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Original article

Evaluation of some essential traditional medicinal plants for their potential free scavenging and antioxidant properties



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ABSTRACT

Objectives: In this study, extracts from different parts of traditionally used medicinal plants were evaluated for their antioxidant activities in vitro.

Methods: The free radical hunting or scavenging activity was measured by sample absorbance at 517 nm using spectrophotometer. Methanol and DPPH were used as a blank and negative control, respectively.

Results: Medicinal plants showed high values of total phenolic (expressed as gallic acid equivalent, GAE) and flavonoid (expressed as quercetin equivalent, QE) contents of ethanolic extracts of *Euphrasia stricta* (*E. stricta*, 58.19 GAE $\mu\text{g}/\text{mg}$) and (42.44 QE $\mu\text{g}/\text{mg}$), *Euphorbia platyphyllos* L. (*E. platyphyllos* L., 46.05 GAE $\mu\text{g}/\text{mg}$) and (43.39 QE $\mu\text{g}/\text{mg}$), *Epimedium brevicomum* Maxim. (*E. brevicomum* Maxim., 51.93 GAE $\mu\text{g}/\text{mg}$), and (39.21 QE $\mu\text{g}/\text{mg}$), respectively. Plants have been found to be rich in phenolic and flavonoid compounds, and their hydroxyl groups are responsible for scavenging free radicals. Highest radical scavenging activity was observed in the *E. stricta* ($\text{IC}_{50} = 38.972 \mu\text{g}/\text{mL}$), *E. platyphyllos* L. ($\text{IC}_{50} = 40.817 \mu\text{g}/\text{mL}$), and *E. brevicomum* Maxim ($\text{IC}_{50} = 46.265 \mu\text{g}/\text{mL}$), medicinal plants for both of their ethanolic and methanolic extracts as compared to the ascorbic acid scavenging activity ($\text{IC}_{50} = 37.337 \mu\text{g}/\text{mL}$).

Conclusions: It was found that the studied plants are capable of acting as important antioxidants that can be used to treat and inhibit extensive degenerative diseases caused by oxidative stress., including cancer, cardiovascular and inflammation diseases, atherosclerosis, dementia, diabetes, asthma, and eye degenerative diseases.

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1. Introduction

Researchers have discovered that reactive oxygen species (ROS) such as H_2O_2 , O_2^- , and OH^- are present in large quantities in the human body. Almost 5 % or more of the oxygen (O_2) that humans breathe is converted into ROS due to univalent (Kontoghiorghes & Kontoghiorghes, 2019; Xu et al., 2017). Various devastating diseases

such as diabetes, cancer, cirrhosis, obesity and cardiovascular disorders may be caused by these free radicals (Wang, Chun, & Song, 2013). So several enzymatic antioxidant barriers like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been efficiently used to neutralize these harmful properties of these free radicals (Khan, 2015; Khan, Busquets, & Azam, 2021; Khan, Busquets, Naushad, & Puignou, 2019; Khan, Naushad, & Alothman, 2017; Khan et al., 2022). Nevertheless, to investigate this oxidative stress, certain factors like ultraviolet rays, unnecessary NADPH stimulation, and cigarette smoke, environmental contaminants/pollutants exposure, mitochondrial electron transport chain, radiation, some parasitic infections or toxic chemicals have been identified and are responsible for causing the overproduction of ROS. The oxidative stress is a change in equilibrium/normal position of an antioxidant or a pro-oxidant process in a living system, leading to mutilation/damage to diverse components like

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DNA, lipids, and membrane proteins and collectively to whole cell structures (Garbi et al., 2015; Shrivastava et al., 2019). Consequently, these diseased conditions can be effectively alleviated by anti-oxidant compounds that can neutralize free radicals (Speakman & Selman, 2011). An extensive diversity of free radical hunting or antioxidant components like phenols, vitamins, terpenoids and flavonoids have been found in plants which possess high antioxidant potentials (Miguel, 2018). The plant derived polyphenolic constituents might be more useful *in-vivo* with their positive effects as these are proved to be more efficient antioxidants as compared to vitamins E or C *in-vitro* (Palacios et al., 2011). Different medicinal plants had been efficiently applied to treat the ROS and are of great importance having antioxidant potential because of such sort of alimentary radical scavenging diet supplement. Medicinal plants have antioxidant properties mainly due to the presence of phytonutrients and ingredients such as phenols, flavonoids, and terpenoids. The antioxidant potentials of many medicinal plants have been studied (D'souza et al., 2014; Y. Li et al., 2019) like anti-cancer and Immunomodulator activity (Madhuri, Pandey, & Verma, 2011), Hepato-protective benefits, hypolipidemic activity (Gopa, Bhatt, & Hemavathi, 2012). Thus, the present study aimed to evaluate and compare various medicinal plants and their free radical scavenging properties by using spectrophotometers (Thilakchand et al., 2013; Uddin et al., 2016).

2. Materials and methods

2.1. Materials

The medicinal plant samples were collected from Himalayan regions of Pakistan. The samples drying were carried out at the University of Virginia, USA, where the antioxidant properties of the medicinal plant samples were evaluated. The chemicals, solvents and reagents used in the preparation of plant extracts were distilled water, ascorbic acid, DPPH (1, 1-diphenyl, 2-picryl hydrazyl), methanol and ethanol. The purity of the reagents was higher than 99 %, were obtained from Sigma-Aldrich (Darmstadt, Germany).

2.2. Sample preparation

For preparing the methanolic extracts, 100 mg of dried powdered plant leaf samples were taken in 1.5 mL of aqueous methanol (40 %) in Eppendorf tubes. The samples were shaken well to dissolve to greater extent with the help of stirrer/shaker. All samples were then incubated in dark for 24 h. The extracts were then filtered to remove undissolved solid particles in form of pellet and the supernatant was obtained for further analysis.

For preparing DPPH (1 mM) solution, 3.94 mg of DPPH was added in 100 mL methanol in a 200 mL flask and was well mixed with the help of stirrer. DPPH gave purple color when dissolved in methanol. The incubation was then done at room temperature for 20 min. The series of dilutions were made for less concentrated DPPH solution, as highly concentrated solution does not give accurate absorbance value by blocking most of light in spectrophotometer system. Different concentrations of plant extracts (5 µg, 25 µg, 50 µg, 75 µg and 100 µg) were added in diluted DPPH solution. The samples were kept in incubation for 30 min in dark again. The purple color of DPPH in methanol solution was faded or completely disappeared because of the activity of antioxidants from medicinal plant samples. The free radical hunting or scavenging activity was measured by taking the absorbance reading of samples at 517 nm using spectrophotometer. Methanol, as the basic and the DPPH were used as a blank and negative control respectively. Ascorbic acid was taken as standard because of its high

antioxidant activity. The increased absorbance indicated the high antioxidant activity of samples. The formula used to calculate the reduction %age,

$$\%age\ reduction = \frac{Ac - As}{Ac} * 100$$

Ac = absorbance of control sample; As = absorbance of real sample.

The ascorbic acid stock standard solution was prepared by adding 0.5 mg of ascorbic acid was mixed in 1 mL of methanol followed by thoroughly mixing to get the clear solution. The %age reduction in absorbance was observed using different concentration of ascorbic acid (10–100 µg/mL) in sample solution to construct an ascorbic acid calibration curve. The Eppendorf tubes were taken with 40 µL of DPPH in 1.5 mL of methanol by mixing it well on stirrer. The initial absorbance of the samples was taken at 517 nm. To measure the absorbance variations, different ranges of this solution (10–100 µL), were added to DPPH sample solution. The DPPH reagent solution was taken as a control in separate Eppendorf tubes. After 5 min of incubation at room temperature, the samples absorbance was measured using spectrophotometer.

The %age reduction/inhibition was calculated by using following formulae,

$$\%age\ Inhibition = \frac{Ac - As}{Ac} * 100$$

Ac = absorbance of control sample; As = absorbance of real sample.

The inhibition concentration (IC₅₀) for ascorbic acid and plant samples was also calculated. The inhibition concentration (IC₅₀) is the value which shows the 50 % inhibition or reduction in initial absorbance by an antioxidant in DPPH Assay. IC₅₀ was calculated by plotting different concentration of extracts vs inhibition/reduction (%). The lower IC₅₀ indicated the antioxidant potential of the sample.

2.3. IC₅₀ value in antioxidant assays

IC₅₀ values in antioxidant assays was estimated using the slope equation:

$$Y = mx + c$$

The IC₅₀ of methanolic, ethanolic and aqueous extracts of all medicinal plants was measured following same DPPH protocol for methanol, ethanol and water with the addition of increasing concentrations of the same solvents DPPH reagent solution. Table 1 and Fig. 1 presented that with the increase in ascorbic acid concentration from 10 to 100 µg/mL, the DPPH absorbance was decreased while the inhibition percentage was increased from top to bottom, respectively at 517 nm. The IC₅₀ value calculated was 37.34 µg/mL.

2.4. Total phenol contents (TPC)

The dried and pulverized plant samples (~100 g) were extracted with 100 mL of 70 % ethanol, 40 % methanol and distilled water (10 %) by means of agitator. The filtration was attained by Muslin cloth, solutions were then centrifuged, and rotary evaporator was used for sample drying through evaporation. The air-tight plastic vials were used to store these collected dried plant samples for further antioxidant investigations. The Folin Ciocalteu reagent was used to measure the TPC of these samples at 765 nm by UV-spectrophotometer. The dilutions of all these ethanolic and methanolic extracts (0.5 mL of 1 µg/L or standard phenolic compound Gallic acid were mixed with Folin Ciocalteu reagent solution, dilution were made by using distilled water (5 mL, 1:10 dilution) and 4 mL of 1 M aqueous sodium carbonate. The mixture was kept for 30 min and then the absorbance was measured at 765 nm for total phenol estimation. The Gallic acid equivalent

Table 1
Reduction in the percentage of DPPH absorbance values of samples at 517 nm with the addition of ascorbic acid.

Concentration ($\mu\text{g/mL}$)	Absorbance (nm)	%age Inhibition	IC ₅₀ ($\mu\text{g/mL}$)	R ²
10	0.944	34.459		
20	0.884	38.623		
30	0.752	47.778		
40	0.691	52.114		
50	0.616	57.223	37.34	0.989
60	0.575	60.069		
70	0.426	70.417		
80	0.394	72.699		
90	0.242	83.195		
100	0.198	86.250		

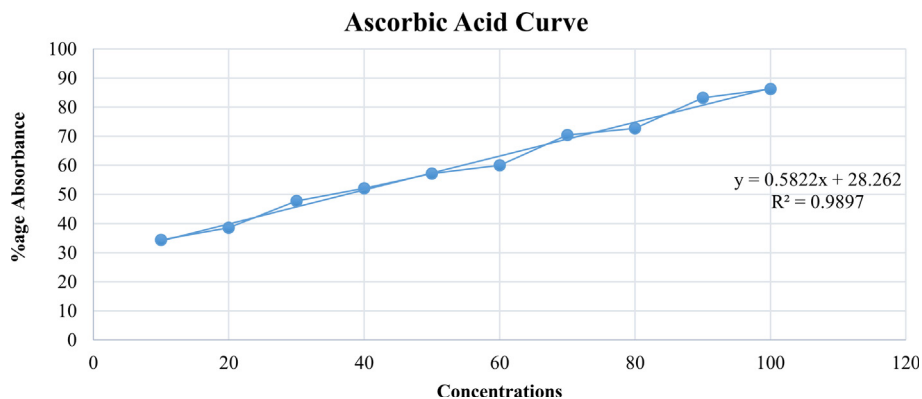


Fig. 1. Calibration curve of ascorbic acid at absorbance 517 nm.

(mg/gm of dry mass) a common reference compound was used to express the TPC. All experiments were achieved as triplicates test ($n = 3$) and the data was analyzed by average of three values.

2.5. Total flavonoid contents (TFC)

To determine the TFC, aluminum chloride assay was applied as reported in earlier study (Uddin et al., 2016). Briefly, a sample comprising of water (4 mL) was added with diverse plant extracts (1.0 mg/mL) and various dilutions (10–1000 $\mu\text{g/mL}$) from Rutin (standard). Then, 0.3 mL of 5 % NaNO_2 was added to the above mixture. 1 M NaOH (2 mL) was added after 6 min and 10 mL total volume was made by using distilled water. The solution was mixed well, and absorbance was taken at 510 nm. The Rutin equivalent (mg/gm of dry mass) a common reference compound was used to express the TFC. All experiments were achieved as triplicates test ($n = 3$) and the data was analyzed by average of three values.

3. Results and discussion

3.1. TPC

Medicinal plants, as well as other plants and fruits, possess antioxidant properties primarily due to their redox properties (Ouml et al., 2013). It was observed that the ethanolic extracts of *E. stricta* showed the higher TPC expressed as gallic acid equivalent (GAE), measured as 58.19 GAE $\mu\text{g/mg}$ followed by *E. platyphyllos* L. 46.05 GAE $\mu\text{g/mg}$ and *E. brevicomum* Maxim. 51.93 GAE $\mu\text{g/mg}$, respectively. The methanolic extracts of *E. stricta*, *E. platyphyllos* and *E. brevicomum* showed the TPC in range of 45.70, 42.00, and 44.06 GAE $\mu\text{g/mg}$, respectively as displayed in Table 2. As the hydroxyl groups (OH^-) is responsible for the free radical scavenging ability in them, so rapid screening of antioxidant activity can

be effectively applied by using on the TPC of these medicinal plants.

3.2. TFC

The flavonoids are a group of secondary metabolites that plants produce that have antioxidant potential. They include flavanols, flavones, and abbreviated tannins. The presence of free OH groups, especially 3-OH group is mainly responsible for the antioxidant activity of these flavonoids. Plant flavonoids can be used as potential antioxidant in vitro as well as in vivo (Mahboubi, Kazempour, & Hosseini, 2013; Tahirovic & Basic, 2017). It was observed that the ethanolic extracts of *E. stricta*, showed the higher TFC expressed as quercetin equivalent (QE), measured as 42.44 QE $\mu\text{g/mg}$ followed by 43.39 QE $\mu\text{g/mg}$ and 39.21 QE $\mu\text{g/mg}$ for ethanolic extracts of *E. platyphyllos* L. and *E. brevicomum* Maxim., respectively. The total phenolic and flavonoid content of the plant extracts of *E. stricta*, *E. platyphyllos* L. and *E. brevicomum* Maxim were evaluated. *E. brevicomum* Maxim. plant ethanolic extracts showed the phenolic and flavonoid contents of 101.89 GAE $\mu\text{g/mg}$ and 265.28 QE $\mu\text{g/mg}$, respectively as shown in Table 2. These medicinal plants possess phenol and flavonoid compounds that contribute to their antioxidant and anti-inflammatory activity. Thus, the tested medicinal plants were observed as a rich source of phenolic and flavonoid compounds. It is, however, necessary to carry out a detailed phytochemical analysis before applying these medicinal plants to the treatment of diseases associated with oxidative stress.

3.3. Evaluation of antioxidant potential of medicinal plants

It was chosen to use DPPH solution in this experiment due to the lack of side effects, such as enzymatic suppression or metal ion chelation, as compared with other free radicals like superoxide

Table 2
TPC and TFC obtained in medicinal plants.

Medicinal plants	TPC, Mean \pm SD (GAE μ g/mg)			TFC, Mean \pm SD (QE μ g/mg)		
	EtOH Extract	MeOH Extract	Aq. Extract	EtOH Extract	MeOH Extract	Aq. Extract
<i>Euphrasia stricta</i>	58.19 \pm 1.74	45.70 \pm 1.48	13.00 \pm 1.20	42.44 \pm 1.26	39.18 \pm 0.74	12.10 \pm 1.62
<i>Euphorbia platyphyllos</i> L.	46.05 \pm 1.10	42.00 \pm 1.54	12.84 \pm 1.24	43.39 \pm 1.05	35.88 \pm 1.34	10.23 \pm 0.44
<i>Epimedium brevicomum</i>	51.93 \pm 1.72	44.06 \pm 0.64	13.26 \pm 0.44	39.21 \pm 1.76	38.62 \pm 1.98	11.90 \pm 1.72
<i>Viscum album</i>	42.84 \pm 0.48	42.50 \pm 0.56	11.08 \pm 0.92	36.92 \pm 1.80	38.96 \pm 0.16	11.72 \pm 0.88
<i>Psoralea corylifolia</i> L.	37.70 \pm 0.62	38.12 \pm 0.06	8.54 \pm 0.88	28.29 \pm 1.34	26.73 \pm 1.91	9.62 \pm 1.54
<i>Equiseti arvense</i>	40.56 \pm 0.32	36.90 \pm 0.18	11.49 \pm 0.70	37.80 \pm 1.98	34.26 \pm 0.69	10.06 \pm 1.88
<i>Veronica officinalis</i>	44.28 \pm 2.00	39.02 \pm 1.24	13.56 \pm 1.20	33.71 \pm 1.36	31.42 \pm 1.50	8.64 \pm 0.96
<i>Artemisia herba</i>	42.24 \pm 0.36	37.16 \pm 0.82	13.04 \pm 0.18	25.85 \pm 0.43	25.15 \pm 0.46	14.46 \pm 0.62
<i>Fagopyrum cymosum</i>	41.82 \pm 1.87	40.30 \pm 1.41	10.03 \pm 1.24	37.53 \pm 1.74	35.76 \pm 1.14	10.16 \pm 1.92
<i>Prunella vulgaris</i>	36.77 \pm 1.84	34.68 \pm 0.54	11.26 \pm 0.69	26.12 \pm 1.65	25.38 \pm 1.74	9.94 \pm 1.76
<i>Hederae folium</i>	34.62 \pm 1.63	32.76 \pm 1.06	14.40 \pm 0.56	24.36 \pm 0.82	22.16 \pm 0.40	11.66 \pm 0.98
<i>Salvia Divinorum</i>	39.40 \pm 1.76	37.08 \pm 0.96	12.00 \pm 1.66	28.93 \pm 1.24	27.88 \pm 1.59	10.33 \pm 1.34
<i>Thymus serpyllum</i> L.	35.23 \pm 1.00	33.28 \pm 1.44	10.03 \pm 0.74	24.44 \pm 0.77	23.26 \pm 0.94	10.85 \pm 0.48
<i>Melissa officinalis</i>	42.00 \pm 1.36	38.12 \pm 1.28	11.20 \pm 1.31	30.21 \pm 1.56	28.57 \pm 0.25	12.43 \pm 0.55
<i>Cassia tora</i> L.	40.94 \pm 1.82	38.74 \pm 0.46	13.02 \pm 0.46	33.78 \pm 1.63	31.00 \pm 0.78	12.58 \pm 1.26
<i>Saussurea lappa</i>	35.79 \pm 1.74	34.33 \pm 1.04	12.20 \pm 1.58	29.35 \pm 1.51	26.94 \pm 1.12	9.00 \pm 1.31
<i>Epilobium parvifolium</i>	40.52 \pm 1.90	37.96 \pm 0.06	9.00 \pm 1.64	25.56 \pm 1.28	24.04 \pm 0.44	10.76 \pm 1.86
<i>Satureja montana</i>	44.30 \pm 1.34	42.12 \pm 1.00	13.44 \pm 0.80	32.41 \pm 0.83	50.72 \pm 1.52	6.64 \pm 1.64
<i>Asperula odorata</i>	38.26 \pm 0.53	36.05 \pm 0.44	11.56 \pm 0.68	27.82 \pm 0.96	25.46 \pm 1.64	9.33 \pm 0.40
<i>Gunnera perpersa</i>	34.15 \pm 1.72	33.46 \pm 1.16	14.66 \pm 1.40	21.60 \pm 0.52	20.58 \pm 0.32	10.49 \pm 0.93
<i>Fritillaria thunbergii</i>	43.72 \pm 1.84	40.78 \pm 1.26	12.14 \pm 1.56	35.28 \pm 0.46	33.44 \pm 0.86	8.61 \pm 0.79
<i>Melissa flava</i>	41.56 \pm 0.88	38.42 \pm 0.34	9.26 \pm 0.68	32.66 \pm 1.35	28.80 \pm 1.41	6.44 \pm 1.96
<i>Ocimum basilicum</i>	34.49 \pm 1.44	32.80 \pm 1.56	11.33 \pm 1.78	26.31 \pm 0.93	25.74 \pm 0.63	8.85 \pm 0.38
<i>Achillea millefolium</i>	39.35 \pm 0.77	36.76 \pm 0.34	8.82 \pm 0.33	28.84 \pm 1.66	24.36 \pm 1.22	10.56 \pm 1.86
<i>Urticae folium</i>	37.06 \pm 1.15	34.76 \pm 0.55	14.53 \pm 0.52	24.38 \pm 1.14	22.04 \pm 0.54	13.34 \pm 1.00
<i>Polygonum aviculare</i>	41.98 \pm 1.86	40.00 \pm 0.98	12.50 \pm 1.80	29.52 \pm 1.44	27.56 \pm 1.68	12.76 \pm 0.58
<i>Lonicera japonica</i> Thunb.	36.75 \pm 1.59	51.64 \pm 1.62	11.00 \pm 1.4	26.60 \pm 1.96	23.64 \pm 1.42	11.58 \pm 1.46
<i>Tinospora cordifolia</i>	39.54 \pm 1.88	37.08 \pm 0.76	12.36 \pm 1.22	24.85 \pm 1.72	22.29 \pm 1.96	11.64 \pm 1.98
<i>Paris polyphilla</i>	35.21 \pm 0.88	34.62 \pm 1.42	13.44 \pm 1.68	28.44 \pm 0.84	27.88 \pm 0.12	9.51 \pm 0.62
<i>Mentha piperita</i> folium	42.92 \pm 0.46	41.36 \pm 1.24	10.31 \pm 0.60	32.16 \pm 0.56	30.33 \pm 1.44	11.00 \pm 1.18
<i>Tephrosia purpurea</i> L.	33.83 \pm 0.57	31.08 \pm 0.92	14.48 \pm 0.43	24.39 \pm 1.88	23.96 \pm 0.80	13.52 \pm 0.86
<i>Marrubium vulgare</i>	40.78 \pm 0.44	39.64 \pm 0.88	13.81 \pm 0.66	31.72 \pm 1.64	28.58 \pm 1.35	11.84 \pm 1.54
<i>Lantana camara</i>	34.56 \pm 1.65	32.50 \pm 1.81	10.04 \pm 1.90	26.30 \pm 1.46	25.06 \pm 0.86	8.40 \pm 1.66
<i>Betulae folium</i>	39.47 \pm 0.46	36.82 \pm 0.53	8.45 \pm 0.94	25.82 \pm 0.70	23.69 \pm 0.53	10.36 \pm 1.48
<i>Teraxaci folium</i>	35.42 \pm 1.56	34.16 \pm 2.12	8.56 \pm 1.48	24.46 \pm 1.58	21.27 \pm 0.67	12.64 \pm 0.76
<i>Rubi idaei folium</i>	43.33 \pm 1.74	42.02 \pm 0.58	14.00 \pm 1.90	34.62 \pm 1.97	32.65 \pm 0.54	10.33 \pm 1.64
<i>Hedyotis diffusa</i>	40.92 \pm 1.78	40.50 \pm 1.82	13.57 \pm 0.74	30.54 \pm 0.69	29.37 \pm 0.96	10.74 \pm 0.41
<i>Smilax glabra</i> Roxb	36.80 \pm 1.76	35.63 \pm 1.51	10.88 \pm 1.64	28.87 \pm 0.78	24.54 \pm 0.45	8.22 \pm 1.20
<i>Trifolium repense</i>	41.45 \pm 1.75	38.59 \pm 1.76	11.24 \pm 1.92	22.40 \pm 1.42	20.16 \pm 1.94	11.08 \pm 0.52
<i>Cantaurii herba</i>	38.24 \pm 0.24	36.18 \pm 0.92	12.44 \pm 0.88	26.63 \pm 0.86	25.94 \pm 0.22	9.70 \pm 0.82

TPC, Total phenol contents; SD, Standard deviation (n = 3); GAE, gallic acid equivalent; TFC, Total flavonoid contents; QE, Quercetin equivalent; EtOH, Ethanol; MeOH, Methanol; Aq., Aqueous.






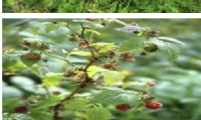






oxides and hydroxyl ions. The deep purple color was shown by freshly prepared DPPH solution with maximum absorbance at 517 nm. The purple color of DPPH was faded up or almost disappeared because of the antioxidants activity present in these plant extracts. Therefore, the free radicals in DPPH can be neutralized by antioxidant molecules (i.e., by providing hydrogen atoms or donating electrons, possibly through an attack on the free radicals present in DPPH molecule) and thus resulting in purple to colorless change in color (e.g., by converting to 2, 2-diphenyl-1-hydrazine, or by replacing corresponding hydrazine molecule), which showed increase in the absorbance at 517 nm as shown in Table 1 and Fig. 1. This DPPH Assay is also very useful as the increase in absorbance of the sample solution can be directly measured by a continuous spectrophotometry in the reaction medium at any time. The consistent information regarding the antioxidant potential of these tested plant samples has been efficiently measured using DPPH assay (Gopinath, Rakesh, Murthy, & Dayananda, 2012; Gülçin, Elias, Gepdiremen, Chea, & Topal, 2010).

In this investigation, the ethanolic, methanol and aqueous plant extracts of 40 medicinal plants (Table 3) were experimented to test their free radical scavenging potential using DPPH assay. Table 4 and Fig. 2 showed the IC₅₀ (the test solution concentration required to increase the absorbance of a sample by 50 % comparing to the blank solution) for different medicinal plants in different extracts.

The results showed that ethanolic extracts of these medicinal plant extracts exhibited the higher level of free radical scavenging or antioxidant properties, followed by methanolic extracts in comparison with the IC₅₀ = 37.34 μ g/mL of standard ascorbic acid. The ethanolic and methanolic extracts of *E. stricta* L. showed the highest antioxidant potential of 38.97 μ g/mL and 43.66 μ g/mL respectively followed by the *E. platyphyllos* L. (40.81 μ g/mL & 42.98 μ g/mL) and *E. brevicomum* Maxim (46.26 μ g/mL & 51.25 μ g/mL) as compare to the ascorbic acid IC = 37.34 μ g/mL (Table 4). The aqueous extracts showed almost similar results for all samples regarding %age inhibition of free radicals. Thus, these medicinal plants with the higher antioxidant potentials can be efficiently used as an efficient source of natural antioxidants to treat various oxidative stress related problems like cancer and other cardiovascular disorders. By evaluating the results of this study obtained by DPPH assay method, a quality control procedure could be designed and potentially used to develop a more effective protocol for investigating the antioxidant, anticancer, and phytochemical properties of medicinal plants.

The *E. stricta* plant was investigated for its antioxidant potentials in this research. It is an important medicinal plant in South Asia. Antioxidant assay was performed in various solvent systems, i.e., water, ethyl ether, 70 % ethanol and 80 % methanol. The highest total phenol content was shown by the methanol extracts among

Table 3
Traditionally used medicinal plants collected from Himalayan regions of Pakistan.

Scientific Name	Plants	Local name	Family	Parts used	Traditional medicinal applications
<i>Trifolium repense</i> L.		White clover, desisiree, saag	Fabaceae	Leaves	Stomach problems, anti-intestinal helminthic worms, anti-cestodal properties
<i>Cassia tora</i> Linn		Kikkar, cassias	Fabaceae	Leaves, seeds	Phyto-chemical and pharmacological properties
<i>Psoralea corylifolia</i> L.		Babchi or bakochi	Fabaceae	Leaves, seeds	Antibacterial, antitumor, anti-inflammatory and immunomodulatory activity
<i>Urticae folium</i>		Nettles or stinging nettles,	Urticaceae	Seeds, leaves	Antimicrobial, antiulcer and analgesic activities
<i>Teraxaci folium</i>		Dandelion	Asteraceae	Whole plant extract	Anti-inflammatory, anti-carcinogenic and antioxidative activities
<i>Rubiidaeii folium</i>		Akhriyar, jamaro	Phragmidiaceae	Fruits, leaves	Antimicrobial properties
<i>Rhus potaninii</i>		Desidrawa, tunn	Anacardiaceae	Fruits, leaves	Anti- hypertension to control high BP, anti-diabetic properties
<i>Lantana camara</i>		Big-sage, wild-sage, red-sage, white-sage, tick-berry	Verbenaceae	Whole plant extracts	Antimicrobial, fungicidal, insecticidal, anti- cancer, skin itches, leprosy, rabies, chicken- pox, measles, asthmaand anti-ulcer properties.
<i>Lonicera japonica</i> Thunb.		Chanbha. Japanese honeysuckle and golden and silver honeysuckle	Caprifoliaceae	Dried leaves, stem and flowers	Anti-inflammatory, to treat fever, headache, cough, thirst, and sore throat
<i>Epilobii herba</i>		Willow herbs	Onagraceae	Whole plant extracts	Used for prostate hyperplasia (BPH),bladder and hormonedisorders
<i>Thymus serpyllum</i> L.		Breckl and thyme, Breckl and wild and creeping thyme	Lamiaceae	Aerial parts	Antimicrobial, treating fever and antitumor activity
<i>Salvia officinalis</i>		Garden sage, common sage, or culinary sage	Lamiaceae	Whole plant extract	Treating Alzheimer's disease as neurotoxic

(continued on next page)

Table 3 (continued)



Scientific Name	Plants	Local name	Family	Parts used	Traditional medicinal applications
<i>Euphrasiae herba</i>		Eyebright	Orobanchaceae	Whole plant extract	For eyestrain and to relieve inflammation caused by colds, coughs, sinus infections, sore throats and hay fever.
<i>Equiseti herba</i>		Horsetail, snake grass, puzzle grass	Equisetaceae	Aerial parts	Antimicrobial and geno-toxicity
<i>Millefolii herba</i>		Yarrow or common yarrow	Asteraceae	Leaves, flowers	Antimicrobial activity
<i>Mentha piperita folium</i>		Wild mint	Lamiaceae	Whole plant extract	Antimicrobial activity
<i>Euphorbia platyphyllos L.</i>		Dhoodal	Euphorbiaceae	Aerial parts	Cytotoxic and apoptotic activities
<i>Marrubium vulgare</i>		White horehound	Lamiaceae	Aerial parts	Treating stomach problems
<i>Melissae folium</i>		Lemon balm, balm, common balm, or balm mint	Lamiaceae	Whole plant extract	Antimicrobial activities
<i>Hederae folium</i>		Jal bail	Araliaceae	Leaves, fruit	Antimicro, antioxidative, hepato-protective and antimutagenic activities
<i>Fritillari athunbergii</i>		Lilly	Liliaceae	Whole plant extract	Antimicrobial activities
<i>Satureja montana</i>		Mountain savory, winter savory	Lamiaceae	Whole plant extract	Antimicrobial activities
<i>Gunnera perperna</i>		River pumpkin, wild rhubarb, wild ramenas, nalcas.	Gunneraceae	Stem, leaves	Anti-microbial, anti-inflammatory and anti-oxidative properties
<i>Absinthii herba</i>		Absinthe, absinthim, absinthe wormwood, grand wormwood, wormwood	Asteraceae	Stalk, leaves	Antidepressant and antioxidant activities
<i>Viscum album</i>		Mistletoe	Santalaceae	Leaves, fruit, stalk	Antihyperglycemic activity
<i>Asperula Herba</i>		Woodruff, golden rod	Rubiaceae	Leaves, stalk, flowers	Antibacterial activities

Table 3 (continued)

Scientific Name	Plants	Local name	Family	Parts used	Traditional medicinal applications
<i>Tephrosia purpurea</i> L.		Sarphonk, Sharpunkha, fish poison, wild indigo	Fabaceae	Leaves	Anticarcinogenic and anti-lipid-peroxidative effects
<i>Tinospora cordifolia</i> (Willd.)		Heart-leaved moonseed, guduchi and giloy	Menispermaceae	Leaves	Anti- bacterial, antifungal properties
<i>Ocimum basilicum</i>		Basil, sweet basil	Lamiaceae	Leaves	Antioxidant and anticancer properties
<i>Saussurea lappa</i>		Costus or kuth	Asteraceae	Whole plant	For stomach problems
<i>Betulae folium</i>		Silver birch, warty birch, European white birch, or East Asian white birch	Betulaceae	Bark, leaves	Anti-inflammatory, antiviral and anti-cancer properties.
<i>Cantaurii herba</i>		Centaurry, centory, starthistles, knapweeds, centaureas	Asteraceae	Whole plant	Cytotoxic, antifungal and antimicrobial properties
<i>Prunella vulgaris</i>		Common self-heal, wound wort, carpenter's herb, brown-wort and blue curls	Lamiaceae	Leaves, flower	Cytotoxic and immunomodulatory activities
<i>Echter ehrenpreis</i>		Speedwell, common gypsy-weed	Veroniceae	Whole plant	Antimicrobial properties
<i>Paris polyphilla</i>		Love apple, satuwa	Melanthiaceae	Leaves	Antimicrobial properties
<i>Melissa officinalis</i> L.		Lemon balm, balm, common balm, or balm mint	Lamiaceae	Leaves, stalk	Genotoxicity and cytotoxicity
<i>Hedyotis diffusa</i>		White flower, snake-tongue grass.	Rubiaceae	Whole plant extract	Anti-inflammatory, cytotoxic and antibacterial activities
<i>Polygonum aviculare</i>		Common knotgrass, Prostrate knotweed, bird-weed pigweed and low-grass	Polygonaceae	Leaves, stalk	Antimicrobial and anti-inflammatory properties

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Table 3 (continued)

Scientific Name	Plants	Local name	Family	Parts used	Traditional medicinal applications
<i>Salviae off. folium</i>		Sage, also called garden sage, common sage, culinary sage.	Lamiaceae	Aerial parts	Cytotoxic properties
<i>Fagopyrum cymosum</i>		Buckwheat, tartary buckwheat	Polygonaceae	Aerial parts	Anti-inflammatory also to treat fever, headache

all the extracts; so, these were used for additional research. To evaluate the antioxidant potential of these methanolic extracts various antioxidant assays were used like DPPH, metal ion chelating assay and FRAP Assay. Antibacterial activity and superoxide radical anions were taken as parameters. The antioxidant potential in the methanol extracts of *E. stricta* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration (Aker et al., 2016). This study is in accordance with the previous study where *E. stricta* was investigated to verify the potential radical scavenging properties in its methanolic, petroleum ether, water, ethyl acetate and diethyl ether extracts using DPPH, DNA and cytotoxic activity in extracts of MCF-7 breast cancer cells from test excretion test exclusions assay DPPH trypan blue exclusion, Hoechst 33,258 and comet assay double staining with iodide propidium, respective. From this study it was observed that all extracted samples showed a dose–response relation. Moreover, this study also showed that antioxidant properties were possessed by various extracts of *E. stricta* which caused DNA fragmentation. These results also proved that the breast cancer can be treated by using this plant as a potential source of anticancer agent. Further studies are needed to isolate and identify individual phenolic compounds in extracts (Ouml et al., 2013).

The similar study was carried out where *E. stricta* extracts were evaluated to measure the total phenol content (TPC) and total flavonoids contents (TFC) through spectrophotometry methods. The higher antioxidant and antimicrobial activity was observed by the methanolic extract as compared to the ethanolic or water extracts. The methanol extract also showed high value of (149 and 36.6 mg /g) for TPC and TFC of *E. stricta* extracts. The ethanolic extracts showed antioxidant potential of (137.20) and 19.50 mg/g) followed by (86.2 and 8.4 mg/g) of water extracts. The lower IC value of 200 µg /mL was shown by methanolic extracts and then the (250 µg /mL) and (400 µg/mL) of ethanol and water extracts respectively. It was observed that there was a positive correlation found amongst antioxidant, antimicrobial activity, TPC and TFC in *E. stricta* extracts (Mahboubi et al., 2013).

The same results were shown when the potential antioxidant properties of methanol extracts of *Viscum album* ssp. (mistletoe) were investigated by using the 1.1-diphenyl-2-picrylhydrazyl (DPPH) assay to check the radical scavenging potential, FRAP and thiobarbituric acid were used to testify the lipid peroxidation inhibitory effect. The maximum activity was shown by the mistletoe extract grown in the lime tree in summer. It was also observed that harvest time and host tree had great influence on the plant's antioxidant capacity (Tahirovic & Basic, 2017).

Similarly, this study is also supported where ethanol extracts of *Psoralea corylifolia* seeds was studied for detection of their phytochemical properties. The total flavonoids and polyphenols were studied to check the presence of polyphenols. To determine the antioxidant activity 1.1-diphenyl-2-picrylhydrazyl (DPPH) assay

and superoxide radical assay were used. The concentrations of flavonoids and ethylene derivatives of polyphenol *Psoralea corylifolia* were 60.63 QE mg/g and 74.35 QE mg/g respectively. The stronger antioxidant activity was shown by the extracts with a lower value of IC₅₀ for DPPH and the elimination of superoxide. The value of IC₅₀ for DPPH and removal of phosphorus oxide was 166.61 mg/mL and 177.69 mg/mL, respectively. The strongest antioxidant activity of ethanolic extract was observed to be due to presence of flavonoids and phenols (Nabi & Shrivastava, 2017).

The similar finding was also observed when the antioxidant effects of different extracts of the *Euphorbia platyphyllus* L. (horse tail) were studied by using various antioxidant assays during lipid peroxidation of lipid particles. Antidepressant activity was studied in the human tumor cells of HLA, HT-29 and MCF7 using the sulforodamine B assay. It was confirmed from the analysis of these results that the ESR extracts suppress the formation of both lipid peroxidic roots and the investigation of adjuvant dosing systems. The results indicated that the extracts of ethyl ether, methanol, butanol and water extracts have been very effective in removing radical peroxigenes. These findings showed that the *E. platyphyllus* extracts were a significant basis of voluntarily accessible accepted antioxidants and latent phytochemicals source (Četojević-Simin et al., 2010).

This study also follows the other previous studies where the *V. teucrium* L., *V. officinalis* L. and *V. Orchidia* L. family Plantaginaceae were the three species tested for their potential phenolic, sterolic, antioxidant and antimicrobial activities. The quantification and identification of phenol compounds and other phytosterol were calculated using the p-coumaric and LC/MS techniques, folic acid, luteolin, and cytosterol acid the main components. More than this, Hesperdolina, Yupatorean and Epatoline were first discovered in the genus Veronica. However, the content of the phytosterol of most of the Veronica genus were not examined. Antioxidant potentials that were examined through Trolox (TEAC) and EPR antioxidant assays showed that the *V. officinalis* and *V. Orchidea* showed comparable antioxidant potentials, while *V. Teucrium* extracts were recorded for lower values. These findings can be helpful promote the best use of genus Veronica as antioxidants and antimicrobial source (Mocan et al., 2015).

The present study is in accordance with the previous study where the *Equiseti arvense* herba was tested for its potential antioxidant activity of its extracts by using the self-protective effect of powder against oxidation induced by alkanes in diabetic mice. The four groups were designed for mice random division: the first group receiving control of a saline solution of 9%, the second group 150 mg of alloxan was used to treat with administered peritoneal. The 400 mg of AHA/kg (body weight) was used treat the third group of mice while Aloxan and AHA were to treat the Group IV animals. The management of AHA aquatic extraction improved these criteria. These results indicated that AHA improves oxidative

Table 4
Free radical scavenging activity of medicinal plants using different extract solutions.

Plants	Sample extracts	Radical scavenging activity (RSA) IC ₅₀ (ug/mL)	R ²
<i>Euphrasia stricta</i>	Ethanol	38.972	0.968
	Methanol	43.665	0.962
	Water	110.057	0.985
<i>Euphorbia platyphyllos</i> L.	Ethanol	40.817	0.983
	Methanol	42.988	0.979
	Water	121.512	0.997
<i>Epimedium brevicomum</i>	Ethanol	46.265	0.978
	Methanol	51.249	0.996
	Water	98.605	0.981
<i>Viscum album</i>	Ethanol	52.279	0.984
	Methanol	54.463	0.991
	Water	141.227	0.979
<i>Psoralea corylifolia</i> L.	Ethanol	51.821	0.988
	Methanol	52.665	0.995
	Water	124.134	0.999
<i>Equiseti arvense</i>	Ethanol	55.246	0.958
	Methanol	58.781	0.993
	Water	128.427	0.983
<i>Veronica officinalis</i>	Ethanol	51.594	0.998
	Methanol	54.159	0.983
	Water	113.361	0.984
<i>Artemisia herba</i>	Ethanol	53.036	0.954
	Methanol	55.514	0.996
	Water	107.481	0.976
<i>Fagopyrum cymosum</i>	Ethanol	54.801	0.981
	Methanol	57.882	0.991
	Water	125.838	0.981
<i>Prunella vulgaris</i>	Ethanol	53.667	0.985
	Methanol	54.531	0.996
	Water	104.822	0.964
<i>Hederae folium</i>	Ethanol	56.797	0.982
	Methanol	59.797	0.998
	Water	109.527	0.981
<i>Salvia Divinorum</i>	Ethanol	60.961	0.979
	Methanol	65.356	0.999
	Water	111.201	0.977
<i>Thymus serpyllum</i> L.	Ethanol	52.559	0.996
	Methanol	57.329	0.994
	Water	134.297	0.972
<i>Melissae officinalis</i>	Ethanol	51.768	0.952
	Methanol	59.436	0.997
	Water	59.436	0.995
<i>Cassia tora</i> L.	Ethanol	54.768	0.991
	Methanol	58.971	0.998
	Water	149.891	0.982
<i>Saussurea lappa</i>	Ethanol	55.289	0.988
	Methanol	58.978	0.999
	Water	112.452	0.995
<i>Epilobium parvifolium</i>	Ethanol	57.381	0.982
	Methanol	59.831	0.999
	Water	116.097	0.994
<i>Satureja montana</i>	Ethanol	57.335	0.991
	Methanol	61.972	0.996
	Water	113.874	0.987
<i>Asperula odorata</i>	Ethanol	64.561	0.973
	Methanol	66.287	0.974
	Water	117.113	0.988
<i>Gunnera perpensa</i>	Ethanol	57.705	0.987
	Methanol	59.522	0.998
	Water	144.375	0.974
<i>Fritillaria thunbergii</i>	Ethanol	55.551	0.997
	Methanol	58.624	0.998
	Water	123.577	0.977
<i>Melissa flava</i>	Ethanol	52.658	0.987
	Methanol	54.856	0.999
	Water	114.268	0.982
<i>Ocimum basilicum</i>	Ethanol	58.759	0.984
	Methanol	60.446	0.999
	Water	91.913	0.986
<i>Achilleamille folium</i>	Ethanol	57.279	0.979
	Methanol	58.336	0.981
	Water	118.324	0.998

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Table 4 (continued)

Plants	Sample extracts	Radical scavenging activity (RSA) IC ₅₀ (ug/mL)	R ²
<i>Urticae folium</i>	Ethanol	51.986	0.996
	Methanol	53.122	0.997
	Water	126.186	0.991
<i>Polygonum aviculare</i>	Ethanol	57.452	0.994
	Methanol	62.838	0.998
	Water	132.052	0.979
<i>Lonicera japonica Thunb.</i>	Ethanol	66.194	0.992
	Methanol	82.037	0.991
	Water	123.601	0.969
<i>Tinospora cordifolia (Willd.)</i>	Ethanol	50.966	0.989
	Methanol	53.372	0.998
	Water	127.955	0.991
<i>Paris polyphilla</i>	Ethanol	55.081	0.979
	Methanol	56.098	0.997
	Water	137.321	0.999
<i>Menthapiperita folium</i>	Ethanol	51.739	0.995
	Methanol	52.919	0.999
	Water	120.492	0.986
<i>Tephrosia purpurea L.</i>	Ethanol	50.955	0.981
	Methanol	54.951	0.999
	Water	117.435	0.981
<i>Marrubium vulgare</i>	Ethanol	55.541	0.998
	Methanol	65.398	0.997
	Water	113.849	0.983
<i>Lantana camara</i>	Ethanol	51.546	0.992
	Methanol	53.722	0.992
	Water	131.394	0.983
<i>Betulae folium</i>	Ethanol	52.297	0.991
	Methanol	56.297	0.991
	Water	129.645	0.999
<i>Teraxaci folium</i>	Ethanol	57.496	0.989
	Methanol	61.474	0.998
	Water	117.948	0.985
<i>Rubiidae folium</i>	Ethanol	58.889	0.988
	Methanol	63.138	0.999
	Water	110.017	0.995
<i>Hedyotis diffusa</i>	Ethanol	54.642	0.987
	Methanol	58.837	0.999
	Water	121.351	0.989
<i>Smilax glabra Roxb</i>	Ethanol	56.638	0.994
	Methanol	60.301	0.998
	Water	109.511	0.999
<i>Trifolium repense L.</i>	Ethanol	52.734	0.998
	Methanol	56.442	0.999
	Water	123.492	0.973
<i>Cantaurii herba</i>	Ethanol	52.487	0.992
	Methanol	58.836	0.998
	Water	134.361	0.981

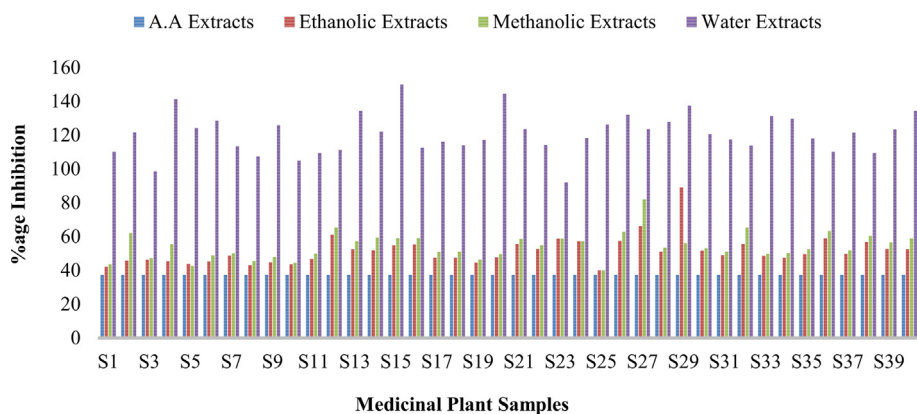


Fig. 2. Free radical scavenging potential of plant extracts in comparison with ascorbic acid.

damages, hyperlipidemia and hyperglycemia in alloxan-induced diabetes in mice (Sekiou, Boumendjel, Taibi, Boumendjel, & Messarah, 2019).

These finding were also supported with the findings where three different types of buckwheat Spp. like *Fagopyrum cymosum*, *Fagopyrum tataricum* and *Fagopyrumes culentum* were tested for

their anti-oxidant and antimicrobial potentials of volatile oils (VOs) extracts from their flowers. The hydro-distillation was used to obtain VOs of fresh buck wheat flowers and to analyze the chemical composition of extracts gas chromatography-mass spectrometry (GC-MS) was used. A remarkable antioxidant capacity of $IC_{50} = 353.15$ mg/mL, 264.92 gm/mL and 210.63 gm/mL from the 1,1-dichényl 2-pycryl hydrazil (DPPH) measured as 174.13 g/mL, 243.16 gm/mL and 216.11 mg/mL, respectively was also shown by the VOs extracts from *F. cymosum*, *F. esculentum* and *F. tataricum* flowers, when β -carotene-linoleic bleaching method was applied. Thus, the finding showed that the buckwheat flowers VOs extracts can be effectively used as natural antioxidants and antimicrobial agents (Zhao et al., 2018).

The similar results were also observed previously where *Prunella vulgaris* Linn (*P. vulgaris*) was investigated for its antioxidant activity of several water-soluble polysaccharides extracts by using the DEAE-Sepharose flow column for different rinsing water (PV-P1), 0.2 M NaCl (PV-P3) and NaCl 0.1 M (PV-P2). As compared to PV-P2 and PV-P3A the higher degree of branching and a higher molecular weight was shown by Structural analysis of PV-P1. The all three extracts PV-P1, PV-P2 and PV-P3 against RA 264.70 in the tested concentrations no cellular toxicity was observed. So, it was shown by that common *P. vulgaris* polysaccharides can be inspected as possible antioxidant source, medicine, immunoglobulins or functional foods (C. Li et al., 2015).

The study is also in accordance where the antioxidant activity of various extracts of dried *Polygonum aviculare* L. was investigated by using various assays by FRAP assay, lipid peroxidation and analysis of DNA-induced cleavage sequences. The IC_{50} value was measured by the results for different extracts which was 50 μ g/mL, 0.9 μ g/mL and 15 μ g/mL for the DPPH antioxidant or radical scavenging assay, H_2O_2 superoxide radical assay and for lipid-peroxidation assay, respectively. Moreover, these extracts also showed a protective effect in hydroxyl radical-induced DNA strand assays. The value of TPC and TFC observed were 677.4 +/- 52.7 μ g/g and 122.7 +/- 14 μ g/g for these extracts. So, the significant antioxidant effects were shown by these findings of *Polygonum aviculare* L. extract (Sung et al., 2013).

Similar findings were also found where various species of *Salvia* medicinal plant e.g., *Salvia macrosiphon*, *Salvia sahendica*, *Salvia chloroleuca*, *Salvia xanthocheila*, *Salvia hydrangea*, *Salvia atropatana*, *Salvia ceratophylla*, *Salvia sclarea* and *Salvia glutinosa* species were evaluated for their antiproliferative and antioxidant potentials. The phytochemical properties, TPC and TFC were also observed. The highest antioxidant activity of $IC_{50} = 8.20$ mg $^{-1}$ by *S. ceratophylla* was shown against C32 cells followed by *S. glutinosa* with an IC_{50} value of 5.29 mg $^{-1}$ compared with ACHN cell lines. However, the *S. glutinosa* also showed higher uptake activity for DPPH roots with IC_{50} than 3.2 μ g $^{-1}$. The highest concentration of phenol and total flavonoids were shown by these species. So, these results specified the importance of *salvia* species as healthy plant food (Loizzo et al., 2014).

The results were also observed previously where the *Hederae folium* extracts were evaluated for their antioxidants effects in soybean oil is susceptible using thermal oxidation. About 3000 mg/kg of organo, thyme olese oil and their mixtures was found in Soybean oil, it also contains tributyl hydroquinone (TBHQ; 50 mg/kg) and soybean without oil exposed to thermal oxidation. Thus, physical, chemical and fatty acids were evaluated. A greater protective effect was applied by organo and thyme separately, which prevented the increase in the formation of TBHQ, showing that by adding of 3000 mg/kg ensures better protection against oxidative oxidation. The increased absorption of urea by adding the thyme and oregano extracts gave a greater protective effect (Jorge, Veronezi, & Del Ré, 2015).

Similarly, this study is also supported where antioxidant potentials of various new plant like (*V. rhodopaea* L., *Veronica bellidioides* L., *V. bccabunga* L., *V. kellereri*, *V. vindobonensis*, *V. austriaco*, *Clinopodiumvulgare* L., *Stachysrecta* L., *Xeranthemumannuum* L. and *Clematis vitalba* were investigated in this research. The potential of antioxidants for new varieties comparable to plants reference drugs. This study showed the antioxidant potential and importance of various traditionally used medicinal plant species (Nikolova, 2011).

This study also follows the other previous studies where the antioxidant potentials of *C. tora* L. aqueous extracts were investigated in this study. It was noted that at a dose of 0.2 mg/mL, the *C. tora* (unroasted) showed 94 % hang-up of linoleic acid peroxidation even greater than the alpha-tocopherol (82 %). The roasting of water extracts of *C. tora* L. was achieved at 200 °C for 5 min and 175 °C for 5 min with inhibition of linoleic acid peroxidation results of 82 % and 83 %, respectively. The WEUCT- IC_{50} value was higher in the lipid formulations caused due to fenton reaction as 0.41 mg/mL, as compared to alpha tocopherol (IC_{50}) value of 0.55 mg/mL. Moreover, in the non-enzymatic and enzymatic systems of oxidization system, WEUCT also demonstrated a good anti-oxidant activity. The roasted *C. tora* L. aqueous extracts showed the high degree of staining resulting from coloring compared to the non-roasted sample (Supare & Patil, 2015).

This study is also supported by previous study where two local medicinal plant species *Saturja montana* L. and *S. subspicata* L. were investigated for eight potential phenolic components (p-coumaric, quercetin, rutin, protocatehúic, caffeine, rosmarinic, ellagic and jeringic acid). The HPLC of ethanolic and methanolic extracts was also measured. The chelating and radical-scavenging assays were used, and the results indicated that the polyphenols and other antioxidant compounds were possessed by both species. A wide range of antimicrobial activity was also observed for various microbial species tested *in-vitro* like (*Candida albicans*, *C. krusei*, *Microsporium gypseum*, *C. dubliniensis*, *C. glabrata*, *Staphylococcus aureus*, *C. parapsilosis* and *Escherichia coli*) by the extracts from both species (Kremer et al., 2015).

The present study is also supported where the antimicrobial and antioxidant potentials of *Satureja montana* L. against seven species of bacteria were assessed here. It was observed that against *Salmonella typhimurium*, the ethanolic extracts were not effective and also no antibacterial activity was shown by water extracts. The main volatile components of essential oils were the thymol (141 g/L), carvacrol (306 g/L) and methyl ether (63 g/L) of these tested extracts. The hot water extracts of *S. montana* showed the strongest antioxidant capacity and also the essential oil with highest percentage of phenol of plant was measured. So, by these findings it was observed that the *S. montana* can be used as an efficient biologically active extract as natural antioxidants and antimicrobial source (Serrano et al., 2011).

Similar findings were also observed where the antioxidant potentials of various extracts of *Equiseti arvense* L. were evaluated. The DPPH free radical scavenging assay was used to measure antioxidant potential of these extracts. The water extract was relatively better in Wound reduction and tissue standards (90.68 ± 6.13 %, 97.18 ± 4.37 % for water extracts 15 % and 30 % compared to 79.29 ± 9.16 % and 91.94 ± 4.14 % for 15 % and 30 % methanol extracts, respectively). The substantial antioxidant potential was noticed with $IC_{50} = 148$ μ g/mL and 83 μ g/mL, for both methanol and aqueous extracts respectively, in the DPPH test. So, assumption was that both extractors have the potential antioxidant potential, as well as empirically and surgically demonstrated the relatively well-burned wound healing activity in the water extract (Kahkeshani et al., 2012).

The similar results were also observed where *Veronica officinalis* L. was evaluated in this research for its total phenol content, antioxidant capacity of flavonoids, free radicals and potential effects of high blood pressure were studied for the water extract. The total phenol contents in TPC were measured as 2008.34 ± 10.5 mg/L from GAE, and the rosmarinic and caffeic acids were the major phenolic compounds. The absorption activity of nitrogen oxide in vitro was 1 mg/l TE 63.43 % showing an IC₅₀ value of 124.40 µg/mL. It was noticed that in all experimental mice, after treatment with TE the heart index was same. No significant activity was shown by the dose given by TE in the uptake of nitric oxide in vivo. It was suggested by these results that TE can be used as antioxidant and also to protect from hyper-tension (Mihailovic-Stanojevic et al., 2013).

This study is also in accordance with the previous study where 70 medicinal plant extracts were experimented for evaluation of their antioxidant potential and total phenol content (TPC). For human consumption infusions were prepared like tea. Folin-Ciocalteu test was applied to measure the TPC (total phenol contents) of the extracts. FRAP (ferric reducing antioxidant power) assay was followed to testify the total antioxidant potentials of these extracts. This medicinal plants infusion showed the total phenolic contents values in ranges from 10 to 2016 mg/L. The antioxidant activity was in range of 0.18 to 26 mm/L FRAP assay. The phenolic of *M. folium* were highly effective ABTS free radical scavengers when compared to vitamin C and Trolox. Finally, from these findings the importance of *M. folium* can be concluded as a vital source of phenolic and antioxidant as compared to red wine or beverages such as tea (Ulewicz-Magulska & Wesolowski, 2019).

4. Conclusions

Various medicinal plant samples were tested for their ability to eliminate free radicals using synthetic DPPH in order to measure their free radical scavenging activity. The reactivity of different compounds with the stable free radicals was because of the odd number of electrons present in them. According to the results, ethanolic extracts of different medicinal plants have the highest levels of free radical scavenging or antioxidant activity, followed by methanolic extracts when measured against the ascorbic acid standard IC₅₀ of 37.34 mg/mL. Maximum absorbance was observed in the *E. stricta* (IC₅₀ = 38.97 µg/mL), *E. platyphyllos* L. (IC₅₀ = 40.817 µg/mL) and *E. brevicomum* Maxim. (IC₅₀ = 46.26 µg/mL), medicinal plants for both of their ethanolic and methanolic extracts. Among these medicinal plants, polyphenols and other phytochemical components contributed to their total antioxidant activity. Comparing the activity of the aqueous extracts with Ascorbic acid, they showed very similar and comparable results. Moreover, all tested medicinal plant samples exhibited significant levels of free radical scavenging activity, even though it was comparatively less than that of ascorbic acid. In conclusion, this study demonstrated that all medicinal plants, particularly *E. stricta*, *E. platyphyllos* L., and *E. brevicomum* Maxim., possess significant antioxidant properties. These compounds can be effectively used as antioxidants for treating and inhibiting diseases caused by oxidative stress, including cancer, cardiovascular diseases, inflammatory joint diseases, atherosclerosis, dementia, diabetes, asthma, and eye diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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