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A COMPREHENSIVE STUDY TO IDENTIFY NOVEL ACTIVE COMPONENTS, AMINO ACIDS, AND ANTIBIOTIC ACTIVITY OF *Acmella oleracea* (L.) R.K. Jansen

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ABSTRACT. *Acmella oleracea is a medicinal herb that belongs to the family Asteraceae and is used in folk medicine. It began to expand globally as a medicinal and ornamental plant in the early 21st century. Because of its prospective medical and cosmetic characteristics derived from its active components, it is likely to be exploited in the pharmaceutical and cosmetic sectors. As a result, it is critical to have a comprehensive understanding of the plant's active compounds and their possible effects as soon as feasible. In our research, we aimed to detect secondary metabolites in the leaves and inflorescence and investigate the antibiotic activity of the plant. In total, 77 active components were detected. Eight substances were only in the flowers, and four substances were only in the leaves. Several of the newly discovered active components have promising medicinal significance. The antibiotic activity was tested on five different microorganisms: Staphylococcus aureus (two strains), Klebsiella pneumoniae, Klebsiella aerogenes, and fungi: Candida krusei and Candida glabrata. Our experiment demonstrated that Acmella oleracea extract has a strong inhibitory effect against the microscopic fungus Candida krusei. Based on our experiments, further phytotherapeutic use of the plant is recommended.*

KEYWORDS: *Acmella oleracea, biological activity, Candida krusei, secondary metabolites.*

INTRODUCTION

There are three major types of regulatory structures that can shape the orientation of a state's medical culture: exclusive, tolerant, and integrated (Murray 1996). The Central and Eastern European countries under Soviet authority, following the norms of the Soviet Union's Cultural Revolution, regarded folk medicine as a dubious, pseudo-scientific activity and so prosecuted it (Souček 2020). However, the richer countries and countries with higher health expenditure are more likely to have integrated Complementary and Alternative Medicine (CAM) treatments into their health care system, in recent years, the Eastern Central European countries have also returned to using the tools of clinical medicine and CAM together. CAM refers to medical and healthcare systems, methods, and products that are not now considered part of traditional medicine (Jupaneant et al. 2014). The widespread popularity of various complementary and alternative medicines for human health has increased public awareness of several extensively advertised species. However, herb-drug interactions with these popular products remain poorly studied (Quave et al. 2012).

Acmella oleracea (L.) R. K. Jansen, a medicinal plant often known as *Jambú*, has just arrived on the Central Eastern European market and is currently considered not only as an herb but also as an ingredient material for cosmetic and pharmaceutical products (Savant & Kareppa 2022, Uthpala & Navaratne 2021). Its floral extract is used as a topical muscular relaxant and may help minimize facial wrinkles produced by mimetic strain (Belfer 2007). The plant is also known as the toothache plant since its leaves are used to treat toothaches (Oliver-Bever 1986, Savant & Kareppa 2022, Rondanelli et al. 2020). Chewing the plant's leaves produces a comparable numbing effect on the gums as lidocaine. Injections of 20% extracts of the aerial parts of *Acmella* produced 87% local anesthesia, while 2% lidocaine showed 97% local anesthesia (Paulraj et al. 2013, Tsuchiya 2017).

In both cases, the analgesic and facial muscle paralyzing endpoints are disclosed due to the inclusion of spilanthol, which is an isobutylamide, an alkylamide-class pain reliever (Cheng et al. 2015, Savant & Kareppa 2022). There are more and more *Acmella oleracea*-based preparations and products on the market of the cosmetics industry. Face creams and anti-aging oil serums are actively developed as a possible natural

substitute for botox (Lalthanpuii et al. 2020). It's extract is also used in chewing gums indicated for toothache (Leng et al. 2011, Santana de Freitas-Blanco et al. 2016). Its culinary application is also important due to the effect of spilanthol and its lookalikes on the ternate nerve, which causes specific taste effects (Ramsewak et al. 1999). Using a formulation containing spilanthol and its isomers as a biological control agent could also be promising (Kadir et al. 1989, Spinozzi et al. 2022). Recent studies have demonstrated that the bioactive compounds from the plant are effective in producing biodegradable packaging films, which extend the shelf life of packaged products (Moura et al. 2023).

Medical research has reported an anti-inflammatory, anti-edema effect (Stein et al. 2021). Studying the influence of the hydroethanolic extract of jambú on malignant cells of stomach cancer, Pinheiro et al. (2023) concluded that spilanthol has an inhibiting potential for recombinant proteins (JAK1 and JAK2 – Janus kinase 1 and 2) of human (Maria-Ferreira et al. 2014, Pinheiro et al. 2023). Jerônimo et al. (2024) studied the effects of essential oils from *Acmella oleracea* on various human cancer cells. Their findings demonstrated effectiveness against malignant cell lines, including those from gastric ascites, melanoma, and lung carcinoma. However, they also noted higher toxicity levels in healthy embryonic kidney cells (Jerônimo et al. 2024).

Acmella oleracea has entered the medicinal plant market in Central and Eastern Europe (Bellumori et al. 2022, Rondanelli et al. 2020). This annual plant is relatively easy to cultivate; however, before it can be widely adopted as a therapeutic product, it is essential to gain a thorough understanding of it.

Bioactive compound profile

Acmella oleracea is already known to be a rich source of important bioactive compounds (Paulraj et al. 2013, Uthpala & Navaratne 2021). Lalthanpuii & Lalchhandama (2020) identified nineteen chemicals in the extract of the aerial parts of the *Acmella* plant. The main compounds were fatty alcohols, including 3,7,11,15-tetramethylhexadec-2-en-1-ol and (9Z)-9-hexadecen-1-ol, important bioactive substances such as an alkylamide, N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide, and a triterpenoid, lupeol (Lalthanpuii & Lalchhandama 2020). While Neves et al. (2019) detected amino acids such as asparagine, glutamic acid, valine,

and isoleucine in the jambu plant. White et al. (1986) determined the following amino acids in raw and processed jambu: alanine, aspartic acid, glutamic acid, serine, hydroxyproline, arginine, glycine, cysteine, proline, tyrosine, valine, threonine, methionine, histidine, isoleucine, leucine, phenylalanine, tryptophan, lysine, and the aminosulfonic acid taurine. Nascimento et al. (2020) identified 45 bioactive components in *Acmella oleracea*, while Aktar et al. (2024) reported 48 components in their review.

Biological activities

The antibacterial activity of several extracts of *Acmella oleracea* and the standard antibiotic, doxycycline, was evaluated for various bacterial strains (Arora et al. 2011). With *Escherichia coli*, ethyl acetate extracts exhibited equivalent efficacy to doxycycline (Arora et al. 2011). In the case of *Bacillus cereus,* none of the extracts showed very excellent activity; however, the *Pseudomonas aeruginosa* water extract showed extremely high activity (Arora et al. 2011). Doxycycline, the usual medicine, was ineffective against *Micrococcus luteus*, while extracts of methanol, petroleum ether, and ethyl acetate showed moderate activity (Arora et al. 2011). Finally, with *Klebsiella pneumoniae*, ethyl acetate demonstrated good performance by having a larger inhibitory zone than doxycycline (Arora et al. 2011). The *A. oleracea*-rich spilanthol extracts have shown outstanding antibacterial activity against the cariogenic *Streptococcus mutans*, suggesting that they could be employed as an economical coadjuvant in dental treatments to prevent and control dental caries (Peretti et al. 2021).

Arora et al. (2011) investigated the antifungal activity of different *Acmella oleracea* extracts and the conventional medication *fluconazole* on various fungus strains. Only the ethyl acetate extract showed mild action against *Aspergillus niger*, whereas methanol, petroleum ether, and ethyl acetate all showed activity against *Penicillium chrysogenum (*Arora et al. 2011). In the instance of *Rhizopus arrhigus*, the ethyl acetate demonstrated a larger inhibitory zone than the standard medicine (Arora et al. 2011). Finally, with *Rhizopus stolonifer*, the water extract showed outstanding activity, having a larger inhibitory zone than the conventional drug *fluconazole* (Arora et al. 2011). Rani & Murty (2006) tested the antifungal activity of *Acmella* flower extract at various doses. It had the largest inhibition zones against *Fusarium oxysporium* and *Fusarum moniliformis* followed by *Aspergillus niger* and *Aspergillus parasiticus*

(Rani & Murty 2006).

To ensure the safe use of any traditional herb, it is essential to identify potential toxicity and understand any synergistic or antagonistic effects with other substances or active ingredients. This necessitates a thorough investigation of secondary metabolites, which was one of the primary objectives of our experiment. Additionally, we aimed to more precisely explore the antibiotic effects, including testing new resistant strains and previously untested microorganisms.

MATERIALS AND METHODS

Secondary metabolite profile

The *Acmella oleracea* plant used in the experiment was taken from the Medicinal and herb garden of the Institute of Agricultural and Engineering Sciences of the University of Nyíregyháza/Hungary. The plant sample was washed three times with distilled water and dried. The dry plants were ground and stored in the freezer until the measurements. We put the samples in the freezer to prevent the degradation of secondary metabolites. The samples were packed in airtight bags. Secondary metabolite profiles of the *Acmella* leaves and flowers were determined using the UHPLC-MS/MS method.

Sample preparation: 2 grams of the dried samples were shaken at 150 rpm with 50 ml of methanol and water (70:30 v/v) at room temperature for 2 hours. All extracts were filtered before injection using 0.22 µm PTFE syringe filters (Kaszás et al. 2020).

Dionex Ultimate 3000RS (Thermo Scientific) UHPLC equipment was used for the separation of the secondary metabolites. The compounds were separated on a Phenomenex Kinetex XB-C18 column (100 mm × 2.1 mm i. d. 2.6 µm) thermostated at 25 °C (±1 °C). Water (A) and methanol (B) (both were acidified with 0.1% formic acid) were used as eluents. The flow rate was maintained at 200 µL/min. The gradient elution was performed as follows: 0–3 min. 95% of mobile phase A; 3–43 min. → 0% A; 43–61 min. 0% A; 61–62 min. → 95% A; 62–70 min. 95% A. The UHPLC equipment was connected to a Thermo Q Exactive Orbitrap hybrid mass spectrometer equipped with an electrospray ionization source (Thermo Scientific). MS data were collected in both positive and negative ion modes in different runs. The following settings were used for MS analyses: resolution: 70000 in the cases of full scans and 35000 in the cases of fragmentation scans; collision energy: 30 NCE; scan range: 100 to 1500 *m*/*z.*

Trace Finder 3.1 (Thermo Scientific) software was used to analyze the raw files.

The secondary metabolites were identified based on previously published works (Zengin et al. 2018, 2020), our and online databases (Metlin, Massbank of North America, m/z Cloud). In every case, the exact molecular mass, isotopic pattern, characteristic fragment ions, and retention time were used to identify the secondary metabolites. The difference between the measured and calculated monoisotopic molecular masses was less than 5 ppm in every case.

Microbial analysis

Dried *herba of Acmella oleracea* were ground in a mortar with sand and extracted with methanol by cold percolation method (Agarwal & Paridhavi 2009). The alcohol was then evaporated by vacuum distillation.

The extract of *A. oleracea* was dissolved in 10 ml of sterile water. The study was carried out on 96-well polystyrene plates. A 150 µl nutrient medium (Muller-Hinton agar) was poured into the Petri dish, which contained a solution of *A. oleracea* extract in three concentrations: 25%, 50%, and 75%. The control cup did not contain extract.

We performed clinical susceptibility screening and created a database of typing collections of microorganisms isolated from wound surfaces of the military patients treated at the Uzhhorod City Clinical Hospital, Ukraine. We used ten of these clinical isolates as test cultures characterised by high multidrug resistance to antibiotics and antifungals, namely *Staphylococcus aureus* (two strains), *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Klebsiella aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*; and fungi: *Candida albicans*, *Candida krusei* and *Candida glabrata*.

A 10 µl suspension of microorganisms (0.5 McFarland's standard) was added to the wells of the plates. The plates were incubated at 37°C for 24 h (for bacterial isolates) and 48 h (for fungi), respectively. After the incubation, they were inoculated onto beef extract agar (bacterial isolates) and Sabouraud medium (fungi), respectively, in a volume of 10 µl. The microbial growth was checked for 24 h (for bacterial isolates) and 48 h (for fungi), respectively, after the incubation (Balouiri et al. 2016).

RESULTS

Secondary metabolite profile

In total, 77 substances were identified in the leaves and flowers, 74 substances in the flowers and 70 substances in the leaves, of which 8 substances were only in the flowers, 4 substances were only in the leaves,

and 66 substances were both in the flowers and in the leaves (Table 1).

In particular, the following substances were identified in the leaves but not in the flowers: vicenin-2 (apigenin-6,8-di-C-glucoside); 3-O-methylrosmarinic acid; hydroxyoctadecatrienoic acid isomer 1; and hydroxyoctadecatrienoic acid isomer 2. The following substances were identified only in the flowers: quercetin-O-dihexoside-O-rhamnoside; quercetin-O-(rhamnosyl) dihexoside; quercetin-O-dihexoside; N-p-coumaroyl-3-hydroxytyrosine; quercetin-O-pentoside; astragalin (kaempferol-3-O-glucoside); isorhamnetin-3-O-glucoside; and dihydrospilanthol isomer.

When analyzing Figure 1 and Table 1, we can see that the spilanthol peak has the highest relative intensity in positive ion mode. In the leaves, the phaselic acid (2-O-caffeoylmalic acid) peak has the highest relative intensity in negative ion mode (Fig. 1B); in the flowers, it was registered in small quantities only (Fig. 1D). For the flowers, the malic acid peak has the highest relative intensity in negative ion mode (Fig. 1D).

According to our research, the following metabolite peaks have the highest relative intensities in the leaves (in descending order): spilanthol; phaselic acid (2-O-caffeoylmalic acid); malic acid; 2-O-feruloylmalic acid; citric acid; N-(2-methylbutyl)-deca-2,6,8-trienamide; hydroxyoctadecatrienoic acid isomer 2; octadecatrienoic acid; and petasiphenol (3-(3,4-dihydroxyphenyl)-2-oxopropyl caffeate) (Figs. 1 A, B). The following are the metabolite peaks with the highest relative intensities in the flowers (Figs. 1 C, D): spilanthol; malic acid; 2-O-feruloylmalic acid; hydroxyoctadecadienoic acid; pinellic acid (9,12,13-trihydroxy-10E-octadecenoic acid); N-(2-methylbutyl)-deca-2,6,8-trienamide; and rosmarinic acid (labiatenic acid). It is noteworthy that a significant share of the substances identified in the measured samples showed a considerable abundance only in the concrete part of the plant, which fact must not be neglected when applying them in medicine and cosmetology.

We detected 14 amino acids in our experiment. Six of them are essential amino acids, such as phenylalanine, histidine, leucine or isoleucine, lysine, threonine, and tryptophan. The rest, arginine, asparagine, proline, serine, aspartic acid, glutamic acid, glutamine, and tyrosine are protein-building amino acids.

This is the first study to report the presence of 35 additional active components of the plant that have never been detected before (Table 1).

Table 1. Chemical composition of leaf and flower extracts from *Acmella oleracea* ([1]Confirmed by standard; *detection: F - flowers, L – leaves)

No.	Name	Detection site*	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Reference
1	Lysine	L; F	C6H14N2O2	1.49	147.11336		130.0501	84.0814	56.0502			White et al. (1986)
2	Arginine	L; F	C6H14N4O2	1.70	175.11951		158.0926	130.0978	116.0709	70.0658	60.0564	
3	γ-Aminobutyric acid	L; F	C4H9NO2	1.70	104.07116		87.0446	86.0606	69.0341	58.0658		No Reference
4	Histidine	L; F	C6H9N3O2	1.75	156.07731		110.0716	95.0608	93.0453	83.0609		White et al. (1986)
5	Proline	L; F	C5H9NO2	1.80	116.07116		70.0658	68.0502				
6	Serine	L; F	C3H7NO3	1.80	106.05042		88.0398	70.0294	60.0451			
7	Threonine	L; F	C4H9NO3	1.80	120.06607		102.0555	84.0450	74.0607	56.0503		
8	Asparagine	L; F	C4H8N2O3	1.82	133.06132		116.0345	88.0398	87.0558	74.0243	70.0294	
9	Quinic acid	L; F	C7H12O6	1.84		191.05557	173.0447	171.0289	127.0390	109.0281	85.0280	No Reference
10	Aspartic acid	L; F	C4H7NO4	1.86	134.04534		116.0346	88.0399	74.0243	70.0295		White et al. (1986)
11	Glutamic acid	L; F	C5H9NO4	1.86	148.06099		130.0501	102.0554	84.0450	56.0502		
12	Glutamine	L; F	C5H10N2O3	1.86	147.07697		130.0501	102.0555	101.0715	84.0450	56.0503	
13	Malic acid	L; F	C4H6O5	1.96		133.01370	115.0023	89.0229	87.0073	72.9916	71.0123	
14	Leucine or Isoleucine	L; F	C6H13NO2	2.62	132.10246		86.0970	69.0706				No Reference
15	Tyrosine	L; F	C9H11NO3	2.70	182.08172		165.0548	147.0441	136.0759	123.0443	119.0495	White et al. (1986)
16	Citric acid	L; F	C6H8O7	2.76		191.01918	173.0082	129.0182	111.0074	87.0072	85.0280	
17	Indoline	L; F	C8H9N	4.71	120.08133		103.0547	93.0703	91.0547			No Reference
18	Phenylalanine	L; F	C9H11NO2	4.77	166.08681		149.0599	131.0495	120.0811	103.0548	93.0701	White et al. (1986)
19	Tryptophan	L; F	C11H12N2O2	10.51	205.09771		188.0708	170.0603	159.0917	146.0602	118.0654	
20	Uralenneoside	L; F	C12H14O8	14.67		285.06105	153.0181	152.0103	109.0281	108.0203		
21	Caffeic acid-O-hexoside	L; F	C15H18O9	16.78		341.08726	179.0341	135.0440	107.0487			No Reference
22[1]	Chlorogenic acid (3-O-Caffeoylquinic acid)	L; F	C16H18O9	17.20	355.10291		163.0390	145.0285	135.0441	117.0336	89.0384	No Reference
23[1]	Caffeic acid	L; F	C9H8O4	17.42		179.03444	135.0440	107.0488				No Reference
24	Quercetin-O-dihexoside -O-rhamnoside	F	C33H40O21	20.24		771.19839	625.1432	446.0875	301.0349	300.0279	299.0201	No Reference
24	Phaselic acid (2-O-Caffeoylmalic acid)	L; F	C13H12O8	20.59		295.04540	179.0342	135.0439	133.0130	115.0023	71.0122	Nascimento et al. (2020)
25	Vicenin-2 (Apigenin-6,8-di-C-glucoside)	L	C27H30O15	20.73	595.16630		559.1420	523.1228	457.1130	325.0706	295.0600	No Reference
26	trans-Clovamide	L; F	C18H17NO7	20.94		358.09268	222.0403	178.0502	161.0228	135.0440		Nascimento et al. (2020)
27	Quercetin-O-(rhamnosyl)dihexoside	F	C33H40O21	21.71		771.19839	301.03540	300.0280	271.0252	255.0302	151.0026	No Reference

Table 1 – continued next page

Table 1 – continuation

No.	Name	Detection site*	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Reference
28	Quercetin-O-dihexoside	F	C27H30O17	21.99		625.14048	301.03590	300.0280	271.0253	255.0301	151.0025	No Reference
29	N-p-Coumaroyl-3-hydroxytyrosine	F	C18H17NO6	22.49		342.09777	222.04030	178.0502	145.0284	119.0489		No Reference
27	N-trans-Caffeoyltyrosine (Deoxyclovamide)	L; F	C18H17NO6	22.83		342.09777	206.0449	163.0388	161.0234	135.0441	119.0488	Nascimento et al. (2020)
28	2-O-Feruloylmalic acid	L; F	C14H14O8	23.33		309.06105	193.0499	178.0262	149.0596	134.0361	71.0123	
29	Quercetin-O-rhamnoside-O-rhamnosylhexoside	L; F	C33H40O20	23.65		755.20347	609.1468	447.0903	446.0860	301.0353	299.0198	No Reference
30	Quercetin-O-glucuronide-O-rhamnoside	L; F	C27H28O17	23.71		623.12483	447.0936	301.0358	300.0277	271.0254	151.0024	No Reference
31	Quercetin-O-acetylhexoside-O-rhamnoside isomer 1	L; F	C29H32O17	24.44		651.15613	505.0999	447.0933	446.0843	301.0351	299.0201	No Reference
32	Quercetin-O-acetylhexoside-O-rhamnoside isomer 2	L; F	C29H32O17	24.78		651.15613	505.0996	447.0954	446.0863	301.0356	299.0201	No Reference
33	Hyperoside (Quercetin-3-O-galactoside)	L; F	C21H20O12	24.93		463.08765	301.0359	300.0282	271.0248	255.0301	151.0025	No Reference
34	Quercetin-O-acetylhexoside-O-rhamnoside isomer 3	L; F	C29H32O17	24.98		651.15613	505.0964	447.0940	446.0841	301.0343	299.0201	No Reference
35[1]	Isoquercitrin (Quercetin-3-O-glucoside)	L; F	C21H20O12	25.12		463.08765	301.0366	300.0281	271.0256	255.0291	151.0023	No Reference
36[1]	Rutin (Quercetin-3-O-rutinoside)	L; F	C27H30O16	25.17		609.14557	301.0357	300.0278	271.0252	255.0299	151.0025	Nascimento et al. (2020)
37	Miquelianin (Quercetin-3-O-glucuronide)	L; F	C21H18O13	25.57		477.06692	301.0357	255.0298	178.9977	163.0026	151.0025	
38	Quercetin-O-acetylhexoside-O-rhamnoside isomer 4	L; F	C29H32O17	25.58		651.15613	505.0969	447.0924	446.0860	301.0355	299.0199	No Reference
39	Rosmarinic acid (Labiatenic acid)	L; F	C18H16O8	26.20		359.07670	197.0450	179.0342	161.0233	135.0440	72.9916	No Reference
40	Quercetin-O-malonylhexoside	L; F	C24H22O15	26.25		549.08805	505.0971	301.0358	300.0277	271.0251	255.0298	No Reference
41	Quercetin-O-((acetyl)rhamnosylhexoside) isomer 1	L; F	C29H32O17	26.27		651.15613	301.0351	300.0276	271.0254	255.0304	151.0024	Nascimento et al. (2020)
42	Quercetin-O-acetylhexoside isomer 1	L; F	C23H22O13	26.61		505.09822	463.0901	301.0345	300.0279	271.0252	255.0301	No Reference
47[1]	Astragalin (Kaempferol-3-O-glucoside)	F	C21H20O11	26.85		447.09274	285.0410	284.0330	255.0301	227.0349		No Reference
48[1]	Isorhamnetin-3-O-glucoside	F	C22H22O12	27.04		477.10330	315.0521	314.0436	285.0408	271.0252	243.0297	No Reference
43	Quercetin-O-acetylglucuronide-O-rhamnoside isomer 1	L; F	C29H30O18	27.07		665.13539	447.0938	301.0358	300.0284	271.0252	151.0024	Nascimento et al. (2020)
44	Quercetin-O-acetylhexoside isomer 2	L; F	C23H22O13	27.09		505.09822	463.0909	301.0357	300.0280	271.0254	255.0301	No Reference
45	Kaempferol-O-glucuronide	L; F	C21H18O12	27.24		461.07200	285.0410	229.0496	113.0229			No Reference
46	Quercetin-O-acetylglucuronide-O-rhamnoside isomer 2	L; F	C29H30O18	27.70		665.13539	605.1132	447.0929	446.0854	301.0356	299.0201	Nascimento et al. (2020)
47	Quercetin-O-((acetyl)rhamnosylhexoside) isomer 2	L; F	C29H32O17	27.74		651.15613	301.0357	300.0279	271.0253	255.0297	151.0026	Nascimento et al. (2020)
48	3-O-Methylrosmarinic acid	L	C19H18O8	28.06		373.09235	197.0450	179.0340	175.0394	160.0154	135.0440	No Reference

Table 1 – continued next page

Table 1 – continuation

No.	Name	Detection site*	Formula	Rt	$[M + H]^+$	$[M - H]^-$	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Reference
49	Quercetin-O-((acetyl)rhamnosylhexoside) isomer 3	L; F	C29H32O17	28.07		651.15613	301.0350	300.0279	271.0250	255.0299	151.0024	Nascimento et al. (2020)
50	Petasiphenol (3-(3,4-Dihydroxyphenyl)-2-oxopropyl caffeate)	L; F	C18H16O7	28.20		343.08178	181.0499	163.0390	161.0233	135.0440	133.0283	
51	Quercetin-O-((acetyl)rhamnosylhexoside) isomer 4	L; F	C29H32O17	28.44		651.15613	301.0358	300.0280	271.0253	255.0298		
52	Quercetin-O-acetylglucuronide isomer 1	L; F	C23H20O14	28.50		519.07748	301.0358	300.0281	255.0301	178.9978	151.0025	
53	Quercetin-O-acetylglucuronide isomer 2	L; F	C23H20O14	29.18		519.07748	459.0601	301.0358	300.0275	178.9977	151.0025	
54	N-Isobutyl-2-nonene-6,8-diynamide isomer 1	L; F	C13H17NO	29.83	204.13884		148.0758	126.0914	120.0809	105.0704	103.0547	
55	N-Isobutyl-2-nonene-6,8-diynamide isomer 2	L; F	C13H17NO	30.75	204.13884		148.0757	131.0494	120.0811	105.0704	57.0707	
56	N-Phenylethyl-2,3-epoxy-6,8-nonadiynamide	L; F	C17H17NO2	31.75	268.13376		250.1232	148.0758	105.0703	103.0548	79.0549	
57	N-Isobutylundeca-2,5-diene-8,10-diynamide isomer 1	L; F	C15H19NO	33.01	230.15449		174.0916	131.0858	129.0701	128.0622	91.0548	Nascimento et al. (2020)
58	Dodecenedioic acid isomer 1	L; F	C12H20O4	33.26		227.12834	209.1176	183.1383	165.1274	111.0801		
59	N-Isobutylundeca-2,5-diene-8,10-diynamide isomer 2	L; F	C15H19NO	33.27	230.15449		174.0915	131.0858	129.0701	128.0622	91.0548	
60	Malyngic acid (9,12,13-Trihydroxy-10E,15Z-octadecadienoic acid)	L; F	C18H32O5	33.48		327.21715	309.2092	291.1973	229.1440	211.1336	171.1017	
61	Hydroxydodecenoic acid	L; F	C12H22O3	33.69		213.14907	195.1390	183.1381				No Reference
62	Dodecenedioic acid isomer 2	L; F	C12H20O4	33.74		227.12834	209.1185	183.1383	165.1275	111.0805		Nascimento et al. (2020)
63	N-Isobutylundeca-2-ene-8,10-diynamide	L; F	C15H21NO	34.08	232.17014		176.1071	133.1012	131.0858	105.0704	91.0548	
64	Pinellic acid (9,12,13-Trihydroxy-10E-octadecenoic acid)	L; F	C18H34O5	34.68		329.23280	311.2232	293.2119	229.1442	211.1335	99.0881	
65	N-(2-Methylbutyl)-2-undecene-8,10-diynamide	L; F	C16H23NO	35.81	246.18579		176.1072	159.0804	131.0857	105.0704	91.0548	
66	Spilanthol	L; F	C14H23NO	36.01	222.18579		168.1386	141.1150	126.0916	81.0705	57.0707	Barbosa et al. (2016)
67	N-Isobutyl-2,7-tridecadiene-10,12-diynamide	L; F	C17H23NO	36.37	258.18579		157.1018	142.0780	129.0701	117.0702	105.0703	Nascimento et al. (2020)
73	Dihydrospilanthol isomer	F	C14H25NO	36.77	224.20144		168.1386	156.1384	113.1013	109.1016	74.0971	No Reference
68	N-Isobutyl-7-tridecene-10,12-diynamide	L; F	C17H25NO	37.08	260.20144		187.1115	159.1167	145.1013	117.0702	105.0703	Nascimento et al. (2020)
69	Dihydrospilanthol	L; F	C14H25NO	37.69	224.20144		168.1381	141.1149	126.0917	109.1017	57.0707	
70	N-(2-Methylbutyl)-deca-2,6,8-trienamide	L; F	C15H25NO	37.71	236.20144		182.1540	168.1384	155.1306	126.0916	81.0705	
71	Dodeca-2,4,8,10-tetraenoic acid isobutylamide	L; F	C16H25NO	38.75	248.20144		167.1310	166.1228	152.1067	107.0859	57.0706	
72	Decen-2-oic acid isobutylamide	L; F	C14H27NO	39.50	226.21709		170.1541	153.1277	83.0863	57.0706		
73	Hydroxyoctadecatrienoic acid isomer 1	L	C18H30O3	40.60		293.21167	275.2026	183.1021	171.1014	155.1067	121.1007	No Reference
74	Hydroxyoctadecatrienoic acid isomer 2	L	C18H30O3	40.79		293.21167	275.2022	235.1694	223.1335	195.1385	179.1431	No Reference
75	Hydroxyoctadecadienoic acid	L; F	C18H32O3	41.83		295.22732	277.2176	195.1385	171.1017	59.0123		No Reference
76	Octadecatrienoic acid	L; F	C18H30O2	45.02		277.21676	233.1906	59.0124				No Reference
77[1]	Linoleic acid	L; F	C18H32O2	45.94		279.23241						No Reference

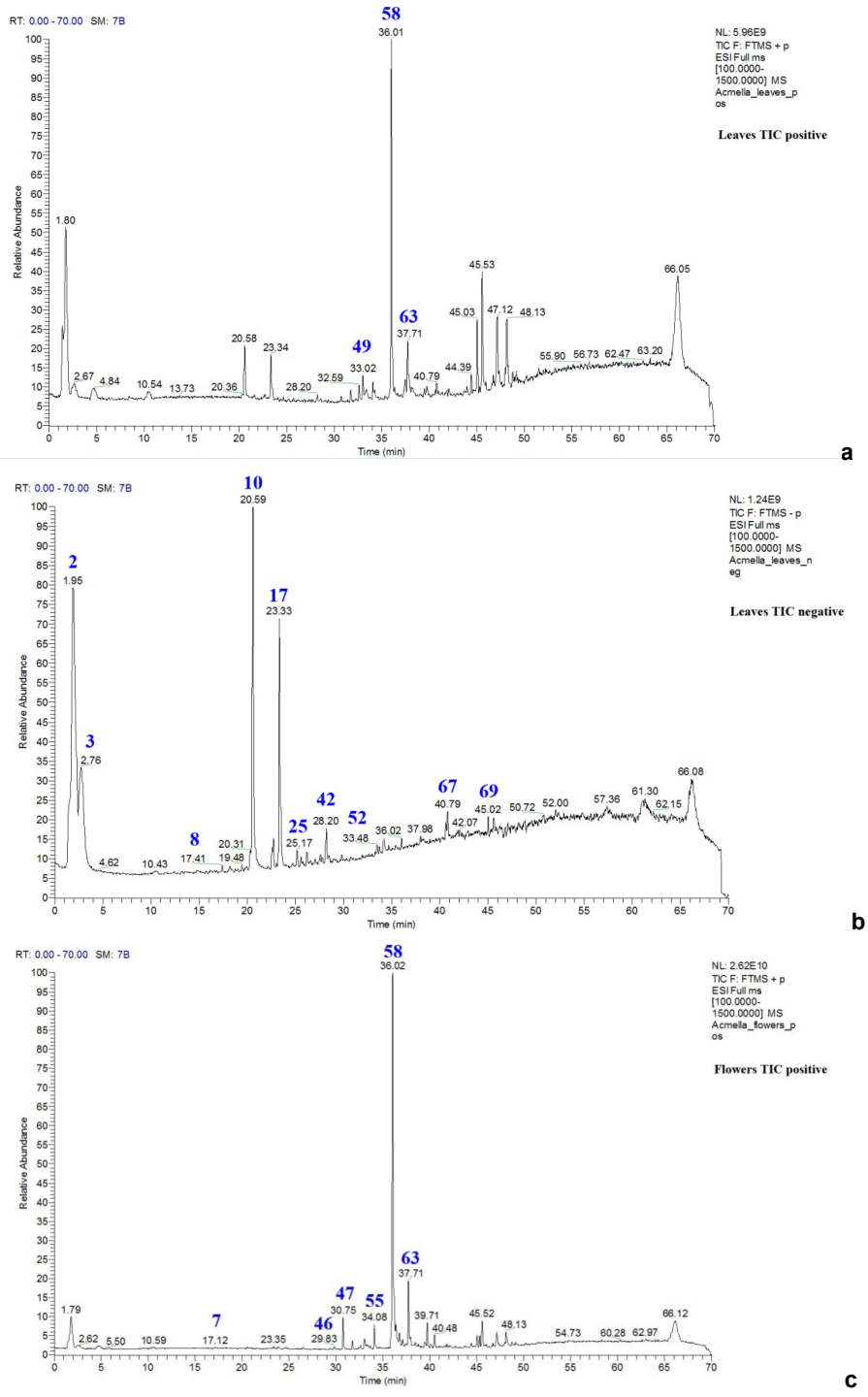
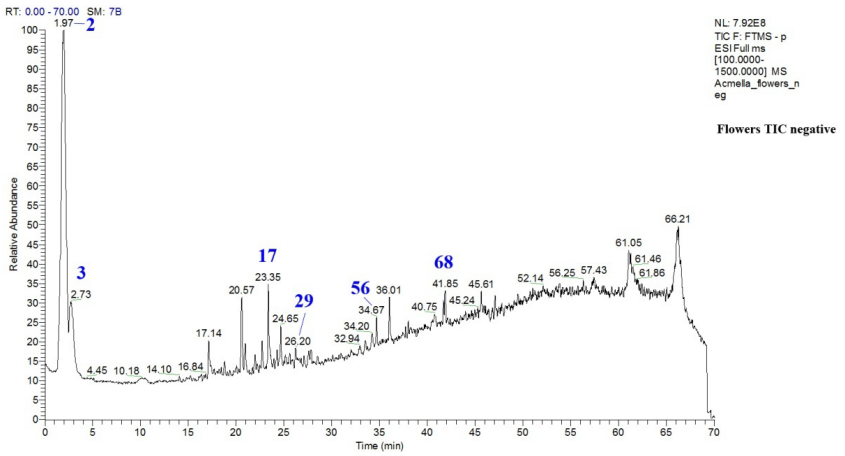


Figure 1 a, b, c. Total Ion Chromatograms for *Acmella oleracea* a.–c– experiment variants. Deciphered peaks are highlighted in blue; substance numbers comply with the values shown in Table 1.



d

Figure 1 d. Total Ion Chromatograms for *Acmella oleracea* - experiment variant. Deciphered peaks are highlighted in blue; substance numbers comply with the values shown in Table 1.

Biological activities

The studied extract of the *herba* of *A. oleracea* in the selected concentrations showed insignificant antibacterial activity against gram-positive cocci and no effect on gram-negative bacteria. High concentrations of the extract (75%) showed a slight bacteriostatic effect against selected strains of *Staphylococcus aureus* and *Streptococcus pyogenes*. Thus, it can be assumed that as the concentration of *A. oleracea* extract increases, its activity against Gram-positive cocci will increase (Table 2).

Table 2. Effect of the *Acmella oleracea* plant on the microorganisms studied as a function of extract concentration.

The microorganism tested	The concentration of the extract		
	25%	50%	75%
Staphylococcus aureus (two strains)	-	-	+
Streptococcus pyogenes	-	-	+
Klebsiella pneumoniae	-	-	-
Klebsiella aerogenes	-	-	-
Escherichia coli	-	-	-
Pseudomonas aeruginosa	-	-	-
Candida albicans	-	-	-
Candida krusei	-	-	++
Candida glabrata	-	-	-

The studied concentrations of *A. oleracea* extract did not show antifungal

activity against clinical isolates of *C. albicans* and *C. glabrata* (Table 2). However, the extract was found to have an inhibitory effect on clinical isolates of the microscopic fungus *Candida krusei.* With an increase in the concentration of the extract in the culture medium, its inhibitory effect increases in direct proportion (Figure 2).

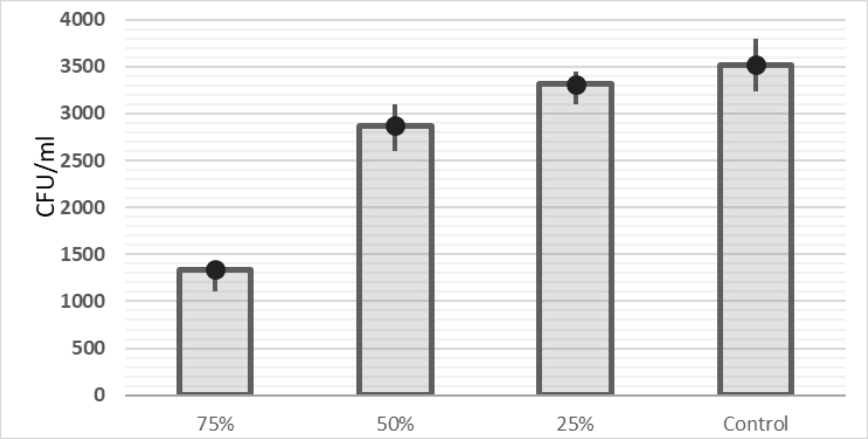


Figure 2. Diagram of the inhibitory effect of various concentrations of *Acmella oleracea* extract on clinical isolates of *Candida krusei* in experimental conditions. Mean value and limits.

DISCUSSION

Secondary metabolite profile

Nascimento et al. (2020) did a previous brief literature study on the biologically active components identified in *Acmella oleracea,* including 45 active components. We described a total of 77 substances. The three most abundant active components are spilanthol, malic acid, and phaselic acid. We also know the health benefits of many of the newly described active substances. Y-aminobutyric acid, called GABA (γ-aminobutyric acid), is a four-carbon, nonprotein amino acid (Bown & Shelp 1997). It was first discovered in potato (*Solanum tuberosum*) tubers about 80 years ago (Steward 1949). It is a neurotransmitter, a chemical messenger in the brain that slows down brain activity by inhibiting certain signals in the central nervous system (Bowery & Smart 2006). GABA is known to produce a relaxing effect (Abdou et al. 2006). GABA levels fluctuate in response to a

variety of medical disorders. Therefore, many drugs target the GABA receptor. More research is needed to determine whether GABA supplements and GABA-rich foods can help prevent or treat disease depression or anxiety (Boonstra et al. 2015).

Quinic acid, a strong antioxidant, is released during roasting and contributes to the pleasant aroma of the coffee (Gigl et al. 2021). Malic acid contributes to the sour taste of fruits and is used as a food additive (Marques et al. 2020). Citric acid occurs naturally in citrus fruits. In foods, it is mainly used as an antioxidant, a sourness regulator, and a flavoring agent, known as E330 (Kane et al. 2023). Indoline is anti-inflammatory and hepatoprotective and can effectively improve metabolic health (Knudsen et al. 2021, Zhang et al. 2022). Uralenneoside is antioxidant and enzyme inhibitory (Youssef et al. 2023). According to Birková et al. (2020), caffeic acid has anti-inflammatory and antioxidant effects; its antioxidant qualities also help prevent cancer, diabetes, inflammation, and neurological illnesses.

Furthermore, we were able to identify several common and some less common flavonoids. Quercetin is a flavonoid with numerous health benefits, including antioxidant, antimicrobial, anti-inflammatory, antiviral, and anticancer properties and it is a part of many dietary supplement products (Aghababaei & Hadidi 2023). Vicenin-2 has anticancer activity and could be a good candidate for future therapeutic use to inhibit chemically induced liver cancer (Zhang et al. 2020). N-Coumaroyl-3-hydroxytyrosine is a natural product found in *Theobroma cacao*, on which the literature is extremely limited, with no health effects or uses mentioned (National Center for Biotechnology Information 2024a). We found some derivatives of quercetin, such as quercetin-O-rhamnoside-O-rhamnosylhexoside, quercetin-O-glucuronide-O-rhamnoside, The composition and quantity of flavonol glycosides are crucial indications of the quality and health potential of the berries, for example, buckthorn, grape, and blueberry, and they have been shown to have protective effects on heart disease (Ma et al. 2016, Xing et al. 2021). Quercetin-O-acetylhexoside-O-rhamnoside isomers 1, 2, 3, and 4 are flavonoids found in pears (Kolniak-Ostek 2016). Hyperoside (quercetin-3-O-D-galactoside) is a flavonoid glycoside from various plants with multiple pharmacological effects, including anti-inflammatory, antidepressant, neuroprotective, cardioprotective, antidiabetic, anticancer, antifungal, radioprotective, gastroprotective, and antioxidant properties (Raza et al. 2017). Isoquercitrin displayed potent antiviral activities with no significant cytotoxic effects (Kim et al. 2020) and also delayed denervated soleus

muscle atrophy by inhibiting oxidative stress and inflammation (Shen et al. 2020). Rutin, also known as vitamin P, was first discovered in *Ruta graveolens*, popularly known as rue, and has since been identified naturally in some regularly consumed plant species (Gawlik-Dziki 2012). The pharmacological potential of rutin includes anti-inflammatory, antidiabetic, cardiovascular, hepatoprotective, anticancer, and neuroprotective actions, making it highly relevant (Siti et al. 2020, Tobar-Delgado et al. 2023). Quercetin-O-pentoside has been found in blueberry, pomegranate, and strawberry (Xing et al. 2021), and quercetin-O-malonylhexoside has been detected in *Atriplex hortensis* var. *rubra* (Tran et al. 2022), *Moringa oleifera* (Pagano et al. 2020), and *Coleostephus myconis* (Bessada et al. 2016), attributing antioxidant activity to them. Also, antioxidant activity is attributed to quercetin-O-acetyl hexoside and its isomers, which were detected in native Hungarian oak (*Quercus*) species (Hofmann et al. 2022), *Moringa oleifera* (Llorent-Martínez et al. 2023), kiwifruit (*Actinidia chinensis)*, and kiwi berry (*Actinidia arguta*) (Yu et al. 2020). The biologically active and therapeutically useful substance "astragalin" has been recognized to possess a broad range of pharmacological properties such as anticancer, anti-inflammatory, antioxidant, neuroprotective, antidiabetic, cardioprotective, antiulcer, and antifibrotic (Riaz et al. 2018). Little is known about the biological actions of isorhamnetin-3-O-glucoside (I3G). Lee et al. (2021) revealed that I3G improved insulin secretion, suggesting that it could be used to treat diabetes problems. However, bioavailability must be considered when determining clinical significance because I3G is poorly absorbed in its basic form (Kim et al. 2010). Kaempferol-O-glucuronides have been suggested to possess antioxidant and antibacterial properties as it is found in numerous plants, including *Thymus*, *Salvia*, and *Cichorium* species (Petropoulos et al. 2017, Semaoui et al. 2021, Ziani et al. 2019).

Rosmarinic acid and 3-O-methylrosmarinic acid are water-soluble phenolic compounds found in many plants, including those from the *Boraginaceae* and *Lamiaceae* families (Guan et al. 2022). It has a variety of pharmacological actions, including anti-oxidant, anti-apoptotic, anti-tumorigenic, and anti-inflammatory properties (Luo et al. 2020). Hydroxydodecenoic acid is a fatty acid and one of the active, measurable components of royal jelly (National Center for Biotechnology Information 2024b). More spilanthol-related amides were found in *Acmella ciliata* (Martin & Becker 1984). Both spilanthol and its 2,3-dihydro derivative bear ester

groups, a novelty for unsaturated amides (Martin & Becker 1984). Lineolic acid is an important fatty acid. Dietary LA has been shown to reduce atherosclerosis and coronary heart disease (CHD) (Farvid et al. 2014). In contrast to plants, most of the animals lack the enzymes required to produce LA (Malcicka et al. 2018). Essential fatty acids (EFAs) must be obtained through our diet. Adults need at least 7.5 grams of linoleic acid per day. When linoleic acid is lacking, the proportion of triglycerides carried by very low-density lipoproteins decreases (Collins et al. 1971). Additionally, a deficiency in alpha-linolenic acid (α-LNA) can result in reduced learning and visual function (Kinsella 1991). Hydroxyoctadecadienoic acids (HODEs) are stable oxidation products of linoleic acid that rise with oxidative stress, such as in diabetes (Vangaveti et al. 2016) or in the case of a very strenuous sporting activity (Nieman et al. 2016). Octadecanoids are enzymatic and non-enzymatic metabolites of monounsaturated or polyunsaturated fats. In recent years, these chemicals have been discovered to mediate a variety of biological processes, including nociception, tissue modulation, cell proliferation, metabolic regulation, inflammation, and immunological regulation (Quaranta et al. 2022).

No new amino acids were discovered when compared to previous literature. White et al. (1986) detected 20 amino acids in the *Acmella oleracea* plant, 14 of which we confirmed.

Biological activities

Previous studies have demonstrated several antibiotic effects of the plant *Acmella oleracea* (Table 3), but no one has included *Candida krusei* in their study. *Candida krusei* is a less virulent species than *Candida albicans*, but its importance is not negligible given its recent emergence as a nosocomial infection (Abbas et al. 2000). It is particularly common in leukemic and HIV-infected patients (Coppola et al. 1995). The introduction of human immunodeficiency virus infection and the widespread use of the newer triazole fluconazole to suppress fungal infections in these patients have contributed to a significant increase in *C. krusei* infection, especially given the fungus high resistance to this drug (Samaranayake & Samaranayake 1994). The currently known plant-based inhibitors of *C. krusei* fungus are the leaf of *Tecoma stans* (Patriota et al. 2016), flavonoids from *Psidium guajava* and *Plinia cauliflora* (Souza-Moreira et al. 2019, Fernandes et al. 2014), and the water-insoluble component of *Uncaria tomentosa* (Gómez-Gaviria & Mora-Montes 2020, Moraes et al. 2017).

Table 3. The antibiotic effect of *Acmella oleracea* extract based on different literature (Indications of the level of antibiotic activity: - no activity, + some activity, ++ strong activity).

Microorganism	The level of the activity	Reference to literature
Escherichia coli	+	Peretti et al. (2021)
	-	Present research
Bacillus cereus	-	Peretti et al. (2021)
Pseudomonas aeruginosa	++	Peretti et al. (2021)
	-	Present research
Micrococcus luteus	+	Peretti et al. (2021)
Klebsiella pneumoniae	+	Peretti et al. (2021)
	-	Present research
Klebsiella aerogenes	-	Present research
Staphylococcus aureus (two strains)	+	Present research
Streptococcus pyogenes	+	Present research
Streptococcus mutans	++	Peretti et al. (2021)
Aspergillus niger	+	Arora et al. (2011)
	++	Rani & Murty (2006)
Penicillum chrysogenum	+	Arora et al. (2011)
Rhizopus arrhigus	++	Arora et al. (2011)
Rhizopus stolonifer	++	Arora et al. (2011)
Fusarium oxysporium	++	Rani & Murty (2006)
Fusarium moniliformis	++	Rani & Murty (2006)
Candida albicans	-	Present research
Candida krusei	++	Present research
Candida glabrata.	–	Present research

In conclusion, thanks to the newly discovered active components of *Acmella oleracea*, the plant's medicinal potential is much broader than previously thought. Antidepressant and sedative effects are suspected. Its use in diabetes treatment requires further investigation, and it is also suggested to explore its cancer-preventive properties. We have also discovered a new plant-based *Candida krusei* inhibitor. Investigations into the medicinal use of plant-based medicines in nosocomial infections, leukemia, and HIV-infected patients may be recommended.

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