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Chemical variation, antimicrobial activity and toxicity of three South African species of *Salvia* used in Cape Herbal Medicine

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ABSTRACT

Salvia is the largest genus in Lamiaceae and includes many species of medicinal and pharmacological value. In South Africa, three blue-flowered species are used in Cape Herbal Medicine, yet *S. dentata* remains poorly studied. This study profiles and compares the phenolic composition of these three species and evaluates the antimicrobial activity and toxicity of *S. dentata.* Phenolic compounds were identified using UHPLC-MS, with carnosic acid and carnosol confirmed by NMR. The UHPLC-MS analysis revealed a distinct chemical profile for *S. dentata*, marked by high levels of rosmarinic acid (**7**), acetoxycarnosic acid (**13**), epirosmanol methyl ether (**16**), carnosol (**14**), and carnosic acid (**18**), closely resembling the profile of Mediterranean rosemary (*S. rosmarinus*). Antimicrobial activity was assessed using the microwell broth dilution assay, and toxicity by the brine shrimp lethality assay. Organic extracts of *S. dentata* showed notable antimicrobial activity, with MIC values of 0.13 mg/mL (against *Streptococcus pyogenes*) and 0.15 mg/mL (*Enterococcus faecium*), and moderate activity against *Staphylococcus aureus, Listeria monocytogenes* and *Streptococcus agalactiae* (MIC values of 0.57 mg/mL, 0.25 mg/mL and 0.23 mg/mL respectively). The extract of *Salvia dentata* was noted to be non-toxic. Out of the three species studied, *S. dentata* demonstrated the greatest promise as an anti-infective agent. However, the activity is not solely attributed to carnosic acid or carnosol, suggesting synergistic interactions between phenolics. The application of UHPLC-MS was key to revealing the distinct chemical profile of *S. dentata*, particularly its strong similarity to *S. rosmarinus*, an important new insight that expands the potential use of *S. dentata* beyond the traditional role in Cape Herbal Medicine.

1. Introduction

Throughout history, plants have been extensively documented in various roles, serving as both poisons for hunting and remedies for different ailments. The latter evolved from credible observations of animals self-medicating by consuming specific plant species, with this valuable knowledge transmitted across generations (Huffman, 2022; Patrício et al., 2022). *Salvia* L., the largest genus within the Lamiaceae, includes numerous medicinal species used worldwide in folk medicine to treat a wide range of minor illnesses. The well-known species, *Salvia officinalis* L., was used medicinally by ancient Egyptians, Greeks, and Romans, for purposes including improving memory, increasing fecundity, platelet aggregation, healing minor skin wounds, and treating throat concerns or coughs (Blumenthal et al., 2000). For that reason, members of this genus have been candidates for continual investigation for their therapeutic action and value.

Natural products, particularly those derived from medicinal plant species, continue to play an important role in the search for new and novel antimicrobial agents, especially those effective against antibiotic-resistant pathogens. In this context, *Salvia* species are notable for their broad-spectrum antimicrobial effects, as evidenced by their use in various herbal tea mixtures recommended to patients afflicted by tuberculosis and bronchitis, which helped alleviate symptoms and reduce perspiration (Kamatou et al., 2005).

South Africa is home to 24 indigenous *Salvia* species (14 of which are endemic), and 14 have documented ethnomedicinal uses by various cultural groups across the country (Rattray and Van Wyk, 2021). In the Cape region, three blue-flowered species (namely *S. africana* L., *S. chamelaeagnea* Berg. and *S. dentata* Aiton; **Fig. S1**) are used to treat a variety of ailments, which include coughs, colds, chest ailments, stomach complaints, topical afflictions, and influenza (Rattray et al.,

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2022). While *S. africana* and *S. chamelaeagnea* have been investigated chemically and pharmacologically, *S. dentata* remains poorly studied and is often assumed to be interchangeable with the other two species due to their morphological similarities and overlapping traditional uses.

This study addresses this knowledge gap by providing the first report on the non-volatile chemistry, antimicrobial activity, and toxicity of *S. dentata*. We further compare its chemical profiles and biological activity to the other two blue-flowered Cape species to clarify whether *S. dentata* is distinct in its phytochemical and pharmacological properties.

2. Material and Methods

2.1. Plant material collection

Fresh aerial parts of *Salvia africana* (n = 6), *S. chamelaeagnea* (n = 6), and *S. dentata* (n = 13) were collected from natural populations in the Cape region of South Africa. Sample localities, verification, and voucher information have been described in Rattray et al. (2022). The relevant collecting permit number from CapeNature is CN35-28-16005 and FLORA 0074/2021 from the Northern Cape Provincial Government.

2.2. Non-volatile (phenolic) extraction and characterization

A mass of 0.5 g of pulverized leaf material was extracted in 10 mL of methanol. The extraction was facilitated with agitation using a vortex for 60 s and put into an ultrasonic bath for 60 min. The ultra-high performance liquid chromatography (UHPLC-MS) analysis was conducted without dilution after the samples were removed from the centrifuge (14,000 rpm, 5 min).

The LC-MS analysis was performed at Stellenbosch University's Central Analytical Facility (CAF) in South Africa. The apparatus used was a Waters Synapt G2 Quadrupole time-of-flight (qToF) mass spectrometer (MS), which was integrated with a Waters Acquity ultra-high performance liquid chromatograph (Waters, Milford, MA, USA) for high-resolution UHPLC-MS analysis. The instrument method/parameters followed were identical to that used by Van Wyk et al. (2017).

The ionization technique employed was electrospray, functioning in both negative and positive mode with a set cone voltage of 15 V. The parameters included a desolvation temperature of 275 °C, and desolvation gas flow at 650 L/h. Data capturing spanned from m/z 150 up to 1500 in both resolution and MS^E configurations. The MS^E mode generated two streams of MS data; the first with a mild collision force of 6 V and the latter using an energy gradient (20–70 V) to produce fragmentation data. Leucine enkaphalin functioned as the lock mass for precise mass assessment, and sodium formate was used for calibrating the apparatus. The chromatographic step used a Waters HSS T3 column, measuring 2.1 × 150 mm with a 1.8 μm particle size. An aliquot of 5 μL was injected, and the solvent system comprised water with 0.1 % formic acid (designated solvent A) and acetonitrile with an identical formic acid concentration as solvent B. The solvent gradient was initiated with 90 % of solvent A for 20 s, transitioning linearly to 90 % B in 11 min. This was followed by a shift to 100 % B in 60 s, a cleansing phase of 1.5 min at 100 % B, and a subsequent return to the initial conditions over 4 min. The solvent flow was set at 0.3 mL/min, and the column's temperature was consistently held at 55 °C. The analysis lasted for a total duration of 15 min.

2.3. Antimicrobial screening

The plant material was ground to a fine powder and extracted using a 1:1 (v/v) blend of dichloromethane and methanol (HPLC grade, Sigma-Aldrich) in a shaker incubator maintained at 30 °C for 24 h. Following filtration, the solvents were evaporated under reduced pressure to yield crude organic extracts. The dried extracts were reconstituted in acetone to reach a final concentration of 32 mg/mL. To ensure the solubility of all constituents, 12.5 % DMSO (anhydrous, Merck) was added.

Bacterial strains were selected to reflect common respiratory and gastrointestinal pathogens relevant to the ethnobotanical uses of *Salvia* species. The test panel (Davies Diagnostics, South Africa) included Gram-positive strains: *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19111), *Streptococcus agalactiae* (ATCC 27953), *Streptococcus pyogenes* (ATCC 12344), and *Enterococcus faecium* (ATCC 27270), along with Gram-negative strains *Citrobacter freundii* (ATCC 44846), *Enterobacter cloacae* (ATCC 13406), and *Acinetobacter baumannii* (ATCC 19606). Each strain was cultured in Tryptone Soya broth (Oxoid) and incubated for 24 h at 37 °C.

Minimum inhibitory concentration (MIC) values of the extracts and isolated compounds were determined through a broth microdilution assay in a 96-well microtiter plate based on the protocols by Eloff (1998) and Van Vuuren and Naidoo (2010). Each well was filled with 100 μL of sterile broth, followed by 100 μL of plant extract at 32 mg/mL or isolated compounds at 1 mg/mL, added to the first row, followed by a serial dilution. Bacterial suspensions were standardized to a 0.5 McFarland turbidity and 100 μL of the broth added per well. To prevent contamination and evaporation, plates were sealed with sterile adhesive sealers (AEC Amersham) and incubated under conditions optimized for each bacterial strain.

Ciprofloxacin (0.01 mg/mL) served as positive control, while acetone, DMSO, and plain broth were used as negative controls. All tests were conducted in triplicate, and bacterial purity was confirmed by streak plating.

After incubation, 40 μL *p*-iodonitrotetrazolium iodide (INT; 0.20 mg/mL) solution (Sigma-Aldrich) was added to each well. Active bacterial growth resulted in a red colour change. The MIC was defined as the lowest concentration preventing this colour change.

Following Van Vuuren and Holl (2017), MIC values ≤ 0.16 mg/mL were interpreted as strong antibacterial activity, while those with MICs between 0.16 and 1.00 mg/mL were considered moderately active.

2.4. Toxicity screening

The toxicity of *S. dentata* organic extracts was evaluated using the brine shrimp lethality assay (BSLA) based on Hübsch et al. (2014). While *Artemia*-based assays are considered preliminary, they offer a cost-effective and ethically acceptable first-pass model for general toxicity, widely used in phytochemical studies. *Artemia franciscana* cysts (Ocean Nutrition™) were hatched in artificial saltwater (Tropic Marine™) under continuous illumination (220–240 V; Kiho) for 48 h.

Hatched nauplii were transferred to a 48-well microtiter plate, with each well receiving 400 μL of the saltwater and at least 40 live nauplii. The extracts were dissolved in 2 % DMSO to yield a starting concentration of 1.00 mg/mL. Potassium dichromate (1.60 mg/mL, Sigma-Aldrich) served as a positive control, while the negative control consisted of saltwater at 32 g/L. The absence of immediate toxicity was confirmed under a light microscope (Olympus, 40x magnification).

Mortality was recorded at 24 and 48 h. To confirm mortality, 50 μL of glacial acetic acid (100 % v/v, Sigma-Aldrich) was introduced to each well. Samples producing >50 % mortality were classified as toxic according to Bussmann et al. (2011) and were subsequently tested at serially diluted concentrations of 0.50, 0.25, 0.125, and 0.062 mg/mL. Mortality data were averaged over three independent replicates.

2.5. Isolation of carnosic acid, carnosol, and NMR analysis

Dried pulverized leaves (300 g) from *S. dentata* were extracted with a 1:1 (v/v) mixture of dichloromethane and methanol (DCM: MeOH) for 24 h at room temperature. The extract was filtered through Whatman 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator to yield a crude extract.

The crude extract was subjected to flash chromatography on silica gel (250 mL bed volume). The elution was performed sequentially with 500 mL of 20 % ethyl acetate in hexane, followed by 250 mL of 30 % ethyl acetate in hexane. Fractions were collected and monitored using thin-layer chromatography, observation under UV light and vanillin-sulphuric acid staining. Fractions exhibiting similar TLC profiles were combined.

Table 1
Peak assignment and identification of compounds in methanol extracts of *Salvia* species using literature data as a reference. Bold text represents the most prominent compounds across the three species as determined through inspection of the chromatograms (> 30 % peak area).

Peak	RT	m-H	ppm Error	DBE	Formula [m-H]	MS^E fragments	UV max	Tentative ID	Level of ID	Compound class	Reference
1	1.18	341.1084	2.3	2.5	$C_{12}H_{21}O_{11}$	89.0232, 119.0377, 179.0551	too low	**Caffeic acid 3-glucoside**	3	HCA	(Lim Ah Tock et al., 2021)
2	2.28	197.0425	4.6	9.5	$C_9H_9O_5$	135.0467, 87.0108, 357.1213, 197.0312	280	Danshensu	3	PAD	(Zhong et al., 2021)
3	3.24	387.1655	2.1	5.5	$C_{18}H_{27}O_9$	387.1666, 59.0140, 119.0370, 179.0411, 207.1042	300	Unknown 1	–	–	–
4	3.39	305.072	−4.6	0.5	$C_8H_{17}O_{12}$	161.0257, 305.0636, 197.0475, 225.1101, 96.9629	too low	Gallocatechin	3	FV	(Sharma *et al*., 2020)
5	4.12	461.0720	0.7	13.5	$C_{21}H_{17}O_{12}$	285.0410, 461.0738, 161.0259, 133.0302	280	Luteolin-3-glucuronide	3	FOG	(Hickl *et al*., 2018)
6	4.47	609.1819	1.6	12.5	$C_{28}H_{33}O_{15}$	299.0580, 284.0341, 607.1711, 161.0260, 135.0446	267sh, 326	Hesperidin	3	FOG	(Hickl *et al*., 2018)
7	4.67	359.0767	−1.4	11.5	$C_{18}H_{15}O_8$	161.0237, 197.0444, 359.0764	282sh, 331	**Rosmarinic acid**	3	PAD	RS
8	5.20	553.2649	−1.3	8.5	$C_{28}H_{41}O_{11}$	553.2562, 387.1638, 161.0174, 113.0210, 89.0234	321	Unknown 2	–	–	–
9	5.48	557.2598	−4.3	-1.5	$C_{27}H_{41}O_{12}$	455.3477, 135.0500, 161.0625, 183.0999, 285.0358	too low	Unknown 3	–	–	–
10	7.43	345.1702	−3.8	8.5	$C_{20}H_{25}O_5$	301.1802, 283.1674, 345.1684	275sh, 335	Rosmanol	3	TP	(Sharma et al., 2020; Zhong et al., 2021)
11	7.70	345.1702	−4.3	8.5	$C_{20}H_{25}O_5$	285.1866, 329.1746, 201.0906	332	Rosmanol isomer	1	TP	This study?
12	8.96	387.1808	0	9.5	$C_{22}H_{27}O_6$	327.1611, 343.1990, 283.1776, 487.3402, 227.1109	too low	**Unknown 4**	–	–	–
13	9.42	389.1964	0.8	8.5	$C_{22}H_{29}O_6$	345.2081, 285.1863, 389.1981, 801.3856, 203.1073, 59.0412	too low	**Acetoxycarnosic acid**	3	TP	(Castañeta et al., 2022)
14	9.74	329.1753	3	8.5	$C_{20}H_{25}O_4$	285.187	283	Carnosol	3	TP	(Sharma et al., 2020)
15	9.91	345.1702	1.2	8.5	$C_{20}H_{25}O_5$	301.1823, 231.1376, 201.0927	286	**Unknown 5**	–	–	–
16	10.21	359.1858	1.1	8.5	$C_{21}H_{27}O_5$	300.1730, 315.1948, 359.1850	too low	**Epirosmanol methyl ether**	3	TP	(Lim Ah Tock et al., 2021)
17	10.38	389.1964	1.8	8.5	$C_{22}H_{29}O_6$	329.1768, 301.1814, 229.1259	too low	**Unknown 6**	3	–	(Lim Ah Tock et al., 2021)
18	10.84	331.1909	1.5	7.5	$C_{20}H_{27}O_4$	287.2013, 331.1906, 288.2052	221	**Carnosic acid**	3	TP	RS
19	11.00	403.2121	0.7	8.5	$C_{23}H_{31}O_6$	343.1836, 403.2119, 328.1701, 59.0140, 344.1971	too low	**Unknown 7**	–	–	–
20	11.55	317.2117	0.6	6.5	$C_{20}H_{29}O_3$	317.2131, 179.1074, 318.2152	too low	Unknown 8	–	–	–
21	11.99	317.2117	−1.6	6.5	$C_{20}H_{29}O_3$	287.2016, 317.2117, 318.2148	226	Unknown 9	–	–	–
22	12.32	299.2011	−4	7.5	$C_{20}H_{27}O_3$	299.2006, 300.2042, 243.1358	225	Unknown 10	–	–	–
23	13.08	455.3525	−2.2	7.5	$C_{30}H_{48}O_3$	455.3531, 933.7006, 934.7024, 457.3690	none	Ursolic acid	3	TP	RS

RT = Retention time (min); Level of ID: 1 = Formula and mass correspond to literature, 2 = Formula, mass, and one fragment ion mass correspond to literature and 3 = Formula, mass, and two or more fragment ion masses correspond to literature.

Carnosol and carnosic acid were isolated as major constituents and subjected to nuclear magnetic resonance (NMR) spectroscopy for structural confirmation. Carnosic acid was dissolved in deuterated chloroform ($CDCl_3$), and carnosol in deuterated methanol (CD_3OD). NMR spectra were acquired on a Bruker 600 MHz spectrometer. Compound identities were confirmed by comparing the ^1H and ^13C NMR data with the published values reported by Schwarz and Ternes (1992).

2.6. Data analyses

Data processing and organisation were conducted in Microsoft Excel. Peak determination and preliminary compound identification from the UHPLC-MS data were carried out using MassLynx™ v4.1 (Waters, Milford, MA, USA), utilising mass spectral data for analysis. Multivariate analyses were performed in RStudio (RStudio Team, 2023; RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA, http://www.rstudio.com/) alongside MetaboAnalyst 4.0 (www.metaboanalyst.ca). The dataset was normalised based on the sample median and underwent log transformation. For data scaling, the Pareto scaling method was applied.

3. Results and discussion

3.1. Liquid chromatography–mass spectrometry

A total of 23 peaks of interest were identified in the chromatograms and their corresponding tentative identifications are listed in Table 1. Of the 23 peaks, 10 were tentatively identified, three were confirmed through reference standards, and 10 remain unknown.

The tentative identifications of the compounds were made through the consideration of molecular ions and the major fragments in most of the cases. The classes of compounds represented include a hydroxycinnamic acid **(1)**, flavonoid **(4)**, flavonoid-O-glucuronides **(5)** and **(6)**, phenolic acid derivatives **(2)** and **(7)**, and terpenoids **(10), (11), (13), (14), (16), (18)** and **(23)**. The unknown compounds and their classes include **(3), (8), (9), (12), (15), (17)**, and **(19–22)**. Prominent compounds both definitively and tentatively identified across the three species include rosmarinic acid **(7)**, acetoxycarnosic acid **(13)**, epirosmanol methyl ether **(16),** carnosol **(14),** and carnsoic acid **(18)**. Four prominent unknown compounds include **(12), (15), (17)** and **(19)**.

Salvia africana and *S. chamelaeagnea* presented similar chromatographic profiles, while *S. dentata* was dissimilar from the other two (Fig. 1). Notable compounds in *S. africana* and *S. chamelaeagnea* include rosmarinic acid **(7)**, acetoxycarnosic acid **(13)**, unknown 5 **(15)**, epirosmanol methyl ether **(16)** and unknown 6 **(17)**. These data correspond to other studies conducted on these two species by Kamatou and Viljoen (2007); Lim Ah Tock et al. (2021) and Lim Ah Tock (2022).

Notable compounds in *S. dentata* include carnosol **(14)**, carnosic acid **(18)**, unknown 9 **(21)** and unknown 10 **(22)** and lower levels of rosmarinic acid **(7)** as compared to the other two species.

3.1.1. Investigation of interspecies variation using UHPLC-MS data

A multivariate analysis of the non-volatile compounds of the *Salvia* data revealed three clusters, as seen in the PCA plot in Fig. 2 A. *Salvia africana* (red group) and *S. chamelaeagnea* (green group) share two clusters along the positive and negative axes of PC 1, while *S. dentata* (blue)

Cluster along the positive axis of PC 1, with a variation of 41.30 % across the three species. Four samples of *S. chamelaeagnea* and one of *S. africana* cluster along the negative axis of PC 2, while *S. dentata* cluster along the positive axis of PC 2. It is evident from the overlap of the clustering that *S. africana* and *S. chamelaeagnea* share a similar, though variable, chemical profile [as was previously also demonstrated by Lim Ah Tock (2021, 2022)], while samples of the hitherto unstudied *S. dentata* have their own distinct chemical profile that differs from those of the other two species. These relationships are further corroborated by the dendrogram seen in Fig. 2 B, with *S. dentata* all clustering in a single group (Cluster III). Cluster II, though sister to Cluster I, contains four *S. chamelaeagnea* samples and one *S. africana* sample. Cluster I includes five *S. africana* and two *S. chamelaeagnea* samples and is sister to Clusters I and II. Based on these patterns, *S. africana* and *S. chamelaeagnea* share similar chemical profiles, though the chemistry of *S. chamelaeagnea* is seemingly closer to that of *S. dentata* than *S. africana*.

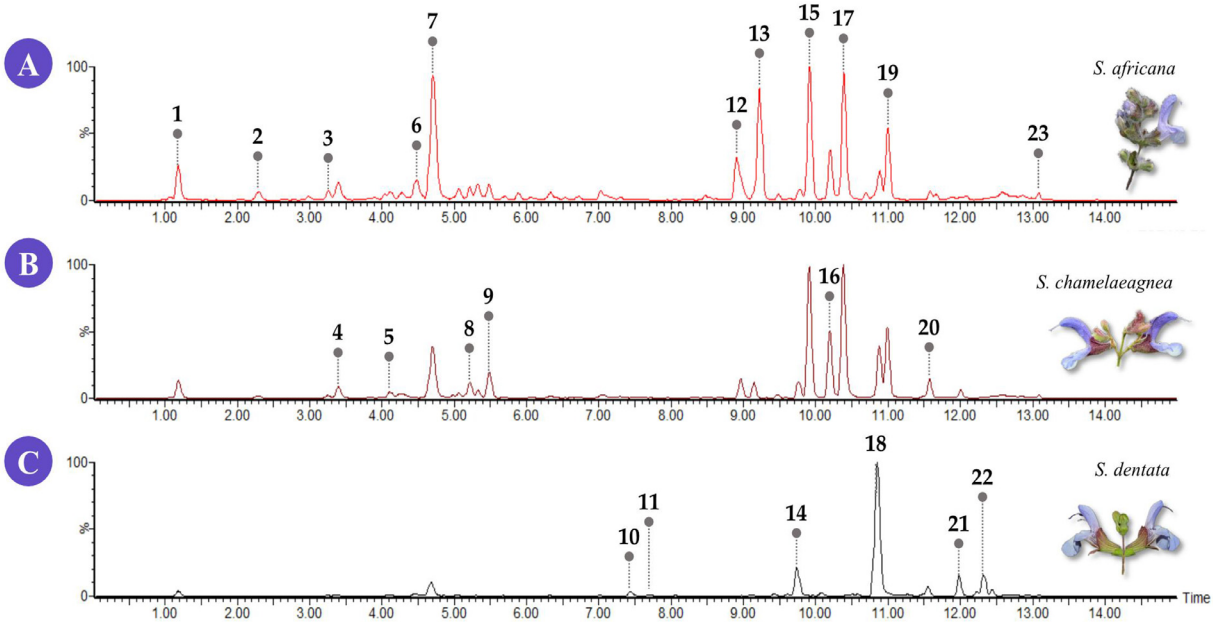

Fig. 1. UHPLC-MS chromatograms of the methanolic extracts of *Salvia* species. A) *S. africana*, B) *S. chamelaeagnea* and C) *S. dentata*. Major compounds are numbered above the corresponding peak, and identities are listed in Table 1.

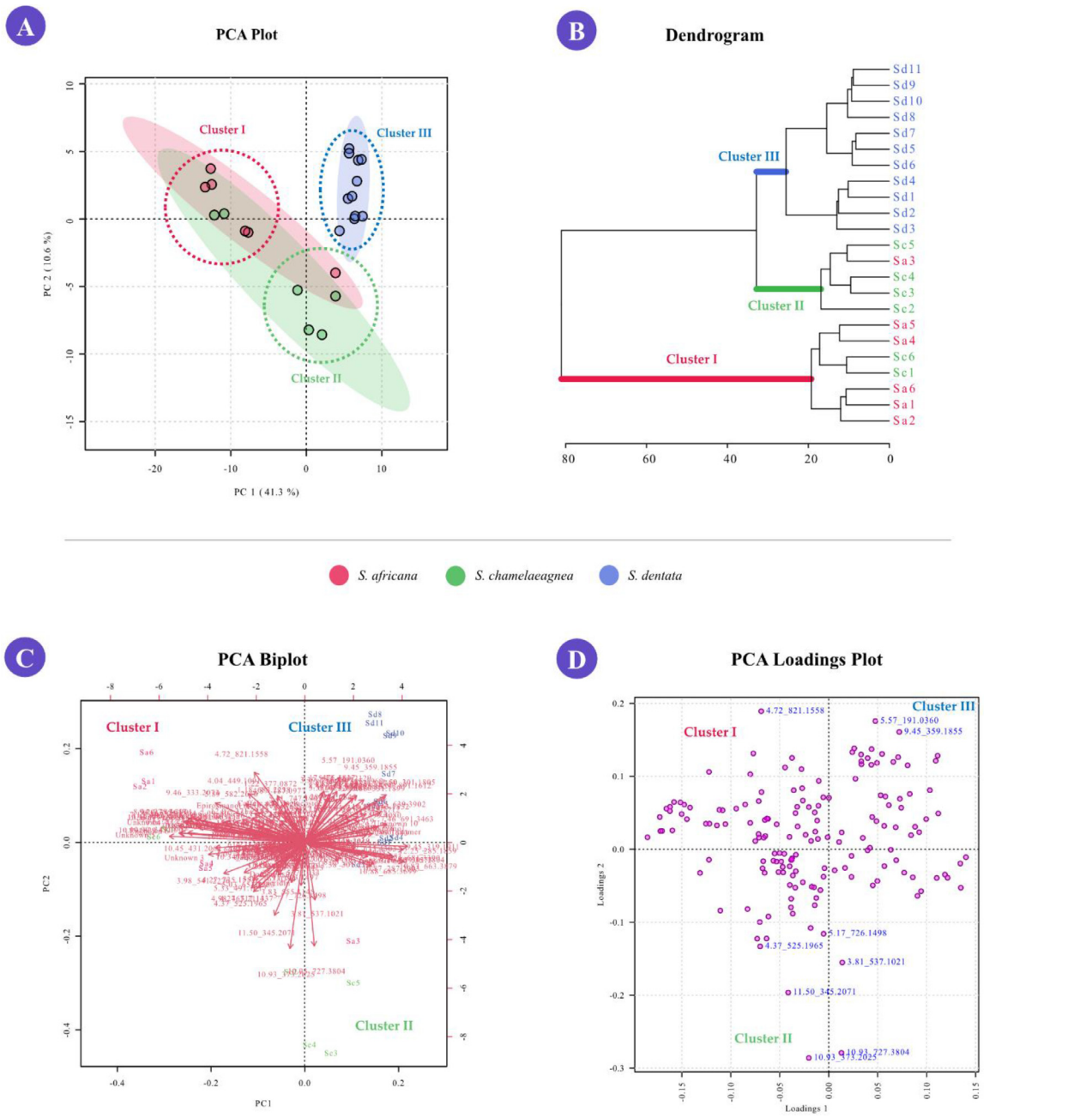

Fig. 2. Multivariate analyses of the non-volatile compounds of three *Salvia* species using UHPLC-MS data. A) PCA Plot and B) Dendrogram, both show the clustering of three clusters, namely Cluster I, II and III, C) PCA Biplot and D) PCA loadings plot.

The PCA biplot and loadings diagram in Fig. 2 C and D, highlight the non-volatile compounds responsible for the groupings seen in Fig. 2 A and B. The identities of many of the compounds remain unknown and require further analyses.

The concentrations of carnosic-, rosmarinic- and ursolic acids were quantified using reference standards, and the results are shown in Fig. 3 A–C and Table 2, respectively. *Salvia dentata* was observed to have the highest concentration of carnosic acid (CA) with a mean value of 10.00 mg/g, followed by *S. chamelaeagnea* (5.30 mg/g) and *S. africana* (4.50 mg/g). *Salvia africana* had the most variability, with concentrations ranging from 0.19 to 10.52 mg/g, followed by *S. chamelaeagnea*, with concentrations ranging from 0.17 to 10.44 mg/g. For rosmarinic acid (RA), *S. africana* exhibited the highest concentration with a mean value of 11.20 mg/g, followed by *S. chamelaeagnea* at 9.70 mg/g and *S. dentata* at 9.60 mg/g. *Salvia dentata* was the most variable for RA, with a 2.20–18.40 mg/g range. Ursolic acid (UA) concentrations were similar between *S. africana* (4.7 mg/g) and *S. chamelaeagnea* (5.0 mg/g). In comparison, *S. dentata* had the lowest mean concentration of 3.30 mg/g and was the most variable, with a range of ND to 7.40 mg/g.

These data are similar to previous reports on quantifying phenolic acids in southern African *Salvia* species. Lim Ah Tock et al. (2021) quantified phenolic acids in three species and observed an average of 4.65 mg/g RA, 4.81 mg/g CA and 15.40 mg/g UA in *S. chamelaeagnea*, 4.64 mg/g RA, and 19.60 mg/g UA in *S. lanceolata* and, 6.27 mg/g RA, and 22.00 mg/g UA in *S. aurea* – values more or less consistent with the data from our study. Kamatou et al. (2012) quantified RA in several *Salvia* species indigenous to southern Africa using HPTLC and HPLC techniques. They reported relatively high concentrations of RA ranging from 9.52 to 145.71 mg/g using HPTLC and 10.40–135.24 mg/g across 11 species. For *S. africana* and *S. chamelaeagnea,* they reported RA concentrations of 71.44 mg/g (HPTLC),

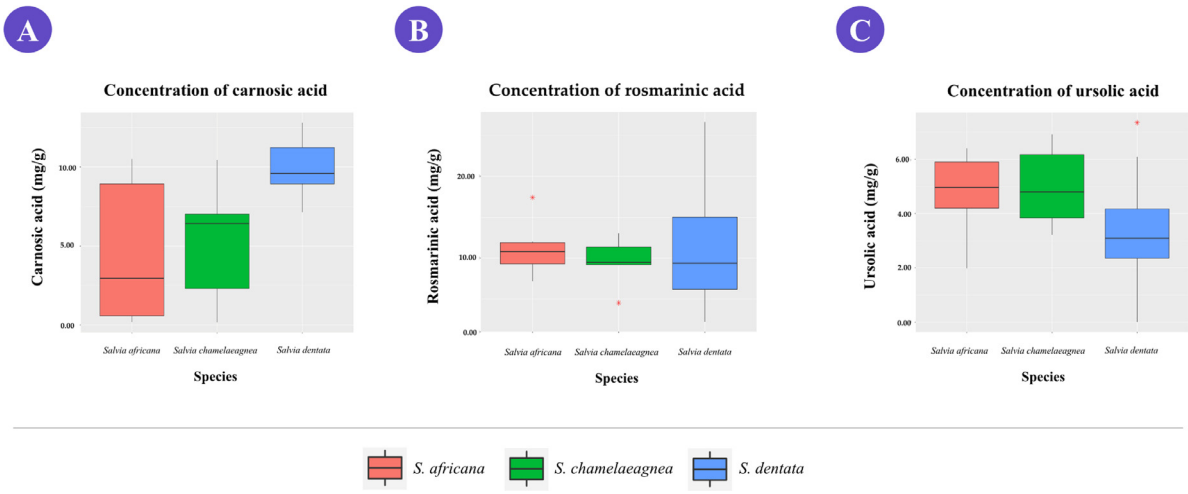

Fig. 3. Quantification of A) carnosic acid, B) rosmarinic acid and C) ursolic acid in the three *Salvia* species.

Table 2
Concentration of carnosic-, rosmarinic- and ursolic acid in the three *Salvia* species.

Species	Carnosic acid (mg/g)	Rosmarinic acid (mg/g)	Ursolic acid (mg/g)
S. africana	4.50 ± 1.60	11.20 ± 6.70	4.70 ± 1.90
S. chamelaeagnea	5.30 ± 4.80	9.70 ± 3.50	5.00 ± 1.60
S. dentata	10.00 ± 3.90	9.60 ± 2.90	3.30 ± 1.60

66.43 mg/g (HPLC), 76.53 mg/g and 71.46 mg/g (HPLC), for both species respectively – concentrations far higher than those reported from this study. The disparity in these results may be a result of the solvent systems used for phenolic acid extractions, the variation within each species as well as the environmental conditions from where each sample was collected.

Salvia limbata C.A.Mey. is a well-known medicinal species utilised to treat various ailments in the Middle East. A study by Jahani et al. (2022) quantified the RA content in *S. limbata* and reported a concentration average ranging from 6.27 to 8.67 mg/g, 4.07–6.62 mg/g and 3.93–5.53 mg/g across vegetative, flowering and seed setting stages respectively. Furthermore, various extraction methods yielded varying concentrations of RA, such as 67.00 mg/g from aqueous, 150.73 mg/g for hydroalcoholic and 120.28 mg/g for methanolic extracts.

It is evident from the comparisons that *S. dentata* may be a rich source of both CA and RA and could be utilised for further studies, industries, and applications.

Some biological and therapeutic activities of *Salvia*, Lamiaceae and other medicinal species have been ascribed to various compounds, such as phenolic acids produced by these species. Carnosic acid is the major phenolic diterpene that has been documented from rosemary (*S. rosmarinus* Spenn.) and has purported anti-tumour, antioxidant, antimicrobial and anti-obesity activity. Further, CA may undergo oxidative degradation and rearrangement cascade, increasing other anti-oxidative rosemary compounds, such as carnosol, gladosol, rosmanol and rosmariquinone. Carnosol's biological activity resembles CA and is reported to exhibit anti-inflammatory, anti-cancer, neuroprotective, and antioxidant activity (López-Jiménez et al., 2013; Mirza et al., 2023).

Rosmarinic acid, an ester formed from caffeic- and 3,4-dihydroxyphenyllactic acid, is commonly found across many plant species, particularly in medicinally significant herbs from the Lamiaceae and Boraginaceae families (Nunes et al., 2017). This compound is known for its diverse pharmacological properties, with studies documenting its antioxidant, antimicrobial, anticancer, anti-inflammatory, and cardioprotective effects (Nunes et al., 2017).

Ursolic acid is a pentacyclic triterpenoid widely distributed in nature and has been isolated from a wide variety of species, including rosemary, calendula (*Calendula officinalis* L.), melaleuca [*Melaleuca leucadendra* (L.) L.], apple [*Malus domestica* (Suckow) Borkh.], and eucalypts (*Eucalyptus* L'Hér. spp.) (Pironi et al., 2018). In recent years (by the time of this publication), the pharmacology and biological activity of UA have been widely investigated and reported to exhibit anti-inflammatory, anti-cancer, oxidant and antimicrobial activities (Khwaza et al., 2020).

3.2. Antimicrobial screening

The average MIC values of the organic extracts of *S. africana, S. chamelaeagnea,* and *S. dentata* are represented in Fig. 4. The MIC values ranged from 0.13 to 1.86 mg/mL across the three species. Organic extracts of *S. africana* exhibited the weakest activity against the pathogens, with MIC values >1.00 mg/mL against six of the eight pathogens (*S. aureus,* L. *monocytogenes, S. agalactiae, C. freundii,* and *E. faecium*) and two moderate values of 0.81 mg/mL (*S. pyogenes*) and 1.00 mg/mL (*E. cloacae*), respectively.

Salvia chamelaeagnea exhibited moderate antimicrobial activity against seven of the eight pathogens, with a range of 0.53–1.00 mg/mL for *S. aureus,* L. *monocytogenes, S. agalactiae, S. pyogenes, E. cloacae, E. faecium* and *A. baumannii.*

Salvia dentata demonstrated the most notable antimicrobial activity among the three species, with 0.13 mg/mL against *S. pyogenes* and 0.15 mg/mL against *E. faecium*. Furthermore, MIC values of 0.57 mg/mL, 0.25 mg/ML, and 0.23 mg/mL were observed for *S. aureus,* L. *monocytogenes,* and *S. agalactiae,* respectively. For *C. freundii, E. cloacae* and *A. baumannii* 1.00 mg/mL, 1.00 mg/mL, and 1.05 mg/mL were documented, respectively.

The antimicrobial activity of the extracts is similar to those reported by other studies for *S. africana* and *S. chamelaeagnea* (Table 3). In some cases, the MIC values reported from this study indicated stronger activity than those reported by the other studies. This is likely due to the choice of pathogens tested and the samples' provenance; as previously stated, various factors may influence the

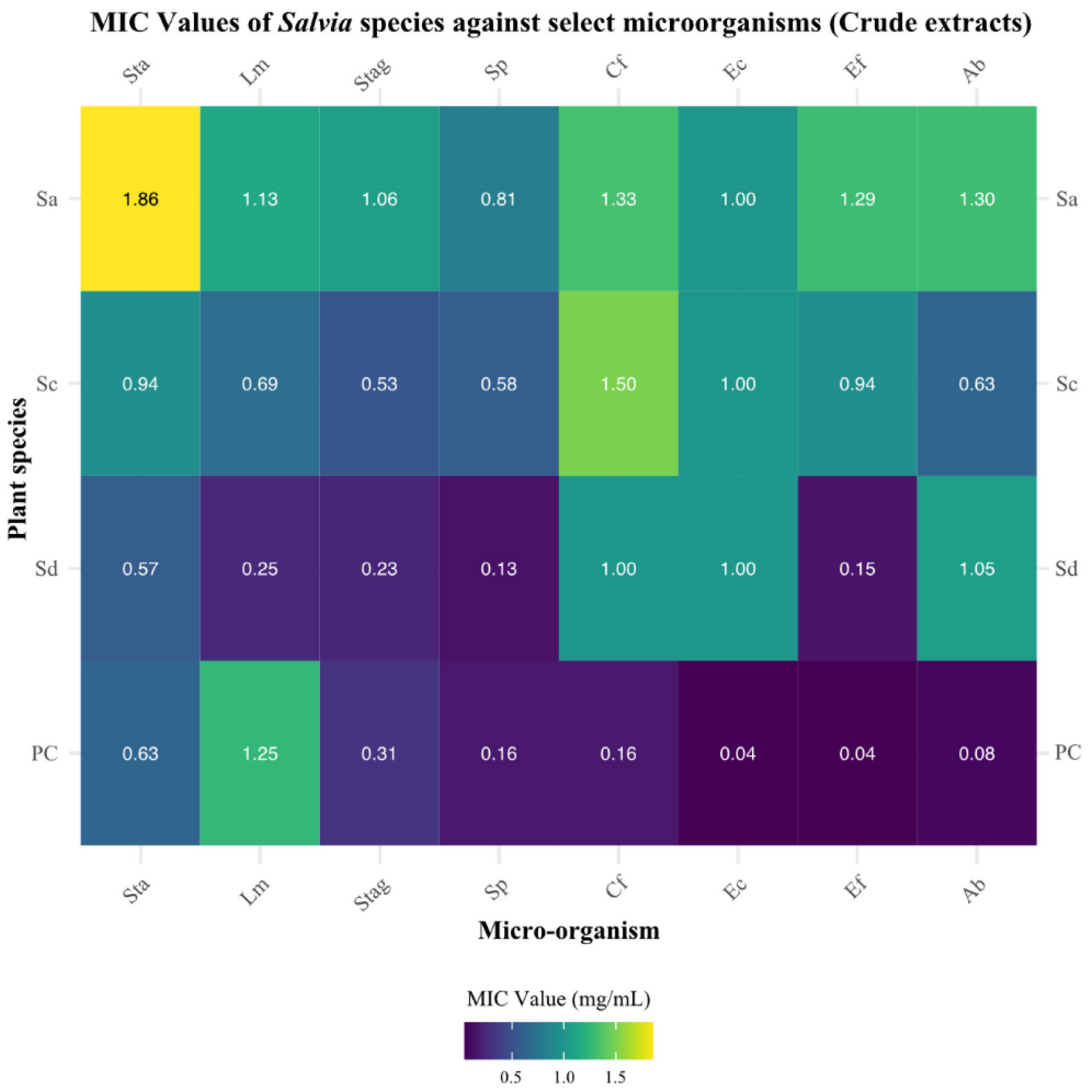

Fig. 4. Antimicrobial activity of *Salvia* extracts tested against respiratory, topical, throat and urinary tract pathogens (MIC values in mg/mL). Sa = *S. africana; Sc* = *S. chamelaeagnea*; Sd = *S. dentata*; PC = positive control (Ciproflaxin), NC = negative control (DMSO); Sta = *S. aureus*; Lm = *L. monocytogenes*; Stag = *S. agalactiae*; Sp = *Streptococcus pyogenes*; Cf = *Citrobacter freundii*; Ec = *E. cloacae*; Ef = *E. faecium*; and Ab = *A. baumannii*; noteworthy activity highlighted in purple; moderate activity highlighted in dark blue.

Table 3
Antimicrobial activity of southern African *Salvia africana* and *S. chamelaeagnea* extracts reported from other studies.

Pathogen tested	Sa	Sc	Reference
Escherichia coli	8.00	–	(Kamatou et al., 2008a)
K. pneumoniae	4.00	–	(Kamatou et al., 2008a)
B. cereus	0.40	–	(Kamatou et al., 2008a)
S. aureus	0.40	–	(Kamatou et al., 2008a)
E. coli	7.00 ($\bar{x}$)	–	(Kamatou et al., 2008b)
K. pneumoniae	5.12 ($\bar{x}$)	–	(Kamatou et al., 2008b)
B. cereus	2.38 ($\bar{x}$)	–	(Kamatou et al., 2008b)
S. aureus	1.53 ($\bar{x}$)	–	(Kamatou et al., 2008b)
B. cereus	<0.50	–	(Fisher, 2005)
B. cereus	<0.50	–	(Fisher, 2005)
S. aureus	–	0.24 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
B. cereus	–	1.38 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
E. faecium	–	0.86 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
B. subtilis	–	1.20 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
A. baumannii	–	0.43 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
P. aeruginosa	–	0.53 ($\bar{x}$)	(Lim Ah Tock et al., 2022)
E. coli	–	1.08 ($\bar{x}$)	(Lim Ah Tock et al., 2022)

Sa = *Salvia africana* and Sc = S. *chamelaeagnea*; ($\bar{x}$) = mean values calculated from studies.

activity, such as chemical composition of plant species, collection times, soil conditions, climate and genetics.

Based on the data, *S. dentata* demonstrated greater noteworthy and moderate antimicrobial activity than *S. africana* and *S. chamelaeagnea* against several pathogens tested. This may further corroborate its use as a medicinal plant for treating respiratory-related illnesses. However, carnosic acid, or carnosol, did not explain this antimicrobial activity against a selection of the organisms tested (Table 4). Carnosic acid and carnosol tested in this assay were isolated from *S. dentata*, as described in Section 2.5. The MIC values presented in Table 4 reflect the activity of the isolated, structurally confirmed compounds.

Table 4
MIC values of carnosic acid and carnosol (μg/mL).

–	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
Unidentified	–	312.5	–
Carnosic acid	250	156.3	78.1
Carnosol	650	650	650

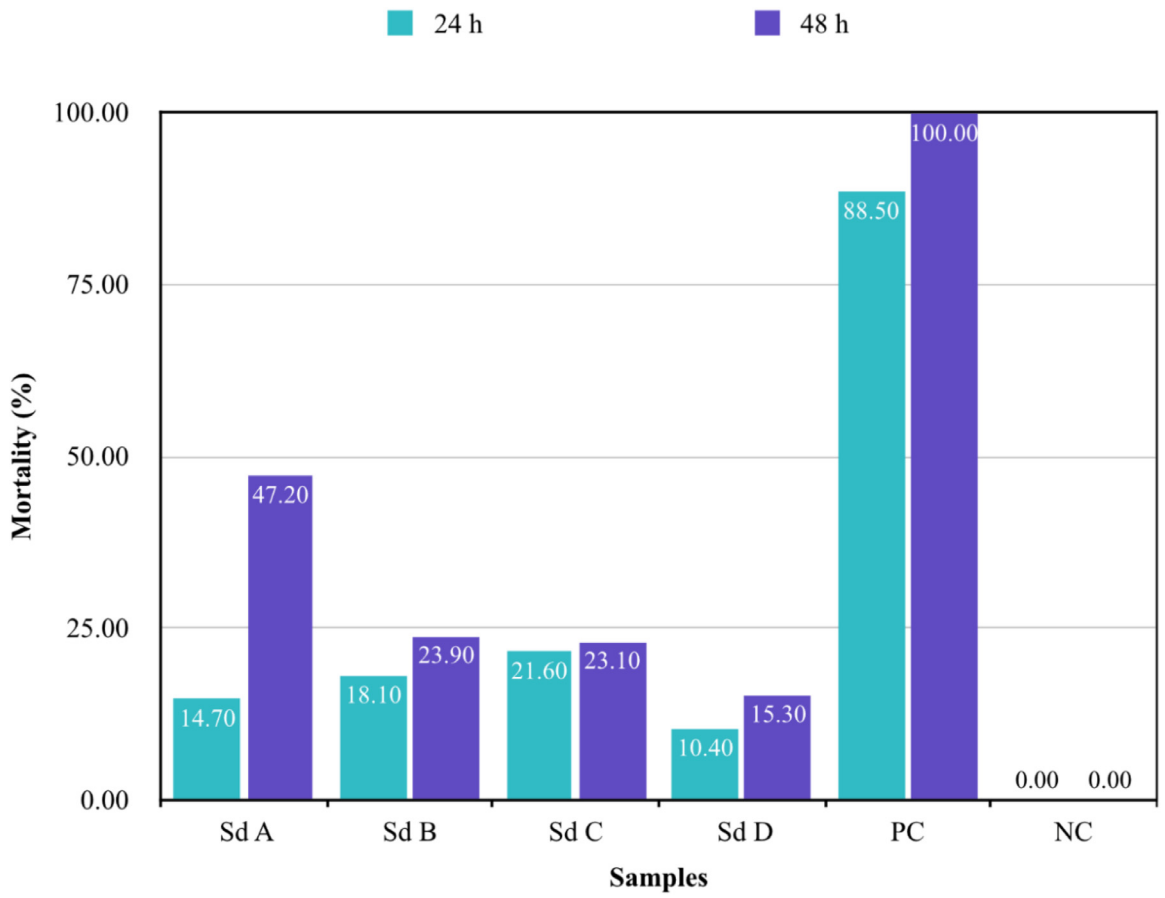

Fig. 5. Percentage mortality of *Artemia franciscana* tested against *Salvia dentata* extracts (Sd A-D. PC = positive control (potassium dichromate), NC = Negative control (sea water).

For example, pure carnosic acid produced an MIC value against *S. aureus* of 250 μg/mL, which is only half the value of the crude extract, yet its concentration in the crude extract is only 1 %. Similarly, carnosol gave a MIC value of 650 μg/mL. Lastly, ursolic acid (a known potent inhibitor of Gram-positive organisms) was present at a concentration significantly lower than the other two species of *Salvia* broached in the current study. Yet, these other species demonstrated substantially lower potency in the antimicrobial screening. Therefore, to understand the antimicrobial potency of *S. dentata* against the select organisms, further work is encouraged, examining synergisms, or screening for compounds with higher potency not identified in the current study. Furthermore, *S. dentata* is traditionally used for treating illnesses such as colds, influenza, and coughs – ailments likely associated with respiratory-afflicting pathogens, such as those tested in this study. These data suggest that the traditional uses of this species may likely be a direct result of the non-volatile constituents' ability to inhibit pathogens and exhibit other biological responses (i.e., anti-inflammatory, analgesic, and antioxidant activities) within the human body.

3.3. Toxicity screening

Fig. 5 presents the mortality rate of *Artemia nauplii* exposed to dichloromethane extracts from *Salvia dentata* for 24 and 48 h. All tested extracts showed mortality rates below 50.00 % for both time periods. While the negative control showed no mortality, the solvent control reached a 100.00 % mortality rate after 48 h. This suggests that the *Salvia dentata* extracts are generally non-toxic and safe.

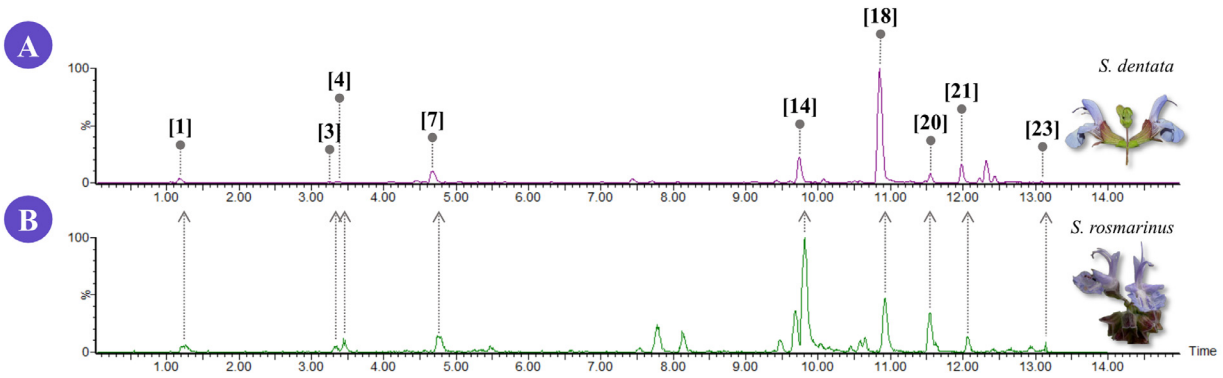

Fig. 6. UHPLC-MS chromatograms of A) *Salvia dentata* and B) *S. rosmarinus*. Compounds indicated in square brackets correspond to those listed in Table 1.

3.4. Salvia dentata - *southern Africa's indigenous rosemary?*

For comparative purposes, the non-volatile UHPLC-MS profile of *S. dentata* was compared to that of *S. rosmarinus,* as seen in Fig. 6. This data revealed a significant overlap in their phytochemical profiles, with several compounds shared between the two species. These compounds include caffeic acid 3-glucoside **[1]**, unknown 1 **[3]**, gallocatechin **[4]**, rosmarinic acid **[7]**, carnosol **[14]**, carnosic acid **[18]**, unknown 8 **[20]**, unknown 9 **[21]** and ursolic acid **[23]**. This overlap is significant, as rosemary is well-known for its culinary and medicinal uses, which include the treatment of various ailments such as headaches, epilepsy, stomach aches, spasms, and depression (Malvezzi et al., 2020; Rahbardar and Hosseinzadeh, 2020).

These findings appear to validate the use of *S. dentata* as a traditional medicine in the Cape region of South Africa (Rattray et al., 2022), especially for respiratory-associated ailments, as noted by the antimicrobial results. However, there are almost no similarities between the more diverse traditional and contemporary commercial uses of rosemary, suggesting that both the herb and the essential oil of *S. dentata* may have a much wider range of applications than the traditional medicinal uses would suggest.

4. Conclusions

This study presents the first account of the non-volatile chemistry and biological activity of *S. dentata*, with comparative insights into its close relatives, *S. africana* and *S. chamelaeagnea. Salvia dentata* was found to contain higher levels of rosmarinic acid, carnosol, and carnosic acid–bioactive compounds with known antioxidant and antimicrobial properties–positioning it as a viable candidate for targeted phytochemical extraction and use in the nutraceutical and cosmeceutical industries. *Salvia dentata* exhibited pronounced antimicrobial activity, particularly against *Streptococcus pyogenes* and *Enterococcus faecium*, supporting traditional medicinal uses in treating respiratory infections and may justify including this species in developing plant-based antimicrobial formulations. These findings not only validate traditional medicinal knowledge associated with *S. dentata* but also provide opportunities for possible commercial development and integration into evidence-based phytomedicine and quality-controlled products.

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Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the study's design; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

CRediT authorship contribution statement

R.D. Rattray: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **N.J. Sadgrove:** Writing – review & editing, Validation, Software, Methodology, Investigation, Formal analysis. **M. Oyedeji-Amusa:** Writing – review & editing, Methodology, Investigation, Formal analysis. **M.A. Stander:** Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Formal analysis. **S.F. Van Vuuren:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **B.-E. Van Wyk:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2025.06.014.

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