

Article

Exploring the Aerial Parts of *Tetracera madagascariensis* as Potential Health-Promoting Ingredient in Herbal Beverages: Phytochemical Insights, Pharmacological Evidence, and Multitarget Effects

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Abstract

The main objective of this study is to assess the potential antioxidant property, anti-inflammatory activity, and broncho-dilatating effect of *Tetracera madagascariensis*, a species traditionally used in the treatment of asthma. Qualitative and quantitative analyses on phytochemical composition and biological properties were performed to evaluate its potential as a bioactive ingredient in plant-based food applications and health-promoting beverages. DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) models were used for antioxidant capacity. The bronchorelaxant activity of METM and its fractions was evaluated on an in vitro experimental model using isolated guinea pig trachea ($n = 5$) pre-contacted with histamine, while the action mechanism of EFTM was determined by using specific contracting reagents and antagonists. The analgesic and anti-inflammatory activities were evaluated in murine models ($n = 5$), specifically using an acetic acid-induced nociception assay and paw inflammatory oedema induced by carrageenan, respectively. Potential toxicity of the extract was evaluated in mice ($n = 6$). Organic acids and phenolics, particularly quinic acid and quercetin, have been detected as the main compounds. METM showed antioxidant activity ($SC_{50} = 7.52 \pm 0.26 \mu\text{g/mL}$ and $FRAP = 228.00 \pm 18.68 \text{ mmol FIE/kg DW}$). METM exerted a concentration-dependent bronchorelaxant activity ($EC_{50} = 562.85 \pm 38.00 \mu\text{g/mL}$) as well as EFTM ($EC_{50} = 128.88 \pm 27.9 \mu\text{g/mL}$), the most active fraction, partially mediated through β_2 -adrenergic pathways with additional non-competitive mechanisms. METM demonstrated dose-dependent anti-inflammatory and analgesic effects, with no toxicity in mice. These findings may support the traditional uses of *T. madagascariensis* as a bioactive ingredient in herbal beverages and highlight its potential as a source of phytochemicals to be used as health-promoting agents against inflammatory and respiratory disorders.

Keywords: traditional herbal beverages; plant-based food applications; Madagascar; pharmacological properties; HPLC; medicinal plants; health-promoting agents



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1. Introduction

According to the Global Initiative for Asthma [1], asthma is a multifactorial disease characterised by chronic inflammation and structural remodelling of the airways, leading to narrowing of the respiratory tract culminating in an asthmatic episode. Clinically, asthmatic patients show some chronic respiratory symptoms such as breathing difficulties and persistent cough, the intensity of which is mainly influenced by environmental factors, along with varying degrees of respiratory impairment. Despite the presence of conventional treatments such as corticosteroids and bronchodilators, the therapies with these drugs have several limitations, including long-term side effects and variable efficacy among patients. In addition, few people living in low-income countries, such as Madagascar, have access to these therapies. Therefore, most Malagasy patients rely on plant-based therapies, justifying the focus on new therapeutic approaches using natural products of plant origin [2]. Medicinal plants represent a promising alternative due to their richness in bioactive compounds with anti-inflammatory, antioxidant, and bronchodilator properties already used by Malagasy people for breathing problems, such as *Euphorbia hirta*, *Vetiveria zizanoides*, and *Phymatodes scolopendria* [3,4]. Asthma has a genetic component, but environmental exposures, such as air pollution and allergens, also contribute to airway inflammation. These exposures increase the production of reactive oxygen species (ROS), which act as key mediators of damage in inflammatory and allergic diseases. Airborne particles, including diesel emissions and pollens, can generate ROS and promote mitochondrial dysfunction, contributing to allergic inflammation [5]. In addition, oxidative DNA damage results in the formation of 8-oxo guanine (8-oxoG), which is generated and processed by the enzyme 8-oxo guanine-DNA glycosylase-1 (OGG1) during DNA repair. The association of 8-oxoG and OGG1 forms a signalling complex that activates NF- κ B, promoting airway inflammation [6]. Several studies have highlighted a close relation between oxidative stress and the occurrence of asthma attacks, suggesting that free radicals like ROS play a physiopathological role in airway inflammation and asthmatic exacerbations [7,8].

Tetracera madagascariensis Willd. ex Schltdl. (*Dilleniaceae* family), also known as “Vahimaranga”, “Laingomantatsimo”, and “Vahimarana”, is an endemic liana from Madagascar. According to the Plants of the World Online [9] and Tropicos databases, *T. madagascariensis* is the accepted name, while *Ttriceris* Thouars ex Baill is listed as a synonym. The species could be found along the eastern and the northwestern coast of the island and used by the local community to treat various inflammatory and respiratory disorders, including asthma [10,11]. Its stem can reach 8 to 15 cm in diameter at the base. Its leaves are variable in size from 4 to 20 cm long, with 7 to 15 pairs of lateral veins. The flowers with white petals are small and grouped in cymes. The herbal tea prepared by leaves infusion of this species is empirically used by the population of the eastern parts of Madagascar to treat several diseases (such as stomachache and ulcers), and reduce pain and fatigue, while the decoction is used by the northern and southeastern population to treat asthma [2,12,13]. The stem decoction is used to treat sore throats [10]. The current ethnobotanical data present a large spectrum of medicinal and health-promoting applications, reinforcing the relevance of *T. madagascariensis* as a potential source of bioactive constituents. Its integration into traditional beverages not only aligns with local uses and cultural heritage. Additionally, it presents an excellent opportunity to obtain natural bioactive agents and develop functional herbal drinks. Such applications may contribute to preventive healthcare and the management of some common ailments in resource-limited countries, similarly to other species commonly used, including *Camelia sinensis* or *Coffea* spp. [14,15]. Consequently, phytochemical and pharmacological investigations have been performed in this study to confirm the traditional claims and explore the potential of this plant as a bioactive ingredient in the formulation of innovative health-promoting beverages.

This study focused on evaluating the potential antioxidant capacity, anti-inflammatory activity, and bronchorelaxant effect, and explaining the anti-asthmatic properties and relative phytochemical composition of the plant extract derived from the aerial parts of *T. madagascariensis*. This research represented preliminary studies to provide a better understanding of the potential applications of this plant as a bioactive ingredient in functional beverages used in managing asthma-related conditions. Additionally, the study also evaluated the extract's toxicity to assess its safety for future potential uses. Overall, this study aimed to provide preliminary scientific results justifying the potential health-promoting effects, benefits, and safety of *T. madagascariensis* as a therapeutic option for asthma particularly in contexts such as Madagascar, where diseases have traditionally been treated by the use of local medicinal plants instead of synthetic active substances due to historical, cultural, and socio-economic reasons, also promoting the sustainable use of local natural resources.

2. Results and Discussion

2.1. Extraction Yield, Fractionation, Phytochemical Screening, and TPC

Maceration of *T. madagascariensis* powder yielded 24.16 g of methanol extract (METM), corresponding to an extraction yield of 12.08%. Table 1 reports the phytochemical screening results of the aerial parts of *T. madagascariensis* as well as the phytochemicals in the ethyl acetate fraction (EFTM). Several secondary metabolites, including polyphenols, flavonoids, tannins, steroids, unsaturated sterols, cardiotonic glucosides, saponins, and polysaccharides, were detected in the studied plant material.

Table 1. Phytochemical families in the aerial parts of *T. madagascariensis*.

Phytochemical Family	<i>T. madagascariensis</i>	
	Aerial Parts	Ethyl Acetate Fraction
Alkaloids	—	—
Flavonoids	+	+
Anthocyanins	—	—
Leucoanthocyanins	—	—
Polyphenolic compounds	+	—
Tannins	+	—
Quinones	—	—
Anthraquinones	—	—
Triterpenoids	—	—
Steroids	+	+
Lactonic steroids	—	—
Unsaturated sterols	+	+
Cardio-tonic glucosides	+	—
Polysaccharides	+	—
Saponins	+	—

—: absence of phytochemicals. +: presence of phytochemicals.

Liquid–liquid fractionation of METM (10 g) allowed the obtaining of 332 mg of hexane fraction (HFTM), 1.44 g of dichloromethane fraction (DFTM), 2.5 g of ethyl acetate fraction (EFTM), and 5.22 g of aqueous fraction (AFTM). Fractionation grouped compounds according to their polarity; these fractionation yields reflect the distribution of major chemical classes composed of polar identified molecules, particularly phenolic compounds. Moreover, the Folin-TPC level of the aerial parts of this species is calculated to $10,821.84 \pm 1066.54$ mgGAE/100 g of dried weight (DW) (mean $\pm$ SD, $n = 3$ replicates), confirming qualitative screening. This value exceeds those reported for other beverage-utilised species, including the aerial parts of *Imperata cylindrica*, the leaf and stem of

Schefflera bojeri and *Uapaca bojeri* under the same experimental conditions [16–18], showing that *T. madagascariensis* is rich in phenolic compounds.

2.2. Bioactive Compounds Fingerprint

Bioactive compounds and nutritional substances listed in Table 2 have been selected as biomarkers for HPLC fingerprint because of their health-promoting value in humans [19,20]. The amounts of each selected compound in *T. madagascariensis* aerial parts are reported in Table 2 along with their total sum, which represents the total bioactive compound content (TBCC).

Table 2. Phytochemical fingerprint compounds of *T. madagascariensis* aerial parts.

Class	Compound	Amount (mg/100 g DW)	Relative Proportion (%)	
Cinnamic acids	Caffeic acid	n.q.	-	0.12
	Chlorogenic acid	n.d.	-	
	Coumaric acid	n.d.	-	
	Ferulic acid	9.93 ± 1.61 ^a	0.12	
Flavonols	Hyperosides	n.d.	-	1.76
	Isoquercetrin	n.d.	-	
	Quercetin	33.74 ± 0.32 ^b	0.41	
	Quercitrin	111.62 ± 6.89 ^{bc}	1.35	
	Rutin	n.d.	-	
Benzoic acids	Ellagic acid	769.70 ± 24.20 ^d	9.29	9.29
	Gallic acid	n.d.	-	
Catechins	Catechin	n.d.	-	0.00
	Epicatechin	n.d.	-	
HPLC phenolics (HPLC PHs)		924.99 ± 33.02		11.17
Organic acids	Citric acid	1728.49 ± 246.06 ^e	20.85	93.45
	Malic acid	n.d.	-	
	Oxalic acid	n.d.	-	
	Quinic acid	5484.29 ± 331.12 ^f	66.16	
	Succinic acid	533.74 ± 340.08 ^d	6.44	
	Tartaric acid	n.d.	-	
Vitamin C	Ascorbic acid	14.36 ± 0.06 ^a	0.17	0.17
	Dehydroascorbic acid	n.d.	-	
Sugars	Fructose	n.d.	-	1.76
	Sucrose	n.d.	-	
	Glucose	147.14 ± 3.50 ^c	1.76	
Total bioactive compound content (TBCC)		8289.26 ± 613.77	100	100

Results are expressed as mean value ± standard deviation (SD) of 3 independent experiments. Significant statistical differences for $p < 0.05$ are reported with different letters (a–f). n.q. = not quantified. n.d. = not detected.

Nine compounds, belonging to six chemical classes, have been quantified during the HPLC analysis (Figure S1). Quinic acid was the main relative compound, followed by citric acid with relative abundances of 66.16% and 20.85%, respectively, relative to TBCC. By adding the rate of succinic acid (6.44%), quantified organic acids from the aerial parts of *T. madagascariensis* were the most dominant class (93.45% of TBCC). Organic acids were often dominant in various beverages that were previously studied using similar protocols [16–18]. Ellagic acid, with a relative abundance of 9.29%, represented the class of benzoic acid, while quercitrin (1.35%) and quercetin (0.41%) represented flavonols. These compounds have also been reported as active ingredients of previous species used as

beverages [16–18]. Glucose (1.76%) was quantified among the sugars, and traces of ascorbic acid (0.17%) and ferulic acid (0.12%) were also detected.

TBCC represented the amounts of total bioactive compounds (phenolics, organic acids, vitamin C, etc.), expressed as mg/100 g of dried weight, TPC represented the total phenolics evaluated by Folin–Ciocalteu reagent assay, expressed as mg of gallic acid equivalent (GAE) on 100 g of dried weight, and HPLC phenolics represented total phenolics as sum of single polyphenols quantified by HPLC, expressed as mg/100 g of dried weight. The TPC (total phenolic content) observed spectrophotometrically with the Folin–Ciocalteu reagent assay did not quantitatively correspond to the total amount of phenolics obtained by HPLC (Table 2), because the Folin–Ciocalteu test is not specific for phenolics, while HPLC analysis selectively identifies and quantifies individual phenolic compounds. To date, no published scientific studies are available on this plant species. In addition, the aerial parts of *T. madagascariensis* contain several phenolic compounds beyond the polyphenols quantified as phytochemical markers in this study, potentially contributing to its antioxidant and medicinal properties. The identification and quantification of small flavonols and phenolic/organic acids only based on UV–Vis spectra and retention time (Figures S1 and S2) may be affected by co-elution effects, impacting the structure–activity considerations and robustness of chemotaxonomic inferences. However, the present study was only a preliminary and exploratory work, as its main aim was to investigate and characterise the phytochemical profile of *T. madagascariensis* aerial parts rather than to verify predefined mechanistic hypotheses. This analytical research focused on describing the distribution of phenolic and other bioactive compounds, together with nutritional constituents, to identify patterns that may hold functional and/or taxonomic relevance. The research emphasised a descriptive structure–activity profiling approach, without implying definitive or direct causal-effect relationships. The present approach potentially highlighted the amounts of selected phytochemical markers and provided a base for more targeted studies in the future. For this reason, further research based on more advanced and combined analytical techniques (e.g., HPLC-MS/MS or GC-MS) is necessary to confirm the preliminary results by adding more markers for a full fingerprint evaluation of *T. madagascariensis*.

2.3. Antioxidant Capacity of *T. madagascariensis* Aerial Parts

The antioxidant activity of *T. madagascariensis* was assessed by the radical DPPH scavenging capacity of secondary metabolites in METM. This is evidenced by the transformation of the stable radical DPPH in methanol into its reduced form in the presence of radical scavengers [21]. The median scavenging capacity (SC_{50}) of the METM was calculated to be 7.52 ± 0.26 $\mu\text{g/mL}$, which is about three times more potent than that of gallic acid with an SC_{50} of 20.06 ± 1.01 $\mu\text{g/mL}$ ($p < 0.001$), used as a Positive control (Figure S3). The higher apparent antioxidant activity of plant extract compared with pure gallic acid may be attributed to matrix effects, assay interferences, or the synergistic action of multiple antioxidant constituents in the extract [22]. This antioxidant activity was then confirmed by the FRAP assay. For this second method, the antioxidant active compounds can reduce ferric ions to ferrous ions [23]. The FRAP value was calculated to be 228.00 ± 18.68 mmol Fe^{2+} /kg of DW.

Compared to other extracts of some endemic plants from Madagascar, such as the methanol extract of the aerial parts of *Vaccinium secundiflorum* Hook. [18], leaf and bark of *U. bojeri* Bail. [16], or aerial parts of *Lygodium lanceolatum* Devs. [24], using the same protocol, the aerial parts of *T. madagascariensis* demonstrated superior antioxidant power. The correlation between phenolic and organic acids content and the antioxidant capacity is well documented [17,25–27]. Several compounds identified in METM, including

succinic acid, quinic acid, quercitrin, and quercetin, possess these functional groups on their chemical structure, suggesting their contribution to the antioxidant properties of the *T. madagascariensis* aerial parts, as reported by several studies [28–31].

2.4. Bronchorelaxant Activity of METM and Its Fractions

2.4.1. Effects of METM and Its Fractions on Histamine Pre-Contracted Trachea

On the histamine pre-contracted isolated guinea pig trachea, METM exerted a concentration-dependent relaxation effect with an EC_{50} of $562.85 \pm 38.00 \mu\text{g/mL}$ (examples of organ bath traces were reported in Supplementary Materials). After liquid–liquid fractionation, the obtained fractions were tested using the same protocol. The EC_{50} s of each fraction are reported in Table 3. According to their EC_{50} value, EFTM is the most active fraction, followed by DFTM and then AFTM. HFTM exerted a relaxation on the pre-contracted guinea pig trachea, but the EC_{50} was higher than that of METM ($p = 0.030$).

Table 3. EC_{50} of METM and its fractions on histamine pre-contracted guinea pig trachea.

Sample	<i>n</i>	EC_{50} ($\mu\text{g/mL}$)	<i>p</i> vs. METM
METM	10	562.85 ± 38.0	
AFTM	6	186.81 ± 9.2^b	0.000
EFTM	10	128.88 ± 2.79^b	0.000
DFTM	4	170.25 ± 6.8^b	0.000
HFTM	4	690.90 ± 33.7^a	0.030

Values are expressed as mean $\pm$ SEM of *n* rings. Significant statistical differences are highlighted by different letters (a,b).

These results suggested that the active compounds may possess polar functional groups in their structures. Phenolics and organic acids are often found in ethyl acetate and/or aqueous fractions that could be at the origin of this activity. These compounds are known for their implications in the treatment of various respiratory diseases as bronchodilators [32,33]. The relaxant effect observed on isolated tracheal tissue may be attributed to the presence of ferulic acid [34], a well-documented phenolic compound known for its protective effects on the respiratory tract. Specifically, one study demonstrated that ferulic acid enhances tracheal epithelial function by activating the cGMP/PKGII pathway, thereby regulating epithelial sodium channels (ENaCs) and reducing bronchial hyperreactivity [35]. The difference could be due to the concentration of the active substance in the different fractions. Therefore, EFTM was selected for the action mechanism determination.

2.4.2. Effect of EFTM on Acetylcholine and KCl-Induced Contractions

EFTM effects on pre-contracted guinea pig trachea were concentration-dependent, whether the contraction was induced with histamine, acetylcholine, or KCl. The EC_{50} increased slightly when contraction was induced by acetylcholine, with a value of $164.28 \pm 3.70 \mu\text{g/mL}$, compared to histamine ($128.88 \pm 27.90 \mu\text{g/mL}$, $p = 0.224$), while the EC_{50} increased significantly when the contraction was induced by KCl with the value of $515.67 \pm 20.60 \mu\text{g/mL}$ when compared to histamine ($p = 0.000$). Histamine is a mediator capable of causing the contraction of smooth muscle fibres by stimulating specific histamine type 1 receptors [36], while acetylcholine is a mediator that causes contraction of bronchial smooth muscle cells by stimulating specific muscarinic acetylcholine type 3 receptors [37].

For histamine and acetylcholine, smooth muscle contraction is due to the activation of phospholipase C β via Gq protein coupling. This signalling pathway leads to the production of inositol-1,4,5-triphosphate (IP3), which in turn stimulates its receptors (IP3Rs) in the sarcoplasmic reticulum, which is a calcium storage site. IP3 causes the release of calcium from these reservoirs by opening calcium channels to the cytoplasm [38]. This increase

in cytoplasmic calcium concentration promotes the formation of a calcium–calmodulin complex, which activates myosin light-chain kinase. This activation leads to phosphorylation of the ATPase of the myosin light chain, enabling actin–myosin interaction and resulting in bronchial smooth muscle contraction [39]. On the other hand, by using KCl for the trachea pre-contraction, the broncho-relaxing effect of EFTM is less potent than that of acetylcholine or histamine pre-contracted isolated trachea. Indeed, KCl causes depolarisation of tracheal smooth muscle cells that open voltage-gated calcium channels (VOCs), leading to calcium influx, which stimulates the calcium-induced calcium release (CICR) pathway, the calcium release from the storage site [40,41]; allowing for reaching the cytosolic calcium concentration required to initiate muscle contraction [42]. EFTM was more active when broncho-constriction was mediated by activation of coupled Gq receptors. It could mean that the bioactive principle of EFTM could modify the affinity of these agonists on their respective receptors.

2.4.3. EFTM Effects on the Histamine Contractile Activity

Figure 1 reports the relationship between the contractile effect of histamine and its concentration in the absence or the presence of EFTM (100 and 200 µg/mL) on the isolated guinea pig trachea, while the EC₅₀ and the maximal effects (E_{max}) are reported in Table 4.

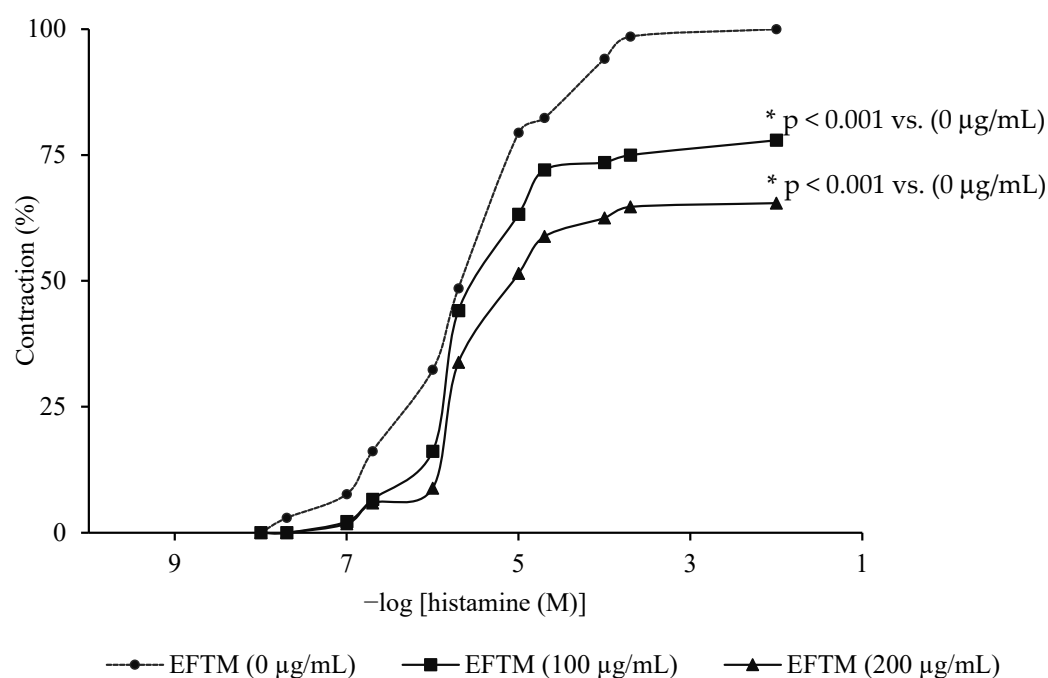


Figure 1. Effects of EFTM on the contractile response to histamine ($n = 4$). * $p < 0.001$ vs. 0 µg/mL (significant reductions in the maximal effect).

Table 4. EC₅₀ and E_{max} of histamine contractile response in the absence and the presence of EFTM (100 and 200 µg/mL).

EFTM Concentration (µg/mL)	EC ₅₀ (µM)	E _{max} (%)
0	1.53 ± 0.20	100
100	2.14 ± 0.05 ^a	77.94 ± 1.47 ^a
200	2.26 ± 0.10 ^a	65.44 ± 1.47 ^a

Values are expressed as mean ± SEM of 4 rings. a: $p < 0.05$ vs. 0 µg/mL.

When tested alone, histamine induced contraction with an EC₅₀ of 1.53 ± 0.20 µM. In the presence of EFTM 100 or 200 µg/mL, its EC₅₀ was increased significantly ($p = 0.026$ and

$p = 0.024$, respectively). However, significant reductions in the maximal effects were also observed at both concentrations ($p < 0.001$), suggesting an effect on maximal efficacy rather than a purely competitive interaction.

2.4.4. EFTM Effects on CaCl_2 -Induced Contraction

Figure 2 shows the concentration-response curves of the external CaCl_2 on the guinea pig trachea without and in the presence of EFTM (100 or 200 $\mu\text{g/mL}$). The corresponding EC_{50} and E_{max} were reported in Table 5. The presence of EFTM significantly increased the EC_{50} of CaCl_2 ($p < 0.001$ for 100 $\mu\text{g/mL}$ and $p = 0.001$ for 200 $\mu\text{g/mL}$). In contrast, the maximal contractile response (E_{max}) was significantly reduced at both concentrations ($p < 0.001$). Its increasing concentration showed a non-competitive antagonism characteristic, suggesting a mechanism that does not involve direct blockade of calcium channels.

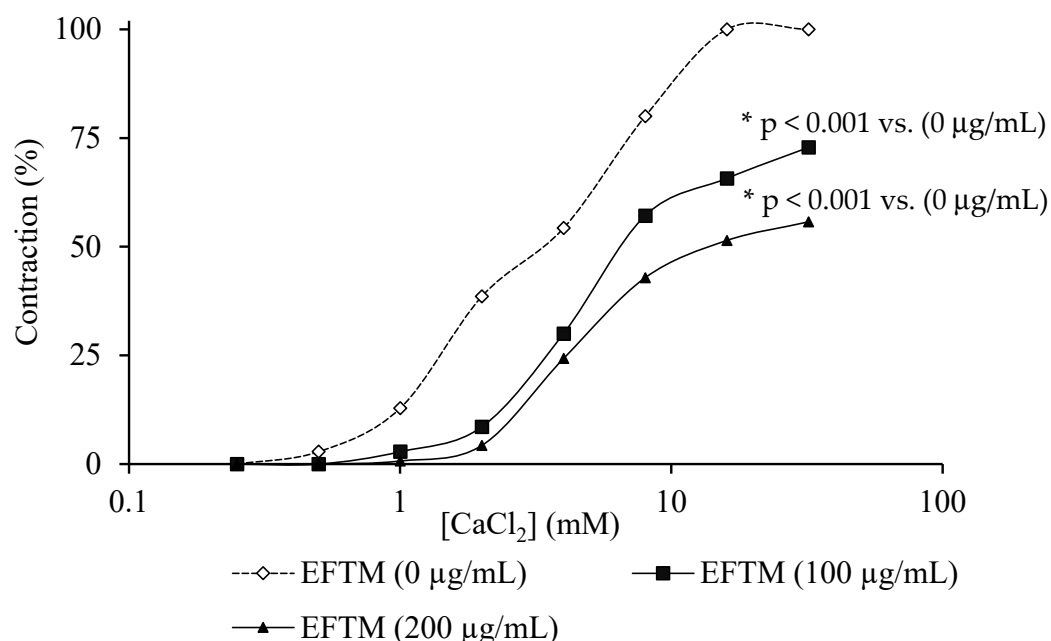


Figure 2. Effects of EFTM on the contractile response to CaCl_2 ($n = 4$). * $p < 0.001$ vs. 0 $\mu\text{g/mL}$ (significant reductions in the maximal effect).

Table 5. EC_{50} and E_{max} of CaCl_2 contractile response in the absence and the presence of EFTM (100 and 200 $\mu\text{g/mL}$).

EFTM Concentration ($\mu\text{g/mL}$)	EC_{50} (μM)	E_{max} (%)
0	1.82 ± 0.15	100
100	3.52 ± 0.15^a	72.86 ± 1.48^a
200	5.93 ± 0.30^a	55.71 ± 1.43^a

Values are expressed as mean $\pm$ SEM of 4 rings. a: $p < 0.001$ vs. 0 $\mu\text{g/mL}$.

2.4.5. Propranolol Effects on the Bronchodilator Activity of EFTM

Curves showing the relationship between the concentration of EFTM and its effect on histamine pre-contracted guinea pig trachea in the absence and the presence of propranolol at 10^{-6} and 10^{-8} M are illustrated in Figure 3. EFTM showed a significant increase in the EC_{50} value from 128.88 ± 2.79 to 166.22 ± 2.20 $\mu\text{g/mL}$ ($p \leq 0.000$) in the presence of 10^{-8} M propranolol and to 183.40 ± 7.90 $\mu\text{g/mL}$ ($p = 0.004$) in the presence of 10^{-6} M propranolol. However, E_{max} values were not affected by the presence of propranolol. These findings are consistent with a competitive antagonistic mechanism. That indicated that the bioactive ingredients of EFTM may act by stimulating β_2 -adrenergic receptors in bronchial smooth

muscle cells [43]. Indeed, β_2 -receptor stimulation leads to muscle relaxation via Gs protein coupling, which in turn activates adenylate cyclase. This signalling pathway causes the lysis of ATP into cyclic AMP (cAMP). The formation of cAMP causes the activation of protein kinases (PKA) that, on the one hand, activate membrane calcium pumps, leading to the reuptake of calcium in the SRs via the SERCA pump and the Ca^{2+} expulsion by PMCA [44]. By stimulating β_2 -receptors, the intracellular concentration of cAMP increases, leading to the opening of potassium channels, causing hyperpolarisation and leading to the VOCs closing. On the other hand, PKA acts on MLCK to inhibit the actin-myosin interaction, causing broncho-relaxation.

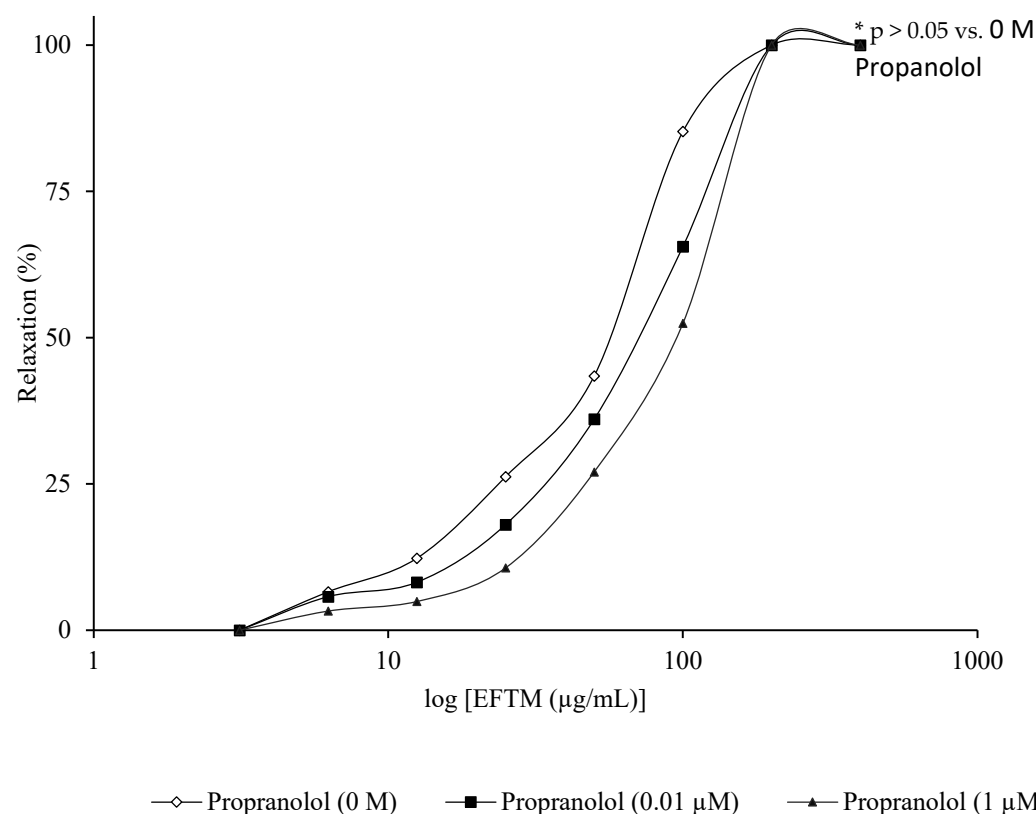


Figure 3. Effects of propranolol on the bronchodilator activity of EFTM ($n = 4$). * $p > 0.05$ (no significant differences in the maximal effect vs. 0 M Propranolol).

2.4.6. EFTM–Theophylline Combination Effects on Histamine Pre-Contracted Guinea Pig Trachea

EFTM (75 $\mu\text{g/mL}$) exerted $26.06 \pm 1.30\%$ relaxation on the histamine pre-contracted trachea, while theophylline (15 $\mu\text{g/mL}$) produced $34.22 \pm 4.00\%$ relaxation. The sum of their relaxation effects was calculated to be $60.04 \pm 2.60\%$. When injected simultaneously, the produced relaxation presented a value of $77.71 \pm 2.10\%$ (Figure 4), which is significantly higher than the calculated sum of the relaxation percentage ($p = 0.002$). Theophylline is a nonspecific inhibitor of phosphodiesterases, an enzyme capable of degrading cAMP into inactive metabolites [45]. The combination of EFTM with theophylline produced a synergistic effect that may suggest the probable broncho-relaxing activity of EFTM through stimulation of β_2 -adrenergic receptors.

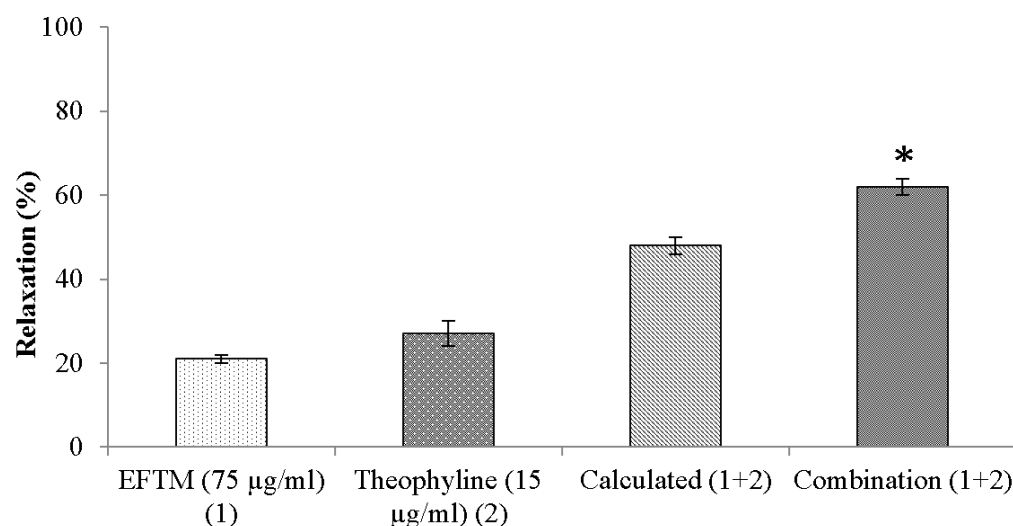


Figure 4. Broncho-relaxation effects of EFTM (75 µg/mL), theophylline (15 µg/mL), and their combination on histamine pre-contracted guinea pig trachea ($n = 4$). * $p = 0.002$ vs. calculated (1 + 2).

2.5. Anti-Inflammatory Activity

2.5.1. Carrageenan-Induced Paw Oedema

The carrageenan-induced hind paw oedema model is the most frequent protocol used for the anti-inflammatory screening of natural products [46]. This inflammatory experimental model presents two distinct phases. The first phase corresponds to the release of amine mediators such as histamine and serotonin, and its duration is 120 min. The second phase, occurring between 180 and 240 min, is mediated by lipid mediators derived from arachidonic acid. The anti-inflammatory effect of METM is reported in Table 6 (individual data points are reported in Table S1, while individual inhibitions of inflammation are highlighted in Figure S5). Compared to the Negative control group, METM significantly reduced the volume of the inflamed paw ($p < 0.05$) at the doses of 100, 200, and 400 mg/kg in mice after 2 h. Moreover, the anti-inflammatory effect increased in a dose-dependent manner ($p < 0.05$). METM also exhibited a significant dose-dependent anti-inflammation effect ($p < 0.001$) on the delayed phase of the oedematogenic response (3 and 4 h after the oedema provocation). Indomethacin significantly inhibited the carrageenan-induced oedema after 3 and 4 h ($p < 0.05$ and 0.001, respectively) with a reduction of about 68.08 and 86.40%, respectively. Indomethacin, by reversibly inhibiting the cyclooxygenase 1 enzyme, reduces the production of prostaglandins, explaining its anti-inflammatory effect in the second phase of the carrageenan inflammation model [47].

As shown in Table 6, at 200 or 400 mg/kg oral dose, the anti-inflammatory effect of METM is significantly more potent than that of indomethacin ($p < 0.05$) after 3 and 4 h. This suggests a possible similar mechanism involving the prostaglandin biosynthesis inhibition. The anti-inflammatory activity of METM may be attributed to several phenolic and organic acids. Quercetin is well recognised for its inhibitory effects on pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, and inhibits the NF- κ B signalling pathway [48–50]. Quinic acid, also present in the plant extract, has been shown to modulate the inflammatory response by suppressing cytokine production and acting on the NF- κ B and MAPK pathways [51,52]. In any case, the preliminary results of the present research need to be confirmed in their mechanistic interpretation related to the identified phytochemicals by additional receptor studies in the future. However, the early anti-inflammatory effect of METM observed during the first phase could explain its potent anti-inflammatory activity.

Table 6. METM effects on provoked inflammation by carrageenan injection in mice paw ($n = 5$).

Tested Sample	Dose (mg/kg)	Inhibition of Inflammation (%)				
		30 min	60 min	120 min	180 min	240 min
Indomethacin	10	6.81 ± 2.05	20.47 ± 5.17	43.40 ± 3.51	68.08 ± 5.30 ^b	86.40 ± 2.95 ^a
METM	100	15.87 ± 5.32	36.08 ± 2.77 ^{c,d}	51.29 ± 3.77 ^c	70.45 ± 2.86 ^a	77.88 ± 3.91 ^a
	200	33.75 ± 10.15 ^d	50.31 ± 11.01 ^c	57.25 ± 8.02 ^c	87.02 ± 3.91 ^{a,d}	95.46 ± 1.91 ^{a,d}
	400	9.27 ± 1.56	24.94 ± 3.92	48.01 ± 3.29 ^c	80.11 ± 4.29 ^{a,d}	96.08 ± 1.53 ^{a,d}
Negative control	-	13.72 ± 2.94	19.90 ± 3.67	26.42 ± 7.23	29.24 ± 7.10	34.71 ± 6.88

The data represent the mean ± S.E.M. (standard error means). a: $p < 0.001$ vs. Negative control; b: $p < 0.01$ vs. Negative control; c: $p < 0.05$ vs. Negative control. d: $p < 0.05$ vs. indomethacin. Dose-dependent for $p < 0.05$ at $t = 60, 120, 180$, and 240 min (ANOVA followed by Tukey's Post Hoc test).

2.5.2. Acetic Acid-Induced Writhing Test

METM analgesic activity was evaluated using the writhing method, which is the most common peripheral analgesic animal model for the screening of analgesic drugs [53]. Acetic acid induced by the i.p. route provokes irritation and the synthesis and release of several endogenous algogen factors, including histamine, serotonin, bradykinin, P substance, and PGs, which activate primary afferent nociceptors [54]. The analgesic activity of METM and indomethacin expressed as pain inhibition percentage is reported in Table 7 (individual data points were reported in Table S2). The i.p. injection of 1% acetic acid solution into the control group caused 35.2 ± 6.19 writhing between the intervals of 25 min after the 5th min of acetic acid induction.

Table 7. METM effects on acetic acid induction in mice ($n = 5$).

Tested Sample	Dose (mg/kg)	Writhing Number	Pain Inhibition (%)
Indomethacin	10	4.10 ± 1.91 ^a	89.77
METM	100	12.60 ± 2.88 ^b	64.20
	200	8.60 ± 4.62 ^a	75.21
	400	3.40 ± 2.51 ^a	90.20
Vehicle	-	35.2 ± 6.83	-

The data represent the mean ± S.E.M. (standard error means). a: $p < 0.01$ vs. Negative control; b: $p < 0.05$ vs. Negative control.

METM significantly reduced, in a dose-dependent manner, the number of writhes compared to the control group (Table 7). The indomethacin, used as a Positive control, showed a significant inhibition value of pain provoked by the acetic acid induction at 88.35% against the NNNegative control ($p < 0.001$). The inhibition of endogenous pain mediators such as PGs may mediate the peripheral analgesic effect of METM; this action may be largely attributed to quercetin and its glycoside quercitrin, both of which have been reported to suppress prostaglandin synthesis through inhibition of COX-1 and COX-2, thereby reducing peripheral nociceptive signalling [55]. Although less extensively studied, succinic acid has also been linked to anti-inflammatory mechanisms via modulation of the GPR91 (succinate) receptor, potentially contributing to the attenuation of inflammatory pain [56].

2.6. Acute Toxicity in Mice

Signs of toxicity, characterised by decreased motor activity and drowsiness in male and female groups, began to appear at a dose of 0.5 g/kg. This dose is much higher than those typically used by the local population in food applications, herbal medicines, and traditional beverages. This represents one of the administered doses of *T. madagascariensis* extract; several other ones were also considered. This dosage corresponds to about 25 g of

plant extract for a 50 kg individual. Considering 12.08% as the extraction yield, this amount is similar to an intake of about 300 g of dried plant material. At a dose of 500 mg/kg, these signs appeared from the 20th minute of administration and disappeared after 6 h. At 1500 mg/kg, signs appeared from the 10th minute and persisted for 20 h. At 2000 mg/kg, 50% of animals died between the 6th and 22nd hour, yielding an estimated LD₅₀ of approximately 2000 mg/kg (Figure 5). According to OECD 423 and GHS classification, METM falls within Category 4 (LD₅₀ between 300 and 2000 mg/kg). An acute toxicity study indicates that the use of this plant at high doses (>1500 mg/kg) is toxic or even fatal for mice. Considering that the traditional dose used in herbal beverages corresponds to about 5 g of dried leaves, the toxic doses observed in mice were much higher than those reported in ethnomedicinal use.

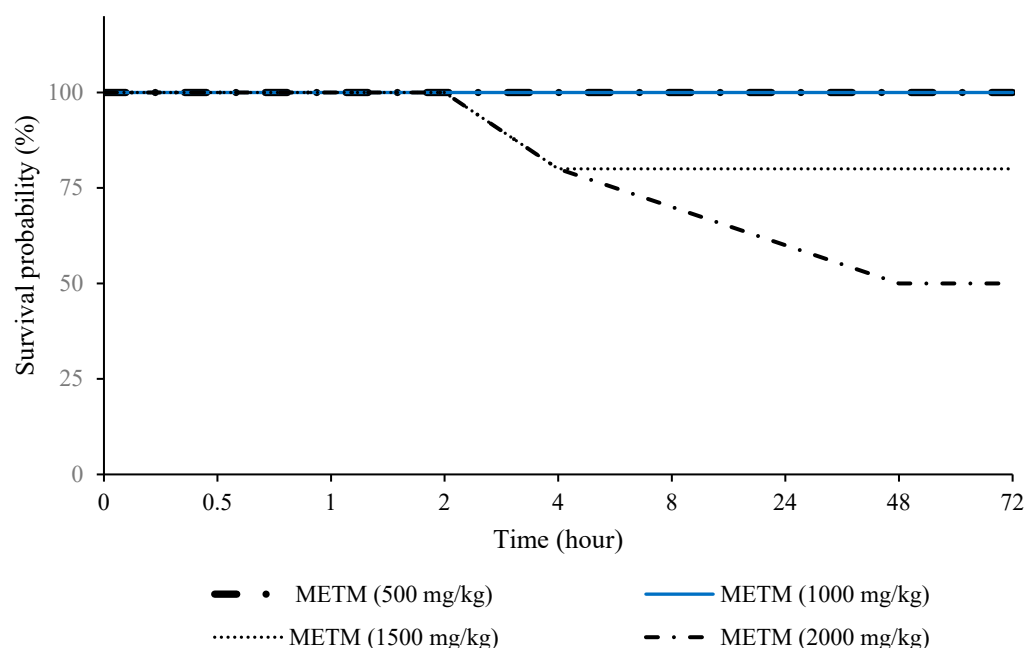


Figure 5. Kaplan–Meier survival curves of Swiss albino mice after a single oral dose of METM (500–2000 mg/kg, $n = 6$ per group) during 72 h of observation.

3. Materials and Methods

3.1. Plant Materials

The aerial parts of *T. madagascariensis* were harvested in January 2023 at Beforona (18°57'54" S 48°34'51" E, 549 m of altitude) in Moramanga District, at the highland part of Madagascar. The collected plant material was dried in a room dedicated to this purpose at room temperature for 20 days before being ground. The voucher specimen was identified by Mrs. Elyna Mahitsoa, the botanist at “Institut Malgache de Recherches Appliquées” (IMRA), by comparing it with the previous voucher (MVT-02/IMRA18–19) and deposited at IMRA herbarium under the identification code MVT-02/IMRA23. The identification was cross-checked with taxonomic databases (Plants of the World Online, POWO 2024; Tropicos 2024) to confirm the accepted name and authority. A photo plate illustrating the collected specimen is provided in Supplementary Materials, Figure S4, to allow external verification.

3.2. Chemicals

Several salts (NaCl, NaHCO₃, KCl, CaCl₂, MgSO₄, and KH₂PO₄) and D-glucose, purchased from Prolabo (Paris, France), were used to prepare the Krebs–Henseleit solution. The reference agonists (histamine, theophylline, and acetylcholine) and antagonists (pro-

pranolol, methylene blue), along with dimethyl sulfoxide (DMSO), were obtained from Sigma-Aldrich (Darmstadt, Germany).

3.3. Extraction and Fractionation

Aerial part powders (200 g) were macerated at room temperature in 95% methanol (500 mL) and stirred continuously for 24 h. The maceration protocol was repeated 3 times to obtain maximum yield, followed by filtration each time. The obtained filtrate was pooled and then evaporated under reduced pressure at 40 °C using a rotavapor (Buchi R114, Essen, Germany) to remove the solvent, obtaining the solid fraction of the *T. madagascariensis* methanol extract (METM).

METM (50 g) was suspended in distilled water and then fractioned by a liquid–liquid partition with successively n-hexane, dichloromethane, and ethyl acetate, to obtain the hexane (HFTM), dichloromethane (DFTM), ethyl acetate (EFTM), and aqueous (AFTM) fractions, respectively.

3.4. Phytochemical Screening

Standard protocols for qualitative phytochemical screening, as described by Tombozara et al. [57], were used to determine the main chemical classes of secondary metabolites in METM and EFTM. These protocols, based on colour changes and precipitate formation according to developed methods by Fong and his collaborators [58], are widely employed for preliminary phytochemical profiling and provide reliable first-line information on the phytochemical composition.

3.5. Chromatographic Analysis

3.5.1. Sample Preparation Protocol for HPLC Analysis

The extract was prepared in triplicate according to the method described by Donno et al. [59]. Basically, 5 g of powders were suspended in 35 mL of a solution of methanol: water: 37% hydrochloric acid (95:4.5:0.5, *v/v/v*) and kept for 24 h in the dark. Samples were then mixed and homogenised for 3 min using a mixer (Ultra Turrax T25 basic, IKA-Werke, Staufen, Germany). Then a second maceration using the same protocol was undertaken for 72 h, followed by an additional 3 min homogenization and incubation in the dark for 24 h. Mixtures were again mixed and homogenised for 3 min. The mixtures were filtered using Whatman filter paper (Hardened Ashless Circles, 185 mm Ø, Cytiva, Marlborough, MA, USA), and the filtrates were collected and stored at 4 °C until HPLC analysis.

3.5.2. Standard Calibration

Standard solutions were prepared in triplicate at the different concentrations listed in Table S3. In total, 20 µL of each solution was injected manually, and the calibration curves were obtained by plotting the peak area (*y*) of the compound at each concentration level versus the sample concentration (*x*).

3.5.3. Apparatus and Chromatographic Conditions

All the analyses were performed by using a High-Performance Liquid Chromatograph (Agilent 1200) coupled to UV-Vis DAD (Agilent Technologies, Santa Clara, CA, USA). Compounds in the samples were separated through a Kinetex C18 column (4.6 × 150 mm, 5 µm, Phenomenex, Torrance, CA, USA) following the chromatographic methods listed in Table S4. These HPLC methods have been previously tested and validated for herbal medicines and food applications [60,61], where each biomarker (performed in triplicate) was identified based on its retention time and UV spectra compared to standard solutions.

3.6. Total Phenolic Content (TPC)

The Folin–Ciocalteu reagent was used to determine the TPC according to the method described by Tombozara et al. [18].

3.7. Free Radical DPPH Scavenging Assay

The radical DPPH assay, highlighted in Tombozara et al. [62], was used to assess the scavenging capacity of METM, where gallic acid was used as a Positive control. The median scavenging capacity was evaluated for METM and gallic acid in three replicates.

3.8. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of METM was evaluated by the FRAP assay according to the method of Benzie and Strain [63] with slight modification of Donno et al. [20]. Each sample was tested in triplicate.

3.9. Bronchorelaxant Study

3.9.1. Effect of METM and Fractions on Histamine Pre-Contracted Trachea

All in vitro experiments were assessed on an isolated trachea from a male guinea pig, approximately three months old and weighing around 250 g. All animals were healthy, showed no visible signs of disease or abnormalities, and were deemed suitable for the experiments. The number of animals used corresponds to n. From each animal, 2 helical strips were prepared, and their responses were averaged to obtain one data point per animal. Briefly, the animal was euthanised by ether suffocation, a method classically employed in our institutional setting. The procedures were performed by trained personnel under an exhaust hood to minimise operator exposure. All the procedures involving animals were performed according to international legislation (Directive 2010/63/EU) and in compliance with institutional animal welfare approval and national legislation. The limitations related to the euthanasia protocol used in this preliminary research have been recognised; approved and updated procedures will be applied in future studies to ensure full safety and compliance with current international ethical standards. A 1.5 to 2 cm segment of the trachea was isolated and placed in pre-heated Krebs–Henseleit solution (37 °C) composed of NaCl (120 mM), NaHCO₃ (25 mM), KCl (4.72 mM), CaCl₂ (2.5 mM), MgSO₄ (0.5 mM), KH₂PO₄ (1.2 mM), and D-glucose (11 mM). The tracheal strip was cleaned, helically cut, and mounted in a 20 mL organ bath with Krebs solution, maintained at 37 °C, and aerated with carbogen (mixture of O₂/CO₂ 95/5 v/v). A 2 g isotonic baseline tension was applied, followed by a 1 h adaptation period with 15 min washes. Viability was confirmed by reproducible contractions to histamine (10^{−6} M) before testing.

Then, tested extracts (METM, HFTM, DFTM, EFTM, and AFTM) were dissolved in DMSO and diluted in distilled water (DMSO < 1%). The organ was pre-contracted with histamine 5 × 10^{−6} M. Cumulative addition of METM (50–1000 µg/mL) or fractions (20–400 µg/mL) was performed to determine the concentration–response relationship.

3.9.2. EFTM Effects on Acetylcholine and KCl-Induced Contractions

The same protocol, described in Section 3.9.1, was used for the study of the broncho-relaxant effect of EFTM. After the equilibration period, the trachea was pre-contracted with 10^{−5} M acetylcholine or 40 mM KCl, and cumulative concentrations of EFTM (20–400 µg/mL for Ach; 50–1200 µg/mL for KCl) were added in the organ bath to cause relaxation; the EC₅₀ values were then calculated.

3.9.3. EFTM Effects on Histamine Contractile Activity

To investigate the potential inhibitory activity of EFTM against histamine, the effect–concentration relationship of histamine alone (10^{−8} to 3.10^{−4} M) or in the presence of EFTM

(100 or 200 µg/mL) was studied. EFTM was incubated for 10 min in the organ bath before the cumulative injection of histamine.

3.9.4. EFTM Effect on CaCl₂ Contractile Activity

The anti-calcic activity was assessed by analysing the CaCl₂ (0.25 to 32 mM) concentration-response curve in the presence and absence of EFTM (100 or 200 µg/mL). CaCl₂-free Krebs–Henseleit solution was used, and contraction amplitudes were recorded.

3.9.5. Propranolol Effects on Broncho-Relaxant Activity of EFTM

Propranolol is a non-selective β-adrenergic inhibitor [64]. The broncho-relaxant effects of EFTM were assessed on histamine pre-contracted guinea pig trachea in the absence or presence of propranolol. Propranolol (10^{−6} or 10^{−8} M) was pre-incubated for 10 min in the organ bath before histamine (5 · 10^{−6} M) addition. At maximal contraction, cumulative concentrations (20 to 400 µg/mL) of EFTM were added. The EC₅₀ was calculated in both conditions.

3.9.6. EFTM and Theophylline Combination Effects on Histamine Pre-Contracted Trachea

Theophylline is a non-selective phosphodiesterase (PDE) inhibitor [45]. The effects of the combination were compared to those of EFTM and theophylline alone. Theophylline (15 µg/mL), EFTM (75 µg/mL), and EFTM-theophylline (75–25 µg/mL) were tested at the plateau of contraction after a pre-contraction with histamine (5 · 10^{−6} M). The percentage of relaxations was calculated for each test with an interval of organ washing between each test.

3.10. *In Vivo* Assays

3.10.1. Animals

Healthy Swiss albino mice, both male and female (weight: 25 ± 5 g; age: 12–14 weeks old), were used during the *in vivo* studies. The animals were raised under controlled conditions (12 h dark and 12 h light cycle, T°: 25 ± 2 °C, humidity: 50 ± 10%) at the IMRA animal house. They were fed with a standard food pellet (1420, Livestock Feed Ltd., Port Louis, Mauritius) and remained fasting for one night before the experiment. The animals were randomly assigned to groups. In the case of an administration error, the animal was replaced and excluded from the analysis. The European Parliament and the Council of 22nd September 2010 on the protection of animals used for scientific purposes (DIRECTIVE 2010/63/EU) were followed as guidance for the protocol execution. All *in vivo* experiments were approved by the local ethics committee (n° 033/CEA-IMRA/2023).

3.10.2. METM Effects on Carrageenan-Induced Paw Oedema

Carrageenan-induced mouse hind paw oedema, as previously described by Razafindrakoto et al. [16], was used to evaluate the anti-inflammatory activity of METM. Acute inflammation was induced by a subplantar injection of 100 µL of 2% λ-carrageenan solution (Commercial Grade, TypeII, batch no. 0000356601, Sigma-Aldrich, Darmstadt, Germany) in 0.9% normal saline solution, into the right hind paw of mice, using a 26-gauge needle. Briefly, fasted animals were divided into 5 groups of 5 mice. Animals of group I orally received the distilled water, used as the vehicle of the tested products, and they formed the Negative control, while the Positive control was treated with indomethacin (10 mg/kg orally administered). Those of groups III–V were treated with METM at a dose of 100, 200, and 400 mg/kg b.w., respectively, 1 h before carrageenan induction. The paw volume was measured on a plethysmometer (Ugo Basile 7140, Gemonio (VA), Italy), calibrated before each use with a marked volume probe, before and at 30th, 60th, 120th, 180th, and 240th min after carrageenan injection. The oedema inhibition (IO%) was calculated using the

formula $IO\% = 100 \times (V_0 - V_t)/V_0$, where V_0 and V_t were the volume of paw oedema at 0 min and t min post-carrageenan solution injection, respectively.

3.10.3. METM Effects on Pain Provoked by Acetic Acid Model

The pain experimental model described in Razafindrakoto et al. [16] was used to determine the METM antinociceptive activity. Fasted animals were divided into 5 groups of 5 mice. Group I was orally administered distilled water (Negative control), while indomethacin (10 mg/kg b.w.) was given to group II (Positive control), and groups II-V were treated with METM (100, 200 and 400 mg/kg b.w.) 1 h before the injection of acetic acid solution (100 μ L, 1%) in 0.9% saline solution by intraperitoneal route to induce writhing. The number of writhings occurring between 5 and 30 min after acetic acid injection was counted. Writhes were defined as characteristic abdominal constrictions with hind limb extension and trunk elongation.

3.10.4. METM Acute Toxicity in Mice

METM oral acute toxicity in mice was evaluated according to the Organisation for Economic Cooperation and Development (OECD) [65] guideline method, slightly modified. Fasted animals were divided into 5 groups, each comprising 3 females and 3 males, separately placed in two different cages ($n = 6$): group I was orally administered distilled water and used as a Negative control, while groups II to V received a single oral dose of METM 250, 500, 1000, and 2000 mg/kg b.w., respectively. The animals were observed for any toxic effects for the first 6 h after treatment. Further observations were investigated for 3 days to evaluate any toxic effects. Behavioural changes and other parameters, such as body weight, urination, food and water intake, respiration, convulsions, tremors, temperature, constipation, changes in eye and skin colour, etc., were systematically recorded. Mortality was also observed and reported as survival per group.

3.11. Statistical Analysis

Results were expressed as the mean $\pm$ standard deviation (SD) or standard error of the mean (SEM) of n (number of used animals on in vivo model or repetition test in vitro). Statistical comparisons were realised on SPSS 20 software using the appropriate method (Student- t test and the one-way ANOVA followed by the HSD Tukey multiple range test). Statistical significance was accepted if the p -value was under 0.05. An example of t -test analysis is shown in the Supplementary Materials.

4. Conclusions

This preliminary study showed significant antioxidant, analgesic, anti-inflammatory, and broncho-relaxant properties of the aerial parts of *T. madagascariensis*, justifying its empirical therapeutic virtues in inflammatory conditions, pain, and asthma. In addition, the broncho-relaxant effect of the ethyl acetate fraction suggests a possible partial involvement of β_2 -adrenergic-related pathways, together with additional mechanisms, although definitive mechanistic conclusions cannot yet be defined. These pharmacological properties may be due to the presence of several phytochemicals, especially the organic acids and phenolics quantified in the extracts. Moreover, METM did not show any toxicity in animal models at a dose for human consumption, supporting the ethnomedicinal use of this plant; however, further toxicological investigations remain necessary (no direct human safety data are available). These findings indicate that the aerial parts of *T. madagascariensis* may be a potential new candidate as a bioactive ingredient for traditional herbal formulations, plant-based food applications, and health-promoting beverages. Finally, further investigations, including bioassay-guided isolation, in silico studies, MS-guided dereplication,

receptor profiling, in vivo asthma models, and bioavailability studies, are necessary to confirm these preliminary findings.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants15050681/s1>. Table S1. Individual data points for anti-inflammatory test; Table S2. Individual data points for analgesic test; Table S3. Calibration curve, R², LOD, and LOQ of each selected bioactive compound; Table S4. Chromatographic conditions of each used method; Figure S1. Chromatographic profiles of *T. madagascariensis* aerial parts; Figure S2. UV-Vis spectra and chemical structure of bioactive compounds and nutritional substances identified in *T. madagascariensis* aerial parts; Figure S3. Free radical scavenging effect of METM (A) and gallic acid (B); Figure S4. Morphological features of *Tetracera madagascariensis* Willd. ex Schltdl. (voucher MVT-02/IMRA23, IMRA Herbarium, Antananarivo, Madagascar); Figure S5. Individual inhibition of inflammation caused by carrageenan injection in mice; Figure S6. EFTM effects on histamine-induced contractile activity; Figure S7. Effect of METM on histamine-pre-contracted trachea; File S1. Author Checklist; File S2. 033-CEA-IMRA-2023.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AFTM	Aqueous Fraction of <i>Tetracera madagascariensis</i>
CICR	Calcium-Induced Calcium Release
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
cGMP/PKGII	Cyclic Guanosine Monophosphate/Protein Kinase G Type II
DFTM	Dichloromethane Fraction of <i>T. madagascariensis</i>
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry Weight
EC ₅₀	Half Maximal Effective Concentration
EFTM	Ethyl Acetate Fraction of <i>T. madagascariensis</i>
ENaC	Epithelial Sodium Channel
FRAP	Ferric Reducing Antioxidant Power
GPR91	G-Protein Coupled Receptor 91 (Succinate Receptor)
HFTM	Hexane Fraction of <i>T. madagascariensis</i>
HPLC	High-Performance Liquid Chromatography
IL-1β	Interleukin 1 Beta
IL-6	Interleukin 6
IMRA	Institut Malgache de Recherches Appliquées
i.p.	Intraperitoneal
IP ₃ Rs	Inositol 1,4,5-Trisphosphate Receptors

LD ₅₀	Lethal Dose for 50% of Test Population
MAPK	Mitogen-Activated Protein Kinase
METM	Methanol Extract of <i>Tetracera madagascariensis</i>
MLCK	Myosin Light Chain Kinase
mmol FIE	Millimole Ferrous Ion Equivalent
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF-κB	Nuclear Factor-Kappa B
OGG1	8-Oxoguanine DNA Glycosylase
PDE	Phosphodiesterase
PKA	Protein Kinase A
PGs	Prostaglandins
PMCA	Plasma Membrane Ca ²⁺ -ATPase
ROS	Reactive Oxygen Species
SC ₅₀	50% Scavenging Concentration
SEM	Standard Error of the Mean
SERCA	Sarco/Endoplasmic Reticulum Ca ²⁺ -ATPase
TBCC	Total Bioactive Compound Content
TNF-α	Tumour Necrosis Factor Alpha
VOCs	Voltage-Gated Calcium Channels

References

1. Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention, 2024 Update*; Global Initiative for Asthma: Genève, Switzerland, 2024.
2. Tombozara, N.; Mahitasoa, F.J.E.; Razafindrakoto, Z.R.; Randriamampionona, D.; Solofoniaina, M.; Tata, P.; Ramanitrahasimbola, D.; Andrianjara, C. Sick Cell Disease: Ethnopharmacological Survey in the Eastern Part of Madagascar. *S. Afr. J. Bot.* **2024**, *174*, 606–623. [\[CrossRef\]](#)
3. Rakoto-Ratsimamanga, A.; Boiteau, P.; Mouton, M. *Éléments de Pharmacopée Malgache. Tome I, Notices 1 à 39*; Société Pour la Promotion de la Pharmacopée Malgache–IMRA: Tananarive, Madagascar, 1969.
4. Ramanitrahasimbola, D.; Rakotondramanana, D.A.; Rasoanaivo, P.; Randriantsoa, A.; Ratsimamanga, S.; Palazzino, G.; Galeffi, C.; Nicoletti, M. Bronchodilator Activity of Phymatodes Scolopendria (Burm.) Ching and Its Bioactive Constituent. *J. Ethnopharmacol.* **2005**, *102*, 400–407. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Zuo, L.; Otenbaker, N.P.; Rose, B.A.; Salisbury, K.S. Molecular Mechanisms of Reactive Oxygen Species-Related Pulmonary Inflammation and Asthma. *Mol. Immunol.* **2013**, *56*, 57–63. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Hosoki, K.; Gandhe, R.; Boldogh, I.; Sur, S. Reactive Oxygen Species (ROS) and Allergic Responses. In *Systems Biology of Free Radicals and Antioxidants*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 3239–3266, ISBN 3-642-30018-9.
7. Truong, L.; Zheng, Y.-M.; Kandhi, S.; Wang, Y.-X. Overview on Interactive Role of Inflammation, Reactive Oxygen Species, and Calcium Signaling in Asthma, COPD, and Pulmonary Hypertension. In *Lung Inflammation in Health and Disease, Volume II*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 147–164.
8. Michaeloudes, C.; Abubakar-Waziri, H.; Lakhdar, R.; Raby, K.; Dixey, P.; Adcock, I.M.; Mumby, S.; Bhavsar, P.K.; Chung, K.F. Molecular Mechanisms of Oxidative Stress in Asthma. *Mol. Asp. Med.* **2022**, *85*, 101026. [\[CrossRef\]](#)
9. *Tetracera Madagascariensis* Willd. Ex Schltdl. | Plants of the World Online | Kew Science. Available online: <http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:317439-1> (accessed on 7 October 2025).
10. Nicolas, J.-P. *Plantes Médicinales du Nord de Madagascar: Ethnobotanique Antakarana et Informations Scientifiques*; Jardins du Monde: Braspars, France, 2012; ISBN 978-2-9543726-0-0.
11. Désiré, O.; Rivière, C.; Razafindrazaka, R.; Goossens, L.; Moore, N.; Andriamadio, P.; Randriantsoa, A.; Raharisololalao, A. Ethnopharmacological Study of Two Plants of Northern Madagascar: Bronchodilator Activity of *Tetracera Madagascariensis* and Antispasmodic Activity of *Mascarenhasia Arborescens*. *Planta Med.* **2009**, *75*, P156. [\[CrossRef\]](#)
12. Humbert, H.; Leroy, J.-F. *Flore de Madagascar et Des Comores: Plantes Vasculaires*; Imprimerie Officielle: Tananarive, Madagascar, 1936.
13. Rabearivony, A.D.; Kuhlman, A.R.; Razafiariso, Z.L.; Raharimalala, F.; Rakotoarivony, F.; Randrianarivony, T.; Rakotoarivelo, N.; Randrianasolo, A.; Bussmann, R.W. Ethnobotanical Study of the Medicinal Plants Known by Men in Ambalabe, Madagascar. *Ethnobot. Res. App.* **2015**, *14*, 123. [\[CrossRef\]](#)
14. Valduga, A.T.; Gonçalves, I.L.; Magri, E.; Delalibera Finzer, J.R. Chemistry, Pharmacology and New Trends in Traditional Functional and Medicinal Beverages. *Food Res. Int.* **2019**, *120*, 478–503. [\[CrossRef\]](#)

15. Maleš, I.; Pedisić, S.; Zorić, Z.; Elez-Garofulić, I.; Repajić, M.; You, L.; Vladimir-Knežević, S.; Butorac, D.; Dragović-Uzelac, V. The Medicinal and Aromatic Plants as Ingredients in Functional Beverage Production. *J. Funct. Foods* **2022**, *96*, 105210. [\[CrossRef\]](#)
16. Razafindrakoto, Z.R.; Donno, D.; Tombozara, N.; Andriamaniraka, H.; Andrianjara, C.; Ramanitrahasimbola, D.; Beccaro, G.L. Antioxidant, Anti-Inflammatory, and Antidiabetic Activities of Leaves and Stems of Uapaca Bojeri Bail. (EUPHORBIACEAE), an Endemic Plant of Madagascar. *Pharmaceuticals* **2020**, *13*, 71. [\[CrossRef\]](#)
17. Razafindrakoto, Z.R.; Tombozara, N.; Donno, D.; Gamba, G.; Nalimanana, N.R.; Rakotondramanana, D.A.; Andrianjara, C.; Beccaro, G.L.; Ramanitrahasimbola, D. Antioxidant, Analgesic, Anti-Inflammatory and Antipyretic Properties, and Toxicity Studies of the Aerial Parts of Imperata Cylindrica (L.) Beauv. *S. Afr. J. Bot.* **2021**, *142*, 222–229. [\[CrossRef\]](#)
18. Tombozara, N.; Razafindrakoto, Z.R.; Donno, D.; Randriamampionona, D.; Ramilison-Razafimahefa, R.D.; Rakotondramanana, D.A.; Andrianjara, C.; Ramanitrahasimbola, D.; Beccaro, G.L. Phytochemical and Pharmacological Activities of Schefflera Bojeri (Seem.) R. Vig. (Araliaceae). *S. Afr. J. Bot.* **2022**, *151*, 514–522. [\[CrossRef\]](#)
19. Silva, B.M.; Andrade, P.B.; Gonçalves, A.C.; Seabra, R.M.; Oliveira, M.B.; Ferreira, M.A. Influence of Jam Processing upon the Contents of Phenolics, Organic Acids and Free Amino Acids in Quince Fruit (Cydonia Oblonga Miller). *Eur. Food Res. Technol.* **2004**, *218*, 385–389. [\[CrossRef\]](#)
20. Donno, D.; Beccaro, G.L.; Mellano, M.G.; Cerutti, A.K.; Bounous, G. Goji Berry Fruit (*Lycium* Spp.): Antioxidant Compound Fingerprint and Bioactivity Evaluation. *J. Funct. Foods* **2015**, *18*, 1070–1085. [\[CrossRef\]](#)
21. Nimse, S.B.; Pal, D. Free Radicals, Natural Antioxidants, and Their Reaction Mechanisms. *RSC Adv.* **2015**, *5*, 27986–28006. [\[CrossRef\]](#)
22. Olszowy-Tomczyk, M. Synergistic, Antagonistic and Additive Antioxidant Effects in the Binary Mixtures. *Phytochem. Rev.* **2020**, *19*, 63–103. [\[CrossRef\]](#)
23. Soobrattee, M.A.; Neergheen, V.S.; Luximon-Ramma, A.; Aruoma, O.I.; Bahorun, T. Phenolics as Potential Antioxidant Therapeutic Agents: Mechanism and Actions. *Mutat. Res./Fundam. Mol. Mech. Mutagen.* **2005**, *579*, 200–213. [\[CrossRef\]](#)
24. Razafin-drabazo, F.; Donno, D.; Tombozara, N.; Razafindrakoto, Z.R.; Rajaonarison, J.F.; Andrianjara, C.; Ramanitrahasimbola, D.; Beccaro, G.L. Phyto-Compounds and Pharmacological Activities of Lygodium Lanceolatum Desv. (*Schizaeaceae*). *S. Afr. J. Bot.* **2020**, *135*, 225–232. [\[CrossRef\]](#)
25. Angonese, M.; Motta, G.E.; Silva De Farias, N.; Molognoni, L.; Daguer, H.; Brugnerotto, P.; De Oliveira Costa, A.C.; Olivera Müller, C.M. Organic Dragon Fruits (*Hylocereus undatus* and *Hylocereus polyrhizus*) Grown at the Same Edaphoclimatic Conditions: Comparison of Phenolic and Organic Acids Profiles and Antioxidant Activities. *LWT* **2021**, *149*, 111924. [\[CrossRef\]](#)
26. Kędzierska-Matysek, M.; Stryjecka, M.; Teter, A.; Skąlecki, P.; Domaradzki, P.; Florek, M. Relationships between the Content of Phenolic Compounds and the Antioxidant Activity of Polish Honey Varieties as a Tool for Botanical Discrimination. *Molecules* **2021**, *26*, 1810. [\[CrossRef\]](#)
27. Zhang, Y.; Li, Y.; Ren, X.; Zhang, X.; Wu, Z.; Liu, L. The Positive Correlation of Antioxidant Activity and Prebiotic Effect about Oat Phenolic Compounds. *Food Chem.* **2023**, *402*, 134231. [\[CrossRef\]](#)
28. Zarubina, I.V.; Lukk, M.V.; Shabanov, P.D. Antihypoxic and Antioxidant Effects of Exogenous Succinic Acid and Aminoethiol Succinate-Containing Antihypoxants. *Bull Exp. Biol. Med.* **2012**, *153*, 336–339. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Chen, J.; Li, G.; Sun, C.; Peng, F.; Yu, L.; Chen, Y.; Tan, Y.; Cao, X.; Tang, Y.; Xie, X.; et al. Chemistry, Pharmacokinetics, Pharmacological Activities, and Toxicity of Quercitrin. *Phytother. Res.* **2022**, *36*, 1545–1575. [\[CrossRef\]](#)
30. Ercan, L.; Doğru, M. Antioxidant and Antimicrobial Capacity of Quinic Acid. *Bitlis Eren Üniversitesi Fen Bilim. Derg.* **2022**, *11*, 1018–1025. [\[CrossRef\]](#)
31. Wang, G.; Wang, Y.; Yao, L.; Gu, W.; Zhao, S.; Shen, Z.; Lin, Z.; Liu, W.; Yan, T. Pharmacological Activity of Quercetin: An Updated Review. *Evid.-Based Complement. Altern. Med.* **2022**, *2022*, 3997190. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Castillo-Arellano, J.; González-Espinosa, C.; Jiménez-Estrada, M.; Reyes-Chilpa, R. Natural Compounds against Allergic and Pseudoallergic Reactions: Molecular Mechanisms of Action. *Stud. Nat. Prod. Chem.* **2024**, *80*, 269–325.
33. Hufnagel, M.; Rademaekers, A.; Weisert, A.; Häberlein, H.; Franken, S. Pharmacological Profile of Dicafeoylquinic Acids and Their Role in the Treatment of Respiratory Diseases. *Front. Pharmacol.* **2024**, *15*, 1371613. [\[CrossRef\]](#)
34. Zhai, Y.; Wang, T.; Fu, Y.; Yu, T.; Ding, Y.; Nie, H. Ferulic Acid: A Review of Pharmacology, Toxicology, and Therapeutic Effects on Pulmonary Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 8011. [\[CrossRef\]](#)
35. Xie, X.; Yu, T.; Hou, Y.; Han, A.; Ding, Y.; Nie, H.; Cui, Y. Ferulic Acid Ameliorates Lipopolysaccharide-Induced Tracheal Injury via cGMP/PKGII Signaling Pathway. *Respir. Res.* **2021**, *22*, 308. [\[CrossRef\]](#)
36. Jo, M.H.; Kim, B.C.; Sung, K.; Panettieri, R.A.; An, S.S.; Liu, J.; Ha, T. Molecular Nanomechanical Mapping of Histamine-Induced Smooth Muscle Cell Contraction and Shortening. *ACS Nano* **2021**, *15*, 11585–11596. [\[CrossRef\]](#)
37. Zhao, L.; Liang, Y.-T.; Tian, D.-B.; Zhang, R.-G.; Huang, J.; Zhu, Y.-X.; Zhou, W.-L.; Zhang, Y.-L. Regulation of Smooth Muscle Contractility by the Epithelium in Rat Tracheas: Role of Prostaglandin E₂ Induced by the Neurotransmitter Acetylcholine. *Ann. Transl. Med.* **2021**, *9*, 313. [\[CrossRef\]](#)
38. Matsui, T. Calcium Wave Propagation during Cell Extrusion. *Curr. Opin. Cell Biol.* **2022**, *76*, 102083. [\[CrossRef\]](#) [\[PubMed\]](#)

39. Chiang, Y.; Chen, H.; Lai, Y.; Ali, M.; Chen, Y.; Hsia, S. Consumption of Artificial Sweetener Acesulfame Potassium Increases Preterm Risk and Uterine Contraction with Calcium Influx Increased via Myosin Light Chain Kinase–Myosin Light Chain 20 Related Signaling Pathway. *Mol. Nutr. Food Res.* **2022**, *66*, 2200298. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Berridge, M.J.; Bootman, M.D.; Roderick, H.L. Calcium Signalling: Dynamics, Homeostasis and Remodelling. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 517–529. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Leybaert, L.; Sanderson, M.J. Interacellular Ca^{2+} Waves: Mechanisms and Function. *Physiol. Rev.* **2012**, *92*, 1359–1392. [\[CrossRef\]](#)
42. Dolphin, A.C. Functions of Presynaptic Voltage-Gated Calcium Channels. *Function* **2020**, *2*, zqaa027. [\[CrossRef\]](#)
43. Paul, A. Adrenergic Agonists. In *Introduction to Basics of Pharmacology and Toxicology: Volume 2: Essentials of Systemic Pharmacology: From Principles to Practice*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 41–53.
44. Suh, D.I.; Johnston, S.L. The Wiser Strategy of Using Beta-Agonists in Asthma: Mechanisms and Rationales. *Allergy Asthma Immunol. Res.* **2024**, *16*, 217. [\[CrossRef\]](#)
45. Stolfa, I.; Page, C. Phosphodiesterase Inhibitors and Lung Diseases. In *Advances in Pharmacology*; Elsevier: Amsterdam, The Netherlands, 2023; Volume 98, pp. 55–81, ISBN 1054-3589.
46. Roome, T.; Dar, A.; Naqvi, S.; Ali, S.; Choudhary, M.I. Aegiceras Corniculatum Extract Suppresses Initial and Late Phases of Inflammation in Rat Paw and Attenuates the Production of Eicosanoids in Rat Neutrophils and Human Platelets. *J. Ethnopharmacol.* **2008**, *120*, 248–254. [\[CrossRef\]](#)
47. Déciga-Campos, M.; Palacios-Espinosa, J.F.; Reyes-Ramírez, A.; Mata, R. Antinociceptive and Anti-Inflammatory Effects of Compounds Isolated from Scaphyglottis Livida and Maxillaria Densa. *J. Ethnopharmacol.* **2007**, *114*, 161–168. [\[CrossRef\]](#)
48. Morikawa, K.; Nonaka, M.; Narahara, M.; Torii, I.; Kawaguchi, K.; Yoshikawa, T.; Kumazawa, Y.; Morikawa, S. Inhibitory Effect of Quercetin on Carrageenan-Induced Inflammation in Rats. *Life Sci.* **2003**, *74*, 709–721. [\[CrossRef\]](#)
49. Heeba, G.H.; Mahmoud, M.E.; Hanafy, A.A.E. Anti-Inflammatory Potential of Curcumin and Quercetin in Rats: Role of Oxidative Stress, Heme Oxygenase-1 and TNF- α . *Toxicol Ind. Health* **2014**, *30*, 551–560. [\[CrossRef\]](#)
50. Azeem, M.; Hanif, M.; Mahmood, K.; Ameer, N.; Chughtai, F.R.S.; Abid, U. An Insight into Anticancer, Antioxidant, Antimicrobial, Antidiabetic and Anti-Inflammatory Effects of Quercetin: A Review. *Polym. Bull.* **2023**, *80*, 241–262. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Jang, S.-A.; Park, D.W.; Kwon, J.E.; Song, H.S.; Park, B.; Jeon, H.; Sohn, E.-H.; Koo, H.J.; Kang, S.C. Quinic Acid Inhibits Vascular Inflammation in TNF- α -Stimulated Vascular Smooth Muscle Cells. *Biomed. Pharmacother.* **2017**, *96*, 563–571. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Ekhtiar, M.; Ghasemi-Dehnoo, M.; Azadegan-Dehkordi, F.; Bagheri, N. Evaluation of Anti-Inflammatory and Antioxidant Effects of Ferulic Acid and Quinic Acid on Acetic Acid-Induced Ulcerative Colitis in Rats. *J. Biochem. Mol. Tox.* **2025**, *39*, e70169. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Negus, S.S.; Vanderah, T.W.; Brandt, M.R.; Bilsky, E.J.; Becerra, L.; Borsook, D. Preclinical Assessment of Candidate Analgesic Drugs: Recent Advances and Future Challenges. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 507–514. [\[CrossRef\]](#)
54. Demsie, D.G.; Yimer, E.M.; Berhe, A.H.; Altaye, B.M.; Berhe, D.F. Anti-Nociceptive and Anti-Inflammatory Activities of Crude Root Extract and Solvent Fractions of Cucumis Ficifolius in Mice Model. *J. Pain Res.* **2019**, *12*, 1399–1409. [\[CrossRef\]](#)
55. Mondal, A.; Maity, T.K.; Bishayee, A. Analgesic and Anti-Inflammatory Activities of Quercetin-3-Methoxy-4'-Glucosyl-7-Glucoside Isolated from Indian Medicinal Plant Melothria Heterophylla. *Medicines* **2019**, *6*, 59. [\[CrossRef\]](#)
56. Liu, H.; Zhang, H.; Zhang, X.; Chen, Q.; Xia, L. Role of Succinic Acid in the Regulation of Sepsis. *Int. Immunopharmacol.* **2022**, *110*, 109065. [\[CrossRef\]](#)
57. Tombozara, N.; Razafindrakoto, Z.R.; Ramanitrahasimbola, D.; Razafimahefa-Ramilison, R.D.; Marchioni, E.; Rakotondramanana, A.D. Isolation of the Gallic Acid in the Butanolic Fraction of Crassula Ovata (Mill.) Druce (CRASSULACEAE) Leaves and Its Vaso-Relaxing Effect. *Am. J. Innov. Res. Appl. Sci* **2017**, *4*, 200–207.
58. Fong, H.H.S.; Tin, W.A.M.; Farnsworth, N. *Phytochemical Screening Review*; Chicago University of Illinois: Chicago, IL, USA, 1977; pp. 73–126.
59. Donno, D.; Beccaro, G.L.; Mellano, M.G.; Torello Marinoni, D.; Cerutti, A.K.; Canterino, S.; Bounous, G. Application of Sensory, Nutraceutical and Genetic Techniques to Create a Quality pro-File of Ancient Apple Cultivars. *J. Food Qual.* **2012**, *35*, 169–181. [\[CrossRef\]](#)
60. Donno, D.; Cerutti, A.K.; Mellano, M.G.; Prgomet, Z.; Beccaro, G.L. Serviceberry, a Berry Fruit with Growing Interest of Industry: Physicochemical and Quali-Quantitative Health-Related Compound Characterisation. *J. Funct. Foods* **2016**, *26*, 157–166. [\[CrossRef\]](#)
61. Fioccardi, A.; Donno, D.; Razafindrakoto, Z.R.; Tombozara, N.; Henintsoa, S.; Mahitasoa, E.; Torti, V.; Solofoniaina, M.; Rosso, L.; Gamba, G.; et al. Assessing a “Least-Concern” Red List Tree Species from Madagascar Used in Traditional Medicine: Morella Spathulata (Myricaceae) Phyto-Compounds and Anti-Inflammatory Properties. *Plants* **2024**, *13*, 2899. [\[CrossRef\]](#)
62. Tombozara, N.; Donno, D.; Razafindrakoto, Z.R.; Randriamampionona, D.; Ramanitrahasimbola, D.; Andrianjara, C.; Ramilison-Razafimahefa, R.D.; Rakotondramanana, D.A.; Beccaro, G.L. The First Assessment on Antioxidant and Antidiabetic Activities of Leaves and Stems of Vaccinium Secundiflorum Hook. (Ericaceae), an Endemic Plant of Madagascar. *South Afr. J. Bot.* **2020**, *130*, 422–429. [\[CrossRef\]](#)

63. Benzie, I.F.F.; Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76. [[CrossRef](#)]
64. Boskabady, M.H.; Teymoory, S. The Influence of Epithelium on the Responsiveness of Guinea-Pig Trachea to -Adrenergic Agonist and Antagonist. *Med. Sci. Monit.* **2003**, *9*, BR336-342.
65. Organisation de Coopération et de Développement Économiques. *Test Guideline 423: Acute Oral Toxicity—Acute Toxic Class Method*; OECD Publishing: Paris, France, 2002.

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