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Variation in Antioxidant Activity and Antioxidant Constituents of *Thymus Serpyllum* L. Grown in Different Climatic Conditions of Uttarakhand Himalayas

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ABSTRACT

In-vitro antioxidant activity of therapeutically important plant *Thymus serpyllum* L. grown at different altitudes viz. foothill areas, lower Himalayan and higher Himalayan regions were evaluated against various radicals such as DPPH, ABTS and reducing power assays. The antioxidant constituents like total phenols, flavonoids and total tannins were also evaluated in the present study. The results revealed that the alcoholic, as well as the aqueous extracts of the plant, showed significant antioxidant potential against all the radicals. Extracts obtained from different altitude cultivation showed variation in IC_{50} values. The alcoholic and aqueous extracts prepared from the plants grown at foothill areas (Haldwani) showed the highest DPPH (IC_{50}: ALC-0.566 mg/ml; AQ- 0.778 mg/ml), ABTS (IC_{50}: ALC-0.484 mg/ml; AQ- 0.533 mg/ml) and reducing power (EC_{50}: ALC- 0.29 mg/ml; 0.42 mg/ml) activities, followed by high altitude cultivated plants, (Auli), with moderate antioxidant activity. The extracts from mid-altitude cultivation (Pithoragarh) exhibited the least antioxidant potential. The results showed that the amount of total phenolics and flavonoids were significantly correlated to the antioxidant activity. Higher the value of phenolics (TS3: 12.63mg CE/g> TS1: 11.51 mg CE/g> TS2: 10.70 mg CE/g) and flavonoids (TS3: 9.30 mg QE/g> TS1: 9.07mg QE/g> TS2: 6.59mg QE/g) in the extracts, greater was the antioxidant activity. Therefore, *T. serpyllum* grown in foothill areas was more beneficial in preparing various herbal formulations.

Keywords: Antioxidant; *Thymus serpyllum* L.; DPPH; ABTS; Reducing power; IC_{50}

1. INTRODUCTION

The toxicity caused by synthetic antioxidants such as butylated hydroxytoluene (BHT), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and butylated hydroxyanisole (BHA) has diverted the attention of consumers towards natural antioxidants that are of plant origin i.e. essential oils (EOs) and plant extracts[1-3] and can protect the brain tissues of the human body from the oxidative damage caused by free radicals. Extracts prepared from aromatic plants can be used as food preservatives to increase their shelf lives and in preparing various herbal formulations. Considering the use of such aromatic plants in multi-disciplinary areas, there is an urge for such germplasm conservation through traditional cultivation methods. The plant under study is an aromatic plant of the Himalayan region i.e. *Thymus serpyllum* L. (Family: Lamiaceae). Traditionally, the shrub is used to prepare herbal formulations such as cough syrups, oils, tinctures and infusions[4]. The aerial parts of the plant are also used as condiments, spices and herbal tea[5]. *Thymus* species also show antiviral, antifungal, anti-parasitic, antioxidant and antimicrobial activities[5-7]. These properties are attributed to the terpenoid constituents present in the oil such as thymol, carvacrol, linalool, p-cymene, etc. while phenolic acid (rosmarinic acid) and flavonoids (quercetin, eriocitrin, luteolin, apigenin) are the polyphenolic compounds which are considered to be responsible for the antioxidant effects of aqueous tea infusions[8-9]. The Physician's Desk Reference (PDR) for herbal medicines proposed that wild thyme possesses antitussive effects and is used in various standardised preparations. Along with this, medicines made from alcoholic extracts are used in treating cold and coughs[10].

Considering its biological characteristics and utilisation in food and beverage industries, the comparative study was carried out in the present work to evaluate the effect of altitude on the antioxidant potential of the methanolic and aqueous extracts of this plant cultivated in different agro-climatic zones such as the Higher Himalayas (Auli; 9000 feet asl), Lower Himalayas (Pithoragarh; 5000 feet asl) and at the Foothill areas (Haldwani; 1350 feet asl) under natural climatic conditions.

2. MATERIALS AND METHODS

2.1 Crop Experimental Design

The plant material was collected from Chamoli district, Uttarakhand situated at an altitude of 10170 feet above sea level (asl) (Latitude 30°41'N, Longitude 79°54'E). The plant specimen was identified from the Botanical Survey of India (BSI), Dehradun with an accession number of 118592. After propagating the plants for 2 years at the Defence Institute of

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Bio-Energy Research (DIBER) Field station, Pithoragarh, the saplings were planted (spacing 20 x 20 m^2) in open field condition at DIBER Field station of Auli (TS1; longitude 79.57 °E; latitude 30.52 °N; Altitude 9000 feet asl), Pithoragarh (TS2; longitude 80.22 °E; latitude 29.58 °N; Altitude 5000 feet asl) and Haldwani (TS3; longitude 79.52 °E; latitude 29.22 °N; Altitude 1350 feet asl). The agronomical practices were followed from time to time. 20 t ha^{-1} of Farmyard manure (FYM) was also added in the experimental plots at the time of plantation (March 2018 for TS2 and TS3; April 2018 for TS1) to maintain the soil nutrients. The plants were harvested at their respective flowering stage (July 2018 for TS2 and TS3; August 2018 for TS1), washed, shade dried, powdered and stored in zipped bags for further studies.

2.2 Preparation of Extracts

50 grams of powdered plant material was soaked in 250 methanol (ALC) and double-distilled water (AQ) separately. The solution was placed in an incubator shaker for 24 h. It was centrifuged at 2000 rpm for 10 minutes and filtered using Whatman filter paper. The solvent was evaporated to dryness on the water bath maintained at 60 °C. The dried extracts were weighed accurately and the yield of the extracts was calculated by the following equation:

$$\%\,Yield\ of\ crude\ extract = \frac{Weight\ of\ residue\ obtained\ after\ evaporation}{Weight\ of\ plant\ powder} \times 100$$

2.3 Evaluation of Antioxidant Activity

Antioxidant activity of the extracts was evaluated by measuring the free radical scavenging activity (FRSA) of various radicals such as DPPH (2, 2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and RP (reducing power).

2.3.1 DPPH Radical Scavenging Activity

DPPH is a long-lived stable free radical having deep purple color and is used to evaluate the scavenging activity of *T. serpyllum* methanolic and aqueous extracts[11].

2.3.1.1 Preparation of Reagents

DPPH (0.135 m M) was freshly prepared by dissolving 0.0045 g of DPPH in a small quantity of methanol (MeOH) and the final volume was make up to 100 ml with MeOH in dark. Standard ascorbic acid (AA) was prepared by dissolving 10 mg of AA in a small amount of distilled water and a final volume of 100 ml was made by adding more water.

2.3.1.2 Procedure

Different aliquots of the plant extracts (10 µl, 20 µl, 30 µl, 40 µl, 50 µl) were taken and make the volume up to 1 ml with water. 2 ml DPPH solution was added. The assays were mixed thoroughly, incubated in the dark for 30 min. and the absorbance of the solution was measured at 517 nm with a UV-Visible spectrophotometer (Labindia UV3000) against methanol as a blank. AA was taken as standard with a concentration range of 0, 100, 200, 300, 400 and 500 µl.

IC_{50} values (concentrations that show 50 per cent of radical scavenging activity) were also calculated using plots between free radical scavenging activity (FRSA) versus concentrations of the extracts. DPPH scavenging activity was evaluated by the following equation:

$$FRSA\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ = Absorbance of the control
A_{sample} =Absorbance of the sample

2.3.2 ABTS Radical Scavenging Activity

The blue-green cation radical ($ABTS^{+}$), generated by the reaction between ABTS and potassium persulfate solution, is reduced (decolorise) by the antioxidant compound and the decolorisation can be measured at 734 nm with a UV-Visible spectrophotometer (Labindia UV3000)[12].

2.3.2.1 Preparation of Reagents

- Solution a: 0.1920 g ABTS was dissolved in a small amount of distilled water and the volume of the solution was finally made up to 50 ml with water.
- Solution b: (Potassium persulfate solution; 2.45 mM) 0.3311 g of potassium persulfate ($K_2S_2O_8$) was dissolved in a small amount of distilled water and the volume of the solution was finally made up to 50 ml with water.

These solutions (a+b) were mixed in a 100 ml volumetric flask, covered with silver foil and kept in the dark overnight. This was ABTS reagent.

Standard ascorbic acid (AA) was prepared by dissolving 10 mg of AA in a small amount of distilled water and a final volume of 100 ml was made by adding more water.

2.3.2.2 Procedure

In each aliquot of the plant extracts (10, 20, 30, 40 and 50 µl) and makeup to 1 ml with distilled water, 1 ml of ABTS solution was added and vortexed at 1000 rpm for 5 minutes. The solutions were incubated in the dark for 7 minutes and absorbance was measured at 734 nm against MeOH as a blank. Ascorbic acid was used as standard with a concentration range of 0 µl, 100 µl, 200 µl, 300 µl, 400 µl and 500 µl. IC_{50} values were calculated and compared for each extract and standard. ABTS scavenging activity was evaluated by using the following equation:

$$FRSA\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ = Absorbance of the control
A_{sample} =Absorbance of the sample

2.3.3 Reducing Power

In this method, Fe (III) gets reduced to Fe (II) by the action of antioxidant compound[13].

2.3.3.1 Reagent Preparation

- 0.2 M phosphate buffer (pH 6.6): In a 1 L volumetric

flask, add 8 g of sodium chloride, 1.44 g of disodium hydrogen phosphate, 0.2 g of potassium chloride, 0.24 g of potassium dihydrogen phosphate and 800 mL of distilled water. Adjust the pH 6.6 of the solution using hydrochloric acid and volume with distilled water.

- 1 % (w/v) potassium hexacyanoferrate ($K_3[Fe(CN)_6]$): 0.5 g of $K_3[Fe(CN)_6]$) was weighed and dissolved in distilled water to obtain 50 ml of the total solution.
- 10 % (w/v) Trichloroacetic acid (TCA): 5 ml of TCA was dissolved in distilled water to obtain 50 ml of the total solution.
- 0.1 % (w/v) Ferric chloride ($FeCl_3$) solution: 0.1 g Fe Cl_3 was dissolved in distilled water to obtain 100 ml of the total solution.
- 0.1 mg/ml Ascorbic acid (AA): 10 mg of AA dissolved in a small amount of distilled water and a final volume of 100 ml was made by adding more water.

2.3.3.2 Procedure

For this, 2.5 ml of phosphate buffer was mixed with 1 ml of each concentration of the samples (10 µl, 20 µl, 30 µl, 40 µl and 50 µl), 2.5 ml of potassium hexacyanoferrate solution was added to it and incubated at 50 °C for 20 min. After that, 2.5 ml TCA solution was added and the assays were centrifuged at 3000 rpm for 20 min. Afterward, 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and then the mixture was homogenised with 0.5 ml $FeCl_3$ solution. The absorbance was recorded at 700 nm with a UV-Visible spectrophotometer (Labindia UV3000) against buffer solution and EC_{50} (concentration which shows a 50 per cent decrease in the absorbance) was calculated using a plot between absorbance versus concentration of the plant extracts.

2.4 Antioxidant Constituents of Extracts

2.4.1 Total Phenolics (TP)

The phenolic content in *T. serpyllum* extracts was estimated by Folin-Ciocalteu reagent method[14] with certain modifications.

2.4.1.1 Reagent Preparation

- 10 % (v/v) Folin-Ciocalteu (FC) Reagent
  FC reagent was purchased from Sigma Eldrich and 1 ml was diluted to 10 ml with distilled water.
- 700 mM sodium carbonate solution
  7.42 g of sodium carbonate was dissolved in distilled water to obtain 100 ml of the total solution.
- Catechol standard(1 mg/ml)
  100 mg of catechol was dissolved in distilled water to obtain 100 ml of the total solution.

2.4.1.2 Procedure

Dried plant powder (100 mg) was accurately weighed, transferred to a volumetric flask and made up to the 10 ml volume with methanol. The solution was centrifuged (Eppendorf 5330R) at 10,000 rpm for 5 min. The supernatant (100 µl) was collected and reacted with 200 µl of FC reagent. The assay tubes were vortexed at 1000 rpm for 5 min and then 800 µl of sodium carbonate was added to it. The assay tubes were kept at room temperature for 2 h. The absorbance was noted at 765 nm with a UV-Visible spectrophotometer (Labindia UV3000) and the total phenolic content (mg catechol equivalent/g; mg CE/g) was calculated from a standard curve of catechol (Fig. 1).

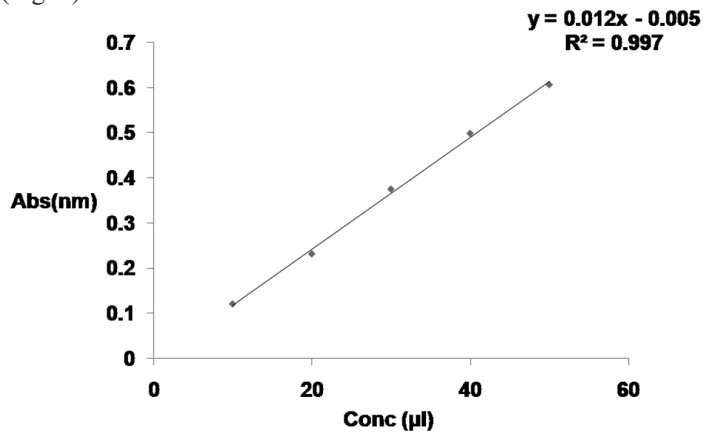

Figure 1. Standard curve of catechol at 765 nm.

2.4.2 Total Flavonoids (TF)

The flavonoid content in *T. serpyllum* was determined according to the method of Chang et al (2002)[15].

2.4.2.1 Reagent Preparation

- 80 per cent methanol: 40ml methanol was diluted to 50ml with distilled water.
- 10 per cent Aluminium chloride ($AlCl_3$): 1 g of $AlCl_3$ was dissolved in distilled water to obtain 10 ml of the total solution.
- 1 M potassium acetate (CH_3COOK) solution: 0.981 g of CH_3COOK was dissolved in distilled water to obtain 10 ml of the total solution.
- Standard solution of quercetin: 100 mg of quercetin was dissolved in distilled water to obtain 100 ml of the total solution.

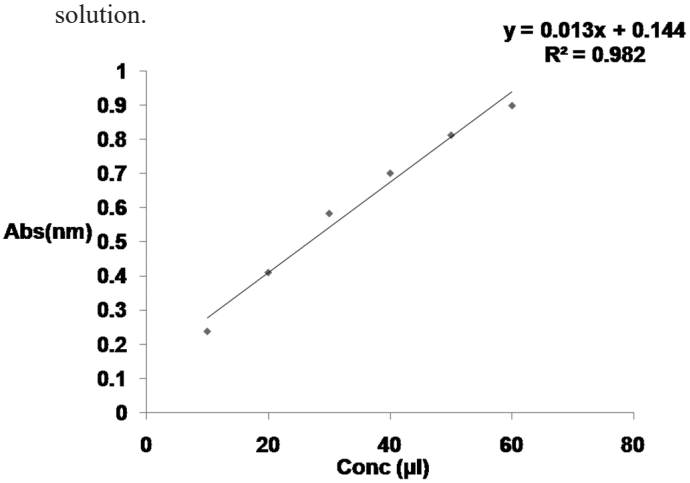

Figure 2. Standard curve of quercetin at 415 nm.

2.4.2.2 Procedure

Dried plant powder (100 mg) was accurately weighed and made up to the volume of 10 ml with methanol. The solution was centrifuged (Eppendorf 5330R) at 10,000 rpm for 5 min. From it, 0.5 ml of the supernatant was taken in an assay tube and 1.5 ml methanol was added to it. Then, 0.1 ml

10 per cent $AlCl_3$, 0.1 ml of CH_3COOK and 2.8 ml distilled water were added to the tube. The resulting sample tubes were incubated for 30 min and finally, absorbance was taken at 415 nm with a UV-Visible spectrophotometer (Labindia UV3000). Total flavonoid content (mg quercetin/g extract; mg QE/g) was calculated using a standard curve of quercetin (Fig. 2).

2.4.3 Total Tannins (TT)

Total tannins in *T. serpyllum* extracts were estimated by the Folin-Denis method[16].

2.4.3.1 Reagent Preparation

- Folin Denis Reagent: In 750 ml distilled water, 100 g sodium tungstate and 20 g phosphomolybdic acid were mixed. 50 ml phosphoric acid was added to this. The whole mixture was refluxed for 2 h and then the volume was make up to 1 L with distilled water.
- Sodium carbonate solution: 350 g sodium carbonate was dissolved in distilled water to obtain 10ml of total solution at 70 °C - 80 °C. It was filtered through glasswool after allowing it to stand overnight.
- Standard tannic acid solution: 100 mg tannic acid was dissolved in distilled water to obtain 100 ml of the total solution.

2.4.3.2 Procedure

For this estimation, 0.5 g of the dried plant powder was weighed accurately and 75 ml water was added to it. The mixture was boiled for 30 min. Then, it was cooled down and centrifuged at 2000 rpm for 20 min. The supernatant was collected in a volumetric flask of 100 ml and the volume was made up with the help of distilled water. 1 ml of this mixture was transferred to a 100 ml volumetric flask containing 75 ml water. To it, 5 ml of Folin–Denis reagent and 10 ml of sodium carbonate solution were added and diluted up to 100 ml with water. After shaking, the absorbance of this solution was taken at 700 nm after 30 min. Total tannin content (mg TA/g) was measured using a standard curve of tannic acid (Fig. 3).

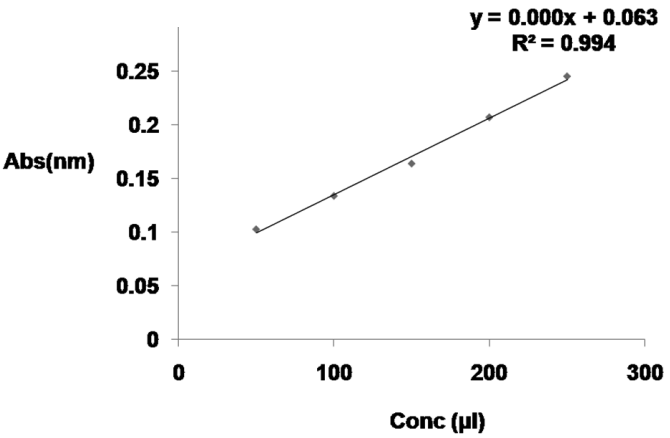

Figure 3. Standard curve of tannic acid at 700 nm.

2.5 Statistical Analysis

MS Excel 2007 was used for the calculation of standard deviation. Experimental data were processed using Origin Pro 8 (graph preparation) and SPSS 16.0 (one-way analysis of variance and correlation). The significance level of the correlation coefficient was checked on probability levels of $p<0.05$ and $p<0.01$.

3. RESULTS

3.1 Crude Extracts

Methanol extracts the higher mass of material from the aerial parts of *T. serpyllum* as compared to the water as the extraction solvent. The percentage yield ranged from 15.71 per cent to 32.53 per cent for methanol extracts and 12.33 per cent to 24.61 per cent for aqueous extracts. The methanol extract of TS3 cultivation had the highest yield (32.53 %), followed by TS2 (21.09 %) and TS1 (15.71 %) respectively. Similarly, the aqueous extracts of TS3 cultivation had the highest yield (24.61 %), followed by TS2 (17.88 %) and TS1 (12.33 %) respectively.

3.2 Antioxidant Activity and Phytoconstituents

The *in-vitro* antioxidant activity was assessed by three different methods viz. DPPH, ABTS and Reducing power assays as all the antioxidant characteristics cannot be expressed by using a single procedure. The capacity of the extracts to inhibit the DPPH, ABTS radicals and reducing power was expressed by calculating the 50 per cent scavenging activity i.e. IC_{50} (mg/ml) values for both the methanolic and aqueous extracts. The DPPH scavenging ability of methanolic as well as aqueous extracts along with ascorbic acid standard can be arranged in the decreasing order i.e. AA (IC_{50}=0.052 mg/ml) > TS3 (ALC) (IC_{50}=0.566 mg/ml) > TS1 (ALC) (IC_{50}=0.626 mg/ml) > TS2 (ALC) (IC_{50}=0.703 mg/ml) > TS1 (AQ) (IC_{50}= 0.778 mg/ml) ~TS3 (AQ) (IC_{50}=0.778 mg/ml) > TS2 (AQ) (IC_{50}= 0.787 mg/ml) (Fig. 4). The data showed that against the DPPH radical, alcoholic extracts of TS exhibited better

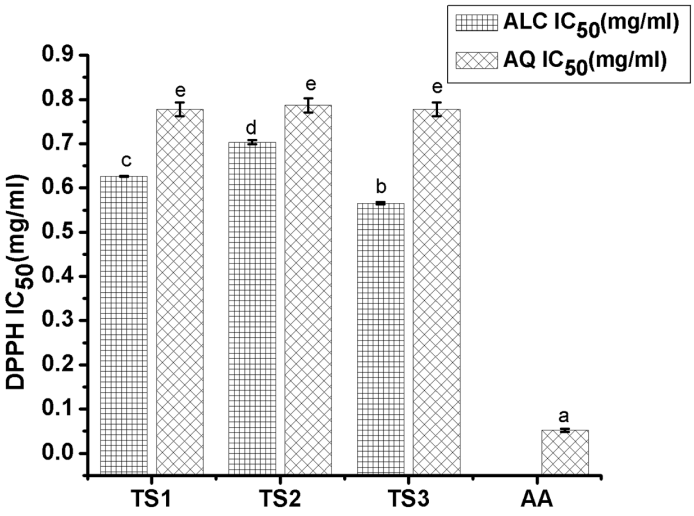

Figure 4. IC_{50} plot of DPPH radical scavenging activity of methanolic (ALC) and aqueous (AQ) extracts of *T. serpyllum* from different locations i.e. Auli (TS1), Pithoragarh (TS2), Haldwani (TS3) [The IC_{50} values with different letters (a-e) are significantly different at a significance level of $p<0.05$ according to Duncan's Multiple Range Test].

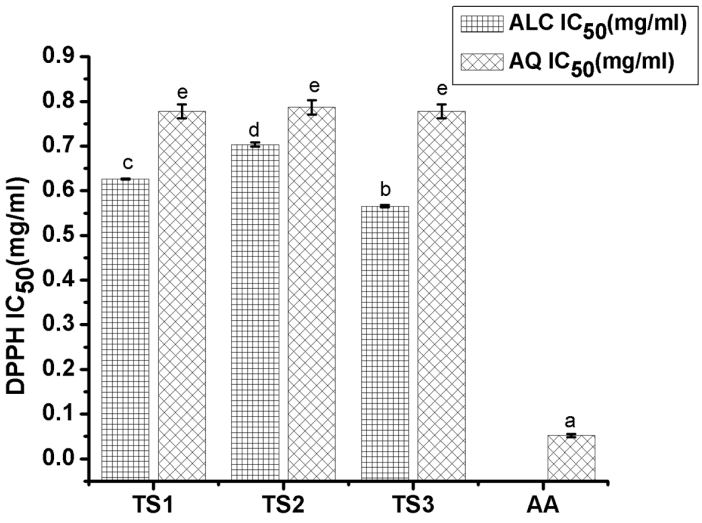

Figure 5. IC_{50} plot of ABTS radical scavenging activity of methanolic (ALC) and aqueous (AQ) extracts of *T. serpyllum* from different locations i.e. Auli (TS1), Pithoragarh (TS2), Haldwani (TS3) [The IC_{50} values with different letters (a-d) are significantly different at a significance level of p<0.05 according to Duncan's Multiple Range Test].

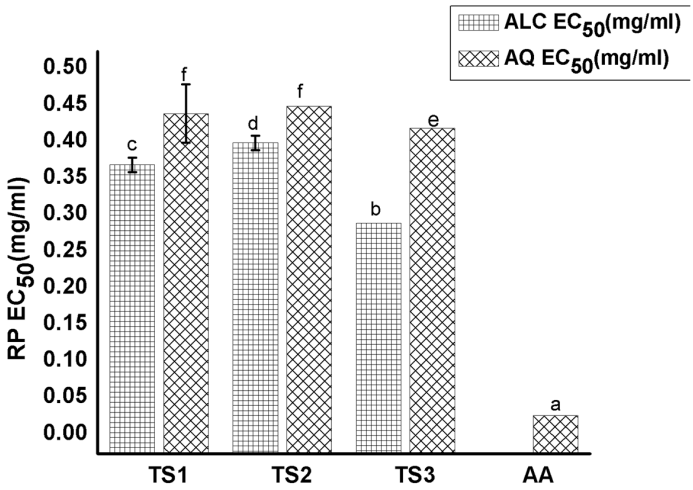

Figure 6. EC_{50} plot of Reducing power assay of methanolic (ALC) and aqueous (AQ) extracts of *T. serpyllum* from different locations i.e. Auli (TS1), Pithoragarh (TS2), Haldwani (TS3)[The EC_{50} values with different letters (a-f) are significantly different at a significance level of p<0.05 according to Duncan's Multiple Range Test].

antioxidant activity than aqueous extracts and on comparing the alcoholic extracts of different cultivations, TS3 i.e. of extract prepared from foothills cultivation showed better antioxidant potential than the other two cultivations. In the similar manner, for ABTS radical, the scavenging effect of both the alcoholic as well as aqueous extracts of TS followed the order AA (IC_{50}=0.032 mg/ml) > TS3 (ALC) (IC_{50}=0.484 mg/ml) > TS1 (ALC) (IC_{50}=0.532 mg/ml) > TS3 (AQ) (IC_{50}=0.533 mg/ml) > TS1 (AQ) (IC_{50}=0.534 mg/ml) ~ TS2 (AQ) (IC_{50}=0.534 mg/ml) > TS2 (ALC) (IC_{50}= 0.589 mg/ml) (Fig. 5). This order revealed that alcoholic extracts possessed better antioxidant activity as compared to the aqueous extracts. A difference was observed that alcoholic and aqueous extract of the mid-altitude cultivation showed the lowest antioxidant potential. The EC_{50} values for the reducing power assay of the extracts followed the order: AA (EC_{50}= 0.03 mg/ml) > TS3 (ALC) (EC_{50}= 0.29 mg/ml) > TS1 (ALC) (EC_{50}= 0.37 mg/ml) > TS2 (ALC) (EC_{50}= 0.40 mg/ml) > TS3 (AQ) (EC_{50}= 0.42 mg/ml) > TS1 (AQ) (EC_{50}= 0.44 mg/ml) > TS2 (AQ) (EC_{50}= 0.45 mg/ml (Fig. 6). The various phytoconstituents such as total phenolics, total flavonoids and total tannins were also analysed in the extracts of thyme. The total phenolic and flavonoid contents were observed highest for TS3 (12.63 mg CE/g and 9.30 mg QE/g respectively), followed by TS1 cultivation (11.51 mg CE/g and 9.07 mg QE/g) and TS2 (10.70 mg CE/g and 6.59 mg QE/g), respectively while total tannin content followed the order TS1 (3.31 mg TA/g)> TS2 (3.10 mg TA/g) and TS3 (2.97mg TA/g) (Fig. 7). The results suggested that *T. serpyllum* of foothill areas had the highest phenolics and flavonoid contents and lowest total tannin content followed by high altitude and mid-altitude samples, respectively. In our previous study on chemical constituents of *Thymus serpyllum* L., cultivated at above mentioned locations, the highest herbage yield, oil % and major constituents in the oil were obtained at the foothill cultivation i.e. Haldwani, followed by Auli and Pithoragarh, respectively[17].

3.3 Correlation Analysis

In this study, high negative significant correlations were

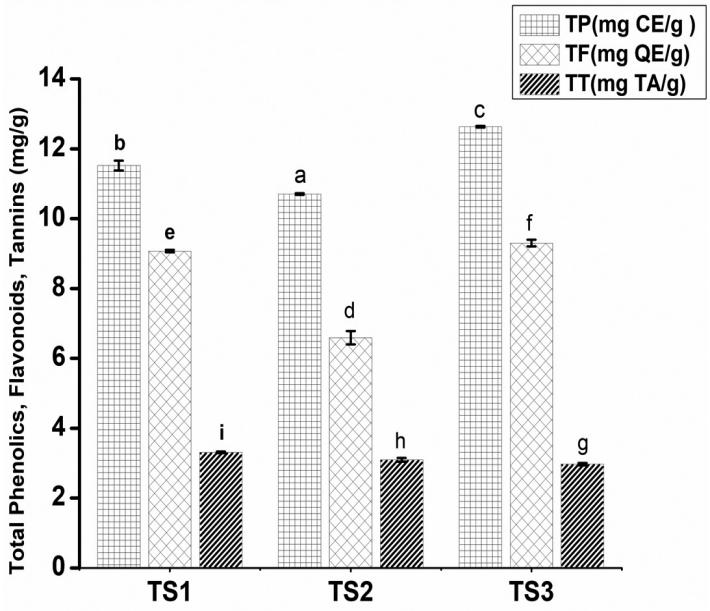

Figure 7. Chemical constituents of *T. serpyllum* from different locations i.e. Auli (TS1), Pithoragarh (TS2), Haldwani (TS3) [Total phenolics, flavonoids and tannins with different letters (a-i) are significantly different at a significance level of p<0.05 according to Duncan's Multiple Range Test].

observed between total phenolics (TP) and IC_{50} (DPPH, r^2 = -.986; p<0.01; ABTS; r^2 = -.985; p<0.01; RP, r^2 = -.963; p<0.01). Similarly, total flavonoids (TF) were also significantly and negatively correlated with the IC_{50} values (DPPH, r^2 = -.913;

p<0.01; ABTS; r^2 = -.912; p<0.01; RP, r^2 = -.704; p<0.05). These correlations suggested that the plant extracts having the high content of TP and TF exhibited high antioxidant potential (lower values of IC_{50}) (Table 1).

Table 1. Correlation between phytoconstituents and antioxidant activity (IC_{50} and EC_{50}) of *T. serpyllum* extracts

	Altitude	DPPH	ABTS	RP	TP	TF	TT
Altitude	1	.438	.443	.723*	-.550	-.050	.968**
DPPH		1	.991**	.919**	-.986**	-.913**	.325
ABTS			1	.924**	-.985**	-.912**	.330
RP				1	-.963**	-.704*	.600
TP					1	.856**	-.444
TF						1	.052
TT							1

*.Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

4. DISCUSSION

Antioxidants are known for delaying or preventing the unwanted oxidation caused by reactive oxygen species (ROS) of the biomolecules[18]. *Thymus serpyllum* is a rich source of such antioxidant compounds. The results revealed the antioxidant potential of the methanolic as well as the aqueous extracts of the thyme. Recent work was done by Sena *et al.* (2018)[19] on another species of thyme i.e. *Thymus vulgaris* to evaluate the antioxidant potential and total phenolic and flavonoid content in the extracts by different extraction procedure i.e. low-pressure solvent extraction (LPSE), soxhlet and ultrasound. IC_{50} values for all the thyme extracts were found to be almost the same (0.34 mg/ml) while the values for total phenols and total flavonoids varied with the extraction procedure. The present work compared the antioxidant potential of the extracts against different radicals. The IC_{50} values of thyme extracts against ABTS varied from 0.484 to 0.589 mg/ml. For DPPH radicals, the IC_{50} values varied in the range of 0.566 mg/ml to 0.787 mg/ml and for reducing power the values varied in the range of 0.29 mg/ml - 0.45 mg/ml. This variation could be attributed to variation in different geographic conditions such as temperature, humidity, rainfall and soil nutrients which bring a change in the chemical constituents of the plants and therefore, varying its antioxidant activity too[20]. The results are in good agreement with the work of Sena *et al.* (2018)[19]. Hmidani *et al.* (2019) also reported the ABTS assay of aqueous extracts of three thyme species (*Thymus atlanticus*, *Thymus zygis* and *Thymus satureioides*) from Morocco and observed that ascorbic acid (IC_{50}: 1.96 µg/ml) had the significantly higher antioxidant activity as compared to the aqueous extracts of these plants[21]. Another report by Chizzola *et al.* (2008) mentioned that the 60 per cent ethanolic extract of thyme had maximum antioxidant activity against DPPH radicals (IC_{50}: 52-55 mg/g)[22]. Total phenolic (TP) and flavonoid (TF) contents of any medicinal plant are considered as the main antioxidant compounds which show scavenging activity against free radicals[23]. In the present study, the highest TP (12.63 mg CE/g) and TF (9.30 mg QE/g) content was shown by foothill cultivation and lowest by mid-altitude cultivation. In comparable to our data, the previous study mentioned that dried flowers of thyme contained TP (15.06 mg GAE /g), TF (10.62 mg CE/g) and condensed tannins (0.66 mg CE/g) respectively[24]. The results of Proestos *et. al.*[25] evaluated the TP (19.2 mg GAE/g) of *Thymus vulgaris* extracts. In the present study, negative correlations were observed between ABTS (IC_{50}) and RP (EC_{50}) assays and total phenolics and total flavonoids which reflected the higher antioxidant activity of *T. serpyllum* having a high content of TP and TF. Chandra *et al.* (2016) evaluated the chemical composition of *Thymus linearis* collected from two different locations of Uttarakhand. The results showed that the EOs as well as the extracts possessed significant antioxidant and pharmacological activities. Also, the TP and TF content found in the two different collections were 5.15-4.65 mg/100 mg, and 0.26-0.52 mg/100 mg respectively[26]. In 2013, Roby and coworkers[27] compared the antioxidant potential of thyme, sage and marjoram. The results of the study showed that thyme was having the strongest antioxidant activity. Also, the higher antioxidant activity of thyme was positively correlated to the polyphenols present. The study also mentioned that the methanolic extracts scavenge free radicals better than the ethanolic extracts. In the literature data, linear correlations were observed for TP and TF and antioxidant potential[28-29]. Our results showed that the antioxidant potential of thyme extracts was the highest in the foothill cultivation followed by the Auli sample further followed by the Pithoragarh sample.

5. CONCLUSIONS

The results revealed the antioxidant potential of the alcoholic as well as the aqueous extracts of the thyme. Cultivating the same plant species at different altitudes brings a change in the chemical composition and activity of the plant extracts. The plant grown at the foothill climatic conditions of the Haldwani region showed the best antioxidant activity against various radicals. It has also been observed that the IC_{50} values of aqueous and alcoholic extracts were different, therefore the *in-vivo* antioxidant potential of the plant extracts should be done using hydroalcoholic extracts. The traditional cultivation methods are recommended for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterise the active constituents responsible for the antioxidant activity of these plants.

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