Research Article

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Study of phytochemical compound and antipyretic activity of *Chenopodium ambrosioides* L. fractions

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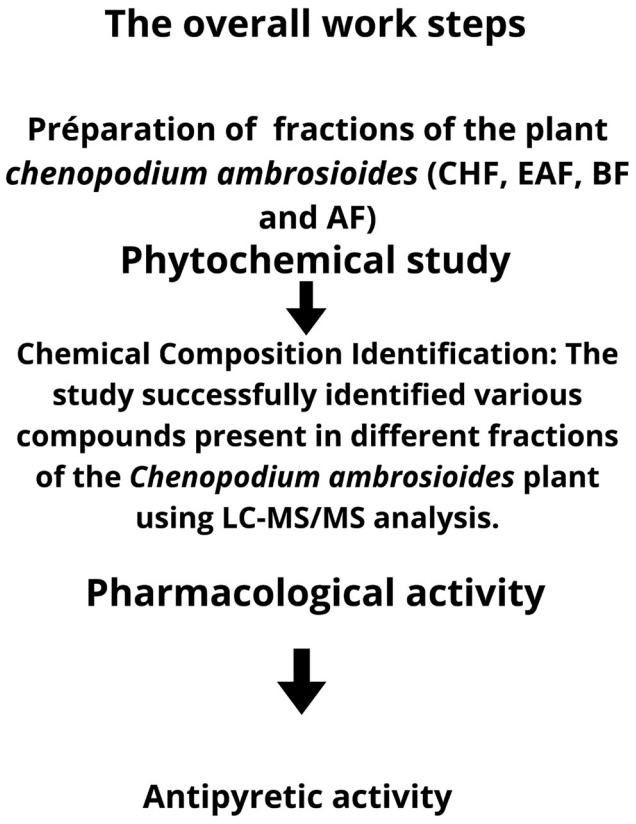
Abstract: This study investigates the chemical composition and potential medicinal properties of different fractions of *Chenopodium ambrosioides* using mass spectrometry. *C. ambrosioides*, commonly known as epazote, has been traditionally used in folk medicine for its purported health benefits. However, there is a lack of comprehensive understanding regarding its bioactive compounds and their physiological effects. Our study aims to fill this gap by analyzing the chemical constituents of three fractions of *C. ambrosioides* – CHF, BF, and AF – and assessing their antioxidant and antipyretic properties. The results reveal



Graphical abstract

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a diverse array of bioactive compounds in each fraction, including protocatechuic acid, vanillin, syringaldehyde, flavonoids, and phenolic acids, which are known for their pharmacological activities. Notably, the CHF fraction exhibits compounds linked to antioxidant effects, suggesting potential therapeutic applications in managing oxidative stress-related disorders. Furthermore, the AF and BF fractions also contain compounds with antioxidant properties, emphasizing the plant's potential health benefits. In addition to chemical analysis, the study evaluates the antipyretic activity of these fractions using a murine model. Significant reductions in rectal temperatures are observed in groups treated with FB and FA fractions, indicating a potential role in modulating body temperature. Conversely, moderate effects are noted in the FCH and ethyl acetate fraction (EAF) groups, suggesting a milder response within safe limits. These findings underscore the importance of further mechanistic studies to elucidate the precise mechanisms underlying the observed effects and ensure the safe utilization of *C. ambrosioides* fractions in medicinal applications. By bridging the gap between traditional knowledge and scientific evidence, this study contributes to advancing our understanding of the therapeutic potential of *C. ambrosioides* and lays the groundwork for future research in this field.

Keywords: *Chenopodium ambrosioides*, fractions, antipyretic activity, phytochemical analysis, LC–MS/MS

1 Introduction

The plant known as *Chenopodium ambrosioides*, commonly called "Mkhinza," is a prevalent annual herb belonging to the Amaranthaceae family. Originally hailing from Central and South America, it has spread extensively throughout tropical and subtropical regions globally, showcasing its remarkable adaptability and resilience. Throughout history, this botanical marvel has held a revered status in traditional medicine, with its leaves and stems serving as potent remedies for a myriad of ailments. From alleviating headaches and abdominal pain to easing joint discomfort and respiratory issues, *C. ambrosioides* has been a stalwart ally in the pursuit of health and wellness for generations. This rich tapestry of traditional usage underscores the plant's cultural significance and underscores its potential as a source of valuable medicinal compounds [1].

Fever, a complex physiological response involving a cascade of intricate mechanisms remains a ubiquitous challenge in medical practice. From infancy to old age, febrile episodes can arise from diverse etiologies, ranging from infectious pathogens to inflammatory conditions. At the heart of this phenomenon lie prostaglandins (PGs), lipid compounds that exert profound effects on thermoregulation and immune function [2]. The synthesis and release of PGs during the immune response play a pivotal role in fever genesis and maintenance, amplifying the body's defenses against invading pathogens. However, unchecked PG production can lead to excessive fever, necessitating pharmacological interventions to restore homeostasis and alleviate discomfort. Thus, understanding the intricate interplay between PGs and fever pathology is crucial for devising effective therapeutic strategies [3].

C. ambrosioides emerges as a captivating subject of pharmacological inquiry, owing to its rich phytochemical composition and potential therapeutic properties. Within its botanical bounty lies a myriad of bioactive compounds, each holding the promise of novel pharmacological applications [4]. This study endeavors to unravel the chemical constituents of *C. ambrosioides* sourced from the Rabat region in Morocco, shedding light on its pharmacological potential, particularly in the context of fever management. By employing a multifaceted approach encompassing chemical analysis and pharmacological assessment, this research aims to bridge the gap between traditional knowledge and scientific inquiry, unlocking the therapeutic treasures hidden within this botanical gem [5].

The exploration of *C. ambrosioides*'s pharmacological potential is not merely an academic pursuit but holds profound implications for public health and medical practice. With the rising prevalence of antibiotic resistance and the limitations of conventional therapies, there is an urgent need to explore alternative sources of therapeutic agents. Natural products, derived from plants and other organisms, have long served as reservoirs of bioactive compounds, offering novel avenues for drug discovery and development. *C. ambrosioides*, with its rich history of traditional usage and its complex chemical profile, presents a tantalizing opportunity for pharmacological exploration. By systematically elucidating its chemical constituents and evaluating its pharmacological effects, this study seeks to unlock the therapeutic potential of *C. ambrosioides*, paving the way for the development of novel fever-reducing agents and antimicrobial therapies [6].

The geographical origin of *C. ambrosioides*, particularly its collection from the Rabat region in Morocco, adds a unique dimension to this study. Environmental factors such as soil composition, climate, and altitude can profoundly influence the chemical composition of plants, leading to variations in their pharmacological properties. By focusing on specimens sourced from a specific geographical region, this study aims to capture the inherent diversity within *C. ambrosioides*'s chemical profile and its

potential implications for pharmacological activity. Moreover, by establishing a connection between traditional usage and scientific inquiry, this research seeks to validate the efficacy of *C. ambrosioides* as a medicinal agent, providing empirical evidence to support its continued use in folk medicine practices [4].

The integration of chemical analysis and pharmacological evaluation represents a synergistic approach to uncovering *C. ambrosioides*'s medicinal potential. Through advanced analytical techniques such as mass spectrometry (LC–MS/MS), researchers can identify and characterize the diverse array of chemical compounds present in *C. ambrosioides*. From polyphenols and flavonoids to terpenoids and alkaloids, each compound holds unique pharmacological properties, contributing to the overall therapeutic profile of the plant. By elucidating the chemical constituents of *C. ambrosioides*, researchers can gain valuable insights into its pharmacological mechanisms of action, laying the foundation for targeted therapeutic interventions [7].

In parallel, pharmacological assessment provides a window into *C. ambrosioides*'s physiological effects, particularly in the context of fever management. By employing animal models and *in vitro* assays, researchers can evaluate the plant's antipyretic properties, elucidating its potential mechanisms of action and therapeutic efficacy. Through meticulous experimentation and data analysis, this research aims to establish a causal relationship between *C. ambrosioides*'s chemical composition and its pharmacological effects, providing a scientific rationale for its traditional usage as a fever-reducing agent.

This comprehensive approach, integrating chemical analysis and pharmacological evaluation, holds immense promise for unlocking the therapeutic potential of *C. ambrosioides*. By elucidating its chemical constituents and evaluating its pharmacological effects, researchers can not only validate its traditional usage but also identify novel therapeutic applications. From fever management to antimicrobial therapy, *C. ambrosioides* offers a treasure trove of pharmacological possibilities, awaiting exploration and exploitation for the betterment of human health [1].

The study of *C. ambrosioides* represents a convergence of traditional knowledge and scientific inquiry, with profound implications for pharmacological research and medical practice. By unraveling its chemical constituents and evaluating its pharmacological effects, researchers can unlock the therapeutic potential of this botanical marvel, paving the way for the development of novel therapeutic agents. From fever management to antimicrobial therapy, *C. ambrosioides* offers many pharmacological possibilities, poised to revolutionize natural product pharmacology. Through interdisciplinary collaboration and rigorous scientific inquiry, we can harness the healing power of *C. ambrosioides* for the betterment of human health and well-being.

2 Materials and methods

2.1 Plant material

The entire plant of *C. ambrosioides* (Chenopodiaceae) was meticulously collected in the Rabat region of Morocco during the period from May to July 2021 (geographical coordinates: 33.970878, −6.814212). To ensure botanical authenticity, rigorous authentication procedures were conducted by the expert floristics team at the esteemed Scientific Institute of Rabat. A representative specimen of this species has been meticulously cataloged and preserved within the herbarium of the Scientific Institute of Rabat, designated with the unique sample identifier RAB113708. Following collection, the harvested plant samples underwent careful drying under ambient conditions within the laboratory premises, preparatory to the subsequent extraction process. The desiccated plant material was then finely powdered utilizing a Binatone Moulinex blender, ensuring optimal consistency for further analytical procedures.

2.2 Fraction preparation

To prepare the fractions, 50 g of the dried aerial part of *C. ambrosioides* underwent a Soxhlet extraction with cyclohexane. Following dry evaporation, the resulting cyclohexane fraction was collected. The residue obtained was then oven-dried for 24 h and subjected to hydroalcoholic maceration using a mixture of ethanol and water (50/50). After filtering the mixture and evaporating the ethanol, successive liquid–liquid separations were performed on the aqueous phase using solvents of increasing polarity (ethyl acetate and *n*-butanol). This process was repeated three times for each solvent (100 mL each time), resulting in ethyl acetate and *n*-butanol fractions after dry evaporation.

The extraction rate was calculated using the following formula:

$$R = (M_i/M) \times 100,$$

where M_i is the mass of the extract and M is the mass of the initial plant material.

2.3 Mass spectrometer and chromatograph conditions

We utilized an ultra-high-performance liquid chromatography (UHPLC) system manufactured by Shimadzu-Nexera, coupled with a tandem mass spectrometer, for the quantitative analysis of 53 phytochemicals. The reverse-phase UHPLC setup included essential components such as an automatic sampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU-20A3R model). To ensure optimal separation and minimize signal suppression, we meticulously fine-tuned the chromatographic conditions. Various columns were evaluated and employed, including the Agilent Poroshell 120 EC-C18 model (150 mm × 2.1 mm, 2.7 µm) and the RP-C18 Inertsil ODS-4 model (100 mm × 2.1 mm, 2 µm). A range of mobile phases, such as acetonitrile and methanol, supplemented with diverse additives including ammonium formate, formic acid, ammonium acetate, and acetic acid, were tested. Column temperatures were systematically adjusted between 25 and 40°C until the optimal conditions for effective separation and detection were achieved.

In the final analysis, chromatographic separation was meticulously executed on an analytical reverse-phase Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 2.7 µm), maintaining the column temperature at a constant 40°C. The eluent gradient consisted of eluent A (water + 5 mM ammonium formate + 0.1% formic acid) and eluent B (methanol + 5 mM ammonium formate + 0.1% formic acid). The elution profile followed a gradient pattern: starting from 20% B and reaching 100% B within 25 min, maintaining 100% B for the subsequent 10 min, and returning to 20% B over the final 10 min. Moreover, the solvent flow rate and injection volume were meticulously set at 0.5 mL/min and 5 µL, respectively. For mass spectrometric detection, a Shimadzu LCMS-8040 tandem mass spectrometer, equipped with an electrospray ionization (ESI) source capable of operating in both negative and positive ionization modes, was employed. LC–ESI-MS/MS data were diligently acquired and processed utilizing the LabSolutions software provided by Shimadzu.

The quantification of phytochemicals was performed using the multiple reaction monitoring mode. This method was precisely calibrated to selectively identify and quantify phytochemical compounds by targeting specific precursor ion-to-fragment ion transitions. Collision energies were meticulously adjusted to ensure optimal fragmentation, maximizing the transmission of the desired product ions. The mass spectrometer's operating parameters were set as follows: a drying gas flow rate (N_2) of 15 L/min, a nebulizing gas flow rate (N_2) of 3 L/min, a desolvation line temperature of 250°C, a heating block temperature of 400°C, and an interface temperature of 350°C.

These conditions, together with the UHPLC system, facilitated the acquisition of high-quality data for the quantitative analysis of the 53 phytochemicals. This precision was crucial for accurately assessing the samples. By carefully adjusting both chromatographic and spectrometric parameters, the method's sensitivity and selectivity were optimized, ensuring the production of reliable and reproducible results.

2.4 Assessment of antipyretic activity

The study of antipyretic activity was conducted following the method outlined by Sawadogo et al. [8]. Hyperthermia was induced by subcutaneous injection at the nape of the mouse with a 20% aqueous suspension of Brewer's yeast at a dose of 10 mL/kg. Mice were selected for the experiment after verifying the stability of their approximate rectal temperature for a week. Subsequently, the mice underwent an 18-h fasting period during which their rectal temperature was measured again using an electronic thermometer. Mice showing an increase in temperature of 0.5°C or more were chosen for further investigation. These selected mice were then administered either plant extracts or a standard treatment, with rectal temperature measurements taken 1 h post-administration and then hourly for 4 h.

2.5 Statistical analysis

The statistical treatment of the data involved the expression of results as mean values accompanied by their respective standard deviations. The analysis was conducted utilizing the one-way analysis of variance method.

3 Results

3.1 Mass spectrometer and chromatograph conditions

The chemical composition of the plant was determined using the LC–MS/MS method. The results of the compounds identified in the plant are presented in Table 1. This table

Table 1: Compounds identified in *C. ambrosioides* extracts and fractions by LC–MS/MS

Compound name	CHF fraction	BF fraction	FA fracton
Fumaric acid	—	0.094	0.139
Aconitic acid	—	0.018	0.025
Protocatechuic acid	0.039	0.477	0.061
4-OH benzoic acid	—	—	—
Caffeic acid	—	0.014	—
p-Coumaric acid	—	0.219	0.037
Salicylic acid	—	0.076	—
Acacetin	0.036	0.03	0.023
Vanillin	0.114	—	
Quercitrin acid	—	—	2.934
Gallic acid	—	0.013	—
Gentisic acid	—	0.147	—
Chlorogenic acid	—	0.206	—
Rutin	—	4.1	—
Isoquercitrin	—	0.182	—
Hesperidin	—	2.1	—
Quercitrin	—	0.039	—
Astragalin	—	0.059	—
Nicotiflorin	—	1.805	—
Quercetin	—	0.127	—
Kaempferol	—	0.019	—
Syringic aldehyde	0.059	—	—

shows the various chemical compounds present in the plant and their respective percentages in the total composition.

LC–MS/MS analysis was employed to identify and quantify compounds present in different fractions of *C. ambrosioides*. The results from this analysis provide valuable insights into bioactive compounds that could contribute to the medicinal properties of the plant.

The CHF fraction revealed the presence of protocatechuic acid, vanillin, syringaldehyde, and acetin. These compounds have been associated with various health benefits, including antioxidant and anti-inflammatory effects. The detection of these compounds in the CHF fraction highlights its potential as a source of bioactive compounds that could undergo further exploration for medicinal applications.

The BF fraction exhibited a diverse range of compounds, including organic acids, flavonoids, and phenolic acids. Rutin, hesperidin, and nicotiflorine, flavonoids with antioxidant properties, were detected in significant quantities, emphasizing the potential health benefits of this fraction. Additionally, quinic acid, gallic acid, and chlorogenic acid were present, contributing to the antioxidant potential of the BF fraction.

It is worth noting that the AF fraction also contained compounds such as quinic acid, fumaric acid, aconitic acid, and various phenolic compounds. This overlap of compounds among the fractions underscores the complexity of *C. ambrosioides* and emphasizes the need for further research to understand the synergistic interactions between these compounds.

In conclusion, LC–MS/MS analysis of fractions from *C. ambrosioides* revealed a rich and diverse chemical composition. The presence of organic acids, phenolic compounds, flavonoids, and fatty acids suggests the potential benefits of this plant for health and culinary applications. These findings lay the groundwork for further research aiming to elucidate the specific bioactive properties and potential therapeutic uses of *C. ambrosioides*. Additionally, the results underscore the importance of exploring natural sources of bioactive compounds that can contribute to human health and well-being.

3.2 Antipyretic activity

Table 2 presents the variations in rectal temperature of mice following the administration of fractions (FCH, FE,

Table 2: Rectal temperature after Brewer's yeast injection of mice treated with *C. ambrosioides* 200 kg/mL fractions (FCH, FE, FB, FA)

	Rectal temperature		Rectal temperature after administration				Total reduction in temperature at the fourth hour
Groupe	Before injection	0 h (18 h after inj)	1 h after gavage	2 h	3 h	4 h	
FCH	37.15 ± 0.07	37.85 ± 0.2	37.5 ± 0.1	37.15 ± 0.7	37 ± 0.6	37.1 ± 0.6	0.75
FE	37.35 ± 0.5	37.85 ± 0.3	37 ± 0.7	37 ± 0.5	36.9 ± 0.5	36.95 ± 0.4	0.9
FB	37.3 ± 0.1	38.1 ± 0.2	37.5 ± 0.1	37.15 ± 0.7	37 ± 0.6	37.01 ± 0.6	1.09
FA	37.32 ± 0.2	38.05 ± 0.26	37.46 ± 0.2	36.96 ± 0.56	37.2 ± 0.6	36.95 ± 0.6	1.1
Control	37 ± 0.1	37.6 ± 0.2	37.55 ± 0.2	37.4 ± 0.2	37.4 ± 0.2	37.45 ± 0.1	0.15
Standard	37.4 ± 0.15	38.1 ± 0.14	37.9 ± 0.14	37.5 ± 0.14	37.3 ± 0.15	37.3 ± 0.15	0.78

Each value is expressed as mean ± standard error of the mean.

FB, FA) of *C. ambrosioides* at a dose of 200 mg/mL. These data provide crucial insights into the thermal response of mice to these treatments, which is an important indicator of the impact of these substances on the organism.

The table reveals several noteworthy points. Initially, before the injection of fractions, the rectal temperature of the mice was relatively stable, ranging between 37.15 and 37.38°C across the different groups. This initial stability is crucial in establishing a baseline reference.

However, of significant interest is the evolution of rectal temperature over subsequent hours. As time progresses, there is a trend towards restoration of temperature across all groups. By the fourth hour, the rectal temperature decreased compared to its initial value in most groups, indicating a gradual adaptation to the initial impact.

The reduction in rectal temperature at the fourth hour is a critical metric for evaluating the impact of the fractions. It is particularly pronounced in groups FB and FA, with reductions of 1.09 and 1.1°C, respectively. These values indicate that these fractions induced significant thermal responses in the mice, suggesting a potential effect on body temperature regulation.

In contrast, groups FCH and FE show slightly less than a 1°C reduction in rectal temperature, suggesting a moderate effect. Importantly, these reductions in temperature remain within limits that do not appear harmful to the mice.

In comparison, the control group exhibited the smallest reduction in rectal temperature at the fourth hour, with only 0.15°C. This indicates that the mice in the control group did not experience a significant impact on their body temperature during the study.

These results highlight the variation in the thermal response of mice to fractions of *C. ambrosioides*. The observed temperature reductions in several groups suggest that these substances could affect the regulation of the mice's body temperature. However, it is essential to note that these effects vary depending on the tested fraction, and further studies are necessary to fully understand their mechanisms of action and their impact on animal health.

Table 3 displays the variations in rectal temperature among mice treated with fractions (FCH, FE, FB, FA) of *C. ambrosioides*, administered at a dose of 500 mg/mL. These data are crucial for evaluating the impact of these substances on the regulation of mice's body temperature.

Initially, before the injection of these extracts and fractions, the rectal temperature of mice in all groups was relatively stable, with initial values ranging between 36.8 and 37.4°C. This stability is essential in establishing a baseline reference.

However, what is particularly interesting is the evolution of rectal temperature over subsequent hours. As time passes, there is a trend toward normalization of temperature in most groups. By the fourth hour, the rectal temperature decreased compared to the initial hour in all groups, suggesting a gradual adaptation to the initial impact.

The reduction in rectal temperature at the fourth hour is a critical parameter for assessing the impact of the fractions. It is particularly pronounced in the FE, FB, and FA groups, with reductions of 1.2, 1.27, and 1.17°C, respectively. These values indicate that these fractions induced significant thermal responses in the mice, showing a potential effect on body temperature regulation.

Both the FCH groups and the control group show rectal temperature reductions slightly less than 1°C, suggesting a moderate effect. It is important to note that these reductions in temperature remain within limits that do not seem to endanger the health of the mice.

These results clearly highlight the variation in mice's thermal responses to the administration of fractions of *C. ambrosioides*. The reductions in rectal temperature observed in several groups suggest that these substances can affect the regulation of mice's body temperature. It is crucial to note

Table 3: Rectal temperature after Brewer's yeast injection in mice treated with the 500 kg/mL *C. ambrosioides* fractions (FCH, FE, FB, FA)

	Rectal temperature		**Rectal temperature after administration**				**Total reduction in temperature at the fourth hour**
Groupe	Before injection	0 h (18 h after inj)	1 h after gavage	2 h	3 h	4 h	
FCH	36.8 ± 0.3	37.63 ± 0.2	37.4 ± 0.2	36.93 ± 0.1	36.8 ± 0.1	36.85 ± 0.2	0.78
FE	37.32 ± 0.4	37.75 ± 0.3	37.05 ± 0.2	37 ± 0.1	36.45 ± 0.2	36.55 ± 0.2	1.2
FB	37.34 ± 0.2	38.32 ± 0.2	37.5 ± 0.5	37.6 ± 0.2	37.35 ± 0.2	37.05 ± 0.1	1.27
FA	37.24 ± 0.1	38.27 ± 0.1	37.9 ± 0.4	37.65 ± 0.3	37.4 ± 0.4	37.1 ± 0.6	1.17
Control	37 ± 0.1	37.6 ± 0.2	37.55 ± 0.2	37.4 ± 0.2	37.4 ± 0.2	36.45 ± 0.1	0.15
Standard	37.4 ± 0.15	38.1 ± 0.14	37.9 ± 0.14	37.5 ± 0.14	37.3 ± 0.15	37.3 ± 0.15	0.78

Each value is expressed as mean ± standard error of the mean.

that these effects vary depending on the tested fraction, and further in-depth studies are necessary to fully comprehend the underlying mechanisms of these responses.

4 Discussion

The use of LC–MS/MS analysis has provided a comprehensive understanding of the chemical composition of different fractions of *C. ambrosioides*. This analysis has revealed a rich assortment of bioactive compounds with the potential to contribute to the medicinal properties of the plant.

The CHF fraction demonstrated the presence of several noteworthy compounds, including protocatechuic acid, vanillin, syringaldehyde, and acetin. These compounds have been associated with various health benefits, particularly antioxidant and anti-inflammatory effects [9].

The detection of these compounds in the CHF fraction underscores its potential as a source of bioactive compounds worthy of further exploration for medicinal applications.

Similarly, the BF fraction exhibited a diverse range of compounds, including organic acids, flavonoids, and phenolic acids. Notably, significant quantities of rutin, hesperidin, and nicotiflorine were detected, all of which are flavonoids known for their antioxidant properties.

Additionally, the presence of quinic acid, gallic acid, and chlorogenic acid contributes further to the antioxidant potential of the BF fraction [10].

The FA fraction also contained notable compounds such as quinic acid, fumaric acid, aconitic acid, and various phenolic compounds. The overlap of compounds among the fractions underscores the complexity of *C. ambrosioides* and highlights the need for further research to understand the synergistic interactions between these compounds.

The LC–MS/MS analysis of fractions from *C. ambrosioides* has revealed a rich and diverse chemical composition. The presence of organic acids, phenolic compounds, flavonoids, and fatty acids suggests the potential health benefits of this plant for various applications [10].

These findings lay the groundwork for further research aiming to elucidate the specific bioactive properties and potential therapeutic uses of *C. ambrosioides*. Additionally, the results underscore the importance of exploring natural sources of bioactive compounds that can contribute to human health and well-being.

The antipyretic activity of *C. ambrosioides* was evaluated by measuring its impact on the body temperature of feverish mice. The results indicate that most of the tested No extracts just fraction caused a slight reduction in the body temperature of the mice compared to the control group.

Comparing the results of the two administered doses, namely 200 and 500 mg/mL of extracts and fractions of *C. ambrosioides*, to assess the concentration's impact on antipyretic activity. At the dose of 200 mg/mL, the data reveals a trend toward normalization of body temperature over time. The rectal temperature significantly decreases at the fourth hour in several groups, suggesting a potential antipyretic effect of these substances.

The reductions in rectal temperature observed at 200 mg/mL indicate a notable thermal response in mice. The EM, FB, FA, and EI groups showed temperature reductions of 1.3, 1.09, 1.1, and 0.78°C, respectively, indicating that these extracts have a significant impact on body temperature regulation. However, the FCH and FE groups exhibited slightly less than 1°C reductions in temperature, suggesting a moderate antipyretic effect. It is essential to note that these temperature reductions remain within limits that do not seem to endanger the health of the mice.

In comparison, at the dose of 500 mg/mL, the reductions in rectal temperature are more pronounced and widespread at the fourth hour. The EI, EM, FE, FB, and FA groups showed temperature reductions of 1, 1.3, 1.2, 1.27, and 1.17°C, respectively. These values indicate a more intense thermal response at these higher concentrations, suggesting a more pronounced antipyretic action.

The data clearly suggest a dose dependency in the antipyretic effect of extracts of *C. ambrosioides*. Higher concentrations appear to have a more significant impact on the regulation of mice's body temperature. However, it is crucial to note that these effects also depend on the tested extract or fraction, emphasizing the complexity of the thermal response.

Pain often accompanies fever, underscoring the importance of assessing antipyretic effects. The induction of fever (hyperthermia) via Brewer's yeast injection is associated with cytokine release (such as TNFα, IL1β, and IL6) that stimulates PG (PGE2) biosynthesis around the hypothalamic thermoregulatory center [11,12]. The aqueous extract derived from *C. ambrosioides* leaves significantly attenuated fever (hyperthermia) two hours after its onset ($p < 0.05$, $p < 0.01$, and $p < 0.001$) [13]. This outcome suggests an antipyretic effect akin to the mechanism of PG synthesis interference, akin to the reference molecule paracetamol. Our findings echo previous studies [14] demonstrating antipyretic effects of the aqueous *C. ambrosioides* leaf extract in mice.

According to Hallal et al. [15], the aqueous extract of *C. ambrosioides* showed a significant degree of antipyretic activity at doses of 500 and 800 mg/kg. The reduction in hyperthermia was pronounced 60 min after administration and lasted for three hours. As per the findings of Lohdip et al. [16], hexadecenoic acid demonstrated antipyretic activity. Notably, it not only exhibited antipyretic

effects but also displayed hypothermic activity, with temperatures dropping below those recorded prior to yeast administration even three hours post-administration [17]. This suggests that hexadecenoic acid maintains its antipyretic properties for a duration of at least three hours after administration.

These results present interesting prospects for future research on the antipyretic activity of *C. ambrosioides*. Further studies are necessary to understand the underlying mechanisms of these thermal responses and to evaluate the safety and potential efficacy of these extracts at different concentrations. Moreover, identifying the specific compounds responsible for antipyretic activity and their mechanism of action would be a significant step toward the potential clinical use of these extracts in fever treatment. In summary, this research provides valuable insights into the influence of concentration on the antipyretic activity of *C. ambrosioides* and paves the way for future investigations.

5 Conclusion

The utilization of LC–MS/MS has yielded a comprehensive understanding of the chemical composition of different fractions of *C. ambrosioides*, revealing a rich assortment of bioactive compounds with potential medicinal benefits.

Analysis of the CHF, BF, and AF fractions unveiled a diverse array of compounds, including antioxidants, agents, organic acids, flavonoids, and phenolic acids. These findings underscore the broad therapeutic potential of *C. ambrosioides* across medicinal and culinary domains.

Furthermore, examination of antipyretic activity in mice following the administration of various fractions yielded intriguing results. Significant reductions in rectal temperature were observed after treatment, indicating potential effects on body temperature regulation. Notably, fractions such as FB and FA exhibited pronounced thermal responses at both 200 and 500 mg/mL doses, suggesting their capacity to modulate body temperature.

However, variations in thermal response intensity were noted among different groups, with some exhibiting moderate reductions and others showing negligible impact compared to the control group.

Chemical analysis has unveiled a diverse range of bioactive compounds in *C. ambrosioides* fractions, hinting at promising medicinal applications. The observed thermal responses in mice underscore the potential impact of these fractions on body temperature regulation, highlighting avenues for further investigation into their specific mechanisms and therapeutic applications.

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