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# Evaluation of Potent Anthelmintic and Antibacterial Activities of Ethanolic Leaf Extracts of *Conocarpus lancifolius Engl.* (Combretaceae)

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## Abstract

The development of novel antimicrobial agents depends heavily on the bioactive secondary metabolites found in traditional medicinal herbs. The therapeutic potential of *Conocarpus lancifolius Engl.* (Combretaceae), a plant used in traditional medicine for a number of illnesses was assessed. The purpose of this study was to use a variety of solvents to examine the anthelmintic and antibacterial properties of *C. lancifolius* leaf extracts *in vitro*. Aqueous, chloroform, methanol, and ethanol solvents were used to macerate leaves that were gathered from Erode, Tamil Nadu. Using Streptomycin as the standard, the disc diffusion method was used to test the antibacterial efficacy against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Time to Paralysis (P) and Death (D) were measured in comparison to piperazine citrate in order to evaluate anthelmintic activity against *Fasciola gigantica* and *Pheretima posthuma*. The ethanolic leaf extract was highly bioactive; it showed good bioactivity against *S. pneumoniae* (29.62 ± 0.04 mm) and *K. pneumoniae* (17.40 ± 0.26 mm) with much greater zones of inhibition than the standard of streptomycin (26.86 ± 0.09 mm and 11.82 ± 0.21 mm, respectively). The ethanolic fraction of 100 mg/mL was effective in inducing paralysis and death of flukes; *F. gigantica* (P: 1.50 ± 0.57 min; D: 2.50 ± 0.05 min) and *P. pasthuma* (P: 2.00 ± 0.23 min; D: 3.00 ± 0.10 min). Both worm species experienced significant reduction in time to paralysis and death in comparison to the control, piperazine citrate, which took 21 min to induce paralysis and 55 min to induce death in earthworms.

**Major Findings:** The study provides scientific support for Tamil Nadu's traditional and folkloric use of *C. lancifolius* for medicinal purposes. It suggests that using a polar solvent, such as ethanol, optimizes the extraction of bioactive secondary metabolites, making *C. lancifolius* a very efficient, natural replacement for creating very effective antibacterial and anthelmintic medications.

**Keywords:** *Conocarpus lancifolius Engl.*, Disc Diffusion Method, Ethanolic Extract, *in vitro* Anthelmintic Activity, *in vitro* Antibacterial Activity

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## 1. Introduction

Secondary metabolites are compounds produced by plants that perform many essential roles beyond aiding in development. Secondary metabolites have a variety of uses, including attracting some helpful insects that help with pollination and protecting the plants from herbivores and harmful insects[1,2]. They play essential functions in the living systems in which they originate and are also necessary medical agents when taken by humans. With these critical features, secondary metabolites are a fascinating and essential study topic[3,4]. One of the two

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species that can be found worldwide on tropical and subtropical shorelines is *C. lancifolius*. It belongs to the Combretaceae family and is one of the two different subspecies in this species group[5,6]. An evergreen tree featuring a wide crown, compact cone-shaped clusters of greenish-blue blossoms in terminal panicles, glaucous medium-green leaves, and greyish or brown trunk bark[7,8]. *C. lancifolius* can reach a vertical height of six meters tall. It is also referred to as buttonwood or push mangrove. The leaves appear glossy, with relatively fewer trichomes on both surfaces[9,10]. There is no information available regarding the anti-diabetic properties of the *C. lancifolius* (Figure 1) used locally in Tamil Nadu. However, much research hasn't been done on phytochemicals or pharmacology up to this point. More new studies on the plant's different pharmacologists have now been produced than have ever been available before with everything from treating fevers, diabetes, and inflammation in traditional medicine all the way through to modern science has identified 53 phytoconstituents using a LC-ESI-MS/MS profiling of *C. lancifolius* (2023) leaf extracts, the scientific findings indicate that these compounds may act as very efficient free radical scavengers and exhibit a significant cardioprotective ability in reducing damage to the heart. Further research continued to investigate its potential in treating diabetes, where the leaves and fruits are the main sources of the extract of the enzyme inhibitors quercetin and vanillic acid. Green synthesis of silver nanoparticles using *C. lancifolius* fruit and leaves has been shown to possess high efficacy against cancer cell lines and multidrug resistant pathogens. Further *in silico* investigations identified bioactive compounds Alnusiin and Egrosine that possess high therapeutic potential in the treatment of hepatocellular carcinoma. Nevertheless, there is still a considerable gap in the comparative study of solvent-specific extraction and its direct influence on respiratory pathogens and helminths. Though there is some evidence of general antimicrobial activity, there is scarce data on the effectiveness of different fractions of leaf extracts on *S. pneumoniae* and *K. pneumoniae*. Moreover, though there is considerable evidence of the antioxidant and anti-inflammatory potential of chloroform extracts, there is still scarce data on the potency of the ethanolic fraction in comparison with standard pharmaceuticals such as Piperazine Citrate. As we previously reported[11,12] the chloroform leaf extract demonstrated the highest antioxidant, antimicrobial, and anti-inflammatory activity levels. In light of our previous research, we have conducted in-depth studies on the antibacterial activity of specific strains of *Klebsiella pneumonia* MTCC 39 and *Streptococcus pneumonia* MTCC 39. These studies used the disc diffusion method and an *in vitro* anthelmintic assay, which involved timing the worm's paralysis and death[13,14].

The novelty of the study is in its scientific comparative analysis of solvent-specific extraction as well as its direct therapeutic impact on highly resistant respiratory and parasitic models. Although the antimicrobial potential of *C. lancifolius* is recognised, there is still an urgent need for more scientific data on the relative effectiveness of various fractions of diverse leaf extracts on MTCC strains of *S. pneumoniae* and *K. pneumoniae*. Moreover, there is still no scientific validation that not only does the ethanolic extract of *C. lancifolius* have inherent anthelmintic properties but also that it is highly effective in comparison with the standard pharmaceutical agent Piperazine Citrate in causing rapid paralysis and death in *F. gigantica* and *P. posthuma*. The novelty of this study is in its scientific validation of the folkloric use of *C. lancifolius* in Tamil Nadu, but also indicates a specific bioactive path for the development of high-efficacy antimicrobial and anthelmintic agents. Based on the high concentration of polar polyphenolic secondary metabolites typically found in this species, the authors hypothesise that the ethanolic leaf extract of *C. lancifolius* will exhibit significantly higher antibacterial and anthelmintic potency compared to aqueous, methanolic and chloroform fractions, potentially surpassing the efficacy of standard pharmaceutical controls in rapid-onset paralysis and bacterial inhibition.

## 2. Materials and Methods

### 2.1 Collection and Extraction

The plant materials were gathered from different locations throughout India. After being taken off the collected branches, the impurities present in the *C. lancifolius* leaves were removed by tap water rinsing. The leaves were then dipped in water again by placing them in a clean surface and kept in the shade of Millipore water for three weeks to air dry. The material of crop was first ground to a coarse powder and a fine powder was obtained. For 48 h, this powder was extracted using the straightforward maceration method at room temperature and with intermittent shaking. To achieve the best extraction results, 900 mL of each solvent chloroform, aqueous,

ethanol and methanol were added to 1 kg of weighted plant fibre stored in large, tightly capped carriers. After that, the flask containing the mixture was submerged in an ultrasound chamber for about half an hour every day. Filtration was done after the solvent was added. The same procedure was run three times to obtain the maximum extracted volume. Separate sample bottles were used to collect the water-based (PA), methanol (P-Me), ethanol (P-ET), and chloroform (P-CH) extracts, and each was assigned a unique code. Plant chemical analyses were carried out using a standard protocol and were documented in previous research[15,16].

## 2.2 Worms Collection

*Taenia solium* (tapeworm, 2.4-2.8 g) and *F. gigantica* (liverfluke, mean weight of 0.05-0.07 g) were collected from recently slaughtered cows in the Trichy city of Tamil Nadu. Earthworm *P. posthuma*[17] specimens weighing between 0.06 and 0.60 g were collected from the wet areas of the Salem dam and the Vellalar Educational Trust (VET) campus in Erode. Dr. S. Kavipriya of Vellalar College of Pharmacy's (VCP) Department of Anatomy and Zoology's Parasitology Research Unit attested to the validity of the three worm species.

## 2.3 Bacterial Studies

Plant extracts antimicrobial activity assessment was conducted using disc diffusion methods against *Streptococcus pneumonia* MTCC 655 and *K. pneumoniae* MTCC 39 [18,19]. The microorganism inoculum in culture was prepared using the bacterial cultures. In sterile petri plate, 15 mL nutrient agar (Hi media) medium was placed in the plates, and allowed to cool and solidify. With the help of spreading rod, evenly distribute 100 µL of the bacterial strain broth over the medium until completely dried. Using an uncontaminated cork borer, 6M diameter wells were bored. A DMSO solution containing 1 mg/mL of each extract was created. The wells were filled with 100 µL of solutions containing chloroform extracts. For 24 h, the petri plates were incubated at 37°C [20,21]. Streptomycin (1 g/mL) was prepared as a positive control, and DMSO was used as a negative control. The Zone of Inhibitions (ZI) diameters was used to measure the antibacterial activity. All measurements were made in triplicate.



**Figure 1.** *C. lancifolius* fruits and leaves.

## 2.4 Antibacterial Disc Diffusion Test

Bacterial strains were grown on the potato dextrose agar and allowed to grow at 35°C in 24 h and 5 days to form mould bacteria. Pure bacterial colonies were moved into a tube containing sterile normal saline using a sterile loop. The colonies were coated and then again suspended in 1 mL of sterile distilled water with 0.1% Tween 20 added specifically for the mould. A hemocytometer adjusted the suspension to 2-5 106 conidia/mL. To obtain 2-5 105 conidia/mL, the solution was diluted by 1:10 to produce the final functional inoculum. The inoculum was placed on top of the MHA supplemented with 2% glucose. Sterile six mM discs impregnated with 1 mg/10 µL of the test compounds, 10 µL of streptomycin hydrochloride (positive control), and 10 µL of water (negative control) were placed over the plate. The samples are incubated at 35°C for 48 h. The inhibitory zone was measured using the millimetre scale[22,23].

## 2.5 Anthelmintic Activity

The same type of worms was given at five distinct concentrations (10, 20, 50, 80, and 100 mg/mL in distilled water) in solutions of crude extracts in 9 cm Petri dishes. For each type of worm, this was done twice. Mean times of paralysis (P, in minutes) were noted every time no motility was observed except in cases where the worms were vigorously shaken. The death time (D in min) of the worms was recorded after it was established that they did not move under the condition of being shaken violently or submerged in warm water (50°C). Distilled water was employed as the control, and piperazine citrate (10 mg/mL) was used as the reference compound[24,25].

# 3. Results

The antibacterial activity of *C. lancifolius* leaves was tested against *K. pneumoniae* and *S. pneumoniae* single

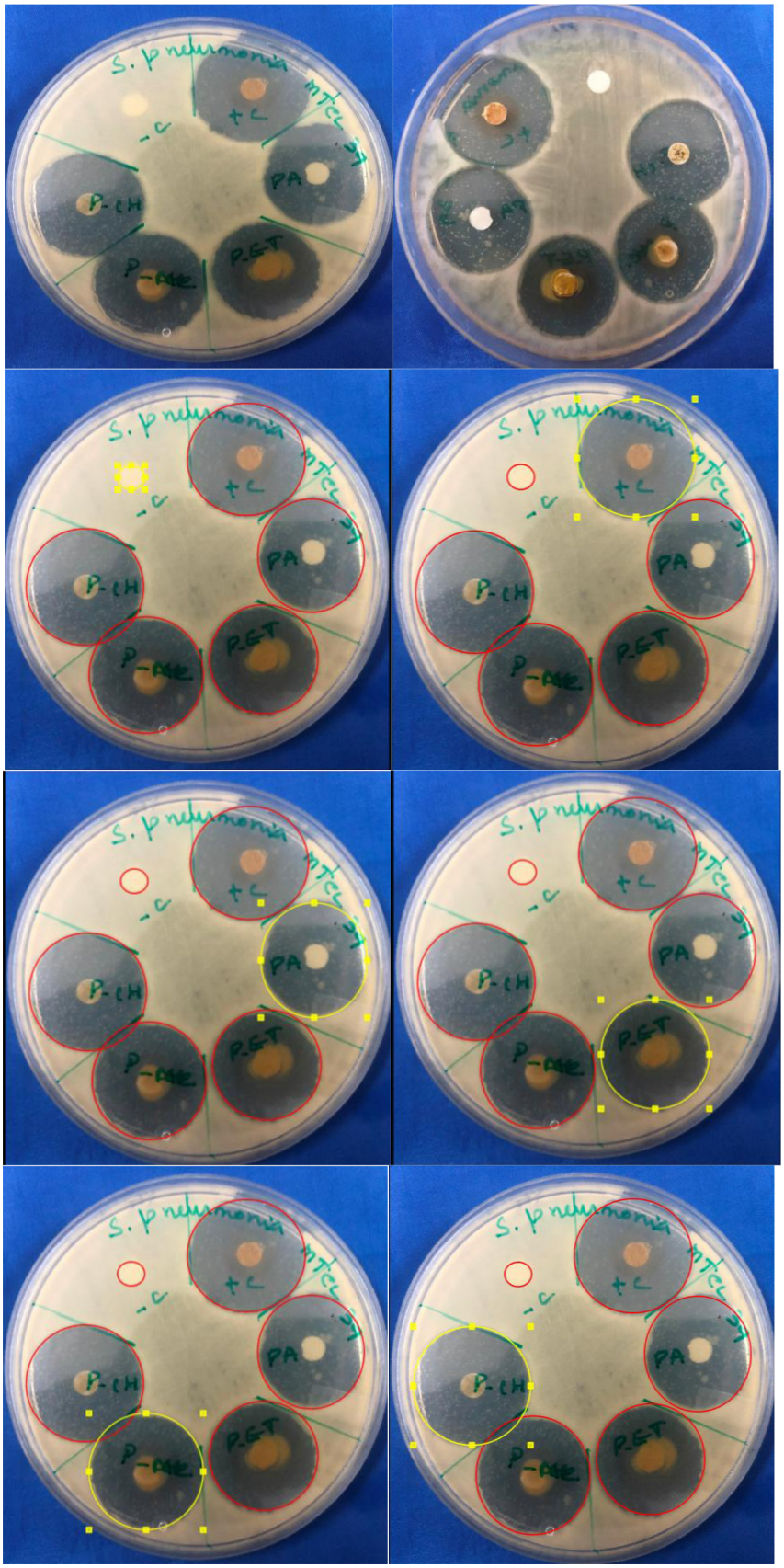



**Figure 2.** Antibacterial potential of different *C. lancifolius* leaf extracts against *S. Pneumoniae*.

species. The total herbal extracts were then dispersed using a nonpolar and extremely polar solution to divide the plant species phytochemical constituents into two categories with distinct chemical constituents and biological properties. In Table 1, Figures 2 and 3, the results are shown as the area of inhibition diameters, or IZD (mm). The inherent anthelmintic characteristics of the leaf extracts are displayed in Table 2, along with the excellent yield of extracts and the findings of the anthelmintic investigations of all extracts of *C. lancifolius* leaves. *Pheretima asthma* and *F. gigantica* were used to test the leaf extracts anthelmintic qualities. $p<0.05$ ($n = 2$) was utilised to test significance using Null's hypothesis. The findings are outlined in Table 2.

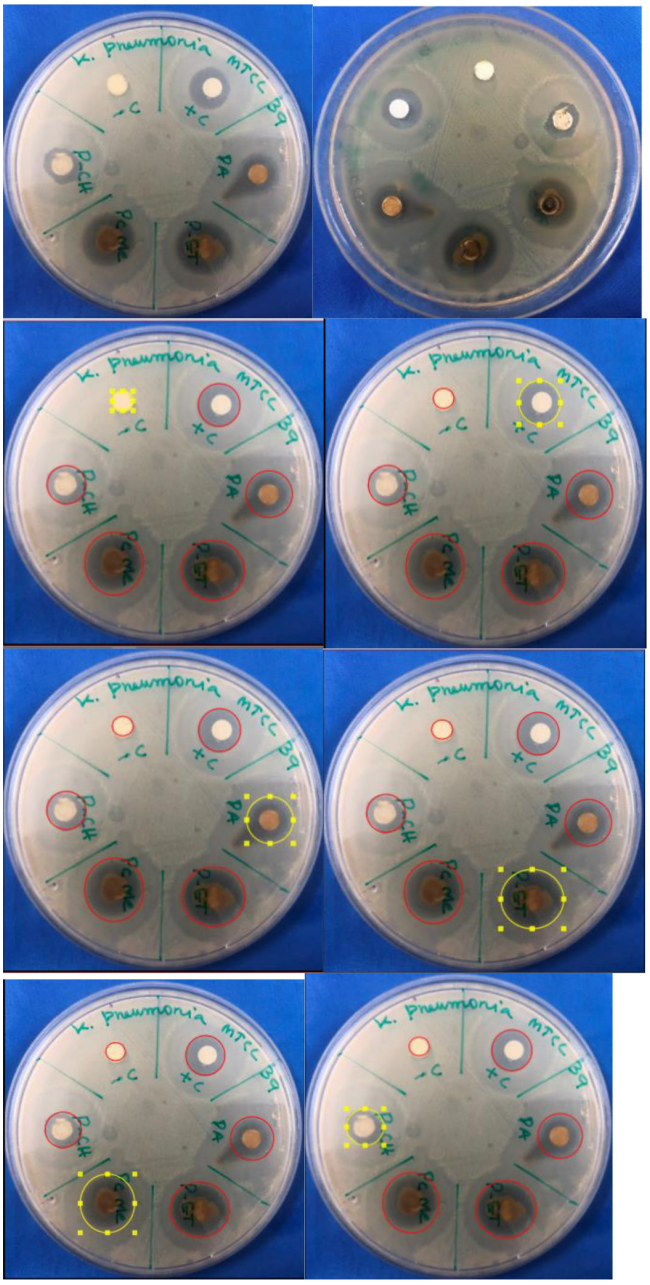


**Figure 3.** Antibacterial potential of different *C. lancifolius* leaf extracts against *K. Pneumoniae.*

## 4. Discussion

The remarkable antimicrobial qualities of chloroform leaf extracts were found to be caused by their high phenolic contents, anti-inflammatory qualities that prevented the denaturation of proteins, and significant antioxidant activity, with an average percentage of inhibition of 62.96% as opposed to 87.96% for ascorbic acid standard, according to our previous research[5]. Through the abovementioned study, we could evaluate the various *C. lancifolius* extracts antibacterial efficacy against the specified organisms. When compared to streptomycin, the drug of choice in our current study, ethanol extract fractions demonstrated exceptional antibacterial activity against *K. pneumoniae* and *S. pneumoniae* in particular. With IZDs of 29.62+0.04 mM and 17.40+0.26 mM, respectively, *S. pneumonia* and *K. pneumonia* were the organisms most susceptible to the ethanol extract. However, compared to both organisms, the chloroform extract showed the second largest IZD against bacteria (27.31+0.17 mM for *S. pneumoniae* and 10.9+10.17 mM for *K. pneumoniae*, respectively). Compared to them, the aqueous extract showed the shortest IZD (25.68+0.11 mM and 13.22+0.17 mM). The methanolic leaf extract showed the more moderate IZD toward the two species

**Table 1.** The antibacterial properties of different *C. lancifolius* leaf extracts were tested in *in vitro* (Inhibition Zone Diameters, IZD)

| S. No. | Inhibition zone diameter (mM)* | | |
|---|---|---|---|
| | Extracts | *K. Pneumoniae* MTCC 39 | *S. pneumoniae* MTCC39 |
| 1 | PA | 13.22±0.17 | 25.68±0.11 |
| 2 | P-CH | 10.91±0.17 | 27.31±0.17 |
| 3 | P-Me | 17.26±0.18 | 26.92±0.08 |
| 4 | P-ET | 17.40±0.26 | 29.62±0.04 |
| 5 | Streptomycin (standard) | 11.82±0.21 | 26.86±0.09 |
| 6 | Water (Negative control) | 5.5±0.26 | 5.5±0.26 |
| 7 | DMSO* | - | - |

*Results are computed after DMSO activity is subtracted

**Table 2.** Anthelmintic activities of *C. lancifolius* extracts

| Extracts | Yield | Conc. (mg/mL) | Paralysis (P) and Death (D) time of worms in min (±SEM) | | | |
|---|---|---|---|---|---|---|
| | | | *P. pasthuma* | | *F. gigantica* | |
| | | | P | D | P | D |
| P-A | 5.2 | 10 | 18±0.52 | 48±0.20 | 25±0.23 | 45±0.15 |
| | | 20 | 15±0.17 | 45±0.26 | 18±0.40 | 41±0.40 |
| | | 50 | 11±0.30 | 25±0.20 | 11±0.11 | 35±0.36 |
| | | 80 | 09±0.11 | 15±0.15 | 09±0.30 | 21±0.20 |
| | | 100 | 07±0.17 | 10±0.11 | 04±0.51 | 15±0.40 |
| P-CH | 4.6 | 10 | 16±0.30 | 35±0.20 | 15±0.11 | 35±0.11 |
| | | 20 | 14±0.40 | 24±0.23 | 10±0.23 | 19±0.17 |
| | | 50 | 11±0.34 | 16±0.20 | 07± 0.30 | 15±0.30 |
| | | 80 | 07±0.06 | 11±0.11 | 05±0.28 | 11±3.30 |
| | | 100 | 03±0.15 | 05±0.20 | 03±0.17 | 09±0.11 |
| P-Me | 3.5 | 10 | 12±0.30 | 24±0.11 | 14±0.30 | 28±0.11 |
| | | 20 | 10±0.41 | 21±0.30 | 09±0.15 | 17±0.23 |
| | | 50 | 09±0.05 | 18±0.15 | 08±0.23 | 11±0.11 |
| | | 80 | 08±0.20 | 11±0.11 | 05±0.15 | 10±0.03 |
| | | 100 | 05±0.28 | 10±0.27 | 03±0.20 | 08±0.28 |
| P-ET | 5.1 | 10 | 09±0.25 | 15±0.11 | 13±0.15 | 11±0.05 |
| | | 20 | 07±0.26 | 11±0.05 | 12±0.17 | 07±0.15 |
| | | 50 | 05±0.17 | 09±0.15 | 08±0.20 | 05±0.11 |
| | | 80 | 03±0.30 | 04±0.25 | 03±0.10 | 03±0.30 |
| | | 100 | 02±0.23 | 03±0.10 | 1.5±0.57 | 2.5±0.05 |
| Piperazine citrate | - | 100 | 21±0.15 | 55± 0.11 | 01±0.05 | 02±0.15 |

(a) Plant extracts/reference drug were dissolved in distilled water.
(b) All values were significant ($p<0.05$). In the control (distilled water treated), *P. pasthuma* lived 48 h, *F. gigantica* lived 5 h.

(26.92+0.08 mM and 17.26+0.18 mM). In addition to their widely recognised antioxidant properties, phenolic compounds possess potent antibacterial properties. Therefore, the remarkable antibacterial activity of ethanolic fraction produced can be attributed to the high polyphenolic content in the plant under study leaves.

The *in vitro* Anthelmintic assay assessed the solvent extracts from the organic matter of *C. lancifolius* leaves, with piperazine citrate serving as a reference. The four extracts of *C. lancifolius* leaves exhibited inherent anthelmintic properties, as indicated in Table 2. The extracts of all the worm types used in the research showed concentration-dependent anthelmintic effects, with 100 mg/mL producing the shortest P and D times of all worm types. The results of both tables showed that the plant leaves were more active in all the types of worms used. Table 2 indicates that earthworms were the most susceptible to the *C. lancifolius* leaf ethanolic extract. Although P and D of the standard drug were 21 min, 55 min and, 1 min, 2 min respectively, in both organisms, it caused paralysis in 2 min and time of death (D) of 3 min against *P. pasthuma* and paralysis in 1.5 min and time of death (D) of 2.5 min against *F. gigantica*. The two earthworms took longer to die when exposed to aqueous leaf extract. Notable was also the activity of some of the leaf extracts against *F. gigantica*. Against *F. gigantica*, the leaf methanol extracts demonstrated significant anthelmintic properties. At 100 mg/mL, worms were paralysed or died in 1.5-2.5 min, and piperazine citrate did the same in 1-2 min. The lifespan of control worms in distilled water was 5-48 h.

The extracts showed the most incredible sensitivity to the earthworms, particularly when contrasted with the

reference medication, piperazine citrate. It was reported that for both earthworms, paralysis began at 1.5 min, and death happened at 2.5 min at 100 mg/mL. The purpose of most worm expellers, such as piperazine citrate, is to paralyse worms so they can be expelled through animal and human faces. The extracts killed the worms in addition to demonstrating this characteristic, especially at 100 mg/mL. The findings of this investigation, our hypothesis that the polar solvent of ethanol would maximise the production of the desired secondary metabolites for the observed bioactivities. Although the aqueous extracts took significantly longer to induce death, the efficacy of the ethanolic extracts supports their potential as a potent alternative to synthetic drugs. This investigation represents the scientific validation of the efficacy of *C. lancifolius* ethanolic extracts as an alternative to conventional pharmaceutical drugs in both antibacterial and anthelmintic therapeutic agents.

## 5. Conclusion

We examined the *in vitro* antibacterial and anthelmintic properties of leaf extracts from *C. lancifolius Engl.* (Combretaceae). The disc diffusion investigation findings showed that the tested plant extracts considerably slowed the growth of *K. pneumonia* and *S. pneumonia*. Previous research revealed that the high phenolic content of chloroform leaf extracts, which prevented protein denaturation, was responsible for their remarkable antibacterial qualities. This study suggests that *C. lancifolius* could be a good candidate for an antibacterial drug. Ethanolic extracts of *C. lancifolius* showed significant anthelmintic activity; for *F. gigantica*, paralysis began at 2 min, and the D was 3 min; in contrast, P and D for reference drug used as a reference against both organisms were 21, 55 min and 1 min, 2 min, respectively. According to the current study findings, the ethanolic component of the leaf extract from *C. lancifolius* has anthelmintic and antibacterial qualities against the corresponding microorganisms. Subsequent phytochemical studies are necessary to pinpoint the precise bioactive substances in the ethanolic fractions of the *C. lancifolius* extracts of leaves that are accountable for these effects. *C. lancifolius* is a highly viable candidate for the production of innovative and unique plant derived antimicrobial and anthelmintic compounds. Further studies should be conducted to isolate and characterise specific bioactive compounds in ethanolic extracts in order to further understand the mechanisms of action.

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