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RESEARCH ARTICLE

Antiaging Effect of Leaves of Different Extract *Salvia Splendens*

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ABSTRACT

The objective of the present work is to study the *in vitro* antioxidant activities of petroleum ether, ethyl acetate, and methanolic extracts of leaves of *Salvia splendens*. The extracts were studied using 1,1-diphenyl-2-picrylhydrazyl, hydrogen peroxide (H_2O_2), total phenolic content (TPC), and total flavonoid content (TFC). The TPC and TFC were estimated taking gallic acid and rutin calibration curve, respectively. All the extracts possess *in vitro* antioxidant activities. However, the order of possessing activities was methanolic > ethyl acetate > petroleum ether extracts of leaves *S. splendens*. The TPC and TFC were highest in methanolic extract. It can be concluded that the extract of the leaves of S. splendens, possess antioxidant activities. The methanolic extract of leaves of *S. splendens* possesses highest anti-oxidant activity *in-vitro*.

Keywords: 1,1-diphenyl-2-picrylhydrazyl, ferric reducing power activity, hydrogen peroxide scavenging, in-vitro anti-oxidant, Salvia splendens, total flavonoid content, total phenolic content

INTRODUCTION

Oxidative damage to cellular biomolecules such as lipids, proteins, and DNA is thought to play a crucial role in the incidence of several chronic diseases.[1-5] Flavonoids are a group of polyphenolic compounds found abundantly in the plant kingdom. Interest in the possible health benefits of flavonoids and other polyphenolic compounds has increased in recent years owing to their potent antioxidant and free-radical scavenging activities.[6-12]

The effects of free radicals on human beings are closely related to toxicity, disease, and aging[1] most living species have an effective defense system to protect themselves against the oxidative stress induced by reactive oxygen species.[2] Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis, and the aging process.[3-5] The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals, and also by acting as oxygen scavengers.

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Salvia splendens of family Lamiaceae/Labiatae (Mint family) is commonly known as scarlet sage.[13-15] It also reported the activities such as analgesic and anti-inflammatory of roots, antiulcerative activity, antimicrobial activity, laxative activity, antioxidant, hepatoprotective, and antihyperlipidemic activity have also been reported.[13-19]

Hence, the present investigation was conducted to study in vitro antioxidant activities of various roots extracts so as to make researcher to route for other pharmacological activities.

METHODS

Plant material, authentication, and extraction procedures

S. splendens plants were collected from Bhopal (Madhya Pradesh) and Hazaribagh (Jharkhand) and was authenticated by Dr. V.P. Prasad, Scientist-C, Botanical Survey of India, Government of India, Howrah, (West Bengal). The specimen no. PY/NIMS 1026/2013 had been submitted. The air-dried roots were made into coarse powder and extracted with methanol, ethyl acetate, and petroleum ether and percentage yield were calculated. The dried roots were extracted with hot continuous soxhlet

apparatus for 72 h with three different solvents, that is, methanol, ethyl acetate, and petroleum ether and concentrated to dryness under reduced temperature.

Preliminary phytochemical analysis

The various extracts of *S. splendens* were tested for different phytoconsituents such as alkaloids, glycosides, saponinins, tannins, terpinoids, phenolic compounds, protein, and carbohydrates using standard procedures.[20]

In-vitro anti-oxidant activity

1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging activity

The DPPH assay of *S. splendens* extract was determined by the method as reported by Patil *et al*. (2009).

The procedure involved UV-spectrophotometric determination. Three solutions, that is, standard, test, and control were prepared.

Preparation of standard ascorbic acid solutions

Different solutions (1–10 µg/mL) of the ascorbic acid were prepared in methanol. 1.5 mL of each solution of ascorbic acid were mixed with 1.5 mL of 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution was taken after 30 min against methanol (as blank) at 517 nm.[21,22]

Preparation of test solutions

Different solutions of the *S. splendens* extract were prepared in methanol to give concentrations (10–100 µg/mL). 1.5 mL of each solution of *S. splendens* extract was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution of *S. splendens* extract was taken after 30 min against methanol (as blank) at 517 nm.[21,22]

Preparation of control solution

For control, 1.5 mL of methanol was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30 min against methanol (as blank) at 517 nm.

Percentage inhibition was calculated using equation (1), while IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n=3).[21,22]

$$I\% = \frac{Ac - (At - Ab)}{Ac} \times 100$$

Where equation (1)

I% = Percentage inhibition

Ac = Absorbance of control (methanol and 200 µM DPPH solution)

At = Absorbance of ascorbic acid/plant extract with 200 µM DPPH solution after 30 min.

Ab = Absorbance of ascorbic acid/plant extract without 200 µM DPPH solution.

Ferric reducing power activity

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates an increase in the antioxidant activity. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium Fe2+, which then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.[23]

Antioxidant = Potassium ferricyanide + ferric chloride → potassium ferrocyanide + ferrous chloride

Preparation of standard ascorbic acid solutions

Different concentrations of the ascorbic acid were prepared in distilled water to give solutions of concentration (20–100 µg/mL). 1 mL of each concentration of ascorbic acid solutions were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The reaction mixtures were incubated for 20 min at 50°C. Afterward 2.5 mL of 10% trichloroacetic acid solution was added and centrifuged at ×560 g for 10 min. After separation, 2.5 mL of upper layer of each solution were

mixed with 2.5 mL of distilled water and 1 mL of 0.1% ferric chloride (freshly prepared solution). Absorbance was recorded for each solution of ascorbic acid against (0.2 M, pH 6.6) phosphate buffer (used as blank) at 700 nm.[23]

Preparation of test solutions

Different solutions of extract were prepared in distilled water to give various concentrations (20–100 µg/mL). 1 mL of each solution of plant part extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The reaction mixtures were incubated for 20 min at 50°C. Afterward, 2.5 mL of 10% trichloroacetic acid solution was added and centrifuged at ×560 g for 10 min. After separation, 2.5 mL of the upper layer of each solution were mixed with 2.5 mL of distilled water and 1 mL of 0.1% ferric chloride (freshly prepared solution). Absorbance was recorded for each solution of plant part extract against (0.2 M, pH 6.6) phosphate buffer (used as blank) at 700 nm.

The absorbance versus concentration curve for ascorbic acid and extract was plotted. The "Y" and "R^2" values obtained in both curve and the cases were comparatively studied to determine the reducing power of extract.[23]

Hydrogen peroxide (H_2O_2) scavenging activity

H_2O_2 is a biologically important oxidant because of its ability to generate the hydroxyl radical (•OH) which is extremely potent. The ability of the •OH to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems.

The ability of the *S. splendens* extract to scavenge H_2O_2 was determined according to the method reported by Panday *et al.* (2011).

The procedure involved UV-spectrophotometric determination of H_2O_2 radical scavenging. Three solutions, that is, standard, test, and control were prepared.

Preparation of standard ascorbic acid solutions

Different concentrations (10–100 µg/mL) of the ascorbic acid were prepared in distilled water. 1 mL of each solution of ascorbic acid was mixed with 2 mL of 0.1 M phosphate buffer solution and 600 µL of 100 mM H_2O_2 solution. After 10 min, absorbance of different concentration of ascorbic acid solutions was taken at 230 nm.[24]

Preparation of test solutions

Various concentrations (10–100 µg/mL) of the *S. splendens* aqueous extract were prepared in distilled water. 1 mL of each solution of aqueous extract was mixed with 2 mL of 0.1 M phosphate buffer solution and 600 µL of 100 mM H_2O_2 solution. After 10 min, (approximately) absorbance of different concentration of *S. splendens* extract solutions were taken at 230 nm.[24]

Preparation of control solution

For control, 2 mL of 0.1 M phosphate buffer solution was mixed with 600 µL of 100 mM H_2O_2 solution. After 10 min, absorbance of control was taken at 230 nm.

The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using equation 1. IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm Tables 1-4 and Graphs 1-4.[24]

$$I\% = \frac{Ac - (At - Ab)}{Ac} \times 100$$

where

I% = Percentage inhibition.

Ac = Absorbance of control (0.1 M phosphate-buffered solution and H_2O_2).

At = Absorbance of ascorbic acid/plant extract with H_2O_2 after 10 min.

Ab = Absorbance of ascorbic acid/plant extract without H_2O_2.

Estimation of total phenolic content (TPR)

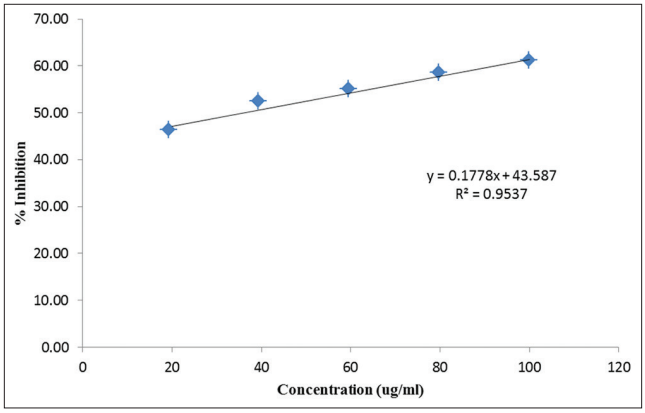
The amount of total phenolic content (TPC) in extracts was determined with the Folin–Ciocalteu reagent. Gallic acid (GA) was used as a standard and the total phenolic were expressed as mg/g GA equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL of GA were prepared in methanol. Concentration of 0.1 and 1 mg/mL

of plant extract were also prepared in methanol and 0.5 mL of each sample was introduced into test and mixed with 2.5 mL of a 10-fold dilute Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The Folin–Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue color was measured spectrophotometrically.

Line of regression from GA was used for estimation of unknown phenol content. From standard curve of GA line of regression was found to be

$y = 0.005x + 0.065$ and $R^2 = 0.976$

Thus, the goodness of fit was found to be good for selected standard curve. By putting the



Graph 1: Regression curve of ascorbic acid by H_2O_2 assay method

Table 1: % Inhibition of H_2O_2 by ascorbic acid

Concentration (µg/ml)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.708	1.302	45.62	36.27
40	0.619		52.46	
60	0.592		54.53	
80	0.546		58.06	
100	0.513		60.60	

Table 2: % Inhibition of H_2O_2 by petroleum ether leaves extract of *S. splendens* (S1)

Conc. (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	1.069	1.302	17.90	170.85
40	1.032		20.74	
60	0.982		24.58	
80	0.922		29.19	
100	0.837		35.71	

S. splendens: Salvia splendens

Table 3: % Inhibition of H2O2 by ethyl acetate leaves extract of *S. splendens* (S1)

Concentration (µg/ml)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.905	1.302	30.49	122.06
40	0.817		37.25	
60	0.793		39.09	
80	0.744		42.86	
100	0.714		45.16	

S. splendens: Salvia splendens

Table 4: % Inhibition of H_2O_2 by methanolic leaves extract of *S. splendens* (S1)

Concentration (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.834	1.302	35.94	68.54
40	0.774		40.55	
60	0.673		48.31	
80	0.598		54.07	
100	0.537		58.76	

S. splendens: Salvia splendens

absorbance of test sample (y = absorbance) in line of regression of above mentioned GA.[25]

Total flavonoids determination

Total flavonoids were measured by a colorimetric assay according to Dewanto *et al*. An aliquot of diluted sample or standard solution of quercetin was added to a 75 µL of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content (TFC) was expressed as mg quercetin/g dry weight (mg quercetin/g DW), through the calibration curve of quercetin. All samples were analyzed in three replications.

Line of regression from quercetin was used for estimation of unknown flavonoid content. From the standard curve of quercetin line of regression was found to be

$y = 0.001x - 0.118$ and $R^2 = 0.985$

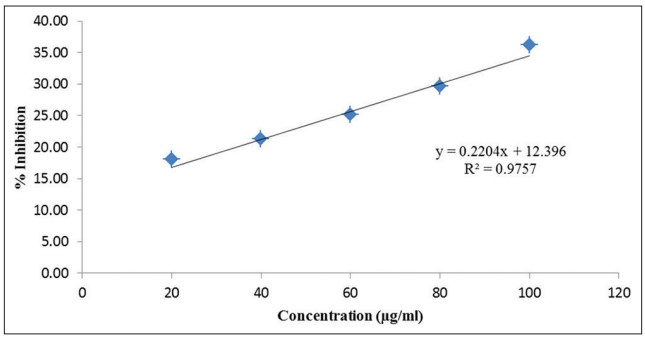
Thus, the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned quercetin.[26-28]

TPC [Tables 5-7 and Graph 5]

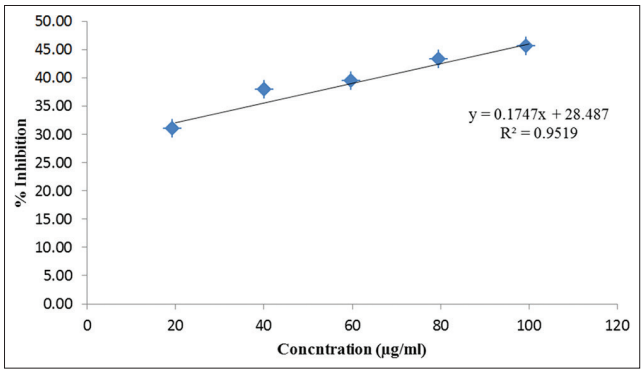
The total phenolic contents (TPC) is determined by comparing with the standard Gallic acid having different concentrations (10-50 µg/ml) with ethyl acetate extract leaves of Salvia splendens (EAESS) and methanolic extract leaves of the Salvia splendens (MESS) having concentration (1mg/ml). The absorbance were taken and compared with the standards.

TFC [Tables 8-10 and Graph 6]

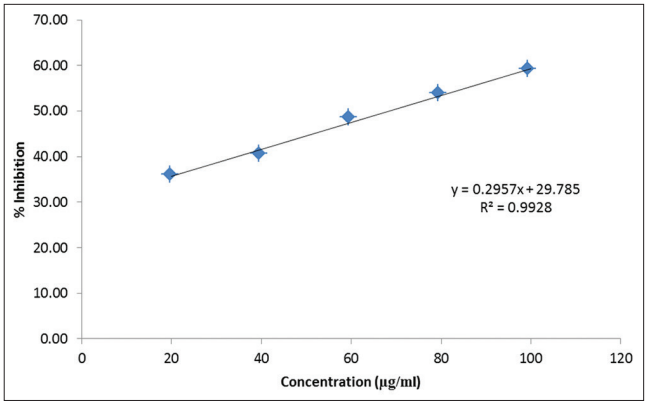
The total flavonoids contents (TPC) is determined by comparing with the standard Quercetin having different concentrations (10-50 µg/ml) with ethyl acetate extract leaves of Salvia splendens (EAESS) and methanolic extract leaves of the Salvia splendens (MESS) having concentration (1mg/ml). The absorbance were taken and compared with the standards.



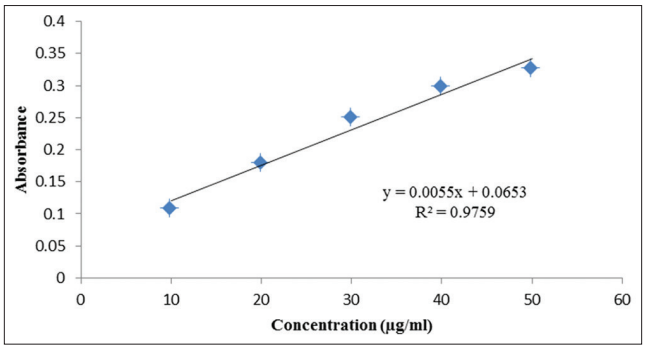
Graph 2: Regression curve of petroleum ether leaves extract of *Salvia splendens* by H_2O_2 assay method (S1)



Graph 3: Regression curve of ethyl acetate extract of *Salvia splendens* by H_2O_2 assay method (S1)



Graph 4: Regression curve of methanolic extract of leaves of *Salvia splendens* by H_2O_2 assay method (S1)



Graph 5: Standard curve of gallic acid

Table 5: Standard curve of GA

Concentration (µg/mL)	Absorbance
10	0.1098
20	0.1763
30	0.2471
40	0.2979
50	0.3258

GA: Gallic acid

Table 6: Total phenolic content in ethyl acetate leaves extract of *S. splendens* (S1)

Absorbance	Concentration	Total phenolic content in mg/g equivalent of GA
0.944	1 mg/mL	175.8
0.934	1 mg/mL	173.8
0.938	1 mg/mL	174.6
Mean ± SD		174.73 ± 1.006

GA: Gallic acid, SD: Standard deviation, *S. splendens: Salvia splendens*

Table 7: Total phenolic content in methanolic leaves extract of *S. splendens* (S1)

Absorbance	Concentration	TPC in mg/g equivalent of GA
1.029	1 mg/mL	192.8
1.021	1 mg/mL	191.2
1.019	1 mg/mL	190.8
Mean ± SD		191.16 ± 1.058

GA: Gallic acid, *S. splendens: Salvia splendens,* SD: Standard deviation, TPC: Total phenolic content

Table 8: Standard curve of quercetin

Concentration (µg/mL)	Absorbance
10	0.136
20	0.152
30	0.163
40	0.177
50	0.198

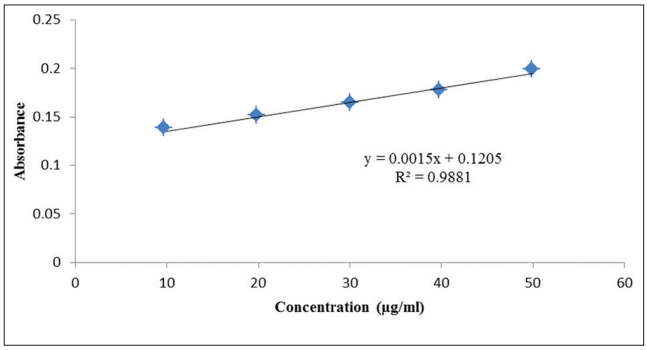
Table 9: TFC in ethyl acetate leaves extract of *S. splendens* (S1)

Absorbance	Concentration	TFC in mg/g equivalent of quercetin
0.213	1 mg/mL	93
0.215	1 mg/mL	95
0.209	1 mg/mL	89
Mean ± SD		92.33 ± 3.055

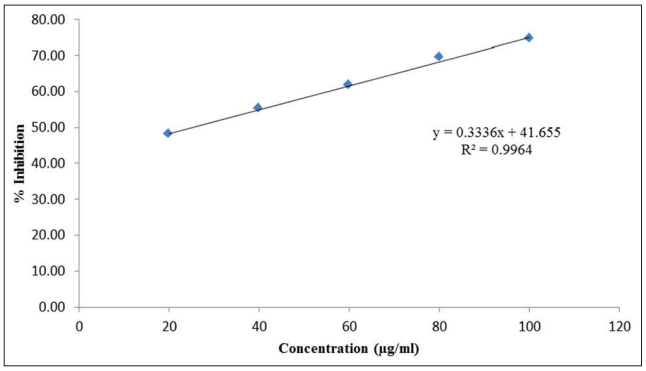
S. splendens: Salvia splendens, SD: Standard deviation, TFC: Total flavonoid content

RESULTS

The successive solvent extraction was done using petroleum ether, ethyl acetate, and methanol using the standard procedure. The behavior of various extracts such as texture and color and extractive yield were calculated.



Graph 6: Standard curve of quercetin



Graph 7: Regression curve of ascorbic acid by 1,1-diphenyl-2-picrylhydrazyl assay method

DPPH free-radical scavenging activity

The DPPH radical scavenging activity of petroleum ether extract of *S. splendens* (PEESS), ethyl acetate extract of *S. splendens* (EAESS), and methanol extract of *S. splendens* (MESS) leaves were detected and compared with ascorbic acid. The percentage inhibition (% inhibition) at various concentrations (10–100 µg/mL) of PEESS, EAESS, and MESS as well as standard ascorbic acid (1–10 µg/mL) were calculated and plotted [Graphs 7-10]. The IC_{50} values of ascorbic acid were 25.07 µg/mL), PEESS (187.74 µg mL), EAESS (112.22 µg/mL), and MESS 95.30 µg mL). Hence, MESS gives a good result of DPPH free-radical scavenging activity.

Ferric reducing power activity

The reductive capabilities of PEESS, EAESS, and MESS leaves were detected and compared with ascorbic acid. The mean absorbance at

various concentration (20–100 µg/mL) of PEESS, EAESS, and MESS as well as standard ascorbic acid (20–100 µg/mL) were calculated in the Tables 11 and 12. The reductive capabilities were found to increase with increasing of concentration in various extract as well as standard ascorbic acid. Hence, MESS gives a good result.

H_2O_2 scavenging activity

The H_2O_2 scavenging activity of PEESS, EAESS, and MESS leaves was detected and compared with ascorbic acid. The percentage inhibition (% inhibition) at various concentration (10-100 µg mL) of PEESS, EAESS, and MESS as well as standard ascorbic acid (10–100 µg/mL) was calculated and plotted in Graph 1. The IC_{50} values are calculated from graph and were found ascorbic acid (36.27 µg/mL), PEESS (170.85 µg mL), EAESS (122.06 µg/mL), and MESS (68.54 µg/mL). Hence, MESS gives a good result.

Table 10: TFC in methanolic leaves extract of *S. splendens* (S1)

Absorbance	Concentration	TFC in mg/g equivalent of quercetin
0.236	1 mg/mL	116
0.234	1 mg/mL	114
0.236	1 mg/mL	116
Mean ± SD		115.33 ± 1.154

S. splendens: Salvia splendens, SD: Standard deviation, TFC: Total flavonoid content

Table 11: Reducing power assay of standard ascorbic acid sample

Concentration (µg/mL)	Ascorbic acid (standard)
10	0.082
20	0.107
40	0.122
60	0.139
80	0.156
100	0.182

TPC

The TPC in PEESS, EAESS, and MESS roots were estimated using standard GAE of phenols. The various concentration of GA (10–50 µg mL) calibration curve was plotted and the results were given in Table 13 and Graph 5. The TPC for PEESS, EAESS, and MESS were obtained for 1 mg/mL of extracts from TPC calibration of GA and the result are given in Table 14. The phenolic compounds are absent in the petroleum ether. The TPC for EAESS and MESS were calculated using standard calibration curve ($y = 0.007x + 0.056$, $R^2 = 0.995$) and found to have 202.06 ± 0.611 and 213.0 ± 0.721 mg/g equivalent of GA, respectively. Hence, MESS gives a good result.

TFC

The TFC in PEESS, EAESS, and MESS roots were estimated using standard quercetin equivalent of phenols. The various concentration of quercetin (25–100 µg/mL) calibration curve was plotted and the results were given in Table 15 and in Graph 6.

Table 12: Reducing power assay of leaves extract of *Salvia splendens* (S1)

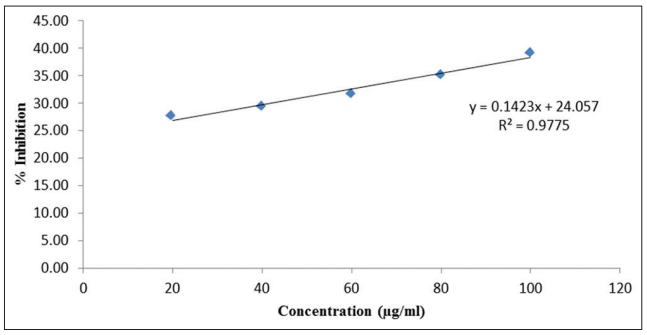
Concentration (µg/ml)	Petroleum ether leaves extract	Ethyl acetate leaves extract	Methanolic leaves extract
10	0.029	0.035	0.049
20	0.036	0.046	0.063
40	0.057	0.069	0.088
60	0.078	0.094	0.112
80	0.105	0.119	0.137
100	0.113	0.126	0.144

Table 13: % Inhibition of DPPH by ascorbic acid

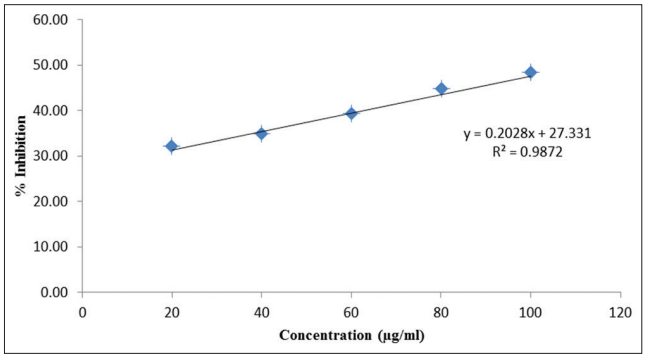
Concentration (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.296	0.569	49.23	26.10
40	0.254		57.98	
60	0.218		63.39	
80	0.175		70.20	
100	0.147		75.38	

DPPH: 1,1-Diphenyl-2-picrylhydrazyl

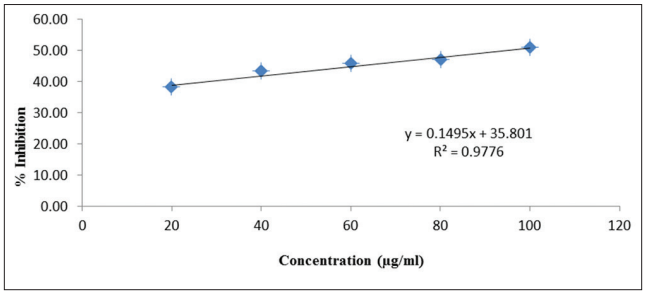
The TFC for PEESS, EAESS, and MESS were obtained for 1000 µg/mL of extracts from TFC calibration of quercetin and the result are given in Table 16. The phenolic compounds are absent in the petroleum ether. The TFC for EAESS and MESS were calculated using standard calibration curve ($y = 0.001x + 0.120$, $R^2 = 0.998$) and found to have 92.33 ± 3.055 and 115.33 ± 1.154 mg/g equivalent of quercetin, respectively. Hence, MESS gives a good result.



Graph 8: Regression curve of petroleum ether leaves extract of *Salvia splendens* by 1,1-diphenyl-2-picrylhydrazyl assay method (S1)



Graph 9: Regression curve of ethyl acetate leaves extract of *Salvia splendens* by 1,1-diphenyl-2-picrylhydrazyl assay method (S1)



Graph 10: Regression curve of methanolic leaves extract of *Salvia splendens* by 1,1-diphenyl-2-picrylhydrazyl assay method (S1)

DISCUSSION

The *S. splendens* leaves, roots, and stem were made coarse powder and extracted with using petroleum ether, ethyl acetate, and methanol as solvent using standard procedure (hot process).

The various extracts of *S. splendens* leaves, roots, and stem were tested for different Phytoconstituents such as alkaloids, glycosides, saponins, tannins, terpenoids, reducing sugars, phenolic compounds, flavonoids, protein, carbohydrates, and volatile oils. The phenolic and flavonoids are widely distributed secondary metabolites in plants having antioxidant activity and have wide range of biological activities as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities.[29,30] Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C and thus might contribute significantly to the protective effects *in vivo*.[31]

In vitro antioxidant studies are widely carried to screen various plant containing phenolic and flavonoids constituents. The *S. splendens* leaves, root, and stem have antioxidant compounds, flavonoids and phenolics which gives a effect such as antioxidant, anti-inflammatory, antitumor activities, and low toxicity.[32,33]

DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses color stoichiometrically with the number of electrons taken up.[34] Such

Table 14: % Inhibition of DPPH by petroleum ether leaves extract of *S. splendens* (S1)

Concentration (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.410	0.578	29.58	188.34
40	0.398		30.55	
60	0.388		30.68	
80	0.369		34.24	
100	0.349		37.98	

DPPH: 1,1-Diphenyl-2-picrylhydrazyl, *S. splendens: Salvia splendens*

Table 15: % Inhibition of DPPH by ethyl acetate leaves extract of *S. splendens* (S1)

Conc. (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.381	0.562	32.21	112.22
40	0.368		34.52	
60	0.343		38.97	
80	0.314		44.13	
100	0.294		47.69	

DPPH: 1,1-Diphenyl-2-picrylhydrazyl, *S. splendens: Salvia splendens*

Table 16: % Inhibition of DPPH by methanolic leaves extract of *S. splendens* (S1)

Concentration (µg/mL)	Absorbance of sample	Absorbance of control	% Inhibition	IC_{50} (µg/mL)
20	0.347	0.562	38.26	95.30
40	0.323		42.53	
60	0.308		45.20	
80	0.299		46.80	
100	0.275		51.07	

DPPH: 1,1-Diphenyl-2-picrylhydrazyl, *S. splendens: Salvia splendens*

reactivity has been widely used to test the ability of compounds/plant extracts to act as free-radical scavengers.[35] In the present study, the DPPH radical scavenging activity of MESS, PEESS, and EAESS leaves, roots and stem were detected and compared with ascorbic acid. The IC_{50} values for DPPH assay of for methanolic extract was found maximum, followed by ethyl acetate extract and for petroleum ether extract was minimum. Although the extracts (MESS) showed good DPPH scavenging activity, it was less effective than standard ascorbic acid. The difference of activity is due to the presence of phenolic components in different extracts. Thus, choosing the appropriate solvent is one of the most important factors for obtaining extracts with a high content of bioactive compounds and antioxidant activity.[35]

In ferric-reducing antioxidant power assay, a yellow color of the test solution changes to various shades of green and blue is depending on the reducing power of each compound. The presence of radicals (i.e., antioxidant) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of pearls Prussian blue spectroscopically, the Fe^{2+} concentration can be monitored; a higher absorbance indicates a higher reducing power. The reductive capabilities of PEESS, EAESS, and MESS leaves, roots, and stem were detected and compared with ascorbic acid. The methanolic extract showed highest reducing power, followed by ethyl acetate extracts and petroleum ether extracts, respectively. The increased reducing power in the extracts indicated that some components in the extract were electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction. Antioxidants are strong reducing agents and this is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure.[36,37]

H_2O_2, a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes. H_2O_2 which in turn generate $^{\bullet}OH$ resulting in initiation and propagation of lipid peroxidation.[37] The H_2O_2 scavenging activity of PEESS, EAESS, and MESS leaves, roots, and stem was detected and compared with ascorbic acid. The IC_{50} values for H_2O_2 scavenging activity for methanolic extract was found maximum followed by ethyl acetate extract and for petroleum ether extract was minimum. Although the methanolic extracts showed good H_2O_2 scavenging activity, it was less effective than standard ascorbic acid. The ability of the extracts to quench OH^- seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.[38]

The TPC in PEESS, EAESS, and MESS leaves, roots, and stem were estimated using standard GAE of phenols. The phenolic compounds are absent in the petroleum ether. The TPC for EAESS and MESS were found to have 202.06 and

213.00 mg/g equivalent of GA, respectively. The methanolic extract was found to have maximum phenolic components and which may be one the reason of its to possess maximum antioxidant activity than other two extracts.[39]

But in TFC, it was found methanolic extract to possess maximum 148.66 mg/g equivalent of quercetin then other ethyl acetate (121.66 mg/g Eq). Flavonoids play some important pharmacological roles against diseases, such as cardiovascular disease, cancer, inflammation, and allergy and other oxidative stress-related diseases.[39]

From above discussion, it was clear that the most powerful antioxidant extract is methanolic extract of leaves of *S. splendens*.

CONCLUSION

It can be concluded that methanolic extract of the leaves of *S. splendens* possess anti-oxidant activities and the potency of anti-oxidant activities depends on the type of extract. The methanolic extract of the leaves of *S. splendens* possesses highest antioxidant activity *in vitro*. This antioxidant power depends on total phenolic and flavonoid contents on particular extract.

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