

Research article

Evaluation of *in vitro* antioxidant, anti-inflammatory and antibacterial activities of chloroform leaf extracts of *Conocarpus lancifolius* Engl

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ABSTRACT

Introduction and Aim: Antibacterial substances are abundant in medicinal herbs, which are used in many extremely effective traditional medical therapies. *Conocarpus lancifolius* Engl. a plant species, has a wide range of therapeutic uses for the handling of haemophilia, inflammation, illness, eye ailments, fever, diabetes and headache. The study's goal was to assess the antibacterial efficacy, *in vitro* antioxidant, anti-inflammatory properties of the solvent-based extract of *C. lancifolius* leaf material.

Methodology: The investigation started in March 2023 and *C. lancifolius* leaves were collected in Tamil Nadu, India. To make the extracts, the maceration technique was employed. The phytochemical constitutions were identified by phytochemical test results. Using the agar diffusion method, the antibacterial capacity of *C. lancifolius* extracted from leaves was evaluated.

Results: At the concentration of 100 microgram/mL level of probability, Gram-positive bacteria including *Staphylococcus aureus* in addition to *Bacillus cereus*, respectively (18 and 21 mm) and Gram-negative microorganisms *Escherichia coli* combined with *Klebsiella pneumoniae* (23 and 20 mm) were considerably inhibited. Additionally, it has the strongest antifungal activity (20 mm) against *Candida albicans*. While the ethanol extract of the leaves significantly inhibited both Gram-negative bacteria like *Escherichia coli* as well as the pneumonia-causing bacteria *Klebsiella pneumoniae* (22 and 19 mm) and Gram-positive bacteria like *Staphylococcus aureus* and the yeast *Bacillus cereus* (18 and 20 mm).

Conclusion: When compared to normal ascorbic acid, which had an antioxidant activity of 87.96 percent, *C. lancifolius* extract with chloroform exhibited a substantial antioxidant activity of 62.96 percent inhibition. When compared to a larger dose of normal diclofenac (84.11 percent), the more potent quantity of *C. lancifolius* extract with chloroform significantly inhibited inflammation by 75.12 percent under similar circumstances. Because there are biologically active compounds in the herbal preparation, the current investigation found that *C. lancifolius* may be a good therapeutic candidate.

Keywords: *In vitro* antioxidant activity DPPH method; *in vitro* antimicrobial activity; anti-inflammatory activity.

INTRODUCTION

The substances that plants create known as secondary metabolites serve a variety of vital functions other than assisting in development. Protection against herbivores and dangerous insects, as well as luring some beneficial insects that aid in pollination, are some of the functions of secondary metabolites. They serve crucial roles in the biological systems from which they are created, but when administered by humans, they also serve as vital medicinal agents. Secondary metabolites are an intriguing and crucial subject for research because of all of these significant characteristics (1-3). *Conocarpus lancifolius* is one of two species found on tropical and subtropical shorelines throughout the world. It is one of the two distinct subspecies in the species group and a member of the *Combretaceae* family. An evergreen tree with a broad crown,

glaucous medium-green leaves, greyish or brown trunk bark, and compact cone-like clusters of greenish blossoms in terminal panicles (4). *C. lancifolius*, sometimes known as buttonwood or button mangrove, grows to a height of 6 meters. The leaves have a glossy look and both surfaces have comparatively less trichomes (5). There is no information available regarding the *C. lancifolius* (Fig.1) used locally in Tamil Nadu's antidiabetic properties. But only a few pharmacological or phytochemical research has been done up to this point. In the current study, antioxidant activity (6, 7) is measured using the DPPH test method, Agar well diffusion was used to measure the antimicrobial effects, and anti-inflammatory activity (8, 9) measured using the protein denaturation inhibition method.



Fig. 1: *C. lancifolius* fruits and leaves

MATERIALS AND METHODS

Collection and extraction

The plant material was gathered in the Tamil Nadu city of Erode surrounds. *Conocarpus lancifolius* Engl. was the name of the plant according to Dr. Mutheeswaran, a scientist at the Xavier Research Foundation in Tirunelveli, Tamil Nadu, India. The *C. lancifolius* leaves were removed off the gathered branches and properly cleaned with regular tap water to get rid of the contaminants. The leaves were then given a second washing with Millipore water and allowed to air dry for three weeks by being laid out on a spotless floor in the shade. The crop material was first processed into a coarse powder and subsequently into a fine powder. This powder was subjected to extraction using the simple maceration method at ambient temperatures with periodic shaking for 48 hours (10). 1 kg of weighted plant fibre was kept in large, tightly-capped carriers having 900 mL of each kind of solvent chloroform, aqueous, ethanol, and methanol - added in order to get the best extraction results. The mixture-containing flask was then immersed in an ultrasonic bath for roughly 30 minutes each day. After the solvent was added, filtering was carried out. The same process was carried out three times to get the maximum extracted volume. The aqueous, methanol, ethanol, and chloroform extracts were gathered in individual sample bottles and given unique codes.

Plant chemical analysis

The method (11, 12) screened for the presence of alkaloids, including glycosides, tannins, flavonoids, anthraquinones, and saponins. Tables 1 and 2 present the findings of this study.

Alkaloids

A small amount (about 5 g) of powdered drug was cooked in weak hydrochloric acid. Adding dilute ammonia solution filtered it and made it alkaline. Five millilitres of chloroform were used to extract this alkalized solution. After that, the chloroform layer

was extracted using 10 mL of dilute acetic acid. The acetic acid extract was given a few droplets of Dragendorff's reagent (the number of alkaloids is denoted by a precipitate of orange or turbidity).

Tannins

Five grams of the substance were extracted with methanol for Braemer's test for tannins. A 10% alcohol solution of ferric chloride was added to methanolic extract. A high level of tannin in the medication is indicated by the solution's intense blue or bluish grey colouring.

Saponins

In order to detect the presence of saponins, 5 g of dried drug powder was vigorously shaken with water. A 20-minute-long persistent froth is a sign that saponins are present. Borntrager's testing for free anthraquinones involved extracting 5 g of powdered medication using hot water and filtering it while it was still hot. It was then extracted using carbon tetrachloride after cooling. The layer composed of CCl_4 was separated, rinsed with water, and then shaken with diluted ammonia solution (the concentration of free anthraquinones is indicated by the ammonia layer's pink to cherry-red colour).

Borntrager's assay for bound anthraquinones with modifications

Hydrochloric acid and ferric chloride solution were used to extract 5 g of drug powder. It was cooked in a water bath for ten minutes before being filtered. It was extracted using carbon tetrachloride after cooling. After being separated, the carbon tetrachloride was rinsed under water and shaken with diluted ammonia solution colour (strong pink to cherry-red colouring of ammonia layer indicates an existence of anthraquinones glycosides).

Shinoda test for flavonoids

Methanol was used to extract 5 g of the substance. A single part of the magnesium band, 1 ml of strong HCl, and the methanol extract were combined. The quantity of flavonoid in the medication is indicated by the pink to crimson or red colouring of the solution.

Studies on the phytochemistry of the chloroform concentrate of *C. lancifolius*

Antimicrobial assay

In vitro tests were done to determine whether the unprocessed extracts for the *Conocarpus lancifolius* leaf beneath research had any antibacterial properties. Utilizing Mueller Hinton Agar (MHA) solution aimed at the bactericidal analysis and Sabouraud Dextrose agar containing (SDA) media for the fungicidal assay, the initial screening was carried out utilizing the agar well dissemination technique (13).

Table 1: Components in *C. lancifolius* leaf extracts

Constituents	Chloroform extract	Methanolic extract	Aqueous extract	Ethanolic extract
Alkaloids	+++	+++	+++	+++
Anthraquinones free	+++	+++	+++	++++
Anthraquinones bound	+++	-	-	-
Saponins	-	+++	+++	+++
Flavonoids	-	+++	+++	+++
Tannins	-	+	+++	++++

+++ fully presented; + partially presented

Table 2: Quantification of *C. lancifolius* leaf extracts

Weight of powdered leaves (g)	Chloroform Extract (g)	Methanolic Extract (g)	Aqueous Extract (g)	Ethanolic Extract (g)
150	1.23	1.85	1.91	1.89

Melted agar was combined with a microbial inoculum (50 L) containing 1 10⁶ CFU/mL, which was then placed into 15 cm plates and given time to set. The rivers (6 mm in diameter) were made using a cork borer. The test solution for the sample was added to each well after the extract made from chloroform had been disintegrating in DMSO at 100 g/mL concentrations. The plates remained then raised for 24 hours at 37°C. After subtracting the activity of DMSO, the circumferences of the area of growth zones of inhibition were measured in millimetres, and the findings were calculated. The standard antibacterial medicine used was ampicillin (Sigma-Aldrich, St. Louis, MO, US), while the standard antifungal drug used was amphotericin B (Sigma-A St. Louis, MO, US).

Antioxidant assay

DPPH radical scavenging activity method

The molecular structure is determined by the spare delocalization of the electron over the entire molecule 1, 1-diphenyl-2-picrylhydrazyl (also known as, α , α -diphenyl-bicrylhydrazyl; DPPH) is a radical that is stable. Therefore, unlike most other free radicals (14,15), the molecule in question does not dimerize. Electron delocalization, which gives all extract solutions an absorption band with a center wavelength of 520 nm, is what gives the deep violet color. A DPPH solution forms the reduced form and loses its violet color when mixed with another substance (AH) that can donate a hydrogen atom. In a 96-well plate, 100 μ l of 1000 g/ml herbal extract and 100 μ l of 0.2 mmol/L methanol dissolved DPPH were combined. In the standard, vitamin C (1000 g/ml in 100 μ l) was utilized. Thirty minutes of fermentation at 37°C produced the desired optical density, which was restrained. Using the following formula, the proportion of activity associated with scavenging was determined.

% Inhibition= Absorbance of control-Absorbance of sample X 100/Absorbance of control

Anti-inflammatory assay

Inhibition of protein denaturation method (16)

The reaction mixture (5 ml) included 0.2 ml of fresh hen's egg albumin, 2.8 ml of a solution of phosphate-buffered saline (PBS), and 0.8 ml of the mixture in addition to 2 ml of various solvents extraction quantities of *C. Lancifolius*, which produced the ultimate concentrations of 50, 100, 200, and 400 g/ml. The same amount of water that had been double distilled was utilised as a control. Upon 15 minutes of incubating at 370°C in the BOD incubators (Labline Technologies), the mixtures were subjected to heating for 5 minutes at 700 °C. Using a vehicle as a blank, their ability to absorb was evaluated at 660 nm (SHIMADZU, UV 1800) after cooling, and the Ostwald viscometer was used to evaluate their viscosity. For the purpose of determining absorbance and viscosity, diclofenac sodium was processed similarly and utilized as a reference medication at final concentrations of (100 and 200 g/ml). The percentage inhibition of the denaturation of proteins was calculated using the formula below:

% inhibition = 100 x (Vt/Vc-1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

RESULTS

Conocarpus lancifolius leaves were used for the extraction of all of the extracts. To categorize the phytochemical components of the species into two main groups with different chemical compounds and biological characteristics, the total extracts were next fractionated using a nonpolar and a highly polar solution. The results are displayed as the inhibitory zone diameters, IZD (mm), in Table 3 and Fig. 2 (represented by a bar diagram).

C. lancifolius leaf extract was tested for its *in vitro* antioxidant activity, and the findings are shown in Table 4 and Fig. 3. In contrast to standard ascorbic acid's 87.96% antioxidant activity, chloroform extract showed a significant oxidative activity of 62.96% suppression of free radicals. Recent research on the *in vitro* anti-inflammatory effects of denaturing egg

albumin against *C. lancifolius* leaf extracts was conducted, and Table 5 summarizes the results. Chloroform extract revealed a significant anti-inflammatory effect of 75.12% less egg albumin breakdown as compared to other solvent extracts. All

of the information was compared to standard Diclofenac, which prevented protein denaturation by 77.10% and 84.11%, respectively, at 100 and 200 g/ml.

Table 3: Different *C. lancifolius* leaf extracts were tested for their antibacterial properties *in vitro* (Inhibition Zone Diameters, IZD)

Extract	Inhibition zone diameter (mm)*				
	Gram (-) bacteria		Gram (+) bacteria		Fungi
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>C. albicans</i>
DMSO	-	-	-	-	-
Ampicillin	26+0.346	16+0.346	23+0.520	25+0.305	#nt
Amphotericin B	#nt	#nt	#nt	#nt	21+0.531
Chloroform	23+0.305	20+0.115	18+0.115	21+0.346	20+0.480
Aqueous	21+0.529	19+0.230	17+0.2	19+0.115	17+0.305
Methanol	20+0.230	18+0.115	16+0.305	18+0.230	16+0.346
Ethanol	22+0.305	19+0.230	18+0.115	20+0.305	19+0.200

*Results are computed after DMSO activity is subtracted. # nt: not tried

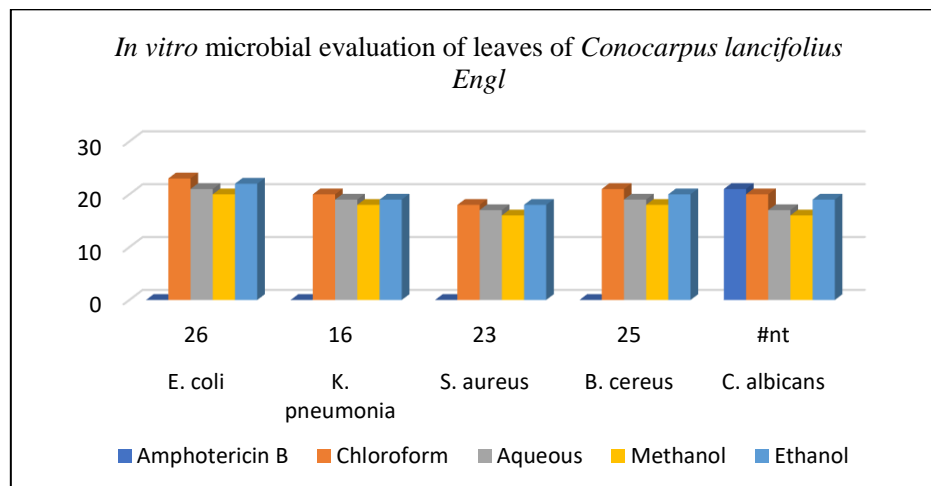


Fig. 2: *Conocarpus lancifolius* leaves recovered in chloroform and *in vitro* microbial assessment

Table 4: DPPH Method (100 mg/mL) *in vitro* antioxidant efficacy of *C. lancifolius* leaf extracts

S. No.	Extract(s)	nm	COD	SOD	% Inhibition	Average
01	Alcohol	520	0.36	0.13	63.89	56.48 %
			0.36	0.14	61.11	
			0.36	0.20	44.44	
02	Chloroform	520	0.36	0.13	63.89	62.96 %
			0.36	0.13	63.89	
			0.36	0.14	61.11	
03	Ethanol	520	0.36	0.14	61.11	59.25 %
			0.36	0.15	58.33	
			0.36	0.15	58.33	
04	Methanol	520	0.36	0.12	66.67	61.11 %
			0.36	0.14	61.11	
			0.36	0.16	55.56	
05	Ascorbic acid (Standard)	520	0.36	0.04	88.89	87.96 %
			0.36	0.05	86.11	
			0.36	0.04	88.89	

* SOD means standard optical density; COD means Control optical density

Antioxidant capacity is monitored by the DPPH method expressed by mg equivalent to ascorbic acid /g extract

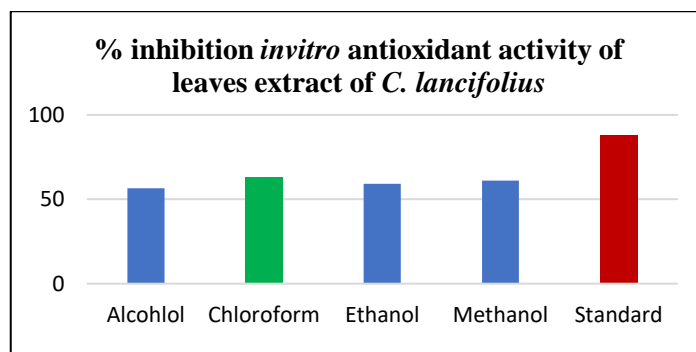


Fig. 3: DPPH method *in vitro* testing of the antioxidant capacity of *C. lancifolius* leaf extracts

Table 5: Different *C. lancifolius* extracts *in vitro* anti-inflammatory effects on the prevention of protein denaturation

S.No	Group	Concentration (µg/ml)	% Inhibition of denaturation	Viscosity (cp)
1	Control	--	--	1.41
2	Chloroform	50	26.20	0.39
		100	45.10	0.42
		200	59.19	0.61
		400	75.12	0.89
3	Aqueous	50	23.21	0.29
		100	40.72	0.35
		200	55.12	0.46
		400	70.21	0.59
4	Methanol	50	17.01	0.21
		100	28.12	0.30
		200	39.19	0.41
		400	47.25	0.50
5	Ethanol	50	24.15	0.31
		100	41.11	0.40
		200	56.12	0.51
		400	73.10	0.69
6	Diclofenac	100	77.10	0.85
		200	84.11	1.18

DISCUSSION

The antimicrobial efficacy of the various *Conocarpus lancifolius* extracts was assessed. In general, the leaf extract fractions in chloroform had the strongest antibacterial and antifungal effects. The most vulnerable organisms to the chloroform extract were the Gram-negative microbes *E. coli* and *K. pneumonia* (with IZDs that of 23 and 20 mm, respectively), as well as the contagious yeast-like fungi *C. albicans* (20 mm). The ethanol extract, on the other hand, demonstrated the second-highest IZD against bacteria such as *S. aureus* and *B. cereus* in comparison (18 and 20 mm, correspondingly). The methanolic extract demonstrated the least IZD (20, 18 & 16, 18 mm and 16 mm) against fungi and bacteria alike. The more moderate IZD towards the two species. (21,19 & 17, 19 mm & 17 mm) was demonstrated by the aqueous leaf extract. Phenolic chemicals have strong antibacterial action additionally to their well-known antioxidant effects (17,18). Therefore, the high polyphenolic content of the plant leaves can be credited with the exceptional antibacterial activity of the chloroform fractions.

Using ascorbic acid as a reference, the DPPH technique was utilized to evaluate the extracted form of chloroform of *C. lancifolius* leaf for *in vitro* antioxidant activities. When compared to the antioxidant activity of conventional ascorbic acid, which was 87.96%, chloroform extract demonstrated a substantial oxidative activity of 62.96% suppression of free radicals (Table 4 & Fig. 3).

In the current investigation, it was determined whether *C. lancifolius* received an *in vitro* anti-inflammatory effect on denaturing egg albumin. Table 5 provides a summary of the findings. By preventing protein (albumin) denaturation, *C. lancifolius in vitro* anti-inflammatory effect was demonstrated. Significant anti-inflammatory activity was demonstrated by *C. lancifolius* in a concentration-dependent manner. When compared to other solvent extracts, chloroform extract demonstrated a considerable anti-inflammatory effect of 75.12% reduction of decomposition of egg albumin. All of the data were compared to regular Diclofenac at 100 and 200 g/ml, which inhibited protein denaturation by 77.10% and 84.11%, respectively.

CONCLUSION

We looked at the in vitro antibacterial, antioxidant, and anti-inflammatory properties of *Conocarpus lancifolius* Engl. (*Combretaceae*) leaf extracts. According to the results of the agar well diffusion investigation, the tested plant extracts significantly inhibited the growth of both Gram positive (*S. aureus* and *B. cereus*) and Gram negative (*E. coli* and *K. pneumonia*) bacteria. The pathogenic yeast *C. albicans* was likewise resistant to their antifungal activities. This study showed that the extraordinary antibacterial properties of chloroform leaf extracts were due to high phenolic contents and that chloroform extract anti-inflammatory properties inhibited the protein denaturation process. *C. lancifolius* may be a good option for an antibacterial medication, according to this study. *C. lancifolius* extracts containing chloroform demonstrated considerable antioxidant activity, with an average % inhibition of 62.96% compared to 87.96% for ascorbic acid standard. The results of the current investigation showed that the chloroform ingredient in the *C. lancifolius* leaf extract has antibacterial and antifungal properties against both Gram positive and Gram negative microorganisms. Future phytochemical investigations are required to identify the specific bioactive compounds in both *C. lancifolius* leaf extracts of chloroform fractions that are responsible for these actions.

CONFLICT OF INTEREST

There are no potential conflicts of interest throughout the course of this inquiry, according to the authors.

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