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Phytochemical study and structural determination of the isolated product of *Ricinus communis* (Euphorbiaceae)

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Abstract:

A phytochemical and structural study of the leaves of Ricinus communis (castor bean plant) was conducted to identify and characterize its active compounds. The plant material, sourced from Madagascar, was subjected to sequential maceration with solvents of increasing polarity, yielding hexane, dichloromethane (DCM), and methanolic extracts. Phytochemical screening revealed the presence of polysaccharides as the most abundant components, with moderate amounts of steroids and low concentrations of alkaloids and saponins. The DCM extract was further purified using thin-layer chromatography and column chromatography, leading to the isolation of a pure compound. Structural elucidation was performed using mass spectrometry (EI-MS) and one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy, including 1H, 13C, and DEPT-135 experiments. The spectroscopic data established a molecular formula of C28H46O and a tetracyclic steroid structure with two degrees of unsaturation. A comparison of the obtained chemical shifts with those reported in the literature confirmed the compound's identity as Brassicosterol, a key phytosterol.

Keywords:
Phytochemical, Ricinus communis, Chromatography, NMR Spectroscopy, Brassicosterol.

I. Introduction

Approximately 250,000 of the world's estimated several hundred thousand plant species have been described and cataloged. These organisms are vital for synthesizing essential primary metabolites, such as amino acids, proteins, sugars, and nucleic acids (**Dewick, 2002**). Over recent decades, there has been a significant surge in the use of medicinal plants and plant-based preparations. The World Health Organization (WHO) estimates that approximately 80% of the global population relies on traditional medicine for their primary healthcare needs (**WHO, 2019**). The widespread adoption of herbal remedies is attributed to various factors, including the high cost of conventional pharmaceuticals, entrenched sociocultural practices, the pressing need for alternative therapeutic agents to combat drug-resistant pathogens, and the absence of effective treatments for certain diseases.

The pharmaceutical industry has increasingly invested in research and development of plant-derived medicines. Natural compounds isolated from plants often serve as crucial structural and functional templates for the rational design of new drugs with analogous molecular architectures (**Rates, 2001**). It has been documented that more than 1,300 medicinal plant species are used as traditional remedies across diverse cultures globally (**Fabricant & Farnsworth, 2001**). This has led to the establishment of extensive research programs, such as

the one in Madagascar, which seeks to inventory all plant species with potential therapeutic properties (**Robinson, 1995**). In Madagascar, both urban and rural populations are increasingly using medicinal plants to address their health concerns.

The therapeutic efficacy of these plants is due to their phytochemical composition, which includes diverse compounds that can be extracted, isolated, and their structure elucidated using a range of sophisticated analytical techniques. These methods allow for the identification and confirmation of the presence of specific active substances responsible for the observed medicinal properties.

Our research is entitled "evaluation of soils and its covers before and after a disaster natural disasters in Madagascar", an application project which aims to understand the impact of natural disasters on the floors and their coverings. We aim to assess Madagascar in the face of natural disasters, to start the study, we start the assessment with a region most affected by natural disasters in 2 cases. The project includes two phases: a pre-disaster assessment phase and an assessment phase after disaster. (FAO, 2022)

Pre-disaster assessment phase: The pre-disaster assessment phase involves collecting data on soils and their covers in areas at risk of natural disasters. These data are collected using remote sensing, geophysics and pedology techniques. The data collected is used to create maps of soils and their covers. These cards are used to identify areas most vulnerable to natural disasters. (Catry et al., 2020)

Post-disaster assessment phase: The post-disaster assessment phase involves collecting data on soils and their covers in areas that have been affected by a natural disaster. These Data is collected using the same techniques used in the evaluation phase before disaster.

The data collected is used to compare the condition of soils and their covers before and after the disaster. This comparison allows us to understand the impact of the disaster on the soil and its cover. We expect research results to show that natural disasters can have a significant impact on soils and their coverings or not. Cyclones can cause soil erosion, degradation of plant covers and soil contamination. These results are important for management natural disaster risks in Madagascar. They will allow us to better understand the impacts of natural disasters on soils and their covers and to develop more risk management strategies effective. (Fidan et al., 2023)

II. Research Method

2.1 Plant material

Ricinus communis, commonly known as the castor bean plant, is a large, perennial shrub belonging to the family Euphorbiaceae . Although often cultivated as an annual in temperate climates, its natural habitat is tropical and subtropical regions, likely originating in East Africa (**Jansen et *al.*, 2007**). This fast-growing plant is characterized by a distinctive and striking morphology.

The stems of *R. communis* are typically stout and hollow, with a smooth surface that can range in color from green to reddish-purple, often with a waxy bloom. The leaves are a key identifying feature, being large, palmate, and deeply lobed with 5 to 11 toothed segments. They are arranged alternately on the stem and have long petioles. The leaves themselves can

vary in color from a bright green to a deep reddish-bronze or purplish-black, depending on the cultivar (**Weiss, 2000**).

The plant is monoecious, bearing both male and female flowers on the same inflorescence, which is a large terminal panicle (**Tetenyi, 2002**). The male flowers are located on the lower part of the panicle and are numerous, with prominent yellow stamens. The female flowers, situated at the top of the panicle, are characterized by red, spiky stigmas. This arrangement ensures efficient wind pollination.

The fruit of *R. communis* is a spiny, spherical capsule, typically green or reddish, that dehisces explosively upon maturity to release the seeds (**He et *al.*, 2011**). The seeds are oval and have a smooth, mottled surface with various patterns of brown, black, white, and gray. A small, fleshy appendage called the caruncle is present at one end of the seed. These seeds are the source of castor oil, but they also contain the highly toxic protein **ricin**, a potent ribosome-inactivating protein (**Olsnes & Pihl, 1982**).

Leaves Fruits

Figure 1. *Ricinus communis*

Ricinus communis is extensively used in traditional medicine in Madagascar to treat a variety of ailments (**Rakotoarison et *al.*, 2020**). The plant's properties are harnessed to alleviate headaches and manage certain open wounds, demonstrating its local application as an analgesic and wound care agent. Furthermore, its efficacy as a natural insecticide is recognized, highlighting its versatile use beyond medicinal purposes in local communities (**Ramihantaniariany et *al.*, 2022**). These traditional practices underscore the plant's significant role in indigenous healthcare systems.

2.2 Plant extraction methodologies

The isolation of bioactive compounds from plant material is a multi-stage process that begins with a suitable extraction technique. Solid-liquid extraction is a fundamental method used to separate a soluble compound from a solid matrix using a liquid solvent. This is typically achieved by immersing the solid in a solvent at various temperatures, ranging from ambient to boiling, to dissolve the target constituents (**Dewick, 2002**). Common variations of this method include maceration, infusion, and decoction.

To comprehensively fractionate the diverse phytochemicals within a plant based on their polarities, a systematic successive maceration protocol is frequently employed. This technique utilizes a series of solvents in a polarity gradient, typically starting with a non-polar

solvent like hexane, followed by a moderately polar one such as dichloromethane, and concluding with a highly polar solvent like methanol. This sequential approach ensures that compounds are selectively extracted according to their solubility characteristics.

Prior to extraction, the plant material requires meticulous preparation. Drying of the leaves away from direct sunlight is crucial to eliminate water content and prevent the degradation of heat-sensitive compounds (**Sasidharan et *al.*, 2011**). Subsequent grinding of the dried material into a fine powder significantly increases the surface area, thereby maximizing the efficiency of the solvent's interaction with the plant matrix during the extraction process.

Each maceration step in the successive protocol is typically performed for a prolonged duration, such as 48 hours. The resulting heterogeneous mixture is filtered, and the solid residue is then re-extracted with the next solvent in the polarity series. To optimize compound recovery, this process is generally repeated three times for each solvent. The collected filtrates are then combined and concentrated using a rotary evaporator at a moderate temperature, usually below 40 °C, to yield a dry extract.

2.3 Phytochemical screening

Phytochemical screening is a qualitative analysis technique used to identify the presence of specific chemical compound classes within a plant extract or drug. This preliminary assessment is based on characteristic reactions that produce a colored complex or an insoluble precipitate (**Harborne, 1998**).

a. Alkaloid identification

The presence of alkaloids can be determined by precipitation reactions with specific reagents. The procedure involves acidifying a dry plant powder with 10% hydrochloric acid for 10-15 minutes, with agitation, followed by filtration. The resulting filtrate is divided into four test tubes, with one serving as a control. The remaining tubes are treated with Wagner's, Mayer's, and Dragendorff's reagents. A positive result is indicated by the formation of a precipitate: a yellowish precipitate with Wagner's reagent, an orange precipitate with Mayer's reagent, and a reddish-orange precipitate with Dragendorff's reagent (**Khadem & Naser, 2018**).

b. Screening for coumarins

Coumarins are typically detected using a colorimetric reaction under ultraviolet (UV) light. A paper strip is saturated with ammonium hydroxide and an ethanolic plant extract is spotted onto it. The paper is then examined under a UV light source at a wavelength of 365 nm. The appearance of a yellow fluorescence around the spot indicates the presence of coumarins in the plant extract.

c. Detection of flavonoids

Flavonoids are identified using color-based tests such as the Wilstater and Bath-Smith tests (**Khadem & Naser, 2018**). A dried plant powder is macerated in ethanol, and after solvent evaporation, the residue is washed with hexane to remove pigments. The remaining residue is dissolved in 30 mL of ethanol and filtered. The filtrate is then divided into two test tubes. Heating one portion with concentrated HCl produces a violet-red color, indicating the presence of leucoanthocyanins. The appearance of a red color upon the addition of concentrated HCl to the other portion at room temperature signifies the presence of anthocyanins.

d. Assessing polyphenols and tannins

The presence of polyphenols and tannins is revealed through precipitation reactions with protein-based solutions. An aqueous alcoholic extract of the plant powder is prepared by heating and agitating the mixture with distilled water and a 10% sodium chloride solution. The filtrate is then divided into three test tubes. The formation of a precipitate after adding 1% gelatin solution to one tube indicates the presence of polyphenols. A separate test for tannins involves observing the formation of a precipitate upon the addition of a gelatin-salt solution to another tube.

e. Detection of polysaccharides

Polysaccharides are identified through a simple precipitation test. A decoction of the dried plant material is prepared and filtered. When a small volume of the filtrate is combined with three volumes of ethanol, the formation of a precipitate confirms the presence of polysaccharides.

f. Screening for quinones

The presence of quinones is typically confirmed by a color change in the alkaline phase of an extract. A hydro-alcoholic extract of the plant is dissolved in distilled water and filtered. The filtrate is then extracted with a 3:1 petroleum ether-chloroform mixture. Upon the addition of a 50% diluted ammonium hydroxide solution, the lower, alkaline phase turns purple, which is indicative of quinones.

g. Saponin characterization

Saponins are characterized by their foaming properties when agitated in an aqueous solution. A small amount of powdered plant material is placed in a test tube with distilled water and shaken vigorously for 30 seconds. The formation of a stable foam with a height of at least 3 cm indicates the presence of saponins in the plant.

h. Steroids and terpenoids profiling

The detection of steroids and triterpenoids involves distinct colorimetric reactions. A hydro-alcoholic extract is prepared, and pigments are removed by repeated washing with petroleum ether. The residue is dissolved in chloroform or dichloromethane. After drying the solution with anhydrous sodium sulfate and filtering, the filtrate is subjected to two key tests.
The Liebermann-Burchard test involves adding acetic anhydride and concentrated sulfuric acid to a portion of the filtrate. A purple coloration suggests the presence of triterpenoids, while a violet or blue-green color indicates steroids.

The Salkowski test is performed by adding concentrated sulfuric acid to another portion of the filtrate, observing the interface between the two liquid layers. A red ring at the point of separation confirms the presence of unsaturated sterols.

2.4 Chromatography analysis

a. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a fundamental separation technique that relies on adsorption phenomena, where a mobile phase—a solvent or a mixture of solvents—travels along a stationary phase fixed on a glass or aluminum plate. After a sample is applied to the stationary phase, its components migrate at a rate determined by their inherent properties and the composition of the mobile phase (**Zubairi & Sarwono, 2021**).

In this study, TLC was employed to separate and identify the chemical compounds present in the three extracts. This approach aimed to determine if there were differences in the efficiency of the various extraction methods used.

b. Principle of Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a straightforward and widely used technique for separating and identifying compounds in a mixture. The process relies on a few key components and a series of well-defined steps to achieve separation.

The fundamental setup for a TLC separation consists of four main elements. First, a chromatographic chamber is a glass container with a tight-fitting lid, used to create a sealed, solvent-saturated atmosphere that ensures consistent separation. Second, the sample, a small volume of a concentrated solution (typically 2-5% by mass) is applied as a small, precise spot on the TLC plate. Third, the eluent serves as the mobile phase. This is a pure solvent or a solvent mixture that slowly moves up the plate, carrying the sample's components with it. Finally, the TLC plate itself is the stationary phase, a small rectangular aluminum sheet coated with a 0.2-mm-thick layer of an adsorbent, most commonly silica gel.

Elution is the core process where separation occurs. After the sample is spotted, the plate is placed inside the sealed chamber, allowing the eluent to ascend the stationary phase via capillary action. The solvent's journey up the plate is referred to as the solvent front. Elution is stopped when the solvent front reaches a predetermined height, usually about 1 cm from the top of the plate. The plate is then removed, and the solvent front is immediately marked with a pencil to calculate the retention factor (R_f) value. After marking, the plate is dried to remove all residual solvent.

Once the compounds have been separated, they need to be visualized. While some compounds are naturally colored and can be seen with the naked eye, most are colorless. Two primary methods are used for visualization. The first is a physical method, where the dried plate is placed under an ultraviolet (UV) lamp at wavelengths of 254 nm or 375 nm, causing the separated compounds to appear as dark or fluorescent spots against the background. The second is a chemical method, which involves spraying the plate with a universal chemical stain, such as p-anisaldehyde, followed by heating in an oven at 110–120 °C for approximately 10 minutes (**Sarkar et *al.*, 2020**). This heating step causes a chemical reaction that makes the compounds visible as colored spots.

2.5 Spectral analysis

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure of unknown organic molecules. This method provides detailed information through the analysis of both proton and carbon-13 spectra.

a. Proton NMR (1H NMR) Spectroscopy

1H NMR spectroscopy provides crucial data for structural elucidation, including the number of signals, chemical shifts, signal integration, and signal multiplicity.
The number of signals observed in a 1H NMR spectrum directly corresponds to the number of sets of chemically equivalent protons within a molecule.

The chemical shift, denoted by δ and expressed in parts per million (ppm), provides information about a proton's chemical environment. All equivalent protons resonate at the same chemical shift. The specific δ value is a key indicator of the functional group to which the proton is attached and can be referenced against standard data tables.

The area under an NMR signal is directly proportional to the number of protons responsible for that signal. The integration curve of the spectrum appears as a series of steps, where the height of each step is proportional to the number of equivalent protons corresponding to the signal. This allows for the determination of the relative ratio of different types of protons in a molecule.

The multiplicity of a signal—the number of peaks it contains—is determined by the influence of neighboring protons. Protons with no neighboring equivalent protons appear as a single peak, called a **singlet**. When neighboring equivalent protons are present, their mutual influence causes the signal to split into a multiplet. According to the (n+1) rule, a group of equivalent protons with n neighboring equivalent protons will display a signal as a multiplet with (n+1) peaks.

b. Carbon-13 NMR (^{13}C NMR) spectroscopy

^{13}C NMR spectroscopy is essential for structural determination as it reveals the number and types of carbon atoms (primary, secondary, tertiary, and quaternary) within an organic molecule.

c. Proton-decoupled ^{13}C NMR

This type of spectrum is obtained by applying a radiofrequency pulse that decouples all proton-carbon spin-spin couplings during acquisition. This process simplifies the spectrum, as each unique carbon atom produces a single signal, provided there is no signal overlap. The intensity of the signals is enhanced by the presence of attached protons, though signal intensity in a decoupled spectrum is not directly proportional to the number of equivalent carbons. This technique is primarily used to determine the total number of distinct carbon environments.

d. Distortionless Enhancement by Polarization Transfer (DEPT) spectroscopy

DEPT spectroscopy is a powerful technique that differentiates carbon signals based on the number of attached protons. It utilizes multi-pulse sequences that transfer polarization from protons to carbons. By varying the pulse angle (θ), specific carbon types can be identified:

DEPT-45 (θ=45∘): Displays positive signals for **CH_3**, **CH_2**, and **CH** groups.

DEPT-90 (θ=90∘): Only shows positive signals for **CH** groups.

DEPT-135 (θ=135∘): Provides positive signals for **CH_3** and **CH** groups, while **CH_2** groups appear as negative signals. Quaternary carbons, which have no attached protons, do not appear in any DEPT spectrum.

III. Result and Discussion

To analyze the phytochemical composition of a plant, a common approach involves a multi-step extraction process followed by qualitative phytochemical screening. The following results illustrate this methodology.

3.1 Extraction Results

A 20 g sample of powdered plant material was sequentially extracted with three solvents of increasing polarity: hexane, dichloromethane (DCM), and methanol. The yields for each extraction step were measured and are summarized in Table 1.

The initial extraction with 250 mL of hexane yielded 1.13 g of crude extract, corresponding to a 5.65% yield. Subsequent extraction with 250 mL of DCM provided a

crude extract of 1.12 g, a 5.60% yield. Finally, the methanolic extraction, also using 250 mL, resulted in a 1.09 g crude extract, a 5.45% yield. The data shows relatively consistent yields across all three solvents, indicating a diverse distribution of chemical compounds with varying polarities within the plant matrix.

Table 1. Summary of sequential extraction yields.

Solvent	Volume (mL)	Crude Extract Yield (g)	Yield (%)
Hexane	250	1.13	5.65
DCM	250	1.12	5.60
Methanol	250	1.09	5.45

3.2 Phytochemical screening results

Qualitative phytochemical analysis was conducted to confirm the presence or absence of key secondary metabolites. The results were graded based on a standardized scale for precipitation, coloration, and foam height, as shown in Table 2.

Table 2. Scoring scale for phytochemical analysis results.

Notation	Precipitation	Coloration	Foam Index (cm)
-	Negative	No Change	0 to 2
+	Weak	Weak	2 to 4
++	Abundant	Clear	4 to 5
+++	Strong	Intense	Above 5

This scoring key provides a standardized framework for the semi-quantitative assessment of phytochemical screening results. The scale, ranging from negative to strong, correlates qualitative observations, such as precipitation, coloration, and foam index, with a graded notation for consistent data interpretation (Harborne, 1998 ; Khadem & Naser, 2018).

a. Alkaloids, coumarins, and flavonoids

Tests for these compound classes yielded predominantly negative results. The Mayer's reagent test for alkaloids showed a weak positive result (+), indicated by slight precipitation, suggesting a low concentration of these compounds. However, tests with Wagner's and Dragendorff's reagents were negative. No characteristic yellow fluorescence was observed under UV light, confirming the absence of coumarins. Similarly, the Wilstater and Bath-Smith tests for flavonoids were negative, with no color change observed, indicating their absence.

b. Polyphenols, tannins, and quinones

The tests for polyphenols and tannins, which rely on precipitation with gelatin, yielded negative results. Similarly, the test for quinones was also negative, as no color change was observed in the alkaline phase of the extract. These results suggest these secondary metabolites are not present in the plant.

c. Polysaccharides

A precipitation test with ethanol yielded an abundant precipitate (+++), strongly confirming the significant presence of polysaccharides in the plant.

d. Saponins

The foaming test for saponins produced a stable foam with a height of 2 to 4 cm (+), verifying the presence of these compounds in the plant material.

e. Steroids and Terpenoids

The Liebermann-Burchard test, performed to identify these compounds, resulted in a clear violet coloration (++), which is indicative of steroids. However, the Salkowski test for unsaturated sterols was negative, as no red ring formed at the interface between the two liquid phases. These results confirm the presence of steroids but the absence of unsaturated sterols.

f. Summary of Phytochemical Results

The phytochemical screening revealed the presence of several key secondary metabolites. As summarized in Table 3, the plant is rich in polysaccharides, with steroids and saponins also present. Alkaloids are found in low concentrations, while coumarins, flavonoids, polyphenols, tannins, quinones, and terpenoids were not detected.

Table 3. Summary of phytochemical screening results.

Chemical Family	Result
Alkaloids	+
Coumarins	-
Flavonoids	-
Polyphenols	-
Polysaccharides	+++
Quinones	-
Saponins	+
Steroids	++
Tannins	-
Terpenoids	-

Analysis of the phytochemical screening results reveals the presence of several key secondary metabolites in the plant. Polysaccharides were detected in high abundance (+++), while steroids were moderately present (++). Alkaloids and saponins were also identified, albeit in lower concentrations (+). Conversely, the absence of coumarins, flavonoids, polyphenols, tannins, quinones, and terpenoids was confirmed through the conducted tests. These findings collectively establish the plant's unique phytochemical profile (Sasidharan et al., 2011).

3.3 Chromatography separation and analysis

a. Thin Layer Chromatography (TLC) results

Thin-layer chromatography (TLC) is a crucial preliminary step in natural product chemistry, enabling the optimization of solvent systems for larger-scale separations. For this analysis, a dichloromethane (DCM) extract from the plant's leaves was examined on pre-coated Merck 60F254 plates, which feature a 0.2 mm layer of silica gel on an aluminum backing. Several solvent systems were tested to achieve the most effective chromatographic resolution, ensuring that the extract's chemical constituents were clearly separated (Zubairi & Sarwono, 2021). The resulting chromatogram provided the basis for the subsequent preparative-scale separation .

Figure 2. Analyse chromatographique d'extrait DCM

The resulting chromatogram provided the basis for the subsequent preparative-scale separation

b. Open-Column Chromatography

Following the preliminary TLC analysis, a preparative open-column chromatography procedure was performed to isolate the compounds. A glass column, measuring 50 cm in length and 1.2 cm in diameter, was dry-packed with silica gel to a height of 42 cm. Approximately 120 mg of the DCM extract was loaded onto the column. Elution was conducted using a gradient solvent system that began with pure DCM and transitioned to a mixture of DCM and methanol (99:1 v/v).

A total of 122 fractions were collected from the column's outlet. The chemical composition of each fraction was monitored using analytical TLC, and fractions with similar chromatographic profiles were pooled together. This systematic approach allowed for the efficient separation of the extract's components into distinct groups, which were then prepared for further structural characterization .

Fractions N° 34 - 37 (F34-37) revealed with H_2SO_4 Fractions N° 94-105 under UV lamp

Figure 3. Fractions from column chromatography of DCM extract of *Ricinus communis* leaves

A successful chromatographic analysis hinges on the selection of an optimal solvent system. The TLC results confirmed that a dichloromethane/methanol (DCM/MeOH) gradient, specifically at ratios of 99:1 or 98:2, provided excellent chromatographic separation of the sample's constituents. Subsequent column chromatography, monitored by TLC, revealed that fractions 34–37 and 94–105 each contained a single, pure compound. These

fractions were pooled and concentrated, yielding two distinct products. The first pooled fraction (F 34–37) weighed 3 mg and exhibited an Rf value of 0.75. The second (F 94–105) weighed 9 mg with an Rf of 0.51 (**Vogel, 1989**). These findings underscore the effectiveness of the chosen eluent system in isolating pure compounds from the crude extract.

3.4 Structural determination of isolated compounds

Structural determination of isolated compounds is a critical step in natural product chemistry, typically achieved through a combination of spectroscopic techniques. The primary methods employed for this purpose are mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

a. Mass Spectrometry (EI-MS)

Mass spectrometry is a powerful analytical tool used to determine the molecular weight of a compound and provide information on its fragmentation patterns. Electron Ionization Mass Spectrometry (EI-MS) is a common technique where a small quantity of the sample is introduced into the mass spectrometer. The sample molecules are then bombarded with a stream of high-energy electrons, causing them to ionize and fragment.

Following bombardment, some molecules remain intact, forming the molecular ion (M+), which corresponds to the compound's molecular weight. Other molecules undergo fragmentation, producing a unique set of fragment ions. The resulting mass spectrum displays a plot of ion abundance versus mass-to-charge ratio (m/z), providing a distinctive fragmentation fingerprint that is crucial for structural elucidation and confirmation (**Smith & Busch, 1999**).

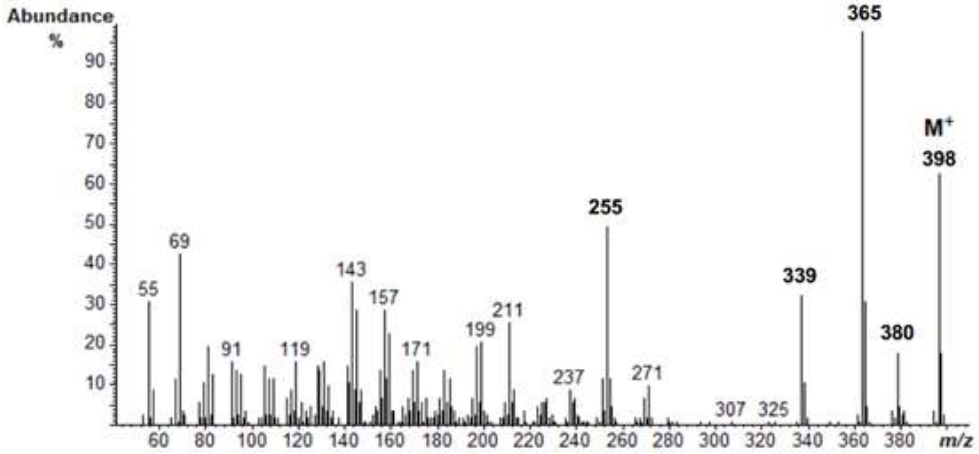

Figure 4. EI-MS mass spectrum of isolated product

The mass spectrum of the isolated compound reveals a molecular ion peak (M+) at a mass-to-charge ratio (m/z) of 398, establishing the molecular mass of the molecule. Analysis of the fragmentation pattern provides crucial insight into the compound's structural features. A prominent fragment at m/z=380 corresponds to a loss of 18 mass units, which is consistent with the elimination of a water molecule (H_2O). Furthermore, the fragment at m/z=365 indicates a loss of 33 mass units, signifying the sequential elimination of both a water molecule and a methyl group (CH_3) from the parent ion (**McLafferty & Tureček, 1993**).

b. Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR spectrum

The isolated product was dissolved in deuterated chloroform ($CDCl_3$) for analysis, with chemical shift values referenced relative to tetramethylsilane (TMS) as an internal standard.

The chemical shift, denoted by δ and expressed in parts per million (ppm), is a fundamental parameter that characterizes a proton's unique electronic environment and provides direct information about its associated chemical group. Furthermore, the signal's multiplicity reveals details about the proximity of neighboring protons. A group of equivalent protons with n neighboring protons will manifest as a multiplet of (n+1) peaks, a phenomenon known as the (n+1) rule, which is essential for determining the connectivity within a molecular structure (**Kemp, 1991**).

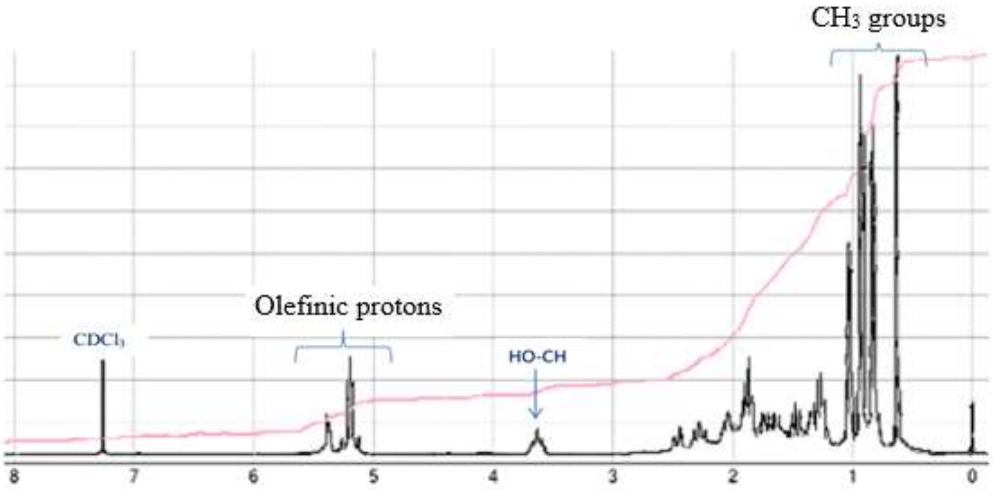

Figure 5. ^{1}H NMR spectrum (in $CDCl_3$)

The NMR spectrum is consistent with a steroidal structure, with key signals providing clear evidence of its functional groups. In the highly shielded, upfield region between 0.7 and 1.1 ppm, prominent signals are observed, which are characteristic of the angular methyl (CH_3) groups commonly found on the steroid core (**Silverstein et al., 2005**). The signal near 3.6 ppm is indicative of a proton geminal to a hydroxyl group (CH−OH), confirming the presence of an alcohol moiety. Furthermore, the downfield signals between 5.2 and 5.4 ppm correspond to olefinic protons, suggesting the presence of at least one carbon-carbon double bond within the molecule's ring system.

Decoupled ^{13}C NMR spectrum

Analysis of the ^{13}C NMR spectrum, in conjunction with Distortionless Enhancement by Polarization Transfer (DEPT) data, is essential for a detailed structural assignment. The fully decoupled ^{13}C NMR spectrum provides signals for every carbon atom in the molecule, while the DEPT-135 experiment distinguishes between protonated carbons. In the DEPT-135 spectrum, signals for CH and CH_3 groups appear in the positive phase, whereas CH_2 groups are observed in the negative phase. An initial inspection of the DEPT-135 spectrum reveals six signals between 0 and 20 ppm, which are unambiguously assigned to six CH_3 groups (**Kemp, 1991**).

^{13}C NMR and DEPT analysis

The decoupled ^{13}C spectrum shows four signals in the 120−165 ppm region, corresponding to two C=C double bonds. The DEPT-135 spectrum further clarifies the protonation state of the carbons, identifying:

Eleven CH groups, with one signal at approximately 72 ppm, indicating a carbon bonded to a hydroxyl group (CH−OH).

Eight CH_2 groups.

Six CH_3 groups.

A comparison of the DEPT-135 data with the fully decoupled ^{13}C spectrum reveals the presence of three quaternary carbons, which are visible in the decoupled spectrum but absent in the DEPT spectra.

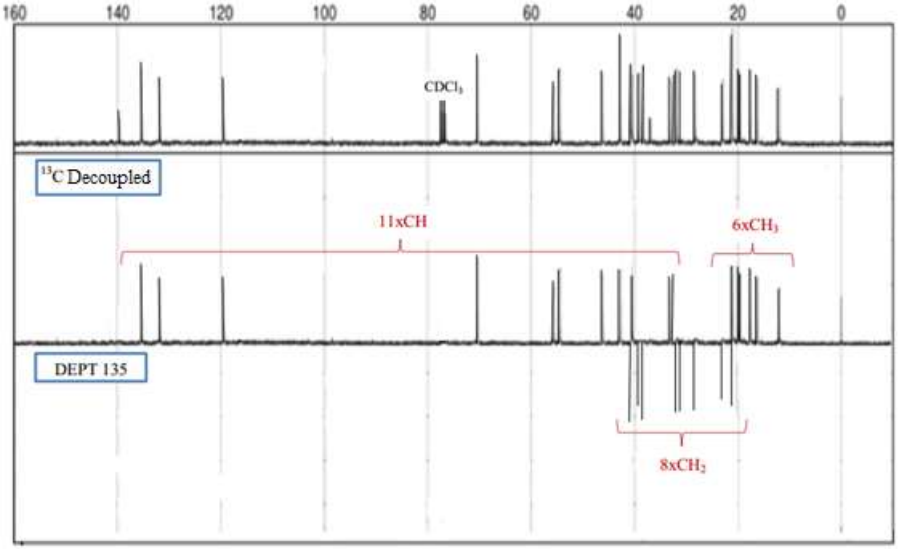

Figure 6. ^{13}C NMR spectrum fully decoupled and DEPT 135 spectrum

Carbon chemical shift assignments

The chemical shifts for the identified carbons are as follows:

Methyl Carbons (CH_3): δ 12.4, 17.9, 19.7, 20.0, 20.3, 21.4 ppm.

Methylene Carbons (CH_2): δ 21.3, 24.6, 28.8, 29.9, 31.7, 37.5, 40.0, 42.6 ppm.

Methine Carbons (CH): δ 32.2, 33.3, 40.5, 43.1, 50.4, 56.3, 57.2, 72.1, 122.1, 132.5, 136.2 ppm.

Quaternary Carbons (Cq): δ 36.9, 37.7, 141.1 ppm.

Molecular formula determination

The cumulative information from the ^{13}C NMR and DEPT experiments provides the basis for determining a partial molecular formula, as summarized in Table 4.

Table 4. Summary of partial molecular formulas from ^{13}C NMR and DEPT spectra.

Spectrum	Signal	Number of peaks	Partial formula
DEPT-135	CH	10	$C_{10}H_{10}$
DEPT-135	CH−OH	1	CH
DEPT-135	CH_2	8	C_8H_{16}
DEPT-135	CH_3	6	C_6H_{18}
Comparison	Cq	3	C_3
Total	**C/H**		**$C_{28}H_{45}O$**

The spectroscopic data collectively suggest a gross formula of $C_{28}H_{46}O$, which aligns precisely with the molecular ion peak (M+) at m/z=398 observed in the mass spectrum, thus confirming the molecular formula of the isolated compound (**Silverstein et *al.*, 2005**).

Degree of unsaturation

The degree of unsaturation, also known as the Hydrogen Deficiency Index (HDI), for the isolated compound was determined using the molecular formula $C_{28}H_{45}O$. The calculation, performed with the standard formula for molecules containing carbon, hydrogen, and oxygen, yielded a value of six (6). Given that the ^{13}C NMR spectrum indicated the presence of four

signals in the 120−165 ppm region corresponding to two C=C double bonds, the remaining four degrees of unsaturation must be attributed to a ring structure. This finding is fully consistent with the characteristic tetracyclic skeleton of steroids.

Proposed Structure

The combined analysis of the mass spectrum and the NMR data, which collectively established a molecular formula of $C_{28}H_{45}O$ and identified a tetracyclic core with two degrees of unsaturation, guided a targeted literature search. This search for tetracyclic steroids with the same elemental composition and spectroscopic properties led to the identification of the isolated product as **Brassicosterol** . The chemical shift values from the ^{1}H and ^{13}C NMR spectra of the isolated compound were found to be in excellent agreement with those reported in the literature for Brassicosterol, confirming its structural identity (**Khadem & Naser, 2018 ; Martínez et *al.*, 2021**).

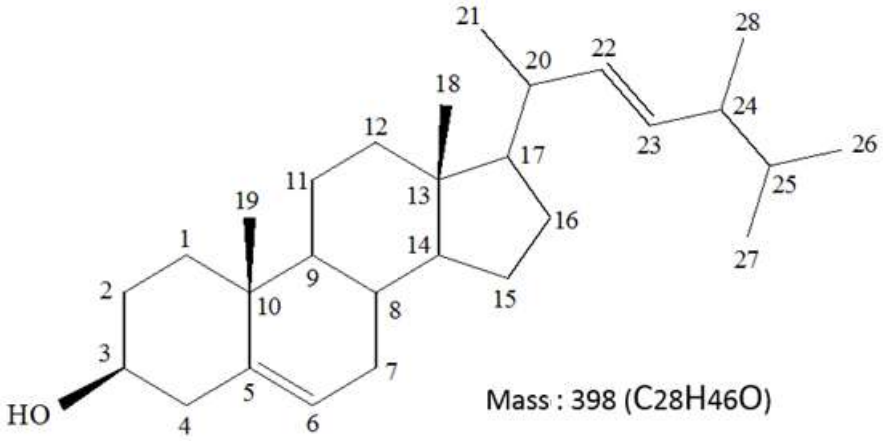

Figure 7. Structure de la molécule isolée

(**Khadem & Naser, 2018 ; Martínez et *al.*, 2021**)

The following table compares the 13C chemical shifts of the isolated product with those reported in the literature (**Khadem & Naser, 2018 ; Martínez et *al.*, 2021**).

Table 5. Comparison of chemical shifts of isolated product with literature

Number of Carbon	Carbon type	Isolated compound (δ ppm)	Literature (δ ppm)
1	CH_2	31.7	31.8
2	CH_2	37.5	37.4
3	CH	72.1	72.0
4	CH_2	42.6	42.4
5	Cq	141.1	140.9
6	CH	122.1	121.9
7	CH_2	29.9	29.9
8	CH	32.2	32.1
9	CH	50.4	50.3
10	Cq	36.9	36.7
11	CH_2	21.3	21.2
12	CH_2	40.0	39.8
13	Cq	42.6	42.5
14	CH	56.3	56.2
15	CH_2	28.8	28.7
16	CH_2	24.6	24.4
17	CH	57.2	57.0
18	CH_3	12.4	12.2

19	CH_3	21.4	21.1
20	CH	40.5	40.3
21	CH_3	17.9	17.8
22	CH	132.5	131.9
23	CH	136.2	136.0
24	CH	43.1	43.0
25	CH	33.4	33.3
26	CH_3	20.0	19.8
27	CH_3	19.7	19.6
28	CH_3	20.3	20.1

The comparison of the ^{13}C NMR chemical shifts for the isolated compound with values from the literature demonstrates a strong correlation. The observed data precisely matches the expected shifts for Brassicosterol, providing conclusive spectroscopic evidence for the molecule's structural identity **(Silverstein et *al.*, 2005).**

IV. Conclusion

Madagascar is globally recognized for its exceptional biodiversity, particularly its rich flora and fauna. In this unique environment, medicinal plants hold significant cultural and practical importance in the daily lives of the Malagasy people, in both rural and urban areas. Among these, *Ricinus communis* is a robust plant that can grow to heights of 3 to 12 meters and withstand drought. It is traditionally used for medicinal purposes, such as alleviating headaches and treating certain open wounds, in addition to its ornamental value.

This study focused on the medicinal plant *Ricinus communis*, with a specific aim to perform a comprehensive phytochemical analysis of its leaf extracts. The investigation involved sequential maceration using solvents of increasing polarity—hexane, dichloromethane (DCM), and methanol—to obtain three distinct extracts. This method not only yielded separate extracts (hexanic, DCM, and methanolic) but also allowed for the estimation of their respective yields from the plant's leaves.

The phytochemical screening conducted on the leaves provided a profile of the major chemical families present. The plant was found to contain several chemical compounds, including alkaloids, polysaccharides, saponins, and steroids, which are often associated with therapeutic properties. Polysaccharides were the most abundant, while steroids, alkaloids, and saponins were present in smaller quantities.

Following the initial extractions, the DCM leaf extract was chosen for further analysis. It was first examined by thin-layer chromatography (TLC) to optimize a suitable solvent system before being subjected to preparative column chromatography.

This two-step chromatographic process, using a DCM/methanol (99:1) solvent system, successfully yielded two pure compounds: F(34-37) and F(94-105), weighing 4 mg and 11 mg, respectively. The larger 11 mg sample, F(94-105), was selected for structural determination. This was accomplished using one-dimensional nuclear magnetic resonance (NMR) spectroscopy, including ^{1}H NMR, ^{13}C NMR, and DEPT-135 experiments. The analysis of the spectral data, combined with a comparison to established literature values, confirmed the molecule's identity as Brassicosterol, a steroid with the molecular formula $C_{28}H_{46}O$.

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