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Original Article

FREE-RADICAL SCAVENGING ACTIVITY LEAF EXTRACT OF *LITSEA LAEVIGATA* GAMBLE

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ABSTRACT

Objective: In the present study, antioxidant activity in the leaf of the pet-ether, chloroform, acetone and methanolic extracts from *Litsea laevigata* Gamble**.** Leaf was investigated by employing established *in vitro* studies. *L. laevigata* belongs to the Lauraceae family.

Methods: The capability of the plant extract to act as hydrogen/electrons donor or scavenger of radicals were determined by *in vitro* antioxidant assays using 2,2-diphenyl-2-picrylhydrazyl free radical ($DPPH^{\cdot}$) scavenging, reducing power assay, superoxide radical ($O_2^{\cdot-}$) scavenging activity, phosphomolybdenum assay, FRAP, ABT and metal chelating activity were performed to know the antioxidant potency of the plant extract of leaves of *L. laevigata.*

Results: Results are evaluated higher in leaf extract of *L. laevigata* recorded total phenol, total flavonoid, and tannin. The present state of work was designed to evaluate the phytochemical, antioxidant in the plant leaf extracts of *L. laevigata*. The plant *L. laevigata* methanolic extract of leaf showed greater IC_{50} antioxidant activity of DPPH assay (5.264 µg/ml) and compare to other extract, higher phosphomolybdenum reduction (164.36 mg/g), better Reducing power activity leaf in methanol (0.711%), higher ferric reducing power (4060.66MmolFe(II)E/mg), and higher in superoxide radical scavenging activity in (78.12 mg/ml). However, the better metal chelating ability was shown by the water extracts of the leaf (5.145 EDTAE/100g) compared to other solvent extracts.

Conclusion: The result indicates the total phenol and antioxidant activity potential of *L. laevigata.*

Keywords: *Litsea laevigata*, DPPH assay, Reducing antioxidant power and antioxidant activity

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INTRODUCTION

Oxygen gives us force by oxidation of nourishment which is crucial for the living. Amid this procedure, exceedingly responsive and destructive oxygen species are additionally created which can harm living beings. Organisms control a complicated structure of antioxidant molecules and enzymes that work together to obstruct oxidative damage of cellular components such as DNA, proteins and lipids [1]. Oxidative stress is among the major causative factors in the enlistment of numerous incessant and degenerative illnesses including atherosclerosis, ischemic coronary illness, maturing, diabetes mellitus, tumor, immunosuppression, neurodegenerative sicknesses and others [2].

Antioxidant-based medication details are utilized for the anticipation and treatment of complex ailments like atherosclerosis, stroke, diabetes, Alzheimer's malady and malignancy [3]. The phenolic compounds, which are broadly present in plant parts, were express to play a significant role as an antioxidant mixture for the prevention of oxidative damage in the living organism [4]. Flavonoids are a broad group of polyphenol compounds, more present in the phenolic compounds, which are widely taken from the plants, it were depended to play a very important role as the dietary antioxidant component for the prevention and decrease the cell damage of living things [5].

Antioxidant specialists can be sufficient for taking out ROS thusly, keeping the cells from negative effect. The arrangement of receptive oxygen flavours (ROS) in typical cell happened under tight homeostatic control by cancer prevention agents, be that as it may, when ROS levels surpass the cancer prevention agent limit of the cell, a malicious condition known as oxidative pressure happens. An incredible number of sweet-smelling, therapeutic, spice and different plants contain synthetic mixes displaying cancer prevention agent properties. An oxidative process is an important process of developing the free radicals in foods, drugs and even in living organism [6].

The role of free radical counteraction in effect of disease is well accepted and known to be concerned in many acute and chronic diseases in human beings, such as diabetes, cardiovascular disease, aging, immunosuppression, breast, and colon cancer [7]. An inequality between ROS and the essential antioxidant capacity of the body, manage the use of dietary and/or medicinal supplements especially in the middle of the disease assault. Research on medicinal plants, vegetables, and fruits has suggested the presence of antioxidants such as phenolic, flavonoids, and tannins. The antioxidant substance of medicinal plants may subscribe to the protection they offer from the disease. The absorption of natural antioxidants has been conversely connected with morbidity and mortality from degenerative disorders [8].

In living cells causes damaged of free radicals that incorporate both reactive oxygen species (ROS) and reactive nitrogen species (RNS) and theses are produced in a regulatory manner that helps to sustain oxidation-reduction homeostasis at the cellular equalize in the ordinary health tissues [9]. Free radicals beginning from absorption or biodegradable resources interact continuously in natural systems, and their unconstrained generation associate produce an immediately molecular level of the cause by so many diseases [10]. The most active method of an antioxidant agent to eradicate and decrease the action of free radicals because which produces the oxidative stress. Antioxidants are such essences in life which possess free radical continue reaction of smash their properties. Currently, there has been an improvement of concern in the remedial potential of highly contain medicinal plants as antioxidants and decrease the oxidative stress. It has caused tissue damage of living being [11]. Based on the numerous naturally appear antioxidants like carotenoids, vitamin E, vitamin D, ascorbic acid, and phenolic compounds, those are much effective of reduction [12].

Many plants include a description of phytochemical property determined to be important in the fields of cultivation, social and veterinary drug. Natural products represent a powerful role in the extension of innovative drug leading to treatment and inhibition of diseases [13]. Hence, the present study was measured by the quantification of phenol, flavonoid and antioxidant activity leaf

extracts of *L. laevigata*. The plant has highly habitat for semi-Evergreen forests. Description of the plant has the occurrence of endemic in nature, to the Western Ghats-South and Central Sahyadris to Southern Western Ghats.

MATERIALS AND METHODS

Plant material

The leaves part of *L. laevigata* was collected from Gudalur of Western Ghats, during April 2017. The collected plant material was identified and authenticated by Botanical survey of India, Southern Circle, Coimbatore (NO. BSI/SRC/5/23/2017/Tech./17) and the voucher specimen has been deposited in Bharathiar University Herbarium, Department of Botany, and Coimbatore. The collected fresh plant for leaves was cleaned thoroughly with running tape water to remove dust and shade dried for a week at room temperature. The powders were in the airtight container.

Chemicals and reagents

Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl(DPPH), potassium persulfate, sodium phosphate, ammonium molybdate, 2,4,6-tripyridyl-s-triazine (TPTZ), polyvinyl polypyrrolidone (PVPP), trichloroacetic acid, riboflavin, ethylenediaminetetraacetic acid (EDTA), nitro blue tetrazolium(NBT), sodium nitrite, aluminium chloride, ferrous sulfate, gallic acid, rutin, tannic acid, ascorbic acid, quercetin, safarnine, fast green. All other reagents used were of analytical grade.

The drugs and fine chemicals were purchased from Sigma. Aldrich chemical company, St. Louis, U. S. A. All other chemicals and solvents were obtained from Himedia, SRL and SD fine chemicals Mumbai, India and were of the highest purity and analytical grade.

Plant extracts preparation

The powder plant material was extracted as one of the extraction method of Soxhlet extractor based extracted continuously with petroleum ether, chloroform, acetone, and methanol. Each time before extricating with the following dissolvable, the thimble was dried in hot air stove underneath 40 °C. The distinctive dissolvable concentrates were thought by rotating vacuum evaporator and after that air dried. The dried concentrate got with every dissolvable was weighed. The rate yield was communicated as far as air-dried weight of plant material.

Quantification of total phenolic, tannin and flavonoid

Quantification of total phenolic and tannin

The total phenol content was determined according to the method described by [14]. 100 µl aliquots for plants extracts (5 mg/ml) were taken in the test tubes and made up to the volume of 1 ml with distilled water. Then 500 µl of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added basically in each tube. forthwith vortex the reaction mixture, then the test tubes were located in dark room for 40 min and after the absorbance was recorded at 725 nm against blank. Response blend without plant remove was taken as clear. The examination proceeded done in triplicate and the results obtained signified by gallic acid equivalents.

Investigated the extract use the tannins were estimated after analysis [14], with polyvinylpolypyrrolidone (PVPP) 75 mg of PVPP was weighed into a 2 ml eppendorf tube and to this 900 µl distilled water and then 750 µl of the sample extracts were added. The content was vortexes and kept in the test tube at 4 °C for 4 h. Then the sample was centrifuged at 4000 for 10 min at room temperature and the supernatant was collected. This supernatant has just basic phenolic other than the tannins (the tannins would have been encouraged alongside the PVPP). The phenolic substances of the supernatant were measured and show up as the substance of non-tannin and phenolic. From the above results, the tannin substance of the example was figured as takes after:

Tannin %= Sum of phenolic %-Non tannin phenolic %

Quantification of total flavonoid

The flavonoid content of the considerable number of concentrates was evaluated as it goes about as a noteworthy antioxidant in plants diminished oxidative anxiety. Assessed according to portrayed by [15]. Initially, 150 µl of all the plant extracts were taken in different test tubes. To each extracts 2 ml of distilled water was added. Then 150 µl of $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation 150 µl of $AlCl_3$ (10%) was added to all the test tubes. The test tubes were incubated for 6 min at room temperature. Then 2 ml of NaOH was added to all the test tubes which were made up to 5 ml using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 min at room temperature. The pink colour developed due to the appearance of flavonoids was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

In vitro antioxidant studies

DPPH radical scavenging activity

The antioxidant movement of the concentrates was resolved regarding hydrogen giving or radical searching capacity, utilizing the steady radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) discribed by Blois, 1958[16], as indicated by the strategy for leaf sample extracts at various concentrations (20-100 µl) was added to 3 ml of 0.1 mmol methanolic solution of $DPPH^{\cdot}$ and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured using the spectrophotometer at517 nm. Methanol was filled in as blank and arrangement without remove filled in as a control. The mixture of methanol, DPPH, and standard (ascorbic acid) filled in as a positive control. Radical scavenging activity was communicated as the restraint percentage of free radical by the sample was figured utilizing the formula. More significantly the IC_{50} of the extracts were also calculated.

Phosphomolybdenum assay

The antioxidant capacity of the extracts has been determine with the phosphomolybdenum reduction assay discribed method of Prieto *et al.* 1999 [17]. The assay was based on the reduction of the extract and subsequent formation of a complex. 500 µl of plant leaf extract added with 3 ml of reagent arrangement (0.6 M sulphuric acid, 28 mmol sodium phosphate, and 4 mmol ammonium molybdate) was hatched at 95 °C for an hour and a half. The absorbance was taken at 695 nm using a spectrophotometer. The results were calculated in ascorbic acid equivalents.

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance without a sample, and as is absorbance with a sample.

Metal chelating activity

Iron II chelating activity was calculated by the check of the creation of Iron-(II)-ferrozine complex following preincubation of the sample. The chelating of ferrous particles by different concentrates in the plant was assessed by the technique for Dinis *et al.,* 1994 [18]. The chelating of ferrous ions by various extracts of *L. laevigata* was estimated. Initially, about 100 µl the extract sample was added to 50 µl of 2 mmol $Fecl_2$ solutions. At that point, the response was started by the expansion of 200 µl of 5 mmol ferrozine and the test tubes were vortexes well and left remaining at room temperature for 10 min. The reaction mixture containing deionized water in place of a sample was considered as the negative control absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). EDTA was against the standard metal chelating specialist and the outcomes were communicated as mg EDTA counterparts/g extricate chelate the ferrous particle was computed by,

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance without a sample, and as is absorbance with a sample.

Reducing power assay

The reducing power of different solvent extracts of *L. laevigata* was determined by the method reported by Oyaizu *et al.* 1986 [20]. 500 µl of the extract was taken in 2.5 ml of 0.2 M phosphate buffer (pH 6.6) was added. To this, 2.5 ml of 1% Potassium ferricyanide solution was added and the mixture was incubated at 50 °C for 20 min. After the incubation, 2.5 ml of 10 % TCA was added. The content was centrifuged at 3000 rpm for 10 min. The upper layer of the supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride. The absorbance of the reaction mixture was measured spectrophotometer at 700 nm.

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance without a sample, and as is absorbance with a sample.

Assay of superoxide radical (O_2^{*-}) scavenging activity

The activity review was found in the limit of the concentrate think to check advancement via seeking superoxide radicals start in the riboflavin-light-NBT system by Beauchamp *et al.,* 1971[19]. Every 3 ml response blend contained 50 mmol sodium phosphate buffer (pH 7.6), 2.33µg riboflavin and 12 mmol EDTA, and 11.55 g NBT. Response was begun by lighting up the response blend with of test extricates (100 µl) for 90 seconds. Response blend with extricate kept in dull filled in as a negative control while the blend without separate was taken as clear. Promptly after brightening, the absorbance was measure at 590 nm. The action was contrasted with ascorbic acid. The rate lessening of superoxide anion creation was figured utilizing the accompanying equation:

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance without a sample, and as is absorbance with the sample.

FRAP-ferric reducing antioxidant power

The antioxidant contents of phenolic extracts of samples were consideration according to the procedure explained by Pulido *et al.,* 2000 [21]. FRAP reagent (2.7 ml), make freshly and incubated at 37 °C, it was mixed with 270 µl of distilled water and 50 µl of extract or methanol (for the reagent blank). The test sample and reagent clear were brooded at 37 o C for 30 min in a water shower. The FRAP reagent making 2.5 ml of 20 mmol/l TPTZ (2,4,6-tripyridyl-s-triazine) arrangement in 40 mmol/l HCl in addition to 2.5 ml of 20 mmol/l $FeCl_3$ • 6H2O and 25 ml of 0.3 m/l acetic acid derivation support (pH 3.6) clarified by Siddhuraju and Becker, (2003). Toward the finish of hatching, the absorbance readings were taken quickly at 593 nm. Results were ascertained in ferrous sulphate counterparts.

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance without the sample, and as is absorbance with the sample.

Antioxidants activity by radical cation ($ABTS^{++}$)

The total antioxidant activity of the samples was measured by $ABTS^{++}$ radicalcation decolourization assay according to the method of Siddhuraju *et al.,* 2007 [22]. $ABTS^{++}$was produced by reaction 7 mmol $ABTS^{++}$aqueous solution with 2.4 mmol of Potassium persulfate in the date for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 V/V) and equilibrated at 30 °C to give an absorbance at 734 nm. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of trolox concentration. The unit of total antioxidant activity (TEAC) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µM/g sample extracts on dry matter.

Hydroxyl radical ($OH^{\bullet}$) scavenging activity

The ability of the extract to scavenge hydrogen peroxide was determined according to the method given of Ruch *et al.,* 1989 [23]. The solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Plant leaf extract (1-10µg/ml) were added to hydrogen peroxide solution (0.3 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

$$(\%)\text{Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where, Ac is the absorbance without a sample, and as is absorbance with the sample.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P<0.05$) using statistical (stat soft Inc., Tulsa, USA). Values expressed fig. are means±SEM (n=4).

RESULTS AND DISCUSSION

Quantification assays

Quantification of total phenolic and tannin

In the naturally phenolic present in the plant parts have gotten impressive consideration as a result of their potential natural action. Phenolic mixes, for example, flavonoid, phenolic, and tannin have various organic exercises including calming, against cancer-causing and anti-atherosclerotic exercises. These exercises may be identified with their cell antioxidant action. These exercises may be identified with their antioxidant activity movement [24]. The yield percentage, add up to phenolic, tannin and flavonoid substance of the concentrates got from *L. laevigata* leaf. The results of total phenol and tannin contents are showed in table 1. Methanolic extracts of *L. laevigata* leaf revealed maximum phenolic (41.83±3.76 GAE/100 g extract) and tannin (7.348±0.48 g/100g extract). Phenolic mixes are omnipresent auxiliary metabolites in plants. They are known to have antioxidant action and it is likely that the movement of these concentrates is because of these mixes [25, 26]. The results obtained in this study showed a significant level of phenolic compounds in methanol and acetone extracts of the leaves of *L. laevigata* (table 1).

Table 1: Determination of total phenolic, flavonoid and tannin content

Plant parts	Sample extracts	Phenolic (GAE/g)	Flavonoid (RE/g)	Tannin (GAE/g)
Leaf	Petroleum ether	18.86^{f}±0.11	9.523^{f}±0.58	1.818^{f}±0.04
	Chloroform	19.23^{d}±0.14	13.73^{d}±0.16	2.334^{d}±0.34
	Acetone	40.08^{b}±4.01	20.47^{b}±1.90	6.886^{b}±0.17
	Methanol	41.83^{a}±3.76	23.41^{a}±0.51	7.348^{a}±0.48
	Water	20.67^{c}±0.30	19.11^{c}±0.04	5.691^{c}±0.31

Values are mean of replicate determination (n=3) mean±standard deviation. GAE-Gallic acid equivalence, RE-Rutin equivalence. a–e = means within a column with the different letter were significantly different ($p<0.05$)

Quantification of total flavonoid

The results of the flavonoid content are presented in table 1. In this estimation of the acetone extract of *L. laevigata* leaf methanolic revealed maximum amount of flavonoid content (23.41±0.51g/100g). Flavonoids are one class of optional plant metabolites that are otherwise called Vitamin P. These metabolites are generally utilized in plants to create yellow and different shades which assume a critical job in the shades of plants. Furthermore, Flavonoids are promptly ingested by people and they appear to show critical militating, against unfavourably susceptible and hostile to disease exercises [27].

Antioxidant assays

DPPH radical scavenging activity

The free radical-scavenging activities in stem and leaf of the plants *L. laevigata* samples along with standards vitamin C was determined by the DPPH radical scavenging assay and the results are shown in fig. 1. The reduction in absorbance of the DPPH radical caused by antioxidant was because of the rummaging of the radical by hydrogen ability. The colour change from purple to yellow is visually evident. A lower value of IC_{50} (inhibitory concentration at 50%) indicates a higher antioxidant activity. Generally, the acetone and methanol extracts of all the part showed a significant reduction of DPPH radical. However, *L. laevigata* is the highest free radical scavenging activity was exerted by petroleum ether and chloroform extract of leaf (IC_{50} value were 5.26µg/ml). It has been generally used to test the capacity of mixes as free-radical scavengers or hydrogen contributors and to assess the antioxidative action of plant concentrates and nourishments [28].

Phosphomolybdenum assay

The total antioxidant capacity of different solvent extracts of the leaf of *L. laevigata* was analysed and shown in fig. 2. Among *L. laevigata* showed higher activity in most of its solvents compared to the methanolic extracts of the leaf. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. Essence nature and free of other antioxidant estimations regularly utilized and examine were reached out to plant polyphenols. In the ranking of the antioxidant capacity obtained by this method, the methanol extract of *L. laevigata* showed higher phosphomolybdenum (164.36 mg AA equivalents/100g), as followed by acetone extract (107.48 mg AA equivalents/100g). This may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants [29].

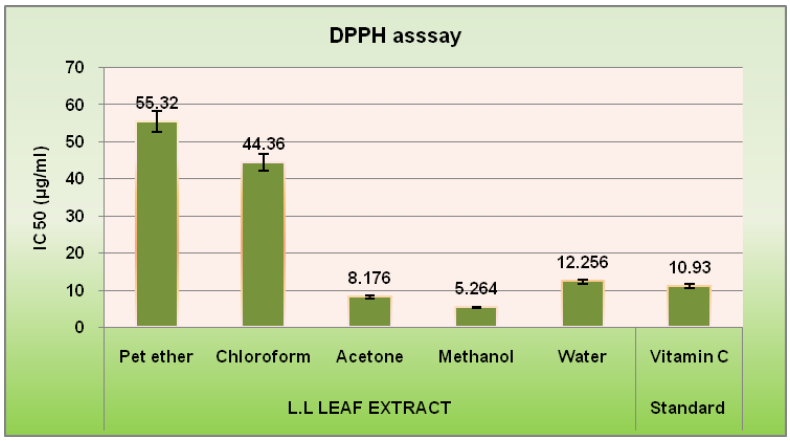

Fig. 1: DPPH assay of leaf extract of *L. Laevigata*, *values expressed fig. **are concentration-based as IC_{50}±SEM (n=4)**

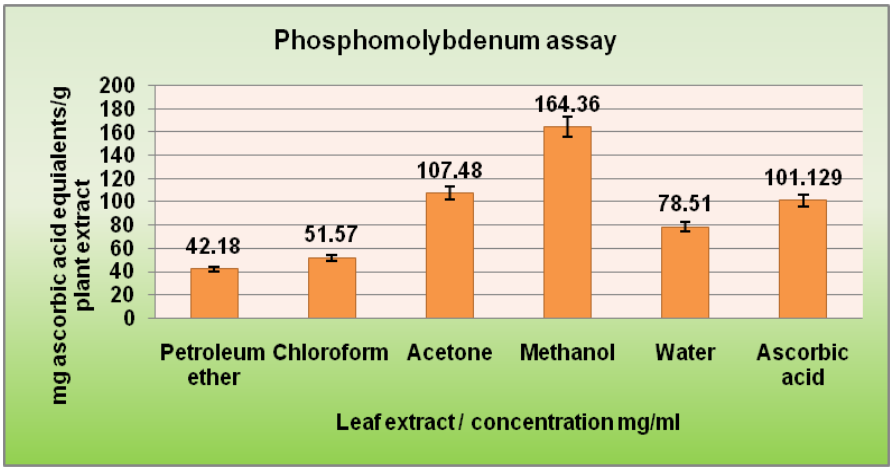

Fig. 2: Phosphomolybdenum assay of leaf extract of *L. Laevigata,* each value in the table is represented as mean±SEM (n=4)

Metal chelating activity

The Fe^{+}chelating capacity of different solvent extracts of leaf and stem of *L. laevigata* were analysed and shown in fig. 3. In *L. laevigata* maximum chelations were observed for the methanol extract of leaf (5.145g EDTAE/100g) extract. Ferrozine can shape a complex with red shading by framing chelates with Fe2+. This response is limited within the sight of other chelating agents and results in a lessening of the red shade of the ferrozine-Fe2+buildings. Estimation of the colour decrease decides the chelating movement to contend with ferrozine

for the ferrous particles [30]. From the iron chelating data, it is evident that the extracts may be able to play a protective role against oxidative damage by sequestering Fe (II) ions that may otherwise catalyse Fenton type reactions or participate in metal catalysed hydroperoxide decomposition reactions. The scavenging potential and the metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups [31].

Reducing power assay

The reducing power of solvent extracts of *L. laevigata* leaf extract samples are shown in fig. 4 respectively. A strong reducing power was noted for the samples of *L. laevigata*, the methanol extract of leaf (0.625 % extract) as follow acetone (0.609 mg/ml) extract. Present in the maximum reducing power based on the concentration, these observed a dose and time-dependent activity which resulted in greater reducing ability. In this prove the yellow colour of the test end of the solution changes to assorted shades of green and blue, depending on the reducing power of each compound. The appearance of reducers (i.e. antioxidants) causes the decrease of the Fe3+/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration [20]. Dietary antioxidant such as ascorbic acid was used for contrast. Compounds with reducing power evidence that they are electron donors and can classify the oxidized agent of lipid peroxidation method, so that they can behave as primary and secondary antioxidants [32].

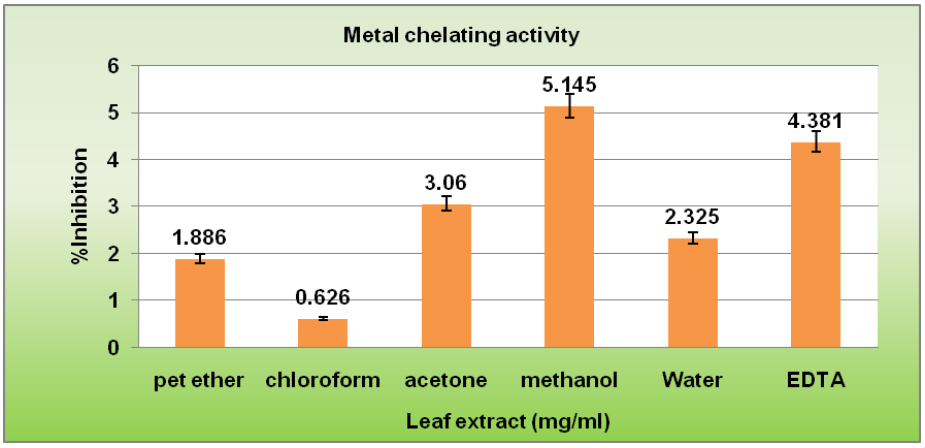

Fig. 3: Metal chelating activity of leaf extract of *L. Laevigata*, each value in the table is represented concentration based as mean±SEM (n=4)

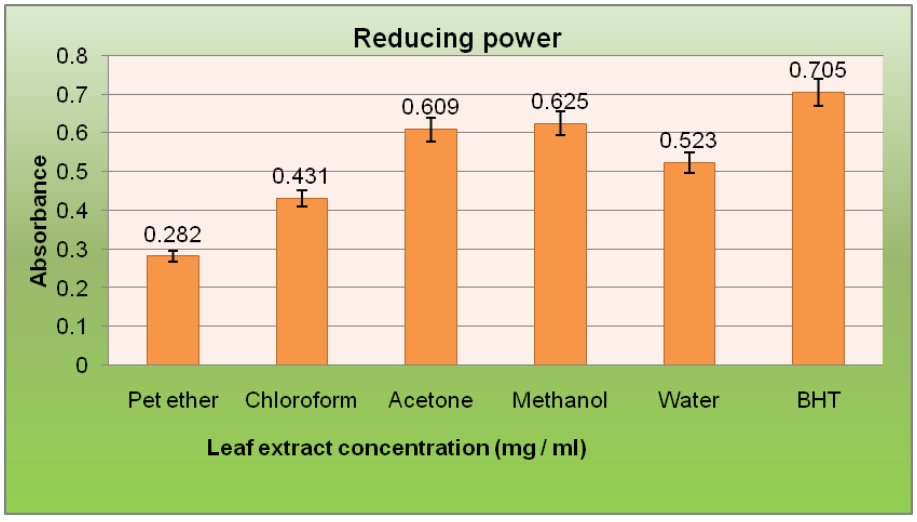

Fig. 4: Reducing power assay of *L. laevigata* leaf extract, each value in the table is represented concentration based as mean±SEM (n=4)

Superoxide radical scavenging activity

Superoxide dismutase (SOD) is a metalloenzyme that catalyse the dismutation of superoxide radical into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) and as a result, provide an important defense mechanism against superoxide radical toxicity [33]. The results of superoxide anion scavenging of different extracts of leaf of *L. laevigata* were analysed and shown in fig. 5, and the significant activity was view by methanol extract and (78.12%), the lower scavenging activity was noticeable in pet ether extract of leaf (41.1%). These results were compared with Vitamin C antioxidants.

FRAP assay

The FRAP assay is determined by the ferric reducing ability of plant crude extracts. The ferric reducing ability of different solvent extracts of leaf of *L. laevigata* was analysed and shown in fig. 6. The result of *L. laevigata* shows that the ferric reducing capacity of methanol leaf extract was much higher (4060.66Mm/g) and as follows in acetone (3555.34Mm/g). FRAP assay was used by several authors for the assessment of the antioxidant activity of various food product samples [34]. The secondary metabolites are redox-active compounds that will be picked up by the FRAP assay [37].

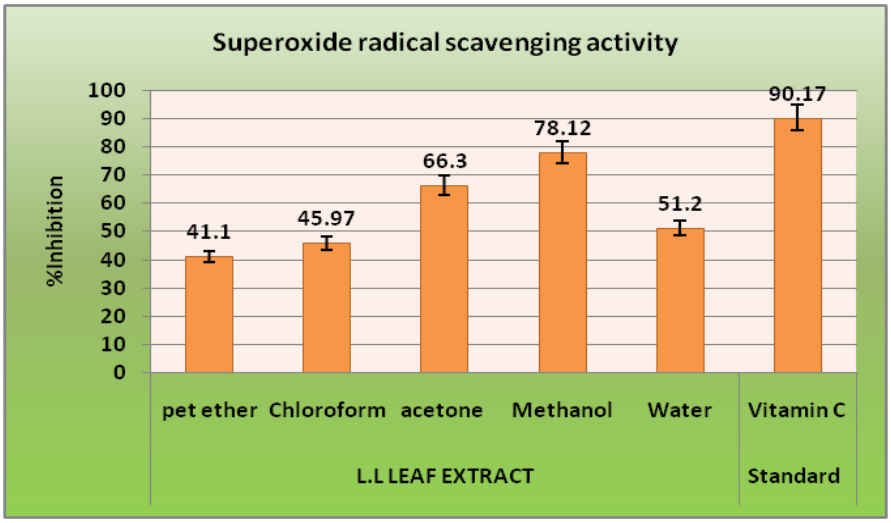

Fig. 5: Superoxide radical activity of leaf extract of *L. Laevigata*, each value in the table is represented concentration based as mean±SEM (n=4)

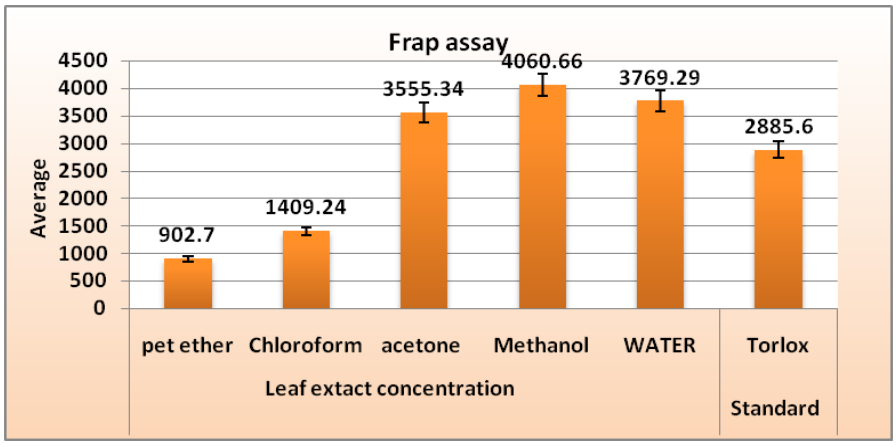

Fig. 6: FRAP assay of leaf extract of *L. Laevigata,* each value in the table is represented as mean±SEM

Antioxidant activity by the ABTS$^{\bullet+}$assay

The chloroform, acetone, and methanol extracts from the leaves of *L. laevigata* was a fast and effective scavengers of the ABTS radical (table 7). In ABTS$^{\bullet+}$scavenging activity the values are varied significantly high ranged from (17954.1 µmol BHT/g) methanol extract. Infect, the ABTS radical cation scavenging activity also consider hydrogen-donating capacity [35] and reported that the high molecular weight phenolic (tannins) have more ability to quench free radicals (ABTS$^{\bullet}$+). Since, the extracts from various samples have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential nutraceuticals when ingested along with nutrient.

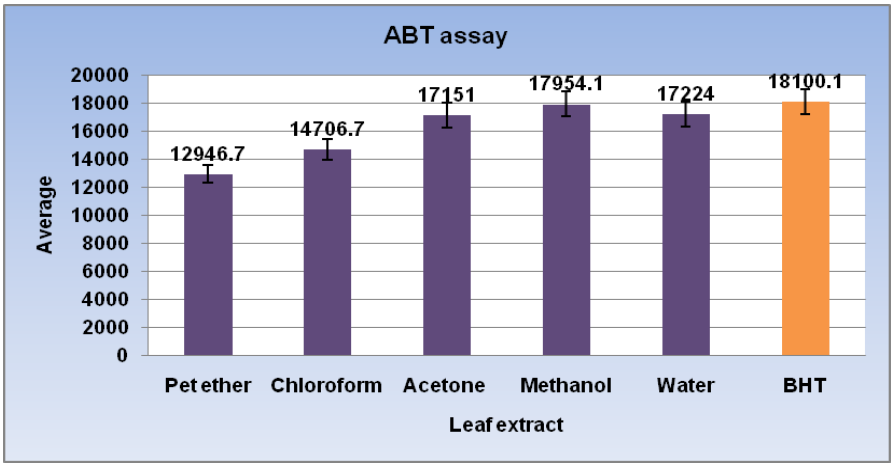

Fig. 7: ABTS$^{\bullet+}$ assay of leaf extract of *L. Laevigata*, each value in the table is represented concentration based as mean±SEM (n=4)

Hydroxyl radical (OH·) scavenging activity

Scavenging of hydroxyl radical is a critical antioxidant movement as a result of the high reactivity of the OH radical, empowering it to respond with an extensive variety of particles found in living cells, for example, sugars, amino acids, lipids, and nucleotides [36]. Along these lines, expelling OH• is critical for the insurance of living frameworks. The hydroxyl radical searching capability of different dissolvable concentrates of *L. laevigata* leaves is appeared in fig. 8. Each concentrate indicating hydroxyl radical searching action was expanded with expanding centralization of test removes. In the present examination, the hydroxyl radical scavenging activity was observed a maximum of in methanolic extract (488.27 %). As follow the acetone extract (474.13%).

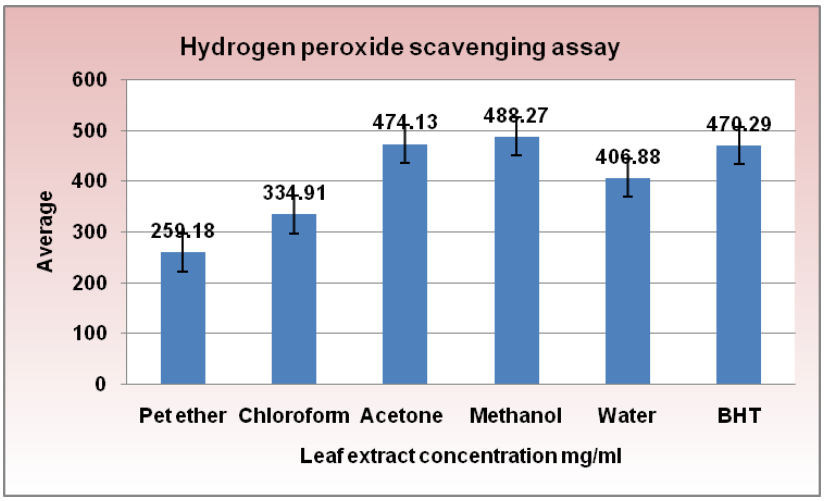

Fig. 8: Hydroxyl radical (OH·) scavenging activity of leaf extract of *L. Laevigata*, each value in the table is represented concentration based as mean±SEM (n=4)

CONCLUSION

L. laevigata leaf can be valuable natural high antioxidants properties source which seemed to provide potential therapeutical value for human health. Further, detailed exploration chemical studies and screening for medicinal and Anticancer against properties with providing a cost-effective and reliable source of medicine for the welfare of humanity.

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AUTHORS CONTRIBUTIONS

Sujatha designed the experiments performed in laboratory analysis, experiments, data analysis and participated in the writing of the manuscript. Dr. T. Sekar helped in paper writing and correction. Authors discussed the results and commented on the manuscript.

CONFLICTS OF INTERESTS

We have no conflicts of interest to declare

REFERENCES

1. Sies H. Oxidative stress, oxidants, and antioxidants. Exp Physiol 1997;82:291-5.
2. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-86.
3. Devasagayam TPA, Tilak JC, Boloor KK. Review: free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 2004;53:794-804.
4. Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 2009;53:75-100.
5. Gao CY, Tian CR, Zhou R, Zhang RG, Lu YH. Phenolic composition, DNA damage protective activity and hepatoprotective effect of free phenolic extract from *Sphallerocarpus gracilis* seeds. Int Immunopharmacol 2014;20:238-47.
6. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans 2007;35:1147-50.
7. Harman D. Free radical theory of aging. Elsevier 1998;20:3–7.
8. Gulcin I. Antioxidant activity of food constituents, an overview. Arch Toxicol 2012;86:345–91.
9. Sireesha K, Rao SP. Oxidative stress and diabetes: an overview. Asian J Pharm Clin Res 2015;8:15-9.
10. Huang DJ, Chen HJ, Lin CD, Lin YH. Antioxidant and ant proliferative activities of water spinach (*Ipomea aquatica* Frosk.) constituents. Bot Bull Acad Sin 2005;406:99-106.
11. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity phenols, flavanoid contents of selected Iranian medicinal plants. S. Afr J Biotechnol 2006;5:1142-5.
12. Duh PD, Tu YY, Yen GC. Antioxidants activity of aqueous extract of harnjyur (*Chrysanthemum morifolium* Ramat). Lebensmwiss Technol 1999;32:269-77.
13. Priyanga S, Mary MR, Hemmalakshmi S, Devaki K. Antihyperlipidemic effect of aqueous extract of *Aegle marmelos* and *Camellia sinensis* in oil-fed hyperlipidemic rats. Int J Pharm Pharm Sci 2014;3:338-41.
14. Makkar HPS. Quantification of tannin in tree and shrub foliage: a laboratory manual Dondrecht. The Netherlands: Kluwer Academic Publishers; 2003.
15. Zhishen J, Mengecheng T, An Jianming W. The determination of flavonoids contents on mulberry and their scavenging effects on superoxide radical. Food Chem 1999;64:555-9.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;29:1199-200.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
18. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetoaminophen, salycilate and 5 aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Bio Phys 1994;315:161-9.
19. Beauchamp C, Fridovich I. Superoxide dismutase, Improved assay applicable to acrylamide gels. Anal Biochem 1971;44:276-7.
20. Oyaizu M. Studies on products of browning reactions, antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutr 1986;44:307–15.
21. Pulido R, Bravo L, Sauro-Calixo F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/

antioxidant power assay. J Agric Food Chem 2000;48:3396-402.

22. Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem 2007;105:950-8.
23. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-8.
24. Chung KT, Wong TY, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr 1998;38:421–64.
25. Okudu T, Yoshida T, Hatano T. Food phytochemicals for cancer prevention II. In: Ho C, Osawa T, Huang MT, Rosen RT. Chemistry and antioxidative effects of phenolic compounds from licorice, tea, and Compositae and Labiateae herbs; Washington, DC: American Chemical Society; 1994. p. 132-43.
26. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six salvia species from turkey. Food Chem 2006;95:200-4.
27. Crozier A, Ashihara H, Clifford MN. Plant secondary metabolites and the human diet. Blackwell Publishing. Oxford. England; 2006.
28. Porto CD, Calligaris S, Celloti E, Nicoli MC. Antiradical properties of commercial cognacs assessed by the DPPH test. J Agric Food Chem 2000;48:4241-5.
29. Loo AY, Jain K, Darah I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem 2008;107:1151–60.
30. Soler Rivas C, Espin JC, Wichers HJ. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochem Anal 2000;11:330–8.
31. Pazos M, Gallardo JM, Torres JL, Medina I. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and fish muscle. Food Chem 2005;92:547–57.
32. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Microbiol Res 2009;13:981-96.
33. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 1972;46:849-54.
34. Pellegrini N, Serafini M, Colombi B, Rio DD, Salvatore S, Bianchi M, *et al*. Total antioxidant capacity of plant foods, beverages and oils consumed in italy assessed by three different *in vitro* assays. J Nutr 2003;133:2812–9.
35. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887–92.
36. Wang H, Gao XD, Zhou GC, Cai L, Yao WB. In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chem 2008;106:888–95.
37. Halvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, Jacobs DR, *et al*. Content of redox-active compounds (i.e., antioxidants) in foods consumed in the United States. Am J Clin Nutr 2006;84:95–135.