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# PHYTOCHEMICAL ANALYSIS AND PHARMACOLOGICAL EVALUATION OF LEAF OF *LANNEA COROMANDELICA LINN.*

**Tekeshwar Kumar[1*] and Vishal Jain[2]**

[1]MJ College of Pharmacy, Bhilai, Durg, 490 023, Chhattisgarh

[2]University Institute of Pharmacy, Pt. Ravishankar University, Raipur, Chhattisgarh, 492 010, India

**Corresponding Author:** tekeshwarverma@gmail.com

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## ABSTRACT

WHO is highly recommended the customary exploitation of traditional therapeutics of medicinal plants and is consequently in pipeline for development of monograph and exploring their uncultivated pharmacological properties. Presently, correct identification of medicinal plants by phytochemical fingerprints is a speedy tool to ensure reproducible quality of herbal drugs. In our study, the ethyl acetate and methanol extract of leaves of *Lannea coromandelica (L. coromandelica)* was subjected for chemical fingerprint as well therapeutic aid as antifilarial agents. High performance thin layer chromatography (HPTLC) was employed to develop chemical fingerprint and filariciadal activity was assessed by motility inhibition and MTT reduction assay with concentrations range 1000 to 25µg/ml. The HPTLC analysis of ethyl acetate and methanol extract was carried out using hexane: ethyl acetate: formic acid: methanol (5:4:1:0.5 v/v/v/v) for fingerprinting and quantification ellagic acid and quercetin. The HPTLC method was found to give compact spots for Rf = 0.54 and 0.51 ellagic acid; Rf = 0.91 and 0.87 quercetin for ethyl acetate and methanol extract respectively. The HPTLC method was validated as per the ICH guidelines. Inhibitory concentration ($IC_{50}$) for the ethyl acetate extract was found to be 284.5µg/ml. In motility assay, complete inhibition of motility was observed for all concentrations. Hence, our study could be valuable for inventing strategies for quality control parameter and justifies the ethnic uses of plant in folkloric medicines.

**Keywords**: **:** *WHO, Lannea coromandelica, HPTLC, filariciadal activity.*

## INTRODUCTION

Natural sources are dealing presently to treat various disease since past to present. In future it needs proper justification of claimed traditional uses. At present innumerable antifilarial drugs DEC, albandazole, Ivermactin and other combination of drugs are not capable to control the filaria disease. So in the present day several medicinal plants have been used as therapeutic aid as antifilarial agents. Experiments on antifilarial activities of various such phytochemicals are coming up with the constant effort made by the researcher from various parts of the world [1]. One of the important methods for identifying leads for drug development is to screen medicinal plants for the antifilarial activity [2]. *Lannea coromandelica* Houtt. (Anacardiaceae) commonly known Jingini, is deciduous large trees, upto 15-20 m tall. Bark & leaves of *Lannea coromandelica* is commonly used in ulcerative stomatitis, dyspepsia, general debility, gout, cholera, dysentery, sore eyes, leprosy, sprains and bruises, wound, elephantiasis, snakebite, stomachache and nerve muscle inhibition [3]. It contains triterpenoids, tannin, alkaloids [4], phenolics, flavonoids [5]. Literature

survey of its activities revealed that there was no studied for its in-vitro anti-filarial activity and phyto-chemical analysis with sophisticated instruments. Hence the objective of our experiment is to quantify phyto-constituents and evaluate antifilarial effect of ethyl acetate and methanolic extract of *Lannea coromandelica* leaves against Setaria cervi in-vitro.

## MATERIALS AND METHODS

### Collection of plant material

Leaves of plant *Lannea coromandelica* were collected from Barnawapara forest near Raipur managed by Government of Chhattisgarh State Forest Division. The collected plant was botanically recognized by Dr. V. P. Prasad. A voucher specimen (CNH/Tech.II/2014/70/139) was submitted to the Central National Herbarium, Howrah, India.

### Preparation of extract

Shade dried *Lannea coromandelica* leaf was powdered (50 gm) and extracted using buchi speed extractor with 90% methanol. The supernatant were evaporated to dryness. The crude dry extract (5%v/v) was then suspended in 2% acetic acid and partitioned with ethyl acetate (30 ml X 4). The pooled ethyl acetate fractions were then evaporated to get dry powder (2.8 g) and stored at $4^0$C for further use. All the chemicals are the analytical grade purchased from Hi-media, Merck India.

### Chromatographic analysis

- **Preparation of standard solution**

Accurately weighed 10 mg of ellagic acid standard was dissolve in 10 mL of methanol in a volumetric flask to prepare stock solution of 100µg/mL. In the same way quercetin stock solution was prepared. These stock solutions were further diluted to prepare the concentration range of 200-800 µg/mL by serial solution method.

- **Preparation of sample solution**

Ellagic acids from four different sources were extracted by hydrolysis of 10 g of the sample with 30 ml of methanol, refluxed for 1 hour. Extract obtained was filtered using Whatman filter paper No 42. 10 mL of distilled water was added to the filtrate and evaporated to a volume of 10 mL. Samples were analyzed immediately after extraction in order to avoid possible chemical degradation. The experiments were carried out in triplicate [6].

- **Chromatography method**

Chromatography was performed on an aluminum backed silica gel 60 $GF_{254}$ TLC plates pre-washed with methanol. The standard and four different sample solutions were applied to TLC plates as 8.0 mm band with 9.0 mm space between two band using a Camag Linnomat IV sample applicator. The plate were developed with a mobile phase of Hexane: ethyl acetate: formic acid: methanol (5:4:1:0.5) in a TLC twin trough chamber previously saturated with the solvent for 30 minutes. After development the plates were dried at 60°C for 5 min. and quantification of the standards and samples were performed by mean of Camag TLC scanner III controlled by WinCATS 1.4.3 version software at 225 nm. The amount of ellagic acid and quercetin in the sample solution were computed from calibration plot.

- **Validation of the method**

After the development of HPTLC method for the simultaneous estimation of the samples, Validation of the method was carried out according to the ICH guidelines with respect to Linearity, Accuracy, Precision, Limit of Detection and Limit of Quantification [7,8].

**In vitro activity**

- **Collection of worms**

Adult S. cervi, a nematode parasite was obtained from the peritoneal cavity of freshly slaughtered cattle. The worms were washed repetitively with normal saline (8.5 g/L) to free them from any extraneous material and used for experiment.

- **In-vitro motility inhibition assay**

The worms (one male and one female) were transferred immediately to DMEM (Dulbecco's modified eagle's medium) with 0.01% strepto-penicillin and supplemented with 10% (v/v) heat-inactivated fetal calf serum. The concentrations range made in DMSO for extract (1000/500/250/100/50/25µg/ml) was used. The worms were incubated at 37℃ for 24 h in 5% (v/v) $CO_2$ incubator and motility observed after 2 to 24h in fresh medium without the test solution and test solution. Each experiment was repeated in triplicates [9].

- **MTT assay**

Effect of the plant extract on adult female Setaria worms was studied by MTT farmazan reduction assay with some modification. High values of absorption correlate with high viability of the worms [10]. Viability of the worms was estimated as percentage inhibition in formazan formation relative to solvent controls and heat killed worms by following the formula:

$$\text{Percentage inhibition} = 100-[(T-H)/(C-H)] \times 100$$

## RESULTS AND DISCUSSION

The development of solvent system for chromatography (HPTLC) analysis was carried out by using different solvents in hit and trial method. A wavelength of 225 nm was chosen for quantification. The Rf value of Ellagic acid and Quercetin after development with the mobile phase Hexane: ethyl acetate: formic acid: methanol (5:4:1:0.5 v/v/v/v) was 0.54 and 0.91 for ethyl acetate extract and 0.51 and 0.87 for methanolic extract respectively. When the concentrations of Ellagic acid and Quercetin and their respective peak areas were subjected to regression analysis by least squares method. Calibration curve of standard and a good linear relationship ($r^2$ = 0.997; 0.991) was observed between the concentrations of Ellagic acid and Quercetin and the respective peak areas in the range 200 - 800 ng / spot. The regression of Ellagic acid and Quercetin was found to be Y= 6.451X+ 1345 and Y= 4.451X+ 1290 respectively, where 'Y' is the peak area and 'X' is the concentration of Ellagic acid and quercetin respectively are shown in Table 1. The chromatograms containing peaks of Ellagic acid and quercetin of standard and in extract were shown in figure 1-3. The concentration of flavonoid is higher in ethyl acetate extract as compare to methanol extract. Hence it was subjected for only Filaricidal effect.

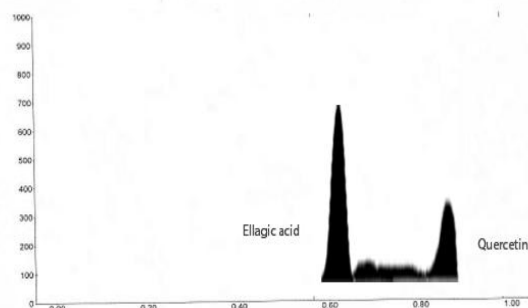


Figure 1. Typical HPTLC Chromatogram of Ellagic acid & Quercetin standard Ellagic acid & Quercetin

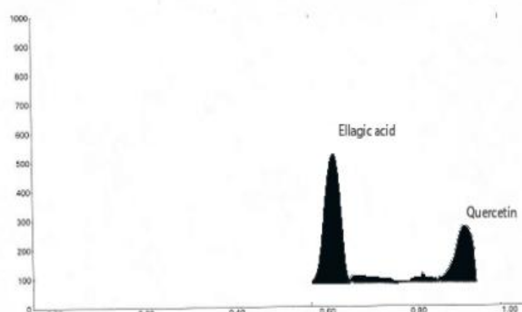


Figure 2. Typical HPTLC Chromatogram of ethyl acetate extract

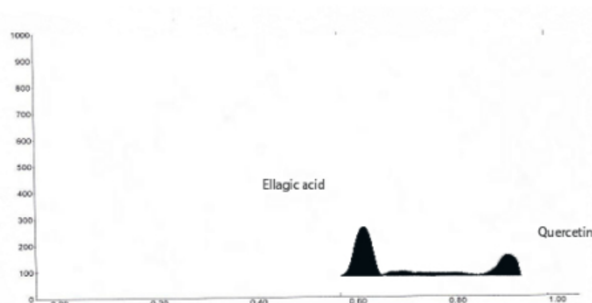


Figure 3. Typical HPTLC Chromatogram of methanol extract

Table 1. System suitability studies for HPTLC method

| S. No. | Parameters | Ellagic acid | Quercetin |
|---|---|---|---|
| 1 | Linearity range | 200-800ng | 200-800ng |
| 2 | Detection wavelength | 225 nm | 225 nm |
| 3 | Mobile phase Hexane: ethyl acetate: formic acid: methanol | 5:4:1:0.5 | 5:4:1:0.5 |
| 4 | Rf | 0.54 | 0.91 |
| 5 | Regression Equation | Y= 6.451X+ 1345 | Y= 4.451X+ 1290 |
| 6 | Correlation coefficient | 0.997 | 0.991 |
| 7 | LOD(ng) | 0.89 | 1.34 |
| 8 | LOQ(ng) | 2.75 | 4.56 |

The Filaricidal effects of this plant extracts shown in dose dependent manner. Antifilarial activity at lower concentration recorded in terms of loss of motility in comparison to the suitable controls indicate that these can be considered as potential drug, though this should be further confirmed by studying corresponding actual loss of viability of the parasites (Table 2) .In motility assay, complete inhibition of motility was observed and in MTT reduction assay which gave >50% reduction for concentrations 1000, 500 and 250μg/ml at 12, 7 and 4 h incubation periods respectively in a dose dependent manner ($P<0.05$) (Table 3). An antifilarial effect imparted by plant extract was found to be a function of their relative concentrations. The very low absorbance value (0.298) was observed for the heat-killed worms due to the least production of formazan in dead worms. Inhibitory concentration ($IC_{50}$) for the ethyl acetate extract was found to be 284.5μg/mL.

Table 2. In vitro antifilarial activity of ethyl acetate extract of *Lannea coromandelica* leaves against adult filarial parasite in terms of motility inhibition.

| **Test Con. (μg/ml)** | **Incubation Period (h)** | **Worm motility (test )** | **Worm motility (control)** |
|---|---|---|---|
| 30 | 24 | 100 | 0 |
| 60 | 21 | 100 | 0 |
| 120 | 16 | 100 | 0 |
| 250 | 12 | 100 | 0 |
| 500 | 7 | 100 | 0 |
| 1000 | 4 | 100 | 0 |

Table 3. In vitro antifilarial activity of ethyl acetate extract of *Lannea coromandelica* against adult filarial parasite in terms of MTT reduction assay.

| **Treatment** | **Test con. (μg/ml)** | **Incubation Period (h)** | **Absorbance at 492 nm** | **Percentage reduction (%)** |
|---|---|---|---|---|
| ʆPositive control | - | 24 | 1.134 ±0.13 | - |
| Negative control | - | 0.5 | 0.470±0.05 | - |
| ethyl acetate extract | 30 | 24 | 1.054±0.03* | 9.51 |
| | 60 | 21 | 0.912±0.12* | 19.83 |
| | 120 | 16 | 0.786±0.41* | 36.62 |
| | 250 | 12 | 0.598±0.02* | 43.32 |
| | 500 | 7 | 0.496±0.14* | 75.34 |
| | 1000 | 4 | 0.401±0.08* | 93.4 |

ʆAdult worms that had previously been heat killed and incubated with MTT served as negative control.
*P value represents the level of significance $P < 0.05$ when comparing the mean value of absorbance observed for the formazan formed between treated and control worms.

The present study chemical profile partially correlated with its pharmacological activity and showed significant antifilarial effect of ethyl acetate extract of *L. coromandelica* in vitro. This study contributed toward the development of database for novel drug candidates for lymphatic filariasis in near future.

## CONCLUSIONS

The present study revealed the presence of significant content of ellagic acid and quercetin and significant antifilarial effect of ethyl acetate extract of *L. coromandelica*. This study contributed toward the development of database for novel drug candidates for lymphatic filariasis in near future.

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