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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTICANCER ACTIVITY OF NEOLAMARCKIA CADAMBA

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ABSTRACT

The present research provides a comprehensive phytochemical and biological investigation of Neolamarckia cadamba, a medicinally significant plant known for its diverse therapeutic properties. This study focuses particularly on evaluating its anticancer potential through in vitro methods and the isolation of specific bioactive compounds. In Study1, the groundwork of the research is laid by introducing the ethnopharmacological importance of N. cadamba, highlighting its traditional uses and potential in modern drug discovery, especially in oncology. Study2 presents an exhaustive review of literature, summarizing previous phytochemical and pharmacological studies on the plant and similar taxa, and identifying research gaps addressed in the current study. Study3 outlines the materials and methodologies used, including the collection of aerial parts (leaves, bark, flowers, petioles, fruits, and seeds), solvent extraction, qualitative and quantitative phytochemical screening, and various in vitro bioassays for anticancer activity. In Study4, a detailed phytochemical profile of different aerial parts is presented. The extracts were found to be rich in secondary metabolites such as flavonoids, phenolics, sterols, and terpenoids. Biological evaluation revealed that methanolic and ethyl acetate extracts exhibited significant cytotoxic effects against selected cancer cell lines, thus establishing a foundation for further compound isolation. Study 5 focuses on the isolation, structural elucidation (using IR, NMR, and MS techniques), and biological evaluation of (24R) and (24S) stigmasterol (stigmaster-5-en-3-ol isomers) from the leaf extract. The isomers demonstrated moderate anticancer potential through cell viability and enzyme inhibition assays.

Keyword: - Neolamarckia cadamba, phytochemical screening, anticancer activity, MTT assay, cytotoxicity, medicinal plants, secondary metabolites.

INTRODUCTION

Cancer, which is one of the leading causes of death on the planet, continues to be a daunting challenge for the many health care systems that are in place across the world. In spite of the fact that targeted therapies, radiation therapy, and chemotherapy have made significant progress, the search for anticancer medications that are both safer and more effective is always continuing. Natural compounds, particularly those derived from medicinal plants, are the source of a variety of anticancer drugs, including vincristine and paclitaxel, amongst others. There is a significant likelihood that novel molecules with high bioactivity and minimal

adverse effects might be discovered, and the plant kingdom is rife with opportunities for such chemical discovery.

A member of the family Rubiaceae, the Kadamba tree, also known as Neolamarckia cadamba (Roxb.) Bosser, is a deciduous tree that is on the verge of experiencing rapid expansion. Southeast Asia and the Indian subcontinent are its natural habitats, and it plays a vital part in traditional medicinal practices such as Ayurveda and Unani that date back thousands of years. For a very long time, practitioners of traditional medicine have depended on the many therapeutic characteristics of this plant to treat a broad variety of illnesses, such as fever, inflammation, wounds, diarrhoea, and liver disorders. In the early research that were conducted, it was discovered that N. cadamba contains a variety of phytochemicals, including alkaloids, flavonoids, triterpenoids, and phenolic compounds. A significant number of these chemicals has anti-inflammatory, antibacterial, and antioxidant properties.

In light of the fact that oxidative stress is increasingly becoming recognised as a significant factor in the development of cancer, plant extracts that are abundant in antioxidants are becoming more relevant in the treatment of cancer. This research aims to profile the phytochemicals of various N. cadamba extracts and their anticancer properties in vitro against selected human cancer cell lines. This research endeavour takes into account the ethnomedicinal history of this plant as well as its phytochemical abundance. The objective is to identify cytotoxic compounds that have the potential to serve as first steps in the process of producing anticancer drugs derived from plants. In order to provide the platform for future pharmacological and mechanistic studies of Neolamarckia cadamba, the purpose of this study is to establish a scientific foundation for the traditional claims. This will be accomplished via the use of systematic extraction, chemical characterisation, and biological evaluation.

OBJECTIVE

1. To find out medicinally active constituents from crude fraction of the selected plant.
2. Structural elucidation of the isolated pure constituents using chemical degradation and spectral analysis (UV, IR, NMR and Mass etc.)

MATERIAL AND METHOD

Glass-wares

Borosil and J-Sil Pvt. Ltd. supplied all of the glassware that was used. Three chromatography columns made of glass (Borosil): one with dimensions of 5.0×75 cm, two with dimensions of 2.5x30 cm, and two with dimensions of 2x30 cm.

Chemicals

Sigma-Aldrich, S.D. Fine Chemicals, and Sisco Research Laboratories, all located in Mumbai, were the suppliers from whom all of the chemicals were obtained. Since all of the reagents and solvents were of analytical grade (AR), they did not need any further purification before being used. Every single chemical that was used was of the A.R. grade, which is the highest possible purity.

1. SDF, Mumbai's 3,5-Dinitrosalicylic Acid

2. SDF, Mumbai, sodium hydroxide

3. SRL, Mumbai-based glucose A.R.

4. SDF, Mumbai's Sodium Potassium Tartarate

5. SRL, Mumbai's Bovine Serum Albumin (BSA)

6. Coombassie Brillant Blue G-250 (Mumbai, SRL)

7. SDF, Mumbai's di-sodium hydrogen phosphate AR

8. SDF, Mumbai's sodium dihydrogen phosphate AR

9. A.R. Starch (SRL, Mumbai)

10. SDF, Mumbai's A.R. sodium acetate

11. Dry, A.R. Glacial Acetic Acid (SDF, Mumbai)

12. 99% Absolute Ethanol (Merck)

13. Aspergillus niger glucoseamylase (EC 3.2.1.3) (SRL, Mumbai)

14. Porcine pancreatic a-amylase (EC 3.2.1.1) (SRL, Mumbai)

15. 60-120 mesh silica gel for column chromatography (SDF, Mumbai)

16. TLC-grade silica gel H (SDF, Mumbai)

17. Sigma-Aldrich, India's 2,2-Diphenyl picrylhydrazyl (DPPH)

18. Ascorbic Acid (Mumbai, SDF)

19. SDF, Mumbai's Butylated Hydroxy Toluene (BHT)

20. 98% A.R. sulphuric acid (SDF, Mumbai)

In contrast, there are two kinds of bacteria: Gram-negative (Salmonella typhi ATCC 23564 and Escherichia coli - ATCC 25922) and Gram-positive (Staphylococcus aureus ATCC 25923, Bacillus subtilis - ATCC 6633).

Solvents

Various products were double-distilled for the purpose of performing research. These included methanol, chloroform, petroleum ether (60-80), diethyl ether, ethyl acetate, acetone, and hexane.

Instruments

The following tools were used over the study's duration:

Antech's GT SONIC ultrasonicator

(Elico LI 120) pH meter

Thermostat for boiling water bath (Meta-lab)

Shimadzu, Japan, UV-2450, UV-Vis spectrophotometer (software: UV Probe 2.32)

FTIR (Perkin Elmer, FTIR/ FIR Spectrometer, Frontier, Pike technologies, Gladi ATR, Software: Perkin Elmer Spectrum)

NMR (Bruker, Avance-300, Software: Topspin 2.1)

GC-MS (Thermo Fischer Scientific, Trace GC Ultra, Triplus, Polarise Q, Direct Probe Controller, Software: X-calibur)

LC-MS (TOF/Q-TOF Mass Spectrometer)

RESULT AND DISCUSSION

Preparation Of Neolamarckia Cadamba Bark Extracts

For three days at room temperature, solvents such as pet ether, chloroform, ethyl acetate, methanol, and water were used to subject the dried and powdered bark of Neolamarckia cadamba to serial maceration. A total of 445 grammes of bark was collected. The volume-to-weight ratio of the solvent to the sample was 1:10. An improvement in the extraction process was achieved by using ultrasonication. The procedure consisted of removing the liquid, filtering it using Whatman filter paper Grade 1, and then letting it evaporate until it was dry at room temperature. Biological activities, including antioxidant and anti-diabetic tests, were conducted, and percentage yields were calculated.

Figure 1 Bark of Neolamarckia cadamba

Extract Yield Percentage

Table 1: Extracts from Neolamarckia cadamba Bark: A Yield Measurement

Extracts	% Yield	Colour	Consistency
Hexane	0.20	Orange brown	Sticky
Petroleum Ether	0.07	Orange	Sticky
Chloroform	0.75	Yellowish brown	Solid
Ethyl Acetate	0.29	Dark brown	Solid
Methanol	6.55	Dark brown	Solid
Aqueous	0.26	Dark brown	Solid

Phytochemical Screening

Table 2 Ecological analysis of Neolamarckia cadamba tree bark samples

Phytochemical Test	Test Used	Pet Ether Extract	Methanolic Extract	Aqueous Extract
Alkaloids	Dragendorff's, Mayer's	–	+	+
Carbohydrates	Molisch's, Fehling's	–	+	+
Flavonoids	Shinoda's, Zn-HCl reduction	–	+	+
Resins	—	–	+	–
Saponins	—	–	+	+
Steroids	Libermann's, Salkowski's	+	+	–
Triterpenoids	Libermann's	+	+	–
Tannins and Phenolics	5% $FeCl_3$, Lead acetate, Acetic acid	–	+	+

When it comes to establishing the existence of phytochemicals, FT-IR spectroscopic investigations consistently show to be of great assistance. The infrared spectra of many different extracts of Neolamarckia cadamba leaf are shown in the following table.

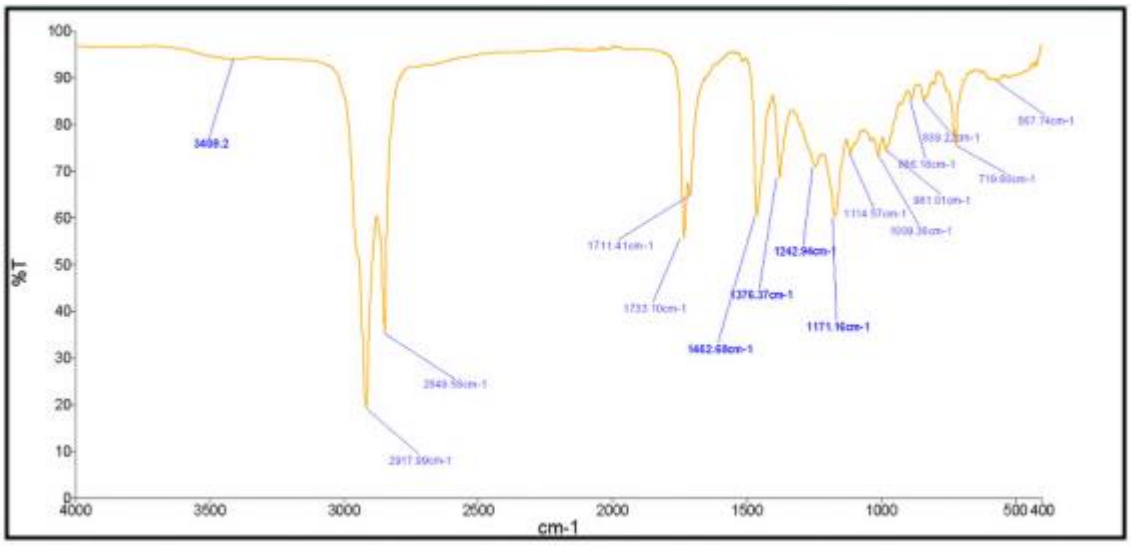

Figure 2 Pet ether Neolamarckia cadamba bark extract IR spectrum

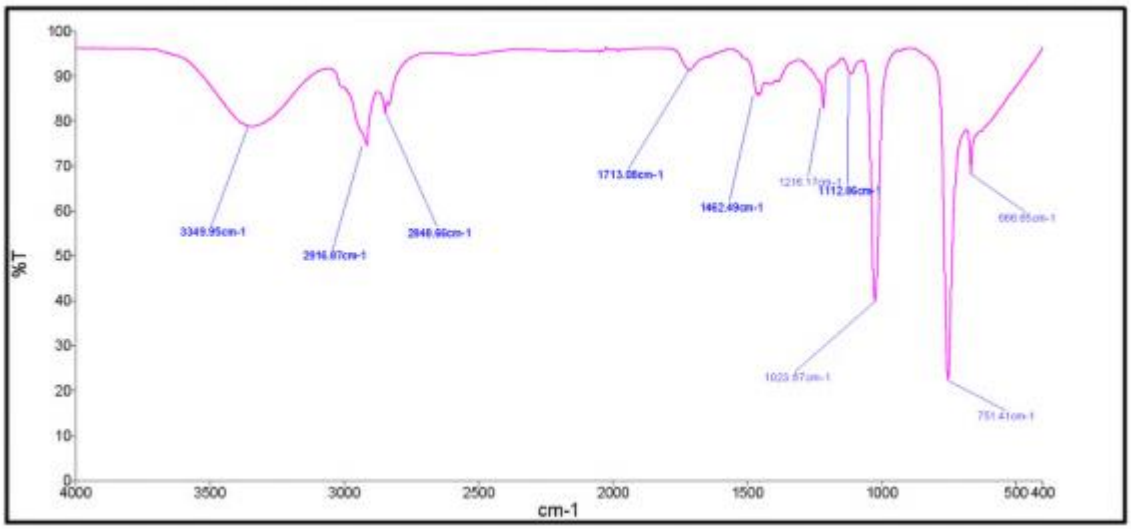

Figure 3 Neolamarckia cadamba bark chloroform extract IR spectrum

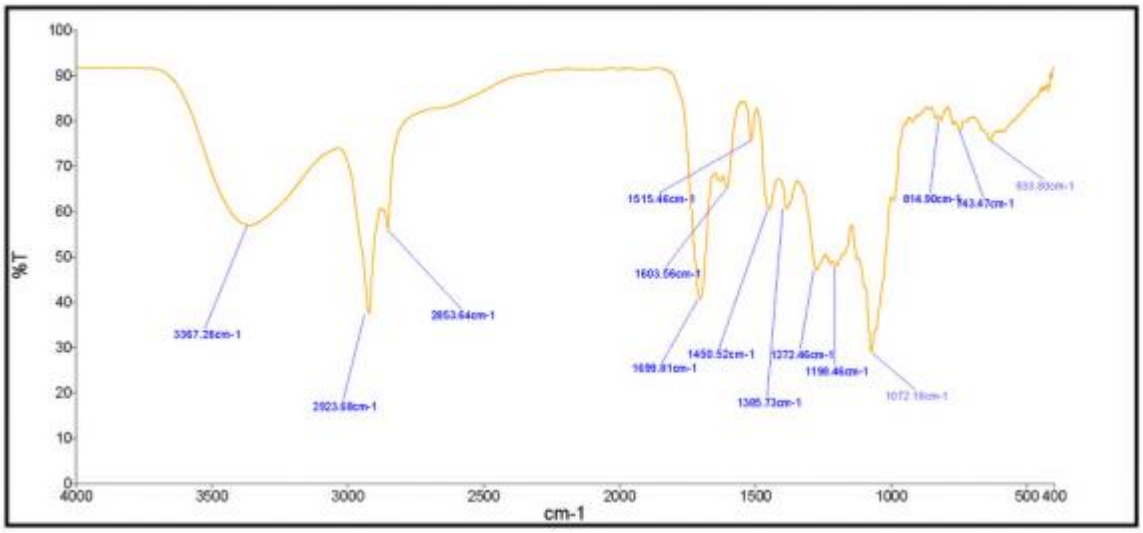

Figure 4 Neolamarckia cadamba bark ethyl acetate extract IR spectra

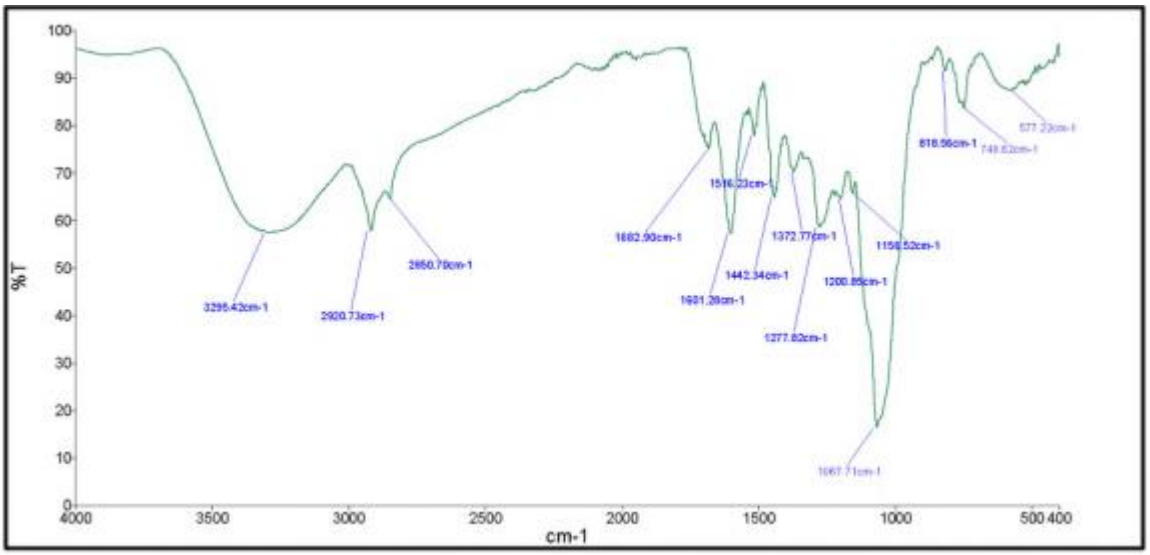

Figure 5 Neolamarckia cadamba bar methanolic extract IR spectrak

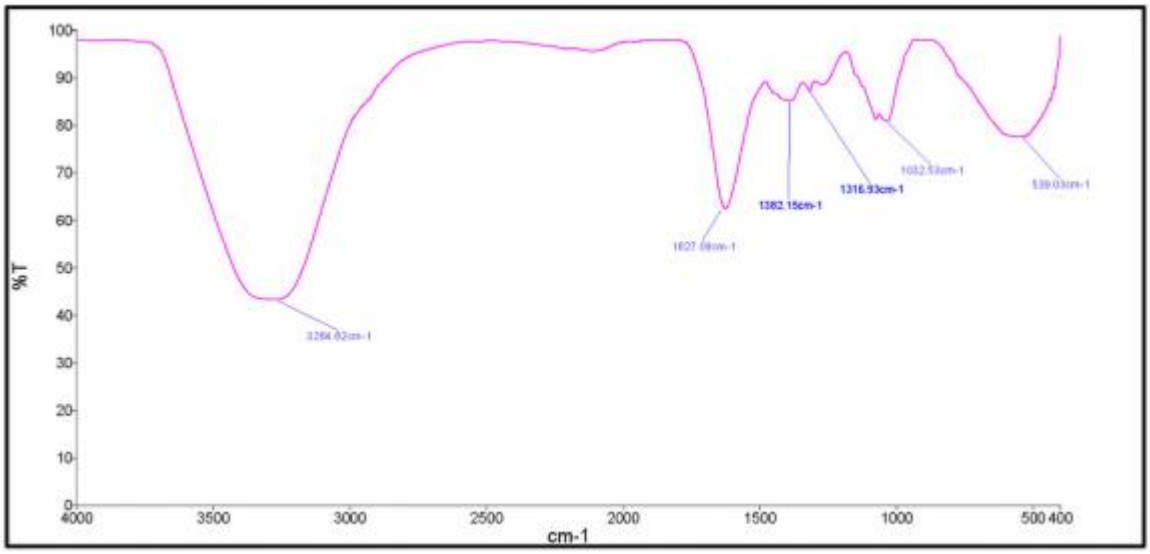

Figure 6 Neolamarckia cadamba bark aqueous extract IR spectra

Table 3: Neolamarckia cadamba Bark Extract FT-IR Spectral Peak Values and Functional Groups

Extract	Peak Values (cm^{-1})	Functional Groups	Secondary Metabolites Present
Pet Ether Extract	1242, 1376, 1733, 2849, 2917	C–O stretching, C–H bending, C=O (carbonyl), C–H stretching	Sterols, triterpenoids, ethers or sterols
Chloroform Extract	751 (sharp), 1023 (sharp), 2916, 3349	C–H bending, C–O stretching, C–H stretching, –OH group	Polyhydroxy compounds, triterpenoids, and sterols
Ethyl Acetate Extract	1072, 1699, 2923, 3367 (broad)	C–O stretching, C=O (carbonyl), C–H stretching, –OH group	Polyhydroxy compounds, alkaloids, saponins, esters, triterpenoids, and long-chain alkenes
Methanolic Extract	1067, 1601, 1682, 2920, 3295 (very broad)	C–N stretching, C=C stretching, C=O (carbonyl), C–H stretching, –OH group	Polyhydroxy substances, phenols, triterpenoids, sterols, amines, and amides
Aqueous Extract	593 (broad), 1032 (broad), 1627, 3264 (very broad)	C–X stretching, C–O stretching, C=C stretching, –OH group	Alkenes, saponins, sterols, polyhydroxy compounds, halogens

Bioactivities

After analysing the bark extracts, an antioxidant test was conducted.

1. Antioxidant activity

The extracts were subjected to a DPPH free radical scavenging experiment in order to carry out antioxidant research.

Table 4 Neolamarckia cadamba bark extracts' in vitro antioxidant activity

Concentration (µg/mL)	Ethyl Acetate Extract	Pet Ether Extract	Chloroform Extract	Methanolic Extract	Ascorbic Acid (AA)	BHT
100	29.13	70.50	54.13	34.20	97.00	42.44
200	58.84	82.35	60.27	61.19	97.11	74.10
300	78.81	85.70	64.91	82.33	96.92	81.90
400	88.10	85.73	66.38	91.83	97.05	89.85
500	90.34	85.25	69.02	91.98	97.22	89.50
IC_{50} (µg/mL)	**174.44**	**53.34**	**76.93**	**168.88**	**5.22**	**34.31**

Anticancer Activity of Bark Extracts

The MTT test was conducted in vitro on two human cancer cell lines to ascertain the anticancer effects of Neolamarckia cadamba bark extracts:

- **MCF-7** (human breast adenocarcinoma)
- **HeLa** (human cervical carcinoma)

Extract Preparation

Bark extracts were prepared by subjecting the material to serial solvent extraction with the same solvents (methanol and ethyl acetate). We further reconstituted the dried extracts in DMSO for testing purposes.

CONCLUSION

The purpose of this research was twofold: first, to identify which Neolamarckia cadamba aerial parts had biological and anticancer activities; and second, to examine which parts have which phytochemical

composition. Through the use of time-honoured extraction methods, a number of plant extracts were retrieved. These extracts were derived from various sections of the plant, including bark, flowers, petioles, fruits, seeds, and flowering tops. These extracts included many valuable bioactive components, according to the findings of an initial phytochemical analysis. Among the substances included in this group were alkaloids, flavonoids, phenolics, tannins, saponins, and steroids. The importance of these compounds to plants' healing abilities is well-known. The concentrations of these components differed depending on the plant part and solvent utilised for analysis.

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