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Nutritional evaluation, Phytochemicals, Antioxidant and Antibacterial activity of *Stellaria monosperma* Buch.-Ham. Ex D. Don and *Silene vulgaris* (Moench) Garcke: wild edible plants of Western Himalayas

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Abstract

Wild plants of Western Himalayas occupy an essential role to the inhabitant's livelihood. The present study evaluates the nutritional composition, phytochemicals and antioxidant activity of *Stellaria monosperma* and *Silene vulgaris*, used commonly as food and medicine by folks of Western Himalayas. These two species are facing extinction as per the IUCN Red List. The present study focused on these two plants which are used by the Gaddis (shepherds) of Western Himalayas as food. Major findings revealed that the aerial parts had high amount of carbohydrate, proteins, sodium, potassium, crude fibre and crude fat. Phytochemicals like phenols, flavonoids, tannins, terpenoids, amino acids, ascorbic acid, tocopherols, carotenoids, alkaloids and phytates were in sufficient amount, helping to deactivate and absorb free radicals. Thus, both the plants have high antioxidant activity and are good radical scavengers. These plants also exhibited antibacterial potential. Amount of nutrition, phytochemicals, antioxidant and antibacterial activity was observed more in *Silene vulgaris* as compared to *Stellaria monosperma*. The need is to bring under control these plants species for harnessing their potentials of nutrition and pharmaceutical industry.

Keywords: Antioxidant, Nutritional, Phytochemicals, *Stellaria monosperma*, *Silene vulgaris*.

1. Introduction

Constantly increasing population pressure and fast reduction of natural resources has become more important to diversify the present-day agriculture yield (Deb, 2013). Utilization of wild edibles, mainly those recognized as under-utilized, are known to improve health and nutrition, livelihoods and environmental sustainability. These plants provide vitamins, trace elements and minerals, which are the source of nutraceuticals (Leonti, 2012). Wild edible plants play significant role in the functioning of the body and suitable growth (Afolayan and Jimoh, 2009; Ali-Shtayeh *et al.*, 2008). According to FAO information, in developing countries about 1billion population depend on wild plants for their food (Taylor, 2015). Traditionally, these edible plants are used as food as well as medicines (Ubwa, 2014). Many ethnobotanical studies suggested that these plants play major role in maintenance of life, especially of the rural community as they are used wild plants as drugs and food (Verma and Kaushal, 2014). Researcher's enthusiasm lies to re-examine each plant with a new approach regarding their probable use for food and medicine. Primary metabolites such as carbohydrate, proteins, vitamins, sterols and lipids occurs in plants and provides food with nutrition (Verma and Kaushal, 2014), while secondary metabolites, such as phenols, flavonoids, tannins, alkaloids, terpenoids, lignin, quinones, coumarins and amines are the best antioxidants (Zheng and Wang, 2001; Cai *et al.*, 2003). Similarly, many wild plants provide essential biochemical and energy besides supplementary resources of vitamins and minerals that sustain the suitable physiological equilibrium of the body. It has been seen that sometimes nutritional potential of uncultivated plant species is superior compared to the cultivated variety (Ebert, 2014). Recently, several researches focused on wild edibles as a source of nutraceuticals, used for the cure of several diseases such as jaundice, diabetes, wounds, cancer etc. as recognized from various ethno pharmacological studies (Mir, 2014). Foods which are obtained from plant are abundant resources of bioactive compounds, which have been found to possess a great variety of biological activities including antioxidant potential. Epidemiological studies revealed that the utilization of fruits and vegetables as food is coupled with decline possibility of chronic and neurodegenerative diseases, mostly due to the occurrence of antioxidants (phenolic compounds and tocopherols) that are concerned in the interruption or prevention of oxidative reactions (Gerber *et al.*, 2002; Di Matteo and Esposito, 2003). The occurrence of diseases caused by microbes are constantly increasing on the entire earth and mostly are cured by the use of antimicrobials. Antibacterials, which are obtained from plants, are safer as compare to synthetic drugs due to

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their natural source. Secondary metabolites of plants such as tannins, flavonoids, quinones, coumarins, terpenoids, alkaloids and polypeptides are frequently responsible for their antimicrobial activity (Savoia, 2012).

Stellaria monosperma Buch.-Ham. ex D. Don, and *Silene vulgaris* (Moench) Garcke are the wild, herbaceous plant of the *Caryophyllaceae* family which are used as vegetable (aerial parts) by the people of Western Himalayas (Fig. 1). *Stellaria monosperma* is perennial herb about 10-60 cm tall, erect stem, leaves simple and opposite. *Silene vulgaris* is a perennial herb up to 10-60 cm length and occurred in weedy areas, semi-dry and open dry areas (Karamian and Ghasemlou, 2013). The present study planned to analyze the nutritional composition; phytochemicals, antioxidant and antimicrobial analysis of these two species from the wild, keeping in mind its importance and lack of literature.

Stellaria monosperma Silene vulgaris

Figure 1. Plants of two species in wild growing in Bharmour region of Chamba district in **Himachal Pradesh**

2. Materials and methods

The aerial parts of *Stellaria monosperma* and *Silene vulgaris* were collected in June 2018 from the Bharmour region of district Chamba, Himachal Pradesh. This area lies at 32°11' 35" to 32° 41' 54" N latitude 76° 31' 35" to 76° 53' 71" E longitude. Collected plants were cleaned with distilled water, dried with blotting paper, chopped to small pieces before drying at 60°C for 72 hours until constant weight was obtained. The dried chopped pieces were ground into powder by mortar pestle and put in the glass bottle and kept at 4°C in refrigerator and used for the further analysis.

2.1. Extract preparation

2 g of the fine powder of each sample was soaked in 20 ml methanol/water (50:50, v/v) and kept at orbital shaker for 48 hrs, filtered and filtrate evaporated and dried at 40°C at water bath and used for extract to evaluate antioxidant and antibacterial activity.

2.2. Nutritional composition

2.2.1. Determination of Carbohydrate content

Weighed 100 mg of sample, homogenized by 2 ml of 2.5 N HCl in test tube, and boiled in water bath for two hours, cooled at room temperature. In 200µl of supernatant added 500µl of distilled water and 3 mL of anthrone reagent. The reaction mixture was heated in water bath for 10 minutes and cooled. UV /visible spectrophotometer read mixture of green color at 630 nm. The carbohydrate content was calculated by calibration curve equation ($y=1.297x-0.050$; $R^2=0.998$) of glucose and results are expressed as 1g of glucose/mg of sample (Hedge and Hofreiter, 1962).

2.2.2. Determination of Protein content

Weighed 100 mg of sample and ground it with a pestle and mortar in 2mL of phosphate buffer. Centrifuged and used the supernatant for protein estimation. In 200µl, added 3 mL of $CuSO_4$ mixed well and kept for 10 minutes and then added 500µl of Folins-Ciocalteau's reagent and mixed properly. Total mixture was kept at room temperature under dark condition for 30minutes. Reaction mixture of blue colour was observed at 660nm by UV/visible spectrophotometer. The protein content of sample was calculated by calibration curve equation ($y=1.041x+0.080$; $R^2=0.999$) of Bovine Serum Albumin (BSA) and the results are expressed as 1g of BSA /mg of sample (Lowry *et al.,* 1951).

2.2.3. Estimation of Sodium and Potassium

Potassium and sodium in the acid-digest of plant sample was determined using flame photometer. A 500mg of the plant sample was weighed and put into 100 ml of conical flask containing 10 ml of concentrated HNO_3. It was kept for about 24hrs in a covered place for pre-digestion. After pre-digestion the solid sample was no more visible. A 10 ml of concentrated HNO_3 and 2-3 ml of $HClO_4$ were added. The mixture was heated, in acid proof chamber having fume exhaust system, at about 100°C for first 1 hr and then raised the temperature to 200°C. Digestion was continued until the mixture became colorless and only white dense fumes appeared. The acid contents were reduced to about 2-3 ml, by continuing heating at the same temperature and filtered through Whattman No. 42 filter paper into a 100 ml volumetric flask. Gave 3-4 washings of 10-15 ml distilled water and made volume 100 ml. Measured Na^+ and K^+ concentrations in the supernatant by flame photometer. Readings were recorded of the standards of Na and K after adjust blank to zero and then drawn a standard curve (Chikhale and Chikhale, 2017).

2.2.4. Determination of Crude Fibre

Sample (2g) was digested by boiling with50 mL of 1.25% H_2SO_4 solution for 30 min, and then filtered under pressure. The residue was rinsed three times with boiled water. This process was repeated using 50 mL of 1.25% Sodium hydroxide. The deposit was dehydrated at 100°C, cooled in room temperature and weighed. It was thereafter crucible put on muffle furnace at 550°C, for 3 h, then cooled at room temperature and reweighed (Aina *et al.,* 2012). The percentage crude fibre was calculated as:

% Crude fibre = Weight of residues – weight of ash / Weight of original sample× 100

2.2.5. Determination of Crude Fats

Sample (5g) was extracted in 25 mL of diethyl ether and placed on shaker for 24 h. The extract was filtered and put into a formerly weighed (W1) beaker. Then 100 mL of diethyl ether was added and shaken for 24 hours. The filtrate was collected in the same beaker (W1). Ether containing beaker was put on the water bath to dry at 40–60°C and the beaker was again weighed (W2) (Unuofin, 2017). The crude fat content was calculated as:

% Crude fat = W2−W1/Weight of original sample× 100

2.3. Determination of Phytochemical content

2.3.1. Determination of Phenols

100 mg of sample was homogenized by 2ml of methanol. Centrifuged for 10 minutes, at 10,000 rpm, collected the supernatant. Took 200 µl of supernatant, added 1ml of Folin-Ciocalteau reagent and kept for 2 min. After that 1000µl of 35% Na_2CO_3 solution added to the mixture and made 10 ml of final volume by distilled water. Reaction mixture was kept for 30 min in dark and OD was observed at 750nm against reagent blank. The amount of phenol was calculated by calibration curve equation (y=0.741x+0.132; R^2=0.995) by expressed in terms of mg of gallic acid (GAE)/g of sample (Jia *et al.,* 1998).

2.3.2. Determination of Flavonoids

100 mg of sample was homogenized by 2ml of methanol and centrifuged for 10 minutes at 10,000 rpm and and the supernatant was collected. 200µl of the plant extracts were made up to 1.5 ml using distilled water and added 75µl of 5% NaNO2. The reaction mixture was kept to stand for 5 min and 150 µl of 10% AlCl3 was added to it. The mixture was mixed well and allowed to stand for 5 minutes at room temperature. Thereafter, 0.5ml of 1M NaOH was added and the OD observed against to reagent blank at 510nm. Results are expressed as mg of rutin equivalents (RE) /g of sample by calibration curve equation (y=0.965x+0.036; R^2=0.999) (Jia *et al.,* 1998).

2.3.3. Determination of Tannins

100 mg of sample was homogenized by 2ml of methanol. Centrifuged for 10 minutes at 10,000 rpm and collected the supernatant. To 1ml of supernatant mixed with 0.5 ml Folin's phenol reagent and 35% Na_2CO_3 of 5ml added and the mixture was kept at room temperature for 5 minutes. The blue color of reaction mixture was observed at 640 nm by UV/visible spectrophotometer. Content of tannin was calculated by calibration curve equation (y=1.501x+0.102; R^2=0.996) of gallic acid and the results expressed as (mg/g) (Schanderl, 1970).

2.3.4. Determination of Terpenoids

100 mg of sample was homogenized by 2ml of methanol. Centrifuged for 10 minutes at 10,000 rpm, collected the supernatant. In 100µl of supernatant, added 3ml of the chloroform. Added 200µl of the concentrated sulphuric acid and solution kept at room temperature for 1.5-2hour in dark, for the duration of incubation a reddish-brown color precipitate was formed. Supernatant was decanted without disturbing the precipitate. 3ml of the 95% methanol added and vortex thoroughly until all the precipitate completely mix in methanol. The absorbance was observed at 538 nm against blank, i.e. 95% methanol. Linalool was used as the standard for estimation. Terpenoid content was calculated by the calibration curve equation (y=1.018x+0.047; R^2=0.997) of linalool and results expressed in mg/g (Ghorai *et al.,* 2012).

2.3.5. Determination of Amino acid

Weighed 500mg of plant sample and ground it in pestle and mortar with 5-10ml of 80% ethanol. Centrifuged and to 0.1 ml of extract, added 1 ml of ninhydrin solution and made the volume to 2ml with distilled water. Heated the tube for 20 minutes in water bath. Add 5ml propanol and mixed the contents. After 15 minutes read the absorbance of purple color against a blank in a spectrophotometer at 570 nm. Standard was prepared using 50mg leucine dissolved in 50ml of distilled water. Took 10 ml of this stock and diluted to 100 ml in another flask for working standard solution. Standard solution of 0.2. 0.4, 0.6, 0.8 and 1.0 ml gave concentration range of 20, 40, 60, 80 and 100 mg. Proceeded as that of the sample and read the colour. Determined the concentration of amino acid in the sample by calibration curve equation (y=0.748x+0.063; R^2=0.988) and expressed as mg/g equivalent of leucine (Moore and Stein, 1948).

2.3.6. Determination of Ascorbic acid

Ascorbate was extracted into 4% TCA by homogenizing 1g of sample in it, and total volume was made 10 ml with 4% Tri-carboxylic acid. The supernatant obtained after centrifugation for 10 min at 2000 rpm was treated with a pinch of activated charcoal, shaken well and kept for 10 min. Centrifugation process was continual for removing the residue of charcoal. 1ml of supernatant was taken for the assay. Total volumes were made to 2 ml with 4% Tri-carboxylic acid. 0.2 to 1.0 ml of the working standard were pipetted into clean test tubes, the total volumes made in test tube to 2ml with 4% Tricarboxylic acid. 500 µl DNPH reagent was added in all test tubes, by addition of 2 drops of 10% solution of thiourea. Formation of osazones after incubation at 37°C for 3 hours was seen. These osazones dissolve in 2.5ml of 85%H_2SO_4, after 30 minutes of incubation, absorbance of samples observed at 540 nm and the content of ascorbic acid in the samples was calculated using calibration curve equation (y=0.988x-0.028; R^2=0.996) and expressed as ascorbate mg/g (Roe and Kuether, 1943).

2.3.7. Determination of Carotenoids

Plant sample was taken 5-10g for saponification in a shaking water bath at 37°C for about 30 minutes. After extracting the KOH transferred, the saponified extract was transferred into a separating funnel contained 10 to 15ml of petroleum ether and mixed well which took carotenoid pigments into the layer of petroleum ether. The lower aqueous layer was decanted to one more separating funnel. Repeated the extraction of the aqueous phase with petroleum ether until it was colorless. After that the aqueous layer was discarded and added a pinch of Na_2SO_4 in petroleum ether layer to eliminate turbidity. The final volume of the petroleum ether extract was recorded. The OD at 450nm was noted in a spectrophotometer by petroleum ether as a blank. (Zakaria *et al.,* 1979).

Carotenoids (µg) = P x 4x V x 100 W

P= Sample optical density

V= Sample volume

W=Weight of the sample

2.3.8. Determination of Tocopherol

100 mg sample was mixed slowly with 0.1 N sulphuric acid and kept for overnight at room temperature, then filtered. To 1.5 ml of tissue extract, 1.5 ml of xylene was added and centrifuged. Then 1.0 ml of xylene was separated and mixed with 1.0 ml of 2, 2-pyridyl and the OD was noted at 460 nm. In the beginning, 0.33 ml $FeCl_3$ added with blank and mixed well. After 15 min, the test and standard solution read against the blank at 520 nm (Rosenberg, 1992). The tocopherol content in the sample was determined by the formula.

Tocopherol (µg) = Reading at 520nm – Reading at 450nm / Reading of standard at 520nm X 0.29 X 15

2.3.9. Determination of Alkaloid

5g of sample was mixed with 25 ml of 10% acetic acid in ethanol. The mixture was enclosed and kept for 2 h. Mixture was filtered and filtrate placed on water bath to a quarter of its original volume. Concentrated NH_4OH was added in drops to the extract when precipitation was finished. The solution was allowed to stand, washed with diluted NH_4OH, filtered, and the collected dehydrated residue was weighed (Omoruyi *et al.,* 2012). Alkaloid content was determined by:

% Alkaloid =Weight of precipitate/Weight of original sample ×100

2.3.10. Determination of Phytate

2g sample was weighed and added 50 mL of 2% HClkept for 2 h and then filtered. 25 ml of the filtrate was put in a 250 ml conical flask with 5 ml of 0.3% NH_4SCN used as indicator and added 53.5 ml of distilled water. This was then titrated with standard iron III chloride solution (0.001 95 g of iron per ml) until a brownish yellow colour developed for 5 min. (Damilola *et al.,* 2013).

Phytic acid was calculated as:

Phytic acid (%) = titer value × 0.001 95 × 1.19 × 100

2.4. Antioxidant activity

2.4.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

In order to know radical scavenging behavior of the extract, DPPH solution was prepared by dissolving 20 mg 2, 2-diphenyl-1-picrylhydrazyl in 100 ml (stock solution) methanol. 3ml was taken from this solution, and its absorbance at 515 nm (control solution) was set to 0.75.To prevent free radicals, the DPPH stock solution was coated with aluminum foil and kept in dark condition for 24 hours. A 5mg of extract was weighed and dissolved in a 5ml methanol for stock solutions preparation. Different dilutions (25, 50, 75 and 100 µl/ml) were prepared from stock solutions by serial dilution. Approximately 2 ml of each dilution was mixed with a solution of 2 ml DPPH and kept at darkness for 15 minutes. Ascorbic acid was used as a typical antioxidant compound in all the assays for comparative analysis. The inhibition percentage of DPPH free radical by extracts was determined by following formula (Barros *et al.,* 2007).

% Inhibition = (Ac-As/Ac) x 100

Where Ac is the OD of the control and As is the OD of the extract/standard. The scavenging activity of samples was expressed as IC_{50} value, which represented the inhibitory concentration of extract/standard essential to scavenge 50% of DPPH radicals.

2.4.2. 2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) assay

The 2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) free radicals scavenging activity by determine the antioxidant potential of extracts. Solutions in 100 ml methanol were prepared for ABTS (7 mM) and potassium persulphate (2.45 mM). These two solutions were thoroughly mixed for the preparation of free radicals and kept in the dark overnight. Around 3 ml of stock solution was taken and its absorbance at 745 nm was set to 0.76 (control solution). Approximately 300µl of the test sample was mixed with 3ml of 2,2-azino-bis(3-ethylbenzothiazoline-6-suplhhonic acid solution and kept at 25°C for 15 min. The optical density of the solution was read at 745 nm. For the preparation of various ascorbic acid dilutions, same method was used as for sample (Re *et al.,*1999). The data was collected in triplicates, and the formula used to measure the percentage of ABTS free radicals scavenging activity was:

% Inhibition = (Ac-As/Ac) x 100

2.4.3. Ferric reducing antioxidant power (FRAP) assay

The antioxidant potential of the edible plants was estimated by procedure of (Benzie and Strain, 1996). The process is based on the reduction of Fe3+ TPTZ complex (colorless complex) to Fe2+-tripyridyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. Freshly prepared FRAP reagent and 2-3 ml was added to the sample and properly mixed. Blue color complex formed when ferric tripyridyltriazine (Fe3+ TPTZ) complex was reduced to ferrous (Fe2+) form and the absorbance read at 593 nm against reagent blank. Solution was kept at 37°C for 30 minutes. The calibration curve was prepared by plotting the absorbance against concentrations of ferrous sulphate. The antioxidant activity of extract was determined by linear calibration curve ($y=0.091x+0.062$; $R^2=0.960$) of $FeSO_4$ and expressed as mM $FeSO_4$ equivalents/g of extract.

2.5. Antibacterial activity

2.5.1. Collection of test organisms

The bacterial strains used such as *Escherichia coli* (MTCC 82), *Staphylococcus aureus* (MTCC 96) were obtained from parasitology laboratory of Shoolini University of Biotechnology and Management Sciences, Solan, India. These strains were grown in nutrient broth and were incubated at 37 °C for 14-16 h.

2.5.2. Antibacterial activity of plants extract

The disk diffusion method is used to evaluate antimicrobial activity of each plant extract (Razmavar *et al.,* 2014)**.** Methanolic plant extracts (50mg) were dissolved in 1 ml of 10% DMSO, then loaded on sterile filter paper discs (8 mm in diameter) to obtain final concentration of 50µl/disc. Filter paper discs loaded with 10µl of streptomycin were used as positive control. The plates were kept in the fridge at 4°C for 2 hr to permit plant extracts diffusion then incubated at 37°C for 24 hrs. The occurrence of inhibition zones was recorded by measuring scale and showed as antibacterial activity of extract.

2.5.3. Statistical analysis

At the end of the experiment, data were subjected to analysis of variance (ANOVA) and mean separation. The statistical analysis was done using Graph Pad Prism® 5.2.The least significant difference (LSD) at 5% level was used to compare the means of two species. Data are mean±SD, of three replicates (n=3) were examined by two way ANOVA followed by Bonferroni multiple comparison post-test.

3. Results

3.1. Nutrition composition

The results of nutrition composition in *Stellaria monosperma* and *Silene vulgaris* are shown in Table 1.Carbohydrate content in *S.vulgaris* was higher by 12% compare to the *S.monosperma*. Protein content in *S.vulgaris* was higher by 71% from the *S.monosperma*. Sodium and potassium content was also higher in the *S.vulgaris* by 12% and 7% respectively. Similarly, crude fibre and crude fat were high in the *S.vulgaris* by 13% and 35% from the *S.monosperma.* It is evident from the above results that *Silene vulgaris* had high nutritional properties as compared to *Stellaria monosperma*.

Table 1. Nutrition composition of *Stellaria monosperma* Buch.-Ham. ex D. Don, *Silene vulgaris* (Moench) Garcke. Data as mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.001*** significance level. Different lower case letters between columns in a table indicate significant difference between two species.

Parameters	*S.monosperma*	*S.vulgaris*
Carbohydrate (mg/g)	5.51±0.065a	6.15 ± 0.234a
Protein (mg/g)	4.53±0.393a***	7.73±0.113b***
Sodium (mg/g)	2.85±0.507a	3.18±0.025a
Potassium (mg/g)	31.11±0.862a***	33.37±0.682b***
Crude fibre (%)	3.23±0.251a	3.66 ± 0.152a
Crude fats (%)	0.34±0.030a	0.46±0.020a

3.2. Phytochemicals determination

The presence of phytochemicals in *Stellaria monosperma* and *Silene vulgaris* studied is shown in Table 2. Phenol and flavonoid content were high by 14% and 15% in *S. vulgaris* respectively. The tannin and terpenoid content was higher by 38% and 82% in *S. vulgaris* from *S.monosperma*. The quantity of amino acid and ascorbic acid was higher by 74% and 25% in *S. vulgaris* as compared to the *S.monosperma*. Tocopherol and carotenoids content was also high in the *S. vulgaris* by 23% and 19% respectively. Alkaloid and phytate content was higher in *S.monosperma* by 7% and 26% as compared to the *S.vulgaris*.

Table 2. Phytochemical composition of *Stellaria monosperma* Buch.-Ham. ex D. Don and *Silene vulgaris* (Moench) Garcke. Data as mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.001*** significance level. Different lower case letters between columns in a table indicate significant difference between two species.

Parameters	*S. monosperma*	*S. vulgaris*
Phenols (mg/g)	8.20 ± 0.434a***	9.33 ± 0.056b***
Flavanoids (mg/g)	1.63 ± 0.110a	1.87 ± 0.073a
Tannins (mg/g)	0.84 ± 0.019a	1.16 ± 0.004a
Terpenoids (mg/g)	2.691 ± 0.074a***	4.91 ± 0.098b***
Amino acids (mg/g)	0.939 ± 0.109a***	3.37 ± 0.172b***
Ascorbic acid (mg/g)	0.546 ± 0.033a	0.68 ± 0.048a
Tocopherol (µg/g)	7.52 ± 0.554 a***	9.27 ± 0.543b***
Carotenoids (µg/g)	205.95±0.563a***	245.60 ± 0.687b***
Alkaloid (%)	0.28±0.05a	0.26±0.03a
Phytate (%)	1.17±0.11a	0.87±0.10a

3.3. Antioxidant assay

Methanolic extract of *S.vulgaris* showed the strongest ABTS activity with low IC_{50} value 4.0375µg/ml while *S.monosperma* showed lower antioxidant activity with high IC_{50} value 5.483 µg/ml. Similarily, *S.vulgaris* showed the strongest DPPH activity with low IC_{50} value 2.303 µg/ml while *S.monosperma* showed lower antioxidant activity with high IC_{50} value4.152 µg/ml.IC_{50} value of FRAP assay of *S.monosperma* was high 129.855 µM as compared to *S.vulgaris* 71.760µM. IC_{50} value of ascorbic acid was low as compared to studied species shown in Table 3. High IC_{50} value indicates low antioxidant activity while lower IC_{50} value means higher antioxidant activity of plant extract. Therefore, the antioxidant activity of the *S. vulgaris* was higher as compared to *S. monosperma*.

Table 3. IC50 value of *Stellaria monosperma* Buch.-Ham. ex D. Don, *Silene vulgaris* (Moench) Garcke by ABTS, DPPH and FRAP assay.

Parameters	Ascorbic acid	*S. monosperma*	*S. vulgaris*
ABTS (IC50 µg/ml)	0.682	5.483	4.0375
DPPH (IC50 µg/ml)	0.851	4.152	2.303
FRAP (IC50 µM)	42	129.855	71.760

Inhibitory concentration at which 50% of ABTS and DPPH radicals scavenged.

3.4. Antibacterial activity

Antibacterial activity of methanolic extracts of *Stellaria monosperma* and *Silene vulgaris* against *E.coli* and S.aureus was shown in Table 4. Zone of inhibition observed in the *S. monosperma* and *S.vulgaris* against *E.coli* was 13.4±0.1 and 23.166±0.152mm, while against *S.aureus* was 14.133±0.152 and 13.533±0.057mm respectively. Streptomycin was used as positive control and zone of inhibition observed against *E.coli* and *S.aureus* was 32.166±0.208 and 28.233±0.208 mm respectively.

Table 4. Antibacterial activity of *Stellaria monosperma* Buch.-Ham. ex D. Don, *Silene vulgaris* (Moench).Data as mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and species.

Bacterial strain	Positive control	*S. monosperma*	*S. vulgaris*
E.coli	32.166±0.208a	13.4±0.1b***	23.166±0.152c***
S.aureus	28.233±0.208a	14.133±0.152b***	13.533±0.057c***

4. Discussion

The present study investigated the nutritional, phytochemical, antioxidant and antimicrobial capacity of two underutilized wild edible plants, i.e. *Stellaria monosperma* and *Silene vulgaris.* These species are threatened and facing extinction as per the IUCN Red List (http://www.iucnredlist.org). Both species, besides food value, have medicinal value (Chopra *et al.*, 1986). *Stellaria monosperma* is known as a folk remedy for asthma, blood disorder, conjunctivitis, constipation, inflammation, skin ailments and obesity. In food it is eaten in salads or served as cooked greens. *Silene vulgaris* is known as emollient and is used in baths or as a fumigant. Aerial parts are eaten

raw as salad or cooked like spinach. Juice obtained from *Silene vulgaris* is used in cure of ophthalmia and is a general antidote in the remedy of poisoning and a remedy against constipation and intestinal pains. The present studies indicate that for the properties studied, *Silene vulgaris* is better than the other species. The high carbohydrate content makes it a rich source of energy, and this could be used to enrich the energy content of diets (Anital *et al.,* 2006). The carbohydrate content of *Stellaria monosperma* were comparatively less than *Silene vulgaris*. The total carbohydrates in the two species ranged between 5.5 to 6.5 mg/g. The recommended carbohydrate values for humans are 130g (Datta *et al.,* 2019). It implies that daily requirement could be reached when nearly 200g of those dried plants are consumed. Amount of protein ranged between 4.5 to 7.7 mg/g and amount studied in these species was nearly same to some leafy vegetables that are underutilized (Datta *et al.,* 2019). Proteins are essential in the manufacturing and defense of certain organic materials necessary for the proper functioning of the human body (Hayat *et al.,* 2014). The rich protein content of *Silene vulgaris* could provide necessary supplement to diets. Sodium and potassium content are considered to play an important role in human diet for physical and mental growth, as well as essential constituents of teeth, bones, muscles and blood (Unuofin *et al.,* 2017). Sodium plays a central role in the transportation of metabolites and potassium necessary for its diuretic nature. In the present study, in both species sodium content was comparatively high compared to cultivated vegetables and fruits where it ranged between 0.03-1.2 (Gopalan *et al.,* 2004). Similarly, the content of potassium was quite high in our study. The ratio of Na and K in any diet constitutes an important role in hypertension and arteriosclerosis. Sodium enhances the blood pressure whereas potassium decreases blood pressure (Saupi *et al.,* 2009). The ratio of potassium/sodium in our study occurred to quite low (10.9 in *Stellaria monosperma* and 10.49 in *Silene vulgaris*) compared to *Amaranthus viridis* (32.4), *Achyranthes aspera* (67.0). In addition, some common fruits like Papaya (11.5) tomato (11.3), and Amla (45.0) have very high ratio as reported by Sundriyal and Sundriyal (2004) and Datta et al. (2019). High fiber contained food help to reduce diseases related with metabolic disorders (Ikewuchi and Ikewuchi, 2009). *Stellaria monosperma* and *Silene vulgaris* have the rich amount of crude fibre (3.23 and 3.66% respectively) indicating that these plants decline the risk of several diseases as well as provide good supplement to human diet.

Excess intake of fat in diet is a main reason of cancer, aging and cardiovascular diseases, (Aruah *et al.,* 2011). In this context, the low content of crude fat in both the species studied (0.34 and 0.46% respectively) revealed that it could avoid certain chronic ailments in humans. Phenols are important aromatic secondary metabolite in plants, which hold ROS scavenging capability and have many biological properties like antioxidant, anti-aging, anti-inflammation, anti-cancer, and anti-atherosclerosis (Han *et al.,* 2007). Phenol content in *Stellaria monosperma* and *Silene vulgaris* was 8.2 and 9.3 mg/g, respectively, in contrast to Prasad and Chandra (2018) who reported 3.3 mg/g in *Euphorbia thymifolia* and 2.5 mg/g in *Pouzolzia hirta*, both edible medicinal plants of Western Himalayas. Total polyphenols present in the leaves of *Silene vulgaris* was 3.35 mg/g as reported by Smahane et al. (2015). Mamadalieva *et al.* (2014) reviewed the genus *Silene* whereby they isolated more than 450 compounds important classes include phytoecdysteroids (which mimic insect molting hormones), triterpene saponins (with detergent properties), volatiles, other phenolics and terpenoids. Similarly, flavonoids have been reported as antioxidants, scavengers of a large variety of free radicals and lipid peroxidation inhibitor (Williams *et al.,* 2004). The flavonoids content recorded in *Stellaria monosperma* and *Silene vulgaris* was 1.63 and 1.87mg respectively, which is in sufficient amount and provides added advantage to these species. Tannins are beneficial for the remedy of cancer and ulcerated tissues (Sun *et al.,* 2012). The tannin content in leaves of *Silene vulgaris* was 0.28 mg/g (Smahane *et al.,* 2015). The occurrence of tannins 0.48 and 1.16mg in both the species seems to be beneficial in the curing of several diseases. Presence of ascorbic acid (0.546 and 0.680mg), tocopherols (7.52 and 9.27µg) which is in sufficient quantity in these wild edible plants plays a significant role in deactivating and absorbing free radicals and protects the human body (Islary *et al.,* 2016). Alkaloid is a capable remedial bioactive substance in plants. Consumption of high alkaloids containing food may cause paralysis and rapid heartbeat which is not good for health. Intake of a high dose of alkaloids will lead to the damage of blood vessels, muscles and other soft tissues and death (Gemede and Ratta, 2014). The alkaloid content recorded in *Stellaria monosperma* and *Silene vulgaris* was of low amount, which justifies its uses by folks. The alkaloids and phytic acids are considered as anti-nutrition. The phytate content of *Stellaria monosperma* and *Silene vulgaris* was low, 1.17% in former and 0.87% in later species. Phytate content in diet of 1-16% over a long time decreases the bioavailability of mineral elements (Oke, 1969). Excessive consumption of phytic acid contained food causes diseases, e.g. osteomalacia rickets. However, the small quantity of anti-nutrient that is alkaloid and phytate could decrease by boiling, soaking and frying (Ekop, 2005). IC_{50} value of methanolic extracts of *P. esculenta* and *S. nigrum* by DPPH and ABTS was 1.37±0.02, 1.03±0.001 mg/g and 0.18±0.01, 0.19±0.001 mg/g (Seal, 2015). The IC_{50}value in studied species by ABTS, DPPH and FRAP in *S.monosperma* and *S.vulgaris* was high as compared to that reported by previous studies. Antibacterial activity of ethanolic extract of the *Catunaregam spinosa* against *E.coli* and *Klebseila pneumonia* was 18mm and 24mm, respectively. It is a wild edible fruit used by people of Tamil Nadu, India (Anand *et al.,* 2017). Zone of inhibition observed in present study was relatively similar to the previous study. Therefore, *Stellaria monosperma* and *Silene vulgaris* can be considered plants with free radical scavengers, besides a great potential in the food, nutritional and pharmaceutical industries. Further studies related to their cultivation (domestication) practices need to be taken up.

5. Conclusion

Wild edible plants *Stellaria monosperma* and *Silene vulgaris* collected from Bharmour region of District Chamba, Himachal Pradesh, were found to have an appreciable amount of carbohydrate, protein, sodium,

potassium, crude fiber and crude fats. These wild plants are rich in phytochemicals, alkaloid, phenol, flavonoids, tannins, terpenoids, amino acid, ascorbic acid, tocopherols and carotenoids, which have the antioxidant capacity. In addition, the present study revealed that the *Silene vulgaris* has a high amount of nutritional, phytochemical and antioxidant potential was observed as compared to *Stellaria monosperma*. The antibacterial activity of the *Silene vulgaris* was high as compared to the *Stellaria monosperma* against *Escherichia coli* and antibacterial activity of *Stellaria monosperma* was high as compared to the *Silene vulgaris* against *Staphylococcus aureus.* Both studied plants showed lowest antibacterial activity as compared to the streptomycin. Therefore, these plants are recommended for their domestication, food industry and biomedicical application.

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