BloEx - Journal
Britain International of
EXACT Sciences Journal
Publish: | January - May - September |
E-ISSN: 2686-1208
P-ISSN: 2686-1216
http://biarjournal.com/index.php/bioex

Phytochemical Analysis of a Medicinal Plant *Senna Alata* (Fabaceae)

Andrianarijaona Mamy[1], Ralaivaon-Dratsitonta Jumaël Edith Fabrice[1,2], Fatiany Pierre Ruphin[1], Robijaona Rahelivololoniaina Baholy[3,4]

[1]Doctoral School of Geosciences, Physics, Environmental Chemistry and High-Pathogen Systems (GPCEHP), University of Toliara, Toliara, Madagascar

[2]Androy Regional University Center (CURA), University of Toliara, Toliara, Madagascar

[3]Polytechnic High School of Antananarivo, University of Antananarivo, Antananarivo, Madagascar

[4]Doctoral School of Industrial, Agricultural and Food Process and Systems Engineering, University of Antananarivo, Antananarivo, Madagascar

Email: robijob111@gmail.com

Abstract:

A preliminary phytochemical study was conducted on the bark of Senna alata, a medicinal plant widely used in Madagascar, to identify and isolate its chemical constituents. This plant is traditionally used for various ailments, including dermatological and respiratory issues. A sequential solid-liquid extraction with solvents of increasing polarity – hexane, dichloromethane (DCM), and ethanol – was performed, with the polar ethanol extract yielding the highest mass. Phytochemical screening of the hydroethanolic extract confirmed the presence of several compound classes. Abundant polysaccharides and flavonoids were detected, along with low concentrations of alkaloids, steroids, saponins, and polyphenols. The DCM extract was then subjected to column chromatography, with fractions monitored by thin-layer chromatography (TLC), which was also used to optimize the solvent system for separation. This process isolated two pure fractions, F28-34 and F61-69. The more abundant fraction, F61-69 (8 mg), was selected for structural elucidation. Mass spectrometry revealed a molecular ion peak at m/z 400, establishing the compound's molecular weight. Subsequent analysis by 1H NMR and 13C NMR spectroscopy revealed a characteristic steroid skeleton. The compound contains 28 carbons and its Double Bond Equivalence (DBE) was calculated as 5, corresponding to four rings and one double bond. Based on the comprehensive spectroscopic data and a comparison to literature, the compound was definitively identified as Campesterol.

Keywords:

Phytochemical, Senna alata, Steroid, Campesterol, NMR

I. Introduction

For millennia, the domestication of flora has been a primary objective of human societies, driven by the fundamental need for sustenance and healing. The majority of early pharmaceuticals were derived from botanical sources (Newman & Cragg, 2020). While the advent of Western civilization introduced modern medicine, its high cost often renders it inaccessible, particularly in developing nations, leading to a persistent reliance on traditional medical practices. This phenomenon is not limited to any single culture ; a significant portion of the global population, including populations in rural Madagascar, relies on plants for medicinal purposes (Farnsworth et *al.*, 1985).

Madagascar, a global biodiversity hotspot, possesses an exceptional floral diversity. The island is home to approximately 12,000 species of flowering plants, of which a remarkable 85% are endemic. An estimated 3,000 of these species are employed in traditional medicine, a practice deeply embedded in the local culture (Ratsimamanga, 1998). The ready availability of

medicinal plants provides a substantial contribution to healthcare and economic development, offering a crucial alternative for a population with limited access to formal social security systems. The continued prominence of traditional plant-based remedies underscores their significant role in maintaining public health.

Senna alata, a plant known by the vernacular names "Anjananjana," "Andrabay," and "quatre-épingles" in Madagascar, is a prime example of a botanically derived therapeutic agent. Its use in traditional medicine is multifaceted, addressing various ailments from dermatological conditions to respiratory issues (Idu & Osemwegie, 2007). The plant's leaves and bark are integral to traditional preparations used to treat skin infections. Topical applications of crushed leaf poultices are commonly used on wounds, eczema, fungal infections, ringworm, and scabies (Razafimahefa, 2015). Beyond its use as a skin treatment, *Senna alata* is also valued for its antimicrobial and laxative properties. However, its internal consumption requires caution, as large or prolonged doses can lead to toxic effects (Srivastava & Singh, 2010).

II. Research Method

2.1 Plant Material

a. The Botanical Profile of *Senna Alata*

Senna alata (L.) Roxb., a member of the Fabaceae family, is a widely recognized medicinal plant with a rich history of ethnobotanical use. This species is a robust, fast-growing shrub that can reach heights of up to four meters. Its striking morphology is characterized by large, pinnately compound leaves, each composed of numerous oblong leaflets **(Kaur et *al.*, 2011).** The most notable feature is the vibrant yellow-gold inflorescence, which forms a dense, candle-like spike at the terminal ends of the branches. These flowers produce a distinct, pungent scent and are often visited by pollinators.

Leaves Fruits Flowers

Figure 1. Leaves, fruits and flowers of *Senna alata*

The plant's distinctive fruits are flattened, winged pods that transition from green to a dark brown or black upon maturity. The pods are septate, containing numerous small, quadrilateral seeds. The botanical name itself, alata, meaning "winged," refers to the prominent wings that run along the length of the pod. The leaves, often a source of traditional remedies, possess a distinctive texture and arrangement. This morphology, combined with its widespread distribution across tropical and subtropical regions, contributes to its prominence in both ecological landscapes and traditional pharmacopoeias. The plant's adaptability and

rapid growth further establish it as a significant species for sustainable cultivation and research (Owoyele et *al.*, 2012).

1. Ethnomedicinal Applications of *Senna Alata*

The therapeutic properties of *Senna alata* are widely recognized across various cultures. A leaf decoction is traditionally used for its laxative and anti-inflammatory effects and as a cooling agent (Bhattacharya et *al.*, 2021). The pulp derived from its seeds has been prescribed as a purgative. Furthermore, a water maceration of the leaves is used as a gargle to alleviate sore throats, while an infusion is considered a depurative agent for blood cleansing in the West Indies (Morales et *al.*, 2012).

2. Malagasy Traditional Practices

In Madagascar, a leaf decoction is prepared as a purgative to treat constipation. A decoction of the flowers is used to manage respiratory ailments such as asthma and bronchitis. The fresh juice extracted from the leaves is applied topically to wounds. Poultices prepared from crushed leaf paste are also applied directly to the skin to treat wounds, eczema, fungal infections, ringworm, and scabies. The seeds are primarily used as a vermifuge (Ratsimamanga et *al.*, 2018).

For the treatment of constipation, a specific regimen is followed for adults: a glass of a decoction made from 20 grams of dried leaves per liter of water is consumed before bedtime. This treatment is not recommended for prolonged use beyond three days and is contraindicated in children, pregnant women, and the elderly due to potential toxicity. For skin and mucous membrane disorders, allergies, and cutaneous mycoses, a concentrated decoction of the leaves is applied externally through baths, compresses, or direct poultices on the affected areas.

2.2 Methods of Extraction

Extraction is a fundamental process in chemical engineering, involving the selective separation of one or more compounds from a mixture by bringing a substance into contact with a solvent. This technique is based on the chemical and physical properties of the compounds, enabling their isolation from a complex matrix (Lahlou, 2007).

In the context of botanical research, harvested plant material is typically dried away from direct sunlight and humidity to prevent the degradation of active compounds and mitigate enzymatic reactions that could alter their structure. The dried material is then ground into a fine powder using a laboratory mill. This pulverization increases the surface area, maximizing contact with the extraction solvent and thereby improving the overall yield of the desired extract (Chemat et *al.*, 2017).

Several extraction methods exist, but solid-liquid and liquid-liquid extraction are the most commonly employed techniques in chemical processes.

a. Solid-Liquid Extraction

Solid-liquid extraction is a separation technique where chemical species are dissolved from a solid phase into a liquid solvent. If the solvent's extractive power varies for different components, it is possible to achieve selective dissolution by sequentially using solvents of differing polarities.

The various methods for solid-liquid extraction include:

1. Decoction: This method involves boiling the solid in a liquid to extract soluble components. It's often used for hard plant parts like roots and bark.

2. Infusion: This process consists of immersing the solid in a hot liquid, near boiling point, to extract useful constituents. This is typically followed by a cooling period, making it ideal for delicate plant parts like leaves and flowers.
3. Maceration: A process where the solid is steeped in a solvent at ambient temperature for an extended period to extract soluble components. This method is suitable for a wide range of plant materials.
4. Digestion: A method similar to maceration, but conducted at an elevated temperature to enhance the extraction process.

b. Liquid-Liquid Extraction

Liquid-liquid extraction is a technique used to partition a soluble compound between two immiscible solvents, typically an organic phase and an aqueous phase. The efficiency of this method can be optimized by adjusting the properties of the solvents to achieve a sufficient yield of the target substance (Morales et *al.*, 2012).

For this study, the solid state of the plant material necessitated the use of a solid-liquid extraction method. A maceration approach was chosen, where 30 grams of plant powder were soaked in a series of solvents of increasing polarity—hexane, dichloromethane (DCM), and ethanol—at ambient temperature. This systematic process was designed to effectively isolate the various chemical components of the plant based on their polarities, ranging from non-polar to highly polar.

2.3 Phytochemical Screening

Phytochemical screening serves as a preliminary method for identifying the major chemical families present within a plant extract (Edeoga et *al.*, 2005). The general principle of these tests is based on qualitative analysis, relying on observable phenomena such as the formation of colored complexes or insoluble precipitates through specific chemical reactions. An initial hydro-alcoholic extract, commonly prepared using a solvent like 90% ethanol, is often used as the test medium.

Alkaloids are detected using general reagents that induce precipitation or color changes. Following an acidic maceration of the plant powder, the filtrate is exposed to reagents such as Mayer's, Dragendorff's, or Wagner's. The formation of a distinctive precipitate—yellow-white for Mayer's, orange-red for Dragendorff's, or brownish for Wagner's—indicates a positive result.

The presence of coumarins is determined by observing fluorescence under ultraviolet (UV) light. An extract spotted on paper is exposed to ammonia vapor before being examined at 365 nm, where the appearance of a yellow fluorescence signals a positive test.

For flavonoids, tests such as the Wilstater and Bath-Smith are employed. These involve treating an ethanolic extract with reagents like concentrated hydrochloric acid and magnesium turnings, which result in characteristic color changes—ranging from red to purple, depending on the specific flavonoid subclass (Harborne, 1998).

Tannins and polyphenols are distinguished by their ability to precipitate proteins or react with metallic salts. The addition of a gelatin solution causes precipitation in the presence of tannins. Similarly, a ferric chloride ($FeCl_3$) solution produces a bluish-black color for hydrolyzable tannins and a bluish-green color for condensed tannins.

Saponins are identified through a simple foam test. A plant powder suspension in water is vigorously shaken, and the formation of a stable foam of a certain height (typically ≥ 3 cm) indicates their presence.

The identification of quinones is achieved by treating an organic extract with a diluted base, which produces a red-violet color change. Similarly, polysaccharides are detected by their insolubility in ethanol, leading to the formation of a precipitate upon its addition to an aqueous extract.

Finally, steroids and terpenoids are screened using a colorimetric reaction. A chloroform extract is treated with a combination of sulfuric acid and acetic anhydride in the Lieberman-Burchard test, yielding a blue-green color for steroids and a purple color for triterpenoids. The Salkowski test, using only sulfuric acid, produces a reddish ring at the interface, indicating the presence of unsaturated sterols (Prakash et *al.*, 2017).

2.4 Chromatographic Analysis Methods

Chromatography is a physical separation method based on the differential affinities of substances for two distinct phases : a stationary (or fixed) phase and a mobile phase (Gupta, 2012). The separation of components carried by the mobile phase results from their successive adsorption and desorption onto the stationary phase, or from differences in their solubility within each phase.

The fundamental principle of chromatography involves the movement of a dissolved sample (solute) through a stationary phase by a mobile phase. The stationary phase retains the substances within the diluted sample to varying degrees, depending on the intensity of weak intermolecular forces such as van der Waals forces and hydrogen bonds.
Classification can also be based on the format of the stationary phase :

1. Column Chromatography: The stationary phase is packed inside a column (e.g., HPLC and GC).
2. Planar Chromatography: The stationary phase is coated on a flat surface (e.g., thin-layer chromatography (TLC) and paper chromatography).

a. Thin-Layer Chromatography (TLC)

TLC is a planar chromatographic technique primarily based on adsorption phenomena. The mobile phase, a solvent or solvent mixture, progresses along a stationary phase fixed onto a plate of glass, plastic, or aluminum (**Stahl, 1969**). After a sample is spotted onto the stationary phase, its components migrate at a rate dependent on their inherent properties and their solubility in the mobile phase.

Principle: As the solvent (eluent) ascends the plate via capillary action, each component of the sample travels at its own unique velocity behind the solvent front. This velocity is influenced by both the electrostatic forces binding the compound to the stationary phase and its solubility in the mobile phase. Consequently, less polar substances typically migrate more quickly than more polar ones. The finished plate, once the solvent has evaporated, is known as a chromatogram.

Visualization and revelation: After the plate has dried, colored compounds are visible without further action. For colorless compounds, two common visualization methods are employed. One method involves placing the plate under a UV lamp (254 nm), where fluorescent backgrounds show up as green, while compounds that absorb UV light appear as

dark spots. A second method uses a chemical spray, such as anisaldehyde reagent, which upon heating, reacts with various compounds to produce colored spots.

b. Column Chromatography

Column chromatography is a preparative technique used for separating and isolating larger quantities of a mixture's constituents (Dey & Harborne, 2011).

Principle of liquid column chromatography: A glass column with a stopcock is prepared with a porous plug (e.g., cotton) at the bottom. The stationary phase, often silica gel, is packed into the column, and the entire system is thoroughly solvated with the mobile phase. After the sample is loaded onto the top of the column, the eluent is continuously added, causing the components to migrate at different rates and separate into distinct bands. These bands are collected as individual fractions, which can be further analyzed and weighed.

Column Packing: The most critical step is packing the column to achieve a homogeneous stationary phase without air bubbles. Two primary methods are used:

1. Wet Packing: The adsorbent is mixed with the least polar solvent to form a slurry, which is then poured into the column in portions.
2. Dry Packing: The column is partially filled with the solvent, and the powdered adsorbent is added slowly, with continuous tapping to ensure uniform compaction.

Elution: The column must be continuously fed with the eluent, ensuring the stationary phase's surface is never exposed to air, as this can lead to cracking and poor separation. A slow flow rate is typically used, with collected fractions being analyzed and pooled to obtain highly pure substances.

2.5 Spectral Analysis Methods

a. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure of organic molecules. The method is based on the interaction between an electromagnetic wave and atomic nuclei within a strong magnetic field. The resulting NMR spectrum provides detailed information about the chemical environment and connectivity of the atoms in a sample (Silverstein et *al.*, 2014).

1. Proton NMR (^{1}H)

A proton NMR spectrum consists of a series of signals, each corresponding to a unique hydrogen atom or group of equivalent hydrogen atoms. The position of each signal is measured by its chemical shift, a value expressed in parts per million (ppm). This chemical shift is influenced by the electron density around the hydrogen nucleus, which is determined by the surrounding atoms. Analyzing the chemical shift, along with signal integration and splitting patterns, allows for the identification of a molecule's proton framework.

2. Carbon-13 NMR (^{13}C)

Carbon-13 NMR is analogous to proton NMR but focuses on the carbon atoms within a molecule. This technique provides crucial information about the carbon skeleton and the different types of carbon atoms present.

a) Broadband-Decoupled ^{13}C NMR: This type of spectrum shows all carbon signals as single peaks, simplifying the analysis. It is particularly useful for identifying the total number of unique carbon environments, including quaternary carbons (carbons with no attached hydrogens).

b) DEPT (Distortionless Enhancement by Polarization Transfer) Spectra: DEPT is a specialized technique that enhances the signal intensity of certain carbons and provides information about the number of protons attached to each carbon atom. By applying specific pulse angles, DEPT allows for the clear differentiation of CH, CH_2, and CH_3 groups.
   1) DEPT-90 spectra exclusively show signals for methine (CH) carbons.
   2) DEPT-135 spectra show signals for CH and CH_3 groups pointing upwards, while CH_2 signals point downwards.
   3) Quaternary carbons (C) do not appear in DEPT spectra and are identified by comparing the DEPT spectra to a fully decoupled ^{13}C spectrum (Broun & Pouchert, 2011).

III. Result and Discussion

3.1 Results

a. Results of Sequential Solvent Extraction

A common principle in natural product chemistry is "like dissolves like." This concept guided a preliminary separation of chemical compounds from 30 g of powdered plant bark using a sequential extraction method. Solvents of increasing polarity—hexane, dichloromethane (DCM), and methanol—were used to selectively isolate chemical constituents based on their polarities (Lahlou, 2007). The results of the extraction process are summarized in the table below:

Table 1. Extraction from *Senna alata* bark

Powder (30 g)	Solvent (50 ml)	Extract Obtained (g)	Compound Polarity	Yield (%)
Bark	Hexane	0.41	Non- or slightly polar	1.37
	DCM	0.66	Moderately polar	2.20
	Methanol	2.20	Polar and highly polar	7.33

The results indicate that the polar and highly polar fractions exhibit a significantly higher yield (7.33%) compared to the less polar extracts. This suggests that the majority of the extractable compounds from the plant bark possess polar characteristics, which is a common finding for many plant secondary metabolites such as flavonoids, tannins, and certain alkaloids (Chemat et *al.*, 2017).

b. Phytochemical Screening Results

The qualitative phytochemical analysis of the plant bark extract was performed to identify the presence of various chemical families. The results were assessed using a standardized scoring system for color intensity, precipitate quantity, and foam height. A weak positive result is indicated by (+), a moderate positive by (++), a strong positive by (+++), and a negative result by (-).

1. Key findings and Interpretations

Alkaloids: A weak positive result was obtained for alkaloids with Mayer's reagent, evidenced by a slight orange precipitate. This suggests a low concentration of these compounds, which are known for their diverse pharmacological activities, including effects on the nervous system.

Flavonoids: The screening for flavonoids, which are often valued for their antioxidant properties, showed a moderate positive result (++) with the Bath-Smith test. This finding indicates the presence of leucoanthocyanins, a subclass of flavonoids, while other standard

tests (Wilstater and modified Wilstater) yielded negative results, suggesting the absence of other flavonoid types in the extract.

Polyphenols and Tannins: Both polyphenols and tannins were detected at low concentrations (+). This was confirmed by the formation of a weak precipitate in the gelatin and gelatin-salt tests. The presence of these compounds is significant as they are known for their astringent and antioxidant properties, which contribute to the plant's traditional medicinal uses.

Polysaccharides: The plant extract showed a very high concentration of polysaccharides (+++), indicated by the formation of a very abundant precipitate upon the addition of ethanol. This is a common finding for many plant sources and suggests that the extract may have demulcent or immunomodulatory properties, as these are common functions of polysaccharides (**Zhu, 2018**).

Quinones and Coumarins: Both quinones and coumarins were absent from the extract (-). The absence of a color change in the quinone test and the lack of fluorescence under UV light for the coumarin test confirmed their non-detection. This finding is notable as it helps to narrow down the potential bioactive components.

Saponins: The presence of saponins was confirmed by a positive foam test (+), with a foam height of approximately 3 cm. Saponins are known for their detergent-like properties and are often associated with anti-inflammatory and cholesterol-lowering effects (Sparg et al., 2004).

Steroids and Terpenoids: A weak positive result (+) for steroids was observed with the Liebermann-Burchard test, indicated by a faint blue-green color change. Terpenoids were not detected. This suggests that while steroids are present in low amounts, they are not a major class of compounds in the extract.

Table 2. Screening summaries

Chemical Family	**Result**	**Interpretation**
Alkaloids	(+)	Present in low concentration
Flavonoids	(++)	Moderate concentration, specifically leucoanthocyanins
Polyphenols	(+)	Present in low concentration
Tannins	(+)	Present in low concentration
Polysaccharides	(+++)	Present in high concentration
Saponins	(+)	Present in low concentration
Steroids	(+)	Present in low concentration
Terpenoids	(-)	Absent
Quinones	(-)	Absent
Coumarins	(-)	Absent

Phytochemical analysis of the extract reveals a high concentration of polysaccharides, alongside a moderate presence of flavonoids. Minor quantities of alkaloids, polyphenols, tannins, saponins, and steroids were also detected. Coumarins, quinones, and terpenoids, however, were conspicuously absent.

c. Thin-film Chromatography Results

Thin-layer chromatography (TLC) was conducted to optimize the solvent system for subsequent column chromatography, a common preliminary step in natural product isolation (Hostettmann et *al.*, 1997). A dichloromethane (DCM) plant extract was applied to a stationary phase, and a series of mobile phases were tested to assess their separation efficiency. The solvent systems evaluated included 100% DCM, as well as binary mixtures of DCM and methanol (MeOH) at ratios of 99:1 and 98:2.

Figure 2. Thin layer chromatography results

Following elution, the plates were visualized using an anisaldehyde reagent and subsequently heated to 120°C to reveal the separated components (Stahl, 1969). The results indicated that all three solvent systems provided effective separation of the constituents in the DCM extract. This suggests that a range of polarities can be effectively utilized for the chromatographic purification of this particular extract's chemical profile.

d. Column Chromatography Results

The separation of components from a dichloromethane (DCM) extract was performed using column chromatography. Approximately 120 mg of the extract was subjected to this technique on a 50 cm column. For optimal sample loading, the extract was first dissolved in a minimal volume of DCM and mixed with a proportional amount of silica. Following solvent evaporation, the resulting dry powder was carefully loaded onto the top of the column, a method known as dry loading, which enhances separation efficiency (Hostettmann et *al.*, 1997). The column was then eluted with pure DCM, and fractions were collected sequentially in numbered vials.

The purity of the collected fractions was monitored by thin-layer chromatography (TLC). A 100% DCM mobile phase was employed, with subsequent visualization using anisaldehyde reagent and heating. This analysis revealed two distinct groups of fractions that exhibited a single, well-defined spot on the TLC plate, indicating a high degree of purity. These groups were fractions 28–34 and 61–69, respectively (Sherma, 2018).

The pure fractions were then pooled, and their solvent was removed by evaporation. This process yielded two isolated, pure compounds, which were designated F 28-34 (3 mg) and F 61-69 (8 mg). These isolated products are now ready for structural elucidation, which will involve advanced spectroscopic techniques to determine their precise chemical structures.

e. Structural Elucidation of Isolated Compounds

The structural determination of the isolated compounds was conducted using advanced spectroscopic methods, including Nuclear Magnetic Resonance (NMR) and Mass

Spectrometry. This analysis was performed in collaboration with the Institute of Organic Chemistry at the University of Hanover, Germany. The proton (^{1}H) and carbon-13 (^{13}C) NMR spectra were recorded on a Bruker AVANCE 500 spectrometer, a high-field instrument known for its excellent resolution and sensitivity (Silverstein et *al.*, 2014).

The compound under investigation was dissolved in deuterated chloroform ($CDCl_3$), a solvent widely favored for its properties in NMR spectroscopy. $CDCl_3$ is an excellent solvent for a broad range of organic compounds and contains no proton signals that would interfere with the ^{1}H NMR analysis. Its volatility also allows for rapid removal of the solvent after analysis. Chemical shifts for the spectra were referenced to tetramethylsilane (TMS), which serves as the universal internal standard for both proton and carbon NMR due to its unique signal and chemical inertness (Pretsch et *al.*, 2016).

1. Mass Spectrometry (MS)

Mass spectrometry (MS) is a highly sensitive analytical technique used for the detection and identification of compounds by measuring their molecular weight and the mass of their fragments with exceptional precision (Chung et *al.*, 2011). It provides crucial information about a molecule's mass and structural integrity.

A FINNIGAN MAT 312 mass spectrometer was used to record the mass spectrum via electron ionization (EI-MS). This method bombards the sample with a beam of electrons, typically at 70 eV, which ionizes the molecules and causes them to break into smaller, charged fragments. The resulting spectrum is characterized by a molecular ion peak, representing the intact molecule, and a series of fragment ion peaks, which provide clues to the molecule's structure.

The mass spectrum is a plot with the abundance of ions (in percent) on the y-axis and the mass-to-charge ratio (m/z) on the x-axis. The analysis of these peaks and their fragmentation patterns is essential for confirming the identity of a compound.

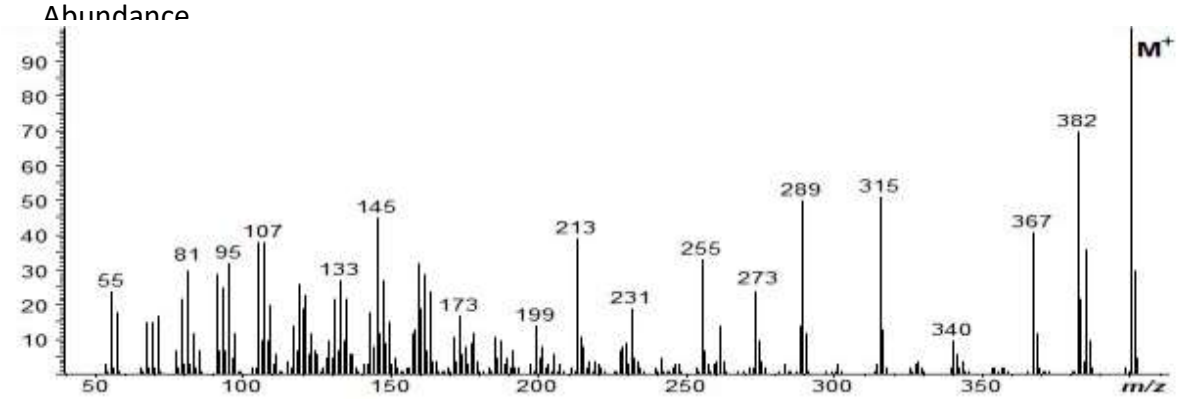

Figure 3. EI-MS mass spectrum of isolated product

Based on the mass spectrometry data, the isolated compound has a molecular ion peak (M+) at m/z 400, which establishes its molecular weight. The fragmentation pattern provides crucial structural information (de Hoffmann & Stroobant, 2007). A fragment ion at m/z 382 corresponds to the loss of 18 mass units (M+−18), consistent with the elimination of a water molecule (H_2O). Furthermore, a prominent peak at m/z 367 indicates a loss of 33 mass units (M+−33), which can be attributed to the combined loss of a water molecule and a methyl group (CH_3).

2. ^{1}H NMR Spectrum Result

The proton Nuclear Magnetic Resonance (^{1}H NMR) spectrum is a fundamental and indispensable tool for the structural elucidation of organic molecules. It provides crucial information regarding the number, type, and connectivity of hydrogen atoms within a compound (Silverstein et *al.*, 2014). This particular spectrum was recorded using deuterated chloroform ($CDCl_3$), a standard solvent in organic chemistry that ensures the absence of a solvent proton signal. Analysis of the chemical shifts, signal integrations, and multiplicities allows for the deduction of the molecular skeleton and the chemical environment of each proton, which is essential for determining the complete molecular structure (Pretsch et *al.*, 2016).

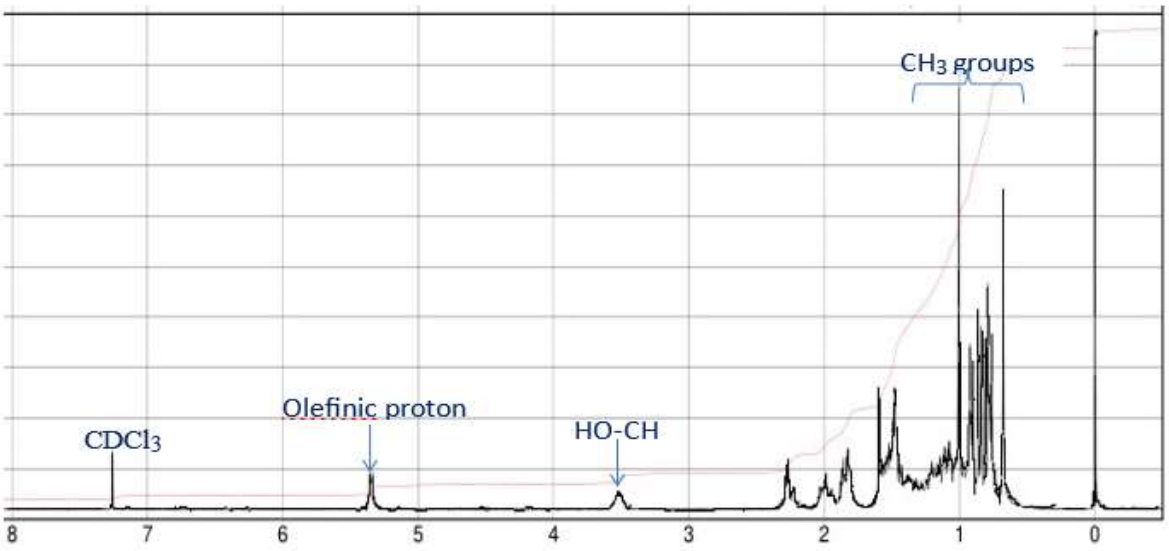

Figure 4. ^{1}H NMR spectrum (in $CDCl_3$)

Based on the interpretation of the ^{1}H NMR spectrum, the isolated compound exhibits several key features characteristic of a steroid skeleton.

- A cluster of signals in the highly shielded region, between 0.7 and 1 ppm, is indicative of the presence of protons from multiple methyl groups (CH_3), which are a hallmark of the steroid framework.
- A signal observed around 3.5 ppm is attributed to a proton on a carbon atom bearing a hydroxyl group (CH−OH). The specific chemical shift of this signal, typically found between 3 and 4 ppm for steroids, strongly suggests the presence of a hydroxyl group at position C-3 of the ring structure (Pretsch et *al.*, 2016).
- A downfield signal at approximately 5.7 ppm corresponds to a vinylic proton (C=CH). The presence of this signal confirms that the compound is an unsaturated steroid, containing at least one double bond in its structure. This information, combined with the other data, is crucial for deducing the full chemical structure of the molecule (Silverstein et *al.*, 2014).

3. ^{13}C NMR Spectrum Result

The carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) spectrum is a cornerstone of modern structural elucidation, providing essential information about the carbon skeleton of a molecule. Each unique carbon atom within a compound produces a distinct signal, with its position (chemical shift) offering key insights into its chemical environment, such as hybridization and neighboring functional groups (Pretsch et *al.*, 2016). This specific spectrum was recorded in deuterated chloroform ($CDCl_3$), a common solvent for organic analysis. The data provided by this spectrum, including the number and chemical shifts of the carbon signals, is crucial for confirming the molecular structure deduced from other spectroscopic methods (Silverstein et *al.*, 2014).

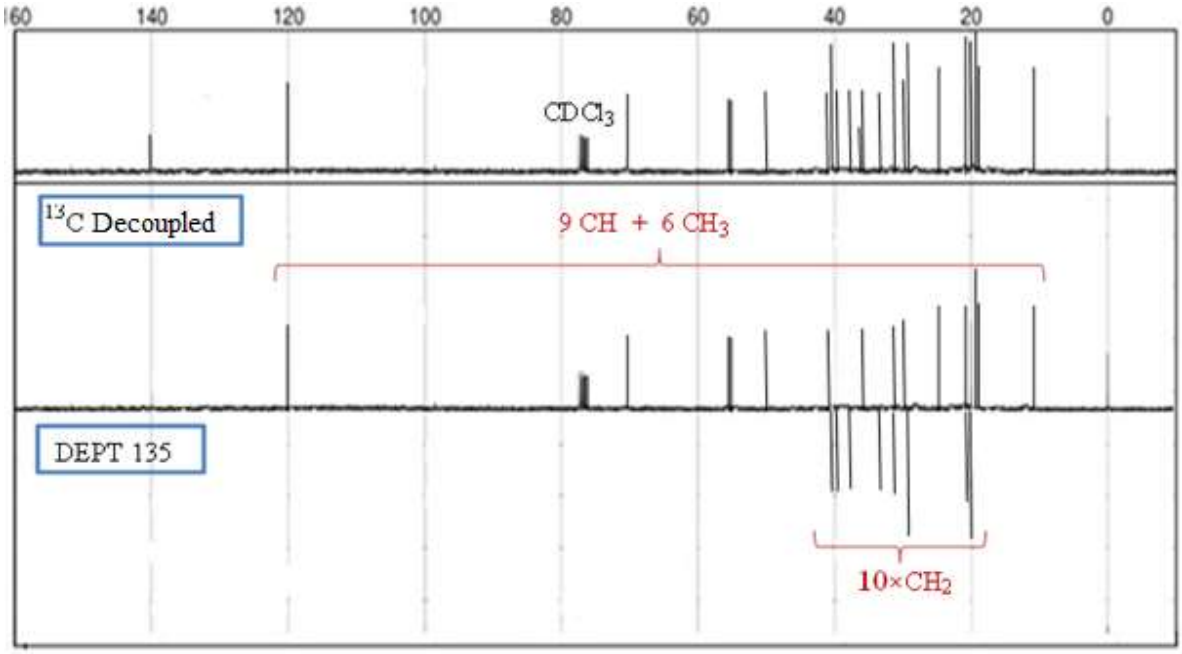

Figure 5. ^{13}C NMR spectrum (in $CDCl_3$)

Based on the broadband-decoupled ^{13}C NMR spectrum, the number of distinct signals corresponds to the total number of carbon atoms in the molecule, revealing a molecular framework of 28 carbon atoms. The chemical shift of each signal provides critical information about the carbon's environment (Pretsch et *al.*, 2016).

✓ **Key Spectral Features**

- **Carbon count and degeneracy:** The spectrum shows a total of 28 signals, which aligns with the typical carbon count for a steroid skeleton (27-29 carbons) and strongly supports the compound's classification as a steroid. The presence of some exceptionally large peaks indicates that multiple carbon atoms share the same chemical shift, a phenomenon known as signal degeneracy. This can involve various combinations of carbon types (CH_2/CH_3, CH/Cq, etc.), and requires further analysis to resolve.
- **Characteristic chemical shifts:** The spectrum shows two distinct signals within the alkene region (sp^2 carbons) at 121.9 ppm and 140.9 ppm, confirming the presence of a double bond in the molecule. Additionally, a signal at 71.9 ppm is characteristic of a carbon atom attached to a hydroxyl (OH) group. This chemical shift range, typically between 60-90 ppm, is often associated with carbons linked to an oxygen atom in natural products (Silverstein et *al.*, 2014).

✓ **DEPT-135 analysis**

The DEPT-135 spectrum, which selectively reveals protonated carbons (CH, CH_2, and CH_3), complements the decoupled ^{13}C NMR data by differentiating between these groups. Signals for CH and CH_3 groups appear in the positive phase, while CH_2 signals are displayed in the negative phase (Broun & Pouchert, 2011).

- **Negative phase:** The DEPT-135 spectrum's negative region contains eight signals, with two exhibiting extra-large intensity, indicating that each belongs to two equivalent CH_2 groups. This translates to a total of ten CH_2 groups in the molecule.
- **Positive phase:** The positive region displays 14 signals. Five of these are located below 25 ppm, a characteristic range for methyl (CH_3) groups. One of these five is an extra-large signal, suggesting two equivalent CH_3 groups, bringing the total to six CH_3 groups. The remaining nine signals, situated above 25 ppm, correspond to nine CH groups.

- **Quaternary carbon assignment:** A comparison between the broadband-decoupled ^{13}C and DEPT-135 spectra reveals that three signals present in the former are absent in the latter. Since the DEPT-135 spectrum only displays protonated carbons, these three missing signals are attributed to quaternary carbons (Cq), which lack attached hydrogen atoms.

Table 3. Summary of DEPT135 and decoupled ^{13}C results

Spectra	Signals	Number of peaks	Formula	Mass
DEPT 135	CH	9	9×CH	117
	CH_2	10	10×CH_2	140
	CH_3	6	6×CH_3	90
Comparison (DEPT 135 vs decoupled ^{13}C)	Cq	3	3×Cq	36
	A Cq is bound to an OH		OH	17
		Final formula	$C_{28}H_{49}O$	400

The comprehensive spectroscopic evidence derived from this NMR analysis (Pretsch et *al.*, 2016 ; Broun & Pouchert, 2011) strongly supports the assigned molecular formula of $C_{28}H_{49}O$. The derived molecular mass of 400, in conjunction with the determined elemental composition, provides a definitive conclusion for the compound's structural identity, completing the spectroscopic analysis.

✓ **Calculating the number of rings and unsaturations**

The number of rings and unsaturations ($N_{R/I}$) in a molecule is the number of rings and multiple bonds, usually double bonds, it contains. Our molecule has double bonds. So let's check the number of unsaturations with the following formula :

$$N_{R/I} = n_C - n_H/2 + n_N/2 + 1$$

For the isolated compound, with a molecular formula of $C_{28}H_{48}O$, the number of rings and unsaturations ($N_{R/I}$) is calculated as follows:

$N_{R/I} = 28 - 48/2 + 0/2 + 1$

$N_{R/I} = 28 - 24 + 1$

$N_{R/I} = 5$

4. Structural Interpretation and Identification

The calculated $N_{R/I}$ of 5 is highly significant, indicating that the molecule contains a total of five rings and/or double bonds. This aligns with the spectroscopic data obtained from ^{13}C NMR, which revealed two distinct signals in the alkene region, confirming the presence of one double bond. Consequently, the remaining $N_{R/I}$ of 4 corresponds to four rings.

This structural profile—a 28-carbon compound with four rings and one double bond—is characteristic of an unsaturated steroid. A comprehensive literature search for such a compound points to the structure of Campesterol, a common phytosterol (Patterson, 1991 ; Akihisa et *al.*, 1991 ; Musa et *al.*, 2015). The combination of the DBE calculation and NMR analysis provides a strong basis for the definitive identification of the isolated product.

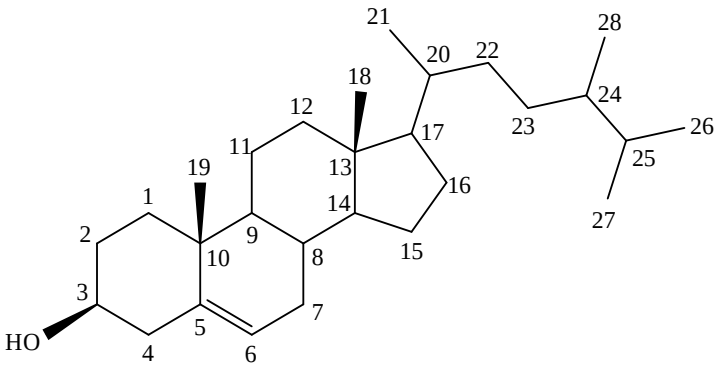

Figure 6. Structure of isolated compound
(Name: Campesterol)

Table 4. Comparison of chemical shifts of isolated molecules with literature **(**Patterson, 1991 ; Akihisa et *al*., 1991 ; Musa et *al*., 2015**)**

Number of Carbons	Carbon type	Isolated compound (δ ppm)	Literature (δ ppm)
1	CH_2	37,3	37,5
2	CH_2	28,4	28,2
3	CH	71,9	71,8
4	CH_2	42,3	42,3
5	Cq	140,9	141,0
6	CH	121,9	121,9
7	CH_2	31,8	31,8
8	CH	29,8	29,7
9	CH	50,1	50,4
10	Cq	36,5	26,4
11	CH_2	20,3	20,4
12	CH_2	39,8	39,8
13	Cq	42,3	42,3
14	CH	56,9	56,8
15	CH_2	20,3	20,4
16	CH_2	28,4	28,2
17	CH	56,1	56,5
18	CH_3	19.5	19,3
19	CH_3	12,0	12,2
20	CH	31,8	31,8
21	CH_3	19,5	19,3
22	CH_2	33,8	33,4
23	CH_2	21,2	21,1
24	CH	42,4	42,1
25	CH	36,1	36,9
26	CH_3	21,2	21,1
27	CH_3	19,0	19,2
28	CH_3	24,4	24,5

3.2 Discussion

The objective of this study was the systematic isolation and structural elucidation of a major chemical constituent from plant bark using a combination of extraction, chromatographic, and spectroscopic techniques.

a. Extraction and Preliminary Screening

A sequential solvent extraction was performed using solvents of increasing polarity to selectively isolate the plant's chemical constituents. The most polar solvent, methanol, yielded the highest percentage of extract, suggesting that the bark is rich in polar compounds. A preliminary phytochemical screening of a hydroethanolic extract corroborated this, revealing the abundant presence of polysaccharides and flavonoids, along with lower concentrations of alkaloids, saponins, polyphenols, and steroids. Thin-layer chromatography (TLC) was utilized to qualitatively confirm these findings and to establish an optimal solvent system for subsequent purification.

b. Isolation and Purification

Chromatographic purification was performed on a dichloromethane extract to isolate individual compounds. All fractions were monitored by TLC, and those showing a single, well-defined spot were selected for further analysis. A specific fraction, designated F61-69, with a mass of 8 mg, met the purity and quantity requirements for structural determination.

c. Spectroscopic Structural Elucidation

The structure of the isolated compound was elucidated through a collaborative effort using advanced spectroscopic methods, including Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS).

- **Mass Spectrometry:** The Electron Ionization Mass Spectrometry (EI-MS) spectrum of the compound showed a molecular ion peak at m/z 400, establishing its molecular weight. Fragmentation analysis revealed key losses, including a loss of 18 mass units (H_2O) and 33 mass units (H_2O+CH_3), providing initial clues to the molecule's composition.
- **^{13}C NMR and DEPT-135:** The broadband-decoupled ^{13}C NMR spectrum revealed a total of 28 carbon signals, a characteristic number for many plant sterols. The spectrum also showed two signals in the alkene region (121.9 and 140.9 ppm) and one oxygen-bearing carbon signal at 71.9 ppm. This data was complemented by a DEPT-135 experiment, which identified nine CH groups, ten CH_2 groups, six CH_3 groups, and three quaternary carbons, confirming the elemental composition.
- **^{1}H NMR:** The ^{1}H NMR spectrum provided detailed information on the proton environment. Key features included signals for methyl groups in the shielded region (0.7-1 ppm), a signal for an oxygen-bearing methine proton at 3.5 ppm, and a vinylic proton signal at 5.7 ppm, confirming the presence of a double bond.

The Double Bond Equivalence (DBE) or number of rings and unsaturations ($N_{R/I}$) for the compound was calculated using its molecular formula ($C_{28}H_{48}O$), yielding a value of 5. This value, combined with the presence of a single double bond identified by NMR, confirms that the molecule contains four rings, which is a key characteristic of the steroid skeleton. The complete spectroscopic data (MS, ^{1}H NMR, ^{13}C NMR, and DEPT) and the DBE calculation together provide definitive evidence for the identification of the isolated product as Campesterol, a common phytosterol.

IV. Conclusion

Plants remain the predominant source of medicine for a significant portion of the world's population, particularly in developing countries, with approximately 40% of all medicines being derived from nature. This study focuses on *Senna alata*, a medicinal plant used in Madagascar to treat various ailments, including asthma, bronchitis, wounds, eczema, and skin infections. The phytochemical investigation aimed to identify the plant's constituents using a systematic approach encompassing solid-liquid extraction, phytochemical screening, and chromatographic analysis.

A preliminary phytochemical screening of a hydroethanolic extract of *Senna alata* bark revealed the presence of several secondary metabolite classes. Weak indications were observed for alkaloids, steroids, and saponins. Conversely, abundant quantities of leucoanthocyanin-type flavonoids and very abundant polysaccharides were detected, alongside a low presence of polyphenols and tannins.

To isolate these compounds, 30 g of powdered bark was subjected to a sequential solid-liquid extraction using solvents of increasing polarity: hexane, dichloromethane (DCM), and ethanol. This process yielded 0.41 g, 0.66 g, and 2.20 g of extract, respectively, indicating a high concentration of polar compounds. The DCM extract was then purified using a combination of thin-layer chromatography (TLC) for method optimization and column chromatography for separation. This process led to the isolation of two pure products, designated F28-34 and F61-69.

The compound F61-69 was selected for structural determination due to its higher mass yield of 8 mg. The structure was elucidated using a suite of spectroscopic techniques, including mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR). Spectral analysis of the isolated product revealed a molecular weight consistent with a steroid from the literature. The compound was identified as Campesterol, an unsaturated sterol.

Future work will focus on isolating and elucidating the structures of other constituents within *Senna alata*. Subsequent research will involve testing the isolated molecules on various biological models, both *in vitro* and *in vivo*, to scientifically validate the traditional medicinal uses attributed to this plant.

References

Bhattacharya, S., Ghosh, A. K., & De, A. (2021). *Senna alata*: A comprehensive review of its pharmacological activities. *Journal of Ethnopharmacology, 273*, 113970. https://doi.org/10.1016/j.jep.2021.113970

Broun, K. & Pouchert, C. J. (2011). *The Aldrich Library of Carbon-13 and Proton FT-NMR Spectra: A Collection of 8710 Spectra of Organic Compounds* (2nd ed., Vol. 3). Aldrich Chemical Company.

Chemat, F., Abert Vian, M., & Cravotto, G. (2017). *Green extraction of natural products: Concepts and principles*. Royal Society of Chemistry.

Chemat, F., Abert Vian, M., Cravotto, G., & D'Alessandro, G. (2017). *Green extraction of natural products: Concepts and principles*. Royal Society of Chemistry.

Chung, S. M., Han, J. Y., & Lee, S. J. (2011). A review on recent advances in mass spectrometry for natural products analysis. *Analytical Science and Technology, 24*(4), 317–328.

de Hoffmann, E., & Stroobant, V. (2007). Mass spectrometry: Principles and applications (3rd ed.). John Wiley & Sons.

Dey, P. M., & Harborne, J. B. (2011). *Plant biochemistry*. Academic Press.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical screening and nutrient composition of three Nigerian medicinal plants. *Journal of Applied Sciences, 5*(10), 1660-1662. https://doi.org/10.3923/jas.2005.1660.1662

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Medicinal plants in therapy. *Bulletin of the World Health Organization, 63*(6), 965–981.

Gupta, V. K. (Ed.). (2012). *Advances in chromatography*. InTech.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.

Hostettmann, K., Marston, A., & Hostettmann, M. (1997). *Preparative chromatography for separation of natural products*. In: *Preparative and Process-Scale Chromatography*. Springer.

Idu, M., & Osemwegie, O. O. (2007). Ethnomedicinal plants used for the treatment of skin diseases in Nigeria: A review. *Journal of Applied Sciences, 7*(11), 1545–1550.

Kaur, S., Singh, K., & Sharma, M. (2011). *Senna alata*: A review on its ethnobotany, pharmacology and phytochemistry. *International Journal of Pharmaceutical Sciences Review and Research, 10*(1), 16–22.

Lahlou, M. (2007). Methods for the evaluation of plant extracts' biological activities. *Molecules, 12*(4), 589–591. https://doi.org/10.3390/molecules12040589

Morales, J. M., Monzote, L., GarcÃa, M., Montalvo, A. M., & SÃ¡nchez, L. M. (2012). Anti-leishmanial and cytotoxic activities of plants traditionally used in the West Indies. *Journal of Ethnopharmacology, 142*(1), 163–170. https://doi.org/10.1016/j.jep.2012.04.015

Musa, W. J. A., Duengo, S., & Kilo, A. K. (2015). Campesterol from MethanoFraction of Brotowali (Tinospona crispa). *Atlantis Highlights in Chemistry and Pharmaceutical Sciences*, *1*, 95–97.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the last 40 years. *Journal of Natural Products, 83*(3), 770–800. https://doi.org/10.1021/acs.jnatprod.9b01090

Owoyele, B. V., Adebayo, J. O., & Akintayo, E. A. (2012). Anti-inflammatory and analgesic effects of ethanolic extract of *Senna alata* leaves in rats. *Journal of Medicinal Plants Research, 6*(32), 4668–4673.

Prakash, M. V., Bhargavi, T., & Vasantha, K. (2017). A review on phytochemical screening of various plant extracts. *International Journal of Pharmaceutical Sciences Review and Research, 43*(2), 162–167.

Pretsch, E., Bühlmann, P., & Badertscher, M. (2016). *Structure determination of organic compounds: Tables of spectral data* (5th ed.). Springer.

Ratsimamanga, A. R. (1998). *Plantes médicinales de Madagascar*. Presses Universitaires de France.

Ratsimamanga, S., & Ratsimamanga, J. (2018). *Plantes médicinales de Madagascar et leurs usages*. Presses Universitaires de France.

Razafimahefa, J. (2015). *Ethnobotanique de quelques plantes médicinales utilisées par les populations du district d'Antananarivo-Atsimondrano*. Université d'Antananarivo.

Sherma, J. (2018). *Thin-layer chromatography*. Academic Press.

Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2014). *Spectrometric identification of organic compounds* (8th ed.). John Wiley & Sons.

Sparg, S. G., Light, M. E., & van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*(2-3), 219–243. https://doi.org/10.1016/j.jep.2004.05.021

Srivastava, S., & Singh, P. P. (2010). A review on medicinal plants and their uses. *International Journal of Pharmaceutical Sciences Review and Research, 3*(1), 1–10.

Stahl, E. (1969). *Thin-layer chromatography: A laboratory handbook*. Springer.

Zhu, F. (2018). Structure, function, and application of plant polysaccharides. *Critical Reviews in Food Science and Nutrition, 58*(16), 2736–2748. https://doi.org/10.1080/10408398.2017.1332768