Int. j. adv. multidisc. res. stud. 2026; 6(1):2727-2730

Received: 02-01-2026
Accepted: 06-02-2026

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Antioxidant Activity of Bark Extracts from *Erythrophleum Couminga* Baill. (FABACEAE)

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DOI: https://doi.org/10.62225/2583049X.2026.6.1.5839
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Abstract

The bark *of Erythrophleum couminga* is used by the population of northwestern of Madagascar to treat promptly infected or fresh wounds. The objective of this study was therefore to verify whether rapid wound healing is linked to the antioxidant activity of this plant. To do this, crude ethyl acetate (AcOEt), methanol (MeOH), and aqueous (H2O) extracts from the plant's bark were used to determine their antioxidant activity using a spectrophotometric method involving free radical scavenging by diphenyl-picrylhydrazil (DPPH). Preliminary phytochemical screening showed that the AcOEt and MeOH extracts are very rich in saponins, triterpenoids, flavonoids, tannins, and steroids. The IC50 values represent the concentration of extracts that inhibit 50% of free radicals. The IC50 values of the AcOEt, MeOH, and H2O extracts were 6.92 µg/ml, 10.35 µg/ml, and 10.99 µg/ml, respectively. The IC50 value of ascorbic acid (reference product) was 3.78 µg/ml. All IC50 values obtained were below 30 µg/ml, indicating strong antioxidant activity in these extracts. This activity could be due to the various chemical families present in the bark of *Erythropleum couminga*, which are known to have powerful antioxidant properties. Further studies will be conducted to isolate and identify the active molecule(s).

Keywords: *Erythropleum couminga,* Triterpenoids, Antioxidant, Free Radicals

1. Introduction

Oxidative stress is the result of an imbalance between systems that produce reactive oxygen species (ROS) and antioxidant systems, such as enzymes such as superoxide dismutases (SOD), glutathione peroxidase (GPx), etc., or non-enzymatic antioxidant systems such as glutathione, vitamins, etc. Several diseases, including cancers, eye diseases (cataracts and macular degeneration), neurodegenerative diseases (ataxia, amyotrophic lateral sclerosis, and Alzheimer's disease) as well as complications and delayed wound healing, are of oxidative origin. The skin is an organ that protects against microbes that want to invade our body (Wells *et al*., 2016) [20]. As a result, damage to the skin, i.e., external wounds, provides an entry point for these microorganisms. Fortunately, after this occurs, our body's self-defense mechanism initiates the healing process, which involves inflammation and epithelial and tissue remodeling (Franco, 2020) [7]. The inflammatory phase of skin tissue regeneration is the crucial stage in normal wound healing (Ross *et al*., 1962) [17]. However, free radicals and reactive oxygen species (ROS) are released from the wound at this stage (Yan *et al*., 2020) [21]. The accumulation of these products increases the level of oxidative stress in cells, disrupts the balance between antioxidants and pro-oxidants, affects redox signal transduction, leads to cellular and tissue damage (De Jager, 2017), and causes a range of diseases, such as infection (Rashaan *et al*., 2019) [15], delayed wound healing (Patel *et al*., 2017), chronic diseases, and cancer (Da Silva, 2010). Oxidative stress is caused by the excessive accumulation of ROS or their metabolites. When ROS are in excess, a series of free radical chain reactions leads to oxidative damage in cells and tissues, severely affecting cellular activity and wound healing (Riley *et al*., 1994). In developing and low-income countries, access to pharmaceutical drugs is difficult and the majority of the population often resorts to using plants for treatment. In this context, the bark of *Erythrophleum couminga* Baill. (Fabaceae) is used by the population of northwestern

Madagascar to quickly treat infected or fresh wounds. The objective of this study is to verify whether this rapid wound healing is due to the plant's antioxidant activity.

2. Materials and Methods

2.1 Plant Material

The bark of the *Erythrophleum couminga* plant (vernacular name Komanga) used in this study was harvested in August 2016. The fresh powder or leaves of this plant are used in traditional Malagasy medicine in the form of a poultice or decoction to treat cuts on the hands or wounds on children after circumcision.

2.2 Extraction

The harvested *Erythrophleum couminga* bark was dried at room temperature in a well-ventilated room for two weeks. It was then ground into a very fine powder using an electronic grinder to facilitate extraction. 300 g of this powder was macerated for one week in 3 liters of pure ethanol. After filtration through filter paper, the macerate was evaporated at 40°C using a rotary evaporator to obtain the dry ethanol extract. Forty grams of this dry extract were then dissolved and macerated in a mixture of 800 ml of methanol and 30 ml of distilled water in a separating funnel. After 2 hours, 500 ml of hexane was added and the mixture was left to macerate and agitate for a few minutes. The hexane macerate was separated, recovered, and evaporated to obtain the dry hexane extract (Hex).

The remaining methanol macerate was evaporated to dryness to obtain dry methanol extract (MeOH). 38 g of this extract was then dissolved in a mixture of 800 ml of distilled water and 1500 ml of ethyl acetate, and the mixture was left to macerate under agitation for a few minutes.

2.3 Phytochemical Screening

Phytochemical screening was performed according to the methods described by Fong *et al*. (1977) [6]. For this purpose, 5 mg of each of the dry extracts obtained (Hex, DCM, AcEt, and MeOH) were used to detect the presence of the various major chemical families present in the bark of *Erythrophleum couminga.*

2.4 Evaluation of Antioxidant Activity

The potential antioxidant activity of each *Erythrophleum couminga* extract was evaluated using qualitative and quantitative methods to measure their antiradical activity using diphenylpicrylhydrazil (DPPH).

2.4.1 Qualitative Evaluation of Antiradical Activity

The antiradical activity of each *Eryhtrophleum couminga* extract was evaluated using the qualitative in situ DPPH bioautography method with thin-layer chromatography (TLC) plates, as described by Andrianarison *et al.* (2015) [2]. For this purpose, X µl of each extract dissolved in 100% methanol was deposited on a 60F 254 aluminum silica gel TLC plate. After elution using a solvent system consisting of DCM/Hexane (80/20) and drying, the plate was developed using a solution of DDPH at 1g/10 ml of methanol, by vaporization. The appearance of yellow-white spots on a purple DPPH background indicates the antiradical activity of the extract tested.

2.4.2 Quantitative Evaluation of Antiradical Activity

The antiradical activity of each *Eryhtrophleum couminga* extract was evaluated using the quantitative DPPH free radical scavenging method described by Kivrak *et al*. (2009) [10] with a few parameter modifications. DPPH is a radical that has specific absorption at a wavelength of $\lambda = 517$ nm. When reduced by an antioxidant, its absorption changes and decreases. To do this, 25 mg of DPPH was dissolved in 100 ml of methanol, protected from light. After dissolution, 10 ml of this solution was taken and added to 45 ml of methanol to obtain a 4.5% DPPH solution. To perform the tests, solutions of each extract at 50, 25, 12.5, 6.25, and 3.125 mg/mL in 4.5% DDPH were prepared in a final volume of 400 µL. The control solution (blank) was prepared by replacing the extract solution with methanol. A solution of ascorbic acid at concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL was used as a reference solution (BLOIS, 1958). After 1 hour of incubation at room temperature in the dark, the absorbance of each solution was measured using a spectrophotometer at a wavelength of 517 nm. The antiradical activity of the extract, which expresses its free radical scavenging capacity, was evaluated by the percentage of discoloration of DPPH in methanol solution (YOO *et al.,* 2008). The inhibitory concentration values for trapping and reducing 50% of moles of the DPPH free radical (IC50) were determined using exponential regressions of graphs representing the percentages of inhibition as a function of different concentrations of the extracts tested. Extracts with an IC50 value < 30 µg/ml were considered to have strong antioxidant activity. Extracts with 30 µg/ml ≤ IC50 ≤ 100 µg/ml were considered to have moderate antiradical activity. Extracts with an IC50 > 100 µg/ml were considered to have weak antiradical activity (Abas *et al.,* 2006) [1].

3. Results

3.1 Extraction Products

Extractions carried out using 300 g of *Erythrophleum couminga* bark powder yielded various crude extracts, including hexane (Hex), dichloromethane (DCM), ethyl acetate (AcEt), and methanol (MeOH). Their respective characteristics and yields are presented in Table 1.

Table 1: Characteristics and yields of *Erythrophleum couminga* bark extracts

Extraits			
Code*	**Masse (g)**	**Appear1ance and color**	**Rendement (%)**
Hex	0.24g	Oily - Brown	0.6%
Aqueous	18g	Powdery - Dark brown	47.36%
AcEt	20g	Rigid - Dark brown	50 %
MeOH	38g	Rigid - Dark brown	52.63%

*Hex: Hexane extract, DCM: Dichloromethane extract, AcEt: Ethyl acetate extract, MeOH: Methanol extract.

3.2 Chemical Composition of *Erythrophleum Couminga* Extracts

Phytochemical screening revealed various major chemical families present in *Erythrophleum couminga* bark extracts (Table 2). The symbols +++ indicate the presence of compounds in very high concentrations, ++, in average concentrations, +, in low concentrations, +/-, and -, not detected by the method used.

Table 2: Chemical composition of *Erythrophleum couminga* bark extracts

	Extracts (Code)*			
Chemical families	**Hex**	**DCM**	**AcEt**	**MeOH**
Alkaloids	-	++	-	++
Coumarins	++	++	++	++
Anthrone	+/-	++	++	++
Anthraquinone	++	++	++	++
Flavonoids	+/-	+/-	+++	+++
Catechin tannins	-	+/-	+++	++
Gallic tannins	-	+/-	++	++
Steroids	++	+++	+++	++
Triterpenoids	-	+	+++	+++

*Hex: Hexane extract, DCM: Dichloromethane extract, AcEt: Ethyl acetate extract, MeOH: Methanol extract.

3.3 Antioxidant Activities of *Erythropheum Couminga* Bark Extracts

3.3.1 Antiradical Activities Obtained Using the Qualitative Method

Thin-layer silica chromatograms of *Erythropheum couminga* bark extracts revealed by DPPH solution showed various spots with different characteristics, indicating the existence of antiradical activities of varying significance. These activities are more significant for AcOEt, MeOH, and H2O extracts, obtained with more polar solvents, marked by streaks of spots of high and medium intensity. Hex and DCM extracts obtained with less polar solvents show less intense spots, indicating less significant antiradical activities (Table 3).

Table 3: Antioxidant activities of Erythropheum couminga bark extracts evaluated by qualitative screening using thin-layer chromatography

Extracts (Code)*	Stain characteristics	Qualitative evaluation of antioxidant activity
H_2O	Medium intensity stain trail	++
AcOEt	High intensity stain trail	+++
MeOH	High intensity stain trail	+++
DCM	Less intense yellow stain	-
Hex	Less intense yellow stain	-

*Hex: Hexane extract, DCM: Dichloromethane extract, AcEt: Ethyl acetate extract, MeOH: Methanol extract, H2O: Aqueous extract.

3.3.2 Antiradical activities obtained using the quantitative method

The IC50 values of *Erythrophleum couminga* bark extracts are shown in Figure 1.

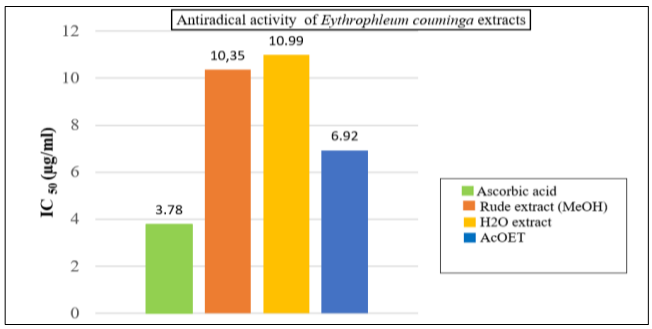

Fig 1: Anti-radical activity of *Erythrophleum couminga* bark extracts against diphenylpicrylhydrazil (DPPH) free radicals: Determination of inhibitory concentration values that trap and reduce DPPH radicals by 50% (IC50) in the absence or presence of AcOEt: Ethyl acetate extract (.6.92 µg/mL), MeOH: methanol extract (10.35 µg/mL), H2O: aqueous extract (10.99 µg/mL.), and ascorbic acid (3.78 µg/ml.).

The IC50 values of AcOEt, MeOH, and H2O extracts are 6.92 µg/mL, 10.35 µg/mL, and 10.99 µg/mL, respectively (Figure 1). The Hexane extract, with a high IC50 value of 482.58 µg/ml, is not shown in Figure 1. Ascorbic acid, used as a reference antioxidant, has an IC50 of 3.78 µg/ml.

4. Discussion

To respond to oxidative stress at the wound site, antioxidants can be used to remove free radicals and restore the wound healing process to normal. After sharing electrons with free radicals, antioxidants thus spare other molecules and tissues from the harmful effects of an oxidation reaction. During the second phase, which is the inflammatory phase, neutrophils and cytokines remove debris from the wound and any harmful microorganisms that may be present in the tissues, such as bacteria (Molnar, 2007) [12]. However, the migration of neutrophils into the wound stops a few days after the initial injury. Monocytes are converted into macrophages in the tissues. This transformation further amplifies the immune response to foreign debris by producing a respiratory burst that releases reactive oxygen species (ROS), which act to eliminate bacterial infection. By capturing free electrons from neighboring molecules, ROS and RNS act as free radicals. Their proliferation in wounds delays wound healing and, at the same time, causes significant damage to healthy cells in the surrounding tissue (Mackay and Miller 2003) [11].

The *Erythrophleum couminga* plant is traditionally used by the local population in northwestern Madagascar to treat fresh and/or infected wounds. The objective of this study is therefore to verify whether the healing of these wounds is linked to the plant's antioxidant activity.

The IC50 values of the AcOEt, MeOH, and H2O extracts of *Erythropleum couminga* that we found are 6.92 µg/mL, 10.35 µg/mL, and 10.99 µg/mL, respectively. The AcOEt extract has the lowest value. According to the work carried out by Bidie *et al.* (2011), plants such as *Mitragyna ciliata*, *Trichilia prieuriana, Chrysophyllum perpulchrum*, and *Disthemonanthus benthamianus*, with respective IC50 values of 4.50 µg/ml, 10.5 µg/ml, 7.5 µg/ml, and 4.00 µg/ml, have been found to possess antiradical and antioxidant activities. These values are similar to those we found with the plant *Erythropleum couminga*. Furthermore, according to the work of Abas *et al.* (2006) [1], natural products with an IC50 < 30 µg/ml are found to have very strong antioxidant activity. In this context, the IC50 values we found with the AcOEt, MeOH, and H2O extracts of *Erythropleum couminga* bark are also < 30 µg/ml, which means that these extracts also have very strong antiradical activity. However, for the Hex extract, the IC50 is 482.58 µg/mL and is well above 100 µg/mL, indicating that it has no antioxidant activity.

The phytochemical screening, we conducted revealed the abundant presence of saponins, triterpenoids, flavonoids, tannins, and steroids in the AcOEt and MeOH extracts. Steroids are present in low concentrations in the DCM extract. These chemical families, including tannins (Di *et al.,* 2012; Karadeniz *et al.,* 2014) [5, 9], phenols (Igor, 2003) [8], triterpenoids (Pollier and Goossens, 2012) [14], and saponins are known for their antioxidant properties (squillaro *et al*., 2018 [18]; Tuyen *et al.,* 2017 [19]; Peng *et al*., 2021). Consequently, these families are most likely involved in the antioxidant activities exerted by *Erythropleum couminga* bark extracts against DPPH free radicals observed in this study.

5. Conclusion

The work carried out as part of this study aimed to scientifically justify the use of the *Erythrophleum couminga* plant in traditional medicine for the rapid treatment of fresh and/or infected wounds. This is due to the abundance of secondary metabolites such as triterpenoids, flavonoids, saponins, and other phenolic compounds in the bark extracts of this plant. This study demonstrated that wound healing through empirical treatment was justified by the antioxidant activity manifested by the ability of *Erythrophleum couminga* to trap DPPH free radicals. However, further studies will be undertaken to isolate and identify the active compounds responsible for this antioxidant activity.

6. Acknowledgements

1. Laboratory of Biotechnology, Environment, and Health for preliminary preparations of plant materials.
2. National Center for the Application of Pharmaceutical Research for product extractions.
3. Laboratory of Pharmacognosy, Paris Cité, France, for the isolation of pure products.

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