



Integrated Phytomineral Synergy: The Essential Role of Oligoelements (Mg, Fe, K) in the Antioxidant and Anti-infective Activities of *Myrothamnus moschatus*

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Abstract: *Myrothamnus moschatus*, commonly known as the "resurrection plant," stands as an emblematic species of Southern Madagascar, traditionally valued as a tonic and an anti-infective remedy. While prior investigations primarily focused on the volatile fraction (essential oils), the present study pioneers the exploration of the non-volatile mineral fraction in relation to the plant's significant biological activities. Elemental analysis of the aerial part's powder revealed a high content of magnesium (1.17%), iron (0.60%), and potassium (0.50%), with no detectable traces of heavy metals (Pb, Cd, Hg, As). Simultaneously, extracts exhibited high antioxidant activity (IC₅₀ = 12.99 µg/mL), a moderate yet significant antiparasmodial inhibition (*Plasmodium berghei*, 34.44% at 500 mg/kg ; $p=0.01395$), and marked antibacterial activity against *Staphylococcus aureus* (16.25 mm). The confluence of a protective mineral profile and measured bioactivities confirms that the plant's efficacy stems not only from its secondary metabolites but also from functional inorganic cofactors, thereby establishing it as a true regenerating tradiphytoremedy. These findings reposition *M. moschatus* as an integrative pharmacognostic resource, paving the way for standardized phyto-mineral formulations for anti-infective and prophylactic purposes.

Keywords: *Myrothamnus moschatus*; tradiphytoremède; bioactive minerals; antiparasmodial; antioxidant; antibacterial

I. Introduction

The global resurgence of infectious diseases, coupled with the alarming escalation of antimicrobial resistance, highlights the critical need for novel therapeutic agents (Newman & Cragg, 2020 ; World Health Organization, 2013, p. 20). In this context, natural substances are re-emerging as credible alternatives to conventional treatments.

Among these, resurrection plants present a unique biological paradigm. These species possess the extraordinary capacity to survive near-total desiccation and fully resume their metabolic functions upon rehydration. Such remarkable desiccation tolerance positions these plants as a major source of inspiration for the exploration and discovery within modern pharmacognosy (Farrant & Moore, 2011).

Myrothamnus moschatus (Baillon) Niedenzu, an endemic species thriving in the arid, rocky formations of Southern Madagascar, is distinguished as one of the few African resurrection plants with documented medicinal applications. Traditionally prepared as a decoction, it has long been utilized in the local pharmacopeia for the management of fever, infections, and inflammatory disorders (Rasoanaivo *et al.*, 2012).

Prior research has predominantly centered on the volatile fraction, specifically its essential oil, revealing noteworthy antibacterial, antioxidant, and insecticidal properties (Pavela *et al.*, 2022; Randrianarivo *et al.*, 2013). However, this exclusive focus overlooks a fundamental dimension of the plant's therapeutic profile : the mineral composition. The role of inorganic elements remains a component rarely integrated into the comprehensive interpretation of the biological activity of traditional medicinal species.

Furthermore, numerous minerals—including magnesium, iron, and potassium—are recognized for their direct or indirect involvement in immune responses, oxidative modulation, and antimicrobial activity (Daglia, 2012 ; Yadav & Fulekar, 2020).

Consequently, the postulation that *M. moschatus*'s therapeutic efficacy relies exclusively on its secondary metabolites represents an incomplete perspective. The pharmacological properties of this species are hypothesized to be substantially supported by a synergy between its organic fractions and elemental components. This integrative action confers a distinctive tradiphytoremedy value, suggesting a therapeutic potential intrinsically linked to the quality and unique conditions of its specific biotope.

Consistent with this integrative pharmacognostic perspective, the fundamental objective of the present investigation is the absolute characterization of the intrinsic mineral composition of *Myrothamnus moschatus* leaf powder, utilizing Total Reflection X-ray Fluorescence (TXRF) spectroscopy.

Concurrently, the study endeavors to evaluate the breadth of the plant's therapeutic potential by subjecting the ethyl acetate extract to a triple biological analysis, encompassing the determination of its *in vivo* antiparasmodial activity, antioxidant capacity, and antibacterial efficacy.

The ultimate step involves establishing an interpretive correlation between these defined chemical and biological profiles and the established traditional uses. This aims to scientifically confirm and validate the full pharmacognosic potential of this valuable Malagasy endemic plant.

II. Research Methods

2.1 Plant material

The aerial parts of *Myrothamnus moschatus* (Myrothamnaceae), an endemic species of Madagascar, were collected in the Ifandana area, Ihosy district, during 2020. Botanical identification was expertly performed by B. Ramandimbisoa from the Tsimbazaza Botanical and Zoological Park and verified by Benja Rakotonirina at the Malagasy Institute of Applied Research (IMRA).

Following collection, the plant material underwent air-drying at ambient temperature, shielded from light exposure. It was subsequently reduced to a fine powder using a mechanical grinder. This resultant powder served as the uniform matrix for both the comprehensive mineral analysis and the subsequent biological extractions.



Figure 1 : *Myrothamnus moschatus* (Tafita Caren)

2.2 Mineral analysis

The elemental composition was determined using Total Reflection X-ray Fluorescence (TXRF) spectroscopy.

The following comprehensive suite of elements was quantified: Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sn, Sb, Ag, Mo, Zr, Rb, Sr, Ba, W, Ta, in addition to the critical heavy metals Pb, Cd, and Hg.

Results for major elements were expressed as a percentage (%), while trace metals were reported in parts per million (ppm).

2.3 *In vivo* antimalarial activity

The antiplasmodial activity was assessed *in vivo* using the four-day suppressive test developed by Peters (Knight & Peters, 1980). Swiss mice infected with *Plasmodium berghei* were administered various oral doses of the extracts (100, 250, and 500 mg/kg). Chloroquine (a quinoline derivative) served as the positive control, while distilled water was employed as the negative control.

The percentage of parasitemia inhibition was calculated from blood smears. Statistical significance was subsequently evaluated using ANOVA, followed by Dunnett's test (GraphPad Prism).

2.4 *In vitro* antioxidant activity (DPPH, 96 wells)

The antioxidant activity was quantified using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay conducted in 96-well microplates (Blois, 1958).

Extracts were tested at escalating concentrations (6.25, 12.5, 25, 50, and 100 µg/mL). Absorbance measurements were taken at 517 nm using a microplate reader. The percentage of inhibition (PI) was calculated using the following formula :

$$PI (\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} * 100$$

2.5 Antimicrobial activity

The evaluation of antibacterial activity was conducted using the agar diffusion method (Kirby & Bauer, 1966). Extracts (20 mg/disk) were applied to sterile disks and subsequently placed on Mueller-Hinton agar plates previously inoculated with the following bacterial strains: *Escherichia coli* (EC), *Bacillus cereus* (BC), *Bacillus megaterium* (BM), *Listeria monocytogenes* (LIST), and *Staphylococcus aureus* (SA).

Following incubation at 37°C for 24 h, the diameters of the inhibition halos (measured in mm) were recorded. Chloramphenicol served as the positive control, and the culture medium functioned as the negative control

III. Results and Discussion

3.1 Results of mineral analysis

To validate the phytomineral synergy hypothesis, the *Myrothamnus moschatus* powder was characterized using Total Reflection X-ray Fluorescence (TXRF). This elemental analysis reveals the plant's crucial non-volatile profile, identifying both major and trace elements essential for interpreting the measured bioactivities and establishing the safety profile of the tradiphytoremedy (Table 1).

Table1. Mineral composition of *Myrothamnus moschatus* powder

Element	Content (%)	Detection limit	Error
Mg	1.17	0.00	0.00
Al	0.58	0.00	0.00
Si	0.27	0.00	0.00
P	0.14	0.00	0.00
S	0.00	0.00	0.00
K	0.50	0.00	0.00
Ca	0.04	0.00	0.00
Ti	0.12	0.00	0.00
V	0.03	0.00	0.00
Cr	0.10	0.00	0.00
Mn	0.00	0.00	0.00
Fe	0.60	0.00	0.00
Co	0.00	0.00	0.00
Ni	0.06	0.00	0.00
Cu	0.02	0.00	0.00
Zn	0.03	0.00	0.00
As	0.01	0.00	0.00
Se	0.00	0.00	0.00
Sn	0.00	0.00	0.00
Sb	0.00	0.00	0.00
Ag	0.02	0.00	0.00
Mo	0.00	0.00	0.00
Zr	0.10	0.00	0.00
Rb	0.03	0.00	0.00
Sr	0.16	0.00	0.00
Ba	0.03	0.00	0.00
W	0.05	0.00	0.00
Ta	0.00	0.00	0.00
Au	0.00 (ppm)	0.00	0.00
Hg	0.00 (ppm)	0.00	0.00
Pb	0.00	0.00	0.00
Cd	0.00	0.00	0.00

TXRF provides spectral visualization of powder composition. The mineral analysis spectrum (Figure 2) identifies and quantifies elements, highlighting the richness of macronutrients (Mg, Fe, K) and the absence of toxic contaminants.

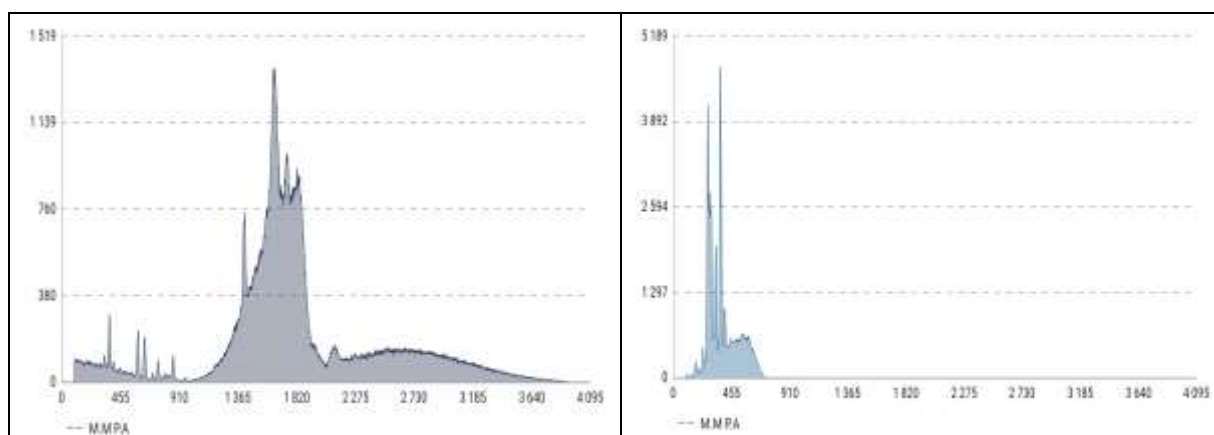


Figure 2. Mineral analysis spectrum of *Myrothamnus moschatus* powder

The elemental profile of *Myrothamnus moschatus* reveals a dominance of specific mineral components, notably magnesium (1.17%), iron (0.60%), and potassium (0.50%), alongside a significant presence of aluminium (0.58%). Other quantified elements occur only as trace constituents.

The substantial content of magnesium, iron, and potassium is particularly noteworthy, given their established roles in antioxidant and antimicrobial mechanisms. This concentration strongly suggests a functional involvement of the mineral fraction in mediating the biological effects previously reported for this species.

3.2 Results of *in vivo* antimalarial activity test

The ethyl acetate extract of *M. moschatus* underwent an *in vivo* screening to objectively validate its antiparasmodial potential, a traditionally documented application. Table 2 details the evaluation of the antimalarial effect against *Plasmodium berghei*, demonstrating a dose-dependent and statistically significant efficacy when compared against a negative control and the reference antimalarial drug, Chloroquine.

Table 2. *In vivo* antimalarial activity of ethyl acetate extract

Species	Dose (mg/kg)	(%) d'inhibition	p-value
<i>M. moschatus</i>	100	20.01	0.07387
	250	27.18	0.03095*
	500	34.44	0.01395*
Chloroquine (positive control)	-	69.09	0.00115**
Negative control	-	0.00	1

*p<0.05 vs. negative control.**p<0.01 vs. negative control.

The ethyl acetate extract of *Myrothamnus moschatus* demonstrated a dose-dependent antiparasmodial activity (Table 2). Specifically :

1. The percentage of parasitemia inhibition peaked at 34.44% at the 500 mg/kg dose, an effect that was statistically significant (p=0.01395).

2. While significant, the measured activity remained lower than that observed for the reference drug, Chloroquine, which achieved 69.09% inhibition.

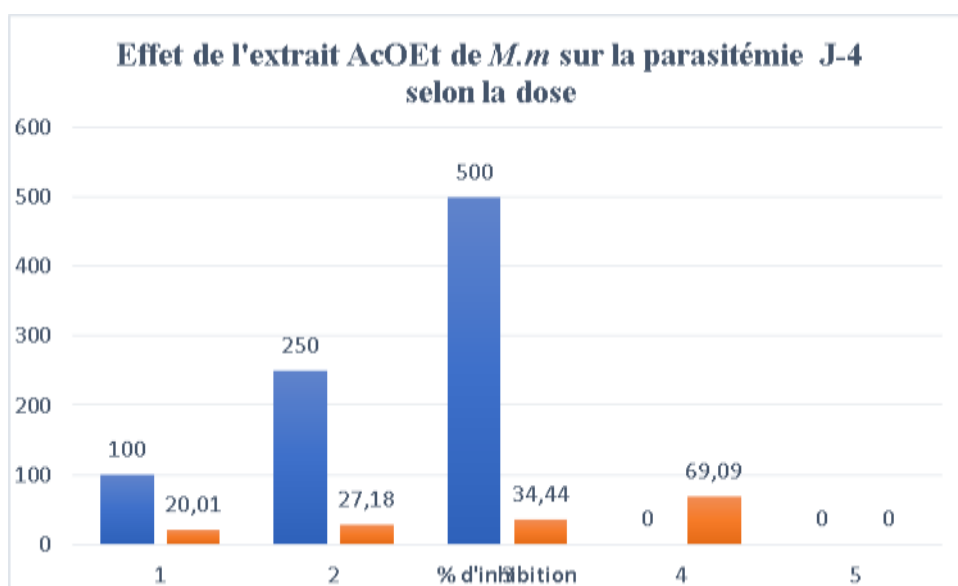


Figure 3. *In vivo* antimalarial activity of *M. moschatus* ethyl acetate extracts

The antimalarial activity of *M. moschatus* ethyl acetate extract was significant and dose-dependent, validating the antiparasmodial potential of this endemic plant.

a. Antimalarial synergy : Iron (Fe), Aluminium (Al) and metabolites

The extract demonstrated a moderate yet significant antiparasmodial inhibition against *Plasmodium berghei* (34.44% at 500 mg/kg). This activity suggests a multi-faceted mechanism involving both secondary metabolites and elemental cofactors.

b. Detailed mechanism of action

1. Secondary metabolites

- 1) Parasiticidal action : Specific compounds within the extract are potentially transported into the parasite's digestive vacuole. Here, they are hypothesized to interact with ferrous iron (Fe^{2+}), a byproduct of hemoglobin degradation. This interaction may catalyze the production of highly toxic free radicals via the Fenton reaction, leading to targeted oxidative stress and subsequent parasite death.
- 2) Synergistic amplification : The observed antiparasmodial efficacy is amplified by the plant's inorganic components. The endogenous Iron (Fe) present in the extract can potentiate this pro-oxidant effect within the parasitic vacuole, while Aluminium (Al^{3+}) stimulates the host's capacity to clear infected cells.

2. Elemental components (Fe et Al^{3+})

- 1) Immune modulation and metabolic stress : The significant presence of Iron (0.60 %) and Aluminium (0.58 %) is functionally relevant. Iron is crucial for host immune metabolism (Cassat & Skaar, 2013). Furthermore, Al^{3+} is a known immune adjuvant that can potentiate the host's innate immune response, notably through macrophage activation and cytokine production (HogenEsch *et al.*, 2018), thereby assisting the host in combating parasitemia.

3.3 Results of antioxidant activity (DPPH assay)

To evaluate the plant's capacity to neutralize oxidative stress, the ethyl acetate extract was subjected to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Table 3 presents the dose-dependent inhibition results, which facilitate the determination of the median effective concentration (IC₅₀). This value serves as a key indicator of the inherent antioxidant potency attributable to *M. moschatus*'s secondary metabolites.

Table 3. Antioxidant activity of ethyl acetate extract (DPPH test, 96 wells)

Species	Concentration ($\mu\text{g/mL}$)	Inhibition (%) (PI)	IC ₅₀ ($\mu\text{g/mL}$)
<i>M. moschatus</i> (AcOEt)	6.25	42.36	12.99
	12.50	50.21	
	25.00	62.48	
	50.00	59.91	
	100.00	63.44	

The tested extracts demonstrated a strong, increasing antioxidant activity (Table 3).

1. The *M. moschatus* (AcOEt extract) exhibited a progressive DPPH inhibition, escalating from 42.36% at 6.25 $\mu\text{g/mL}$ to 63.44% at 100 $\mu\text{g/mL}$.
2. The derived IC₅₀ value of 12.99 $\mu\text{g/mL}$ translates to a high radical scavenging activity. This significant result is indicative of a dual, synergistic defense strategy orchestrated between the plant's antioxidant metabolites and the high content of magnesium (an essential cofactor for numerous antioxidant enzymes).

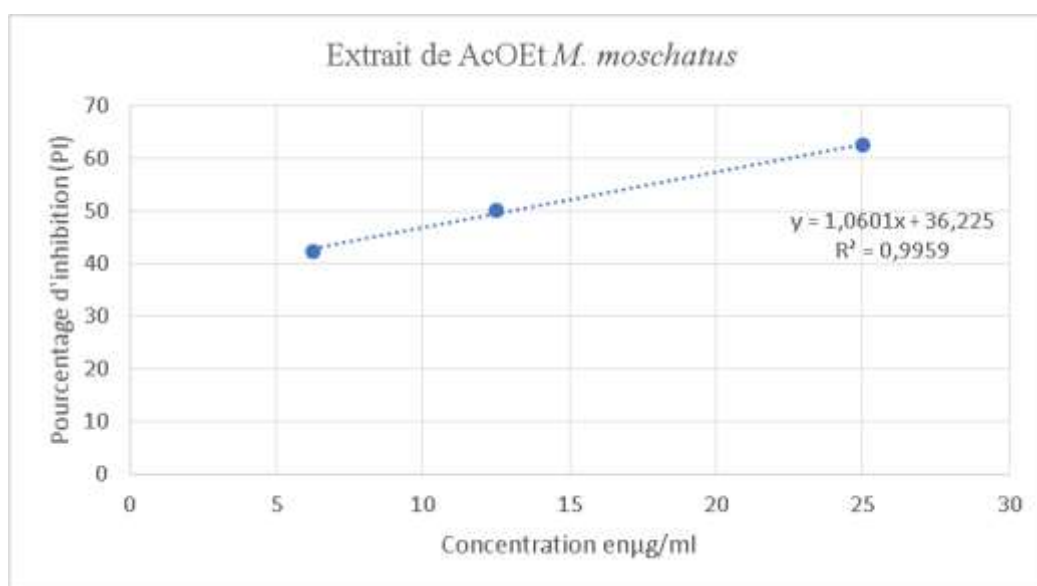


Figure 4. Antioxidant activity of *M. moschatus* ethyl acetate extract (DPPH assay)

The ethyl acetate extract of *M. moschatus* exhibits a powerful free radical scavenging activity, quantified by an IC₅₀ value of 12.99 $\mu\text{g/mL}$. This high potency confirms the extract's richness in antioxidant compounds and underscores its potential for mitigating oxidative stress.

a. Detailed mechanism of action

1. Secondary metabolites (phenolic compounds)

- 1) Mechanistic role : First-Line Defense (Direct Action): These molecules function as efficient radical scavengers, immediately neutralizing Reactive Oxygen Species (ROS), such as DPPH \cdot . This is achieved through mechanisms like Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET), thereby effectively terminating lipid peroxidation chains.
- 2) Synergistic support : The action of secondary metabolites is synergistically enhanced by Mg²⁺. The presence of magnesium ensures the regeneration and support of the body's internal enzymatic antioxidant systems, allowing the secondary metabolites to focus primarily on the immediate neutralization of external oxidative threats.

2. Elemental component (Mg²⁺)

- 1) Second-line defense (indirect action) : The significant presence of Magnesium (1.17%) positions it as a vital elemental component. Mg²⁺ is an essential cofactor (HogenEsch et al., 2018) crucial for the activation and function of primary endogenous antioxidant enzymes, including Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) (Cazzola et al., 2024). Furthermore, magnesium contributes to the stabilization of cellular membranes, reinforcing overall defense against oxidative damage.

3.4 Results of antimicrobial activity test

To determine the anti-infective potential of *M. moschatus*, an evaluation of the antibacterial activity of various extracts was conducted. Table 4 presents the diameters of the inhibition halos obtained against several bacterial strains. This polarity-based analysis provides an essential comparative view for isolating the most active fraction and understanding the contribution of its secondary metabolites to the observed antimicrobial effect.

Table 4. Antimicrobial activity of extracts (inhibition halo diameters, mm)

Species	Extract	EC	BC	BM	LIST	SA
<i>M. moschatus</i>	Hexane	7.00	7.00	7.00	7.25	7.00
	DCM	9.25	9.00	9.50	9.50	12.50
	ACOEt	12.25	11.50	16.00	14.25	16.25
	HydroEtOH	10.50	9.25	9.50	10.25	10.75
Chloramphénicol (positive control)	-	16.00	22.25	20.75	20.75	13.50
Negative control	-	0.00	0.00	0.00	0.00	0.00

(EC : *Escherichia coli* ; BC : *Bacillus cereus* ; BM : *Bacillus megaterium* ; LIST : *Listeria monocytogenes* ; SA : *Staphylococcus aureus*)

The extracts exhibited variable inhibition halos across the tested bacterial strains (Table 4):

1. For *M. moschatus*, the Ethyl Acetate (ACOEt) extract displayed the most potent activity, with the largest diameter reaching 16.25 mm against *S. aureus*.
2. The reference drug, Chloramphenicol (positive control), generally exhibited larger halos, with its highest inhibition measured at 22.25 mm (>20 mm) against *B. cereus*.

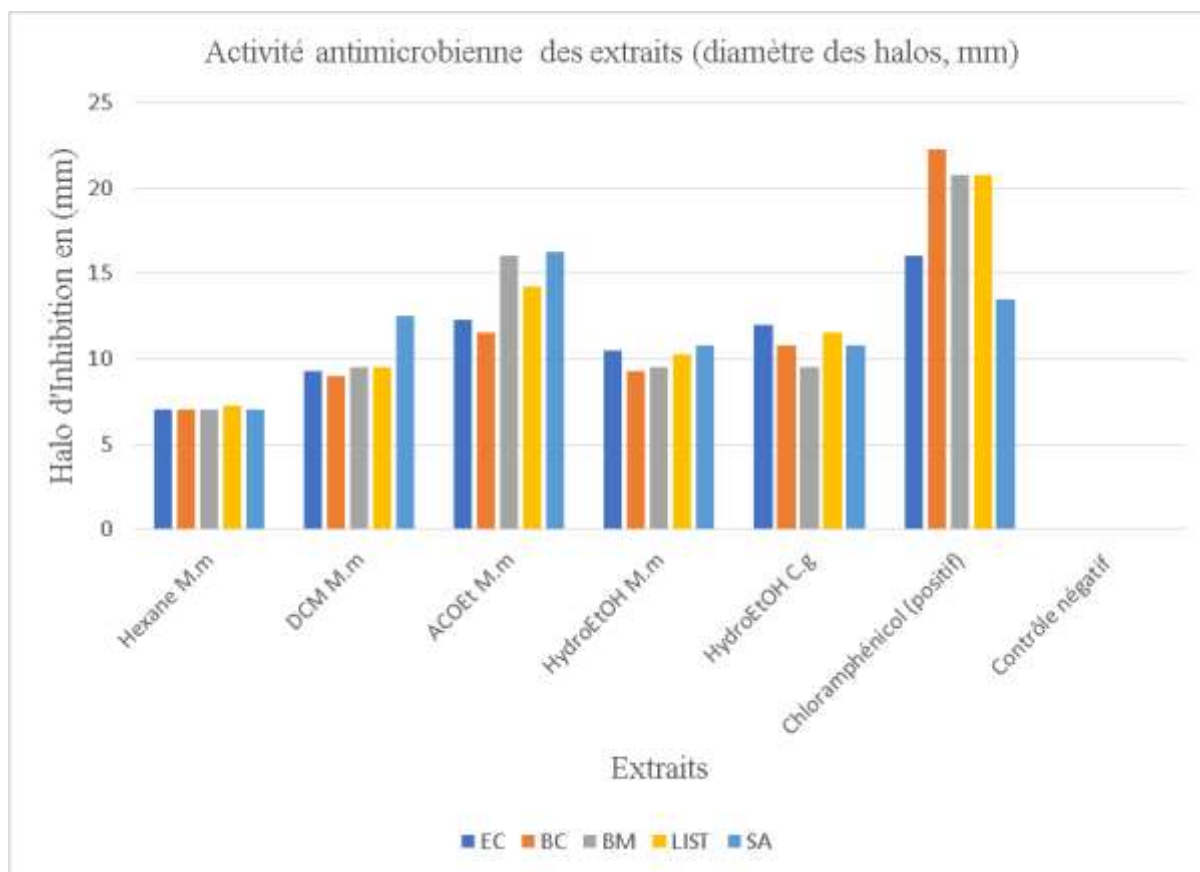


Figure 4 : Antimicrobial activity of *M. moschatus* aerial extract

Ethyl acetate extract has superior antibacterial activity, particularly against *Staphylococcus aureus*, confirming its traditional anti-infectious potential.

3.5 General discussion on phytomineral synergy

The therapeutic efficacy of *Myrothamnus moschatus* is best explained by a phytomineral synergy between its secondary metabolites (notably phenolic compounds) and its mineral fraction (elemental components). This characteristic validates its traditional use as a tradiphytoremedy.

The results confirm that *M. moschatus*, traditionally recognized as a "resurrection plant," possesses a pharmacological profile consistent with its ethnomedicinal applications. In contrast to previous studies focused almost exclusively on the volatile fraction (Pavela *et al.*, 2022; Randrianarivo *et al.*, 2013 ; Rasoanaivo *et al.*, 2012), the current investigation demonstrates for the first time that the mineral fraction actively contributes to the observed biological properties, likely in synergy with the secondary metabolites.

a. Correlation between Magnesium and antioxidant activity

The high magnesium content (1.17 %) suggests a direct role in the plant's antioxidant capacity. Magnesium (Mg^{2+}) is known to contribute to biological membrane stabilization, reduction of oxidative stress, and the support of antioxidant enzymatic systems (Cazzola *et al.*, 2024). The recorded IC_{50} of 12.99 $\mu g/mL$, comparable to that of many powerful natural antioxidants, is therefore interpreted as a combined effect between phenolic compounds and these protective mineral cofactors.

b. Role of Iron and Aluminium in antiplasmodial activity

- a) Iron (0.60 %) is an essential cofactor in immune metabolism and can indirectly disrupt the malarial parasite's cycle, which is highly dependent on intracellular iron for growth (Cassat & Skaar, 2013). The observed dose-dependent inhibition (20.01 to 34.44 %), while moderate compared to chloroquine, confirms a tangible biological effect that justifies the traditional uses against malarial fever.
- b) The notable presence of Aluminium (0.58 %), a component often overlooked in pharmacognostic analyses, may exert an immunostimulatory role as an adjuvant, similar to its function in certain vaccine formulations (HogenEsch *et al.*, 2018), though this potential function warrants cautious interpretation.

c. Antibacterial activity and traditional panacea potential

The inhibition of *Staphylococcus aureus* (16.25 mm) is highly significant, considering this pathogen is frequently associated with the skin and respiratory infections against which *M. moschatus* is traditionally employed. Potassium (0.50 %) is vital for bacterial homeostasis, regulating osmolarity and stress resistance, thus making it a potential factor in microbial growth inhibition (Epstein, 2003).

d. Scientific justification as a resurrection plant

The combination of antiplasmodial, antioxidant, and antibacterial activities, coupled with a complete absence of toxic heavy metals (Pb, Cd, Hg non-detected), confers an exceptional safety profile on *M. moschatus*, a quality rare among wild medicinal plants (Weltgesundheitsorganisation, 2007). This evidence reinforces the concept that the species is not only "resurrectional" from a botanical standpoint but also pharmacologically capable of revitalizing the organism in contexts of infectious or oxidative stress (Kranter *et al.*, 2002).

e. Towards an expanded pharmacognosy : The logic of tradiphytoremedies

Contemporary pharmacognostic research increasingly supports the rehabilitation of the holistic plant preparation concept, underscoring that the efficacy of a traditional plant is not based on a single isolated active compound but on a multi-constituent synergy, including essential inorganic elements (Abubakar & Haque, 2020 ; Heinrich *et al.*, 2022).

This study contributes to an emerging approach proposed here as the Science of Tradiphytoremedies: the rational study of traditional remedies through their elemental components as much as their secondary metabolites.

Prior work confirms that magnesium and iron possess direct immunomodulatory and antimicrobial properties (Cassat & Skaar, 2013 ; Chandrasekaran *et al.*, 2014), while potassium, though often neglected, plays a role in bacterial osmotic pressure and anti-inflammatory cellular signaling (Di *et al.*, 2018 ; Epstein, 2003). These data suggest that ethnomedicinal practices are not based solely on empirical intuition but often on a botanical selection historically aligned with an implicit biochemical logic (Mukherjee, 2019).

The environmental purity of *M. moschatus*, attested by the complete absence of toxic heavy metals, makes it potentially an ecological and therapeutic sentinel species, justifying its valuation as a reference tradiphytoremedy within the integrated healthcare systems of Southern Madagascar (Tsirinjara, 2016).

IV. Conclusion

This investigation provides a major contribution to the pharmacognostic understanding of *Myrothamnus moschatus*, demonstrating that its therapeutic potential is not solely dependent on secondary metabolites, as often suggested in the literature, but also and

significantly on its non-volatile mineral fraction. This fraction is notably rich in magnesium (1.17 %), iron (0.60 %), and potassium (0.50 %).

These elements, recognized for their roles in immune modulation, combating oxidative stress, and metabolic support, emerge as essential cofactors for the observed antiparasmodial (*Plasmodium berghei*, 34.44 % at 500 mg/kg ; $p = 0,01395$), antioxidant (CI₅₀ = 12.99 µg/mL), and antibacterial activities (against *Staphylococcus aureus*, 16.25 mm).

The dual finding of measurable biological efficacy and a complete absence of toxic heavy metals fully justifies the traditional use of the plant as a regenerative tonic within the *tradiphytoremedies* of Southern Madagascar. Consequently, this research scientifically confirms the status of *M. moschatus* as a resurrection plant, not just botanically, but also pharmacodynamically.

This work opens the door to a new direction in therapeutic valorization, shifting the focus from solely volatile extracts to an integrative approach combining phytochemistry with functional geochemistry. Future investigations should explore the bioaccessibility of these elements and their synergistic interactions with phenolic compounds, with the prospect of developing standardized phyto-mineral formulations aimed at the prevention or therapeutic support of chronic infections and inflammatory conditions.

Myrothamnus moschatus thus stands as a model of biological resilience translatable to pharmacology, effectively reconciling traditional knowledge with rigorous scientific validation.

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