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Anti-Microbial Activity of Crude Extract from *Neolamarckia Cadamba(L)* Seed Against Multi-Drug Resistance Bacteria

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KEYWORDS

Di(2-Ethylhexyl) Phthalate, Muller-Hinton agar medium, Nutrient Agar, Antimicrobial resistance, zone of inhibition, minimum inhibitory concentration

Abstract

Introduction:
Neolamarckia cadamba has been widely studied for its various parts; however, research on its seeds remains limited. This study explores a novel approach to seed extraction and evaluates the bioactive potential of the seed extract.

Objective:
To extract and characterize bioactive compounds from *Neolamarckia cadamba* seeds and to assess their antibacterial activity against multidrug-resistant pathogens.

Methods:
Mature fruits were collected, processed, and decomposed over seven days to obtain seeds. The dried seeds were cleaned, ground, and subjected to continuous hot percolation using ethanolic extraction in a Soxhlet apparatus for 24–48 hours. The extracts were analyzed using Gas Chromatography–Mass Spectrometry (GC-MS), Fourier-Transform Infrared Spectroscopy (FT-IR), and UV–VIS spectrophotometry. Antibacterial activity was evaluated using the agar well diffusion method against Escherichia coli and Staphylococcus aureus.

Results:
The extracts contained bioactive compounds such as alkaloids, carbohydrates, tannins, and flavonoids. GC-MS analysis revealed a significant presence of Di(2-ethylhexyl) phthalate (DEHP), which was further confirmed by FT-IR analysis. UV–VIS spectrophotometry showed absorbance peaks between 260–280 nm, indicating polyphenolic compounds. The ethanolic extracts exhibited notable antibacterial activity, with significant inhibition zones at concentrations of 100 and 200 µg/mL and a minimum inhibitory concentration (MIC) ranging from 20 to 25 µg/mL.

Conclusion:
The study demonstrates that *Neolamarckia cadamba* seed extract is a promising source of bioactive compounds, particularly DEHP, with effective antibacterial activity against multidrug-resistant bacteria, suggesting its potential for pharmaceutical applications.

1. INTRODUCTION

The well-known medicinal tree Neolamarckia cadamba, also referred to as Kadam, is a member of the Rubiaceae family. It is primarily found in South Asia, and it is also found in Nepal, Myanmar, Western China, and the temperate Himalayan regions of Garhwal, Assam, Himachal Pradesh, Manipur and Sikkim.[1]. With a straight trunk and a U-shaped canopy, the tree has impressive growth characteristics, reaching heights of about 45 meters. N. cadamba's rapid growth period it reaches maturity in 6–8 years, and flowering starts after 4–5 years of development is a key characteristic [2]. Although the plant is very adaptive to a variety of soil types, it grows best in nutrient-dense soil and is especially hampered in inadequately aerated conditions. N. cadamba is utilized in the pulp, paper, and lumber industries in addition to its therapeutic uses [3].

The fruit of N. cadamba exhibits interesting morphological characteristics, resembling tiny, meaty capsules that cluster densely to produce a vivid yellow-orange infructescence. Each fruitlet has four solid or hollow structures in its upper portion,

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and each cluster of fruits contains about 8000 seeds. [4]. Crucially, the ripe fruits can be consumed uncooked; the seeds are triangular or irregular in shape and lack wings. [5]. From a medicinal perspective, *N. cadamba* has attracted considerable attention in traditional systems of medicine, particularly Ayurveda, as various parts of the plant are valued for their distinct therapeutic properties. Bark extract used orally has demonstrated effectiveness in treating fever, ocular irritation, and cough. Dried stem bark has also been shown to be beneficial for treating uterine problems, anemia, and a variety of skin diseases. [6].

Cadamba is very well-liked by traditional healers, and practically every part of the plant has therapeutic properties and is used to cure conditions including diabetes, fever, anaemia, and tumours, as well as to improve the quality of semen. The biological actions that have already been documented include the hypolipidemic (root), antidiarrheal (flowering top), analgesic (stem bark, leaf), and antidiabetic (stem bark) qualities .fruit (antidiabetes) [7].

Neolamarckia cadamba fruits are known to contain a variety of bioactive secondary metabolites such as flavonoids, tannins, and phenolic compounds, which are widely associated with antimicrobial properties. Preliminary studies indicate that these fruit extracts possess substantial amounts of such phytoconstituents, contributing to their antibacterial efficacy. Plant-derived phenolics are well documented for their ability to inhibit microbial growth through multiple mechanisms, including disruption of cell membranes and enzyme inhibition [8].

However, there is currently no published scientific information about the seed's. Even so, research on fruits has been conducted thus far, and no research has been found on seeds alone. The objective of this study is to evaluate the antimicrobial properties of N. cadamba seed extracts against particular pathogenic microorganisms and perform a comprehensive phytochemical analysis. This study aims to explore potential new applications in modern medicine and provide scientific evidence for current ones.

2. Objectives

The present study aims to systematically investigate the largely unexplored seeds of Neolamarckia cadamba, for which there is currently no well-documented scientific evidence, despite extensive research on other plant parts such as fruits, bark, and leaves. The primary objective is to evaluate the antimicrobial potential of *N. cadamba* seed extracts against selected pathogenic microorganisms, particularly multidrug-resistant strains such as Escherichia coli and Staphylococcus aureus.

In addition, this study aims to conduct a comprehensive phytochemical screening and advanced analytical characterization of the seed extracts to identify and profile bioactive constituents, including alkaloids, flavonoids, tannins, and other secondary metabolites. The research also seeks to correlate the identified phytochemicals with observed biological activities, thereby elucidating possible mechanisms underlying antimicrobial effects.

Furthermore, the study intends to: Standardize the extraction process and optimize conditions for maximum yield of bioactive compounds. Evaluate the minimum inhibitory concentration (MIC) and dose-dependent efficacy of the extracts. Compare the antimicrobial effectiveness of seed extracts with findings reported for other parts of the plant. Assess the potential of isolated compounds for future pharmacological and therapeutic applications. Ultimately, this research aims to generate novel scientific data on *N. cadamba* seeds, validate their medicinal relevance, and contribute to the discovery of new plant-based antimicrobial agents, thereby supporting their integration into modern pharmaceutical development

3. Methods

3.1 Gathering and Identifying Plant Material

Fruits of Neolamarckia cadamba were colleted during the first week of October 2024, and their authenticity was confirmed by the Siddha Central Research Institute, Anna Government Hospital Campus, Arumbakkam, Chennai (Central Council for Research in Siddha; Authentication Certificate No. 1442.27102502). The fruits were then cut into small pieces and allowed to dry. The dried pieces were subsequently crushed, and the seeds were separated after a few days. Approximately 23,000–25,000 seeds per gram were obtained. The separated seeds were further dried in the shade and then subjected to an extraction-like process. [9]

3.2 Collection and Process of Plant Material

Mature and healthy fruits were carefully selected. The fruits were cut into small pieces dried under open sunlight for 7 days, carefully removed the seed and shade dried at room temperature for 3 days at (25 ± 2 °C) until a constant weight was attained [10].

3.4 Crude Extraction by Soxhlet Extraction method:

The above dried seeds of 20gms were crushed using a mortar and pestle as mentioned in (fig .1), the dried seeds crushed in order to expand the surface area. For the Soxhlet method of ethanolic extraction. The thimble wattmann filter paper No.1 should be sufficiently filled with powdered seed . In our studies, we utilize a 25x80mm thimble (wattmann filter paper with an average of 15g NC seed powder. A round-bottom flask was then charged with 250 mL of ethanol as the solvent. A Soxhlet extractor and condenser on a mantle (Fig. 2). A Soxhlet extractor is placed inside a thimble that contains the crushed seed material. Glass wool was used to line the side arm. After being heated, the solvent begins to evaporate and travels through the device to the condenser. After the procedure is completed, a rotary evaporator used to remove the ethanol,

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leaving 2-3 milliliters of the seed powder that was extracted in the glass-bottom flask. [11]

3.5 ANALYTICAL METHODS OF *NEOLAMARCKIA CADAMBA* SEED

3.5.1 *Preliminary Phytochemical Screening*

Phytochemical screening was carried out using standard procedures to identify the presence of various bioactive compounds. Flavonoids were detected by the alkaline reagent test using 10% NaOH and dilute HCl; the formation of a yellow color that disappears upon addition of dilute HCl indicates a positive result (Harborne, 1998).[12] Carbohydrates were identified by Molisch's test, where the addition of Molisch reagent followed by concentrated H_2SO_4 forms a violet ring at the interface, confirming their presence (Trease & Evans, 2009).[13]

Proteins were tested using the Ninhydrin test; heating the extract with Ninhydrin reagent produces a blue or violet color, indicating a positive result (Edeoga et al., 2005)[14]. Anthraquinone glycosides were detected by Borntrager's test, in which hydrolyzed extract is treated with chloroform and ammonia, producing a pink to red color.

Alkaloids were identified using Mayer's and Hager's tests. In Mayer's test, the addition of Mayer's reagent to an acidic extract results in a cream precipitate, while Hager's test produces a yellow precipitate upon addition of picric acid (Harborne, 1998)[12]. Tannins were detected using ferric chloride and lead acetate tests; the formation of a blue-black or green color with 5% $FeCl_3$ and a white precipitate with lead acetate confirm their presence (Edeoga et al., 2005).[14]

Terpenoids were identified by the Salkowski test, where the addition of concentrated H_2SO_4 to the chloroform extract produces a reddish-brown interface, indicating a positive result (Harborne, 1998).[12]

3.5.2 Gas Chromatography Mass Spectrometry (Gc-Ms) Analysis

GC–MS profiling of the crude ethanolic seed extract of *Neolamarckia cadamba* was carried out using a PerkinElmer Clarus 600 gas chromatograph coupled with a Clarus 600 mass spectrometer at Bishop Heber College, Tiruchirappalli. Separation was achieved on an Rtx-5MS fused silica capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness), capable of operating up to 350 °C. Helium (99.99% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The temperatures of the injector, ion source, and transfer line were maintained at 290 °C. Electron impact ionization was performed at 70 eV, and the electron multiplier voltage was regulated through auto-tuning mode. The oven temperature was initially set at 60 °C (held for 2 min) and then increased gradually to 280 °C at a rate of 3 °C/min.

Prior to analysis, the crude extract was diluted (1:100, v/v) with a suitable solvent, and 1 µL of the particle-free solution was injected in split mode with a split ratio of 30:1. Mass spectra were recorded in full-scan mode over a mass range of 40–550 amu. The relative percentage of each component was calculated based on peak area normalization. Identification of compounds was carried out by comparing retention times and mass spectral fragmentation patterns with those stored in standard mass spectral libraries using computerized matching [15,16]

3.5.3 UV-Vis Spectrophotometer Analysis

For proximate evaluation, the optical properties of the extracts were examined using UV–Visible spectroscopy across both ultraviolet and visible regions. Prior to analysis, the samples were centrifuged at 3000 rpm for 10 min to remove particulate matter, followed by filtration through Whatman filter paper (No. 1) under vacuum using a high-pressure filtration system. The clarified extracts were subsequently diluted with the corresponding solvent at a ratio of 1:10 (v/v).Spectral measurements were performed using a PerkinElmer UV–Visible spectrophotometer, scanning across a wavelength range of 200–600 nm. The resulting spectra were recorded, and distinct absorption maxima corresponding to the chemical constituents of the extracts were identified [17],[18].

3.5.4 FT-IR Spectrophotometer Analysis

The most effective method for recognizing functional groups and figuring out whether a substance contains chemical bonds is FTIR . Functional groups containing X-H or C-X type bonds, such as O-H, C≡N, C≡C, C=C, C-H, N-H, C-C, and C=O, can be identified by IR spectra analysis. Samples were collected in clean bottles, and the dried extracts obtained using different solvents were subjected to FTIR was investigated in the ranges of 400–4000 cm^{-1}. FTIR analysis and peak values of several functional groups derived from Neolamarckia cadamba seed ethanolic extract. FTIR analysis was performed using dried extracts of various solvents. The BRUKER Model ALPHA FTIR spectrophotometer, which has a scan range of 400 - 4000 cm^{1}, was used to load the sample of each solvent extract. [19,20].

3.6 *IN-VITRO* METHODS

3.6.1 *In-vitro* antibacterial activity

The antimicrobial activity of solvent extracts produced from plant leaf samples was evaluated for in vitro antibacterial research. Bacterial pathogens obtained from the Institute of Microbial Technology (IMTech) in Chandigarh served as test organisms for this investigation. The lyophilized cells were revived by adding the strains to the nutritious broth. After being grown on Nutrient Agar (NA) slants, the bacterial pathogens were stored at 4°C. List of Bacterial pathogens used in the present study.[21]

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Table 1: Test organisms used for Anti-bacterial Assay

S. No.	Bacterial strains
1	*Staphylococcus aureus* MTCC 87
2	*Escherichia coli MTCC 452*

3.6.2 *Preparation of bacterial Inoculum*

The investigation's active young cultures were created by subculturing a loopful of cells in nutrient broth and then incubating them for 24 hours (37°C) C. After 24 h of incubation, the cultures were diluted in sterile nutrient broth and standardized to match the 0.5 McFarland turbidity, equivalent to ~1 × 10^6 CFU/mL [22].

3.6.3 *Agar wells method*

The standardized bacterial inoculum was evenly spread over Mueller–Hinton agar plates, after which 6-mm wells were aseptically created at uniformly spaced locations using a sterile cork borer. Varying concentrations of the crude extracts obtained from antagonistic bacteria (25, 50, 75, and 100 µg) were dispensed into the respective wells, while ciprofloxacin (20 µg) served as the positive control.

The agar plates containing the inoculated bacterial cultures were maintained at 37 °C for a 24-hour incubation period. After incubation, antibacterial efficacy was assessed by examining the plates for the formation of distinct clear zones around the wells, indicating suppression of bacterial growth. The diameter of each inhibition zone was measured in millimeters at three different orientations, and the mean value was calculated to quantify the antibacterial effect. Three duplicates of each experiment were carried out. [23,24]

3.6.4 Determination of Minimum Inhibitory Concentration (MIC)

A two-fold serial dilution procedure (Ericsson and Sherriel, 1971) using MH Broth in 96-well microtiter plates was used to estimate the MIC. In short, one hundred microliters of Mueller-Hinton broth (Himedia) was made. To get dilutions of the active extract ranging from 25 to 200 µg, several concentrated extracts were made and put into 96-well plates. Following the addition of 10 µL of the test organisms to the well (resulting in a final cell density of 1 x 106 CFU/mL), the plates were incubated at 37 °C (24 hours). The lowest extract concentration required to regulate the organisms' apparent development was known as the MIC.[25]

4. RESULTS

4.1 Preliminary Phytochemical Screening

Several important types of secondary metabolites, including flavonoids, carbohydrates, tannins, alkaloids, and others, were detected by preliminary phytochemical investigation. It was discovered that the ethanolic extract lacked both protein and anthraquinone glycosides.

Phytochemicals	Test used	Ethanolic extract
Flavonoids	Ammonia, alkaline reagent	+
Carbohydrates	Molisch's	+
Protein	Ninhydrin	-
Anthraquinone Glycosides	Nitroprusside	-
Alkaloids	Hager's, Meyer's	+
Tannins	FeCl3, lead acetate	+
Terpenoids	Salkowski (modified)	+

Table 2: The ethanolic extract's initial phytochemical screening results.

Peak	RETENTION TIME	AREA %	COMPOUND	Molecular formula	MEDICINAL USES
1	13.852	2.25	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	Skin Conditioning Agent/Emollient
2	17.458	1.38			

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			1,3-Diphenyl-1-((Trimethylsilyl)Oxy)-1(Z)-Heptene	$C_{22}H_{30}OSi$	Antioxidative, Antimicrobial, or Anticancer activities
3	20.692	0.82	Tri-O-Trimethylsilyl, N-Pentafluoropropionyl Derivative Of Terbutaline	$C_{23}H_{42}F_3No_4Si3$	selective β_2-adrenergic receptor agonist
4	34.743	64.44	Bis(2-Ethylhexyl) Phthalate	$C_6H_4(Co_2C_8H_{17})$	Antimicrobial, or Anticancer activities
5	37.675	0.21	Propanoic acid, 2,2-dimethyl-, cesium salt	Cesium pivalate $C_5H_9CSO_2$	certain oral β-lactam antibiotics
6	37.783	24.63	13-Docosenamide, (Z)	Erucamide $C_{22}H_{43}NO$	Antimicrobial Activity, Antifungal Activity
7	38.826	0.11	Acetamide, N-(Acetyloxy)-N-[2-Chloro-3-Nitro-5-(Trifluoromethyl)Phenyl]	$C_{11}H_8CLF_3N_2O_5$	antifungal and anti-inflammatory properties.
8	38.985	0.16	(3.Alpha.,3a.Beta.,7a.Beta.)-(+-)-3-[(1,2-Dihydro-1-Methoxy-2-Oxo-3h-Indol-3-Ylidene)Methyl]Hexahydro-6-Oxopyrano[3,4-C]Pyrrole	$C_{19}H_{22}N_2O_6$	Antioxidant Activity, Anti-inflammatory Potential
9	39.295	1.21	Dimethyl Bis(3-Iodopropyl)Malonate	$C_{11}H_{18}I_2O_4$	Drug Discovery Intermediate
10	39.449	1.85	Dimethyl Bis(3-Iodopropyl)Malonate	$C_{11}H_{18}I_2O_4$	Drug Discovery Intermediate
11	39.849	0.6	Pregn-5-En-3-Ol, 20-Iodo-, Acetate, (3.Beta.,20r)-	$C_{23}H_{33}IO_2$	Steroid Intermediate Derivative of Pregnenolone Acetate
12	40.161	0.51	5,11[1',2']-Benzeno-5h-Cyclohepta[B]Naphthalene, 5a,11-Dihydro-5-Methyl	$C_{21}H_6$	Anticancer Activities, Neuroprotective or Psychotropic Potentials

Table 3: Chemical Composition of *Neolamarckia cadamba* Extracts Determined by GC–MS

4.2 ***Characterization of Extracts by GC–MS***

GC–MS profiling of the ethanolic crude extract revealed fifteen well-resolved chromatographic peaks, distinguished by their individual retention times on a fused silica capillary column. The detected constituents predominantly belonged to chemical classes such as alcohols, hydrocarbons, ketones and esters. Table 3 represents details of chemical which exhibits important biological activities including anti-microbial activity.

The GC-MS analysis of the ethanolic extract of N.lambara camber seed was found to contain approximately 12 variable compounds. Among that 12 chromatogram the two peaks with an area percentage of 64.44% and 24.63% are Bis(2-Ethylhexyl) Phthalate and 13-Docosenamide . In comparison to other compounds, it has the largest area percentage value. Some compound shown as least area percentage of 1.3 are 1,3-Diphenyl-1-((Trimethylsilyl)Oxy)-1(Z)-Heptene, (3.Alpha.,3a.Beta.,7a.Beta.)-(+-)-3-[(1,2-Dihydro-1-Methoxy-2-Oxo-3h-Indol-3-Ylidene)Methyl]Hexahydro-6-Oxopyrano[3,4-C]Pyrrole , Propanoic acid, 2,2-dimethyl-, cesium salt had a area percentage arpound 1.38 ,0.16,0.2.

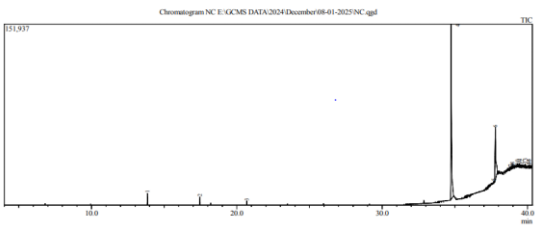

Figure 5: GC-MS chromatogram of ethanolic extract of *Neolamarckia cadamba seed*

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4.3 *FT-IR (Fourier-Transform Infrared Spectroscopy) Analysis of N. cadamba Seed Extract*

FTIR analysis and peak values of several functional groups derived from *Neolamarckia cadamba* seed ethanolic extract. While samples were gathered in bottles, FTIR analysis was performed using dried extracts of various solvents. 1520cm -1 is the peak corresponds to strong nitro

Type of solvent	Peak value cm-2	Functional Groups
Ethanolic Extract of *Neolamarckia cadamba seed*	1073.5	C-O stretching, primary alcohol, strong
	1326.9	S=O stretching, sulfone, strong
	1446.2	C-H bending, alkane, medium
	1520.8	nitro compound, N-O stretching, strong
	1610.2	α,β-unsaturated ketone, C=C stretching, strong
	2079.9	N=C=S, stretching, isothiocyanate, strong
	2855.1	N-H stretching , strong, broad, amine salt
	3227.9	alcohol strong, broad, O-H stretching
	2922.2	C-H stretching, alkane, medium

Table 4: FTIR analysis and peak values of several functional groups derived from Neolamarckia cadamba seed ethanolic extract.

compound, N-O stretching, with a peak of 1610cm-1.Strong, C=C stretching, α,β-unsaturated ketone, peak 2079, 2 cm-2.9 N=C=S, isothiocyanate, strong, stretched, peak 2855.Strong, broad, amine salt, peak 2922, 1 cm-2 for N-H stretching.2 cm-2 for medium, alkane, C-H stretching, 3227 is a summit.9 cm-2 O-H stretching, broad confirmed, powerful alcohol. damba seed extract. The existence of flavonoids, proteins, fats, and carbohydrates as significant functional groups was demonstrated by FTIR spectra analysis. Functional groups for confirming the presence of both polyphenols and chemicals associated to red phthalate were revealed by FTIR analysis of the plant extract. Polyphenolic structures are established by the wide O–H stretch at 3227.9 cm^{-1}, the C–O stretch at 1073.5 cm^{-1}, and the C=C stretch at 1610.2 cm^{-1}. Phthalates are also established by aromatic C-H bending and alkyl C-H stretches at 2855.1 and 2922.2 cm^{-1}. These substances are in charge of the extract's purported bioactivity, which includes possible endocrine-related and antioxidant properties.

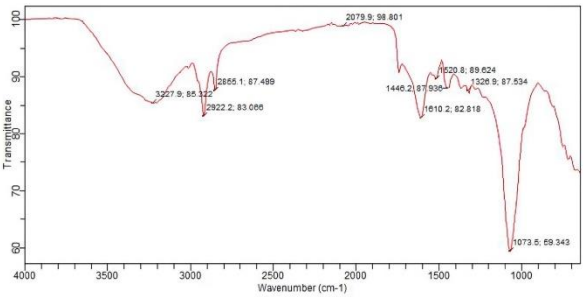

Figure 6: FTIR Spectrum of ethanolic extract of seed of *Neolamarckia cadamba.*

4.5 UV-VIS Spectrophotometer Analysis of Neolamarckia Cadamba Seeds

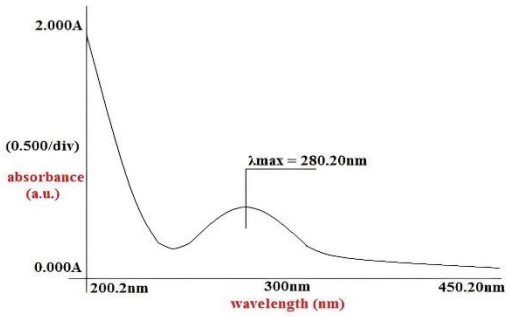

Figure 7: UV-VIS Spectrophotometer shows their absorbance maxima at 280.20nm for crude ethanolic extract of *Neolamarckia cadamba* seeds.

The UV–Visible spectroscopic analysis of the ethanolic extract of *Neolamarckia cadamba* seeds revealed a characteristic absorption profile in the range of 200–450 nm. The spectrum exhibited a prominent absorption maximum (λmax) at 280.20 nm, which is typically associated with the presence of phenolic compounds and flavonoids. These compounds contain aromatic rings and conjugated double bonds that strongly absorb in the UV region, particularly around 270–290 nm. phthalate esters like Bis(2-ethylhexyl) phthalate generally exhibit absorption in the **UV region around 270–280 nm**, due to π→π* transitions of the aromatic benzene ring. This correlates well with observed **λmax at 280.20 nm**, suggesting that this compound may contribute to the major absorption peak in the spectrum.

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4.6 Anti-bacterial activity of crude extracts of Neolamarckia Cadamba seeds

On MHA plates, the bactericidal activity of an all-solvent crude extract of Neolamarckia Cadamba seeds was evaluated against a variety of bacterial pathogens. The zone of inhibition varied significantly depending on the concentration used, and the crude extract from Neolamarckia Cadamba seeds demonstrated the highest growth inhibitory effect against all tested bacterial pathogens. Escherichia coli (15.23±0.06 mm) and Staphylococcus aureus (16.08±0.76 mm) in 100 µg were both successfully suppressed by the methanol crude extract.

Between 17 and 19 mm was the zone of inhibition for the common antibiotic Ciprofloxacin. The lowest MIC values were 25.0±1.2 µg/ml for Escherichia coli and Streptococcus aureus. The range of the MIC values was 25–200 µg/ml. (Table 5).

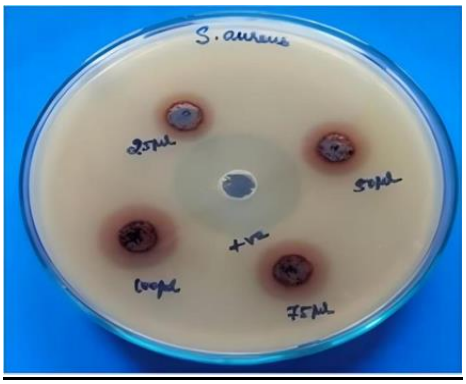
The antibacterial potential of Neolamarckia cadamba seed extract against bacteria that are resistant to many drugs is demonstrated by the statistical analysis and the data interpreted from the antimicrobial assay. Gram +ve as well as Gram -ve. When compared to the standard medication Ciprofloxacin, bacteria exhibit superior Zone of Inhibition (ZOI) values. Additionally, it has better results for both S. aureus and E. coli species, with a MIC value of 25.0 ± 1.2 µg/ml.

Test bacterial pathogens	Zone of inhibition (mm)					MIC (µg /ml)
	25 µg	50 µg	75 µg	100 µg	ciprofloxacin (20 µg)	
S. aureus	08.34±0.21	10.34±0.82	12.84±0.19	16.08±0.76	19.28±0.72	25.0±1.2
E. coli	08.02±0.34	10.42±0.54	13.42±0.44	15.23±0.06	17.25±0.44	25.0±1.2

Table 5: Anti-microbial activity of crude extract of *Neolamarckia Cadamba* seed against Multi Drug Resistant bacteria.

Anti-microbial studies of Neolamarckia cadamba seed

Figure 8: *Neolamarckia cadamba* seed crude extract against *Staphylococcus aureus* MTCC 87 (Positive strain)



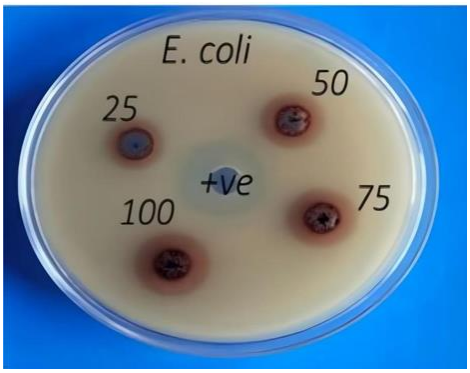


Figure 9: *Neolamarckia cadamba* seed crude extract against *Escherichia coli MTCC 452 (Negative strain*

5.Discussion:

The preliminary phytochemical screening of the ethanolic extract of *Neolamarckia cadamba* seeds confirmed the presence of major secondary metabolites, including flavonoids, tannins, alkaloids, and carbohydrates, while proteins and anthraquinone glycosides were absent. This indicates that the extract is rich in bioactive phytoconstituents, particularly phenolic compounds known for their antioxidant and antimicrobial properties. Similar phytochemical profiles have been reported in previous studies employing FTIR and phytochemical screening, which highlight the abundance of hydroxyl-rich compounds and secondary metabolites (Pharmawati & Wrasiati, 2020; Ragavendran et al., 2011) [26,27].

GC–MS analysis of the ethanolic crude extract revealed fifteen distinct peaks, indicating the presence of diverse chemical constituents. Among them, Bis(2-ethylhexyl) phthalate was identified as the major compound with a retention time of 34.743 min and a peak area of 64.44%, followed by 13-docosenamide (Z) with a retention time of 37.783 min and a peak area of 24.63%. The predominance of these compounds suggests that phthalate derivatives and fatty acid amides may significantly contribute to the biological activity of the extract, which is consistent with earlier reports on natural product analyses and antimicrobial studies (Boyanova et al., 2005) [23].

FTIR spectral analysis further confirmed the presence of characteristic functional groups corresponding to these phytochemicals. The broad O–H stretching peak at 3227.9 cm^{-1} indicates polyphenols and alcohols, which are

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associated with antioxidant activity. Peaks at 2922.2 cm^{-1} and 2855.1 cm^{-1} correspond to alkyl C–H stretching, supporting the presence of hydrocarbons and phthalate-related compounds. Additionally, the C=C stretching at 1610.2 cm^{-1} and C–O stretching at 1073.5 cm^{-1} confirm aromatic and phenolic structures. These findings are in agreement with previous FTIR-based studies on plant extracts rich in flavonoids and phenolics (Ragavendran et al., 2011; Pharmawati & Wrasiati, 2020) [27,25,28].

The presence of these functional groups suggests that the extract contains bioactive compounds such as flavonoids, tannins, and phenolic acids, which play a crucial role in antioxidant and therapeutic activities. Phenolic compounds are particularly important due to their ability to donate hydrogen atoms and neutralize free radicals, thereby preventing oxidative stress (Balasundram et al., 2006) [29]. Moreover, certain phenolic constituents, including chalcones and cinnamic acid derivatives, may contribute to biological activity even without exhibiting characteristic absorption at 280 nm (Alzohairy, 2016) [30].The antimicrobial activity of the extract was evaluated using Mueller–Hinton agar, demonstrating significant antibacterial effects against tested pathogens. At a concentration of 100 µg/ml, the extract showed notable inhibition against *Staphylococcus aureus* and *Escherichia coli*, with zones of inhibition comparable to standard antibiotics, supporting its potential as a natural antimicrobial agent (Nostro & Paparella, 2012) [31]. Furthermore, the minimum inhibitory concentration (MIC) values ranged from 25 to 200 µg/ml, with the lowest MIC observed against *S. aureus* and *E. coli*, indicating strong antibacterial efficacy at relatively low concentrations. The MIC determination followed standardized procedures as described by Wiegand et al. (2008) and CLSI guidelines (2018) [32,33].

Overall, the observed antimicrobial activity may be attributed to the synergistic action of phytochemicals identified through GC–MS and FTIR analyses. Compounds such as phenolics, phthalates, and fatty acid derivatives may exert their effects by disrupting microbial cell membranes, inhibiting enzymes, or interfering with essential metabolic pathways. Similar antimicrobial properties of phenolic-rich natural products have been documented in earlier studies, including the activity of propolis against *Helicobacter pylori* (Boyanova et al., 2005) [23].

6.Conclusion and Future Prospectives:

This study demonstrates that the crude extract of Neolamarckia Cadamba seeds, The extract's bioactive properties were confirmed by a variety of analytical methods, including GC-MS, UV-VIS spectroscopy, phytochemical analysis, and microbiological testing. The findings suggest that this phytochemicals such as . Bis(2-Ethylhexyl) Phthalate and 13-Docosenamide (z), could be a good alternative for treating antibiotic-resistant bacterial infections. With noteworthy concentrations of alkaloids, tannins, flavonoids, and phenolics, the ethanolic extract exhibits a substantial phytochemical diversity. The antimicrobial effect raises the possibility of using it to create natural antibacterial compositions, especially against gram-positive bacteria.

Future studies should focus on isolating and characterizing the key bioactive compounds, such as Bis(2-Ethylhexyl) Phthalate and 13-Docosenamide (Z), to understand their precise antimicrobial mechanisms. Investigating their synergistic effects with conventional antibiotics and assessing safety through in vitro and in vivo toxicity studies will be important for potential therapeutic applications. Additionally, standardizing extract preparation, testing against a broader range of pathogens, and evaluating in vivo efficacy can pave the way for developing natural antibacterial formulations, particularly against antibiotic-resistant bacteria.

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