71 mm), *S. aureus* (21.0 ± 1.41 mm), *E. coli* (17.5 ± 0.71) and *K. pneumoniae* (21.5 ± 2.12) as compared to fruits extracts of other regions and amoxyclav (Fig. 3a and b).

Antioxidant activity of methanolic fruit extract of *P. emblica*

Antioxidant activity of methanolic extracts of *P. emblica* and ascorbic acid by DPPH and FRAP method at various concentrations is shown in Fig. 4a and b. It was found that all the fruits extracts showed free radical scavenging (DPPH) and reducing capacity (FRAP) in a dose dependent manner. Methanolic extracts of fruits from Bilaspur showed higher antioxidant potential as shown by lesser IC_{50} with DPPH assay (4.09 ± 1.34 µg/ml) and FRAP assay [20.80 ± 2.26 µM Fe(II) equivalents] as compared to that of ascorbic acid [(3.86 ± 0.141 µg/ml (DPPH) and 13.43 ± 0.63 µM Fe(II) equivalents (FRAP)] (Table 3).

Quantification of gallic acid levels in methanolic fruit extracts of *P. emblica* by HPTLC

To quantify the amount of gallic acid responsible for antioxidant activity of the *P. emblica* fruit extracts, HPTLC was carried out along with gallic acid followed by staining with p-anisaldehyde. As shown in Fig. 5a and b, a band corresponding to gallic acid was detected in all the samples. The peak purity of gallic acid marker was confirmed by comparing the spectra at three different levels, i.e. start, middle, and end positions of the bands. The gallic acid content was estimated using linear regression of HPTLC densitometry in methanolic fruits extracts of *P. emblica* collected from various regions. The linear regression equation used was $y = 2489.4x + 2980.3$ with $R^2 = 0.996$ (Fig. 5c). HPTLC results indicated the presence of highest gallic acid content in methanolic extracts of fruits from Kangra (765.23 ± 12.79 mg/g, extract), followed by Bilaspur (621.86 ± 2.68 mg/g, extract), Mandi (408.32 ± 4.21 mg/g, extract) and Una (360.34 ± 5.73 mg/g, extract).

TLC bioautography assay for identification of antioxidant compounds in methanolic extracts of fruits collected from different regions of Himachal Pradesh

To screen the antioxidant compounds in fruits extracts of *P. emblica*, a TLC bioautography method was performed. After separation of fruit extracts on TLC plates, the compounds with radical scavenging activity were determined in situ using 0.004% DPPH reagent. The TLC plate was observed under visible light (Fig. 6). The bands producing yellowish color against purple background were considered as antioxidants (Ruiz-Terán et al. 2008; Rumzhum et al. 2012). It was found that the R_f value of antioxidant compounds showing yellow color in all the fruits extracts of *P. emblica* corresponds with R_f value of Gallic acid ($R_f \sim 0.4$), indicating that gallic acid present in all the fruits extracts of *P. emblica* and could be major contributor of antioxidant activity (Fig. 6).

Correlation between TPC, TFC, antioxidant activity, gallic acid content with latitude, longitude and altitude

The Pearson correlation coefficients for latitude, longitude and altitude of the growing site of *P. emblica* and TPC, TFC,

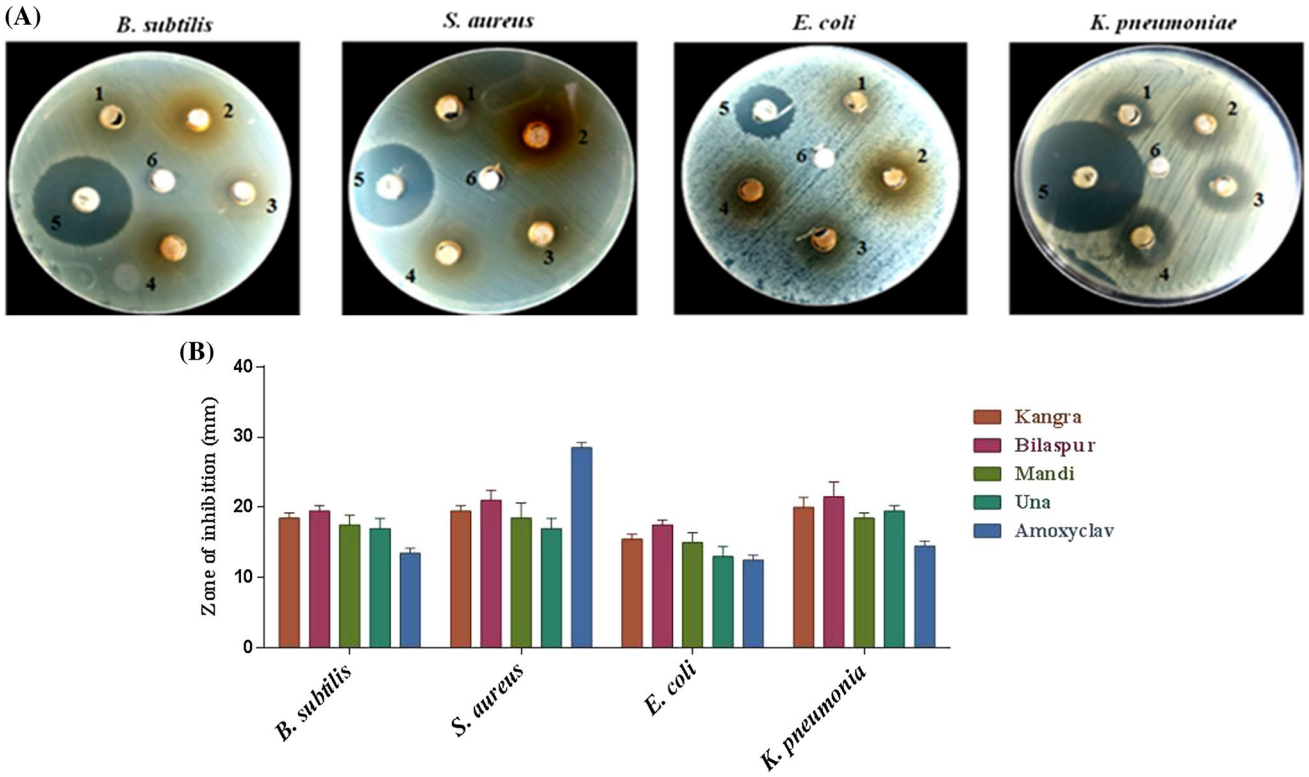

Fig. 3 Antibacterial activity of fruits extract of *P. emblica*. **a** Agar well diffusion method showing antibacterial activity of methanolic fruit extracts of *P. emblica* against Gram positive (*B. subtilis, S. aureus*) and Gram negative (*E. coli*, *K. pneumoniae*) bacteria. Extract were indicated as 1, 2, 3, 4 for fruits extract of Kangra, Bilaspur, Una, and Mandi, respectively. Amoxyclav (5) was used as positive and DMSO (6) was used as solvent control. **b** Antimicrobial activity in terms of diameter of zone of inhibition (mm) against different bacterial strains. Values are expressed as mean ± SD of three independent experiments

DPPH, FRAP, gallic acid content were calculated using IBM SPSS statistics 20 software and summarized in Table 4. It was found that latitude and longitude have no effect on TPC, TFC, DPPH, FRAP, gallic acid content. However, Altitude showed negative correlation with TFC and FRAP activity which is significant at 0.05 and 0.001 levels (*t* test) respectively. Both DPPH and FRAP showed negative non-significant and significant correlation respectively with Gallic acid content, although the correlation was negative, but due to fact that lower the value of IC_{50} in antioxidant assay, higher the antioxidant capacity. Therefore, the correlation become positive for antioxidant assay with gallic acid content. Gallic acid content showed positive correlation with parameters such as latitude, longitude, altitude TPC, and TFC, but they are statistically insignificant.

Discussions

There is an increased demand of phytocompounds and secondary metabolites of medicinal plants that have therapeutic potential. The production and accumulation of phytocompounds and secondary metabolites does not solely depend on genotype, but largely affected by many biotic and abiotic factors. Therefore, it is very important to study the altitudinal variation in phytocompounds of medicinal plants that are industrially relevant (Alonso et al. 2005; Carey and Wink 1994; Choudhry et al. 2014). Himachal Pradesh being part of North-west Himalayas encompasses wide altitudinal and geographical variation and hence a hotspot of biodiversity. Although there are numerous studies on the quantification of medicinally important phytocompounds from different medicinal plants, but correlation of phytocompounds with altitudinal, seasonal and geographical variation remained largely unexplored. The current study was designed to study the effect of longitude, latitude and altitudinal variation in accumulation of phytocompounds in *P. emblica* and compare antimicrobial and antioxidant properties. Phenolic compound such as gallic acid (3, 4, 5-trihydroxybenzoic acid) is one of the major phytoconstituents of fruits of *P. emblica*. Gallic acid is a naturally occurring polyphenolic compound and has been reported as anti-bacterial against a wide range of pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Vaquero et al. 2007). It is also known for number of therapeutics like anti-allergic, anti-inflammatory, anti-mutagenic

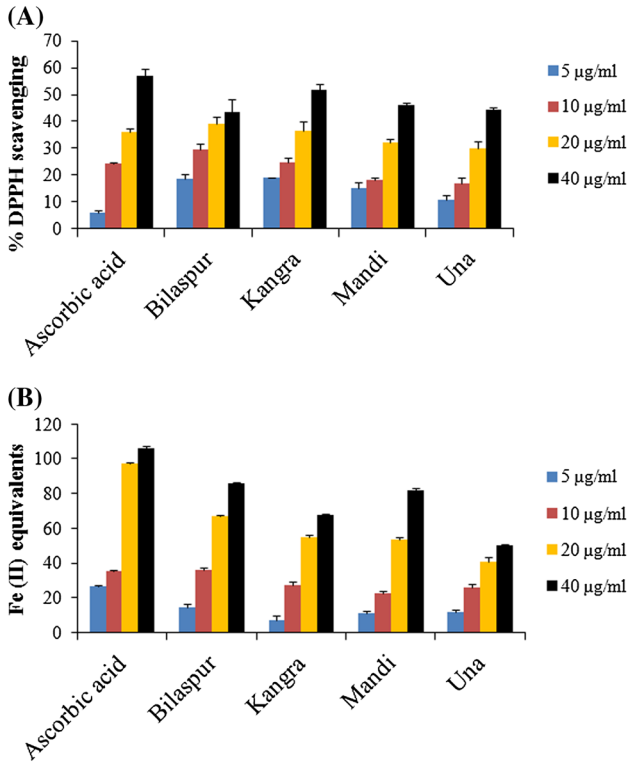

Fig. 4 Antioxidant activity of methanolic extracts of fruits of *P. emblica* collected from different altitudes of Himachal Pradesh, India. **a** DPPH radical scavenging method and **b** Ferric reducing antioxidant power (FRAP) method. Different amounts of extracts (5–40 µg/ml) were used in DPPH and FRAP assay as indicated. Ascorbic acid was used as control. Values are expressed in terms of mean ± SD of two independent experiments

and anti-carcinogenic (Choubey et al. 2015; Gali et al. 1991; Singleton 1981). It can be used as antioxidant to protect human cells against oxidative damage, to treat albuminuria and diabetes and as a remote astringent in cases of internal hemorrhage (Abbasi et al. 2011). It also showed cytotoxic effects against cancer cells without harming normal cells (Beniwal et al. 2013).

In the present study, we found that TPC of *E. officinalis* was marginally higher in fruits extract of Mandi (239.74 ± 39.28 mg/g, GAE), whereas, TFC was higher in fruits extract from Kangra (356 ± 27.63 mg/g, RE) and lowest in fruit extracts of Mandi. On the other hand, methanolic extract of *E. officinalis* fruits of Bilaspur and Kangra showed the highest antibacterial activity against all the tested Gram-positive and Gram-negative bacteria as compared to fruits extracts of other regions and standard antibiotic, Amoxyclav, except antibacterial activity of amoxyclav against *S. aureus*. This may be partially attributed to the presence of high amounts of TPC and TFC in *E. officinalis* fruits extract of Bilaspur and Kangra regions. Antimicrobial potency of fruits of *E. officinalis* was also reported in various studies (Al-Gbouri and Hamzah 2018; Jamil 2017; Khoo et al. 2016). The antioxidant potency of fruits of *E. officinalis* was analyzed using DPPH and FRAP assays. It was observed that methanolic extracts of fruits from Bilaspur showed more antioxidant potential as shown by lower IC_{50} with various antioxidant assays such as DPPH radical scavenging assay (4.09 ± 1.34 µg/ml) and FRAP assay [20.8 ± 2.26 µM Fe(II) equivalents] as compared to that of ascorbic acid (DPPH-3.86 ± 0.141 µg/ml; FRAP-13.43 ± 0.63 µg/ml). The higher antioxidant activity in Bilaspur sample may be due to high amount of flavonoid content, indicating correlation of TFC with antioxidant activity. Several studies have reported the positive correlation of TPC with antioxidant activity (Fidrianny et al. 2018; Gan et al. 2017; Rakholiya et al. 2014; Petridis et al. 2012), however, some studies have also showed the positive correlation of flavonoids with various antioxidant assays (Shan et al. 2019; Hazra et al. 2010). TLC bioautography showed that gallic acid is one of the phytoconstituents present in fruits of *P. emblica,* which is responsible for their antioxidant activity. Thus, gallic acid content could be used as an index for antioxidant potential in the fruits of *P. emblica*. HPTLC chromatogram showed the presence of highest content of gallic acid in methanolic extracts of fruits from Kangra (765.23 ± 12.79 mg/g, extract) followed by Bilaspur (621.86 ± 2.68 mg/g, extract), Mandi (408.32 ± 4.21 mg/g, extract) and Una (360.34 ± 5.73 mg/g, extract). Correlation of TPC, TFC, DPPH, FRAP and gallic acid content with longitude, latitude and altitude was investigated in terms of Pearson correlation coefficient using IBM SPSS statistics 20 software. It was found that TPC, TFC, DPPH, FRAP, gallic acid content was not affected by latitude and longitude, but altitude showed negative significant

Table 3 Half maximal inhibitory concentration (IC_{50}) of methanolic extract of fruits of *P. emblica* collected from different altitudes of Himachal Pradesh. Values are mean ± SD of three independent experiments

Antioxidant assay	Ascorbic acid	IC_{50}			
		Bilaspur	Kangra	Mandi	Una
DPPH[a]	3.86 ± 0.141	4.09 ± 1.34	4.57 ± 1.58	4.87 ± 0.99	4.63 ± 0.24
FRAP[b]	13.43 ± 0.63	20.80 ± 2.26	22.06 ± 1.46	26.87 ± 2.02	39.61 ± 2.1

[a]µg/ml

[b]µM Fe(II) equivalents

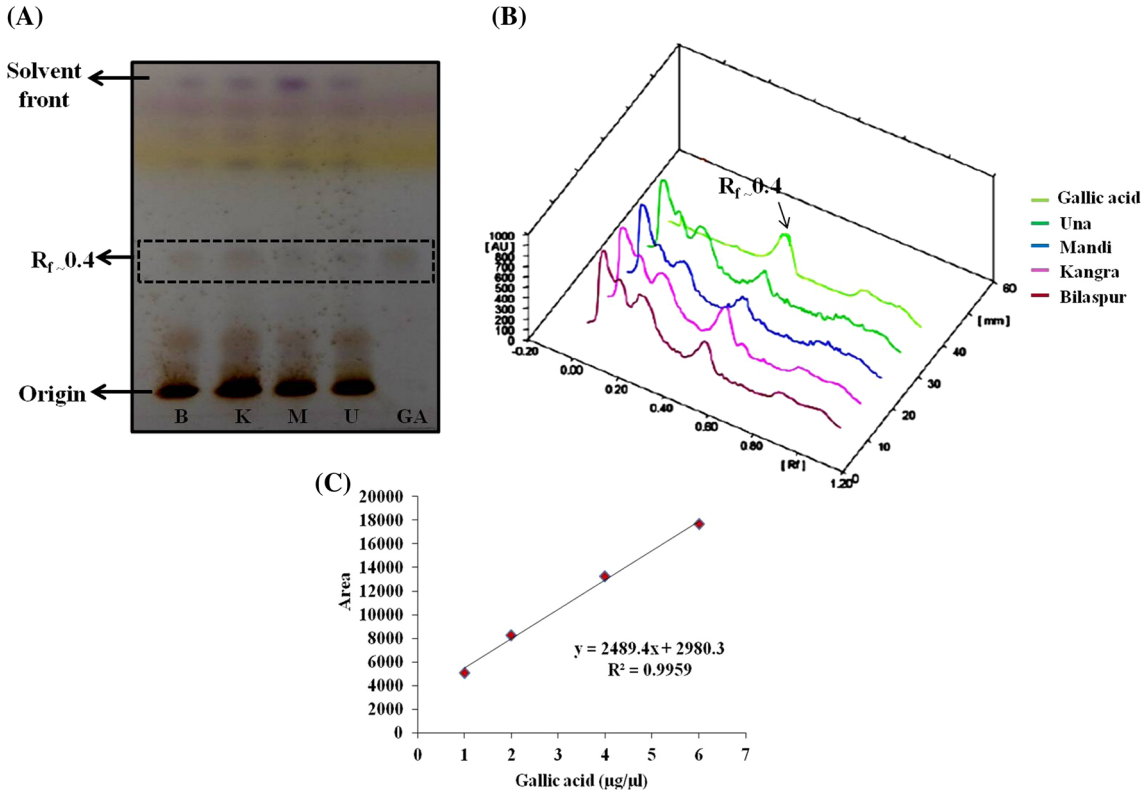

Fig. 5 HPTLC analysis for quantification of gallic acid by HPPTLC method in methanolic fruit extracts of *P. emblica* collected from different altitude of Himachal Pradesh, India. **a** HPTLC fingerprint profile of methanolic extracts of *P. emblica* collected from different attitude of Himachal Pradesh along with gallic acid (standard); Lane 1–4 represents sample collected from Bilaspur (B), Kangra (K), Mandi (M), Una (U). Lane 5 represents gallic acid (GA) as control. **b** Densitometric scanning profile of the spot corresponding to gallic acid in the chromatogram analysis at 254 nm; **c** linear regression graph of gallic acid

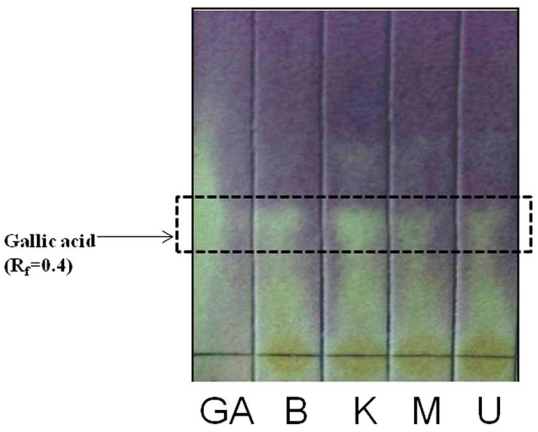

Fig. 6 TLC bioautography of Gallic acid and methanolic fruit extracts of fruits of *P. emblica* from different altitude after sprayed with DPPH. *GA* gallic acid, *B* Bilaspur, *K* Kangra, *M* Mandi, *U* Una as indicated are methanolic extracts of fruits of *P. emblica*. Arrow indicates a yellow spot corresponding to gallic acid with $R_f = 0.4$

correlation with TFC (at 0.05 level, t test) and FRAP activity (at 0.001 level, *t* test). Both DPPH and FRAP showed negative non-significant and significant correlation with gallic acid content, respectively. According to value of IC_{50} in antioxidant assay, these correlations become positive with gallic acid content. Gallic acid content also showed positive correlation with parameters such as latitude, longitude, altitude TPC, and TFC, but all these are statistically insignificant. In contrast to our study, a direct correlation was found between the total phenol and flavonoid content and the altitude (Pandey et al. 2018) on *Thalictrum foliolosum*. Study from Dey and Pandey (2014) on altitudinal variation of stigmasterol, an anti-venom compound in *Rauvolfia serpentina* using HPTLC method showed the altitude is positively correlated with stigmasterol content.

Conclusions

The present study showed that the fruits of *P. emblica* from Bilaspur region showed higher antioxidant and antibacterial potential. Gallic acid was found to be major antioxidant phytocompounds in fruits of *P. emblica* as shown in

Table 4 Correlation of TPC, TFC, antioxidant activity and gallic acid content in fruit extracts of *P. emblica* with latitude, longitude and altitude using Pearson correlation coefficient (r)

		Latitude	Altitude	Longitude	TPC	TFC	DPPH	FRAP	Gallic acid content
Latitude	Pearson correlation	1	−0.414	−0.066	0.236	0.545	0.194	0.176	0.343
	Sign (1 tailed)		0.154	0.438	0.438	0.081	0.323	0.338	0.203
Altitude	Pearson correlation		1	−0.019	−0.429	−0.693*	−0.157	−0.894**	0.026
	Sign (1 tailed)			0.482	0.145	0.028	0.355	0.001	0.476
Longitude	Pearson correlation			1	0.241	0.002	-0.397	−0.068	0.001
	Sign (1 tailed)				0.282	0.498	0.165	0.436	0.499
TPC	Pearson correlation				1	−0.049	0.218	0.555*	0.419
	Sign (1 tailed)					0.439	0.248	0.030	0.087
TFC	Pearson correlation					1	−0.205	0.529	0.208
	Sign (1 tailed)						0.261	0.38	0.259
DPPH	Pearson correlation						1	0.11	−0.222
	Sign (1 tailed)							0.366	0.244
FRAP	Pearson correlation							1	−0.484*
	Sign (1 tailed)								0.050
Gallic acid content	Pearson correlation								1
	Sign (1 tailed)								

*Correlation is significant at the 0.05 (*t* tailed test

**Correlation is significant at the 0.01 (*t* tailed test)

TLC bioautography. The higher antioxidant and antibacterial potency in fruit extract of *P. emblica* from Bilaspur region may be probably due to synergistic effect of other phytoconstituents such as ascorbic acid, quercetin along with gallic acid.

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Compliance with ethical standards

Conflict of interest The authors confirm that they have no conflicts of interest with any parties regarding the content of this article.

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