



## Original Research Article (Experimental)

# Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities



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## ARTICLE INFO

## Article history:

Received 23 November 2016

Received in revised form

8 February 2017

Accepted 11 February 2017

Available online 1 November 2017

## Keywords:

Altitudinal variation

Antimicrobial

Antioxidant

Berberine

Flavonoid

HPTLC

Phenolics

*Thalictrum foliolosum*

## ABSTRACT

**Background:** The quality of herbal medicine is determined by its secondary metabolites, which may vary according to growth, season and altitude etc.

**Objective:** We studied the variation in phytochemistry and biological activities of *Thalictrum foliolosum* (TF) roots collected from four sites at different altitudes.

**Material and methods:** The berberine content in different extracts of *T. foliolosum* roots collected from various altitudes was estimated using HPTLC. Total phenolic and flavonoid contents were determined using Folin-Ciocalteu reagent and aluminum chloride method respectively. The sensitivity of microbes for the extracts was studied using disc diffusion and the MIC was estimated using broth dilution method. Antioxidant capacity of the plant was studied using  $\beta$ -carotene bleaching assay, lipid peroxidation assay using goat liver, reducing power assay and DPPH free radical scavenging activity.

**Results:** Berberine content varied inversely with altitude; while phenol and flavonoid content of TF increased at higher altitudes. All the TF extracts showed moderate to high activity against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Extracts with high berberine content were most effective against *C. albicans* and *S. aureus* and also showed relatively significant anti-lipid peroxidation,  $\beta$ -carotene bleaching and reducing power. TF extracts with higher phenol and flavonoid content showed better scavenging of DPPH free radicals. Berberine was used as a standard in all the antioxidant and antimicrobial experiments performed.

**Conclusion:** *Thalictrum* from lower elevations can be explored as an alternate source of berberine and the plant has high antioxidant and antimicrobial properties owing to its berberine content.

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## 1. Introduction

Medicinal plants are the natural resources of potential biodynamic compounds which can be used for drug development [1]. The secondary metabolites, such as alkaloids, flavonoids, phenols, quinones, tannins and terpenoids, derived from medicinal plants are used worldwide for the treatment of several diseases. Apparently the quality and therapeutic efficacy of medicinal and aromatic plants depend on their secondary metabolites, which in turn depend on the environmental factors [2]. For example, plants in

temperate habitats are exposed to severe environmental stresses including temperature and radiation extremes. Consequently, there is an increase in the antioxidative property and production of UV-B protective compounds (anthocyanins, ascorbic acid, flavonoids and phenolic acid); while a decrease in allelopathic and anti-herbivory substances (alkaloids, iridoids, and sesquiterpene lactones) is observed [3]. With the advent of green pharmacy, it has become imperative to study the effect of altitudinal variation on the production of secondary metabolites and related medicinal properties, so that the effective chemotypes can be harvested.

The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Hence, for many years antibiotics were used to treat infections of millions of people. However, after reports of penicillin resistance became a substantial

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Peer review under responsibility of Transdisciplinary University, Bangalore.

clinical problem, new beta-lactam antibiotics were developed to combat microbial infections. The evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) and subsequent resistance of other microbes to nearly all antibiotics posed a global threat to human health. Later, Vancomycin was introduced into clinical practice in 1972, for the treatment of methicillin resistance in both *S. aureus* and coagulase-negative Staphylococci. Cases of Vancomycin resistance were reported in coagulase-negative Staphylococci in 1979 and 1983 [4]. The new regime of complex and effective antibiotics successfully curbed microbial infection for a long time. However, with increasing use of antibiotics, more and more pathogenic bacteria developed resistance to their inhibitory effects. Consequently, despite their initial effectiveness, most antibiotics have a limited life, and from their first introduction, they select for pathogen variants that have intrinsic or acquired resistance mechanisms [5]. Thus the search for natural biodynamic compounds with antimicrobial activity is extremely important.

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [6]. Generation of free radicals is an integral feature of normal cellular state, however, it increases manifold during pathological conditions [7]. The generation of excess free radicals can damage cellular lipids, proteins or DNA inhibiting their normal function and may cause metabolic imbalances speeding the process of aging [8]. The plant-derived secondary metabolites especially the phenolic compounds are the biggest group of exogenous antioxidants [9]. Studies on the free radical-scavenging properties of flavonoids have permitted characterization of major phenolic components of naturally occurring phytochemicals as antioxidants [10].

*Thalictrum* is an abundant medicinal plant source with almost 200 species distributed worldwide. However, *Thalictrum foliolosum* DC. is found in temperate habitat and its roots are traditionally being used as a tonic, antiperiodic, diuretic, febrifuge, purgative and stomachic [11]. It is also considered as a good remedy for atonic dyspepsia, relieves edema, useful in skin diseases and in convalescence after acute diseases and as an application for ophthalmia [12]. The chemical constituents viz. berberine, columbamine, jatrorrhizine, oxy berberine, thalifendine, palmatine, thalidasine, dehydrodiscretamine, tembetarine, xanthoplanine, and magnoflorine were reported from the root of this plant [13,14]. Berberine, a tetra isoquinoline alkaloid has been used as the traditional medicine of India, China and North America [15]. It is in great demand among the pharmaceutical industries owing to its therapeutic potential which includes antileukaemia, antihepatoma, cardioprotective, anticancer and activation of AMP-activated protein kinase resulting in beneficial metabolic effects in diabetic and insulin-resistant states [16]. Berberine is produced by plants of families Berberidaceae, Ranunculaceae, Menispermaceae, and Rutaceae. Among the plants, *Berberis* species (Berberidaceae), *Coptis* species (Ranunculaceae) and *Phellodendron arvense* (Rutaceae) are mainly being exploited as a source of berberine. Identification of more than one source of phyto-compounds benefits two-fold, firstly, it prevents over-exploitation of a single source and secondly, the phytochemicals become globally available as a result of their detection in more than one plant.

The preliminary antimicrobial activity of leaf, root, and rhizome of *T. foliolosum* was reported [17–19]. However, the altitudinal variation of secondary metabolites of *T. foliolosum* and their correlation with antimicrobial and antioxidant activities are not adequately explored. Thus, the present study was undertaken to analyze variation in berberine, total phenolic and flavonoid content in *T. foliolosum* collected from different altitudes and to understand the relation between its phytochemical variation and related biological activities.

## 2. Materials and methods

### 2.1. Plant material and collection sites

Fresh roots of *T. foliolosum* DC. (TF) were collected from various locations at different elevations, Bhowali (1709 m), Sankri (2031 m), Taluka (2111 m) and Kedarkantha (3086 m) in North India, in the months of October–November. Bhowali is located at 29.38°N 79.52°E. The climate of Bhowali is temperate highland tropical climate with dry winters. The temperature ranged between 18 °C and 33 °C and precipitation was 2127 mm. The climate of Sankri and Taluka is classified as warm and temperate. The average temperature in Sankri was 15.8 °C while that in Taluka was 13.25 °C. About 1328 mm of precipitation falls annually. Sankri is located at 31.07°N and 78.18°E while Taluka is located at 31.02°N and 78.05°E. Kedarkantha is located at 30.31°N and 78.03°E and the average temperature was 8 °C at the time of collection. The plants were identified by Dr. AKS Rawat and a voucher specimen was deposited at CSIR-National Botanical Research Institute (India).

### 2.2. Chemicals and instruments

Chemicals and solvents used in the study were of analytical grade. Ascorbic acid, berberine hydrochloride, and DPPH were obtained from Sigma Chemicals (St. Louis, Mo, U.S.A.). Other chemicals were obtained from Ranbaxy Fine Chemicals Ltd., India. UV spectrophotometer (Shimadzu 160 IPC), homogenizer, centrifuge (Remi, India) and pH meter (Elico Ltd., India) were the instruments used for the study. HPTLC was performed on CAMAG, Switzerland system and TLC silica gel plates were purchased from Merck.

### 2.3. Extract preparation

The TF roots collected from all the locations were washed with fresh water, chopped into small pieces and shade dried for about ten days. The material was ground to a fine powder using an electric grinder. The 95% ethanolic, 50% aqueous ethanolic and aqueous extracts were prepared for detailed studies. A 100 g of material was refluxed with solvents thrice over a period of 72 h and filtered using Whatman filter. The filtrate so obtained was dried using a vacuum rotary evaporator (BUCHI) and lyophilized. The extracts were stored at 4 °C until further use.

### 2.4. Estimation of total phenolic and flavonoid content

The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent [20] and the total flavonoid content (TFC) was estimated using aluminum chloride method [21].

### 2.5. Estimation of berberine content

HPTLC was performed on 10 cm × 20 cm pre-coated silica gel 60 F<sub>254</sub> aluminum sheets. Ten µl extracts (1 mg/ml) of TF were applied as a band of 6 mm width, 10 mm from the bottom edge using Linomat 5 applicator fitted with Hamilton syringe (100 µl). The varying quantity of berberine marker (0.1 mg/ml) was applied on the plate. The plate was developed to a distance of 8.0 cm in 20 ml mobile phase, Isopropanol: Formic acid: Water (9:2:1). In CAMAG twin-trough chamber previously saturated with the mobile phase for 45 min at 24 ± 2 °C. The plates were completely dried after the run and densitometric scanning was done at 340 nm for berberine using Deuterium lamp in a CAMAG TLC scanner 3 with winCATS 3.2.1 software. The calibration curve was prepared over a concentration range of 10–50 ng/band for berberine reference marker. The spectra were recorded to confirm the presence of aforesaid marker

compound in the extracts. The photographs were taken with CAMAG Reprostar 3 video documentation unit by illumination at 366 nm.

### 2.6. In vitro antioxidant analysis

Antioxidant potential of the plant was studied using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [22];  $\beta$ -carotene linoleate model system [23]; reducing power assay [24] and lipid peroxidation assay [25] with some modifications using goat liver. In the modified anti-lipid peroxidation assay 2.8 ml of 10% goat liver homogenate and 0.1 ml of 50 mM, freshly prepared  $\text{FeSO}_4$  were mixed with varying concentrations of the extract. The reaction mixture was incubated at  $37 \pm 2^\circ\text{C}$  for 30 min. One ml of this reaction mixture was mixed with 2 ml of 10% TCA-0.67% TBA in acetic acid (50%) for stopping the reaction. The mixture was then boiled at  $100^\circ\text{C}$  followed by centrifugation at 1000 rpm for 5 min. The absorbance of the supernatant was recorded against blank at 535 nm. The blank contained all the reagents except liver homogenate and extract. A similar experiment was performed using varying concentrations of berberine. The percent inhibition was estimated using the formula  $((A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}) \times 100$ . The assays were carried out in triplicate and the results expressed as mean values  $\pm$  standard deviations. Ascorbic acid and berberine were used as standards.

### 2.7. Antimicrobial activity

The susceptibility of *S. aureus* (ATCC 4944), *E. coli* (ATCC 25922), *C. albicans* (ATCC10231) and *P. aeruginosa* (MTCC 424) to various extracts was studied by using the methods designed by Bauer et al. [26]. The minimum inhibitory concentration (MIC) of different microbial strains was determined using dilution method [27] with slight modification, wherein, the extracts in each well were serially diluted from a concentration of 5 mg/ml to 0.0097 mg/ml. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC. *C. albicans* was grown in Sabouraud dextrose broth and agar, while other bacterial strains were grown in Mueller-Hinton agar and broth. Similar studies were performed to test bacterial sensitivity against berberine and positive control standard antibiotics gentamycin (10  $\mu\text{g}/\text{disc}$ ) and streptomycin (10  $\mu\text{g}/\text{disc}$ ).

### 2.8. Statistical analysis

Results were represented as Average  $\pm$  SE. The effect of altitude on berberine, phenol, flavonoid content and various models of antimicrobial and antioxidant activities, where analyzed by ANOVA

and Pearson correlation coefficient;  $p < 0.05$  was considered significant.

## 3. Results

### 3.1. Total phenolic and flavonoid content

TPC ranged from 1.02 GE/mg to 26.5 GE/mg in various extracts of TF. There is a marked difference in the phenolic content in different extracts with the highest content in 95% ethanolic extract and least content in water extracts. The phenol content is clearly affected by altitudinal variation, wherein samples from higher altitude showed higher phenolic content, as compared to samples from lower altitude; Pearson's correlation coefficient being 0.8542. The TFC varies from 5.09 RE/ to 8.28 RE/mg showing significant variation depending upon the extract type and the altitudinal disparity. The higher altitude samples showed high flavonoid content, while lower altitude samples showed relatively lower content of flavonoid. The direct correlation between altitude and flavonoid content was evident from the Pearson's correlation coefficient 0.9057 (Table 1).

### 3.2. Estimation of berberine content using HPTLC

The peak purity of berberine marker was confirmed by comparing the spectra at three different levels, i.e. start, middle, and end positions of the bands. The berberine (Fig. 1) content was

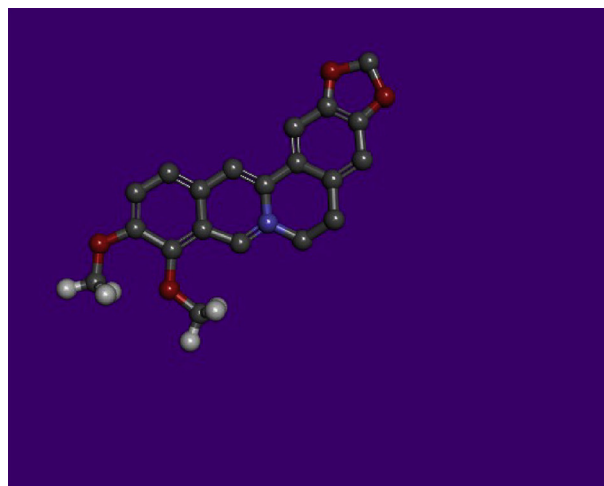


Fig. 1. Three dimensional structure of berberine (Drawn in Discovery studio).

Table 1

Total phenol, flavonoid and berberine content in various TF extracts from different elevations.

Location & Altitude	Extracts	Total phenol (GAE/g extract)	Total flavonoids (RE/g extract)	Berberine content ( $\mu\text{g}/\text{mg}$ ) of crude sample
Kedarkantha (3086 m)	95% ethanolic	26.57 $\pm$ 2.32	8.28 $\pm$ 1.24	7.37 $\pm$ 0.54
	50% ethanolic	18.4 $\pm$ 2.80	6.36 $\pm$ 1.26	6.84 $\pm$ 0.78
	Aqueous	1.54 $\pm$ 0.56	5.64 $\pm$ 1.82	5.1 $\pm$ 0.88
Taluka (2111 m)	95% ethanolic	25.42 $\pm$ 3.28	7.96 $\pm$ 1.24	7.92 $\pm$ 0.85
	50% ethanolic	16.8 $\pm$ 1.84	6.02 $\pm$ 0.84	7.24 $\pm$ 0.15
	Aqueous	1.4 $\pm$ 0.82	5.28 $\pm$ 0.52	5.74 $\pm$ 0.94
Sankri (2031 m)	95% ethanolic	24.7 $\pm$ 2.54	7.2 $\pm$ 1.02	9.76 $\pm$ 1.06
	50% ethanolic	15.62 $\pm$ 1.28	5.63 $\pm$ 0.82	9.42 $\pm$ 1.04
	Aqueous	1.24 $\pm$ 0.72	5.09 $\pm$ 1.05	6.80 $\pm$ 1.02
Bhowali (1709 m)	95% ethanolic	21.27 $\pm$ 2.52	6.94 $\pm$ 1.24	11.1 $\pm$ 1.06
	50% ethanolic	12.52 $\pm$ 1.56	5.24 $\pm$ 1.24	10.8 $\pm$ 1.12
	Aqueous	1.01 $\pm$ 0.23	4.82 $\pm$ .96	7.84 $\pm$ 1.07

estimated using linear regression of HPTLC densitometry in ethanolic, 50% aqueous ethanolic and aqueous extracts of TF collected from various altitudes. The linear regression equation was  $Y = 64.503 + 0.877 * X$  with  $r = 0.994$ . The HPTLC results showed the higher content of berberine in ethanolic extracts while low berberine content was observed in the case of aqueous extracts (Fig. 2). Berberine content in various extracts ranged from 5.09 to 11.1  $\mu\text{g}/\text{mg}$  of crude powder (Table 1). There was a decrease in berberine content with increase in altitude as evident from the Pearson's correlation coefficient,  $-0.912$  ( $p < 0.05$ ).

### 3.3. Antioxidant activity

#### 3.3.1. Scavenging effect on DPPH free radical

A direct correlation was observed between the total phenol content and DPPH free radical scavenging activity of the samples ( $r^2 = 0.6741$ ). The ethanolic extracts scavenged free radicals more effectively as compared to water extracts. In general, the extracts of TF from higher altitude scavenged free radicals more efficiently than samples from a lower altitude, Pearson's correlation coefficient being 0.981; TF extracts from Kedarkantha (3086 m) showed significantly higher scavenging potential as compared to extracts from Bhowali (1709 m) (Table 2). The  $\text{IC}_{50}$  values for TF extracts

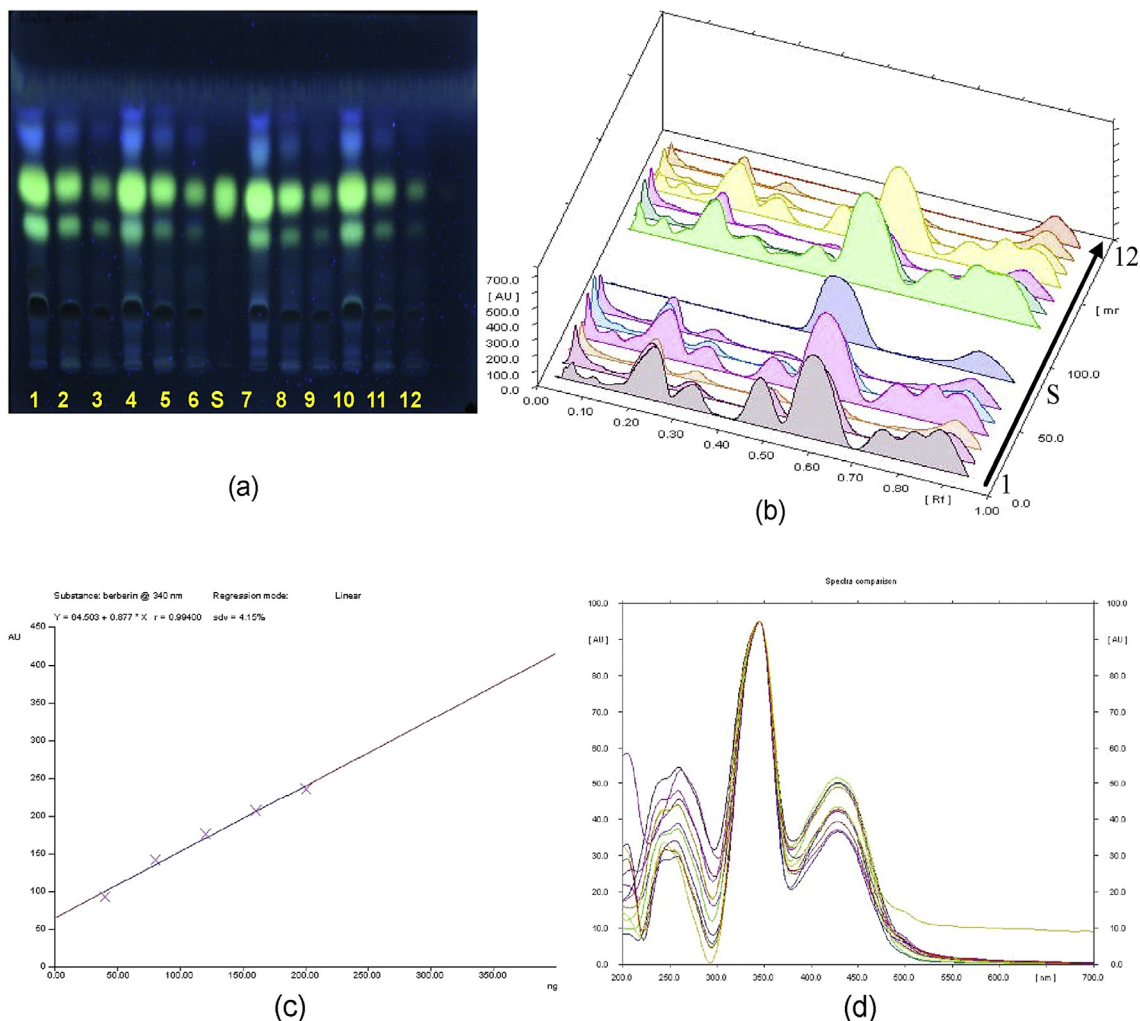
ranged between 24.879  $\mu\text{g}/\text{ml}$  to 295.82  $\mu\text{g}/\text{ml}$ , while berberine standard showed  $\text{IC}_{50}$  of 100.525  $\mu\text{g}/\text{ml}$ , while  $\text{IC}_{50}$  of ascorbic acid was 40.20  $\mu\text{g}/\text{ml}$  (Fig. 3). The DPPH free radical scavenging activity of ascorbic acid was relatively higher than berberine standard and TF extracts.

#### 3.3.2. Anti-lipid peroxidation activity

The anti-lipid peroxidation activity of berberine was higher as compared to various TF extracts. Among the TF extracts, maximum anti-lipid peroxidation activity was observed in ethanolic extracts from Bhowali and least activity was detected in water extracts of TF collected from Kedarkantha. It is to be mentioned here that there was relatively less difference in the anti-lipid peroxidation activity of ethanolic, 50% aqueous ethanolic and aqueous extracts of TF collected from same altitude. Berberine showed markedly higher anti-lipid peroxidation activity as compared to TF extracts (Table 2).

#### 3.3.3. $\beta$ -Carotene bleaching assay

Highest antioxidant potential was observed in an ethanolic extract from Bhowali (1709 m) while lowest antioxidant ability was shown by an aqueous extract from Kedarkantha (3086 m). In general, samples with high berberine content showed significant



**Fig. 2.** (a) HPTLC fingerprint profile of alcoholic, aqueous alcoholic and aqueous extracts of TF collected from different elevations along with berberine (standard); (b) Densitometric scanning profile at 340 nm; (c) Linear regression graph of berberine; (d) Overlaid spectra of berberine and the corresponding peaks in the respective extracts. Lanes: 1–3, Kedarkantha; 4–6 Taluka; 7–9 Bhowali; 10–12 Sankri; S, Standard marker berberine. Samples 1,4,7,10: ethanolic extracts; samples 2,5,8,11: 50% aqueous ethanolic extracts; samples 3,6,9,12: aqueous extracts.

**Table 2**Antioxidant activity of various extracts of TF<sup>e</sup> collected from different altitudes. The results are expressed as Mean ± SE of three determinations.

Collection site	Extract	RP <sup>a</sup> (absorbance @ 100 µg/ml)	BCB <sup>b</sup> (%inhibition @ 100 µg/ml)	ALP <sup>c</sup> (%inhibition @ 100 µg/ml)	DPPH <sup>d</sup> (%inhibition @ 100 µg/ml)
Kedarkantha (3086 m)	K95% ethanolic	0.406 ± 0.05	52.6 ± 2.52	20.34 ± 3.48	62.82 ± 5.24
	50% aqueous ethanolic	0.362 ± 0.06	37.82 ± 2.68	11.4 ± 2.42	55.2 ± 3.82
	Aqueous	0.228 ± 0.04	36.12 ± 1.46	9.8 ± 2.03	52.36 ± 4.34
Taluka (2111 m)	95% ethanolic	0.438 ± 0.08	56.82 ± 3.38	22.4 ± 2.42	56.2 ± 5.02
	50% aqueous ethanolic	0.427 ± 0.07	38.32 ± 2.82	11.64 ± 1.63	42.6 ± 4.83
	Aqueous	0.256 ± 0.06	39.68 ± 3.02	10.2 ± 1.02	42.34 ± 3.42
Sankri (2031 m)	95% ethanolic	0.496 ± 0.05	60.8 ± 4.24	24.82 ± 2.12	53.68 ± 5.47
	50% aqueous ethanolic	0.456 ± 0.08	42.6 ± 2.68	11.82 ± 1.42	38.32 ± 2.31
	Aqueous	0.279 ± 0.04	42.34 ± 4.84	10.4 ± 1.04	39.68 ± 2.82
Bhowali (1709 m)	95% ethanolic	0.724 ± 0.08	62.48 ± 5.34	25.71 ± 3.52	53.72 ± 2.62
	50% aqueous ethanolic	0.578 ± 0.08	55.2 ± 5.82	12.3 ± 1.24	37.82 ± 1.82
	Aqueous	0.32 ± 0.06	52.36 ± 4.02	11.4 ± 1.52	36.12 ± 2.14

<sup>a</sup> RP- Reducing power. <sup>b</sup> BCB- Beta carotene bleaching. <sup>c</sup> ALP- Anti-lipid peroxidation. <sup>d</sup> DPPH- 2,2-diphenyl-1-picrylhydrazyl. <sup>e</sup> TF- *Thalictrum foliolosum*.

activity in a concentration dependent manner as compared to samples with low berberine content,  $r^2 = 0.8956$  (Table 2).

### 3.3.4. Reducing power assay

The ability to reduce ferric ions was recorded highest in berberine which was closely followed by ascorbic acid and ethanolic extract of TF collected from Bhowali. Among the extracts types, the ethanolic extract possessed maximum reducing power while the aqueous extracts showed moderately low reducing power. The reducing power followed the trend similar to anti-lipid peroxidation activity, where low altitude TF extracts possessed potential reducing abilities as compared to high altitude extracts, Pearson's correlation coefficient was  $-0.842$  (Table 3). Additionally, 95% ethanolic extracts of TF with high berberine content showed greater reducing ability as compared to extracts with low berberine content ( $r^2 = 0.9082$ ).

### 3.4. Antimicrobial activity and determination of minimum inhibitory concentration

Susceptibility of microbes was tested (disc diffusion model) against various extracts (ethanolic, 50% aqueous ethanolic and aqueous) of TF samples collected from different elevations. All the extracts showed moderate to significant inhibition of microbial growth, however, the ethanolic extracts were found to be most effective (Table 4). The ethanolic extracts were thus studied further

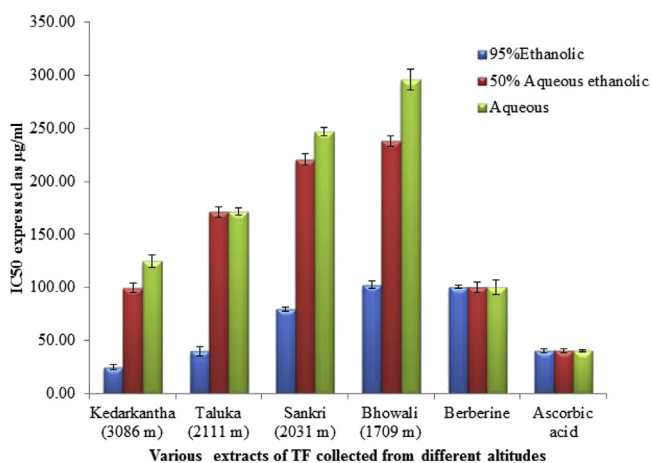
to determine the MIC using broth dilution assay. The ethanolic extract of TF sample from Bhowali showed maximum activity against *C. albicans* and *S. aureus*. Similar results were obtained for berberine standard. The ethanolic extracts of TF samples collected from Kedarkantha, most effectively inhibited the growth of *E. coli* and *P. aeruginosa* (Table 5). We further proved the results using Pearson's correlation matrix (XLSTAT software), wherein the antimicrobial effect on *S. aureus* and *C. albicans* was closely related to berberine content of the extracts, correlation constant between MIC and berberine content being  $-0.801$  ( $p < 0.005$ ,  $r^2 = 0.742$ ). On the contrary, the antimicrobial effect on *E. coli* and *P. aeruginosa* was closely related to phenol content, correlation constant between MIC and phenol content being  $-0.608$  ( $p < 0.005$ ).

### 3.5. Statistical analysis

The correlational analysis of different biochemical parameters with increase in altitude was successfully established through Pearson correlation coefficient. Clearly, there was an increase in the total phenol and flavonoid content and DPPH free radical scavenging property of TF, with increase in altitude. In contrast, a decrease in berberine content, reducing power, beta-carotene bleaching and anti-lipid peroxidation was observed with increase in altitude (Table 3).

## 4. Discussion

The increasing demand of therapeutically important secondary metabolites can be securely met if we are able to map the variation in their content in plants owing to change in seasons and altitudes [28–30]. Thus the evaluation of altitudinal variation in plant secondary metabolites, triggered by ecologic factors varying with the altitude of the growing site, is very important [3]. Though there have been numerous studies on the secondary metabolite content and related therapeutic potentials of medicinal plants, important



**Fig. 3.** The concentration of extracts that can scavenge 50% DPPH free radicals, expressed as IC<sub>50</sub> (µg/ml).

**Table 3**

Correlational analysis of different biochemical parameters with increase in altitude.

Total phenol	0.854294113
Total flavonoids	0.905731139
Berberine content	-0.907267061
Reducing power	-0.842266956
Beta carotene bleaching	-0.822363783
Anti-lipid peroxidation	-0.873738158
DPPH free radical scavenging	0.98100909

**Table 4**  
Antimicrobial activity of 95% ethanolic extract of TF collected from various elevations. Values expressed as, zone of inhibition (Mean  $\pm$  SD, n = 3).

	Concentration ( $\mu$ g/ml)	Bhowali (1709 m)	Sankri (2031 m)	Taluka (2111 m)	Kedarkantha (3086 m)	Berberine
<i>Candida albicans</i> (ATCC10231)	20	–	–	–	–	8.2 $\pm$ 1.2 mm
	40	10.2 $\pm$ 2.4 mm	–	–	–	9.5 $\pm$ 2.4 mm
	60	13.5 $\pm$ 2.2 mm	–	–	–	15.5 $\pm$ 2.5 mm
	80	16 $\pm$ 3.5 mm	8.8 $\pm$ 0.8 mm	8.2 $\pm$ 1.02 mm	8 $\pm$ 0.5 mm	19 $\pm$ 2.5 mm
<i>Staphylococcus aureus</i> (ATCC 4944)	20	–	–	–	–	8.5 $\pm$ 1.02 mm
	40	10.5 $\pm$ 2.5 mm	–	–	–	10.5 $\pm$ 0.8 mm
	60	15.4 $\pm$ 3.2 mm	–	–	–	16 $\pm$ 1.02 mm
	80	20.5 $\pm$ 3.4 mm	9 $\pm$ 1.02 mm	8.5 $\pm$ 0.8 mm	8.2 $\pm$ 0.8 mm	21.5 $\pm$ 3.2 mm
<i>Escherichia coli</i> (ATCC 25922)	20	–	–	–	–	–
	40	–	–	8.4 $\pm$ 1.2 mm	8.4 $\pm$ 1.02 mm	8.2 $\pm$ 1.02 mm
	60	–	–	10.5 $\pm$ 1.2 mm	12.5 $\pm$ 1.2 mm	10.5 $\pm$ 1.2 mm
	80	8.5 $\pm$ 1.2 mm	8 $\pm$ 0.8 mm	15.2 $\pm$ 2.4 mm	16.8 $\pm$ 2.4 mm	13.5 $\pm$ 2.4 mm
<i>Pseudomonas aeruginosa</i> (MTCC 424)	20	–	–	–	–	8 $\pm$ 1.02 mm
	40	–	–	8 $\pm$ 1.02 mm	8.2 $\pm$ 1.4 mm	12.4 $\pm$ 2.4 mm
	60	–	–	10.2 $\pm$ 1.2 mm	11.8 $\pm$ 2.4 mm	16.5 $\pm$ 2.4 mm
	80	9.2 $\pm$ 1.2 mm	8.2 $\pm$ 0.5 mm	14.8 $\pm$ 2.4 mm	15.6 $\pm$ 2.4 mm	19.2 $\pm$ 2.4 mm

factors like the effect of seasonal and altitudinal variation on secondary metabolite production still need to be studied in detail.

Alkaloids, mainly belonging to the class of isoquinoline alkaloids are the major secondary metabolites found in *Thalictrum* species; much of the therapeutic potential of the plant can thus be attributed to them. There exists a compelling evidence about the increasing pressures caused by herbivores at lower elevations; hence the increase in alkaloid content may be regarded an obvious defense measure adopted by the plants at lower elevations [28,31]. Berberine is one of the major alkaloid reported in TF [13]; hence a correlation between the berberine content and related medicinal properties would be helpful in setting the quality parameters of the plant. The berberine content in TF varied inversely with altitude in all type of extracts i.e. ethanolic, aqueous ethanolic and aqueous extracts. The maximum berberine content was obtained in the samples collected from Bhowali (1709 m) as compared to Kedarkantha (3086 m). Similar findings were reported in *Berberis* species collected from different altitudes in Garhwal, Himalaya i.e. lowland species and types were found to have considerably higher berberine content in all plant parts than sub-alpine and alpine species [32,33].

On the contrary, a direct relation was observed between the total phenol and flavonoid content and the altitude. Li et al. [34], Jaakola and Hohtola [35] and many subsequent workers propounded that plants at higher altitudes face higher UV-B radiations which have pleiotropic effects on plant development, morphology, and physiology; the most effective protection mechanism stimulated under this light regime is the biosynthesis of flavonoids and phenols [36]. This explains the higher phenol and flavonoid content in TF of higher altitude as compared to its lower altitude counterparts.

The TF extracts showed moderate to significant inhibition of microbial growth; however, the ethanolic extracts were found to be most effective. Previous reports on *T. foliolosum* and other plants of high altitude area showed the higher antimicrobial activity of alcoholic extracts as compared to water extracts [18,19]. The

ethanolic extracts were thus studied further to determine the MIC using broth dilution assay. The ethanolic extract of TF sample from Bhowali showed maximum activity against *C. albicans* and *S. aureus*. Similar results were obtained for berberine standard. The ethanolic extracts of TF samples collected from Kedarkantha, most effectively inhibited the growth of *E. coli* and *P. aeruginosa*. Thus, extracts with high berberine content were effective against *C. albicans*, *S. aureus*; similar activity was observed in the case of Berberine. The results are concomitant with the earlier reports [37,38]. Berberine has a long history of medicinal use in traditional Chinese, Native American and Indian medicines. Berberine extracts show significant antimicrobial activity against bacteria, viruses, and fungi. The potential mechanisms of its antimicrobial activity include the suppression of cell adhesion and migration and inhibition of microbial enzymes. Recent literature reports have demonstrated that its activity against Gram-positive bacteria mainly targets the cell division protein FtsZ [39]. Another important aspect of the antimicrobial mechanism of berberine is that it non-cooperatively binds to the microbial DNA and is not a potent genotoxic or mutagenic agent [40]. In the case of *E. coli* and *P. aeruginosa*, TF extracts collected from higher elevations (Kedarkantha, 3086 m & Taluka, 2111 m) were found to be more effective. This indicates a possible synergism between phenols and flavonoids along with berberine for the demonstrated anti-microbial effect.

Antioxidant potential of TF was correlated with the secondary metabolite content through various mechanisms which include inhibiting lipid peroxidation, binding metal ions and scavenging of free radicals. Lipid peroxidation has been implicated in the pathogenesis of a number of diseases and clinical conditions which include diabetes, chronic inflammatory diseases, fibrosis, cancer etc. [6,41]. Malondialdehyde formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of lipid peroxidation reaction. Our results suggest an active role of berberine in anti-lipid peroxidation activity of the plant wherein extracts with higher berberine content inhibited lipid peroxidation significantly while extracts with lower berberine

**Table 5**  
MIC expressed as  $\mu$ g/ml of 95% ethanolic extracts, berberine and positive controls.

	Bhowali (1709 m)	Sankri (2031 m)	Taluka (2111 m)	Kedarkantha (3086 m)	Berberine	Gentamycin	Streptomycin
<i>Candida albicans</i> (ATCC10231)	39.07 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	19.51 $\mu$ g/ml	19.51 $\mu$ g/ml	9.70 $\mu$ g/ml
<i>Staphylococcus aureus</i> (ATCC 4944)	39.07 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	19.51 $\mu$ g/ml	19.51 $\mu$ g/ml	19.51 $\mu$ g/ml
<i>Escherichia coli</i> (ATCC 25922)	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	39.07 $\mu$ g/ml	39.06 $\mu$ g/ml	39.06 $\mu$ g/ml	19.51 $\mu$ g/ml
<i>Pseudomonas aeruginosa</i> (MTCC 424)	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	78.13 $\mu$ g/ml	39.07 $\mu$ g/ml	39.06 $\mu$ g/ml	19.51 $\mu$ g/ml	19.51 $\mu$ g/ml

content were relatively weak in inhibiting lipid peroxidation. Reaction of cell membrane phospholipids with polyunsaturated fatty acid moieties produces lipid hydroperoxide that decomposes to produce alkoxy and peroxy radical, which eventually yield numerous carbonyl products such as malondialdehyde. The carbonyl products are responsible for DNA damage, generation of cancer and aging related disease [42]. The decrease in the concentration of malondialdehyde level with increase in the concentration of berberine indicates the antioxidant role of berberine; consequently the extracts from lower altitudes were more active as compared to extracts from higher altitude. The antioxidant property of the plant could also be attributed to the presence of alkaloids like thalictroline, palmatine and jatrorrhizine [43]. Further, reducing power is concomitant with antioxidant activity and may serve as an important reflection of the antioxidant activity. Our results indicate that reducing ability of TF extracts may have positive correlation with berberine content in the plants, while it is inversely related with altitude. Earlier studies have also confirmed the high reducing potential of berberine and other alkaloids [42,43]. The antioxidant activity of carotenoids is based on radical adducts of carotenoids with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated  $\beta$ -carotene. Presence of different antioxidants can hinder the extent of  $\beta$ -carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system [44]. Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their color, and thus absorbance, for a longer period of time. Antioxidant activities of berberine and TF extracts increased with their increasing concentration; highest activity observed in berberine and TF extracts from Bhowali. It is probable that the antioxidative components in the TF extracts can reduce the extent of  $\beta$ -carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system. Both phenols and alkaloids are known to scavenge free radicals generated in case of  $\beta$ -carotene bleaching assay; however, the results of the current study suggest that alkaloids may be more effective in scavenging linoleate free radicals.

The DPPH free radical scavenging activity was concentration dependent and TF extracts with higher phenol content scavenged free radicals more effectively. Earlier studies have reported the presence of protocatechuic acid, caffeic acid, and p-coumaric acids in *Thalictrum* [45]. The TF extracts from Kedarkantha (3086 m) and Sankri (2031 m) showed significantly higher free radical scavenging activity as compared to TF extracts from a lower altitude. In all the models tested, the antioxidant activity of berberine was studied in relation to that of ascorbic acid, a known standard potent antioxidant. Overall, the TF extracts revealed strong antioxidant properties. There was indeed a positive correlation observed between the berberine content and reducing power, lipid peroxidation, and  $\beta$ -carotene bleaching abilities, thus indicating the involvement of berberine in strengthening the antioxidant mechanism of TF. However, the effect of an increase in berberine content of TF at lower elevations is suggestive in its significant antioxidant properties. Similarly, the direct correlation between the phenol and flavonoid content and DPPH free radical scavenging activity indicates the active role of phenols in the robust antioxidant efficacy of the plant.

## 5. Conclusion

The current study has established certain facts; firstly, the berberine content increases with a decrease in altitude; secondly, the plant from lower altitude can be explored as an alternate source of berberine; thirdly, *T. foliolosum* has effective antioxidant and

antimicrobial properties, hence positioning it as a promising therapeutic agent. Further studies require the assessment of berberine content in aerial parts of the plant which may ensure a more judicious use of the plant for harvesting secondary metabolites.

## Sources of funding

The funding to carry out the experimental work was provided by CSIR project “Bioprospection of plants and other natural products” (BSC-106).

## Conflict of interest

None.

## Acknowledgements

The authors extend sincere thanks to the Director CSIR-NBRI, for providing the necessary infrastructure to carry out experiments.

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