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PHYTOCHEMICAL SCREENING AND EVALUATION OF BIOLOGICAL ACTIVITY OF THE CRUD EXTRACT FROM THE STEM BARK, LEAVES AND ROOT BARK OF THE *CANARIUM MADAGASCARIENSIS* (BURCERACEAE) ORIGINATED IN THE SOUTHEASTERN OF MADAGASCAR

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ABSTRACT

The present study centered on the evaluation of the biological activity and the identification of the chemical family present in the plant known by the vernacular name "Ramy" or "Emboka," the Malagasy name, and scientifically called Canarium madagascariensis (Burseraceae). This plant is endemic to the southeastern region of Madagascar and is well known in this region for its therapeutic properties in traditional medicine. This study constitutes a component of the research program of the Doctoral School in Geosciences, Physics, Environmental Chemistry, and Host-Pathogen Systems (GPCEHP) at the University of Toliara. The primary objective of this study is to verify the ethnobotanical data of the plant in question based on traditional uses, using available scientific methods based on biological tests, and to detect the chemical families of the compounds present in this plant. The present study aims to establish a foundation for future research endeavors that will focus on the identification, isolation, and determination of the chemical structures of the active ingredients responsible for these biological effects. The results of phytochemical screening revealed the presence of various chemical families of secondary metabolites, including tannins, polyphenols, saponins, steroids, and terpenoids. In vitro biological tests carried out on extracts from this plant revealed that it has antioxidant effects and antibacterial properties. The presence of phenolic compounds and terpenoids in this plant is indicative of its biological activity, as these two chemical families are well-documented to exhibit such properties. A comprehensive review of the extant phytochemical and biological screening studies of extracts from *Canarium madagascariensis* (Burseraceae) reveals the scientific validation of the traditional uses of this plant.

KEYWORDS: *Canarium madagascariensis*, Burseraceae, Phytochemical screening, antioxidant, antibacterial.

1. INTRODUCTION

Increases in environmental disturbances (e.g., global warming, pollution) and their consequences for the proliferation and mutation of pathogens (Swynghedauw, B. 2009) have led to significant global health challenges. These challenges include the emergence of zoonotic pathologies and growing resistance to antibiotics. Heat stress, with its direct effects on the human organism, is another public health problem posed by climate change (Besancenot, J. P. 2000). Recent epidemiological crises, including the COVID-19 pandemic and the Ebola and monkeypox epidemics, underscore the pressing need to develop innovative and effective therapeutic strategies. In this critical context, herbal medicine, though popular with a large proportion of the world's population and with a long history, often lacks the rigorous scientific validation needed to understand its mechanisms of action and optimize its use. Despite the vast biodiversity of African flora, this encompasses numerous untapped medicinal plants, particularly those endemics to Madagascar due to their abundance and diversity. Secondly, given that over 80% of the population relies on traditional medicine (Diallo et al., 2001; Jiofack et al., 2009, 2010), it is evident that a significant proportion of these resources remain poorly characterized scientifically. This prompts the following question: How can the as yet unexplored potential of traditional medicinal plants are harnessed to address current public health challenges.

The rationale underlying our decision to adopt a scientific approach, integrating Phytochemistry and biology, was to substantiate and empirically validate the traditional applications of *Canarium madagascariensis*.

2. MATERIAL AND METHOD

2.1. Ethno-botanical Survey

Ethno-botanical information about the plant species selected for this study was obtained from fourteen (14) traditional healers during a field work in the Southeastern of Madagascar. Informants were selected for their authentic knowledge on the utilization of medicinal plants Malagasy, the national language of Madagascar was used during anthropological interviews. Traditional healers were, interviewed on a voluntary basis. The study followed principles laid out in the declaration of Helsinki as previously reported.[5,10] Informed consent was obtained from both the Government of Madagascar to collect plant samples and to conduct non-commercial research on Malagasy medicinal plants and the respondents to divulge their knowledge.

2.2. Selection and collection of plant material

The plant species *Canarium madagascariensis* (Burseraceae) was selected based on its relative citation frequency (use values 0.70) and the information consensus factor value (0.421). The leaves, stem bark and root bark of *Canarium madagascariensis* were collected in Mahasoabe village, district of Vohipeno (Southeastern part of Madagascar) on May 2024. The plant sample was identified by comparison with reference specimens available at the department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number RAM-01 was deposited at the herbarium of Laboratory of Applied Chemistry, Lay Flaylle Street University of Toliara-Madagascar.

2.3. Extracts of preparations

The samples (leaves, stem bark, and root bark) of the collected plant were dried in the drying room at room temperature for ten (10) days. Subsequent to desiccation, the specimens were pulverized using a mechanical grinder, and the powder obtained from each part of the plant was stored in a polyethylene bag. 500g of sample powder (leaves, stem bark, and root bark) was extracted by repeated maceration with ethanol 90° (3x4 hrs, 5l) at room temperature. After filtering the mixture, the aqueous-ethanol filtrates were pooled, dried over Na_2SO_4 and evaporated to dryness under reduced pressure using a rotary evaporator to yield crude ethanolic extract yielding from each sample: EF-01 (crude leaf extract), ET-01 (crude extract from stem bark), and ER-01 (crude extract from root bark) Table 1. A quantity of 15 grams of crude extracts per sample was suspended in water and sequentially partitioned with n-hexane, dichloromethane, and ethyl acetate (1:1, v/v) to yield the corresponding extract fractions. The different extracts were evaporated to dryness on an evaporator apparatus and were evaluated for their pharmacological properties to verify and to localize the active fraction. All extracts were stored at +4 °C.

2.4. Phytochemical screening

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol and water (100 mL × 2) for 48 hours. Chemical screening was done as below.[12]

2.4.1. Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.2. Detection of flavonoids

The ethanol extract (5 mL) was added to a concentrated sulphuric acid (1 mL) and 500 mg of magnesium. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

2.4.3. Detection of tannins

Two methods were used to test for tannins. First, about 1 mL of the ethanol extract was added in 2 mL of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration. Second, 2 mL of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric

chloride solution was added . A dark green or blue green coloration indicates the presence of tannins.

2.4.4. Detection of saponins

To 1 ml of aqueous extract was added 0.3 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

2.4.5. Detection of alkaloids

Five mL of the aqueous extract was added to 2 mL of HCl. To this acidic medium, 1 mL of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

2.4.6. Detection of triterpenoids

Ten (10) mg of the aqueous extract was dissolved in 1 mL of chloroform; 1 mL of acetic anhydride was added following the addition of 2 mL of concentrated sulphuric acid. Formation of reddish violet color indicates the presence of triterpenoids.

2.5. Evaluation of antimicrobial activity

2.5.1. Microbial strains

The activity of each crudes extracts samples was tested toward 10 different microorganisms: Gram positive bacteria represented by Bacillus subtilis (B. subtilis ATCC 6633), Staphylococcus aureus (S. aureus ATCC 25923), Streptococcus sinensis, Bacillus cereus (B. cereus ATCC 10876), and Gram negative bacteria: Escherichia coli (E. coli ATCC 25922), Salmonella typhii (S. typhii ATCC 13311), Pseudomonas aeruginosa (P. aeruginosa ATCC 27853), and Enterobacter cloacae (E. cloacae ATCC 13047). The tested strains were obtained from the American Type Culture Collection (ATCC, Rockville MD, USA).

2.5.2. Disc diffusion

The agar disc diffusion method was used to determine the antibacterial activity of the different crude extract samples as follow: A 1 mL of suspension of any tested bacteria containing about 106 UFC/mL were spreaded on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 20 µL of pure each crudes extracts and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters. Chloraphenicol or cycloheximid (10µg/mL) was included as control.

2.5.3. Minimum inhibitory and minimum bactericidal concentration

An aliquot (10 µL) of a 106 CFU/mL overnight culture was added to wells of a sterile 96-well micro-titre plate. Pattern of each crude extract samples (EF-01, ET-01 and ER-01) was diluted in Mueller Hinton Broth (MHB) containing 0.1% (v/v) Tween 80 and added to wells to give final concentrations ranging from 0.03 to 10µL/mL. The positive control wells contained MHB+ bacteria suspension without each crude extract while negative control wells contained MHB only. Optical density (OD) was measured at 630 nm using a microplate reader (Titertek Twin-reader, Finland) and again after incubation for 24 hours at 37°C. The Minimum Inhibitory Concentration (MIC) was determined as the lowest each crude concentration at which the OD after 24 h of incubation of the inoculum remained the same or reduced compared with the initial reading. MTT (30 µL) in aqueous solution (0.01%) was used to evaluate the microorganism viability. For Minimum Bactericidal (MBC) determination, 10 µL was taken from each well after incubation and spot inoculated on to MHB and incubated for 24 hours at 37 °C. The concentration at which no growth observed on subculture was determined as the MBC.[13] The mean MBC/MIC ratio was evaluated for each sample.

2.6. Evaluation of the antioxidant activity of different extracts from *Canarium madagascariensis*

The literature describes two methods for evaluating the antioxidant activity of a compound: bioautography (Tiqwari et al., 2000 and Diarra 2006) and DPPH assay (Luhata et al., 2015; Sanchez-Moreno, 1998; and Mahefarivo, 2018). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method using UV spectrometry was used to evaluate the antioxidant activity of different extracts from samples (leaves, stem bark, and root bark) of Canarium madagascariensis.

2.6.1. The method is straightforward: use a spectrophotometer to measure DPPH

We quantified the antioxidant power of different extracts from samples (leaves, stem bark, and root bark) of *Canarium madagascariensis* using the method described by Brand et al., 1995, and Sanchez Moreno et al., 1998, with some modifications. DPPH (25 mg) dissolves perfectly in 100 ml of methanol. This preparation is guaranteed to stay fresh when stored away from light. Add ten milliliters of this solution to 45 milliliters of methanol. We have prepared concentrations ranging from 2 mg/mL to 0.125 mg/mL of various extracts of Canarium madagascariensis with the utmost rigor. In dry tubes, 200 µL of each concentration was mixed with 3800 µL of the 4.5% DPPH solution. I prepared blanks consisting of 3800 µL of the 4.5% DPPH solution and 200 µL of methanol. The test was meticulously executed six times, ensuring consistency and accuracy. Then, it was incubated in the dark for one hour. The same procedure was applied to the vitamin E (α-tocopherol) control, and their absorbances were measured using a spectrophotometer at a wavelength of 517 nm. The antioxidant activity, defined as the capacity to capture free radicals, is quantitated by the extent of discoloration of DPPH in a methanol solution. The expression (1) is the means by which this is determined:

Percentage of Inhibition:

$$\% = [(Ac - As) / (Ac)] \times 100 \quad (1)$$

Ac: The absorption of the DPPH at a wavelength of 517 nm was measured.

As: absorbance of the test extract

3. RESULTS AND DISCUSSION

3.1. Ethno-botanical survey

During ethno-botanical survey, fourteen (14) traditional healers were interviewed about medicinal plants of ethno pharmacological relevance in Malagasy folk medicine to treat wounds, fever and bacterial infections. The most cited plant *Canarium madagascariensis* (Burseraceae) has the use value and informant consensus factor of 0.710 and 0.421 respectively.

3.2. Phytochemical screening

The results of phytochemical chemical screening of *Canarium madagascariensis* (root bark) revealed the presence of leucoanthocyanins, flavonoids, coumarins, steroids, terpenoids, polysaccharides, polyphenols, and tannins. However, chemical groups such as alkaloids, saponins, and quinones were not found in the investigated plant material, and for the ethanolic extract of the stem bark watch the high presence of coumarins, quinones, antraquinones, flavonones and triterpenoids. Steroids, iridoïdes, polyphenols and flavonols are also present. Alkaloids, polysaccharides, tannins and saponins are notably absent, at the end for the crude extract from the leaves of the *Canarium madagascariensis* indicated the presence of flavonoids compounds, steroids and coumarins. Alkaloids, tannins, polyphenols, triterpènes, polysaccharides, saponins and quinones are notably absent.

The presence of various secondary metabolites in this plant species could justify it ethno-medical use.

3.3. Extract yields

The extract yields of each part of *Canarium madagascariensis* (leave, stem bark and root bark) obtained with different solvents have been recorded in Table I.

Table 1: Extraction results of each part of *Canarium madagascariensis* (Stem bark, leaves, and root bark)

Species	***Canarium madagascariensis***		
Samples studied	**Leaves**	**Stems bark**	**Root bark**
Powder of mass (g)	750	750	750
Ethanolic extract (g)	21.01	23.68	2.32
Ex-Hex (g)	6.23	4.53	2.32
Ex-DCM (g)	7.08	8.04	6.61
Ex-ACoET (g)	5.16	6.30	11.07
Ex-Aq (g)	1.51	1.08	0.30

3.4. Antimicrobial activity

The antimicrobial activity of each crudes extracts samples of *Canarium madagascariensis* against microorganisms was determined. The results are shown in Tables 2 and 3. All bacteria demonstrated some degree of sensitivity to the crude extracts within the concentrations tested. The ethanolic extracts of *Canarium madagascariensis* root bark displayed antimicrobial activity against all bacterial strains tested with the inhibition zones varying from 7 to 16 mm (Table 2). B. cereus, B. subtilis, B. sinensis and S. aureus are the most sensitive strains while E. coli, P. aeruginosa, E. cloacae and S. typhii are the least sensitive. The crude extract from *Canarium madagascariensis* stem bark displayed interesting bioactivity on all tested germs showing the inhibition zones from 10 to 22 mm. All the germs were very sensitive. The Canarium *madagascariensis* leaves crude extract was inactive on E. coli, E. cloacae, P. aeruginosa and S. typhii; but it was bioactive on B. subtilis, B. cereus, *S. aureus* and *S. sinensis*. The inhibition zones varied from 3 to 10 mm. The minimum inhibitory concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values ranged from 0.312 to 10 µg/mL (Table 3).

Table 2: Inhibitory effect of each crude extract against bacteria (expressed as the inhibition zones of bacterial growth)

Bacterial strains	**Diameter of inhibition zones (mm)**		
	EF-01 (leaves extract)	**ET-01 (Stem bark extract)**	**ER-01 (Root bark extract)**
B. subtilis	10.02	22.15	16.03
B. cereus	09.04	21.40	13.42
S. aureus	07.85	17.13	14.05
S. sinensis	08.33	20.51	15.15
E. coli	05.12	12.71	08.13
S. typhii	07.64	14.22	09.20
P. aeruginosa	03.12	10.63	07.50
E. cloacae	04.56	13.80	10.30

Table 3: Inhibitory effect of each crude extract against bacteria (expressed as the Minimum Inhibitory Concentration MIC and the Minimum Bactericidal Concentration MBC).

Bacterial strains	MIC (µL/mL)			MBC (µL/mL)		
	EF-01	ET-01	ER-01	EF-01	ET-01	ER-01
B. subtilis	1.25	0.125	0.312	1.25	0.125	0.312
B. cereus	2.50	0.125	1.25	2.50	0.125	1.25
S. aureus	5	0.625	0.625	5	0.625	0.625
S. sinensis	2.50	0.312	0.312	2.50	0.312	0.312
E. coli	-	1.25	2.5	-	1.25	2.5
S. typhii	10	0.625	2.5	10	0.625	2.5
P. aeruginosa	-	1.25	5	-	1.25	5
E. cloacae	-	0.625	1.25	-	0.625	1.25

3.5. Antioxidant test result

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical that exhibits maximum absorption at 517 nm. It has been utilized to assess the capacity of diverse extracts from plant matrix samples to function as free radical scavengers or hydrogen donors. Additionally, it has been employed to evaluate the antioxidant activity of various extracts of Canarium madagascariensis, employing α-tocopherol (vitamin E) as a reference product. The results of the DPPH radical scavenging activity test on the different extracts of the samples (leaves, stem bark, root bark) of Canarium madagascariensis are presented in Tables 4 and 5.

Table 4 clearly shows that all parts of Canarium madagascariensis exhibit anti-radical activity. However, at a concentration of 75 µg/ml, the hydro-ethanolic extract of the leaves is inactive. Both extracts are still active. The bark extract's anti-radical effect is particularly noteworthy (see Table 5).

Table 4: Percentage inhibition of different extracts from *Canarium madagascariensis* samples.

Concentration (µg/ml)	Percentage of inhibition (%) à n=6			
	Crude extract of leaves	Crude extract of stem bark	Crude extract of root bark	Witness (Vitamin-E)
200	71.02			
150	34.19			
100	19.12			
75	08.95	95.71	83.15	
50	04.12	91.53	70.54	94.87
25		80.05	52.03	92.72
12.5		61.52	38.95	75.01
6.25		36.17	23.47	51.20
3.12		21.08	12.31	44.61
1.56		11.45	05.87	22.12

Table 5: The CI50 values of the different extracts from the *Canarium madagascariensis* samples.

Extracts	CI_{50} (µg/ml) at n=6
Leaves	43.57 ± 0.06
Stems bark	13.21 ± 0.03
Roots bark	21.85 ± 0.05
α-tocopherol (Vitamin E)	08.36 ± 0.07

3.6. DISCUSSION

For a long time, infectious diseases have been a global concern. When penicillin was discovered in 1945, it was believed that major infections had been defeated. However, as humans developed in the industrial world, germs became increasingly resistant. Antibiotic resistance has worsened significantly. The problem of bacterial resistance is reaching alarming proportions worldwide. This is due to the misuse and irrational use of antibiotics in healthcare.

Scientific researchers are facing a constant threat from infectious agents due to bacterial (multi)resistance to antibiotics and the decline in the number of new antibiotics being developed. However, in recent years, several scientific researchers have developed numerous chemical strategies to repel pathogen attacks, based on plants used in traditional medicine [Joosteen L, et al, 2011]. Phytopathogenic microorganisms play a decisive role in the early emergence of secondary metabolite diversity. It is highly unlikely that a microorganism will develop resistance in a plant. Furthermore, for millennia, numerous nations worldwide have utilized plants native

to their regions to treat and cure a wide range of diseases, validating that plants serve as a vast reservoir of potential compounds attributed to metabolites [Fatiany P.R., et al., 2024]. Plants play important roles in human society. In Madagascar, it's clear that plants play a pivotal role in Malagasy society, especially in terms of their practical applications in traditional medicine [Fatiany et al., 2021].

Ethnobotanical surveys in eastern and southeastern Madagascar have revealed that the plant known as "Ramy," scientifically called Canarium madagascariensis, is important in these regions because of its therapeutic properties. The leaves and bark of the stems of this plant are used to treat infections. A decoction is prepared and then three cups a day is drunk. The bark of the roots is used to treat incurable wounds. The bark is scraped and then applied around the wound. Its resin has also been used in the production of incense for traditional religious ceremonies.

Phytochemical screening studies on various extracts from samples (leaves, stem bark, and root bark) of Canarium madagascariensis revealed that not all parts of this plant contain alkaloids and saponins. The root bark and leaves do not contain quinones, but the stem bark contains compounds from the quinone chemical family and their derivatives. Iridoids are present, unlike in the leaves and root bark. The leaves and stem bark do not contain tannins and polysaccharides, which are present in the root bark of this plant.

The results of biological screening on various plant extracts are clear: all extracts exhibit significant anti-radical activity and antimicrobial effects. However, these activities vary depending on the part of the plant from which the extract is obtained.

All extracts demonstrate antimicrobial activity, but their effectiveness differs significantly. The hydro-ethanolic leaf extract is less effective than the bark extracts (stem and root) with inhibition diameters ranging from 3 to 7 mm for Gram-negative bacteria and 7 to 10 mm for Gram-positive bacteria. These inhibition zones definitively show that the leaf extracts are inactive for Gram-negative bacteria and that for Gram-positive bacteria, they show moderate sensitivity. The root bark extract of the plant is more active than the leaf extracts, with an inhibition diameter of between 7 and 16 mm. Stem bark is clearly more active than root bark, as its inhibition zones range from 10 to 22 mm. This explains why the results observed were so promising. Stem bark extracts have shown remarkable antibacterial activity against the microorganisms tested. The results of the studies clearly demonstrate that stem bark extract is more active than leaf and root bark extracts. The hydro-ethanolic extract of stem bark is more active than positive controls such as chloramphenicol and cycloheximide. The hydro-ethanolic extract of stem bark can act in two ways: by inhibiting bacterial growth and/or inducing their death. This activity depends on bacterial strains. It is clear that throughout human history, plants have always interacted with microorganisms. Plants have developed numerous chemical strategies to repel attacks from pathogens, including the production of bactericidal and anti-infective compounds [Joosteen L, et al., 2011]. It is important to note that, despite the decisive role played by phytopathogenic microorganisms in the early emergence of secondary metabolite diversity, it is highly unlikely that a microorganism would be able to develop resistance in a plant.

The results of the antioxidant activity testing are clear: all parts of Canarium madagascariensis exhibit anti-radical activity (see Table 5). However, at a concentration of 75 µg/ml, the hydro-ethanolic extract of the leaves is inactive, while both extracts remain active. The stem bark extract displays a compelling anti-radical effect in comparison to the root bark. The results of the biological screening studies on the different extracts of Canarium madagascariensis samples were consistent with their phytochemical screening results. It is clear that these results are corroborated by the ethnobotanical data for this plant.

CONCLUSION

This work is part of the research program of the Doctoral School of Geosciences, Physics, Environmental Chemistry, and Host-Pathogen Systems at the University of Toliara.

After thorough ethnobotanical surveys in the eastern and southeastern regions of Madagascar, we have identified a remarkable plant with certainty: "Ramy" or Canarium madagascariensis (Burseraceae), as the experts call it. This plant is held in high esteem in this region for its exceptional medicinal properties. It is a pillar of traditional medicine. It is used successfully to treat the most stubborn infections and incurable wounds.

The results of biological studies conducted on hydro-ethanolic extracts from the leaves and bark (stems and roots) are conclusive. Canarium madagascariensis has been shown to possess promising anti-radical and antibacterial properties. Phytochemical screening studies have yielded definitive results, confirming that this plant is a rich source of flavonoid and terpenoid compounds. It is an irrefutable conclusion that quinone compounds are present in the bark of the stems. The presence of this chemical family in the plant is indisputable evidence of its biological activities.

A substantial corpus of scientific studies has repeatedly demonstrated the efficacy of this plant in traditional medicine. This result is extremely promising. The study offers a highly encouraging outlook for future research, particularly with respect to the isolation of bioactive molecules.

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