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Antioxidant, free radical scavenging and *invitro* cytotoxic studies of ethanolic extract of *Leucas indica* var *lavandulifolia* and *Leucas indica* var *nagalapuramiana*

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ABSTRACT

Objective: The main focus of the study is to determine antioxidant, free radical scavenging and cytotoxic effect of ethanolic extract of whole plant of *Leucas indica var lavandulifolia* and *Leucas indica var nagalapuramiana* using various in vitro assay methods as well as to estimate the amount of total phenol and flavonoid contents. **Methods:** *Leucas indica var lavandulifolia* and *Leucas indica var nagalapuramiana* were collected in February 2008 from Thirumala hills and standard procedure from the literature were used to estimate the antioxidant and free radical scavenging activity. **Results:** The results suggest that both the extracts can be considered as a valuable source of antioxidants products as the obtained values are more or less near to the used standard compound. **Conclusions:** The characterization of leucas for antioxidant and free radical scavenging activities are new to literature in this context this extensive *in vitro* study of the selected two species of leucas for antioxidant activity will be a valuable study for further *in vivo* activities.

1. Introduction

The recent scientific investigation suggesting the involvement of oxidative stress in the pathogenesis of atherosclerosis, inflammation, cancer and neurodegenerative disorder like alzheimer, parkinsonism and convulscent. Oxidative stress is mainly induced by the reactive oxygen species. This species include both oxygen radical such as peroxyl, superoxide anion, hydroxyl, peroxynitrite and nitric oxide radical and non radical derivatives of oxygen like hydrogen peroxide, hypochlorous acid and singlet oxygen[1]. These free radicals produced by the biochemical reaction in human body and cause the structural and functional damage to the neuron, protein, lipids, nucleic acid and cellular molecules[2]. In recent years, many plants extracts and different types of secondary metabolite have been shown not only free radical scavenging and antioxidant activities but also antiinflammatory, hepatoprotective[3], antiproliferative[4], neuroprotective[5] and cardio protective activities[6]. The flavonoids are typical phenolic compounds and rich in plants. The common feature of flavonoids is hydroxyl group substituted flavan moiety. These characteristic chemical functionalities are necessary for the flavonoids to scavenge free radical and prevent the oxidation of biological molecules by converting the more reactive oxygen species by donating hydrogen atom into inactive species[7]. Therefore antioxidant and free radical scavenger could be more beneficial in the prevention and treatment of oxidative stress induced disorder and disease.

In the present study, we examined antioxidant, free radical scavenging and cytotoxic effect of ethanolic extract of whole plant of *Leucas indica var lavandulifolia* (LL) and *Leucas indica var nagalapuramiana* (LN) using various in vitro assay methods as well as to estimate the amount of total phenol and flavonoid contents.

LL and LN are the members of the family Lamiaceae. LL is erect herbs; branches appressed pubescent. Leaves are linear- lanceolate, entire, undulate or distantly serrate. Flowers are white, shortly pedicelled in whorls, towards the end of the branches. Calyx is tube slightly curved, 8-toothed, posterior tooth longer than the rest. Corolla is annulate within near the middle. Stamens are 4. LN is erect herbs; branches slender, puberulous. Leaves are linear-

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laceolate, entire, slightly undulate or serrulate, puberulous on both sides. Flowers are white, in whorls at the ends of branches. Nutlets are oblong, trigonous, brownish-black[8].

2. Materials and Method

2.1. Materials

1, 1-diphenyl-2-picrylhydrazyl (DPPH), β - carotene, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (St Louis, MO, United States). Reduced nicotinamide adenine dinucleotide (NADH) and Linoleic acid were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Folin-Ciocalteu reagent and 1, 10- Phenanthroline were purchased from Sd Fine Chemicals Mumbai, India. Phenazine methosulphate and Sulfanilamide were purchased from NR chemicals Pvt Ltd, Mumbai, India. Sodium nitroprusside was obtained from HiMedia Laboratories Pvt Ltd, Mumbai, India. Other reagent and solvents used were of analytical grade and obtained from various other commercial sources.

2.2. Methods

2.2.1. Sample collection and Extraction

The whole plant of LL and LN were collected in February 2008 from Thirumala hills, Andhra Pradesh, India. LL and LN were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The specimens of LL and LN were prepared and submitted in the Department of Botany under the voucher no: 276 and 239 respectively. The entire plants were cleaned, dried at room temperature and stored properly in air tight container. The dried plants material were ground into a fine powder by laboratory mill. About 200gm of the dried powder of plants were packed in a soxhlet apparatus and defatted with petroleum ether (60-80 °C). The defatted marc was percolated in a soxhlet apparatus with 70% ethanol until exhaustion. The resultant extracts were concentrated under reduced pressure at room temperature using a rotary vacuum evaporator. The concentrated extracts were collected and preserved in dessicator until used for further studies.

2.2.2. Determination of total phenolic content

The total phenolic content in the plants extracts were determined by a colorimetric assay method based on Folin and Ciocalteu procedure described by Mohammad Ali *et al* [9]. About 0.5 mL of ethanolic extract was mixed with 2.5 mL Folin and Ciocalteu reagent (diluted 1:10%) followed by 2 mL of sodium carbonate (7.5% v/v) solution. The absorbance was then measured at 765 nm after incubation at 30 °C for 90 min estimation of the phenolic compounds was carried out in triplicate. Results were expressed as gallic acid equivalents.

2.2.3. Determination of total flavonoid content

The total flavonoid content was calculated by the method developed by Mohammad Ali *et al* [9]. About 0.5 mL of ethanolic extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of $NaNO_2$ solution (15%). After 6 min, 0.15 mL of aluminum chloride solution (10%) was added and allowed to stand for 6 min. To the resultant solution 2 mL of sodium hydroxide solution (4%) was added. Immediately water was added to bring the final volume to 5 mL.The mixture was thoroughly mixed and allowed to stand for 15 min. Absorbance was determined at 510 nm versus distilled water as blank. Concentration of sample was detected from standard rutin calibration curve and calculated in mg rutin equivalent.

2.2.4. Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method described by Mohammad Ali *et al* [9]. The ethanolic extract (0.75 mL) of various concentrations were mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexaferricyanide (1% w/v) followed by incubation at 50 °C in a water bath for 20 min. the reaction was stopped by adding 0.75 mL of trichloroacetic acid solution (10%w/v) and then centrifuged at 3 000 rpm for 10 min. A total of 1.5 mL of supernatant was mixed with 1.5 mL of distilled water and 0.1ml of ferric chloride solution (0.1% w/v) for 10 min. The absorbance at 700 nm was recorded as the reducing power. All the tests were run in triplicate. Ascorbic acid was used as the standard.

2.2.5. β -Carotene-linoleic acid assay

The antioxidant activity of extract was measured according to the method described by Kumaran *et al* [2]. A solution of β -Carotene was prepared by dissolving 2 mg of β -Carotene in 10ml of chloroform. About 2 mL of this solution was pipetted in to a 100 mL round bottomed flask. After removing the chloroform under vacuum, 40mg of Linoleic acid, 400 mg of tween 20 as emulsifier and 100 mL of distilled water were added to the flask with vigorous shaking. 2.5 mL aliquots were transferred into different test tubes containing different concentrations of extract (0.2 mL). As soon as the emulsion was added to each test tube, the zero time absorbance was measured at 470 nm. The tubes were placed at 50 °C in a water bath and measurement of absorbance was recorded after 2 h. A blank devoid of β -Carotene was prepared for background subtraction. The same procedure was repeated with trolax as a positive control. All readings were taken in triplicate. Antioxidant activity in terms of % inhibition by,

Antioxidant activity= (β -Carotene content after 2 H of assay / initial β -Carotene content) × 100.

2.2.6. DPPH scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was measured using the method described by Shih-Chuan Liu *et al* [10]. 1 mL of different concentrations of ethanolic extract was added to 3 mL of 0.1 mM methanolic solution of DPPH. The mixture was shaken followed by inhibiting at room temperature for 30 min in

dark. The absorbance against blank was measured at 570 nm. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition was calculated by following equation.

% DPPH radical scavenging capacity= [(A0– A1)/A0] ×100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.7. Super oxide radical scavenging activity

The effect of super oxide radical scavenging activity was determined by the nitroblue tetrazolium reduction method and described by Wei Fu et al [3]. 1 ml of nitroblue tetrazolium (NBT) solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 μM NADH in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of different concentrations of the ethanolic extract were mixed. The reaction was started by adding 100 μL of Phenazine methosulphate (PMS) solution (60μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank samples, containing all the reagents except the PMS. All readings were taken in triplicate and Trolox was used as the standard. The % inhibition was calculated by following equation.

% Super oxide radical scavenging capacity= [(A0– A1)/A0] × 100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.8. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity of ethanolic extract was evaluated by the method described by Olabinri *et al* [11]. 60μL of $FeSO_4.7H_2O$ (1 mM) was added to 90μL of aqueous 1,10 phenanthroline(1 mM), 2.4 mL of 0.2 M phosphate buffer pH 7.8 was added to the above mixture, followed by addition of 150 μL of hydrogen peroxide (0.17 mM) and 1.5mL of different concentrations of sample in sequence. The mixture was incubated for 5min at room temperature. The absorbance of the mixture was read at 560 nm against blank. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition was calculated by following equation.

% Hydroxyl radical scavenging capacity= [(A0– A1)/A0] × 100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.9. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging capacity was measured according to the method performed by Mohammad ali *et al* [9]. 1 mL of different concentrations of ethanolic extract was added to 2 mL of hydrogen peroxide solution (10 mM) in phosphate buffer (50 mM, pH 7.4) and reaction mixture was incubated at 25 °C for 30 min. The unreacted hydrogen peroxide was determined by measuring the absorbance of the reaction mixture at 230 nm against distilled water as a blank.

All readings were taken in triplicate and Trolox was used as the standard. The % inhibition was calculated by following equation.

% Hydroxyl radical scavenging capacity= [(A0– A1)/A0] × 100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.10. Nitric oxide radical scavenging activity

Nitric radical scavenging capacity of ethanolic extract was measured according to the method described by Olabinri et al [11]. 0.1ml of sodium nitroprusside (10mM) in 0.2ml of phosphate buffer (0.2 M, pH 7.8) was mixed with 0.5ml of different concentration of ethanolic extract and incubated at room temperature for 150min. After incubation period, 0.2 mL of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N– (1– Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546nm against blank. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity= [(A0– A1)/A0] × 100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.11. Metal chelating activity

Metal chelating capacity of ethanolic extract was measured according to the method described by Iihami Gulcin et al [12]. 1 mL of different concentrations of ethanolic extract was added to a 0.05ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 mL of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition of ferrozoine– Fe^{2+} complx was calculated by following equation.

% Inhibition of ferrozoine– Fe^{2+} complx = [(A0– A1)/A0] ×100

Where A0 was the absorbance of control and A1 was the absorbance of ethanolic extract or standard.

2.2.12. In vitro cytotoxicity study

In vitro cytotoxicity stydy was performed at Amala Cancer Research centre, Amalanagar, Thrissur, Kerala, India. The ethanolic extract was studied for short term in vitro cytotoxicity using Dalton's Lymphoma Ascites Carcinoma cells. The tumor cells were aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with normal saline and checked for viability using trypan blue dye exclusion method. The cell suspension (1 $\times 10^6$ cells in 0.1 mL) was added to tubes containing various concentrations of the test compounds and the volume was made upto 1 mL using phosphate buffered saline. Control tube contained only cell suspension. These assay mixture were incubated for 3 h at 37 °C and percent of dead cells were evaluated by trypan blue exclusion method.

3. Results

3.1. Extraction yield, total phenolics and flavonoids content

70% ethanol was selected as solvent to extract wide range of phenolics and flavonoids substance. % yield, phenolic and flavoniod contents of extract LL were 20.364%, 30.459±1.023 and 47.418±1.245. % yield, phenolic and flavoniod contents of extract LN were found to be 19.761%, 20.101±0.856 and 36.174±0.347.

3.2. Reducing power assay

Anti oxidants are also reducing agents by donating hydrogen atom. Reducing ability of extracts was given in Figure1 in comparison with the standard Ascorbic acid. The reducing power of LL (0.566±0.008 μg/mL) was almost equal to ascorbic acid (0.579±0.014 μg/ml) at a concentration of 100 μg/mL and was given in Figure 1.

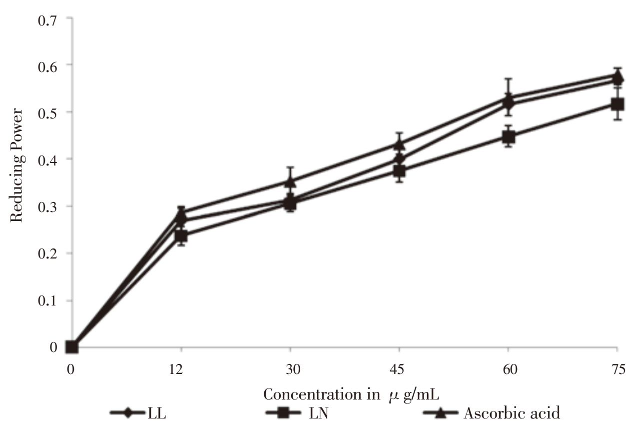

Figure 1. Reducing powers of LL and LN in comparison with Ascorbic acid.

3.3. β – Carotene– Linoleic acid assay

The activity of both the extracts was increasing with the concentration from 4–20 μg/mL. LL and LN were found to have IC_{50} values 32.64±2.02 μg/mL and 33.02±1.85 μg/mL respectively which are higher than Trolax (25.65±1.53 μg/mL). Activity of the extracts in comparision with trolax standard was shown in Table 1.

Table 1

β –Cartotene lineolate activity of the prepared extracts along with standard.

Concentration (μg/mL)	LN	LL	Trolax
4	10.32±0.93	11.95±2.16	16.93±1.68
8	13.98±2.21	15.43±0.93	21.35±2.32
12	19.06±0.92	19.47±2.37	27.43±1.59
16	23.51±1.37	26.16±1.29	33.25±1.56
20	31.96±1.85	32.13±2.02	37.73±1.53
IC_{50}	33.02±1.85	32.64±2.02	25.65±1.53

3.4. DPPH radical scavenging activity

The values DPPH radical scavenging activities were presented in Table 2. Among the extracts, LL (49.57±10.14) was found to have the lesser IC50value than LN (69.32±2.08 μg/mL). These values were significantly ($P<0.05$) less than the DPPH radical scavenging activity of curcumin (55.30±1.70 μg/mL).

Table 2

DPPH radical scavenging activity of the prepared extracts along with standard.

Concentration (μg/mL)	LL	LN	Curcumin
20	41.75±1.19	35.92±0.67	52.37±1.26
40	50.99±0.67	40.78±1.36	53.75±1.67
60	52.88±1.14	49.10±1.88	55.61±1.55
80	56.28±0.86	53.92±0.65	57.90±1.23
100	61.88±1.32	60.22±2.10	64.84±0.575
IC_{50}	49.57±10.14	69.32±2.08	55.30±1.70

3.5. Superoxide radical scavenging activity

The values of superoxide radical scavenging activity were given in Table 3. Both the extracts had a scavenging activity in a concentration dependent manner from 2–10 μg/mL. However the activity of LL with IC_{50} of 6.637±0.342 μg/mL was found to be higher than LN with IC_{50} of 7.100 ± 0.469 μg/mL.

Table 3

Super oxide activity of the prepared extracts along with standard.

Concentration (μg/mL)	LL	LN	Trolax
2	24.670±3.014	22.00±2.70	32.70±1.23
4	35.86±5.63	31.98±4.03	46.34±1.00
6	52.10±3.85	45.32±4.83	54.94±1.04
8	60.47±1.35	57.50±2.78	64.13±1.00
10	64.49±1.69	64.16±2.12	67.58±1.16
IC_{50}	6.630±0.342	7.100±0.469	5.900±0.153

3.6. Hydroxyl radical scavenging activity

The values of hydroxyl radical scavenging activity were shown in Table 4. The IC_{50} values of LL, LN and Curcumin were found to be75.422 ± 3.464, 76.296 ± 4.794 and 61.961 ± 2.178 respectively.

Table 4

Hydroxyl radical scavenging activity of the prepared extracts along with standard

Concentration (μg/mL)	LL	LN	Curcumin
30	46.516±1.573	47.078±2.020	57.153±1.302
60	57.940±2.538	55.168±1.975	61.086±1.484
90	62.247±1.325	62.696±1.011	64.419±2.027
120	64.569±0.915	64.344±2.088	70.973±1.293
150	67.827±1.630	68.089±3.887	79.325±1.296
IC_{50}	75.422±3.464	76.296±4.794	61.961±2.178

3.7. Hydrogen peroxide scavenging activity

The values of hydrogen peroxide scavenging activity were shown in Table 5. The IC_{50} values of LL, LN and trolax were found to be76.502 ± 4.890, 82.702 ± 4.798 and 70.962 ± 2.978 respectively.

Table 5

Hydrogen peroxide scavenging activity of the prepared extracts along with standard.

Concentration (μg/mL)	LL	LN	Trolax
20	25.95±3.04	21.910±2.383	30.930±1.127
40	37.44±1.63	31.59±4.51	40.01±1.91
60	44.66±2.83	40.490±2.653	47.45±2.1
80	50.92±1.72	49.43±2.25	53.62±1.22
100	56.20±1.53	54.58±1.64	60.34±1.70
IC_{50}	76.502±4.890	82.702±4.798	70.962±2.978

3.8. Nitric oxide radical scavenging activity

The values of nitric oxide radical scavenging activity were shown in Table 6. Free radical scavenging ability was increased on increasing concentration from 10μg/mL to 50μg/mL. The IC_{50} values of LL, LN and Curcumin were found to be 23.335 ± 1.57, 26.030 ± 1.753 and 19.284 ± 0.599 respectively.

Table 6

Nitric oxide scavenging activity of the prepared extracts along with standard.

Concentration (μg/mL)	LL	LN	Curcumin
10	46.13±2.98	47.640±2.709	55.550±1.061
20	60.48±2.24	54.340±1.741	61.810±0.993
30	66.188±1.120	58.79±1.49	69.57±1.28
40	68.06±2.26	61.77±1.89	77.740±1.270
50	71.633±3.470	70.71±3.98	83.590±1.106
IC_{50}	23.335±1.570	26.030±1.753	19.280±0.599

3.9. Metal chelating activity

The values of metal chelating activity were shown in Table 7 along with the standard Curcumin. The metal chelating activity was increased on increasing concentration from 20 μg/ml. the IC50 values of LL and LN were found to be 49.22± 1.693 and 54.748±2.738 respectively. The IC50 values of plants extracts were near to the standard Curcumin (IC_{50} = 44.361± 1.496).

Table 7

Metal chelating activity of the prepared extracts along with standard

Concentration (μg/mL)	LL	LN	Curcumin
20	52.816±2.690	44.300±3.070	54.208±1.250
40	54.108±1.510	50.000±1.640	56.693±1.550
60	59.609±2.000	55.89±1.35	63.021±0.810
80	66.13±2.13	63.353±2.400	68.986±1.292
100	70.079±1.400	69.715±1.428	78.561±1.044
IC_{50}	49.220±1.693	54.74±2.73	44.361±1.490

3.10. In vitro cytotoxicity study

The percentage of cell death was 46% at 200μg/mL and 12% at 200μg/mL for LL and LN respectively and the data was given in Table 8. The LL had showed more percentage of cell death at all concentration than LN.

Table 8

Cytotoxic activity of the extracts on Dalton's Lymphoma Ascites carcinoma cells.

Drug concentration (μg/mL)	% cell death DLA (LL)	% cell death DLA (LN)
200	46%	12%
100	32%	8%
50	23%	4%
20	10%	0
10	4%	0

4. Discussion

Aqueous alcoholic solution is the effective solvent to extract phenolic substance and flavonoid substance [13]. Among the two extracts LL was found to have higher content of both phenolic and flavonoid contents.

The reducing power of LL was almost equal to ascorbic acid at a concentration of 100μg/mL. The reducing powers of both the extracts were concentration dependent from 20 to 100 μg/mL. When the reducing powers of both the extracts were considered, LL was found to have more reducing power than LN.

β- Carotene is oxidized by Hydroperoxide formed from Linoleic acid. As a result of oxidation, β- carotene loss its characteristic orange colour, which is detected by spectrophotometrically at 470 nm[14]. β-Carotene- Linoleic acid assay indicates pronounced anti oxidant activity of Trolax than the extracts.

1,1- Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical. DPPH is gained its stability as free radical molecules due to the delocalization of odd electron throughout the molecules. This more stabilized DPPH produce intense violet colour in ethanol solution. The antioxidant present in the extracts reacts with DPPH free radical solution and converts them into reduced form either by donating hydrogen atom or transferring electron followed by proton. This oxidation reaction is accompanied with loss of violet colour which can be measured quantitatively at 517 nm[15]. The activity of both the extracts was found to be lesser than standard curcumin and concentration dependent in both the extracts from 20-100 μg/mL.

The superoxide anion radicals $O_2^{\bullet -}$ scavenging activity of the extract was determined by nitro blue tetrazolium (NBT) method. Phenazine methosulphate (PMS) reacts with Nicotinamide adenine dinucleotide (NADH) to produce superoxide anion radicals. The generated superoxide anion radicals reduce Nitro blue tetrazolium into formazon. Free radicals scavenger present in the extracts compete with Nitro blue tetrazolium for superoxide anion radicals and slowdown the formation of blue color, which is quantitatively measured at 560nm[16]. The activities of both the extracts were near to the standard Trolax with IC_{50} value of 5.908±0.153.

Hydroxyl radical scavenging activity of ethanolic extract was measured by 1, 10 phenanthroline-Fe^{2+} complex oxidation method. Fe^{2+} was formed when ferrous sulphate added to hydrogen peroxide. This formed ferrous ion reacts

with 1, 10 phenanthroline and forms 1, 10 phenanthroline-Fe^{2+} complex which is acts as indicator in oxidation reduction reaction. Simultaneously the hydroxyl radical formed from the H_2O_2-Fe^{2+} reaction mixture oxidize Phenanthroline - Fe^{2+} into Phenanthroline - Fe^{3+} complex. Presence of free radical scavenger in the extract reduces the oxidation reaction accompanied with reduction in the absorbance which can be measured quantitatively at 560 nm[11,17]. Free radical scavenging ability was increased on increasing concentration from 30 μg/mL to 150 μg/mL and the IC_{50} values are near to the standard curcumin.

Hydrogen peroxide absorbs the ultraviolet radiation at 230 nm. The addition of scavenger containing extract to the H_2O_2 solution caused a fast decrease in the concentration of hydrogen peroxide, which is monitored at 230 nm[1]. Hydrogen peroxide scavenging ability was increased on increasing concentration from 20 μg/mL to 100 μg/mL and the IC_{50} values are slightly lesser than the standard Trolax.

Sodium nitroprusside generates nitrous oxide at physiological pH in aqueous solution. Nitrous oxide reacts with oxygen to form stable nitrite and nitrate ion. The free radical scavenger presents in the extract compete with oxygen this leads to decrease in the concentration of nitrite ion. The nitrite ion in the aqueous solution further reacts with sulphanilamide presents in the Griess reagent to produce diazotized molecule. This azo derivative was measured at 546 nm[11,18]. Both the extracts have shown good ability of nitric oxide scavenging activity.

Ferrous ion reacts with ferrozine forms violet color Ferrozoin - Fe^{2+} complex. Chelating compounds present in the extract prevent the formation of Ferrozoine - Fe^{2+} complex, which leads to decrease in the intensity of violet colour[18]. both the extracts were found to have the remarkable metal chelating activity. From the cytotoxic studies, it can be inferred that both the extracts are safe for the administration.

In this study, it was determined that the LL ethanolic extracts contained a higher amount of total phenolic and flavonoid content than the LN ethanolic extracts. When comparing both plants extracts LL extracts exhibited more antioxidants and free radical scavenging in all the experimental models. This can be directly correlated to the high phenolic and flavonoid content which play a major role in controlling the oxidation. This research proves the use of LL and LN extracts as easily accessible and naturally occurring antioxidant. Further studies are required to establish the exact phytoconstituents responsible for the anti oxidant activities of the extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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