

# Phytochemical Screening, HPTLC Finger Print and *in vitro* Antioxidant Activity of Bark Extracts of *Lannea coromandelica* (Houtt.) Merr.

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## ABSTRACT

**Background:** *Lannea coromandelica* Merr (Houtt.) is used as a common medicinal plant among the tribal communities of Bangladesh has long been used in indigenous medicine for curing certain health disorders. Phytochemicals present in the bark are associated with their therapeutic capabilities. **Objectives:** The study was aimed to evaluate the HPTLC finger printing and *in vitro* antioxidant activity of methanol extract (MELC) and aqueous extract (AELC) of *L. coromandelica* bark extracts. **Materials and Methods:** The study consists of parameters which includes moisture content, ash values and ethanol and water soluble extractive values. **Results:** HPTLC finger print assessment will help to identify and monitor the drug effectiveness and ensure therapeutic efficacy. Antioxidant activity was measured using *in vitro* methods such as total phenolic content, total flavanoid content, 1,1 diphenyl 2 picryl hydrazyl (DPPH) radical scavenging potential, ferric ion reduction assay and phosphomolybdenum assay at different concentrations. MELC showed a high phenolic, flavanoid content, higher DPPH free radical scavenging, lower antioxidant ability of ferric ions and a phosphorous molybdenum assay compared to that of standard. **Conclusion:** The presence of natural phytoconstituents which are responsible for antioxidant activity in plant extracts. This may aid in further extensive studies for identifying and isolating compounds with potential therapeutic value in *Lannea coromandelica* (Houtt.) Merr.

**Key words:** *Lannea coromandelica* houtt, Phytochemistry, *in vitro* antioxidant activity, Physicochemical parameters, HPTLC.

## INTRODUCTION

Natural products play a critical function in retaining human health by enhancing the quality of life. Due to the increasing awareness of natural products, demand for medicinal plants is rising, as it has fewer side effects compared to synthetic compounds, low price availability and cost-effectiveness. Bioactive compounds present in plants are alkaloids, flavonoids and terpenoids which are the rich source of antioxidant and have different structures in terms of functional group present in the structure contributes to antioxidant potential.<sup>1,2</sup> Consequently, antioxidants and other phytochemicals present in these plants can prevent oxidative stress and its associated disorders.<sup>3,4</sup>

Various environmental stresses result in excessive ROS production resulting in progressive oxidative damage and eventually cell death. Scavenging or detoxification of excess ROS is accomplished by an efficient antioxidant mechanism consisting of both non-enzyme and enzyme antioxidants.<sup>5</sup> Antioxidants are considered significant for maintaining health, modulating oxidative processes in the body. Despite the availability and regular use of many synthetic antioxidants, the protection and toxicity of these antioxidants are extremely important.<sup>6</sup> Excess of free radicals and reactive oxygen species (ROS) in the current lifestyle will reduce the efficacy of antioxidants.

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Although ROS functions as second messengers, it can cause atherosclerosis, inflammation, neurodegenerative diseases, cataracts, cancer and aging, DNA mutation, protein oxidation and lipid peroxidation in both physiological as well as pathological pathways.<sup>7-12</sup> The production of natural antioxidants by the human body eliminates the free radicals, prevents oxidative stress and associated diseases. Natural antioxidants play an significant role in health care.<sup>13</sup> Phenolic acids, flavonoids, anthocyanins, tannins and carotenoids are the natural antioxidant sources of the plant.<sup>14,15</sup>

In a shorter time, TLC with better resolution varies from TLC only in particle size and the sorbents pore size is a major advancement. HPTLC is a valuable tool for accurate identification of herbal formulations, qualitative determination and quantitative determination of active ingredients of herbal extracts and synthetic drugs, standardization of herbal extracts and formulations, detection of adulteration, herbal extract stability studies and estimation of synthetic/natural drugs in the formulation. HPTLC can also provide chromatographic fingerprints that can be viewed and processed as electronic images and validation of analytical methods plays a crucial role in drug discovery, pharmaceutical development and production.<sup>16</sup>

The *Lannea coromandelica* (Houtt.) Merr. (Anacardiaceae) is a deciduous tropical tree widely distributed in India Bangladesh and other tropical countries. It has a wide scope around the world.<sup>17</sup> The bark is effective in cuts, wounds, ulcers, ophthalmia, gout, ulcerative stomatitis, odontalgia, sprains, heart disease, diarrhoea and dysentery. The astringent bark is used as a lotion in impetiginous eruptions and severe ulcerations.<sup>18</sup> The different parts of the plants have traditionally been used as therapeutic agents for various diseases. The bark of *Lannea coromandelica* was used by the tribal peoples of Bangladesh for the treatment of hepatitis, diabetes mellitus, ulcers, coronary heart disease and dysentery.<sup>19</sup> Leaf juice has been taken orally to alleviate ulcers and pain, while fruit sap has been used to treat cold and cough.<sup>20,21</sup> The bark of *L. coromandelica* was used to treat gout, dyspepsia, dysentery, skin eruption, ulcers and toothache.<sup>22,23</sup> Researchers performed experimental studies focused on these traditional applications of *Lannea coromandelica* against various illnesses. Barks should have anti-inflammatory, hypotensive, anti-hyperglycaemic, antimicrobial, analgesic and antioxidant effects.<sup>24-32</sup> The aqueous extract of *L. coromandelica* antagonizes the glucagon receptor and has the ability to minimize the output of glucagon-mediated liver glucose and antinociceptive activity.<sup>33,34</sup>

Quercetin, (2S,3S,4R,10E)-2-[2'R)-2'-hydroxytetracosanoyl amino]-10-octadecene-1,3,4-triol, aralia cerebroside, 5,5'-dibuthoxy-2,2'-bifuran,  $\beta$ -sitosryl-3 $\beta$ -glucopyranoside-6'-O-palmitate,  $\beta$ -sitosterol palmitate, myricadiol, protocatechuic acid, p-hydroxy benzoic acid ethyl ester, isovanillin, trans-cinnamic acid, palmitic acid and steric acid were isolated from the barks of *Lannea coromandelica*.<sup>35</sup> HPLC revealed the presence of gallicacid, (-) epigallocatechin-3- gallate, catechin, chlorogenic acid and caffeic acid in *L. coromandelica* bark extract.<sup>36</sup> Bioassay guided fractionation and chemical characterization of *Lannea* extracts by MALDI-TOF-MS revealed that the active constituents were angular type of polyflavanoid tannins and exhibit zoosporicidal activity.<sup>37</sup>

In consideration of the traditional use of this drug, antioxidant activity was predicted in preliminary phytochemical studies. Therefore, the purpose of the study is to qualitatively and quantitatively evaluate the existence of different phytochemicals in *Lannea coromandelica* using the HPLTC technique and to investigate the *in vitro* antioxidant capacity of extracts of *Lannea coromandelica* bark.

## MATERIALS AND METHODS

### Collection and authentication of plant material

The *L. coromandelica* bark was collected from the southern region of Tamil Nadu, India. Plants were identified and certified with the help of Dr. V. Chelladurai, Research Officer- Botany, C.C.R.A.S. Govt of India, Tirunelveli, Tamil Nadu and a voucher specimen is retained in the department for future reference.

### Preparation of the plant extracts

Weighing accurately about 500 g of bark of *L. coromandelica* were dried and milled into coarse powder and extracted for 7 days by increasing the polarity of organic solvents such as petroleum ether, chloroform, ethyl acetate, methanol by Soxhlet extractor and water (AELC) by maceration. After each extraction, the marc was collected and dried in the air, then repackaged in the apparatus. The extracts in the rotator evaporator were filtered and concentrated dried and stored in the desiccators under reduced pressure.

### Physico-chemical parameters

Various physicochemical parameters were studied, such as total ash, insoluble acid ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value, foreign organic matter and loss on drying.<sup>38</sup>

### Preliminary phytochemical screening

Phytochemical screening was performed to identify alkaloids, tannins, flavanoids and terpenoids.<sup>39,40</sup>

### HPTLC profile, Development of chromatogram, scanning and detection of spots

It requires standardized manufacturing processes and adequate analytical tools to lay down the required framework for quality control in herbals. High-performance thin-layer chromatography (HPTLC) is widely used separation techniques to establish fingerprints of reference for herbs. HPTLC fingerprint profile of MELC was executed.<sup>41,42</sup> The sample solutions have been prepared and configured for fingerprinting of high quality. Approximately 5 mg / ml of MELC concentration in the respective HPLC grade solvents was made and filtered by Whatman filter paper No. 1. Prewashing for HPTLC plate by methanol and then dried. MELC was applied by Camag (Muttenez, Switzerland) Linomat V automated spray-on band applicator with a band length of 6 mm and 10s  $\mu\text{l}$ -1 application rate of 100.00- $\mu\text{l}$  (Hamilton) syringe. The band width was 6 mm, the plate edge distance was 10 mm and the plate bottom distance was 10 mm. Toluene: ethyl acetate: methanol (60: 30: 10) was the mobile phase used for development. Mobile phase chamber saturation was performed for 20 m in twin trough glass chamber at room temperature (30.5°C). The mobile phase was evaporated from the plate by using an air-dryer after development. The air-dried plate was examined for various secondary metabolites at 254 nm, 366 nm and visible light spectrum after 10 m of spraying with 10 percent v/v vanillin sulphuric acid incubated at 110°C. Deuterium lamp and tungsten lamp have been used in absorbance mode for densitometric scanning at 254 nm, 375 nm and 550 nm.

### Determination of antioxidant activity

#### Estimation of total phenolic content (TPC)

Total phenolic content was evaluated using a slightly modified Folin-Ciocalteu reagent method.<sup>43</sup> The Folin-Ciocalteu method is an electron transfer-based assay and provides reducing capacity expressed as phenolic content. Specified extracts (200  $\mu\text{l}$ ) concentration was combined with Folin-Ciocalteu concentration and incubated for 10 min. At 37°C for 90 m, the reaction mixture was added 1.25 ml of aqueous sodium carbonate and 1 ml of distilled water and the absorbance measured at 760 nm of spectrophotometric and distilled water was used as blank. The standard gallic acid was used and expressed as mg / gallic acid (GAE) equivalent. All experiments were carried out in triplicate for precision

and values were expressed as mg gallic acid equivalent (GAE)/ g of the sample

#### Total flavonoid content (TFC)

Total flavanoid content was determined with slight modification by colorimetric assay of aluminium chloride.<sup>44</sup> A volumetric flask (10 ml volume) was added with 1 ml of test sample and 4 ml water. Add 0.3 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminum chloride after 5 min. After 6 m of room temperature incubation, 1 ml of 1 M sodium hydroxide was added to the reaction mixture. 10 ml of distilled water have been added to make the final volume. The sample absorbance was measured against the blank at 510 nm using UV spectrophotometer. All experiments were repeated three times for precision, total flavonoid content in terms of Quercetin equivalent (QE)/ g of the sample.

#### Free radical scavenging activity (DPPH)

The method described by Brand-Williams was used to evaluate DPPH-free radical scavenging activities with minor modifications for the study of MELC and AELC antioxidant potential.<sup>45</sup> DPPH-free radical scavenging activity was performed at a specified concentration of 100-500  $\mu\text{l}$  of MELC and AELC dissolved in ethanol. DPPH (0.004 %) was prepared by dissolving in ethanol and added separately 1 ml of the above solution to MELC, AELC and standard ascorbic acid. The above mixture was incubated for 30 m in the dark and the absorbance was measured spectrophotometrically at 517 nm. The degree of color change from purple to yellow shows the MELC and AELC free radical scavenging efficiency. Lower absorption of the reaction indicated higher free radical scavenging activity.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Ac}-\text{At}}{\text{Ac}} \times 100$$

Ac is the control absorbance (1 ml of DPPH solution in 1 ml of ethanol) At is the test absorbance

Then, curves were created by plotting concentration in  $\mu\text{g}$  / ml Vs percentage inhibition. The equation of this curve was used to calculate the  $\text{IC}_{50}$  corresponding to concentration of the drug that reduced the DPPH free radical scavenging to 50 %.

#### Ferric ion reducing antioxidant power assay (FRAP)

Ferric ion reduction ability of antioxidant power assay was performed as described by Benzie and Strain with minor modification method.<sup>46</sup> The antioxidant activity of MELC and AELC was obtained by dissolving the different concentrations (100  $\mu\text{l}$  to 500  $\mu\text{l}$ ) with a 2.5 ml of 0.2 mM phosphate buffer (pH 7.4) and 2.5 ml of

potassium ferricyanide (1% w/v) incubated at 50°C for 20 min. Add 2.5 ml of trichloroacetic acid (10 % w / v) and centrifuge for 10 min at 3000 rpm. To 2.5 ml of the upper layer, add 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1 % w / v) and absorbance was measured by spectrophotometer at 700 nm against the standard ascorbic acid. Higher ferric ion reducing ability indicated by higher absorbance values of the reaction mixture.

### Phosphomolybdenum assay

The total antioxidant capacity assay based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of a green phosphate Mo(V) complex at acidic pH is a spectroscopic method for the quantitative determination of antioxidant capacity through the formation of the phosphomolybdenum complex. Phosphomolybdenum (PM) assays were performed to determine total antioxidant activity according to Prieto *et al.*<sup>47</sup> In each tube, MELC and AELC were added to 3 ml of distilled water and 1 ml of molybdate reagent at concentrations of 100 µl and 500 µl. Incubate for 90 m at 95°C. Then they are maintained for 20 to 30 m at room temperature and the absorbance was spectrophotometrically measured against the standard ascorbic acid at 695 nm.

## RESULTS

### Phytochemical analysis

Phytochemical analysis of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts shows the presence of phytoconstituents such as alkaloids, flavanoids, phenol and saponins in EALC, MELC and AELC. Sterols are found to contain in petroleum ether and methanol extracts. Tannins are found to contain in methanol and aqueous extracts. In all the extracts, volatile oil was absent. The results of physicochemical parameters of *L. coromandelica* bark were summarized in Table 1.

### DISCUSSION

Plants are the key source of bioactive compounds for the development of new chemotherapeutic agents. Phytochemical screening and physicochemical parameters were carried out to assess the quantity of soluble constituents and the quality and purity of a crude drug. Preliminary phytochemical analysis of the extracts shows alkaloids, flavanoids, phenol, tannins, sterols and saponins. Preliminary phytochemical studies provide researchers a broad idea for their further research. The total flavanoid content of in MELC and AELC was

completely synchronous with that of the total phenolic content. It has been successfully demonstrated that samples with a high phenolic content also contain large amounts of flavonoids as Table 2. The rich-flavonoid crude drug can be a good source of antioxidants helping to increase the overall antioxidant potential of an organism and prevent lipid peroxidation.<sup>48</sup>

The physico chemical parameters for a plant are almost constant, so they help to set crude drug standards. These parameters are crucial for detecting adulteration or improper drug handling. To determine the purity and quality of drugs, assessing total ash is essential. There are three common methods for determining ash value, such as total ash, acid-insoluble ash and water-soluble ash. Ash value is the residue that remains after the incineration of plant material and is useful in assessing sample quality and purity, as well as providing important qualitative standards. Total ash content analysis is actually burning away organic matter, leaving inorganic minerals behind. This helps to evaluate the quantity and type of minerals in the drug that are useful in determining the physicochemical properties of the drug. Total ash containing both physiological and non-physiological ash typically consists of carbonates, phosphates, silicates and silica. A high ash value means contamination, substitution, adulteration or carelessness occurs, when the crude drug is being prepared for commercialization as shown in Table 1a. Extractive values are performed to assess the identity of the extractable polar or non-polar compounds in the drug. Loss on drying was performed on the drug to determine the moisture and should be excluded from the product absolutely. Microbial growth is possible if the crude drug is stored for a very long period of time and its stability is directly related to the moisture content of the crude drug. The lower the amount of the moisture, the greater the stability of the drug and the lower the risk of microbial growth as depicted in Table 1b.

**Table 1a: Physicochemical parameter.**

Type of ash content	Ash values
Total ash (% w/w)	4.9052
Acid Insoluble (% w/w)	1.2847
Water soluble ash (% w/w)	2.1437

**Table 1b: Extractive values**

Type of extractive values	Extractive values (%w/w)
Alcohol soluble extractives	2.6552
Water soluble extractives	4.3943
Foreign organic matter	0.76
Moisture content	4.36

Results (n=3, Mean ± SD)

MELC had the best possible results in Toluene: ethyl acetate: methanol (60: 40: 10) and solvent system. The plates were scanned and visualized in absorbance mode at both 254 nm, 375 nm, 550 nm and visible light range after spraying with 10 percent v/v vanillin sulphuric acid reagent. The images from the HPTLC Figure 1 specify that all phytoconstituents were clearly separated without any tailings or diffusiveness. The results of HPTLC fingerprint scanned for MELC scanned at 254 nm revealed the presence of eight phytoconstituents. The  $R_f$  values were from 0.13 to 1.00. MELC scanned at 375 nm and 550 nm revealed the presence of nine phytoconstituents (Table 3). The  $R_f$  values were from 0.09 to 0.94 and 0.16 to 0.94 respectively. From Table 3 and chromatogram as shown in Figure 2, of the nine components,  $R_f$  values 0.13, 0.30, 0.45 was found predominantly higher as the percentage area is 30.32 %, 15.43 % and 19.43 % respectively out of eight components. From Table 3 and chromatogram as shown in Figure 3, of the nine components, the component has  $R_f$  values 0.09, 0.23, 0.66, 0.49 was found to be more like 33.56 %, 18.96 %, 15.55 % respectively. From Table 3 and chromatogram as shown in Figure 4, of the nine components, the component has  $R_f$  values of 0.16, 0.78 were found to be more like 46.14 % and 14.91 % respectively. The proposed HPTLC method has been found to be more suitable for the calculating the number of constituents and also used in routine research as a rapid analytical tool to detect losses or variations in plant crude extract phytoconstituents. The HPTLC fingerprints developed will be useful in the manufacturer's quality control and standardization of herbal drugs and will also be useful in differentiating species from adulterants in the pharmaceutical industry and systematic plant studies.

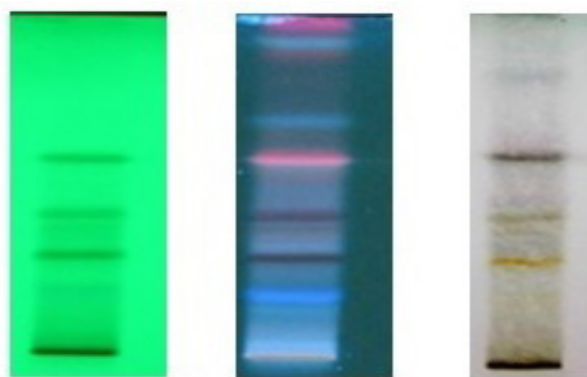
**Table 2: Total phenol content (TPC).**

Extracts	Total phenol content (mg GAE/ g sample)
EALC	11.42 ± 0.45
MELC	54.72 ± 0.21
AELC	45.51 ± 0.25

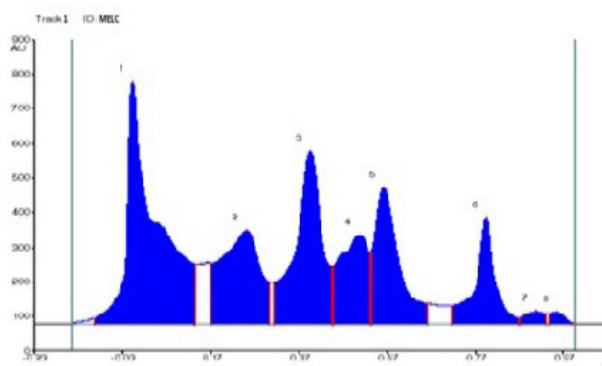
Results (n=3, Mean ± SD)

In MELC, the total phenolic content was higher than in EALC and AELC as shown in Table 2. Phenolic compounds are responsible for their function as antioxidants and also known for their redox properties in free radical absorption and neutralization; singlet and triplet oxygen quenching or peroxide decomposition.<sup>49</sup> In MELC the total flavanoid content was higher than in EALC and AELC as shown in Table 4.

The DPPH assay was widely used as a simple, reliable and reproducible parameter to check the general *in vitro* antioxidant activity of pure compounds and plant extracts.<sup>50</sup> Various concentrations of MELC and AELC were screened for free radical DPPH scavenging assay.



**Figure 1: HPTLC fingerprint profile of MELC at 254 nm, 366 nm and visible for secondary metabolites.**



**Figure 2: HPTLC profile of MELC scanned at 254 nm.**

**Table 3: HPTLC fingerprint of MELC at 254, 375 and 550 nm.**

Extracts	Mobile phase	No. of Peaks	$R_f$ Value	Percentage area (%)
MELC at 254 nm	Toluene : Ethyl acetate :	08	0.13,0.30,0.45,0.53, 0.66, 0.87,0.94,1.00	30.32,15.43,19.43, 10.54,13.91,8.70, 1.02, 0.66
MELC at 375 nm	Methanol (60 : 30 : 10)	09	0.09,0.23,0.29,0.35, 0.49,0.57,0.63,0.77, 0.94	33.56,18.96,5.71, 6.63,15.55,4.33, 2.60,10.62,2.05
MELC at 550 nm		09	0.16,0.22,0.30, 0.41, 0.48,0.56,0.61,0.78, 0.94	46.14,9.27,8.08,4.72, 4.54,3.56,3.05,14.91, 5.74

Results indicated that MELC and AELC were capable of inhibiting DPPH-free radicals. As the extract concentration increased from 100 to 500  $\mu\text{g/ml}$ , it was found that DPPH activity increased. Inhibitory action of was compared with standard ascorbic acid. MELC antioxidant activity was higher than AELC as shown in Figure 5. Iron is essential for transporting oxygen, breathing and many enzyme functions.<sup>51</sup>

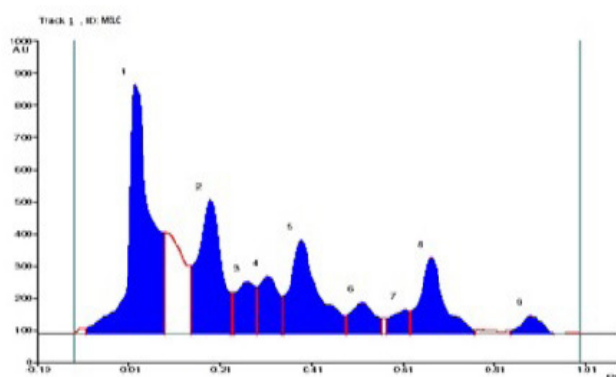
The presence of antioxidants in MELC and AELC will lead to the donation of an electron to reduce  $\text{Fe}^{3+}$  ferri cyanide to  $\text{Fe}^{2+}$  ferrocyanide. Together with standard ascorbic acid, FRAP assay was carried out in MELC and AELC. It was observed from the analysis that MELC displayed higher ferric ion diminishing antioxidant potential than AELC compared to standard ascorbic

acid as Figure 6. The metal chelating ability of MELC was evaluated by the formation of a ferrous ion ferrozine complex and the reducing power of MELC increased in a concentration-dependent manner due to its electron donating ability of secondary metabolites.<sup>52</sup> It involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature. It will give a direct estimation of reducing capacity of antioxidant. Without induction of free metal ions, it forms a green phosphomolybdenum complex. Phospho molybdenum assay was performed with MELC and AELC along with standard ascorbic acid. Compared to standard ascorbic acid, MELC showed the highest activity than AELC as shown in Figure 7. Due to their phenolic constituents, some plants exhibit antioxidant activity. A broad class of low molecular weight, secondary metabolites commonly found in plants are flavanoids. The beneficial effects of flavanoids are attributable to their antioxidant and chelating ability.<sup>53</sup> Our results on *Lansea coromandelica* bark extracts are in accordance with the Tekesh kumar et al reports.<sup>54</sup> Imminent antioxidant properties of various leaf extracts of *Lansea coromandelica* have been

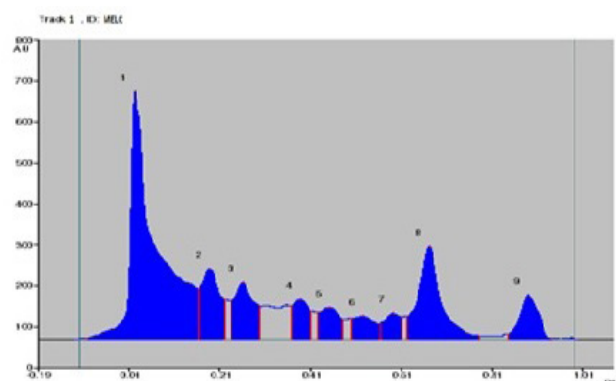
**Table 4: Total flavanoid content (TFC).**

Extracts	Total flavanoid content (mg quercetin/ g sample)
EALC	4.42 $\pm$ 0.45
MELC	19.72 $\pm$ 0.21
AELC	17.51 $\pm$ 0.25

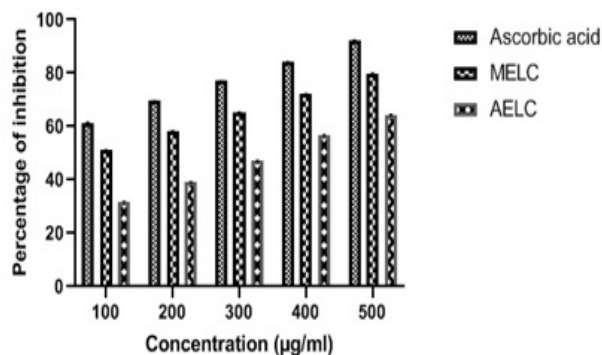
Results (n=3, Mean  $\pm$  SD)



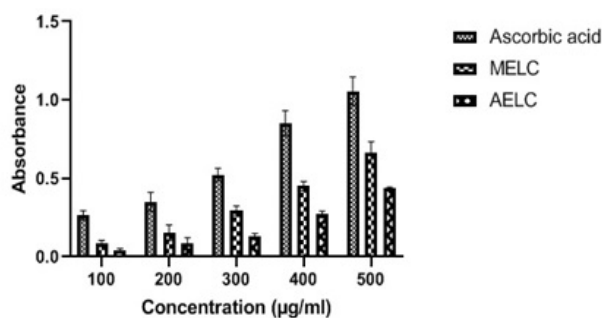
**Figure 3: HPTLC profile of MELC scanned at 375 nm.**



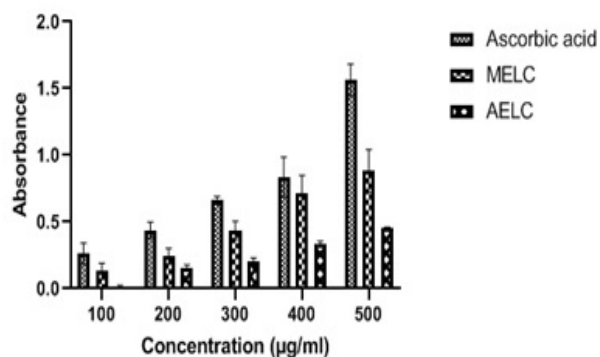
**Figure 4: HPTLC profile of MELC scanned at 550 nm.**



**Figure 5: Free radical scavenging (DPPH) of MELC and AELC. Results expressed as the mean  $\pm$  standard deviation (n=3).**



**Figure 6: Ferric ion reducing antioxidant power assay (FRAP) of MELC and AELC compared to that of standard ascorbic acid. Each value is expressed as mean  $\pm$  standard deviation (n = 3).**



**Figure 7: Phospho molybdenum assay of compared to that of standard ascorbic acid. Each value is expressed as mean  $\pm$  standard deviation ( $n = 3$ ).**

reported. However, antioxidant activities were found to possess in the bark extracts of *Lannea coromandelica* in this study.

## CONCLUSION

Based on the results obtained, HPTLC fingerprinting was one of the possible approaches for medicinal plant identification and quality control to check the bioactive principle and standardization. However further work with marker compounds is necessary to perform quantitative estimation. This plant antioxidant potential also reveals their medicinal importance. The phytoconstituents responsible for antioxidant activity need to be explored and establish the specific mechanism of action for antioxidant activity.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

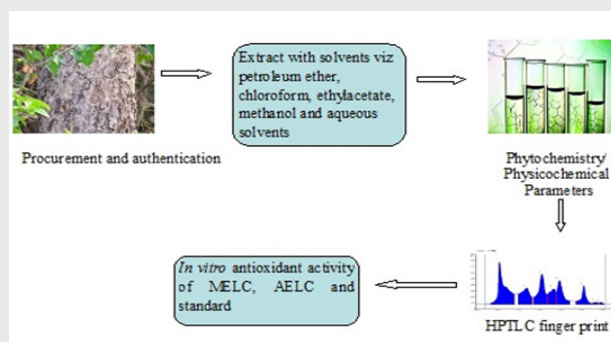
**DPPH:** Free radical scavenging potential of 1,1 diphenyl 2 picryl hydrazyl; **EALC:** Ethyl acetate extracts of *Lannea coromandelica*; **MELC:** Methanol extracts of *Lannea coromandelica*; **AELC:** Aqueous extracts of extracts of *Lannea coromandelica*; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent; **TFC:** Total Flavanoid content; **TPC:** Total phenol content; **FRAP:** Ferric ion reducing antioxidant power assay; **PM:** Phosphomolybdenum assay.

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## PICTORIAL ABSTRACT



## About Authors



**Dr. Rajesh R**, is an Associate Professor and HOD, Department of Pharmaceutical Analysis at Acharya & BM Reddy College of Pharmacy, Bengaluru. He has 21 years of academic and research experience. He has wide experience in the area of chemistry of natural products and pharmaceutical analysis mainly in the area of phytochemistry, HPLC analytical and bioanalytical method development and validation.



### SUMMARY

Phytochemicals are a strong category of compounds that belong to secondary plant metabolites and comprise a number of chemical entities, including polyphenols, flavonoids, steroidal saponins, organosulfur compounds and vitamins. Oxidative stress results from increased ROS and/or reactive nitrogen species (RNS) resulted in autoimmune diseases such as cancer, inflammatory response, vascular disease and neurodegenerative disorder. Antioxidants have a role in neutralizing the excess of free radicals, protecting cells against their harmful effects and helping to prevent disease. While there are many synthetic antioxidants available and due to the risk of causing side effects they have limited use. Recent research activities have therefore been based on using natural plant antioxidants rather than synthetic antioxidants. HPTLC fingerprinting was one of the potential methods for the identification and quality control of medicinal plants in order to evaluate the bioactive principle and standardization. It was confirmed that the presence of poly phenol in the bark of *Lannea coromandelica* possesses antioxidant properties. These findings provide scientific evidence to prove its antioxidant ability and will be used to treat oxidative stress-related diseases in the near future with phytochemical based drugs.

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