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Research Paper

Phenolic compounds and Antioxidant Activities of *Cedrelopsis grevei* Baill. bark, an Endemic Plant of Madagascar from the family Rutaceae.

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Abstract
The study conducted on Cedrelopsis grevei *Baill., an endemic plant of Madagascar belonging to the Rutaceae family, highlighted its richness in secondary metabolites (Mulholland et al., 2003). Thin-layer chromatography analyses combined with various characterization reactions revealed the presence of several chemical families, including coumarins, anthraquinones, leucoanthocyanins, flavones, condensed tannins, triterpenoids, and steroids (Um et al., 2003).*
The crude extract showed an extraction yield of 18.5%. Moreover, its antioxidant activity was particularly remarkable, with a DPPH radical scavenging percentage of 93.33% and an α-tocopherol equivalent of 548.77 µM/mg/mL of extract. These findings suggest that Cedrelopsis grevei *Baill. is a promising source of bioactive compounds with strong antioxidant potential.*
The fractionation of the ethyl acetate extracts led to the isolation of two phenolic compounds: cathecin $^{(P1)}$ *andquercetin* $^{(P2)}$*.*

Keywords: *Rutaceae, chemical screening, antioxidant activity, Cedrelopsis grevei Baill.*

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I. INTRODUCTION

Madagascar boasts an exceptional plant biodiversity, including numerous medicinal species that remain underexplored. Among the Rutaceae family, *Cedrelopsis grevei* Baill., locally known as katrafay , is an endemic tree native to the southwestern regions of Madagascar. Traditionally, its bark and essential oil have been utilized for various therapeutic purposes, such as inflammatory disorders, infections, and premature aging *(Randrianarivelojosia et al., 2003)*, treating muscle fatigue, rheumatism, and as an aphrodisiac *(Rasoanaivo et al., 1992).*

Despite its widespread use in folk medicine, scientific studies on its chemical composition and biological activities are limited. This study aims to assess the antioxidant activity of the methanolic extract of *Cedrelopsis grevei* Baill. and to characterize its chemical constituents. These findings may provide insights into the plant's therapeutic potential and contribute to the validation of its traditional uses*(Um et al., 2003)*. The fractionation of the ethyl acetate extracts led to the isolation of two phenolic compounds. The structural identification of phenolic compounds from this species are described herein.

Fig1: *Cedrelopsis grevei* Baill

II. MATERIALS AND METHODS

2.1. General

Thin-layer chromatography (TLC) was performed on aluminium silica gel 60 F_{254} plates *(0.2 mm thickness, Merck, 2020).* Each extract was dissolved in methanol for sample application. Three solvent systems of different polarities were employed to obtain comprehensive TLC profiles: CH_2Cl_2/1% MeOH (non-polar), CH_2Cl_2/15% MeOH (moderately polar), and AcOEt/MeOH/H_2O (100/15/15) (polar). Spots were visualized under UV light at 254 and 366 nm, and revealed using vanillin-sulfuric acid spray reagent.

Column chromatography was performed on silica gel 60 (particle size 6.3–20 μm, Merck, 2020). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV-400 spectrometer equipped with a cryoprobe for both ^{1}H and ^{13}C nuclei *(Bruker, 2020)*. Chemical shifts (δ) were reported in parts per million (ppm) using $CDCl_3$ residual solvent peak as reference, and coupling constants (J) were expressed in Hertz (Hz).

2.2. Plant material

In this study, the species investigated was *Cedrelopsis grevei* Baill. The bark was collected in Toliara, Madagascar, in October 2024 (herbarium reference TAR). This species was identified at the Department of Botany of the Botanical and Zoological Park of Tsimbazaza (Antananarivo). A voucher specimen was deposited at the Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo, and the Geosciences, Physics, Environmental Chemistry and High Pathogenic System Doctoral School (GPCEHP), University of Toliara, Toliara 601, Madagascar, for future references.

2.3. Extraction

The extractions were carried out by macerating the plant powder at room temperature in methanol, following classical procedures described for phytochemical investigations (Harborne, 1998). The dried plant powder was macerated for 48 hours, after which the extract solution was collected and concentrated. The recovered solvent was then returned to the plant powder for a new maceration cycle. This process was repeated until the plant material was completely exhausted by the solvent.

2.3. Phytochemical screening

Phytochemical screening was carried out using classical methods to detect the chemical families likely to be present, employing specific reactions and reagents*(Trease & Evans, 2002; Harborne, 1998).*These tests were based either on the formation of insoluble complexes (precipitation reactions) or on the formation of colored complexes (coloration reactions).

2.4 Isolation

The ethyl acetate extract (10 g) was fractionated by column chromatography over silica gel (150 g), using a stepwise isocratic elution with mixtures of hexane, ethyl acetate, and methanol (50:40:10 followed by 50:35:15, v/v/v) *(Harborne, 1998).* A total of 135 fractions of 10 mL each were collected. Fractions 8–20 were subsequently subjected to preparative thin-layer chromatography (TLC) using the same solvent system, allowing the isolation of product P1 (8 mg) *(Wagner & Bladt, 1996)*. Fractions 26–67 were further purified through gel filtration on Sephadex LH-20 with DCM/MeOH (1:1, v/v) as eluent, yielding compound P2 (10 mg) *(Hostettmann & Marston, 1990)*. This combination of chromatographic techniques ensured efficient separation of metabolites according to their polarity and molecular size.

2.4. Evaluation of Antioxidant Activity

The strategy was based on a screening of all samples, followed by the quantification of antioxidant activity for those samples that showed positive results during the screening.

2.4. 1 Preliminary Screening

The preliminary screening was carried out by TLC on silica gel (normal phase), eluted with AcOEt / 10% MeOH, and then revealed with DPPH.

2.4. 2 Quantification of Antioxidant Activity

The antioxidant activity was quantitatively assessed using a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the methods described by Brand-Williams et al. (1995), Sánchez-Moreno et al. (1998), and Awika et al. (2003). Briefly, 25 mg of DPPH was dissolved in 100 mL of methanol and stored at -20 °C in the dark until use. For each assay, 200 µL of the test solution was added to 3800 µL of the 25% DPPH solution in dry tubes. A corresponding blank was prepared with 200 µL of methanol plus 3800 µL of DPPH solution. After 30 minutes of incubation in the dark at room temperature, the absorbance was recorded at 517 nm using a UV/Visible spectrophotometer. This method allows precise determination of the free radical scavenging activity of plant extracts.

The antioxidant activity, expressing the ability to scavenge free radicals, was estimated by the percentage of DPPH decolorization in methanol solution. It was calculated using the following formula *(Yoo et al., 2008)*:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

where Abs represents the absorbance at $\lambda = 517$ nm.

The results were expressed as the mean of three measurements ± standard deviation. The inhibition percentage thus calculated was compared using two α-tocopherol calibration curves, corresponding to concentrations ranging from 6.25 µM to 100 µM and from 150 µM to 600 µM.

III. RESULTS AND DISCUSSION

3.1.Phytochemical screening

The methanolic maceration extraction of *Cedrelopsis grevei* Baill. gives promising results. From 200 g of dried plant powder, 37 g of crude extract is obtained, corresponding to an extraction yield of 18.5%. The extract appears brown. These results indicate that the plant contains a considerable amount of extractable compounds, suggesting good potential for further chemical and biological studies.

Table 1: Results of Phytochemical Screening of *Cedrelopsis grevei Baill* bark

Chemical Family	**Characterization Reagents**	***Cedrelopsis grevei* Baill.**
Coumarins	NaOH, UV lamp	+++
Anthraquinones	NH_4OH	++
Anthracenic heterosides	HCl or H_2SO_4, $FeCl_3$	+++
Anthocyanins	Cold HCl	–
Leucoanthocyanins	Hot HCl	++
Flavones	HCl, Mg, isoamyl alcohol	+++
Flavonols		–
Flavanones, flavanonols	HCl, Mg	–
Polyphenols	Gelatin	+++
Tannins	NaCl, gelatin	+++
Hydrolyzable tannins	$FeCl_3$	–
Condensed tannins		+++
Cyanogenic heterosides	$CHCl_3$; sodium picrate	+
Alkaloids	KI, I_2, $HgCl_2$, $Bi(NO_3)_3$, tartaric acid	–
Iridoids	HCl, $CuSO_4$, glycerol	–
Triterpenoids	Acetic anhydride, H_2SO_4, antimony	++
Steroids		+++
Unsaturated sterols	H_2SO_4	+++
Lactonic steroids	Picric acid, KOH or NaOH, 3,5-dinitrobenzoic acid	+++
Cardenolides	H_3PO_4, CCl_3COOH	+++
2-deoxysugar heterosides	H_3PO_4, CCl_3COOH	+++
Saponins	Foam height	+++
Polysaccharides	90% ethanol	–

Legend of Table 1:

- – : no reaction observed (negative test)
- + : faint coloration or slight precipitate formation
- ++ : distinct coloration or abundant precipitate
- +++ : intense coloration, immediate flocculation, or foam height greater than 5 cm

Phytochemical screening of *Cedrelopsis grevei* Baill. showed a rich chemical diversity, with abundant phenolic compounds (coumarins, flavones, polyphenols, tannins) and steroid derivatives (steroids, sterols, cardenolides, saponins). Several classes, including alkaloids, anthocyanins, and iridoids, were absent. The profile suggests potential antioxidant, antimicrobial, anti-inflammatory, and cardiotonic activities, supporting the plant's traditional uses and highlighting its value as a source of bioactive molecules.

3.2 Identification

The structures of the isolates were determined through analysis of their spectroscopic data. The NMR spectra of all isolated compounds are consistent with polyphenol skeletons. By means of 1-D and 2-D NMR spectra and comparison with literature compounds P1 and P2 are recognized as quercetin*(Saeed Tavakoli et al)***,**and Catechin *(Zor et al. BMC 2017 17:229 ; Kwee & Kristanti, 2020)*respectively (Figure 1).

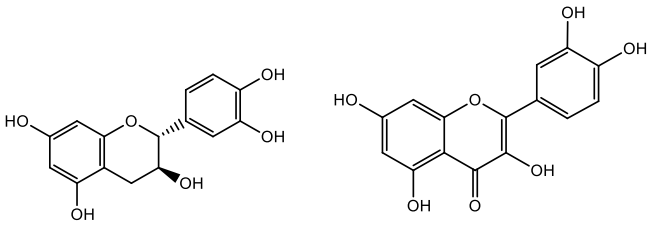
Catechin(P1): yellow amorphous powder

^{1}H NMR (600 MHz, CD_3OD): 4.50 (H-2, d,); 3.90 (H-3, ddd); 2.85 (H-4α, dd); 2.85 (H-4β, dd,); 5.85 (H-6, d,); 5.90 (H-8, d); 6.85 (H-2′, d); 6.79 (H-5′, d); 6.72 (H-6′, dd). ^{13}C NMR (150 MHz, CD_3OD): 83.0 (C-2); 69,0 (C-3); 28.6 (C-4); 156.9 (C-5); 95.5 (C-6); 157.6 (C-7); 96.4 (C-8); 157.9 (C-9); 101.0(C-10); 132.4 (C-1′); 115.1 (C-2′); 146.4 (C-3′); 146.3 (C-4′); 116.0 (C-5′); 120.03 (C-6′).

Quercetin (P2): pale yellow powder

^{1}H NMR (600 MHz, $CDCl_3$):7.60 (1H, H-2′); 7.54 (1H, H-6′); 6.88 (1H, H-5′); 6.50 (1H, H-8); 6.28 (1H, H-6)

^{13}C NMR (125 MHz, $CDCl_3$): 174.7 (C-4); 164.0 (C-7); 160.6 (C-9); 157.0 (C-5); 150.4 (C-4′); 146.9 (C-2); 145.5 (C-3′); 136.4 (C-3); 122.7 (C-1′); 121.0 (C-6′); 116.1 (C-5′); 115.8 (C-2′); 104.0 (C-10); 99.0 (C-6); 94.2 (C-8)



1 **2**

Fig 2: Isolated compounds chemical structures from *Cedrelopsis grevei Baill.* bark.

3.5 Free radical scavenging activity on DPPH.

The crude ethanolic extract of *Cedrelopsis grevei*bark exhibited antioxidant activity in the TLC-DPPH assay, as indicated by a yellow halo comparable to, or even larger than, that of the standard α-tocopherol (Figure 3).

2 : α-tocopherol

3:crude ethanolic extract

Fig 3: Qualitative antioxidant test of *Cedrelopsis grevei*bark extracts

The antioxidant activity of the crude extract of *Cedrelopsis grevei*bark was evaluated using the DPPH assay, a widely employed method based on the ability of antioxidants to quench the stable free radical DPPH°. At a concentration of 1 mg/mL, the extract exhibited a radical scavenging percentage of 93.33%, corresponding

to an α-tocopherol equivalent of 548.77 µM/mg/mL (Table 2). These results indicate that the extract possesses a very strong antioxidant potential, comparable to or even higher than the standard α-tocopherol.

Table 2: Quantification of antioxidant activities of Rutaceae extracts by spectroscopic method (Reading wavelength: 517 nm; Extract concentration: 1 mg/mL).

Plant extract	% DPPH inhibition	α-tocopherol equivalent (µM/mg/mL of extract)
Cedrelopsis grevei Baill. (*R. minor*)	93.33	548.77

The antioxidant assay using the DPPH method revealed a very high radical scavenging activity for the extract of *Cedrelopsis grevei* Baill., with 93.33% inhibition at a concentration of 1 mg/mL. This corresponds to an α-tocopherol equivalent of 548.77 µM/mg/mL, indicating that this extract exhibits strong antioxidant potential, comparable to or even higher than some standard antioxidants. Such results suggest that *C. grevei* could be a valuable natural source of bioactive compounds with antioxidant properties.*(Daniela et al., 2013)*

IV. CONCLUSION

The study carried out on *Cedrelopsis grevei* Baill. (Rutaceae), an endemic species of Madagascar, revealed a significant chemical richness. Phytochemical screening highlighted the major presence of secondary metabolite families such as coumarins, flavones, polyphenols, tannins, steroids, sterols, and saponins, along with moderate amounts of anthraquinones, leucoanthocyanins, and triterpenoids. In contrast, certain classes such as anthocyanins, flavonols, alkaloids, and polysaccharides were absent. These results confirm previous findings indicating that Malagasy Rutaceae are often poor in alkaloids *(André, Galle, & Razafindratsita, 1976).*

Regarding the biological results, the crude methanolic extract of *Cedrelopsis grevei* Baill. exhibited remarkable antioxidant activity, with a DPPH radical scavenging capacity of 93.33%, corresponding to 548.77 µM/mg/mL of α-tocopherol. Chromatographic analysis (TLC) further revealed a diversity of compounds with varying polarity, reinforcing the hypothesis of a correlation between chemical richness and the observed biological activity.

The abundant presence of phenolic and flavonoid compounds, which are well known in the literature for their antioxidant properties *(Lu, Foo, Wong, & Huang, 2000; Hazama, Watanabe, & Nakashima, 2005; Gulsen, Mutlu, & Uzun, 2007),* most likely explains the high potential observed in this extract. These results also validate the traditional uses of katrafay as a medicinal plant, particularly in the treatment of inflammation and premature aging.

The pronounced antioxidant activity observed in *Cedrelopsis grevei Baill.* is mainly attributed to its richness in phenolic compounds, particularly flavones, coumarins, leucoanthocyanins, and condensed tannins, whose hydroxyl groups provide a strong radical scavenging capacity.

In conclusion, *Cedrelopsis grevei Baill.* stands out due to its rich and diverse chemical profile, associated with highly significant antioxidant activity. These characteristics make it a promising species for the isolation of bioactive molecules of pharmaceutical interest and justify further studies to explore its other biological potentials.

V. DISCUSSION

The study's findings indicate that the extract of *Cedrelopsis grevei* bark possesses significant antioxidant properties. The isolated compounds from the ethyl acetate extracts, which are common in many plant species, have not been previously reported in the Rutaceae family. While these compounds were not subjected to bioassays in this study, their biological activities have been documented in prior research *(Mabry & Markham, 1975).*

Literature suggests that flavonoids, including quercetin and catechin, are the primary active components responsible for the antioxidant and anti-inflammatory effects observed in many medicinal plants (Pietta, 2000). Quercetin is well-known for its potent antioxidant capacity, acting by scavenging free radicals and chelating metal ions *(Rice-Evans, Miller, & Paganga, 1996)*. Similarly, catechin is also a powerful antioxidant, protecting cells from oxidative damage *(Terao, Piskula, & Yao, 1994).* The presence of these compounds in the extract likely contributes to the overall antioxidant effect observed.

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