Section: Research Paper

In-Vitro Antioxidant Activity of various extract of *Leonotis nepetifolia, Blumea lacera,* and *Phylanthus acidus*

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Abstract

The antioxidant assay results demonstrated that extracts of all plants, including LNS: *Leonotis nepetifolia* (Stem), BLR: *Blumea lacera* (Roots), and PASB: *Phylanthus acidus* (Stem bark), may significantly reduce the amount of free radicals in the body. Their radicals scavenging effects against 2, 2-diphenyl-1-picrylhydrazyl [DPPH], nitric oxide, Super oxide anion, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) [$ABTS^{\cdot+}$], as well as Lipid peroxidation inhibition activities showed dose-dependent activities. It can be revealed that the presence of important bioactive compounds, which are associated with antioxidant activity.

Keywords: Extract, Anti-oxidant Activity, Radicals

Introduction

Antioxidant-rich plants can shield -cells from reactive oxygen species (ROS), hence preventing diabetes that is brought on by ROS. Exploring traditional medicinal herbs that can be used to decrease blood glucose levels and obesity-related indicators is therefore important. The capacity of antioxidants to protect against the harmful consequences of hyperglycemia while simultaneously improving glucose metabolism and absorption should be considered as a primary therapeutic option for diabetes mellitus. According to research, oxidative stress is a major contributor to hyperglycemia-induced tissue injury, as well as early events contributing to the development of T2DM [1-2]. The present work was undertaken to determine the *In-Vitro* Antioxidant Activity of various extract of *Leonotis nepetifolia*, *Blumea lacera*, and *Phylanthus acidus*

Material and Methods

Collection of herbs and their authentication

The fresh plant parts, which include LNS: *Leonotis nepetifolia* (Stem), BLR: *Blumea lacera* (Roots), and PASB: *Phylanthus acidus* (Stem bark), were collected from neighborhood locations in the Indore, Khandwa, rewa and Malwa Region of Madhya Pradesh, India between July and September 2019. The collected plant materials were identified and authenticated by Dr. S. N. Dwivedi, Professor and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.). A Voucher specimen of all the plants has been preserved in our Laboratory for further references.

Successive extraction of selected herbs

After being broken up, the samples were run through a 40 mesh filter. The shade-dried, finely powdered plant material (250 gm) was extracted using a Soxhlet apparatus utilizing petroleum ether (60-62°C), chloroform, ethanol, and water until the extraction was finished. After extraction was complete, the solvent was eliminated using distillation. The extracts were dried using rotator evaporators. After the residue had been dried. [3-4]

***In-Vitro* Antioxidant activity**

The total antioxidant activity of food extracts may be measured using a variety of assays. Each approach involves the production of a distinct radical that acts through a number of processes, as well as the measurement of a variety of end points at a defined time point or across a range. Two different approaches have been used: inhibition assays, in which the level of scavenging a free radical that has already formed by hydrogen or electron donation is the indicator of antioxidant activity, and assays that involve the presence of an antioxidant system when the radical is being produced.In this investigation, five in vitro procedures were utilized to test the antiradical and antioxidant properties of the chosen plant extracts. Total antioxidant activity was determined using the *$ABTS^{\bullet+}$ radical scavenging, lipid peroxidation inhibitory, DPPH radical, Superoxide anion radical*, and *nitric oxide* radical scavenging assays. [5-10]

DPPH Radical scavenging activity

When doing an antioxidant experiment, DPPH, or 2,2-diphenyl-1-picrylhydrazyl, an organic chemical substance with persistent free-radical molecules, is employed to track the interactions involving radicals. The free radical scavenging abilities of extracts from selected plants were tested for this experiment. This technique depends on the colored DPPH radical being reduced in a methanolic solution. When an antioxidant chemical that may donate hydrogen combines with DPPH, DPPH is decreased and the color changes from deep violet to bright yellow, which were detected at 517 nm. First, make a 0.1mM solution of the DPPH reagent in methanol and measure the absorbance of each test tube. Add 3ml of a methanolic solution containing various extracts at concentrations of 10, 20, 30, 40, 50, and 100 gm to these test tubes (each holding 1mL of solution), incubate for 30 minutes at 517 nm, and then measure the absorbance. As a benchmark medication, ascorbic acid was used. The exam was conducted three times, with the average of the outcomes. The initial and final absorbance of each solution were used to calculate the percentage decrease in absorbance. [7-8]

Percentage scavenging of DPPH radical was calculated using the following formula:

$$\%\ \text{Scavenging of DPPH} = \frac{[Absorbance of Control - Absorbance of Test]}{[Absorbance of Control]} \text{X } 100$$

Nitric oxide radical scavenging effect

When in aqueous solution, sodium nitroprusside produces nitric oxide that interacts with oxygen to form nitrite ions, which may be detected by the Griess reaction at physiological pH levels. Nitric oxide scavengers compete with oxygen, which lowers nitric oxide synthesis.[45-46] The extracts were combined with varying doses of sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) to create the reaction mixture (3 mL). 150 minutes were spent incubating

these combinations at 25°C. The incubated extracts (0.5 mL) should be taken out and the same quantity of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) should be added at intervals of 30 minutes. At an absorbance of 546 nm, the chromophore that was produced was measured. The outcomes were averaged after each analysis was carried out in triplicate. By contrasting the absorbance values of the control and test groups, the percentage inhibition of the nitric oxide production was calculated. Curcumin was employed as a benchmark substance.

Superoxide anion radical scavenging effect

A technique to gauge superoxide anion scavenging activity was described by Nishimikiet.al. We utilized this test with a few minor adjustments. A reaction mixture was prepared by adding 1 mL of nitro blue tetrazolium (NBT) solution (156 μM NBT in 100 mMphosphate buffer, pH 7.4), 1 mL of NADH solution (reduced form of β-nicotinamide adenine dinucleotide) (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of extracts in different concentration (10, 20, 30, 40, 50 and 100 μg) in distilled water, Phenazinemethosulphate (PMS) solution (60 mM PMS in 100 mM phosphate buffer, pH 7.4) was added to start the reaction. This combination was incubated at 25^{o}C for 5 minutes, and in contrast to blank samples, its absorbance was determined at 560 nm. Reduced absorbance of the reaction mixture was a sign of higher superoxide anion scavenging activity. Catechin is utilized as a benchmark substance. The findings of every experiment were averaged after being carried out in triplicate. By comparing the results of the control and test samples, the percentage of inhibition was estimated.

Lipid peroxidation inhibitory activity

In order to assure optimal liposome production for this activity, 300 mg of egg lecithin were sonicated in an ultrasonic sonicator for 30 min with 30 mL of phosphate buffer, pH 7.4. Plant extracts and the standard (ascorbic acid) were added to this sonicated solution at various concentrations (10, 20, 30, 40, 50, and 100 g/mL) and incubated for 10 minutes.Ferric chloride (0.5 mL, 400 mM) and L-ascorbic acid (0.5 mL, 400 mM) were added to induce lipid peroxidation, which was then incubated for an hour at 37^{o}C.Trichloroacetic acid (TCA, 150 mg/mL), thiobarbituric acid (TBA, 3.75 mg/mL), and butylated hydroxy anisole (BHA, 0.50 mg/mL) were added to hydrochloric acid (2 mL, 0.25 N) to halt the reaction. The reaction mixture was heated for 20 minutes at 80^{o}C, cooled, and centrifuged for 10 minutes before the absorbance of the supernatant was measured at 532 nm. The percentage of inhibition at various concentrations was estimated using the following formula after experiments were carried out in triplicate and averaged:

$$\% \text{ Inhibition} = 1 - \frac{[Vt]}{[Vc]} \text{X } 100$$

Where, Vt = mean absorption of test, Vc= mean absorption of control

The IC_{50} value was derived from the % inhibition at different concentration.

Total antioxidant activity

To scavenge the ABTS●+ cation radical, the antioxidant activity of plant extracts was compared to that of the widely used antioxidant TROLOX (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid). The interaction between ABTS and potassium persulfate produces a radical

cation known as ABTS●+ [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]. The amount of the radical's inhibition is assessed in relation to normal TROLOX when this radical combines with a plant extract that contains antioxidants.

Prepare a 7 mM stock solution by dissolving ABTS in water before starting the test. The interaction between ABTS stock solution and 2.45 mM potassium persulfate produced the ABTS●+radical cation. When the absorbance has stabilized after 12 to 16 hours of standing this combination in the dark at room temperature, it is suitable for use.After being diluted with water, the ABTS+ solution equilibrated to 0.70 (0.02) at 30°C. Perform this experiment at least three times. Add 10 mL of plant extracts and 0–15 ml of standard TROLOX individually to 1 mL of ABTS●+ solution. After 6 minutes, analyse the absorbance at 734 nm. Calculate the TROLOX equivalent antioxidant concentration (TEAC) by plotting the % inhibition of absorbance as a function of the standard and sample concentrations. The gradient of the plot for the sample was divided by the gradient of the plot for TROLOX to determine the TEAC, and the scavenging activity of the samples was estimated using the following formula:

$$S\ \% = \frac{[A_{Control} - A_{Sample}]}{[A_{Control}]} X\ 100$$

Where $A_{control}$ is the absorbance of the blank control ($ABTS^{\bullet+}$ solution without test sample) and A_{sample} is the absorbance of the test sample.

Results and Discussion

The antioxidant reacts with stable free radical DPPH and converts it to 1,1-diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH was measured at an absorbance of 516 nm. The DPPH radical scavenging (in %inhibition) by different extract reported in table 1. Ascorbic acid has taken as reference standard which showed 98.52±0.07% inhibitions at dose of 100 µg/mL with IC_{50} value 33.92. Amongst the extracts studied AEPASB, EELNS and EEBLR having more potent activity than other extracts of corresponding plant and showed 84.35±0.03, 77.70±0.25, and 64.52±0.23% inhibition respectively at concentration of 100 µg/mL with correspondence IC_{50} value of 48.98, 67.17 and 88.22 respectively. While all other extracts show the significantly moderate activity. All experiments were performed in triplicate. The overall results of % scavenging of DPPH by each extract and standard were shown in the Table 1 and Graph 1. The IC_{50} values ± SEM are represented in Graph 2.

AEPASB, EELNS, and EEBLR had higher phenolic and flavonoid concentrations than other extractives. Between the phenolic content and the effectiveness of scavenging free radicals (DPPH and •OH), a positive connection (p value 0.001) was found. AEPASB had the greatest activity level among the all studied extracts.

Nitric oxide (NO), a crucial chemical mediator produced by endothelial cells, macrophages, neurons, etc., has a role in the control of a number of physiological processes. Numerous disorders are associated with high NO concentrations. Nitrite and peroxynitrite anions are produced when oxygen and extra nitric oxide combine, acting as free radicals. In the current investigation, the chemicals under test compete with oxygen to react with nitric oxide, preventing the anions from forming. The percentage suppression of nitric oxide generation caused by various tested chemical doses is shown in Table 2 and Graph 3. As a benchmark,

curcumin has been used. EELNS, EEBLR, and EEPASB were shown to provide potent% inhibition values of 55.11±0.17, 52.04±0.14, and 48.92±0.09% of conventional curcumin, which produces 91.42±0.22% inhibition.

The outcomes demonstrated the antioxidant capacity of all the chosen plant components, including LNS: Leonotis nepetifolia (Stem), BLR: Blumea lacera (Roots), and PASB: Phylanthus acidus (Stem Bark). All the tested extracts shows the nitrous oxide scavenging activity in dose dependant manner. The standard employed was curcumin. Nitric oxide synthase induction is inhibited by curcumin, a naturally occurring direct nitric oxide scavenger. It lessens the quantity of nitrite produced when oxygen reacts with the nitric oxide produced by sodium nitroprusside. The outcomes also demonstrate that the ethanolic extract is more effective than the other extracts.

Antioxidants cause the absorbance at 560 nm to drop, which shows that superoxide anion has been consumed in the reaction mixture. Comparing the potent% inhibition value of extracts to standard catechin, which causes 94.14±0.43% inhibition at concentrations of 100 g/mL, it was discovered that the extracts were 60.52±0.24, 60.87±0.14, and 52.47±0.18%. The superoxide scavenging activity of each plant's extract and catechin on the PMS/NADH-NBT system is shown in Table 3 and Graph 4. The concentration-dependent rise in superoxide production inhibition was seen in all the examined extracts of the chosen plants, and the maximum O2 •- scavenging activity was noted at a concentration of 100 g/ml by EEBLR.

Reactive oxygen species, or lipid peroxidation precursors, are created by both endogenous metabolic processes in the human body or in food systems and foreign chemical agents. We examined each extract's ability to scavenge free radicals in combination with the reference medication Ascorbic acid at various concentrations, as indicated in Table 4 and Graph5. EELNS, AEBLR, and AEPASB were shown to inhibit radicals at a concentration of 100 g/mL at 43.65±0.061, 37.51±0.06, and 39.33±0.41%, respectively, as opposed to conventional ascorbic acid, which produces 99.83±0.05% inhibition. These findings suggest LNS: *Leonotis nepetifolia* (Stem), BLR: *Blumea lacera* (Roots), and PASB: *Phylanthus acidus* (Stem bark) extract has a potential as an excellent natural antioxidant agent.

The antioxidant activity of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation was measured and compared to 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (TROLOX). The comparative analysis of results is shown in Table 6 and Graph. At 100 g/mL concentrations, EELNS, EEBLR, and EEPASB inhibited radicals at 46.74±0.85, 52.09±0.13, and 59.60±0.06%, respectively, compared to standard TROLOX, which inhibited radicals at 97.09±0.29%. The antioxidant assay results demonstrated that extracts of all plants, including LNS: *Leonotis nepetifolia* (Stem), BLR: *Blumea lacera* (Roots), and PASB: *Phylanthus acidus* (Stem bark), may significantly reduce the amount of free radicals in the body.

Conclusion

Their radicals scavenging effects against 2, 2-diphenyl-1-picrylhydrazyl [DPPH], nitric oxide, Super oxide anion, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) [$ABTS^{\cdot+}$], as well as

Lipid peroxidation inhibition activities showed dose-dependent activities. It can be revealed that the presence of important bioactive compounds, which are associated with antioxidant activity.

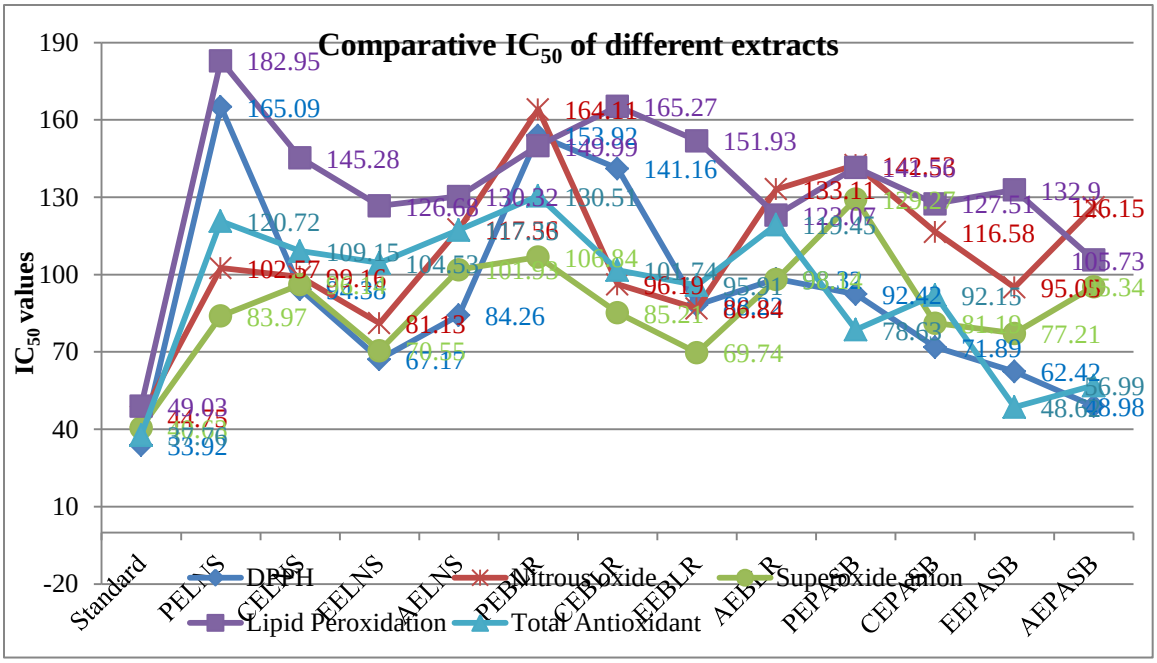


Table 1: DPPH radical scavenging activity

	IC50	100 µg/mL	50 µg/mL	40 µg/mL
Standard	33.92	98.52	71.34	59.8
PELNS	165.09	32.49	23.85	18.47
CELNS	94.38	58.88	29.88	26.34
EELNS	67.17	77.7	48.8	32.25
AELNS	84.26	65.41	34.52	29.13
PEBLR	153.92	31.06	22.89	19.47
CEBLR	141.16	35.42	23.44	18.44
EEBLR	88.22	64.52	46.27	37.58
AEBLR	98.32	51.41	31.2	27.61
PEPASB	92.42	61.29	36.46	29.46
CEPASB	71.89	69.51	44.83	35.38
EEPASB	62.42	77.7	46.49	39.7
AEPASB	48.98	84.35	54.83	45.38

All readings are mean ± SD, n = 3, IC_{50} value reported as

Sample	10 µg/mL	20µg/mL	30 µg/mL
Ascorbic acid	18.46	33.14	47.35
PELNS	4.42	9.17	14.55
CELNS	10.55	16.38	21.06
EELNS	12.11	18.36	25.79
AELNS	11.72	16.34	22.21
PEBLR	3.45	8	14.55
CEBLR	6.07	11.38	13.88
EEBLR	12.11	17.57	26.97
AEBLR	10.55	17.64	22.56
PEPASB	10.21	18.67	22.45
CEPASB	12.23	17.45	28.79
EEPASB	13.4	18.56	25.94
AEPASB	15.23	25.24	33.79

Table 2: Nitrous oxide radical scavenging activity

50 µg/mL	100 µg/mL	IC_{50}
61.35±0.32	91.42±0.22	44.75
35.49±0.06	45.45±0.12	102.57
35.13±0.05	47.76±0.11	99.16
38.70±0.17	55.11±0.17	81.13
28.22±0.02	40.21±0.31	117.56
18.08±0.28	31.96±0.23	164.11
33.21±0.12	50.02±0.02	96.19
36.38±0.07	52.04±0.14	86.84
23.27±0.08	36.74±0.21	133.11
20.83±0.04	34.92±0.02	142.53
31.74±0.04	44.79±0.01	116.58
34.16±0.02	48.92±0.09	95.05
23.29±0.09	38.25±0.21	126.15

All readings are mean ± SD, n = 3, IC_{50} value reported as Conc. ± SEM, $P < 0.01$ Vs Standard

Sample	10 µg/mL	20µg/mL	30 µg/mL	40 µg/mL
Curcumin	16.12±0.24	29.86±0.14	38.42±0.02	48.76±0.04
PELNS	11.93±0.18	20.58±0.16	24.51±0.13	31.96±0.23
CELNS	9.43±0.01	18.37±0.14	23.98±0.02	27.26±0.20
EELNS	8.60±0.49	18.08±0.30	27.30±0.24	33.40±0.30
AELNS	5.22±0.003	11.78±0.09	18.37±0.14	23.98±0.02
PEBLR	6.81±0.02	9.69±0.17	11.93±0.38	15.73±0.28
CEBLR	12.57±0.01	16.47±0.21	21.06±0.08	25.05±0.01
EEBLR	7.31±0.14	15.87±0.05	22.29±0.24	30.91±0.30
AEBLR	4.92±0.22	11.20±0.04	15.89±0.13	19.46±0.15
PEPASB	4.19±0.13	9.72±0.17	11.93±0.38	15.42±0.12
CEPASB	13.51±0.003	18.18±0.01	23.11±0.11	27.67±0.10
EEPASB	11.52±0.19	17.51±0.05	25.76±0.24	31.94±0.01
AEPASB	4.83±0.06	11.86±0.06	15.45±0.01	18.16±0.02

Table 3: Superoxide radical scavenging activity

100 µg/mL	IC_{50}
94.41+0.43	40.63
55.07±0.23	83.97
41.61±0.56	96.14
60.52±0.24	70.55
44.18±0.12	101.93
44.87±0.82	106.84
52.86±0.41	85.21
60.87±0.14	69.74
45.85±0.02	98.14
38.22±0.22	129.27
58.80±0.04	81.19
52.47±0.18	77.21
45.85±0.02	95.34

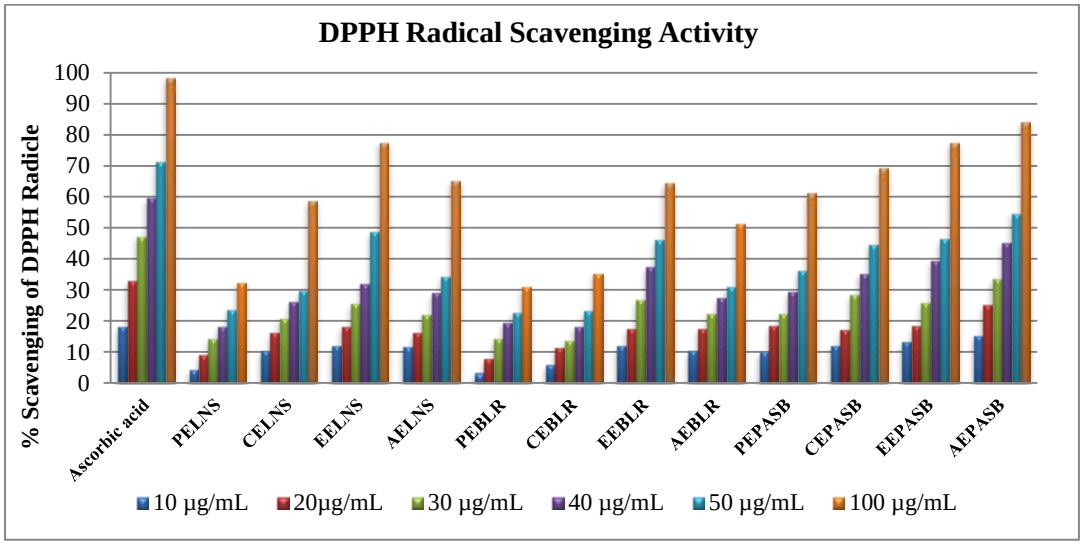
All readings are mean ± SD, n = 3, IC_{50} value reported as Conc. ±

Sample	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
Catechin	32.15+ 0.19	50.82+0.52	66.24+0.14	77.04+1.23	90.32+0.23
PELNS	6.41±0.14	13.32±0.28	21.98±0.36	29.35±0.26	36.85±0.17
CELNS	5.92±0.45	8.05±0.65	20.25±0.58	24.20±0.37	30.36±0.43
EELNS	13.17±0.62	23.20±0.76	31.09±0.21	37.67±0.55	46.68±0.53
AELNS	10.97±0.16	24.82±0.36	29.52±0.29	34.39±0.31	42.02±0.46
PEBLR	6.86±0.08	10.68±0.13	21.90±0.24	26.72±0.33	33.28±0.62
CEBLR	5.92±0.27	11.50±0.31	23.27±0.29	30.47±0.26	38.75±0.09
EEBLR	13.17±0.13	24.63±0.16	32.77±0.18	40.41±0.18	47.53±0.06
AEBLR	10.97±0.15	21.40±0.07	29.53±0.08	33.92±0.16	42.12±0.14
PEPASB	6.95±0.05	10.80±0.28	21.82±0.03	25.58±0.13	27.19±0.33
CEPASB	6.31±0.14	10.83±0.12	25.23±0.18	36.31±0.16	41.5±0.17
EEPASB	10.47±0.05	21.59±0.32	34.82±0.21	38.48±0.11	47.19±0.08
AEPASB	10.66±0.15	22.38±0.09	28.42±0.48	33.73±0.28	36.44±0.35

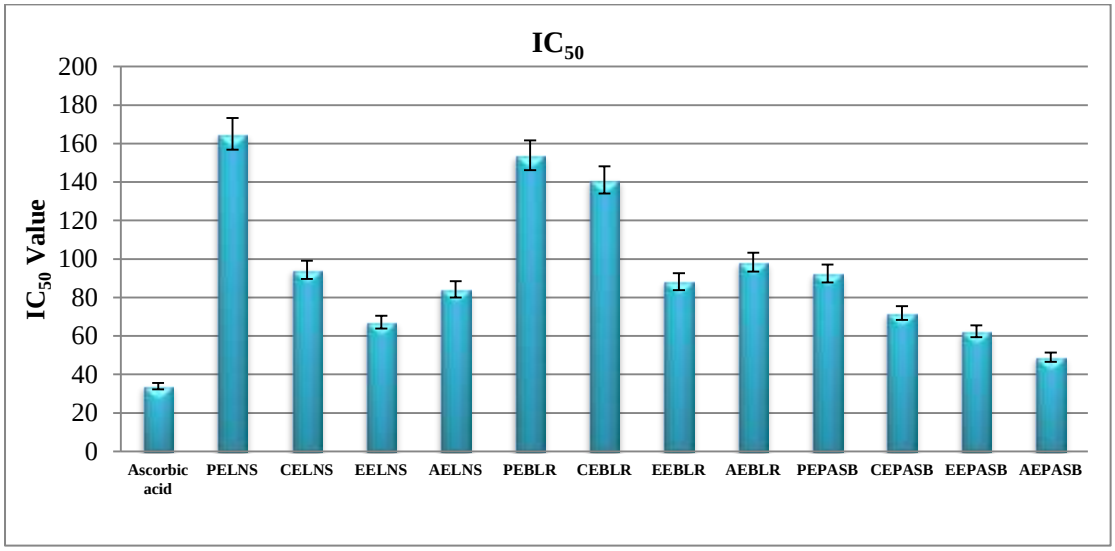
Table 4: Lipid peroxidation inhibitory activity							
Sample	**10 µg/mL**	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**	**100 µg/mL**	**IC50**
Ascorbic Acid	12.04±0.30	21.47±0.03	31.64±0.43	40.60±0.36	53.26±0.36	99.83±0.05	49.03
PELNS	3.60±0.18	6.86±0.40	11.24±0.09	15.15±0.17	19.29±0.41	26.75±0.21	182.95
CELNS	11.36±0.15	18.36±0.23	21.43±0.02	28.48±0.50	34.67±0.76	42.20±0.15	145.28
EELNS	11.36±0.05	16.83±0.02	23.02±0.12	26.89±0.17	30.08±0.37	39.51±0.39	126.68
AELNS	8.30±0.07	16.71±0.03	23.48±0.58	26.95±0.05	30.31±0.60	37.51±0.06	130.32
PEBLR	3.62±0.11	8.65±0.38	12.93±0.27	19.16±0.34	24.02±0.55	31.68±0.55	149.99
CEBLR	6.22±0.18	8.57±0.49	11.93±0.23	18.15±0.33	22.75±0.17	30.00±0.42	165.27
EEBLR	6.76±0.05	10.70±0.02	15.35±0.12	20.75±0.17	24.40±0.13	32.75±0.16	151.93
AEBLR	6.79±0.21	6.86±0.40	13.62±0.09	23.96±0.56	28.48±0.26	39.33±0.41	123.07
PEPASB	8.31±0.06	13.72±0.55	20.02±0.13	26.70±0.17	28.75±0.55	34.67±0.76	141.56
CEPASB	10.73±0.45	16.30±0.36	22.44±0.60	25.47±0.76	30.15±0.55	39.14±0.15	127.51
EEPASB	8.61±0.21	16.36±0.12	23.05±0.76	26.33±0.42	30.19±0.44	37.02±0.17	132.9
AEPASB	8.30±0.07	19.78±0.03	24.35±0.22	30.01±0.045	36.13±0.51	43.65±0.061	105.73
All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard							

Table 5: Total antioxidant activity							
Sample	**10 µg/mL**	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**	**100 µg/mL**	**IC_{50}**
TROLOX	25.51±0.54	34.73±0.43	44.57±0.71	53.87±0.43	62.76±0.79	97.09±0.29	37.76
PELNS	8.17±0.16	15.43±0.12	20.04±1.09	27.03±0.51	33.89±0.51	41.01±0.25	120.72
CELNS	12.97±0.35	18.51±0.47	26.10±0.22	32.09±0.47	36.97±0.65	44.28±0.70	109.15
EELNS	9.78±0.54	16.56±0.84	23.37±0.16	30.85±0.20	35.37±0.85	46.74±0.85	104.53
AELNS	11.07±0.31	16.97±0.94	22.80±0.05	26.27±0.13	33.15±0.62	43.84±0.54	117.33
PEBLR	9.73±0.02	14.66±0.32	19.91±0.64	27.67±0.44	33.15±0.25	41.24±0.55	130.51
CEBLR	13.23±0.61	21.37±0.12	29.34±0.11	36.72±0.15	41.84±0.65	49.25±0.32	101.74
EEBLR	10.42±0.29	17.54±0.83	23.84±0.56	31.68±0.13	38.37±0.58	52.09±0.13	95.91
AEBLR	14.53±0.40	18.57±0.71	23.37±0.15	29.12±0.13	32.51±0.19	44.15±0.47	119.45
PEPASB	14.23±0.65	19.90±0.82	30.41±0.46	37.18±0.12	40.71±0.22	57.80±0.33	78.63
CEPASB	15.08±0.30	21.10±0.51	31.69±0.14	37.53±0.53	42.86±0.27	52.33±0.47	92.15
EEPASB	16.74±0.41	24.06±0.63	34.50±0.56	45.86±0.45	52.30±0.54	59.60±0.06	48.62
AEPASB	19.80±0.34	25.26±0.17	34.71±0.53	42.55±0.10	49.49±0.19	66.27±0.19	56.99
All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard							

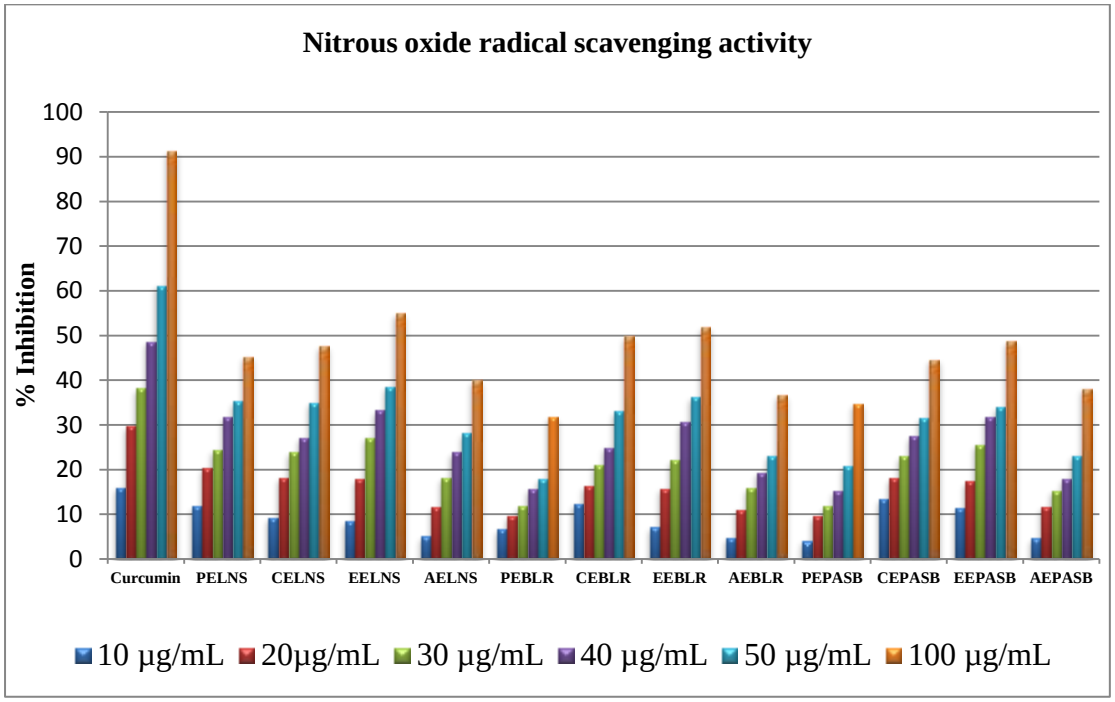
Graph 1: DPPH radical scavenging activity



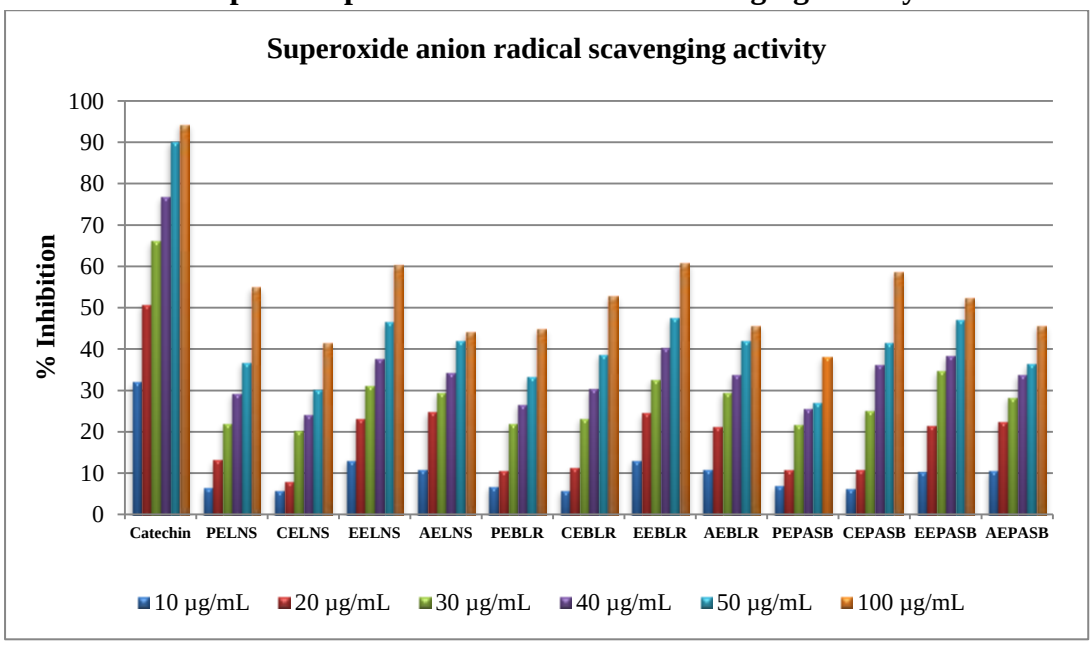
Graph 2: IC_{50} values of DPPH radical scavenging activity by different extracts and standard ascorbic acid



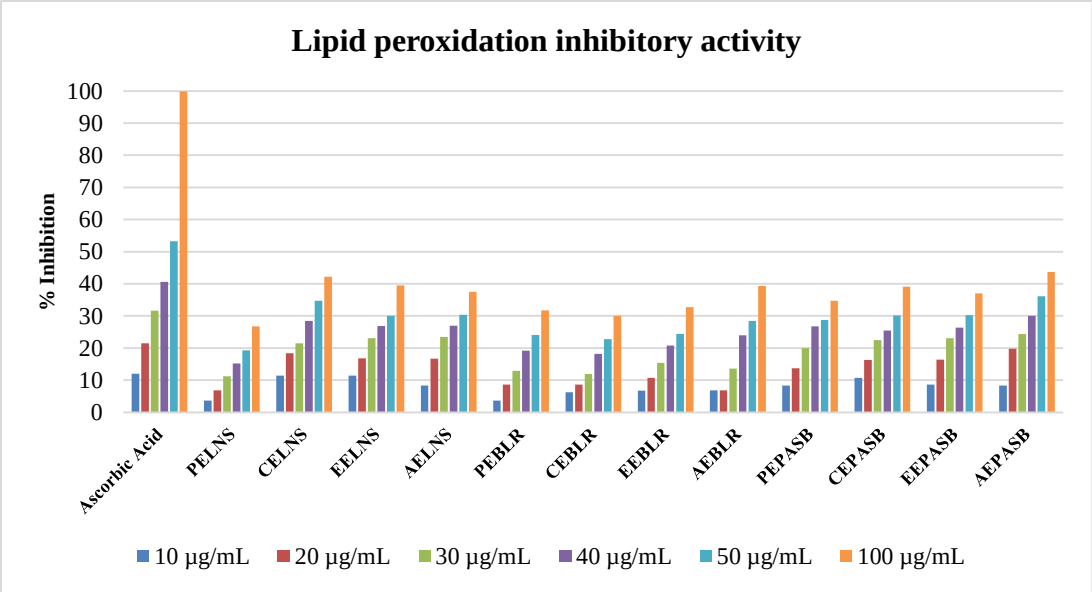
Graph 3: Nitrous oxide radical scavenging activity



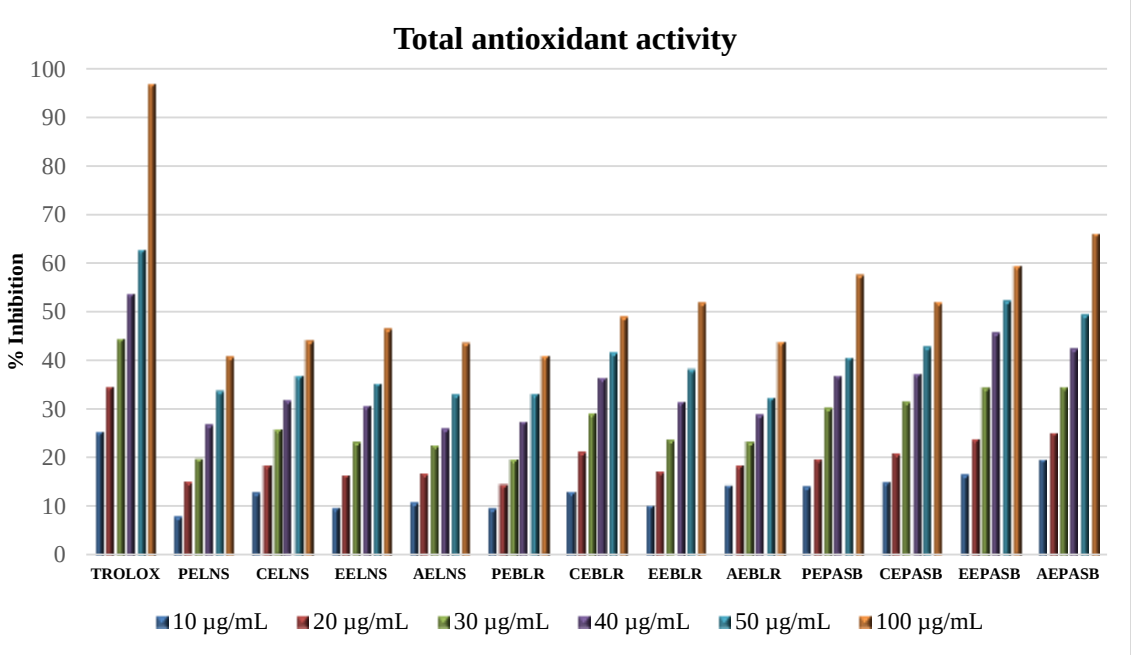
Graph 4: Superoxide anion radical scavenging activity



Graph 5: Lipid peroxidation inhibitory activity



Graph 6: Total antioxidant activity



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