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Identificação e Avaliação de Metabólitos Secundários em Extratos de *Acmella oleracea* (L.) R.K. Jansen (Asteraceae) Utilizando ESI-MS

Identification and Evaluation of Secondary Metabolites in *Acmella oleracea* (L.) R.K. Jansen (Asteraceae) Extracts Using ESI-MS

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RESUMO

O presente estudo teve como objetivo avaliar a atividade antioxidante e caracterizar os constituintes químicos do extrato hidroalcoólico da planta *Acmella oleracea* (jambu), com foco nas diferentes partes da planta. A análise foi conduzida por espectrometria de massas com ionização por eletrospray (ESI-MS), utilizando modos positivo e negativo, e pela técnica de sequestro de radicais DPPH para mensuração da atividade antioxidante. Os resultados revelaram que o extrato do caule apresentou a maior capacidade antioxidante entre as partes analisadas, com valores de EC_{50} significativamente menores (0,03 µg/mL) do que o padrão utilizado (ácido gálico – 3,3 µg/mL), indicando forte eficácia na neutralização de radicais livres. A atividade antioxidante decresceu proporcionalmente à concentração, com o caule mantendo a melhor performance em todas as faixas testadas. A espectrometria de massas identificou uma ampla diversidade de metabólitos secundários. Em modo positivo, destacaram-se flavonoides glicosilados, como kaempferol 3-rutinosídeo-7-glicosídeo (*m/z* 753), kaempferol 3-rutinosídeo-7-arabinose (*m/z* 711), kaempferol 3-metoxi-7-O-glicosídeo (*m/z* 475), e kaempferol 3-O-arabinose-7-metoxi (*m/z* 433), além de kaempferol livre (*m/z* 287). O íon *m/z* 391 foi atribuído a derivados de alquilamidas ou terpenoides oxigenados. No modo negativo, foram detectados compostos altamente polares, como taninos hidrolisáveis (*m/z* 787), ácidos fenólicos conjugados (*m/z* 577), flavonoides glicosilados (*m/z* 751), derivados do ácido elágico (*m/z* 709) e triterpenoides modificados (*m/z* 563), todos conhecidos por suas propriedades bioativas. Esses achados reforçam o potencial farmacológico do jambu como fonte natural de antioxidantes, com aplicações promissoras nas indústrias farmacêutica, cosmética e nutracêutica.

Palavras-chave: *Acmella oleracea*; Antioxidantes naturais; Espectrometria de massas; Flavonoides glicosilados.

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ABSTRACT

This study aimed to evaluate the antioxidant activity and characterize the chemical constituents of the hydroalcoholic extract of *Acmella oleracea* (jambu), with a focus on the different parts of the plant. The analysis was conducted using electrospray ionization mass spectrometry (ESI-MS) in both positive and negative ionization modes, along with the DPPH free radical scavenging assay to assess antioxidant activity. The results showed that the stem extract exhibited the highest antioxidant capacity among the parts analyzed, with significantly lower EC_{50} values (0.03 µg/mL) compared to the standard (gallic acid – 3.3 µg/mL), indicating strong efficacy in neutralizing free radicals. Antioxidant activity decreased proportionally with concentration, with the stem maintaining superior performance across all tested levels. Mass spectrometry revealed a wide diversity of secondary metabolites. In positive ion mode, glycosylated flavonoids were predominant, including kaempferol 3-rutinoside-7-glucoside (*m/z* 753), kaempferol 3-rutinoside-7-arabinose (*m/z* 711), kaempferol 3-methoxy-7-O-glucoside (*m/z* 475), and kaempferol 3-O-arabinose-7-methoxy (*m/z* 433), in addition to free kaempferol (*m/z* 287). The ion at *m/z* 391 was attributed to derivatives of alkylamides or oxygenated terpenoids. In negative mode, highly polar compounds were detected, such as hydrolyzable tannins (*m/z* 787), conjugated phenolic acids (*m/z* 577), glycosylated flavonoids (*m/z* 751), ellagic acid derivatives (*m/z* 709), and modified triterpenoids (*m/z* 563), all known for their bioactive properties. These findings reinforce the pharmacological potential of jambu as a natural source of antioxidants, with promising applications in the pharmaceutical, cosmetic, and nutraceutical industries.

Keywords: *Acmella oleracea*; Natural antioxidants; Mass spectrometry; Glycosylated flavonoids.

1. INTRODUCTION

A. oleracea (L.) R.K. Jansen, commonly known as jambus, Brazil cress, or buttercup, is a herbaceous plant of the Asteraceae family, widely distributed across tropical regions of South America, Asia, and Africa. In Brazil, it holds significant cultural, economic, and medicinal value, particularly in the Amazon region, where its aerial parts—leaves, stems, and inflorescences—are traditionally used in both cuisine and folk medicine (Gilbert; Alves; Favoreto, 2022; Jerônimo *et al*., 2024).

This species exhibits a terrestrial growth habit, with stems ranging from decumbent to erect, and petiolate leaves with ovate to deltate blades, toothed margins, and an acute apex. The capitula are discoid and pedunculate, bearing yellow flowers with a pentamerous corolla and 15 to 18 herbaceous bracts—features characteristic of the species (Nakajima, 2020). The plant typically reaches heights of 30 to 40 cm, thriving in warm and humid environments.

Traditionally, *A. oleracea* is used to treat various ailments, including toothache, oral conditions, fever, anemia, hepatitis, malaria, and throat infections (Jerônimo *et al*., 2024). Its culinary application is linked to its distinctive flavor and sensory effect—a tingling and numbing sensation on the lips and tongue—caused

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by the presence of N-alkylamides, particularly spilanthol, its primary bioactive compound (Choudhary *et al*., 2021; Kalpoutzakis *et al*., 2023).

In recent years, *A. oleracea* has garnered growing interest from the international pharmaceutical and cosmetic industries—particularly in Brazil, the United States, and Japan—due to the broad spectrum of biological activities attributed to its major compound, spilanthol. Reported effects include anti-inflammatory, anesthetic, analgesic, antioxidant, anti-aging, neuroprotective, antimicrobial, antitumor, larvicidal, and wound-healing properties (da Silva *et al*., 2023; Jerônimo *et al*., 2024).

Extracts of *A. oleracea* have demonstrated strong antioxidant and antibacterial activities, along with notable efficacy against *Herpes simplex* virus and oral pathogens, supporting its potential use in oral and topical therapeutic formulations (Pinheiro *et al*., 2020; Weintraub *et al.*, 2020). Phytochemical studies of the species have identified a range of secondary metabolites, including flavonoids, tannins, terpenoids, sterols, saponins, and amino acids, all of which contribute to its diverse and potent bioactivity (Kalpoutzakis *et al*., 2023; Salih *et al*., 2021).

Given this wide array of bioactive properties and the increasing scientific and industrial interest, *A. oleracea* is emerging as a promising source of natural compounds for pharmaceutical, cosmetic, and nutraceutical applications. This study aims to evaluate its antioxidant activity and characterize the chemical profile of its bioactive extracts, thereby contributing to the growing understanding of its potential as a multifunctional natural ingredient.

2. MATERIAL AND METHODS

2.1 Collection and obtaining of plant material

Plant material of *A. oleracea* was obtained from a local grower in the municipality of Manaus, Amazonas, Brazil. The samples were transported to the Biorganics Laboratory at the State University of Amazonas (UEA), where the plant's anatomical parts (leaves, stems, roots, and inflorescences) were manually separated and dried in a forced-air oven at a controlled temperature.

Once fully dehydrated, the dried materials were individually weighed using a high-precision analytical balance, and their respective masses were recorded for

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subsequent extract preparation. Extraction was performed by static maceration using absolute ethanol and an appropriate plant-to-solvent ratio, according to the methodology adapted from Romão *et al.* (2015). After the extraction period, the material was filtered, and the solvent was removed using a rotary evaporator under reduced pressure, resulting in the dry crude extract.

For antioxidant activity assays and chemical analyses by mass spectrometry, 2 mg aliquots of each extract were accurately weighed and solubilized according to the specifications of each analytical protocol.

2.2 Antioxidant Assays and Mass Spectrometric Characterization

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, following the protocol established by Rufino *et al*. (2007). This colorimetric method is based on the absorbance changes resulting from the neutralization of DPPH radicals and is widely employed due to its simplicity, sensitivity, and reproducibility in assessing the antioxidant capacity of natural products.

Chemical profiling was carried out using mass spectrometry on a TSQ Quantum Access™ system (Thermo Scientific) equipped with an electrospray ionization (ESI) source. Samples were analyzed in both positive and negative ionization modes to maximize the detection of metabolites with diverse polarity and structural characteristics. This dual-mode approach allowed for a more comprehensive characterization of the chemical constituents present in the bioactive extracts.

3. RESULTS AND DISCUSSIONS

3.1 Evaluation of Free Radical Scavenging Capacity and EC_{50} Determination

Table 1 presents the results of the antioxidant activity evaluation of aqueous extracts from different parts of *A. oleracea*, tested at decreasing concentrations. The analysis revealed variations in antioxidant capacity among plant organs, reflecting differences in the content and composition of bioactive metabolites.

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Table 1: Antioxidant activity (AA%) of aqueous extracts of different parts of *A. oleracea* in decreasing order.

Concentration (µg/ mL)	AA% - Stem	AA% - Inflorescence	AA% - Leaves	AA% - Root
1000	76.33	53.20	50.12	66.33
500	72.23	51.20	45.24	45.78
250	57.78	43.68	35.72	40.85
125	65.89	39.29	33,33	40.65
62.5	60.03	34.65	26.50	41.48
31.25	53.68	32.36	19.28	38.41
15.6	48.02	31.82	12.01	35.82
7.81	42.80	30.94	4.88	34.11

Antioxidant activity. Gallic acid control AA% = 95.2.

The aqueous extract obtained from the stem of *A. oleracea* exhibited the highest antioxidant activity at the highest concentration tested, reaching 76.33% at 1000 µg/mL. This was followed by the extracts from the roots (66.33%), inflorescences (53.20%), and leaves (50.12%). As the concentrations decreased, a downward trend in antioxidant activity was observed across all plant parts, with the most pronounced reduction occurring in the leaves, which showed the lowest activity at the lowest concentrations. In contrast, the stem extract consistently maintained the highest antioxidant percentages, even at lower concentrations. Gallic acid, used as a positive control, demonstrated a stable antioxidant activity of 95.2%, serving as a benchmark for comparison with the plant extracts.

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These findings are consistent with previous studies reporting variations in the antioxidant activity of *A. oleracea* depending on the plant part analyzed and the solvent employed. Romão *et al*. (2015) observed that flowers and leaves exhibited moderate antioxidant activity in ethanolic extracts, with flowers showing the strongest effect. In the present study, the use of aqueous extraction may have contributed to differences in the profile of bioactive metabolites, as more polar phenolic compounds tend to be better solubilized in water.

Peretti Jr. (2021) also reported greater antioxidant capacity in hydroalcoholic extracts obtained from the stem and leaves, suggesting that the stem contains higher concentrations of spilanthol, flavonoids, and other natural antioxidants. This supports the observation that this plant part was more effective in neutralizing the DPPH radical.

Gomes *et al*. (2022) further support this trend by demonstrating that aqueous extracts of leaves and flowers exhibit lower antioxidant activity compared to those extracted with ethanol or ethyl acetate. Therefore, in addition to the plant part used, the extraction method plays a crucial role in determining the antioxidant efficiency observed.

The overall decrease in antioxidant activity across all plant parts with decreasing concentrations is consistent with previous literature and represents a typical dose-dependent behavior in DPPH assays (Rufino *et al*., 2007). This dose-response pattern strengthens the reliability and reproducibility of the experimental data.

Gallic acid, used as a positive control, consistently exhibited antioxidant activity of 95.2%, a value in line with that reported by Borges (2009) for this standard compound, confirming the adequacy of the experimental methodology employed.

Altogether, the results not only confirm the strong antioxidant potential of *A. oleracea*, but also indicate that the stem may represent the most promising plant part for pharmacological and nutraceutical applications—particularly when subjected to optimized extraction techniques.

The analysis of antioxidant responses at varying concentrations allowed us to define the linear activity range for each extract, which enabled the calculation of

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EC_{50} values (the effective concentration required to reduce DPPH radicals by 50%). These values for each plant part are presented in Table 2, allowing for direct comparison of extract potency.

Table 2. EC_{50} values of aqueous extracts from different parts of *A. oleracea* and gallic acid.

Sample	EC 50 µg/ mL
Stem	0.03
Inflorescence	0.28
Leaves	0.61
Root	0.50
Gallic acid	3.3

The EC_{50} results indicate that the aqueous extract from the stem of *A. oleracea* exhibited the highest antioxidant efficiency, showing the lowest EC_{50} value (0.03 µg/mL), followed by the extracts from the inflorescence (0.28 µg/mL), root (0.50 µg/mL), and leaves (0.61 µg/mL). These values confirm the superior antioxidant performance of the stem compared to other plant parts (Sudevan *et al*., 2015).

Remarkably, all tested extracts demonstrated lower EC_{50} values than gallic acid (3.3 µg/mL), which was used as the positive control. This finding underscores the potent antioxidant potential of compounds present in *A. oleracea*, suggesting it could be an even more effective natural source of antioxidants than some commonly used synthetic standards.

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Despite these promising results, it is important to note that antioxidant activity in this study was assessed solely using the DPPH assay. Therefore, further studies involving additional methods—such as FRAP, ABTS, and ORAC assays, along with in vivo models—will be necessary to validate and expand our understanding of the species' antioxidant efficacy under different biological and experimental conditions.

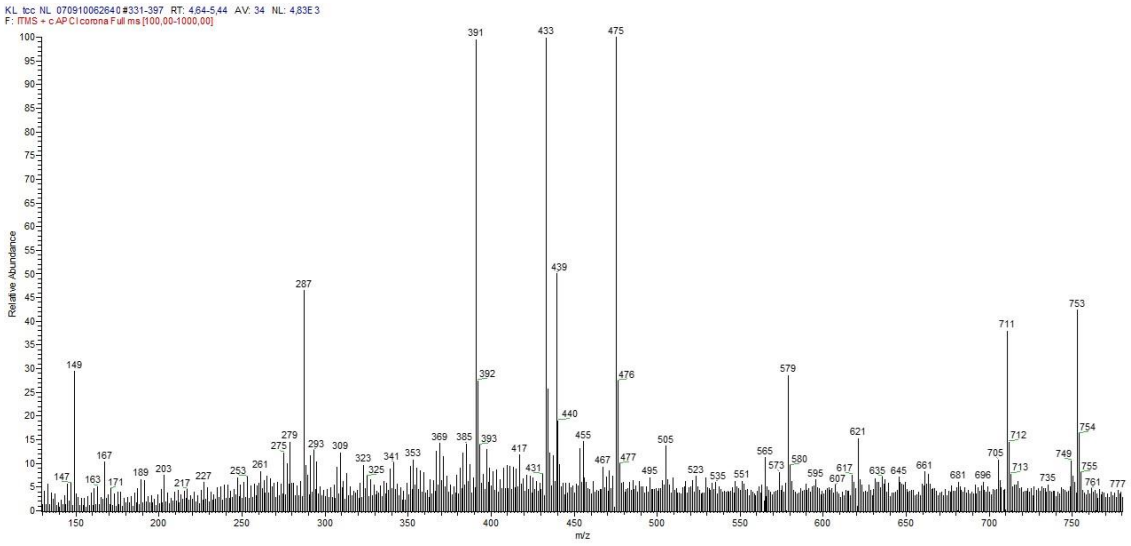
Lower EC_{50} values are directly associated with stronger antioxidant potency, indicating that smaller concentrations are required to achieve 50% DPPH radical inhibition. The fact that all plant extracts outperformed gallic acid suggests the presence of highly reactive antioxidant compounds—potentially flavonoids, N-alkylamides, and terpenoids—as previously reported in studies on *A. oleracea* (Gomes *et al*., 2022; Rodrigues, 2021).

3.2 Characterization of Chemical Constituents by Mass Spectrometry

The mass spectra of *A. oleracea* stem extracts acquired in positive and negative ionization modes are shown in Figures 1 and 2, respectively. These spectra display the distribution and intensity of the detected signals, reflecting the chemical diversity of compounds present in the stem samples.

For this analysis, atmospheric pressure chemical ionization (APCI) was employed, with data acquired in full-scan mode. This method allowed for broad-spectrum metabolite detection in both ionization polarities, facilitating the identification of compounds with varying chemical properties—particularly important when analyzing complex plant matrices, as noted by Meurer (2020).

Figure 1. Full-scan mass spectrum in positive ionization mode of the hydroalcoholic extract from *A. oleracea* stems.



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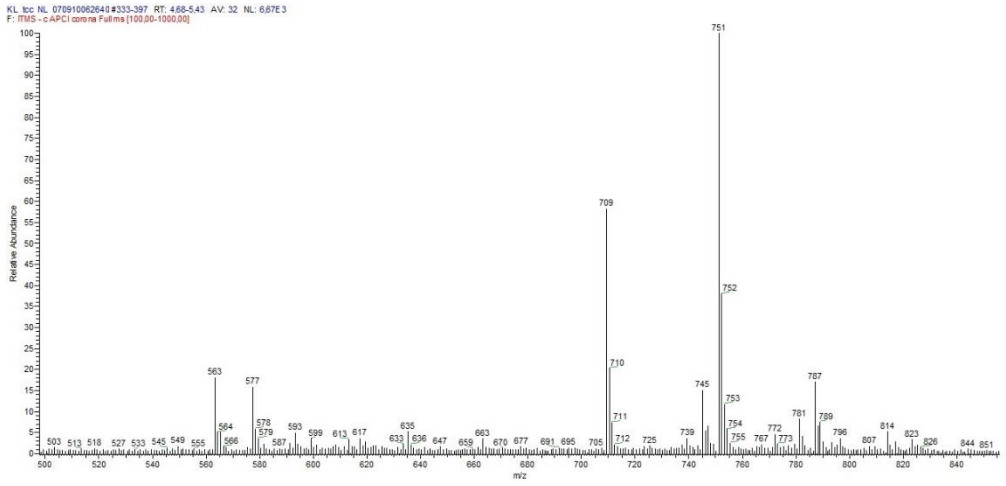
Source: Author (2025).

The signals observed in Figure 1 include pseudomolecular ions $[M+H]^+$ at *m/z* 753, 711, and 579, suggesting the presence of high molecular weight bioactive compounds, likely associated with complex structures such as glycosylated flavonoids, terpenoids, or long-chain *N*-alkylamides. The most intense peaks in the spectrum were observed at *m/z* 475, 433, and 391, indicating a predominance of metabolites with strong affinity for positive ionization mode—possibly reflecting their structural stability and higher concentration in the extract. Additionally, lower-intensity peaks such as *m/z* 287 and 149 were also detected, which may correspond to fragmentation products or smaller molecular weight constituents.

Figure 2. Full-scan mass spectrum in negative ionization mode of the hydroalcoholic extract from *A. oleracea* stems.



Source: Author (2025).

Figure 2 displays the spectral profile obtained in negative ionization mode using APCI. Among the detected signals, the ion at *m/z* 787 is particularly notable, while the most intense peaks were recorded at *m/z* 751 and 709, suggesting a predominance of compounds with higher affinity for negative ionization. Additionally, lower-intensity ions at *m/z* 577 and 563 were also detected, indicating the presence

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of less abundant metabolites that nonetheless contribute to the chemical diversity of the extract.

By analyzing the spectra obtained in both positive and negative ionization modes, it was possible to infer the predominant classes of compounds present in the extract. In positive mode, the detection of $[M+H]^+$ ions at *m/z* 753, 711, and 579, along with intense peaks at *m/z* 475, 433, and 391, suggests the presence of relatively non-polar compounds, including *N*-alkylamides, methoxylated flavonoids, and neutral terpenoids. These classes are commonly associated with pharmacological effects such as local anesthetic, anti-inflammatory, and antioxidant activity (Jerônimo *et al*., 2024; Kalpoutzakis *et al*., 2023).

Conversely, the ions detected in negative mode—such as those at *m/z* 787, 751, and 709, along with lower-intensity peaks at *m/z* 577 and 563—indicate the presence of phenolic acids, acidic triterpenoids, and other polar compounds with acidic functionalities. These metabolites are frequently linked to antioxidant, hepatoprotective, and immunomodulatory properties (Meurer, 2020; Salih et al., 2021; Renai *et al*., 2021).

The use of atmospheric pressure chemical ionization (APCI) mass spectrometry in both positive and negative ion modes enabled a broad and complementary characterization of the chemical constituents of the A. oleracea stem extract. The results obtained reinforce the species' potential as a rich source of bioactive metabolites, consistent with recent findings on phytocomplexes with significant therapeutic potential.

In light of the high-intensity signals observed and their pharmacological relevance, structural characterization of the major compounds was further pursued through fragmentation analysis using tandem mass spectrometry (MS/MS).

3.3 Compound Identification by MS/MS

The compounds described in this section are illustrated in Figure 1. In the mass spectrometry results obtained in positive ionization mode, the ion detected at *m/z* 753 was tentatively identified as kaempferol 3-rutinoside-7-glucoside, a flavonoid commonly reported in metabolomic analyses of medicinal plants.

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This compound consists of a kaempferol backbone (*molecular weight: 286 Da*), conjugated with two sugar moieties: a rutinoside (*308 Da*) at position 3, and a glucose (*162 Da*) at position 7. The combined molecular mass is approximately 756 Da in its neutral state. Its protonated form $[M+H]^+$ appears at *m/z* 753, which is consistent with the observed spectral data and expected neutral losses during ionization.

Recent studies support this identification. Mi *et al*. (2021), for example, used LC-MS/MS to characterize glycosylated flavonoids similar to those found here, including kaempferol derivatives bearing multiple sugar moieties. Their work confirmed fragmentation patterns and mass spectra consistent with the current findings.

Previous investigations have also reported the presence of this type of glycosylated flavonoid in various plant species. Harborne and Williams (1984) noted that the conjugation of glucose and rutinoside units to flavonoids like kaempferol is a common feature in plants with medicinal value. Additionally, Snook *et al*. (1992) provided a detailed description of the mass spectrometric fragmentation of kaempferol derivatives, highlighting the characteristic neutral losses of −162 Da (glucose) and −308 Da (rutinoside), which align with the fragmentation behavior observed in this study (Ruiz *et al*., 2010; Pop *et al*., 2013).

The ion observed at *m/z* 711 in the positive ion mode was tentatively assigned to kaempferol 3-rutinoside-7-arabinose, another glycosylated flavonoid frequently reported in plant extracts. This proposed identification is based on the compound's structural features and is supported by existing literature, which highlights such conjugated flavonoids as key constituents in the metabolomic profiles of various medicinal plants.

The ion observed at *m/z* 711 in the positive-mode mass spectrum was attributed to kaempferol 3-rutinoside-7-arabinose, a glycosylated flavonoid commonly reported in plant extracts. This identification is supported by the compound's structural features and corroborated by scientific literature, which recognizes conjugated flavonoids as key constituents in the chemical profiles of numerous plant species.

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Kaempferol (molecular mass: 286 Da) is a flavonol widely distributed in nature and known for its high propensity to form glycosidic bonds at specific positions, particularly C-3 and C-7. In this case, the presence of a rutinoside moiety (308 Da) at position 3 and an arabinose unit (150 Da) at position 7 yields a total neutral molecular mass of 744 Da. The detection of the $[M+H]^+$ ion at *m/z* 711 is consistent with this structure, considering the expected mass losses during ionization and potential fragmentation.

Previous studies support this structural assignment. Harborne and Williams (1984) described similar glycosylated kaempferol derivatives with multiple sugar attachments. Snook *et al*. (1992) also reported typical fragmentation behaviors for such compounds, including neutral losses of 150 Da (arabinose) and 308 Da (rutinoside), in line with the observed spectrometric data. Additionally, Pop *et al*. (2013) identified kaempferol conjugates with disaccharides and pentoses using mass spectrometry, further reinforcing the plausibility of this proposed structure.

The ion observed at *m/z* 475 in the positive-mode spectrum was attributed to kaempferol 3-methoxy-7-O-glucoside, a glycosylated flavonoid featuring both methoxylation and glycosylation modifications on the kaempferol backbone. This structural proposal is supported by the well-established chemical behavior of kaempferol, which frequently undergoes such modifications in various plant species.

The kaempferol aglycone (molecular mass: 286 Da) possesses hydroxyl groups at key positions that serve as potential sites for substitution. In this case, methylation of the hydroxyl group at position 3 by a methoxy group ($–OCH_3$, +15 Da) increases the molecular mass to 301 Da. The subsequent glycosylation at position 7 with a glucose unit (+162 Da) results in a total neutral molecular weight of 463 Da. In positive-mode ESI-MS, this molecule appears as the $[M+H]^+$ ion at *m/z* 475, which matches the experimental data observed.

The scientific literature provides strong support for this assignment. Harborne and Williams (1984) documented the widespread occurrence of methoxylated flavonoids in medicinal plants. Snook *et al*. (1992) also reported typical fragmentation patterns of methoxylated and glycosylated flavonoids, including neutral losses of 162 Da (glucose) and 15 Da (methoxy group), which are consistent with the fragmentation behavior expected for this compound. The detection of

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kaempferol 3-methoxy-7-O-glucoside in A. oleracea extracts aligns with the known metabolic diversity of the Asteraceae family and further supports the compound's identification.

The ion at *m/z* 433 detected in the positive-mode mass spectrum was attributed to kaempferol 3-O-arabinose-7-methoxy, a glycosylated and methoxylated flavonoid frequently reported in the scientific literature for its structural features and biological activity. This assignment is supported by the compound's molecular mass, typical substitution patterns of natural flavonoids, and previously documented fragmentation behavior.

The core structure is kaempferol (molecular weight: 286 Da), a flavonol commonly found in plant species and known for its capacity to form stable conjugates with sugars and functional groups. Methoxylation at position 7 introduces a methoxy group ($–OCH_3$, +15 Da), increasing the molecular mass to 301 Da. Glycosylation at position 3 with an arabinose moiety (+150 Da) brings the total neutral mass to 451 Da. In the positive ESI-MS spectrum, the ion appears at *m/z* 433, consistent with a $[M+H]^+$ ion that has undergone dehydration (−18 Da) during the ionization process.

This structural hypothesis is well supported in the literature. Harborne and Williams (1984) described the frequent occurrence of flavonoids glycosylated and methoxylated at positions 3 and 7, which often enhance compound stability and bioactivity. Similarly, Snook *et al*. (1992) documented characteristic fragmentation profiles for such compounds, including neutral losses of arabinose (−150 Da) and methoxy groups (−15 Da), in agreement with the fragmentation patterns expected for the proposed structure.

The ion at *m/z* 391, identified in the positive-mode mass spectrum, presented a challenge for definitive structural assignment. Initially, it was hypothesized to correspond to a derivative of N-alkylamides, compounds widely reported in *A. oleracea* due to the presence of spilanthol and its analogs. However, the observed molecular mass exceeds the typical range for simple alkylamides, suggesting the presence of oxygenated derivatives or conjugation with other chemical classes, such as terpenoids.

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Conversely, the ion at *m/z* 287, also detected in positive mode, was confidently assigned to kaempferol, a flavonol broadly distributed across the plant kingdom and well-known for its diverse bioactive properties. This compound, with the molecular formula $C_{15}H_{10}O_6$ and a molecular mass of 286 Da, is frequently found in species of the Asteraceae family, including *A. oleracea* (Harborne & Williams, 1984; Pop *et al*., 2013). In the mass spectrum, kaempferol appears as the $[M+H]^+$ ion at *m/z* 287, fully consistent with the experimental data obtained in this study.

Kaempferol is widely recognized for its antioxidant, anti-inflammatory, and neuroprotective properties, playing a key role in protecting cells against the harmful effects of oxidative stress (Pop *et al*., 2013). Its identification in the hydroalcoholic extract of *A. oleracea* stems further reinforces the pharmacological potential of the species, particularly in relation to the high antioxidant activity percentages (AA%) observed in the assays. This flavonoid emerges as one of the primary bioactive compounds in the extract, contributing significantly to the plant's overall chemical profile.

Moreover, analysis by negative-mode mass spectrometry revealed a broad spectrum of bioactive secondary metabolites. Among the detected ions, the signals at *m/z* 787, 751, 709, 577, and 563 stand out and are likely attributable to hydrolyzable tannins, glycosylated flavonoids, conjugated phenolic acids, and triterpenoids. These compound classes are well-documented in the literature for their antioxidant, anti-inflammatory, and antimicrobial activities, further supporting the therapeutic relevance of *A. oleracea* extracts.

The ion at *m/z* 787 was attributed to the presence of hydrolyzable tannins, likely derived from ellagic acid conjugated with disaccharides. Tannins of this type are widely distributed throughout the plant kingdom and are closely associated with high antioxidant capacity, significantly contributing to defense mechanisms against reactive oxygen species (Salih *et al*., 2021). The *m/z* 751 ion was linked to glycosylated flavonoids, such as kaempferol or quercetin conjugated with sugar moieties. These compounds are well known for their roles in protecting cells from oxidative stress and also demonstrate anti-inflammatory and cytoprotective effects (Dhanani *et al*., 2015). The signal observed at *m/z* 709 was identified as a likely

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ellagic acid derivative—a compound frequently reported in plant extracts due to its potent antioxidant activity (Fecka *et al*., 2015).

The ion at *m/z* 577 suggests the presence of conjugated phenolic acids, such as esterified gallic acid or caffeic acid, which are well known for their free radical scavenging capacity and broad bioactive potential across various biological systems (Renai *et al*., 2021). In turn, the ion at *m/z* 563 was attributed to smaller triterpenoids, likely modified forms of ursolic acid or oleanolic acid—compounds widely recognized for their antioxidant, antimicrobial, and anti-inflammatory activities (Salih *et al*., 2021).

Finally, Table 3 summarizes the ions detected by mass spectrometry in the *A. oleracea* extract, along with the proposed identifications based on structural inference and literature support.

Table 3: Ions detected by mass spectrometry and proposed identifications based on scientific literature.

m/z detected	Proposed compound	Reference
753	Kaempferol 3-rutinoside-7-glucoside	Pop *et al.*, 2013; Ruiz *et al*., 2010
711	Kaempferol 3-rutinoside-7-arabinose	Pop *et al*., 2013; Snook *et al*., 1992
475	Kaempferol 3-methoxy-7-O-glucoside	Snook *et al*., 1992; Harborne & Williams, 1984
433	Kaempferol 3-O-arabinose-7-methoxy	Snook *et al*., 1992; Harborne & Williams, 1984
391	Alkylamide derivative or oxygenated terpenoid	Singh *et al*., 2021
287	Kaempferol	Pop *et al*., 2013; Harborne & Williams, 1984
787	Hydrolyzable tannin (derived from ellagic acid + disaccharide)	Salih *et al*., 2021
751	Glycosylated flavonoid (Kaempferol or Quercetin conjugated with sugar)	Dhanani *et al.*, 2015
709	Ellagic acid derivative	Fecka *et al*., 2015
577	Conjugated phenolic acid (gallic acid or esterified caffeic acid)	Renai *et al*., 2021
563	Modified triterpenoid (ursolic acid or oleanolic acid)	Salih *et al*., 2021

Source: Author (2025).

4. CONCLUSION

The findings of this study confirm the strong antioxidant potential of the hydroalcoholic extract of *A. oleracea*, especially from the stem, which showed the highest activity levels. This effect is associated with flavonoids and other bioactive

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metabolites identified by mass spectrometry, including glycosylated kaempferol derivatives and kaempferol itself (*m/z* 287). The presence of *m/z* 391 suggests additional constituents, such as alkylamides or terpenoids. In negative mode, the detection of polar compounds—like tannins, phenolic acids, and triterpenoids—further highlights the extract's pharmacological value. Altogether, the results position *A. oleracea* as a promising natural antioxidant source for pharmaceutical, nutraceutical, and cosmetic applications

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