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) scavenging, reducing power assay, superoxide radical (O2•−) 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 leaf extracts of L. laevigata. The plant L. laevigata methanolic extract of leaf showed greater IC50 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 (4060.66 MmolFe(II)/mg), and higher in superoxide radical scavenging activity (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 elmination 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 homoeostasis at the cellular level 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. BS/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 tap 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-piryldihydrazyl (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, aluminum chloride, ferrous sulfate, gallic acid, rutin, tannic acid, ascorbic acid, quercetin, safarinine, 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 weighted. 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 extract (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 incubated at 95 °C for an hour and a half. The absorbance was taken at 765 nm using a spectrophotometer. The results were calculated in mmol sodium phosphate, and 4 mmol ammonium molybdate (EDTA, nitro blue tetrazolium(NBT), sodium nitrite, aluminum chloride, ferrous sulfate, gallic acid, rutin, tannic acid, ascorbic acid, quercetin, safarinine, fast green. All other reagents used were of analytical grade.

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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.

\[
\text{Absorbance} = \frac{A_{c} - A_{s}}{A_{c}} 
\]

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

**Hydroxyl radical (OH\(^-\)) scavenging activity**

The ability of the extract to scavange hydroxyl peroxide was determined according to the method given by 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 (0.2 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.

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\text{Absorbance} = \frac{A_{c} - A_{s}}{A_{c}} 
\]

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 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]. However, the results obtained in this study showed a significant level of phenol compounds in methanol and acetone extracts of the leaves of *L. laevigata* (table 1).

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Sample extracts</th>
<th>Phenolic (GAE/g)</th>
<th>Flavonoid (RE/g)</th>
<th>Tannin (GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Petroleum ether</td>
<td>18.86±0.11</td>
<td>9.52±0.58</td>
<td>1.81±0.04</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>19.23±0.14</td>
<td>13.73±0.16</td>
<td>2.33±0.34</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>20.08±2.41</td>
<td>20.47±1.90</td>
<td>6.88±0.17</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>41.83±3.76</td>
<td>23.41±0.51</td>
<td>7.34±0.48</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>20.67±0.30</td>
<td>19.11±0.04</td>
<td>5.69±0.31</td>
</tr>
</tbody>
</table>

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 IC50 (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 (IC50 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].

![](image1)

**Fig. 1: DPPH assay of leaf extract of L. Laevigata,** 'values expressed fig. are concentration-based as IC50±SEM (n=4)

![](image2)

**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 Perls Prussian blue at 700 nm can monitor the Fe2+ 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].

![Image of Metal chelating activity](https://example.com/metal_chelating_activity.png)

**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)**

![Image of Reducing power assay](https://example.com/reducing_power_assay.png)

**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 (H2O2) and molecular oxygen (O2) 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].
Antioxidant activity by the ABTS•+ 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•+ scavenging activity the values are varied significantly high ranged from (17954.1 μmol BHT/g) methanol extract. Indeed, 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•+). 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. 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**

**Fig. 7: ABTS•+ 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.

ACKNOWLEDGMENT

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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

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