Isolation and characterization of catechol derivative of Semecarpus anacardium

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Abstract- ***The traditional Indian systems of medicine, specifically Ayurvedic and Siddha, frequently utilize the fruits and seeds of Semecarpus anacardium for treating cancer and various ailments due to their rich composition of secondary metabolites. This study aims to isolate, characterize, and identify phytochemicals from the seeds of Semecarpus anacardium. Phytochemicals were isolated using a reverse phase C18 column via preparative flash chromatography and characterized as catechol alkenyl derivatives through spectroscopic analyses, including HPLC, FTIR, UV, ^{13}C NMR, ^{1}H NMR, and LCMS. The identified structures of the isolated phytochemicals were determined to be 1,2-dihydroxy-3-pentadec-8-enylbenzene (A) and 1,2-dihydroxy-3-pentadeca-8,11-dienylbenzene (B). This research builds on existing literature regarding the medicinal applications of Semecarpus anacardium as a source of alternative therapies for various conditions. Numerous studies have highlighted its unique properties, demonstrating anti-arthritic, antimicrobial, antihelminthic, antioxidant, and anti-cancer activities attributed to these phytochemicals.***

Indexed Terms- ***Semecarpus anacardium, Catechol Derivative, Preparative Chromatography, TLC, LC-MS, FTIR***

I. INTRODUCTION

For thousands of years, nature has served as a vital source of medicinal agents, with numerous modern drugs isolated from natural sources. Approximately 80% of the global population continues to rely on plant-based traditional medicine as their primary form of healthcare, highlighting its enduring significance [3]. According to the World Health Organization, medicinal plants represent a key resource for a wide range of medications, underscoring the necessity for research to fully understand their characteristics, safety, and effectiveness [3]. This growing demand for plant-derived medications reflects a resurgence of interest in traditional medicine, which remains popular due to its cultural values despite the availability of modern pharmaceuticals. Various scriptures and textbooks document numerous plants and herbs recognized for their medicinal properties [8].

Among the well-known bioactive substances derived from plants are digoxin, salicylic acid, caffeine, and morphine, with these compounds extracted from various parts of plants, including roots, stems, leaves, and fruits, and utilized in systems such as Ayurveda and Homeopathy (Gromova, 2013) [14]. Today, commercially available drug products, including Taxol, Quinine, and Epinephrine, are all obtained from medicinal plants, further emphasizing the importance of studying these resources to develop safe and effective treatments for life-threatening diseases under pharmacological testing protocols for medicinal herbs. In traditional medical systems, the seeds of *Semecarpus anacardium* Linn, a member of the Anacardiaceae family, are highly valued in both Ayurvedic and Siddha medicine for their ability to treat a wide range of illnesses [7,9,11]. In Siddha medicine, the nut milk extract of *Semecarpus anacardium* is referred to as “Kalpaamruthaa,” celebrated for its reported antioxidant, analgesic, antipyretic, and ulcerogenic properties (Arulkumaran et al., 2006; Mythilypriya et al., 2007; Veena et al., 2007) [1]. This deciduous tree, commonly known as Sajivani or Bhallatak, thrives in the Himalayan region as well as tropical and temperate areas of Southeast Asia [8].

The term *Semecarpus anacardium* is derived from Greek, where “simeion” means “marking” and “carpus” means “nuts,” while “anacadium” refers to a heart-like shape [8]. The plant is colloquially known as the "Marking Nut" due to its ability to leave a water-insoluble mark on cloth. In Ayurveda, the seeds of *Semecarpus anacardium* are employed in the treatment of a variety of diseases, including

rheumatoid arthritis, tumors, asthma, epilepsy, leprosy, and for promoting longevity. Recent studies indicate that active compounds from this plant may be effective against life-threatening conditions, such as hepatocellular carcinoma and mammary cancer, making it popular in both tribal and rural communities [8,11]. Given its diverse medicinal properties, it is often referred to as “Ardha Vaidhya” or “Multipurpose Medicine” [8]. As the prevalence of infections and antibiotic resistance escalates, the need for alternative therapeutic options becomes increasingly urgent. Many microorganisms, including bacteria and viruses, have developed significant resistance to a range of commercial antibiotics, prompting scientists to explore other sources, particularly medicinal plants, for healthier and more effective treatment solutions. To elucidate the pharmacological mechanisms of *Semecarpus anacardium*, it is essential to isolate its active ingredients and characterize their respective structures and activities. Standardization—defining qualitative and quantitative parameters to ensure efficacy, safety, and reproducibility—is crucial for confirming the quality of herbal drugs and their therapeutic value in clinical settings [7,10]. This background motivates our efforts to identify the active compounds present in *Semecarpus anacardium* seeds and investigate their potential effects. Preliminary phytochemical studies of the extract have revealed a variety of bioactive compounds, including flavonoids, phenolic compounds, anacardic acid, and several fatty acids [9]. However, the presence of various bioactive compounds with different polarities poses significant challenges for their identification and characterization. Employing a combination of separation techniques—such as TLC, column chromatography, preparative chromatography, flash chromatography, Sephadex chromatography, and HPLC—is essential for isolating pure compounds. These pure compounds can then be utilized for further structural determination and assessment of biological activity.

II. MATERIALS AND METHODS

2.1. Plant Material

Semecarpus anacardium seeds were collected from a field area near the village of Umberwadi, Tal-Gadhinglaj, in the Kolhapur district of Maharashtra, located approximately 60-65 km from Kolhapur city. This plant is closely related to the cashew and is commonly known as Bhallatak in India, as well as "marking nut" due to its distinctive properties.

a. Procedure of Plant material/Oil extraction: -

One to two kilograms of *Semecarpus anacardium* seeds were thoroughly cleaned with water and left to air dry. Due to the seeds' corrosive properties, they were then soaked in milk overnight. After soaking, the seeds were removed from the milk and dried in sunlight, employing a natural method that avoids harmful chemicals or metal-based vessels during the extraction process. The dried seeds were transferred to a pot and subjected to gentle heating, which allowed the oil to be extracted. The resulting oil was collected in a separate pot and stored at room temperature for further analysis. A black, viscous liquid with a pungent odor was obtained from the seeds. This oil was found to be immiscible in polar protic solvents, such as water, methanol, and acetonitrile while being completely miscible in non-polar organic solvents like dichloromethane and n-hexane.

b. Reagents, Chemicals and Analytical Instruments:

Analytical grade solvents were used for extraction, while chromatographic solvents of HPLC grade were sourced from Honeywell. Ultra-pure water, with a resistance of 18 $M\Omega.cm^{-1}$, was obtained using the Xtrapure Lablink water purification system. LC-MS analysis was performed using a Waters Xevo TQ system, coupled with Masslynx software. The separations were carried out on an Acquity BEH C18 column (2.1 mm x 50 mm, 1.7 µm particle size). For mobile phase preparation, LC-MS grade ammonium formate buffer (Honeywell) and acetonitrile (JT Baker) were utilized. Preparative flash HPLC was conducted on a Buchi Flash C-815 system, equipped with a binary pump, a variable wavelength UV detector, and a fraction collector. The separations were achieved using Thermo Scientific Buchi Flash Pure Select Chromatography Cartridges with a C18 stationary phase. For the HPLC analysis of isolated compounds, a Waters HPLC system was employed, featuring a vacuum degasser, a quaternary pump, an automated liquid sampler, and a DAD detector. The separations were performed on a Kromasil C-18 column (250 mm x 4.6 mm, 5 µm particle size) using ammonium formate buffer (Honeywell), formic acid (Honeywell), and acetonitrile (Honeywell) as the mobile phase. The pH adjustments were made using a

Lab India pH meter. The data acquisition and processing were carried out using Chromeleon 7.2 software. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed using a Shimadzu IRSpirit FTIR spectrophotometer, operated with Lab Solution software. NMR spectra were recorded on a Bruker NMR instrument.

c. Evaluation by Chromatography:-

To evaluate the presence of any active components, a chromatographic study was conducted. As S. anacardium oil is immiscible in both polar and polar-protic solvents, the sample was dissolved in a non-polar solvent to facilitate analysis.

i. Thin layer Chromatography:

Thin Layer Chromatography (TLC) was performed using TLC aluminum sheets (20 × 20 cm, silica gel 60 F254), procured from Merck, Darmstadt, Germany. A TLC chamber (21 × 21 × 9 cm) was prepared by lining the walls with filter paper. The mobile phase, consisting of toluene: acetate (4:3:3), was poured into the chamber, saturating the filter paper. A sufficient mobile phase was added to form a solvent layer 5-10 mm deep. The chamber was then closed and allowed to equilibrate for 10-15 minutes at room temperature. The S. anacardium oil sample was dissolved in dichloromethane, and a TLC plate (15 × 4 cm) was run for approximately 12-15 minutes under the described conditions.

ii. Liquid Chromatography (LCMS):

The TLC study of the S. anacardium oil extract revealed two close spots with similar Rf values under UV (254 nm) light, indicating the presence of UV-active compounds in the extract. To further analyze these compounds and detect any additional unknown masses (m/z) not observable in the near UV range (approximately 210 nm), the S. anacardium oil extract was subjected to Liquid Chromatography-Mass Spectrometry (LC-MS). The oil extract was dissolved in dichloromethane to obtain a concentration of 500 ppm for LC-MS analysis. The chromatographic conditions used for the analysis are outlined in Table 1.

Table 1: Chromatographic conditions of Preparative Chromatography

Column:	2.1mm x 50mm x 1.7µ, BEH C18 Waters Acquit		
Mobile Phase A	Weigh about 1.26g/L Ammonium Format in 1000mL of water. Adjust the pH 4.4 with diluted Formic Acid, filter 0.2µ, and degas the solution.		
Mobile Phase B	Pre-mix 50 volumes of acetonitrile and 50 volumes of methanol, sonicate and degas the solution**.**		
Run time	20 mins		
Injection volume	1.0 µL.		
Wavelength	275 nm		
Flow	0.25 ml/min		
Diluent	Dichloromethane		
Gradient Programme	Time (min)	% Mobile Phase A	% Mobile Phase B
	0.0	60	40
	2.0	60	40
	12.0	10	90
	15.0	10	90
	16.0	60	40
	20.0	60	40
3) Mass conditions: -			

Ionization mode	ES (-ve)
Capillary Voltage	3.00 Kv
Cone voltage	30 V
Source temp.	150°C
Desolvation Temp.	400°C
Desolvation gas	800 L/hr
Cone gas flow	50 L/hr
Collision gas flow	0.20 mL/min
Acquisition	Scan Mode
Collision energy	5ev

2.3.3. Gas Chromatography (GC):

As part of the evaluation to investigate the presence of any organic thermally stable/non-volatile compounds in the S. anacardium seed extract, the extract was subjected to Gas Chromatography (GC) analysis. The extract was dissolved in dichloromethane and analyzed using DB-5 and DB-1 non-polar columns (30 m × 0.32 mm × 0.25 µm). The injector temperature was set to approximately 260°C and the detector temperature to 300°C. During the analysis, two co-eluting peaks were observed at a high temperature of around 300°C on the DB-1 column, but with distorted peak shapes. Due to the limitations of the temperature and column operating conditions, further analysis of the S. anacardium seed extract using Gas Chromatography was not feasible.

III. ISOLATION OF ACTIVE COMPOUND BY PREPARATIVE CHROMATOGRAPHY

To achieve full identification and detailed characterization of the components in S. anacardium oil extract, isolation of the active components with the desired purity from the crude mixture was necessary. TLC analysis (Silica) indicated that the extract contained UV-active components, while LC-MS analysis (C18 column) revealed two distinct components, accounting for approximately 50% and 40% area normalization, with different m/z values, retention times, and elution in a strong solvent composition (around 90% acetonitrile). This suggested that the extract comprised two major components.

TLC analysis demonstrated poor resolution between the two spots, while LC-MS showed two separated peaks. This discrepancy is attributed to the different mechanisms employed by the two techniques. In TLC (Silica as the stationary phase), the rate of adsorption and desorption of the components is similar between the silica stationary phase and the non-polar organic mobile phase. Conversely, in LC-MS using a Reversed Phase C18 column with strong polar solvents (acetonitrile), the components' varying functional groups or polarity lead to different interactions with the stationary phase (C18) and the polar mobile phase, causing distinct retention times due to differing rates of adsorption and desorption.

Based on these findings, it was decided to isolate the two components through preparative chromatography. Preparative HPLC is widely used for the isolation and purification of valuable compounds in chemical, pharmaceutical, biotechnology, and biochemical fields. It can isolate natural components or synthesize novel molecules, with the required amount of compound ranging from micrograms (for enzyme isolation) to larger gram quantities for standards, reference compounds, and compounds intended for toxicological or pharmacological testing.

For the identification and structure elucidation of unknown compounds in synthesis or natural product chemistry, it is crucial to obtain pure compounds in the milligram range. Industrial-scale preparative HPLC, used to isolate compounds in kilogram quantities, is increasingly employed for the production of valuable pharmaceutical products. Preparative flash chromatography was employed as a straightforward

method for separating molecules from complex mixtures. After injecting the crude mixture into the column (Silica, Chiral, Reversed Phase C8, or C18), similar to analytical HPLC, the active components interact with the stationary phase, becoming adsorbed on the column and then eluting with the mobile phase. This process enables the separation of the two active components with better resolution. These active components from natural products, such as plant extracts, are then subjected to various physicochemical and functional tests to identify and characterize the peaks and to investigate their biological activity.

3.1. Pre-treatment to S. Anacardium Oil Extract:

During method development for the isolation of active components using preparative chromatography, several challenges were encountered, including sample solubility, column compatibility, and sample loading. To prevent contamination of the preparative column by metallic impurities or undissolved particles, the crude material was dissolved in dichloromethane and filtered through a 0.22 µm filter to remove any particulate matter. The resulting filtrate was then applied to a silica column and sequentially washed with dichloromethane, n-hexane, and ethyl acetate. The filtrates from each solvent were collected separately in rotary evaporation flasks and concentrated under vacuum using a rotary evaporator at 35°C-40°C. To ensure the complete removal of solvent traces, the rotary flasks were stirred under vacuum at 35°C-40°C for an additional 1-2 hours.

3.2. Development of Chromatographic method on Preparative Flash Liquid Chromatography (PLC) Condition: -

The objective of an analytical HPLC run is the qualitative and quantitative determination of a compound. For a preparative HPLC run it is the isolation and purification of a valuable product (Table 2).

Table 2: -Definition of analytical and preparative HPLC

Analytical HPLC	Preparative HPLC
The sample goes from the detector into Waste	The sample goes from the detector into the fraction collector
Goal: Quantification and/or identification of compounds purification of compounds	Goal: Isolation and/or purification of compounds

Optimization of chromatographic conditions for the isolation of active components from natural products, such as plant extracts is challenging, tedious, costly, and time consuming.

3.2.1. Selection of Column and Mobile Phase:-

Isolation of the unknown impurity for identification and characterization in pharmaceutical industries is often initiated using silica columns such as Chromatopak Silica Peerless (250 mm × 21.2 mm, 5 µm), Kromasil Silica (250 mm × 21.1 mm, 10 µm), and Altima Silica (250 mm × 20 mm, 10 µm), with various mobile phases, including n-hexane, n-heptane, and n-hexane alcohol. Additionally, chiral columns like ChiralPak (250 mm × 20 mm, 20 µm) with mobile phases of acetonitrile or acetonitrile were employed. However, in both silica and chiral column trials with different mobile phases and compositions, only a single peak was observed. Based on LC-MS data (retention time/resolution), the experiments for isolating the two components were designed and performed using preparative chromatography with reversed phase C8/C18 columns as the stationary phase. Ammonium formate buffer (10 mM) was used for the separation of active components, but in preparative chromatography, the sample must be in pure form and free of additives to avoid further processing, such as lyophilization to remove the buffer from the pure compounds. For the isolation of the active components from the S. anacardium oil extract, various LC-MS-compatible and easily lyophilizable volatile buffers such as ammonium formate and ammonium acetate, adjusted to specific pH levels, were tested. The final method involved using water as mobile phase A and acetonitrile as mobile phase B, with an initial gradient program. The detailed chromatographic conditions are provided in Table 4.

3.2.2. Sample Loading Technique:

In analytical chromatography, the sample quantity is typically in the mg to µg range or lower, with the compound-to-stationary phase mass ratio on the column being less than 1:100,000. Additionally, the

injection volume is significantly smaller, often less than 1:100 of the column volume. This small sample injection results in improved resolution, peak width, and peak symmetry, with the ideal peak shape closely resembling a Gaussian curve. However, when the injected sample volume exceeds a certain threshold, the adsorption isotherm becomes non-linear, leading to unsymmetrical peaks. It is often said that the column is the heart of liquid chromatography, a statement that holds in both analytical and preparative chromatography.

The choice of the right column and mobile phase conditions is critical in preparative chromatography. Inappropriate choices can significantly impact both the cost and the performance of sophisticated instrumentation. Working with samples that have limited solubility in the injection solvent, diluent, or mobile phase, especially with large injection volumes, can lead to disastrous results. Issues such as sample precipitation can wreak havoc on expensive wide-bore prep columns and cause equipment damage.

For the purification of crude materials, and natural products, and the isolation of low levels of unknown impurities in drug substances or products, large sample amounts can be handled through either concentration overloading or volume overloading techniques. Concentration overloading increases the concentration of the sample while keeping the injection volume constant. This approach is only viable when the compound exhibits good solubility in the mobile phase. For compounds with poor solubility, concentration overloading is unsuitable, and larger sample volumes must be injected. Volume overloading involves injecting a larger volume of the sample. This method is typically used when the compound has limited solubility in the mobile phase.
In the case of isolating the active components from the S. anacardium oil extract using a reversed-phase (C8/C18) column and polar organic solvents such as methanol and acetonitrile in preparative chromatography, there were limitations regarding the choice of diluent and sample loading. Although the oil extract was fully soluble in dichloromethane, it was unsuitable for use in reversed-phase analysis. Attempting to employ concentration overloading with dichloromethane resulted in unsymmetrical peaks, a higher risk of impurity co-elution, precipitation, and potential column clogging or destruction. To mitigate these issues and ensure symmetrical peaks and better resolution, the volume overloading technique was applied. In this case, the sample was loaded at a concentration of 10 mg/mL (1000 ppm), with an injection volume of 2.5 mL to prevent precipitation, and clogging, and to achieve optimal separation.

3.2.3. Detector and Diverter:
The separation of components in both analytical and preparative HPLC relies on similar principles, depending on the stationary phase and mobile phases used. The primary difference between analytical and preparative HPLC lies in what happens to the sample post-detection. In analytical HPLC, the sample typically travels directly into a waste receptacle, while in preparative HPLC, the sample is directed to a fraction collector. In preparative HPLC, based on specific triggering decisions, the fraction collector diverts the flow either to waste or into a fraction container via a fraction collection needle. This diversion is controlled by a diverter valve, which can be programmed to switch based on time intervals or a detector signal. The preparative scale fraction collector is designed to handle flow rates up to 100 mL/min, making it suitable for the isolation of larger quantities of material.

The chromatographic method used was created with peak-based fraction collection, triggered by a UV detector signal. The decision to collect fractions was based on parameters such as threshold and/or slope. A fast gradient method using water and acetonitrile, which covered a wide polarity range (from low to high polarity), was selected as the starting point for method development. Under these chromatographic conditions, peaks corresponding to both Compound A and Compound B in the S. anacardium oil extract were observed, confirming the presence of the desired components.

Table 3: Chromatographic conditions of Preparative Chromatography

Column:	FP Select C18 120g, Particle Size25-35 µm, Pore Size-92-		
Mobile	Water		
Equilibratio	4.0 min		
Instrument	C-815		
Mode	Flash		
Mobile	Acetonitrile		
Run time	30 mins		
Injection	2.5mL.		
Slope	On		
UV Trigger	0.05 AU		
Collection	Collect Peak		
Per-Vial	25 mL		
Wavelength	225 nm & 275nm		
Flow	50.0ml/min		
Diluent	Dichloromethane: Acetonitrile		
Gradient Programme	Time	Mobile	Mobile
	0	30	70
	2	30	95
	8	5	95
	20	5	95
	25	30	70
	30	30	70

3.2.4. Isolation and Concentration of Component:-

A simple reverse-phase Preparative Flash Chromatography method was employed to isolate the two major components of S. anacardium nut. During the isolation process, one peak/component eluted at approximately 15 minutes, while the second peak/component eluted at around 24.0 minutes. The chromatographic method utilized was UV-triggered, allowing for the collection of peak fractions corresponding to the elution times of 15 minutes and 24 minutes. The isolated fractions were subsequently concentrated by removing water, acetonitrile, and dichloromethane at room temperature under a high vacuum using a Buchi Rotavapor Model R124. This concentration process ensured that the fractions were effectively reduced in volume while maintaining the integrity of the isolated components.

3.4. Purity check for isolated compound:

The isolated components were subjected to an analytical method to determine their purity using HPLC. Each isolated component was prepared at a concentration of 500 ppm in an acetonitrile diluent. The chromatographic conditions for the HPLC method are detailed in Table 4.

Table 4: Chromatographic conditions of Analytical Method (HPLC)

Column:	250mm x 4.6mm x 5µm C18		
Mobile Phase A	Weigh 1.26gm of ammonium Formate in 1000 ml water sonicate to dissolve and adjust the		
Mobile Phase	Acetonitrile		
Run time	35 mins		
Injection	10µL.		
Column	30°c		
Wavelength	225 nm & 275nm		
Flow	1.0ml/min		
Diluent	Acetonitrile: Water (98:2)		
Gradient Programme	Time	Mobile	Mobile
	0	30	70
	7	5	95
	25	5	95
	30	30	70
	35	30	70

Identification of active compound from S. anacardium oil extract by Fourier Transform Infrared Spectroscopy:

The isolated, purified, and concentrated components A and B obtained from S. anacardium oil extract via preparative chromatography were utilized for identification studies using FT-IR spectroscopy to predict the corresponding functional groups in the marker compound. The Fourier Transform Infrared Spectrophotometer (FTIR) is recognized as one of the most effective instruments for determining the types of chemical bonds, also known as functional groups, present in compounds. The annotated spectrum illustrates the characterization of chemical bonds by the wavelength of light absorbed. FT-IR spectra of the isolated components A and B were recorded over a wave number range of 4500-450 cm^{-1} using the ATR unit, with a resolution of 2 cm^{-1}.

Identification of active compound from S. anacardium oil extracts Nuclear Magnetic Resonance Spectroscopic Technique:

The ^{1}H and ^{13}C NMR spectra of the isolated, purified, and concentrated components A and B obtained from S. anacardium oil were recorded using a 300 and 100 MHz Bruker spectrometer. $CDCl_3$ (deuterated chloroform) was used as the solvent, and the chemical shifts were recorded in parts per million (ppm) with tetramethylsilane (TMS) serving as an internal reference.

IV. RESULT AND DISCUSSION

4.1. Thin Layer Chromatography

The developed TLC plate was air-dried and visualized under a UV cabinet at 254 nm (short wave) and 365 nm (long wave). Two close spots were observed at 254 nm. Additionally, another TLC plate was exposed to an iodine chamber to check for any other spots that might have developed. The distance of each spot from the application point was measured and recorded, and the Rf value was calculated by dividing the spot's travel distance by the distance traveled by the mobile phase front (solvent front). The obtained Rf values for both spots were approximately 0.78.

4.2. LC-MS Analysis

The LCMS chromatogram of the S. anacardium oil extract revealed the presence of two components at retention times of 12.86 min (approximately 51% area normalization) and 13.63 min (approximately 38% area normalization). These components eluted in a strong organic solvent composition, specifically about 90% acetonitrile, indicating that these two compounds are highly non-polar. A summary of the LCMS results is provided in Table No. 5.

Table 5: Result summary of retention time (min) and Molar mass (m/z)

Sr.	Compound	Retention	[M-	UV
1	Compound	12.86	315.08	225,275
2	Compound	13.63	317.29	225,275

6.3. Isolation by Preparative Chromatography

A simple reverse-phase Preparative Flash Chromatography was employed to isolate the two major components of the S. anacardium nut oil extract. During the isolation process, one peak/component was observed to elute at approximately 15 minutes, while the second peak/component eluted at around 24.0 minutes.

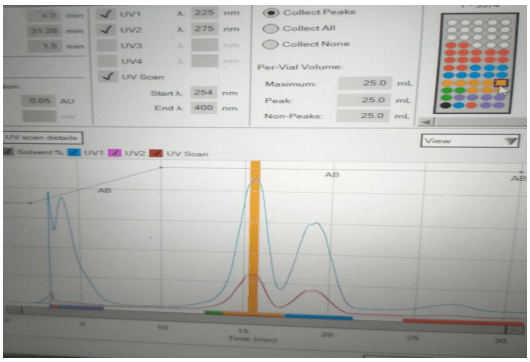

Figure 1: Isolation Peak at about 15.1min RT

Figure 2: Isolation Peak at about 24.3min RT

4.4. Determination of Purity of Isolated compound by High-Performance Liquid Chromatography

The HPLC purity of the isolated compounds, Component A and Component B, was assessed using the % area normalization method. The results are summarized in Table 6, while the corresponding chromatographs are illustrated in Figures 3 and 4.

Table 6: Result of % Purity by HPLC

Sr.No	Peak/ Compound Name	Purity (%)
1	A	91.05
2	B	98.08

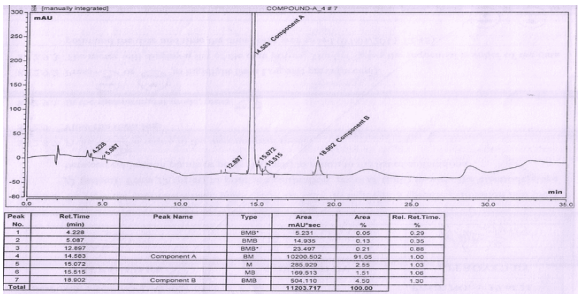

Figure 3: - Purity Chromatograph of Isolated Peak / Compound (A)

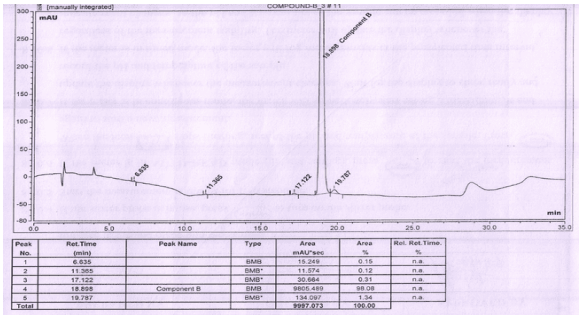

Figure 4: Purity Chromatograph of Isolated Peak / Compound (B)

4.5. FT-IR Interpretation:

FT-IR spectroscopy was employed to identify Component A and Component B, which were isolated through preparative chromatography of the S. anacardium oil extract. This analysis aids in predicting the corresponding functional groups present in the marker compounds.

Table 7: IR Interpretation of Isolated compound A from S. anacardium seed oil extract

Sr. No	Peak value observed (cm^{-1})	Frequency range (cm^{-1})	Assignments
1	3445	3500-	O-H Stretch
2	3008-	3100-	=C-H Stretch
3	2924-	2990-	C-H Stretch
4	1622-1475	1680-	C=C Stretch
		1625-	C=C Stretch
5	1277-	1300-	C-O Stretch
7	961-915	995-685	=C-H bend
8	776-730	770-735	=C-H bend

Table 7: IR Interpretation of Isolated compound B from S. anacardium seed oil extract

Sr. No	Peak value observed (cm^{-1})	Frequency range (cm^{-1})	Assignments
1	3398	3500-	O-H Stretch
2	3005-	3100-	=C-H Stretch
3	2923-	2990-	C-H Stretch
4	1621-1476	1680-	C=C Stretch
		1625-	C=C Stretch
5	1280-	1300-	C-O Stretch
7	966-923	995-685	=C-H bend
8	777-732	770-735	=C-H bend

5.5. NMR Interpretation (1H NMR and 13C NMR):

The proton NMR spectra of isolated Compound A exhibited distinct peaks corresponding to various proton environments. These included: Allylic protons at 2.05 ppm (4H), Homobenzylic protons at 1.64 ppm (2H), Terminal methyl protons at 0.93 ppm (3H), Benzylic protons in the range of 2.61-2.65 ppm (2H), Diallylic protons at 2.80 ppm (2H). An apparent singlet indicative of 1,2,3-substituted protons, along with olefinic and phenolic protons in the range of 5.39-5.42 ppm (5H)

Additionally, the multiplet observed at 1.36-1.14 ppm (11H) and the benzene protons at 6.73 ppm (3H) corroborated the presence of protons bonded to additional carbon atoms in the lipid chain.

The proton NMR spectra of isolated Compound B displayed distinct peaks corresponding to various proton environments, including Allylic protons at 2.04 ppm (4H), Homobenzylic protons in the range of 1.62-1.66 ppm (2H), Terminal methyl protons at 0.93 ppm (3H), Benzylic protons in the range of 2.61-2.65 ppm (2H), Olefinic and phenolic protons at 5.39 ppm (3H). Moreover, the multiplet observed at 1.35 ppm (17H) and the benzene protons at 6.74 ppm (3H) further supported the presence of protons bonded to additional carbon atoms within the lipid chain.

The carbon NMR spectra of isolated Compound A revealed distinct chemical shift values corresponding to various carbon environments. The following peaks were observed, Benzylic and homobenzylic carbons at 29.74 ppm and 29.82 ppm, Diallylic carbon at 25.72 ppm, Allylic carbons at 27.30 ppm and 29.24 ppm,

Terminal methyl carbon at 13.87 ppm, Aromatic carbons with chemical shifts of 143.12 ppm, 113.09 ppm, 120.22 ppm, 122.16 ppm, 130.24 ppm, and 142.02 ppm corresponding to C1, C2, C3, C4, C5, and C6, respectively, Olefinic carbons at 128.07 ppm, 128.26 ppm, and 130.02 ppm, Aliphatic side chain carbons at 29.59 ppm, 29.51 ppm, 29.36 ppm, 29.34 ppm, and 22.87 ppm. These chemical shifts collectively provide insights into the structural characteristics of Compound A.

The carbon NMR spectra of isolated Compound B displayed distinct chemical shift values corresponding to various carbon environments. The observed peaks included; Benzylic carbon at 29.81 ppm, Allylic carbon at 31.84 ppm, Olefinic carbons at 130.01 ppm and 129.93 ppm, Terminal methyl carbon at 14.16 ppm, Aromatic carbons with chemical shifts of 143.10 ppm, 113.01 ppm, 120.19 ppm, 122.11 ppm, 129.61 ppm, and 141.97 ppm, corresponding to C1, C2, C3, C4, C5, and C6, respectively, Aliphatic side chain carbons at 29.58 ppm, 29.50 ppm, 29.05 ppm, 27.28 ppm, and 22.72 ppm. These chemical shifts elucidate the structural features of Compound B, contributing to a comprehensive understanding of its molecular framework.

The probable structures of Component A and Component B, isolated from the oil extract sample, have been deduced based on various analytical data, including LC-MS (m/z values), FTIR (observed wavenumbers), and chemical shift values obtained from ^{1}H NMR and ^{13}C NMR analyses. These proposed structures are illustrated in Figures 5 and 6.

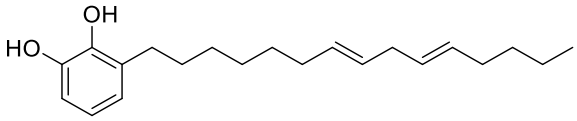

Figure 6: 1, 2-dihydroxy-3-pentadeca-8,11-dienylbenzene

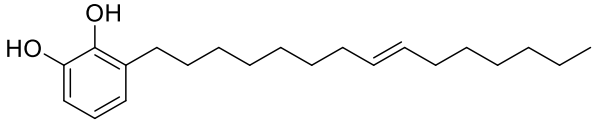

Figure 7: 1, 2-dihydroxy-3-pentadec-8-enylbenzene

CONCLUSION

Infectious bacterial illnesses are a major global contributor to morbidity and mortality, highlighting the urgent need for novel antimicrobial agents to treat bacterial infections. Numerous naturally occurring substances found in plants, spices, and herbs exhibit antibacterial properties, suggesting they could serve as potential sources of antimicrobial drugs against pathogens. In this study, catechol alkenyl derivatives were isolated from S. anacardium oil extract using preparative chromatography and were thoroughly characterized through various techniques, including TLC, FTIR, LC-MS, HPLC, ^{1}H NMR, and ^{13}C NMR. The isolated compounds were identified as 1',2'-dihydroxy-3'-pentadec-8-enylbenzene (A) and 1',2'-dihydroxy-3'-pentadeca-8,11-dienylbenzene (B). Literature indicates that these phytocompounds exhibit inhibitory activity against acetylcholinesterase and butyrylcholinesterase. Additionally, researchers have developed various potential docking sites for these compounds using genetic algorithms. The data supports the docking and interaction of acetylcholinesterase with 1',2'-dihydroxy-3'-pentadec-8-enylbenzene and 1',2'-dihydroxy-3'-pentadeca-8,11-dienylbenzene (Adhami et al., 2012). The phytochemicals present in S. anacardium demonstrate significant potential as anti-cancerous, anti-inflammatory, neuroprotective, antihelminthic, and antibacterial therapeutics. Thus, Semecarpus anacardium stands out as a promising candidate for recognition as a ‘Herbal Drug’.

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