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Phytochemical analysis of *Flacourtia ramontchi* (Salicaceae)

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Abstract: *Flacourtia ramontchi is a medicinal plant deeply rooted in the traditional practices of Madagascar, where a decoction from its leaves and bark is commonly used to treat respiratory ailments such as asthma and dry coughs. The present study was conducted to provide a scientific foundation for these traditional uses by performing a comprehensive phytochemical analysis. The investigation involved the successive maceration of powdered bark and leaf samples using a series of solvents of increasing polarity, namely n-hexane, dichloromethane (DCM), and methanol. This extraction process yielded a variety of crude extracts, with the highest yields for both plant parts obtained from the non-polar hexane solvent, suggesting a rich presence of non-polar constituents. Phytochemical screening of the extracts identified a significant abundance of secondary metabolites with recognized therapeutic value. The bark and leaves were particularly rich in polyphenols, tannins, and flavonoids, compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties, which scientifically validate the plant's traditional medicinal applications. Following the preliminary screening, the DCM bark extract was selected for further separation and purification. Thin-layer chromatography (TLC) was optimized to an ideal hexane/DCM (30:70) mobile phase, revealing four dominant constituents. This strategic approach led to the subsequent isolation of a pure compound via column chromatography. The fraction with the highest yield was chosen for structural elucidation. The structure of the isolated compound was determined using mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, including ^{1}H NMR and ^{13}C NMR. Spectral data analysis, specifically the molecular ion peak in the mass spectrum at m/z 300 and the carbon and proton signals from the NMR data, confirmed the compound to be spruceanol, a diterpenoid. This finding is a significant contribution to the understanding of the plant's chemical composition.*

Keywords: Phytochemical, *Flacourtia ramontchi*, structure elucidation, NMR, EI-MS, Chromatography

I. Introduction

The therapeutic use of plants is a practice deeply intertwined with human history, dating back to prehistoric hunter-gatherer societies who utilized botanical resources not only for sustenance but also for medicinal purposes (**Tadele, 2018**). Over centuries, the application of medicinal plants and herbal remedies has expanded, and today, an estimated 80% of the global population, particularly in developing countries, relies on traditional

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medicine for primary healthcare (**WHO, 2019**). In Madagascar, traditional practitioners are widely authorized and consulted by the local population for healing, a practice supported by the island's high diversity of medicinal plant species (**Randrianarivelo et *al*., 2009**).

To advance this field, a rigorous scientific approach is essential to understand the toxicological, physiological, and pharmacodynamic efficacy of these plants, correlating their effects with the active compounds they contain. This study, titled "Contribution to the Phytochemical Study of *Flacourtia ramontchi* (Lamoty), Family Salicaceae," focuses on a species traditionally used in Madagascar for its anti-rheumatic, anti-allergic, and anti-asthmatic properties (**Patro et *al*., 2013**).

Flacourtia ramontchi is a tree or shrub, typically growing to a height of 3–5 meters, occasionally reaching 10 meters. It is native to tropical Africa, Madagascar, and Asia, and is known by the vernacular name *lamoty* in Madagascar, as well as Governor's plum or Madagascar plum in English (**Patro et *al*., 2013**).

This species was selected due to a notable lack of comprehensive chemical and biological data. The present investigation therefore aims to perform a preliminary phytochemical screening to identify the major secondary metabolites. Subsequent objectives include the isolation of dominant compounds and the elucidation of their chemical structures for potential application in industrial pharmacy.

All experimental work was conducted at the Chemistry Laboratory of the University of Toliara, with a specific focus on the leaves and bark of the plant.

II. Research Methods

2.1 Plant material

Flacourtia ramontchi L'Hér., often treated as a synonym of *Flacourtia indica* (Burm.f.) Merr. (Govaerts et *al*., 2000), is a plant species widely distributed across Madagascar, tropical Africa, and Asia. The plant material for this study, consisting of bark and leaves, was collected in March 2021 from the rural municipality of Mitsinjo Betanimena, located in the Toliara II district of the Atsimo-Andrefana region of Madagascar. A voucher specimen of the plant's leaves and stripped bark was preserved for identification purposes.

a. Plant Nomenclature

The species is scientifically known as *Flacourtia ramontchi*. Its synonym, *Flacourtia indica*, is also widely recognized. The plant is known by various vernacular names depending on the language and region (**PIER, 2018**).

Figure 1. Debarked trunk and leaves

b. Systematic classification

The systematic classification of the plant *Flacourtia ramontchi* places it within the major clades of the plant kingdom. Its taxonomic hierarchy, from the broadest to the most specific category, is as follows: it belongs to the domain Biota and the kingdom Plantae, specifically within the subkingdom Tracheobionta and the supergroup Streptophyta. The plant is further classified under the division Magnoliophyta and the class Equisetopsida. It is situated within the clades Tracheophyta and Spermatophyta, followed by the subclass Magnoliidae and the superorder Rosanae. The species is part of the order Malpighiales, the family Salicaceae, and the subfamily Flacourtioideae, which includes the tribe Flacourtieae. The genus is *Flacourtia*, and the specific epithet is *ramontchi* (**The Angiosperm Phylogeny Group, 2016**).

c. Botanical description

Flacourtia ramontchi is a spiny, bushy shrub that can grow up to 10–15 meters in height. The simple or forked spines often disappear as the plant matures. The branches have a yellowish-gray to brown bark that is fissured and scaly. The leaves are quite variable in appearance, ranging from elliptical to oval, with or without an acuminate tip, and can be glabrous or pubescent. They emerge as a coral red color, become pink when young, and then mature to a dark green. The leaves are typically up to 12 cm long with a petiole of about 2 cm, and have 4–7 pairs of prominent veins on both surfaces.

The flowers are unisexual, arranged in axillary or sub-terminal clusters. The fruit is a globose, dark red to reddish-brown drupe that is edible when fully ripe; otherwise, it is highly astringent. Fruits ripen one to two months after flowering, toward the end of summer, producing large quantities of purple-red or blackish fruits that are approximately one inch in diameter at maturity (**Bourjot et *al*., 2012**).

The species is not demanding to cultivate, thriving in warm climates and exhibiting good drought resistance. A deep, rich, and well-drained soil, along with full sun exposure, is favorable for its growth.

d. Traditional medicine uses

Based on an ethnobotanical review, various parts of this plant—including the root, bark, leaves, and fruit—are used by traditional healers to treat several ailments (**Patro et *al*., 2013**).

In southern Africa, the Lobedude tribe uses a root decoction to alleviate body pain. In India, an infusion of the bark is employed as a gargle to treat hoarseness.

In Madagascar, a decoction made from the leaves and bark is used to treat respiratory difficulties such as asthma and dry coughs. The same decoction is used in baths for children with fontanelle issues (acute dehydration), and a poultice made from the roots is applied around the fontanelle. The green fruits are consumed to treat diarrhea, while the ripe fruits are eaten and are also an ingredient in *toaka gasy* (local rum) (**Galla et *al*., 2015**).

2.2 Methods

a. Extraction methods

The principle of extraction involves separating one or more components from a solid or liquid mixture by using a solvent in which the desired compounds are soluble (**Sasidharan et *al*., 2011**).

In this study, the collected plant material, consisting of both bark and leaves, was dried separately in a well-ventilated area, away from direct sunlight and humidity. After drying, the plant material was pulverized to a fine powder to increase its surface area, thereby enhancing permeability to the extraction solvent. Both solid-liquid and liquid-liquid extraction processes were employed to selectively isolate compounds based on their chemical and physical properties.

1) Solid-liquid extraction

Solid-liquid extraction, specifically successive maceration, was performed at ambient temperature. The process required prolonged contact between the pulverized solid and a series of solvents of increasing polarity: n-hexane, followed by dichloromethane, and finally methanol. For each extraction step, 50g of leaf powder and 50g of bark powder were placed in separate flasks. The maceration was carried out for approximately 48 hours, followed by agitation and filtration through a hydrophilic cotton filter. The resulting filtrates for each solvent were pooled and subsequently evaporated. Evaporation was conducted by heating the filtrates under reduced pressure using a rotary evaporator to remove the organic solvents and obtain the crude extracts.

The extraction yield was calculated using the following formula :

$$Yield(\%) = \frac{M_1 - M_2}{M_3} \times 100$$

Where :

- M_1: Mass of the flask after evaporation
- M_2: Mass of the empty flask
- M_3: Initial mass of the sample

2) Liquid-Liquid Extraction

Liquid-liquid extraction is a technique used to transfer a substance from one solvent in which it is difficult to separate to another in which it is more easily isolated (**Sasidharan et *al.*, 2011**). This method is predicated on two fundamental conditions : the two solvents must be immiscible, and the substance to be extracted must be significantly more soluble in the extraction solvent than in the original solvent (**Pimentel et *al.*, 2016**).

The efficiency of extraction with a given solvent is directly proportional to the number of successive extractions performed. Multiple extractions with smaller volumes of solvent are more effective than a single extraction with a larger volume (**Pimentel et *al.*, 2016**).

The isolation of a substance, whether natural or synthetic, frequently necessitates a solvent extraction followed by washing steps to remove impurities from the mixture.

a) Washing with water removes water-soluble impurities, such as inorganic or organic ions and low-molecular-weight polar compounds.
b) Washing with a diluted acid or basic solution facilitates the removal of residual bases or acids, respectively. The use of diluted solutions minimizes the temperature increase associated with these exothermic reactions.

These operations consistently conclude with a decantation step to separate the layers.

b. Preliminary phytochemical screening

A preliminary step in plant chemical analysis, **phytochemical screening**, employs a range of characteristic tests to identify the major classes of biologically active compounds within a plant extract (Sasidharan et al., 2011). This analysis targets a broad spectrum of secondary metabolites, including **alkaloids**, **coumarins**, **flavonoids**, **tannins**, **polyphenols**, **polysaccharides**, **quinones**, **saponins**, **steroids**, and **terpenoids**.

The primary purpose of these experiments is to generate a comprehensive chemical profile for the specific plant part under investigation. The identification of these diverse chemical families is typically achieved through two main types of qualitative assays: **precipitation-based reactions** and **colorimetric reactions** (Ammar et al., 2019; Rao & Suseela, 2016).

c. Thin Layer Chromatography (TLC)

This technique is a simple, rapid analytical method for separating compounds from a mixture. The principle relies on the differential migration of components between a stationary phase (a thin layer of adsorbent material, such as silica gel, on a solid support plate) and a mobile phase (a solvent or solvent mixture). A small sample is spotted onto the plate, which

is then placed in a chamber with the mobile phase. The mobile phase ascends the plate via capillary action, carrying the components of the mixture with it. Separation occurs because each compound has a different affinity for the stationary and mobile phases, causing them to move at varying speeds. The result is a series of separated spots, with their relative positions on the plate serving as a means of identification (**Tswett, 1906 ; Kirchner, 1978**).

d. Column Chromatography (CC)

Column chromatography is a widely used technique for the purification and isolation of compounds from a mixture. The separation principle is based on the differential partitioning of components between a stationary phase packed in a vertical glass column and a mobile phase that flows through it. The stationary phase is typically an adsorbent like silica gel or alumina. A mixture is loaded onto the top of the column, and the mobile phase is then introduced, causing the components to move down the column at different rates. Compounds with a weaker affinity for the stationary phase move more quickly, while those with a stronger affinity move more slowly. This process results in the separation of the mixture into distinct bands, which are collected as they exit the column at different times (**Tswett, 1906 ; Still et *al*., 1978**).

III. Results and Discussion

3.1 Chemical extraction

The plant extract was prepared from both the leaves and bark samples simultaneously. This process involved maceration of 50 g of powdered material from each part with 200 mL of various solvents arranged in order of increasing polarity. These solvents included hexane, dichloromethane (DCM), and methanol (MeOH). The mixture was agitated frequently over a period of 48 hours to ensure thorough extraction. Following this, the extracts were filtered, and the solvents were evaporated using a rotary evaporator to obtain the crude extracts, which were then subjected to phytochemical screening and chromatographic analysis (**Sasidharan et *al*., 2011; Zhang et *al*., 2018**).

The extraction yield for each sample was determined by weighing the flask containing the dried extract and calculating the percentage based on the initial mass of the plant powder. The results are summarized in the following table :

Table 1. Extraction results

Part Examined (Powder)	Solvents (200 ml)	Extract Yield (in g)	Color	Yield (%)
Leaves (50 g)	Hexane	1.56	Imperial green	3.12
	DCM	1.47	Veronese green	2.94
	MeOH	0.91	Empire green	1.82
Bark (50 g)	Hexane	1.36	Beige	2.72
	DCM	1.42	Brown	2.84
	MeOH	1.02	Brown-reddish	2.04

The data from the extraction reveals notable trends in the chemical composition of the plant's leaves and bark. The highest yields were consistently obtained with hexane, a non-polar solvent, for both plant parts. This suggests that the leaves and bark are richer in non-polar compounds, such as fats, waxes, and certain terpenoids, compared to their polar counterparts (**Abdel-Aal et *al*., 2014**).

The yields generally decreased as the polarity of the solvents increased, with the lowest yield recorded for the methanol extracts. This is an interesting finding, as it is often observed

that polar solvents, like methanol, tend to give higher extraction yields due to their ability to dissolve a broad range of phytochemicals (**Lapornik et *al*., 2005**). The observed trend here indicates a higher concentration of non-polar constituents within these specific plant tissues.
The color of the extracts also provides some insight into their chemical makeup. The green coloration of the leaf extracts from all three solvents is likely due to the presence of chlorophyll and other photosynthetic pigments, which can be extracted by solvents of varying polarities. In contrast, the bark extracts, with their beige to brown hues, suggest a higher concentration of colored compounds like tannins and other phenolic compounds, which are often responsible for brown and reddish pigments in plant tissues (**Singh & Singh, 2016**).

3.2 Phytochemical screening

The phytochemical screening of *Flacourtia ramontchi* organs has revealed a diverse profile of bioactive compounds, with a notable abundance of specific constituents in the bark and leaves. As summarized in the table below, the principal abundant phytochemicals identified were steroids, tannins, flavonoids, and polyphenols in the bark, and flavonoids, polyphenols, and tannins in the leaves. Other compounds that tested positive were detected in low or trace amounts (**Khan et *al*., 2012 ; Semwal et *al*., 2014**).

These findings corroborate the traditional therapeutic applications of this plant in treating various ailments. The rich presence of compounds such as flavonoids, polyphenols, and tannins, known for their antioxidant, anti-inflammatory, and antimicrobial properties, provides a scientific basis for its use in traditional medicine (**Doss, 2009 ; Kaundal & Sharma, 2021**).

Table 2. Phytochemical screening results of *Flacourtia ramontchi* organs

Identified Compound	Reaction Test	Leaves (fFr)	Bark (eFr)
Alkaloids	Dragendorff, Mayer	-	-
Coumarins	Fluorescence to UV	-	-
Flavonoids	Wilstater, Bath-Smith	++	+++
Polyphenols	H_2O + 10% NaCl + 1% Gelatin	++	+++
Polysaccharides	80º EtOH	-	±
Quinones	50% Diluted NH4OH	-	-
Saponins	Foam Test	-	-
Steroids	Lieberman-Burchard	+	++
Tannins	1% Gelatin + 10% NaCl	++	++
Terpenoids	Lieberman-Burchard	-	-

Note : +++ = Very abundant ; ++ = Abundant ; + = Present ; ± = Trace ; - = Absent

The phytochemical analysis of *Flacourtia ramontchi* reveals a clear distinction in the chemical composition between its bark and leaves. Both organs are particularly rich in phenolic compounds, including flavonoids and polyphenols. These compounds are widely recognized for their potent antioxidant and free radical scavenging activities (**Doss, 2009**). The presence of these constituents suggests that the plant extracts possess significant potential for mitigating oxidative stress-related diseases.

The high concentration of tannins in both the leaves and bark is also noteworthy. Tannins are polyphenolic compounds known for their astringent properties, which contribute to their use in treating diarrhea, wounds, and inflammation (**Semwal et *al*., 2014**). This finding provides a direct biochemical explanation for the traditional application of the plant in managing these conditions.

A distinct difference between the two organs is the presence of steroids in both the bark

and leaves, with a more significant abundance in the bark. While some phytochemical studies on *Flacourtia indica* have reported the absence of steroids, this finding highlights potential variations in phytochemical profiles based on the specific plant parts and environmental factors (**Kaundal & Sharma, 2021**). Steroids often serve as precursors for various hormonal and therapeutic compounds, suggesting additional biological activities for the bark extract.

The notable absence of certain compound classes, such as alkaloids, coumarins, and saponins, is also a crucial finding. This specificity of chemical composition allows for a more targeted understanding of the plant's biological actions. The results collectively affirm that the medicinal efficacy of *Flacourtia ramontchi* in traditional medicine is likely attributable to the synergistic effects of its abundant phenolic compounds and other detected constituents.

3.3 Thin Layer Chromatography (TLC)

The optimization of chromatographic separation for the dichloromethane (DCM) extract was conducted using thin-layer chromatography (TLC). A sample of the extract was spotted at a distance of 1 cm from the bottom edge of a TLC plate, and the solvent front was allowed to migrate until it reached 1 cm from the top edge.

Initial efforts to identify a suitable mobile phase involved the use of single-component solvents: 100% hexane and 100% DCM. These systems were unsuccessful, as evidenced by the resulting chromatograms which displayed significant streaking, indicating inadequate separation of the constituents. A refined approach was then employed, exploring binary solvent systems to achieve a better balance of polarity. Promising results were obtained with a hexane/DCM (50:50, v/v) mixture and a DCM/MeOH (98:02, v/v) mixture, both of which demonstrated a clear separation of the extract components.

Following the preliminary trials, a more precise solvent ratio was investigated to further improve resolution. A fifth system, hexane/DCM (30:70, v/v), was tested and proved to be highly effective, yielding four distinct, well-resolved major spots. This mobile phase was subsequently selected for further chromatographic analysis and preparative applications.

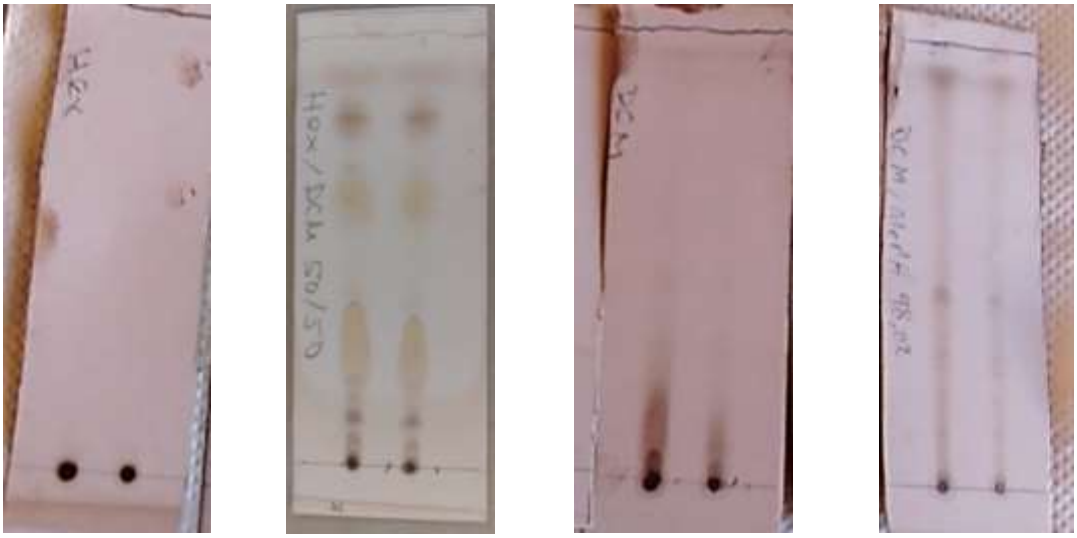
Visualization of the chromatograms was performed by initial exposure to ultraviolet light at wavelengths of 254 nm and 366 nm. Subsequently, the plates were sprayed with 10% sulfuric acid in methanol and heated to 120 °C to reveal additional spots. The major compounds observed under UV light were circled for documentation.

Thin-Layer Chromatography (TLC) results

The following images represent the chromatograms obtained from the initial four solvent systems, illustrating the progressive improvement in separation.

- **(a)** 100% Hexane: A single, long streak from the baseline, indicating no separation.
- **(b)** Hexane/DCM (50:50): A clear separation into multiple spots.
- **(c)** 100% DCM: A single, elongated streak, indicating poor separation.
- **(d)** DCM/MeOH (98:02): Good separation of constituents, with distinct spots.

The chromatograms from systems (a) and (c) demonstrate the failure of single-component eluents to effectively resolve the extract's constituents. The sample appears as a continuous streak on the plate, a result of the inadequate polarity match between the solvent and the compounds (**Sherma & Fried, 2011**). In contrast, chromatograms (b) and (d) show a clear separation of components, confirming the effectiveness of these binary systems.



(a) (b) (c) (d)

Figure 2. Résults of Thin-Layer Chromatography (TLC)

The subsequent optimization with the hexane/DCM (30:70, v/v) system yielded superior resolution, as shown in the following figure.

Eluent : Hexane/DCM : 30/70 ; Reagent :H_2SO_4 + heating to 120 °C

Figure 3. Constituents dominated by the Hex/DCM eluent (30/70).

The chromatogram shows four well-defined and distinct major spots, indicative of an ideal separation for the DCM extract.

The progressive refinement of the TLC mobile phase provides critical insight into the physicochemical properties of the bioactive compounds within the DCM extract. The initial failure to achieve separation with non-polar (hexane) and highly polar (DCM) single-component eluents indicates that the extract contains a complex mixture of compounds with intermediate polarity (**Fried & Sherma, 2015**).

The successful separation achieved with binary solvent systems, particularly the hexane/DCM (30:70, v/v) mixture, confirms that the most abundant compounds in the DCM extract are of moderate polarity. This specific ratio provides the optimal eluting power, allowing for the differential migration of individual compounds and their resolution into distinct bands. The visualization of four major, well-separated spots suggests that these are the primary constituents of the extract, a finding that is crucial for further research. This optimized chromatographic system is a prerequisite for the isolation and purification of these

specific compounds, which can then be subjected to structural elucidation and biological activity assays. This strategic approach streamlines the process of isolating target molecules from a complex plant extract (**Mukherjee, 2019**).

3.4 Column Chromatography (CC)

The dichloromethane (DCM) extract, previously optimized via thin-layer chromatography (TLC), was subjected to silica gel column chromatography for further purification. This technique, a cornerstone of natural product isolation, facilitates the separation of complex mixtures into their individual components based on differential polarity (Fried & Sherma, 2015).

The preparative column, a cylindrical Pyrex glass tube measuring 15 cm in height and 2 cm in internal diameter, was wet-packed with a slurry of silica gel to a height of 42.5 cm. After preparation, the crude extract was carefully applied to the top of the column. A stepwise gradient elution was then initiated to selectively elute compounds based on increasing polarity. The elution began with a hexane/DCM (50:50, v/v) mixture, progressively transitioned to a hexane/DCM (30:70, v/v) system, and was concluded with 100% DCM to ensure the elution of all constituents. A total volume of approximately 800 mL of eluent was used, and 102 fractions were collected.

The collected fractions were subsequently analyzed by TLC to monitor the migration and purity of the separated compounds. Fractions exhibiting similar spot profiles were pooled together to consolidate the isolated compounds. This analysis revealed that four fractions—specifically, fractions 10 to 20, 30 to 40, 45 to 50, and 60 to 65—each contained a single compound, as indicated by the presence of a solitary spot on the TLC plate. These purified fractions were separately collected, and their yields were determined as follows: F10-20 (7 mg), F30-40 (6.5 mg), F45-55 (8.02 mg), and F60-65 (9.82 mg).

For subsequent structural elucidation, the F60-65 fraction was chosen for its superior yield. The quantity of this fraction is deemed sufficient to enable comprehensive analysis by sophisticated spectroscopic techniques, such as nuclear magnetic resonance (NMR) and mass spectrometry, which typically require a minimum quantity of material for accurate and interpretable data (**Siddiqui & Khan, 2020**).

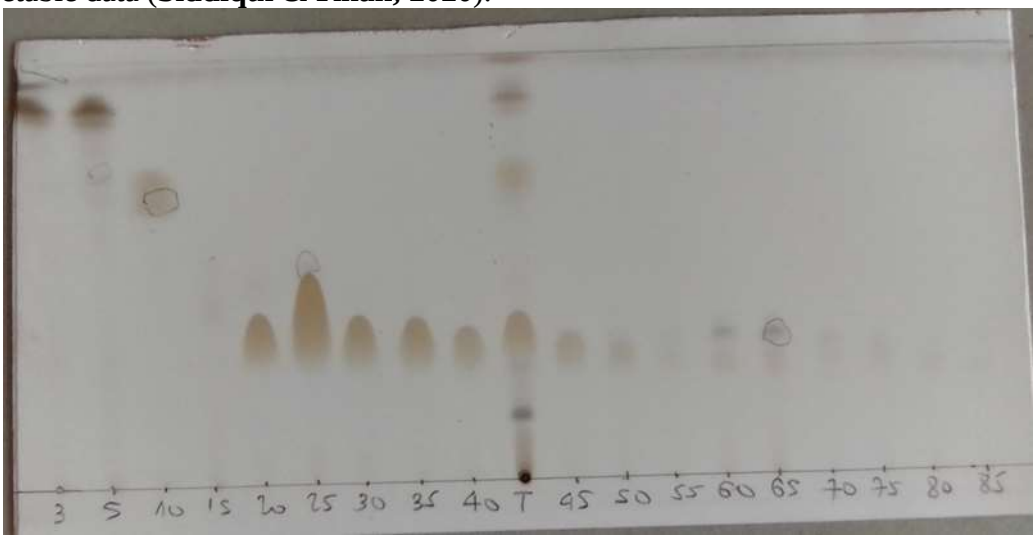


Figure 4. TLC control of the fractions obtained

The successful isolation of four pure fractions via column chromatography validates the optimization strategy employed with TLC. The initial solvent gradient, which began with a non-polar system and incrementally increased in polarity, effectively separated the compounds in the extract. The distinct spot profiles observed in the TLC analysis of the fractions provided clear evidence of successful fractionation. The presence of a single, well-

defined spot for each of the four identified fractions (F10-20, F30-40, F45-55, and F60-65) confirms the isolation of pure compounds. The decision to proceed with the F60-65 fraction for structural analysis is a strategic one, as its higher yield of 9.82 mg provides a greater sample mass, ensuring reliable and high-quality data from advanced spectroscopic instruments. This systematic approach is a standard and essential practice in natural products research for identifying and characterizing novel bioactive molecules.

3.5 Determination of isolated product structure

Analytical techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are indispensable tools for the structural elucidation of isolated compounds. These methods provide complementary information about a molecule's molecular weight, elemental composition, and structural connectivity.

Mass Spectrometry (MS)

Mass spectrometry is a highly sensitive and precise analytical technique used to determine the molecular weight and formula of a compound. The process involves ionizing molecules and separating the resulting ions based on their mass-to-charge ratio (m/z). This technique provides a unique "fingerprint" of the molecule, revealing its structural characteristics.

In this study, a MAT 312 mass spectrometer from the FINNIGAN company was employed for Electron Impact Mass Spectrometry (EI-MS). The sample molecules were ionized by bombarding them with a beam of electrons at 70 eV. This process converts neutral molecules into positively charged ions, primarily through the loss of a single electron, to form the molecular ion [M]+. The mass-to-charge ratio of these ions is then measured to determine the molecular weight of the compound (**Karasek & Clement, 1999 : De Hoffmann & Stroobant, 2007**).

The fundamental principle of mass spectrometry is represented by the following equation, which relates the mass of the ion (m), its charge (z), the magnetic field strength (B), and the velocity of the ion (v) :

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

where:

- m = mass of the ion
- z = charge of the ion
- B = magnetic field strength
- r = radius of curvature
- V = accelerating voltage

This equation allows for the precise determination of the mass-to-charge ratio, providing critical information for identifying the molecular weight and fragments of the compound.

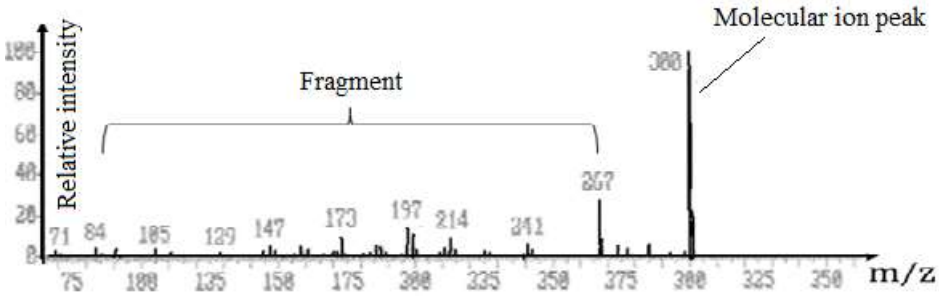


Figure 5. EI-MS mass spectrum

According to this result, the m/z value of the molecular peak is equal to 300, which corresponds to the molecular mass of our molecule. The fragmentation peaks allow us to

reconstruct part of the structure. The fragments at m/z 285 [M+- 15] and m/z 267 [M+- 33] can be interpreted as a loss of CH_3 and (H_2O + CH_3) respectively.

Nuclear Magnetic Resonance (NMR) spectroscopy

Structural elucidation of the isolated compound was performed using nuclear magnetic resonance (NMR) spectroscopy. All spectra were recorded at the Institute of Organic Chemistry at the University of Hanover, Germany.

The measurements were carried out on a Bruker AV 400 spectrometer, operating at a frequency of 400 MHz for proton NMR (^{1}H NMR) and 100 MHz for carbon-13 NMR (^{13}C NMR). The isolated product was dissolved in deuterated chloroform ($CDCl_3$), a common solvent for NMR analysis due to its lack of interfering proton signals (Pavia et *al*., 2015).

The chemical shifts, which are crucial for determining the magnetic environment of individual nuclei, are reported in parts per million (ppm). These values are referenced against the internal standard tetramethylsilane (TMS), a widely used reference compound with a defined chemical shift of 0.00 ppm for both proton and carbon NMR (Silverstein et *al*., 2014).

^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy

The ^{1}H NMR spectrum of the isolated compound provides critical insights into its structural features, with key resonances aligning with those characteristic of a diterpene skeleton. The spectrum, recorded on a Bruker AV 400 spectrometer in deuterated chloroform ($CDCl_3$), was analyzed with reference to tetramethylsilane (TMS) at δ 0.00 ppm.

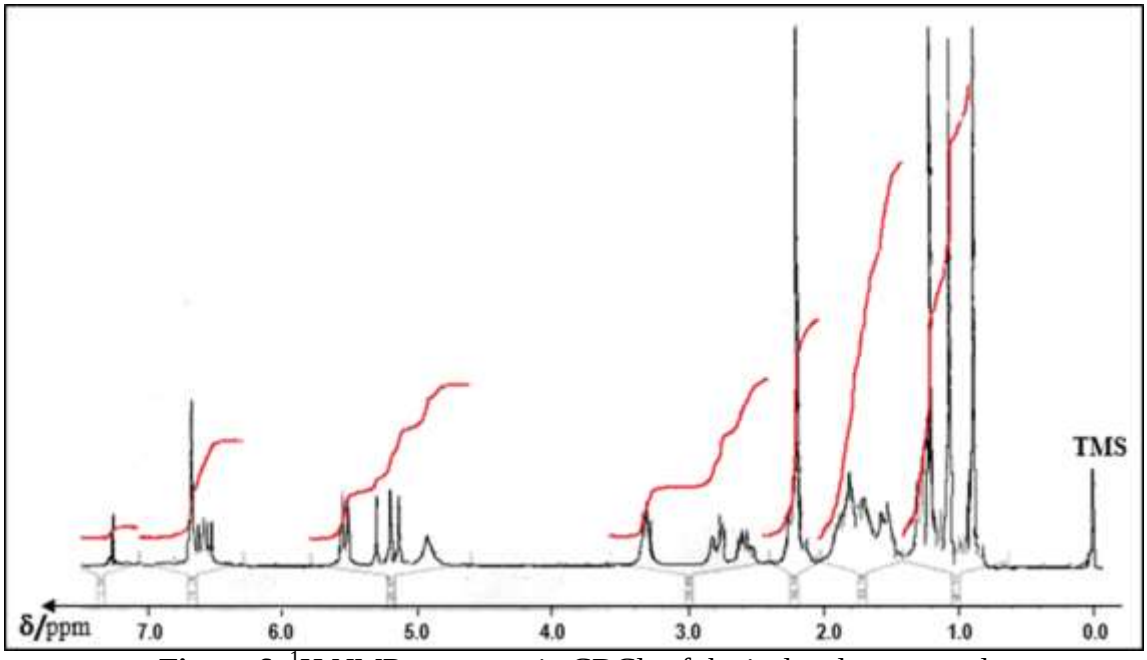


Figure 6. ^{1}H NMR spectrum in $CDCl_3$ of the isolated compound

Analysis of the spectrum (Figure 6) reveals a distinct set of signals attributed to various proton environments within the molecule. Four significant singlets, each integrating to three protons, are present, corresponding to four methyl (CH_3) groups. Three of these methyl resonances are shielded, appearing upfield at chemical shifts of δ 0.98, 1.09, and 1.41 ppm. A fourth methyl singlet is observed at a slightly more downfield position of δ 2.25 ppm.

A triplet resonance, integrating to a single proton at δ 3.3 ppm, is a typical signal for a methine proton (CH) directly bonded to an oxygen atom, specifically in a hydroxyl group (-OH), which is a common motif in diterpene or triterpene structures (**Pavia et *al*., 2015**).

In the olefinic region, between δ 5.2 and 5.7 ppm, a collection of signals integrating to three protons is consistent with the presence of an exocyclic vinyl group (CH_2=CH−).

Additionally, a sharp singlet at δ 6.8 ppm with an integration of one proton is highly suggestive of a proton on an aromatic ring (**Silverstein et *al.*, 2014**). This combination of signals supports the proposed diterpenoid nature of the compound and provides a basis for more detailed structural elucidation.

^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy

The 13C NMR spectrum of the isolated compound reveals the presence of a total of 20 distinct peaks, which confirms the presence of 20 carbon atoms within the molecular structure. This information, while fundamental, is supplemented by a technique known as Distortionless Enhancement by Polarization Transfer (DEPT), which is essential for differentiating between different types of carbon atoms (**Pavia et *al.*, 2015**).

The primary advantage of DEPT spectroscopy is its ability to categorize carbons based on their attached hydrogen atoms: methyl (CH_3), methylene (CH_2), methine (CH), and quaternary (Cq) carbons. This distinction is crucial for constructing the carbon skeleton of a molecule.

DEPT 135: A DEPT experiment with a 135° pulse angle provides key information for structural assignment. In this spectrum, methyl (CH_3) and methine (CH) groups are represented by positive signals, while methylene (CH_2) groups appear as negative signals. Quaternary carbons (Cq), which have no attached hydrogens, are not observed in this spectrum.

DEPT 90: A DEPT experiment with a 90° pulse angle is more selective. It provides positive signals exclusively for methine (CH) groups. All other carbon types—methyl, methylene, and quaternary—are completely absent from this spectrum.

The combination of data from the full ^{13}C NMR spectrum and the DEPT experiments (DEPT 135 and DEPT 90) provides a powerful tool for a detailed structural assignment of the compound. The number of signals in the DEPT spectra can be directly correlated with the number of methyl, methylene, and methine groups, thereby confirming the structural units identified in the ^{1}H NMR spectrum and complementing the molecular weight information from the mass spectrum (**Silverstein et *al.*, 2014**).

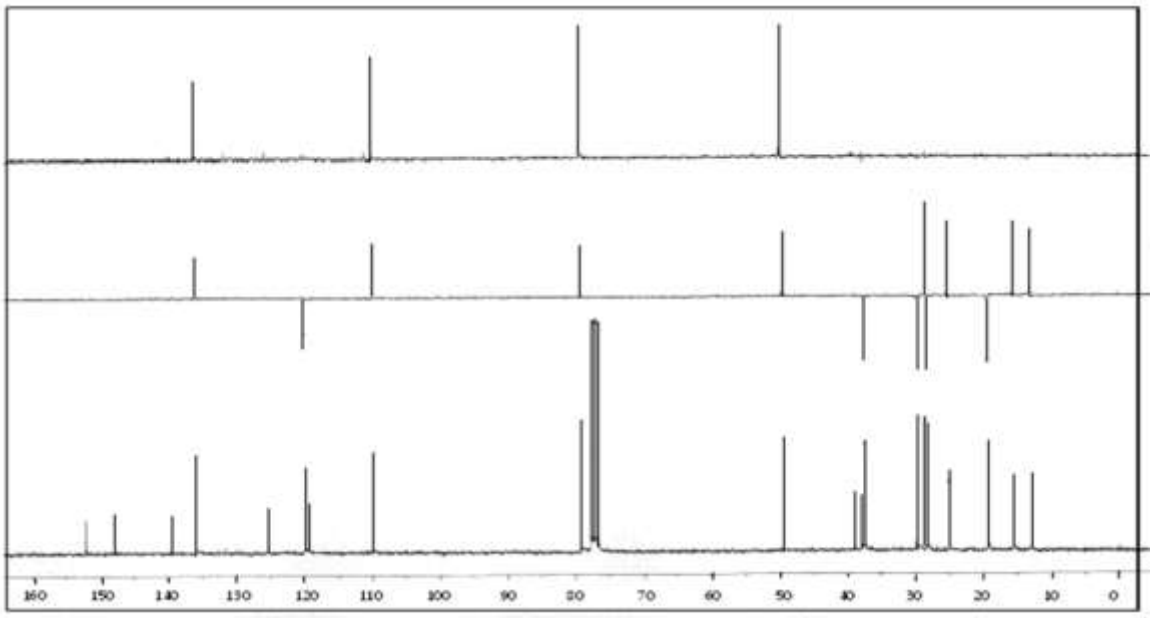


Figure 7. Results of 13C, DEPT (90 and135) NMR spectra of the isolated compound

Detailed analysis of the ^{13}C NMR and DEPT spectra confirms the presence and type of various carbon atoms within the molecule. The full ^{13}C NMR spectrum shows a total of 20 peaks, which is consistent with the presence of 20 carbon atoms.

The DEPT 90 spectrum yielded four signals, confirming the presence of four methine (CH) groups (**Pavia et *al*., 2015**).

The DEPT 135 spectrum further distinguished the carbon types. It showed five signals in the negative region, corresponding to five methylene (CH_2) groups. In the positive region, there were a total of eight signals, comprising four methine (CH) groups and four methyl (CH_3) groups.

The chemical shift values of the carbon signals provide additional structural information:

- **Four methyl carbons** were observed at δ 13.2,16.1,25.3, and 28.8 ppm.
- **Five methylene carbons** were identified at δ 20.0,28.7,30.6,38.7, and 119.3 ppm.
- **Four methine carbons** were found at δ 51.1,79.5,110.5, and 137.3 ppm.
- **Seven quaternary carbons** were detected at δ 38.8,39.9,120.5,124.7,140.0,148.6, and 154.3 ppm.

Notably, the methine signal at δ 79.5 ppm is significantly downfield, suggesting that it is attached to an electron-withdrawing group, likely a hydroxyl (-OH) group. Similarly, the quaternary aromatic carbon at δ 154.3 ppm is consistent with an aromatic carbon bonded to a hydroxyl group (**Silverstein et *al*., 2014**).

Furthermore, the spectrum indicates the presence of an aromatic ring and a double bond. The olefinic region shows two peaks corresponding to one carbon-carbon double bond, while the aromatic region displays six peaks, which is characteristic of a six-membered aromatic ring. Combining the olefinic and aromatic regions gives a total of eight unsaturated carbons (two from the double bond and six from the aromatic ring).

Based on the cumulative data from the ^{13}C NMR and DEPT analyses, the molecular formula of the isolated compound was determined.

Table 3. Procedure for obtaining the general formula

Spectrum	Signal	Number of peaks	Formula
DEPT 90 :	CH	4	C_4H_4
DEPT 135 :	CH_2	5	C_5H_{10}
	CH_3	4	C_4H_{12}
Comparison of DEPT with ^{13}C decoupled	Cq	7	C_7
	OH	2	H_2O_2
	Total		**$C_{20}H_{28}O_2$**

This derived molecular formula, $C_{20}H_{28}O_2$, corresponds to a molecular weight of 300 g/mol. This value is in complete agreement with the parent ion peak observed at m/z=300 in the mass spectrum, providing strong evidence for the correctness of the proposed formula.

Calculating the number of rings and unsaturations ($N_{C/I}$)

The number of cycles and unsaturations ($N_{C/I}$) is a key parameter for determining the structural formula of a molecule. It represents the number of rings and multiple bonds (typically double bonds) present. This value can be calculated from the molecular formula using the following equation:

$$N_{R.I} = n_C + \frac{n_H}{2} + n_N + 1$$

Where:

- C = number of carbon atoms
- H = number of hydrogen atoms
- N = number of nitrogen atoms
- Halogens (X) are treated as hydrogens, and oxygen is ignored.

Based on the determined molecular formula of the isolated compound, $C_{20}H_{28}O_2$:

$$n_R = 20, n_H = 28 \text{ et } n_N = 0$$

Substituting these values into the formula:

$$N_{R/I} = 20 + \frac{28}{2} + 0 + 1$$

$$N_{R/I} = 7$$

The presence of 8 peaks in the C=C double bond zone indicates the presence of 4 unsaturations (4 double bonds).

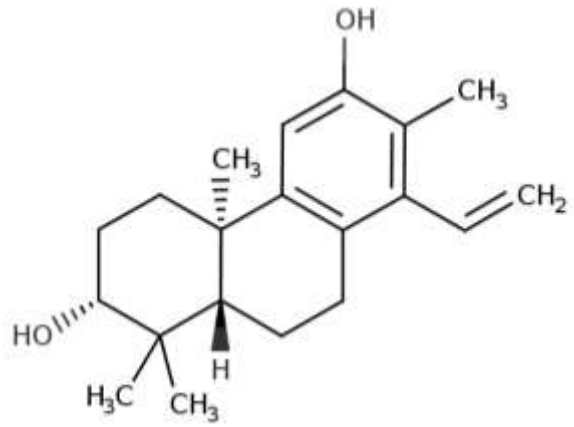
As $N_{C/I}$=7 the molecule contains 4 double bonds and 3 rings.

Structural Proposal

The presence of eight peaks in the olefinic and aromatic regions of the ^{13}C NMR spectrum confirms the presence of an aromatic ring and a double bond, which account for a total of four unsaturations (three from the aromatic ring and one from the double bond). Given that the total unsaturation count is seven ($N_{C/I}$=7), the remaining three unsaturations must be attributed to the presence of three cycles.

Therefore, the molecular structure must contain three rings and four double bonds (one exocyclic double bond and a benzenoid ring). The total number of carbons is 20, which is characteristic of a diterpene. The combination of a tricyclic skeleton and the presence of four unsaturations aligns perfectly with the characteristics of a tricyclic diterpenoid.

By comparing these spectroscopic and computational data with established literature on natural products, the structure was identified as **spruceanol** (IUPAC name: 3,12-dihydroxy-cleistantha-8, 11, 13, 15-tetraene). This compound, with a molecular mass of 300 and a formula of $C_{20}H_{28}O_2$, is consistent with all spectroscopic data obtained.



Trade name : Spruceanol
IUPAC Name : 3,12-dihydroxy-cleistantha-8, 11, 13, 15-tetraene
Molar Mass : 300
Molecular Formula : $C_{20}H_{28}O_2$
Figure 8. Spruceanol structure

The structural identity of the isolated compound was conclusively confirmed by comparing its 13C chemical shifts with reported values from the literature (**Gunasekera et *al.*, 1989**). The following table presents a side-by-side comparison of the observed chemical shifts for each of the 20 carbon atoms with those previously published for the compound

spruceanol.

Table 4. Comparison of chemical shifts of isolated compounds with those in the literature (**Gunasekera et al,1989**)

Carbon number	Carbon type	Isolated compound δ (ppm)	Literature δ (ppm)
1	CH_2	38.7	38.6
2	CH_2	28.4	28.5
3	CH	79.5	80.0
4	Cq	39.9	40.1
5	CH	51.1	51.1
6	CH_2	20.0	20.3
7	CH_2	30.6	30.5
8	Cq	124.7	124.5
9	Cq	148.6	148.6
10	Cq	38.8	39.0
11	CH	110.5	110.2
12	Cq	154.3	153.9
13	Cq	120.5	120.4
14	Cq	140.0	140.4
15	CH	137.3	138.0
16	CH_2	119.3	118.9
17	CH_3	13.2	15.1
18	CH_3	28.8	28.9
19	CH_3	16.1	16.2
20	CH_3	25.3	24.6

The data presented in the table demonstrates a remarkable consistency between the 13C chemical shifts of the isolated compound and those reported for spruceanol. The observed values are in excellent agreement, with only minor deviations (typically less than 1 ppm), which are well within the accepted range for minor variations due to solvent, concentration, and instrument calibration (**Pavia et *al*., 2015**). This close match across all 20 carbons, including the distinct methine, methylene, methyl, and quaternary carbon types, provides definitive proof of the molecule's identity.

The comparison of chemical shift values is a cornerstone of natural product chemistry, as each carbon atom in a specific chemical environment resonates at a unique frequency. The precise correlation of the measured δ values to the literature-reported values confirms not only the molecular formula but also the complete stereochemical arrangement of the atoms. This final piece of evidence, combined with the earlier mass spectrometry and ^{1}H NMR data, unequivocally establishes the structure of the compound isolated from *Cynometra madagascariensis* as spruceanol.

IV. Conclusion

Flacourtia ramontchi, a small, spiny, deciduous tree native to Madagascar, has a rich history in traditional medicine. It's used by the Malagasy population to treat a variety of ailments, including **asthma, dry cough, pain, pneumonia, and intestinal worms**. This ethnobotanical context highlights the plant's potential therapeutic value and provides a strong foundation for its scientific investigation. The study of this species is a valuable contribution to the national efforts to conserve medicinal plants and preserve traditional local medicine.

Phytochemical analyses were conducted on bark and leaf samples. Both were subjected to maceration using a series of solvents with increasing polarity: hexane, dichloromethane (DCM), and methanol. The extraction yields were 1.56 mg (hexane), 1.76 mg (DCM), and 2.01 mg (methanol) for the bark, while the leaves yielded 1.25 mg, 1.32 mg, and 1.64 mg, respectively.

Phytochemical screening of the extracts revealed a high concentration of **polyphenolic compounds, tannins, and terpenoids**. These secondary metabolites are known for their significant therapeutic properties. Following the extraction and screening, the DCM bark extract was chosen for further analysis. Thin-layer chromatography (TLC) using a hexane/DCM (30/70) eluent showed four dominant spots, suggesting the presence of major constituents.

The extract was then separated using column chromatography. Fractions 60 to 65 yielded the highest mass (9.82 mg) and were selected for structural determination. The isolated compound was characterized using mass spectrometry and one-dimensional nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR followed by DEPT). Spectral data comparison with existing literature confirmed the compound to be **spruceanol**, a diterpene from the terpenoid family.

To build on this research, it would be beneficial to expand the scope of investigation. The methodology could be applied to other plant species to continue the valuable ethnobotanical and phytochemical studies in Madagascar. Furthermore, the isolated compound and other fractions should be tested in various biological models, both *in vitro* and ***in vivo***, to confirm the therapeutic activities traditionally attributed to *F. ramontchi*.

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