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Phytochemical Analysis of *Cynometra Madagascariensis* (Fabaceae), a Malagasy Medicinal Plant

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Abstract:

A comprehensive phytochemical and physicochemical investigation was conducted on the bark of Cynometra madagascariensis, a plant of the Fabaceae family traditionally used in Madagascar to treat and heal wounds and burns. Sequential maceration extractions were performed with solvents of increasing polarity (hexane, dichloromethane, and methanol) to obtain a range of crude extracts. Subsequent phytochemical screening revealed a diverse chemical profile, with a significant presence of tannins and terpenoids, and lower concentrations of quinones and saponins. Alkaloids, coumarins, flavonoids, and steroids were notably absent from the bark. Chromatographic methods were then employed for the separation and isolation of constituents from the hexane extract. Thin-layer chromatography (TLC) was utilized to optimize the mobile phase and monitor the fractionation process. This led to the successful isolation of a pure compound, designated F58-60, through column chromatography. The structure of this molecule was elucidated using one-dimensional NMR spectroscopy (1H and 13C NMR). A comparative analysis of the spectroscopic data with published literature confirmed the compound's identity as di(2-ethylhexyl) phthalate. This research provides a foundational understanding of the chemical composition of this medicinal plant and paves the way for further studies on the biological activities of its constituents.

Keywords:
Cynometra madagascariensis, phytochemical screening, chromatography, NMR spectroscopy

I. Introduction

Across human history, plants have stood as a foundational source of remedies and healing. Their significance endures today, with a substantial portion of the global population, particularly in developing nations, relying exclusively on traditional plant-based medicines. In Africa alone, these botanical resources fulfill the healthcare needs of more than 80% of the population. Consequently, the value of these therapeutic systems and the plant-based remedies they employ is receiving increasing recognition in both policy development and the scientific literature (Sartor et *al*., 2018).

The valorization of traditional medicinal plants fundamentally involves the isolation and identification of their therapeutically active molecules. Discovering new natural compounds and elucidating their biological activities remains a compelling challenge. Furthermore, a growing imperative exists to preserve our natural heritage, and it is incumbent upon the scientific community to contribute to its conservation. For millennia, humanity has drawn upon the environment for survival and well-being, and plants continue to provide a

precious source of sustenance and remedies for the treatment of various diseases in traditional medicine (Luo et *al*., 2020).

Despite the vast number of species—including microorganisms, plants, and marine organisms—that inhabit the Earth, only a small fraction has been subjected to detailed chemical or biological investigation. This rich biodiversity presents an immense opportunity for natural product chemists, as it promises a correspondingly significant diversity in chemical structures. The efficacy of natural products is highlighted by famous anticancer drugs like Taxol and Taxotere, and it is worth noting that more than 50% of pharmaceutical agents have some connection to nature (Newman & Cragg, 2020). Nature, therefore, remains a consistent source of medicines, whether they are directly isolated from natural sources or synthesized using natural molecules as scaffolds.

In an effort to contribute to the valorization of Malagasy medicinal plants, a phytochemical study was conducted on a plant with the scientific name *Cynometra madagascariensis*. This plant is traditionally used in the southeastern region of Madagascar to treat burns, staunch bleeding, and dry wounds.

The objective of this work is to proceed with the characterization of the plant's various chemical constituents and to perform a physicochemical study to prospectively identify the active compounds with therapeutic properties.

II. Research Method

2.1 Plant Material

The plant material was collected in August 2018 from Sasinaka, a region within the Vatovavy Fitovinany district. The botanical identification was performed at the Parc Botanique et Zoologique de Tsimbazaza in Antananarivo.

The plant specimen was formally identified as *Cynometra madagascariensis*, a species belonging to the Fabaceae family. Vernacular names for this plant include "Mapagne," "Mapange," and "Arivo Ravy."

Figure 1. *Cynometra madagascariensis*

Ethnobotanical surveys conducted among local villagers revealed the traditional use of *C. madagascariensis* for medicinal purposes. A decoction of the plant is employed to treat and dry wounds, with a specific application for those resulting from burns. The plant's properties are highly valued for their perceived ability to heal and desiccate affected tissues.

2.2. Methods

a. Plant Material Preparation

Freshly harvested barks were dried in a dry, well-ventilated area away from direct sunlight for a period of two weeks (Eloff et *al.*, 2008). The dried plant material was subsequently ground into a fine powder to maximize the surface area for solvent contact, thereby ensuring an optimal extraction yield (Laware & Laware, 2015).

b. Sequential Extraction Procedure

A 50 g sample of the dried and pulverized bark was subjected to successive maceration using a series of three solvents of increasing polarity: hexane, dichloromethane, and methanol. This sequential approach, a type of serial exhaustive extraction, was employed to obtain a broad range of phytochemicals, from non-polar to polar compounds (Lawsonia inermis, 2012; Ammar et *al.*, 2019). Each maceration step was conducted for 72 hours at ambient laboratory temperature and in the absence of light (Agatonovic-Kustrin et *al.*, 2018). The extraction process was repeated three times for each solvent, with 300 ml of solvent utilized in each replicate. Following each maceration, the mixture was filtered through paper to separate the marc from the liquid filtrate.

The filtrates from each respective solvent were combined and subsequently concentrated using a rotary evaporator to yield dry extracts (Rao & Suseela, 2016). The mass of each resulting dry extract was then measured to determine the extraction efficiency.

c. Phytochemical Screening

Phytochemical screening serves as the preliminary stage in the chemical analysis of a plant. This procedure involves a series of characteristic tests designed to detect the principal families of active compounds present within a plant extract (Sasidharan et *al.*, 2011). The targeted compounds for this type of analysis typically include a wide range of secondary metabolites, such as alkaloids, coumarins, flavonoids, tannins, polyphenols, polysaccharides, quinones, saponins, steroids, and terpenoids.

The primary objective of these experiments is to provide a comprehensive overview of the chemical constituents of a specific plant part. The presence of these diverse chemical classes is commonly ascertained through two main types of qualitative tests: those based on precipitation reactions and those based on colorimetric reactions (Ammar et *al.*, 2019; Rao & Suseela, 2016).

d. Thin-Layer Chromatography (TLC) Analysis Protocole

The following details the standard procedure for performing Thin-Layer Chromatography (TLC) for the analysis of plant extracts (Sasidharan et *al.*, 2011).

The preparation of the chromatographic chamber begins by introducing the eluent or solvent mixture. The solvent level is adjusted to approximately half a centimeter from the bottom of the chamber. The container is then sealed to allow the chamber to become fully saturated with solvent vapor. To accelerate both the saturation and the elution process, a strip of filter paper can be placed against the inner walls of the chamber (SiliCycle, n.d.).

For sample spotting on the plate, a clean TLC plate is used if necessary. The sample is dissolved in an appropriate solvent to create a solution between two and five percent concentration. Approximately half a microliter of this solution is spotted onto the plate at a point located one centimeter from the bottom edge. The diameter of the spot should be around two millimeters, especially when multiple samples are being applied (Patra & Panigrahi, 2018). The spot is then dried with a dryer, and new applications may be performed as needed to concentrate the sample.

Once the sample is spotted, the chromatogram development can proceed. The plate is placed vertically in the prepared chamber, and the container is resealed. The elution continues until the solvent front reaches approximately one centimeter from the top edge of the plate. At this point, the plate is removed, and the final position of the solvent front is marked. A line can also be drawn in advance to serve as a reference point for stopping the elution (Deep Blue Repositories, n.d.).

After development, the plate is dried with a dryer. The separated spots are then visualized using either a UV lamp or a chemical staining agent. The spots are circled, and their centers are marked to allow for quantitative analysis. The retention factor, or R-f value, for each compound is calculated by dividing the distance traveled by the solute by the total distance traveled by the solvent (Deep Blue Repositories, n.d.).

For visualization, if the components of the analyzed sample are colored, their separation is easily observable on the plate. If they are colorless, however, they must be made visible through a revelation process. There are two primary methods for this. The first is UV lamp revelation, where the plate is exposed to UV light at two different wavelengths: 254 nm and 366 nm (ResearchGate, 2015). At the 254 nm wavelength, certain compounds with conjugated or aromatic systems appear as brilliant spots against a dark background. At the 366 nm wavelength, other components become visible as luminous, colored spots.

The second method is chemical revelation, where a specific reagent is sprayed onto the plate. This reagent reacts with the separated spots to produce a colored product. For certain revealing reagents, the TLC plate must be heated after the spray application. Some of the most commonly used reagents include Dragendorff's reagent, sulfuric acid, vanillin-sulfuric acid, phosphomolybdic acid, and anisaldehyde-sulfuric acid, all of which require heating (SiliCycle, n.d.; EPFL, n.d.). Sulfuric acid is a particularly versatile revealer because it is capable of visualizing a wide range of natural products, including alkaloids, steroids, unsaturated sterols, saponins, polysaccharides, lipids, and retinoids (EPFL, n.d.).

e. Column Chromatography

Column chromatography is a widely used physicochemical technique for separating and isolating the components of a mixture. The homogeneity and packing of the stationary phase are critical for an efficient separation. Two primary packing methods exist: wet packing and dry packing. The wet-packing method, however, is often preferred for its ability to produce a more uniform and compact column (Chemistry LibreTexts, 2024).

The wet-packing procedure begins by creating a homogeneous slurry. This involves thoroughly mixing the solid stationary phase, typically silica powder, with an appropriate amount of the mobile phase. The goal is to create a lump-free, air-free mixture. A small plug of absorbent material, such as hydrophilic cotton, is placed at the bottom of the column to retain the stationary phase. The slurry is then carefully poured into the glass column in small portions to ensure maximum settling and compaction. Once packed, the excess mobile phase

is allowed to drain through the column's stopcock, but a small amount is intentionally left above the adsorbent's surface. This is a crucial step to prevent the stationary phase from drying out or cracking, which would compromise the separation (Microbe Notes, 2022).

The sample, in this case, a plant extract, is first prepared for loading. A weighed amount of the extract is dissolved in a minimal volume of solvent. This solution is then mixed with a small, equal mass of silica, and the solvent is removed completely using a water bath. The resulting dry, powdered mixture is then carefully and evenly layered onto the top surface of the packed adsorbent. The eluent is then added slowly, ensuring that the deposited extract and the adsorbent remain submerged in the solvent throughout the entire separation process. Finally, the stopcock is opened, and the eluted fractions are collected in a series of numbered vials for subsequent analysis (Biotage, n.d.).

f. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique employed for elucidating the molecular structures of chemical compounds. Its primary application lies in the structural determination of organic molecules, with the most commonly studied nuclei being ^{1}H, carbon ^{13}C, phosphorus ^{31}P, and nitrogen ^{15}N (Lowe & Keeler, 2021).

The fundamental principle of NMR relies on the intrinsic magnetic properties of atomic nuclei. Certain nuclei, such as ^{1}H and ^{13}C, possess a nuclear magnetic moment, which causes them to behave as microscopic magnets characterized by a quantum property known as spin (Lambert et *al.*, 2010).

In the presence of an external magnetic field, these nuclear spins align in discrete energy levels. The energy difference between these levels is directly proportional to the strength of the applied magnetic field (Pretsch et *al.*, 2009). By irradiating the sample with a suitable electromagnetic radiation, transitions between these energy levels can be induced. This absorption of energy at specific frequencies provides the basis for an NMR spectrum.

For analysis, samples are typically dissolved in a deuterated solvent, such as deuterated methanol, chloroform, or pyridine, which do not produce signals in the spectral region of interest (Mahan, 2011). Each of these solvents has a characteristic chemical shift that serves as a reference point. The sample tube is then placed within a strong magnetic field to obtain the spectra necessary for structural elucidation.

III. Result and Discussion

3.1 Results

a. Results of the Extractions

Dried bark from the plant was ground into a fine powder. A 50 g sample of this powder was then subjected to a sequential extraction process using solvents of increasing polarity. The extraction was performed successively with hexane, followed by dichloromethane, and finally methanol. To ensure exhaustive extraction, the process was repeated three times for each solvent. The resulting extracts, rich in different chemical constituents based on their solubility, were collected for subsequent analysis (Barchan et *al.*, 2014 ; Nawaz et *al.*, 2020 ; Wagutu et *al.*, 2022).

The various extracts obtained were collected and stored in individual vials. The following table provides a summary of the mass and yield for each crude extract, highlighting the results of the sequential extraction process.

Table 1. Summary of crude extract masses and yields

Extracts	Mass (g)	Yield (%)
Hexane	1.15	2.30
Dichloromethane (DCM)	0.82	1.64
Methanol (MeOH)	1.53	3.06

b. Phytochemical Screening

1. Scales used

The following table provides a qualitative grading scale for the assessment of various phytochemical reactions. This standardized notation allows for a consistent and reproducible interpretation of assay results.:

Table 2. Grading Scale for Phytochemical Assays

Notation	Precipitation	Coloration	Foam index
-	Negative	No change	0 to 2cm
+	Slight	Faint	2 to 4cm
++	Abundant	Distinct	4 toà 5cm
+++	Significant	Intense	> 5cm

A comprehensive phytochemical screening was conducted on the plant's bark to identify the presence of key secondary metabolites. The results, summarized below, reveal the diverse chemical profile of the plant material. The assays utilized a qualitative grading system to indicate the relative abundance of each chemical family.

Table 3. Phytochemical screening results

Chemical Family	**Part used**	**Results**
Alkaloids	Bark	-
Coumarins	Bark	-
Flavonoids	Bark	-
Polyphenols	Bark	++
Tannins	Bark	+++
Polysaccharides	Bark	++
Quinones	Bark	+
Saponins	Bark	+
Steroids	Bark	-
Terpenoids	Bark	+++

The screening indicates a significant presence of tannins and terpenoids, both marked with a "+++" rating, suggesting they are major constituents of the bark. Polyphenols and polysaccharides were also found in moderate abundance ("++"), while quinones and saponins were present in lower concentrations ("+"). In contrast, the absence of alkaloids, coumarins, flavonoids, and steroids was noted, as indicated by a negative result ("-"). This profile provides valuable insight into the biological and pharmacological properties of the plant.

c. Chromatographic analysis results

1. Thin-Layer Chromatography (TLC)

Chromatographic analysis was performed using pre-coated silica gel plates on aluminum sheets (MERCK, type 60 F254, 0.2 mm thickness). The hexane extract of the plant bark was selected for analysis.

Prior to column chromatography, thin-layer chromatography was used to screen various solvent systems to identify the one that provided the best separation. Pure dichloromethane (100%) was ultimately chosen as the optimal mobile phase.

The chromatogram was visualized using a two-step process. First, the plate was exposed to a UV lamp at 254 nm and 366 nm, and any visible spots were marked with a pencil. The plate was then sprayed with a 10% sulfuric acid solution in methanol and heated at 110 °C to reveal additional compounds. The image below shows the resulting chromatogram of the hexane extract.

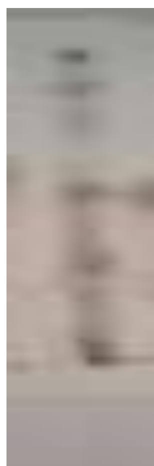

Figure 2. TLC of the hexane extract. Mobile phase: Dichloromethane.
Visualization: H_2SO_4 spray followed by heating at 110 °C.

2. Column Chromatography of the Hexane Extract

Column chromatography was employed to fractionate the hexane extract. The separation was performed using a glass column with a height of 50 cm and a diameter of 1 cm. The column was packed with silica gel to a height of 46 cm. The silica gel had a particle size ranging from 0.02 to 0.04 mm.

Approximately 100 mg of the extract was loaded onto the column. Elution was performed using 100% dichloromethane as the mobile phase. A total of 65 fractions were collected, with each fraction having a volume of approximately 1 mL. The migration of the different components of the crude extract was monitored by analyzing the fractions using thin-layer chromatography (TLC) (Odebiyi & Sofowora, 1978; Yadav et *al.*, 2017). The resulting TLC chromatogram is shown below.

Figure 3. TLC analysis of the collected fractions. Mobile phase: Dichloromethane. Visualization: H_2SO_4 spray followed by heating at 110 °C.

Upon analysis by thin-layer chromatography (TLC), fractions 58 through 60 (F58-60) showed a single, well-defined spot, indicating the presence of a pure compound. These fractions were combined, and the solvent was removed by evaporation. The isolated pure product weighed 8 mg and was subsequently set aside for structural analysis.

Figure 4. TLC analysis of fractions 58-60 (F58-60). Mobile phase: Dichloromethane. Visualization: UV light at 254 nm.

d. Structural Elucidation of the Isolated Compound

Structural determination of the purified compound (F58-60) was carried out at the Institute of Organic Chemistry at the University of Hanover, Germany. The sample was prepared by dissolving it in deuterated chloroform ($CDCl_3$). NMR spectra were recorded on a Bruker AV 400 spectrometer, operating at 400 MHz for 1H NMR and 100 MHz for ^{13}C NMR. Chemical shifts were referenced to TMS as an internal standard. High-resolution mass spectrometry (HRMS) was also performed at the same institute, yielding a molecular formula of $C_{24}H_{38}O_4$.

1. NMR ^{13}C Spectral Analysis

The fully decoupled ^{13}C NMR spectrum reveals a total of 12 signals, indicating the presence of 12 unique carbon atoms. Analysis of the DEPT spectra further clarified the carbon types : three methine (CH) groups, five methylene (CH_2) groups, and two methyl (CH_3) groups were identified. Comparison of the DEPT data with the fully decoupled spectrum confirmed the presence of two quaternary carbons.

Several key signals were assigned based on their chemical shifts :

- A signal at 167.6 ppm is characteristic of a quaternary carbonyl carbon (C=O) within an ester group.
- The aromatic region displays a quaternary carbon signal at 132.4 ppm and two methine (CH) signals at 130.8 ppm and 128.7 ppm.
- A signal at 68.1 ppm is consistent with a methylene carbon (CH_2) bonded to an oxygen atom in an ester.

The presence of only 12 unique carbon signals, while the molecular formula ($C_{24}H_{38}O_4$) suggests 24 carbon atoms, strongly indicates that the isolated compound possesses a symmetrical structure with a $C_{12}H_{19}O_2$ moiety as a repeating unit.

The degree of unsaturation (or double bond equivalents, DBE) was calculated using the formula:

$$DBE = C - \frac{H}{2} + \frac{N}{2} + 1$$

For the molecular formula $C_{24}H_{38}O_4$, the calculation is as follows:

$$DBE = 24 - \frac{38}{2} + 0 + 1 = 24 - 19 + 1 = 6$$

A DBE of 6 suggests the presence of both rings and/or multiple bonds. Based on the NMR data, three of these unsaturations correspond to an aromatic ring, while the remaining two are attributed to two ester carbonyl groups. This leaves a remaining DBE of 1, which corresponds to the presence of a single ring.

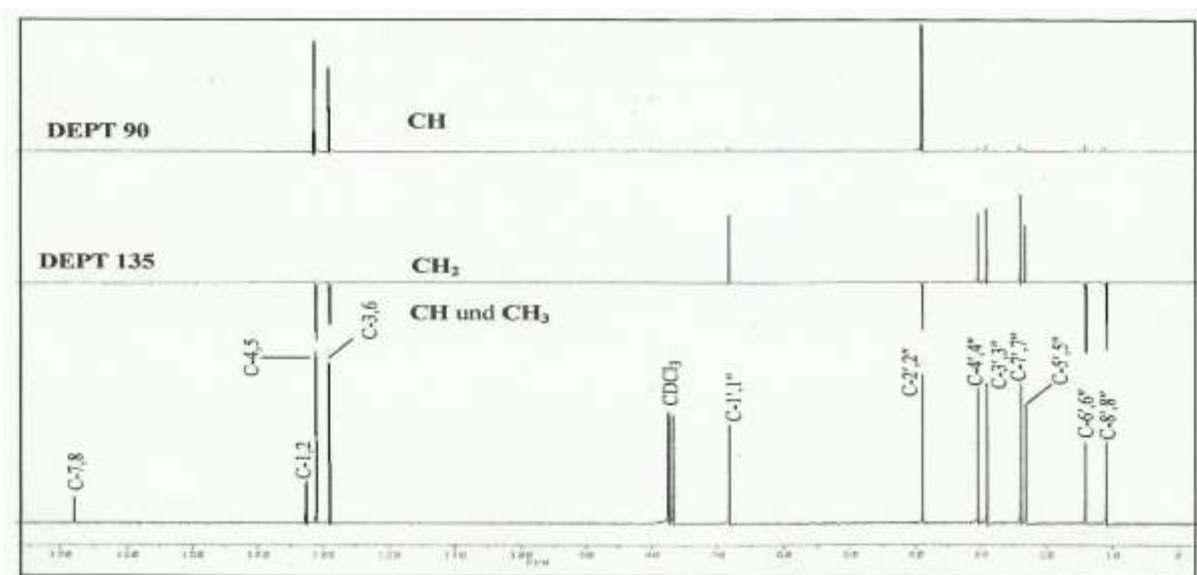

Figure 1. ^{13}C NMR spectra of the isolated compound

The bottom spectrum is the fully decoupled ^{13}C NMR spectrum, which shows all carbon signals as singlets. The middle spectrum is the DEPT 135 spectrum, which reveals the presence of CH and CH_3 groups as positive signals, and CH_2 groups as negative signals. The top spectrum is the DEPT 90 spectrum, which exclusively shows signals for CH groups.

By combining the information from these three spectra, the number of each type of carbon can be determined.

- The DEPT 90 spectrum (top) shows two signals, indicating the presence of two CH groups. These correspond to the peaks labeled C-1,2 and C-3,6 in the DEPT 135 spectrum.

- The DEPT 135 spectrum (middle) shows multiple signals. The negative signals (pointing down) correspond to CH_2 groups. There are five distinct negative signals, indicating the presence of five CH_2 groups.
- The DEPT 135 spectrum also shows positive signals. By subtracting the two CH signals identified in the DEPT 90 spectrum, the remaining positive signals are attributed to CH_3 groups. There are two such signals, confirming the presence of two CH_3 groups.

Finally, by comparing the fully decoupled ^{13}C spectrum with the DEPT spectra, the signals that are present in the former but absent in the latter correspond to quaternary carbons. Two such signals are clearly visible in the fully decoupled spectrum, confirming the presence of two quaternary carbons.

In summary, the spectroscopic data indicates the isolated compound contains:

- Two quaternary carbons
- Two CH groups
- Five CH_2 groups
- Two CH_3 groups

This detailed analysis of the carbon environment is crucial for the full structural elucidation of the compound.

2. ^{1}H NMR spectral analysis

The ^{1}H NMR spectrum of the isolated compound exhibits a total of six signals. The integration curve, with its six plateaus, presents a proton ratio of 1:1:2:1:8:6. When scaled to the molecular formula of $C_{24}H_{38}O_4$, these integrations correspond to the following proton counts: 2H, 2H, 4H, 2H, 16H, and 12H, respectively.

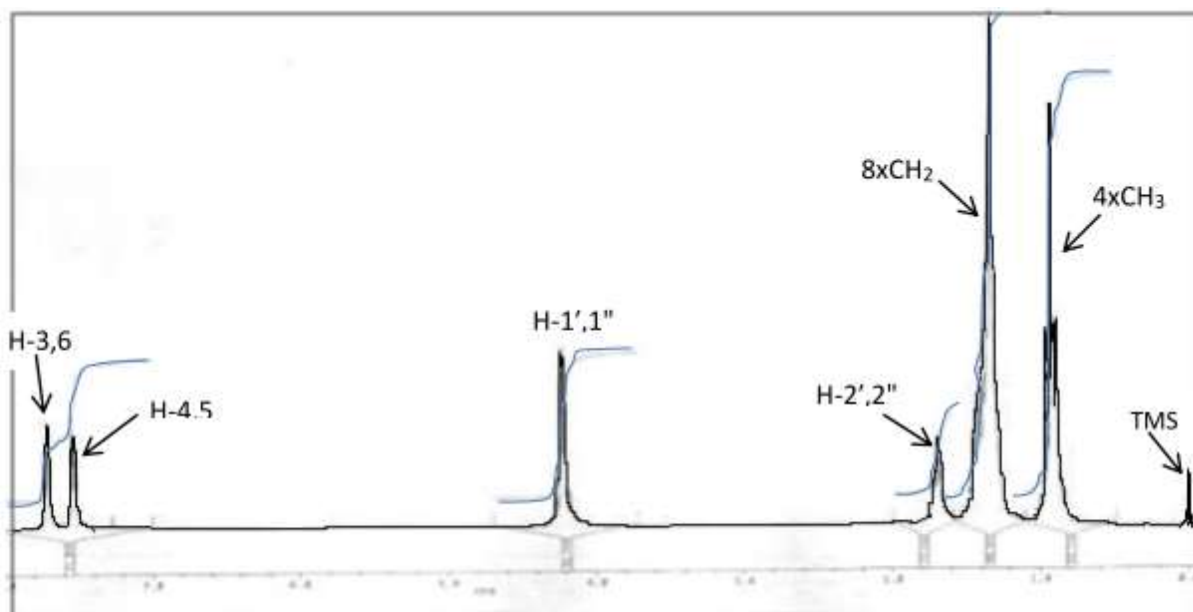

Figure 6. ^{1}H NMR spectrum of the isolated compound (F58-60)

3. Signal Assignments

- Two signals observed in the aromatic region, at 7.52 ppm and 7.70 ppm, are each integrated for two protons (2H). These are consistent with the presence of four aromatic CH groups, which is characteristic of a symmetrical disubstituted aromatic ring.
- A signal at 4.22 ppm integrates for four protons (4H) and is attributed to two equivalent CH_2 groups adjacent to an oxygen atom (CH_2−O), likely part of an ester linkage.
- A broad multiplet centered around 1.38 ppm integrates for 16 protons (16H). This large signal is highly indicative of multiple, chemically equivalent CH_2 groups in a long alkyl chain.
- Finally, a large triplet signal at 0.92 ppm integrates for 12 protons (12H), which corresponds to four equivalent CH_3 groups. The triplet multiplicity suggests these methyl groups are coupled to an adjacent CH_2 group.

These assignments align with the symmetrical structure inferred from the ^{13}C NMR data and provide compelling evidence for the presence of an aromatic core, ester functionalities, and long alkyl chains with terminal methyl groups.

Comparison of the spectral data of the isolated compound with those in the literature (Sadtler Research Laboratories, 1970; Sadtler Research Laboratories, 1978) leads to the structure of di(2-ethylhexyl) phthalate.

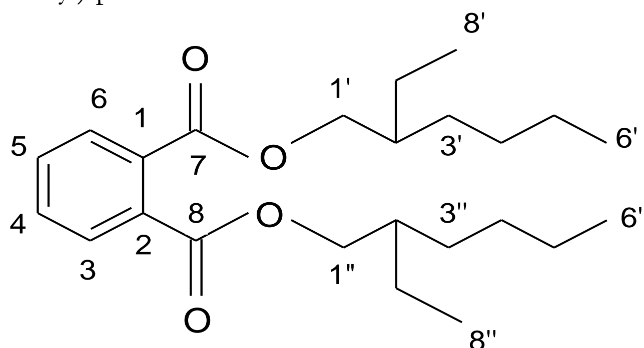

Figure 2. Structure of di(2-ethylhexyl) phthalate

Here is a refined, eloquent, and scientific rephrasing of the provided text and table.

Table 4. ^{13}C NMR data: Isolated Compound vs. Literature

Carbon Atoms	Isolated compound in $CDCl_3$ (ppm)	Literature (in $CDCl_3$) (ppm)
C-1,2	132.4	132.8
C-3,6	128.7	128.9
C-4,5	130.8	130.9
C-7,8	167.6	167.6
C-1’,1”	68.1	68.1
C-2’,2”	38.7	39.0
C-3’,3”	28.8	29.1
C-4’,4”	30.8	30.6
C-5’,5”	22.9	23.1
C-6’,6”	13.7	14.1
C-7’,7”	23.7	23.9
C-8,8”	10.9	11.9

The strong correlation between the experimental and literature chemical shifts provides conclusive evidence for the compound's structure. The minor variations observed

are within the typical range for such measurements and can be attributed to slight differences in sample concentration, temperature, or instrument calibration. This comparative analysis confirms the successful isolation and identification of the target compound.

3.2 Discussion

a. Phytochemical Screening Discussion

The results of the preliminary phytochemical screening of the plant's bark provided a comprehensive profile of its secondary metabolite composition. The analyses revealed the presence of a diverse range of compounds, including quinones, saponins, polyphenols, polysaccharides, tannins, and terpenoids. The findings indicate that tannins and terpenoids are particularly abundant, suggesting they may be the predominant chemical classes responsible for the plant's biological activity. Conversely, the concentration of quinones and saponins was found to be relatively low, with some appearing to be present only in trace amounts. These results provide a foundational understanding of the chemical nature of the plant, guiding subsequent targeted isolation and structural characterization efforts.

b. Chromatographic analysis discussion

1. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) played a crucial role in the analytical workflow, serving as both a preliminary screening tool and a control mechanism. First, it was used to identify an optimal solvent system for the separation of the constituents within the hexane extract. This exploratory phase led to the selection of pure dichloromethane as the ideal mobile phase, as it provided superior resolution and distinct separation of the different components.

Second, TLC analysis helped validate some of the phytochemical screening results. The use of a universal spray reagent like 10% sulfuric acid in methanol allowed for the visualization of a wide range of natural products, including alkaloids, steroids, saponins, and lipids, providing an initial chromatographic confirmation of their presence or absence as indicated by the initial spot tests.

2. Column chromatography

The successful isolation of a pure compound from the crude extract was achieved through column chromatography. This separation was conducted with a high degree of precision. Fractions collected during the elution process were systematically monitored using TLC. This meticulous approach ensured that fractions containing a single, distinct spot—and in sufficient quantity (over 4-5 mg) for further analysis—were pooled for subsequent structural elucidation.

A key factor in the successful isolation was the use of 100% dichloromethane as the eluent. The efficacy of this mobile phase, determined during the preliminary TLC trials, was critical for achieving a clean and efficient separation. Furthermore, the practice of collecting a large number of small-volume fractions enhanced the resolution, preventing the co-elution of components and maximizing the purity of the isolated compound. This refined approach demonstrates the importance of both thoughtful solvent selection and meticulous fraction collection in achieving high-purity isolation from complex natural product extracts.

IV. Conclusion

This study focused on the phytochemical investigation of *Cynometra madagascariensis*, a plant of the Fabaceae family native to southeastern Madagascar. Locally known as Mapagne, Mapange, or Arivo Ravy, this plant is traditionally used to treat and dry burns and other wounds. The present work aimed to conduct a comprehensive physicochemical analysis, including extraction, phytochemical screening, chromatographic separation, and structural analysis of isolated compounds.

Successive maceration extractions with solvents of increasing polarity—hexane, dichloromethane, and methanol—yielded crude extracts, the mass percentages of which were determined. Phytochemical screening of the bark revealed the presence of several key secondary metabolites, notably quinones, saponins, polyphenols, polysaccharides, tannins, and terpenoids. The analysis indicated that tannins and terpenoids are present in high concentrations, while quinones and saponins are in low or trace amounts.

Chromatographic analysis was instrumental in the separation and purification of the plant's constituents. Thin-layer chromatography (TLC) was used to optimize a solvent system for the hexane extract, which subsequently led to the successful isolation of a pure compound via column chromatography. The structure of this isolated compound, designated F58-60, was elucidated using one-dimensional NMR spectroscopy (1H and 13C NMR). Comparison of the spectroscopic data with literature values confirmed the compound's identity as di(2-ethylhexyl) phthalate.

This research has provided valuable insights into the chemical profile of *Cynometra madagascariensis* and served as a foundation for future studies. Future work should prioritize the isolation and structural determination of other compounds within the extracts. Furthermore, a detailed investigation into the biological activities of the isolated compounds and the crude extracts is warranted to validate the traditional medicinal uses of the plant. Continued research on other related plant species is also recommended.

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