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An investigation of antiproliferative and antioxidant properties of crude extracts from *Sedum nicaeense All.* (Crassulaceae).

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Abstract: This study evaluated the antioxidant and antiproliferative effects of aqueous, ethanolic, and methanolic extracts of *Sedum nicaeense* flowers and leaves. The MTT assay assessed cytotoxicity against colorectal cancer cells (Caco-2, HCT-116), breast cancer cells (T47D, MCF-7) and normal fibroblasts (MRC-5), while the ferric-reducing antioxidant power (FRAP) assay measured antioxidant capacity. Essential oils from flowers and leaves were analyzed using gas chromatography-mass spectrometry (GC-MS). Methanolic and ethanolic flower extracts exhibited potent antiproliferative effects against HCT-116 cells, with IC_{50} values of 8.62±9.21µg/mL and 17.41±9.54µg/mL, respectively. The methanolic leaf extract showed strong activity against T47D cells (IC_{50}: 27.33±6.43 µg/mL). The antioxidant activity of the flower's methanolic extract and the leaves' methanolic and ethanolic extracts was comparable to ascorbic acid. GC-MS analysis revealed isopropyl myristate (21.63%) as the main component in flower oil, while 6,10,14-trimethyl-2-pentadecanone (18.09%) predominated in leaf oil. These findings highlight the therapeutic potential of *S. nicaeense*, with significant antiproliferative and antioxidant effects. The study provides a foundation for further exploration of its extracts and essential oils as candidates for biomedical applications.

Keywords: *Sedum,* antiproliferative, antioxidants, essential oil, cell lines.

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INTRODUCTION

Global health and the economy suffer significant threats from cancer, a disease that is a primary concern. It is responsible for nearly 1/6th of all recorded deaths in 2020, with roughly 10 million fatalities (Piñeros *et al*., 2021). Although chemotherapy, immunotherapy and targeted therapy have progressed in recent years, they have also been associated with many unavoidable side effects and complications (Liu *et al*., 2022).

Medicinal plants have long been used to address various human health conditions. Recently, natural products have emerged as pivotal sources for discovering life-saving drugs (Mahgoub *et al*., 2023). Medicinal herbs offer both effectiveness and safety compared to synthetic medicines, making them reliable natural remedies used for centuries as both food and medicine (Süntar, 2020).

Medicinal plants are rich in valuable components that exhibit diverse pharmacological effects, including antimicrobial (Mayyas *et al*., 2021; Althaher *et al*., 2024), anti-inflammatory (Al-Kafaween *et al*., 2023), hepatoprotective (El-Elimat *et al*., 2023), anticancer, and antioxidant properties (Althaher *et al*., 2021; Oran *et al*., 2022; Althaher and Mastinu, 2023; Althaher *et al*., 2024). This diversity underscores their immense potential in treating various ailments, offering hope for the future of healthcare.

They are unlocking medicinal plant potential for new anticancer drug discoveries. Vincristine, vinblastine, topotecan, docetaxel and paclitaxel exemplify their immense therapeutic potential (Asma *et al*., 2022). Despite persisting challenges in treating cancer and metabolic disorders such as diabetes mellitus and cardiovascular complications, there is a determined effort to develop more effective medications sourced from natural compounds (Sikalidis, 2019; Maity *et al*., 2022).

Sedum, a genus of the Crassulaceae family, comprises almost all herbaceous or shrubby plants that are typically succulent. These species are widely distributed across the northern hemisphere, including regions such as the Mediterranean, Asia, Africa and America (Nikulin *et al*., 2016). Sedum species have been recorded in Jordan in several areas, including Mafraq, Tafila, Jarash, Ajloun, and Amman (Al-Eisawi, 2013).

Sedum species are utilized in conventional medicine to manage persistent viral liver inflammation due to their rich content of secondary compounds such as tannins, alkaloids, flavonoids and arbutin. These compounds are predominantly present in the leaves and flowers of the plant. The medicinal properties of these plants have been

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well-documented in various studies (Kang *et al*., 2000; Moon *et al*., 2009; Szewczyk *et al*., 2012; Boriollo *et al*., 2020; Canli *et al*., 2021; Zong et al., 2023; Dalla Costa *et al*., 2024). Beyond their therapeutic uses and biological activities, *Sedum* species are also considered a valuable food source in East Asia, where they are highly regarded for their delicious taste in salads and soups. In particular, the leaves and flowers of *S. sarmentosum* are widely consumed as popular vegetable dishes (Jung *et al*., 2008).

Several studies have provided evidence of different *Sedum* species' antioxidant and cytotoxic properties (Huang *et al*., 2010; Mo *et al*., 2011; del Carmen Beltrán-Orozco *et al*., 2013; Bai *et al*., 2016; López-Montemayor *et al*., 2021; Qiu *et al*., 2022; Choi *et al*., 2023; Lim *et al*., 2023). These investigations, documented in various research papers, suggest that *Sedum* plants may have therapeutic potential for treating various ailments.

Exploring the potential of natural compounds to fight against life-threatening diseases is a noble and inspiring pursuit. With that in mind, the study sought to examine the antioxidant properties of various extracts derived from the leaves and flowers of *S. nicaeense*, as well as their potential antiproliferative effect on breast cancer (T47D and MCF-7), colorectal cancer (Caco-2 and HCT-116), and fibroblast cell lines.

MATERIALS AND METHODS

Chemicals and instruments

The MCF-7 human adenocarcinoma cells, T47D breast cancer cells, Caco-2 human colorectal adenocarcinoma cells, colon cancer cells HCT116 and MRC-5 human skin fibroblast cell lines were all sourced from the American Type Culture Collection®, which is the world's most extensive general service culture collection. The chemicals utilized in this study were commercially obtained and used without further purification. These included fetal bovine serum (Gibco, USA), Dulbecco's modified eagle medium (DMEM), L-glutamine, penicillin-streptomycin, trypsin-EDTA, and EDTA from Euro-clone, Italy, trypan blue stain, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and ascorbic acid from Sigma, USA. Other solvents and chemical reagents were procured from commercial vendors and employed following standard procedures, ensuring the reliability of the results. Anhydrous sodium sulfate (Analar, England. The essential oils were extracted from hydro-distillation using a Clevenger-type apparatus (JSGW, India). The chemical components of the oil were then analyzed through GC/MS/MS-200 (Satum, Netherlands) using a Variant chrompack CP-3800.

Plant materials collection

In spring 2019, *Sedum nicaeense's* leaves and flowers were gathered from the western mountains in Amman City, Jordan (31°58'18.9 N, 35°48'48.9" E). Prof. Sawsan Oran, a plant taxonomist, identified and authenticated the plant. The voucher specimen (SN-2019-04-02) was stored at the herbarium of the Department of Biological Sciences at the University of Jordan in Amman.

Preparation of crude extracts from flowers and leaves of S. nicaeense

The plants were air-dried for 3-4 weeks at room temperature (23-25°C) in the dark and then powdered. Forty grams of the powdered plant material were soaked in 400mL of distilled water, absolute ethanol, or absolute methanol. The mixtures were stirred using a hotplate magnetic stirrer, a crucial step that ensures thorough mixing and efficient extraction and allowed to stand for three days. The extracts were then filtered through the Whatman No. 1 filter paper. Solvents were eliminated using a rotary evaporator at 60 °C, and the crude extracts were dried thoroughly and stored at 4 °C for future experiments (Habtom *et al*., 2019). These extracts were then examined for their antioxidant capacity and their antiproliferative activity.

Sedum nicaeense essential oils extraction and GC-MS analysis

Separately, 250 grams of dried leaves and flowers from *S. nicaeense* were subjected to hydrodistillation for three hours using a Clevenger-type apparatus to extract essential oils. After getting the essential oils, they were dried with anhydrous sodium sulfate (Na_2SO_4) and kept at 4°C in sealed, opaque vials until they were analyzed. The percentage of essential oils (weight/weight) was determined via the following equation [1].

$$\text{The yield of extracted oil}\,\% = \frac{\text{Weight of extracted oil}}{\text{Weight of dry matter}} \times 100$$

The analysis of oil samples from *S. nicaeense* used a Varian Chrompack CP-3800 GC-MS-200 instrument with a DB-5 capillary column (30 m length, 0.25mm diameter, 0.25µm film thickness) composed of 5% diphenyl and 95% diphenyl polysiloxane. Helium was used as the carrier gas at a 1mL/min flow rate. The GC was equipped with an FID and an electron ionization source at 70 eV and 180°C. The initial temperature was 60°C, ramping at 3°C/min to 246°C and held for 3 min. Mass spectra were compared against NIST, Wiley and Adam-2017 libraries (Adams, 2017) and retention indices were also used for compound identification.

Ferric reducing antioxidant power assay (FRAP)

The study aimed to assess the antioxidant capacity of extracts from *S. nicaeense* leaves and flowers using a method based on Alabdallat and Bilto (2015). Extracts in methanol (1mL) (concentration range 50-1000µg/mL) were mixed with phosphate buffer (2.5mL) and potassium ferricyanide (2.5mL), incubated and treated with trichloroacetic acid (2.5mL). After centrifugation (10 min, 3000 rpm), supernatants were mixed with distilled water (2.5mL) and ferric chloride (0.5mL) and absorbance at

700 nm was measured. Increased absorbance indicated higher reducing activity, correlating with greater antioxidant capacity. The absorbance of the blank, containing phosphate buffer without plant extracts, was compared. Ascorbic acid was used as a positive control to compare antioxidant activity.

Cell culture

On a variety of human cancer cell lines-including colorectal adenocarcinoma (CaCo-2), human colon cancer (HTC-116), mammary gland carcinoma (MCF7), breast cancer (T47D) and normal human skin fibroblast (MRC-5), the cytotoxic effects of various plant extracts were assessed. 10% of the cells were grown in Dulbecco's modified eagle medium (DMEM), which contained 5mL of 1% L-glutamine (2mM), 5mL of penicillin-streptomycin (100µg/mL) and heat-inactivated fetal bovine serum (FBS). Until they reached 75-80% confluence, the cells were incubated in culture flasks at 37 °C with 5% carbon dioxide and 95% humidity. Phosphate buffered saline (PBS) was utilized to rinse the cells and then subjected to trypsin-EDTA treatment in preparation for single-cell suspension isolation. Ultimately, the number of viable cells was determined using the trypan blue dye exclusion assay (Bustanji *et al*., 2012).

Cell viability assay (MTT)

The assessment of cell viability using the MTT assay is a crucial process. In a 96-well plate, cells were cultured for 24 hours at 37°C and 5 percent CO_2 with a density of $1x10^4$ cells per well. One hundredµL quantities of plant extracts ranging from 3.125 to 200µg/mL in DMSO were applied, while the assay controls contained fresh media only and culture medium supplemented with 0.01% DMSO. Doxorubicin (0.05-50µg/mL) served as a positive control. To create formazan crystals, 20µL of 5mg/mL MTT in PBS was applied to each well and incubated for three hours, following a 72-hour incubation period. Following the removal of MTT, 200µL of DMSO was added to dissolve the crystals. A micro plate reader was used to measure the absorbance at 570 nm using a reference wavelength of 630 nm. In order to evaluate viability, absorbance measurements were normalized to assay control cells and background correction was applied by subtracting the absorbance of the negative control (Bustanji *et al*., 2012).

The following formula [2] was used to determine the percentage of survival cells:

$$\text{Percentage of survival cell} \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Where Abs: absorbance

The half-inhibitory concentrations (IC_{50}) were determined by measuring the extent to which the growth of each examined cell line was reduced by 50%. Three independent IC_{50} values were determined and averaged.

STATISTICAL ANALYSIS

Each experiment was replicated three times independently, and the results were presented as mean± SEM. The IC_{50} values for the different assays were ascertained by applying linear regression analysis. Using GraphPad Prism 5.0 (San Diego, USA), a one-way analysis of variance (ANOVA) was conducted. Subsequently, a Dunnett's multiple comparison test was performed to compare the IC_{50} of the tested plant extracts against the cancer cell lines with that of the normal cell line. The results were validated at a significance level of p-value < 0.05.

RESULTS

Sedum nicaeense oil extraction and phytochemical investigation

The hydro distillation process, meticulously conducted on the air-dried leaves and flowers of *S. nicaeense*, yielded a precise amount of colorless oil, measuring at 0.039% and 0.042% (w/w), respectively.

The essential oils extracted from *S. nicaeense* were analyzed using GC/MS to identify their chemical components. The examination revealed that the oil comprised twenty-two compounds in the flowers, accounting for 97.58% of the total. In contrast, the leaves contained thirty compounds, which constituted 97.81%. The extracts' GC-MS spectra are illustrated in figs. 1A and B.

The results indicated that isopropyl myristate is the primary constituent of the essential oil of *S. nicaeense* flowers, comprising 21.63% of the oil. Musk xylol is the second most abundant component, with a percentage of 12.65%. The volatile constituents are mostly oxygenated sesquiterpenes, representing 36.26% of the oil and containing musk xylol (12.65%), 2-hexyl-(E)-Cinnamaldehyde (5.43%) and ar-turmerone (3.64%). Non-aromatic compounds were also present in relatively high amounts (29.14%), with isopropyl myristate (21.63%), pentadecanoic acid (6.26%), and nonadecane (1.25%) being the most prevalent (table 1).

The essential oil extracted from *S. nicaeense* leaves contains the primary components 6,10,14-trimethyl-2-pentadecanone, accounting for 18.09% of the total composition. This is followed by β-N methyl ionone, which constitutes 10.14% of the oil. Most of the volatile constituents are oxygenated sesquiterpenes, representing 48.87%, with the main components being β-N methyl ionone (10.14%), Geranyl acetone (7.94%) and 2-hexyl-(E)-Cinnamaldehyde (5.43%). The non-aromatic compounds account for 39.32% of the oil, with 6,10,14-trimethyl-2-pentadecanone (18.09%) and 1-Undecyne (6.23%) being the most prevalent (table 2).

Table 1: Chemical composition of *S. nicaeense* flowers' essential oil analyzed by GC-MS

KI ^{Exp}	KI^{Lit}	Compound	% Peak area	Identification method
1033	1026	1,8-Cineole	1.44	MS-KI
1106	1099	α-Pinene oxide	1.64	MS-KI
1585	1577	Spathunelol	1.17	MS-KI
1600	1592	Veridiflorol	1.31	MS-KI
1615	1607	5-epi-7-α-Eudesmol	1.17	MS-KI
1669	1668	ar-Turmerone	3.64	MS-KI
1673	1672	5-iso-Cedranol	1.30	MS-KI
1678	1674	Salicylic acid, hexyl ester	1.17	MS-KI
1751	1748	2-hexyl-(E)-Cinnamaldehyde	5.43	MS-KI
1767	———	Octyl phenol isomer	1.36	MS
1774	1775	Cedryl methyl ketone	2.51	MS-KI
1787	———	Isopropyl myristate	21.63	MS
1805	1807	2-ethylhexyl-Salicylate	2.18	MS-KI
1826	———	Musk ambrette (dinitro type)	1.76	MS
1845	1837	Galaxolide (Musk derivative)	11.63	MS-KI
1856	———	1-(2-Fluoro-phenyl)-4,6-dimethyl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile	3.14	MS
1865	1866	Musk xylol	12.65	MS-KI
1877	1864	Benzyl salicylate	3.98	MS-KI
1883	1869	Pentadecanoic acid	6.26	MS-KI
1888	1879	Laurenene	2.76	MS-KI
1894	———	Unknown	2.43	-
1900	1900	Nanadecane	1.25	MS-KI
1911	1905	Isopimara-9(11),15-diene	8.20	MS-KI
Monoterpenes (MT)				
Hydrocarbons MT: 0				
Oxygenated MT: 3.08%				
Sesquiterpenes (ST)				
Hydrocarbons ST: 0				
Oxygenated ST: 36.26 %				
Diterpenes (DT)				
Hydrocarbons DT: 10.96%				
Oxygenated DT:11.63%				
Non-terpenoid non-aromatic compounds: 29.14%				
Non-terpenoid aromatic compounds: 6.51%				
Total identified%: 97.58%				

KI ^{exp}: retention indices relative to (C_8-C_{20}) n-alkanes, KI ^{lit} retention indices from literature. The identification method uses retention indices (KI) of authentic compounds on the DB-5 column; MS was identified by matching mass spectra with literature data and repositories maintained by NIST, Wiley and Adam's libraries.

In vitro antioxidant potential of S. nicaeense flowers and leaves crude extracts

The FRAP assay results for *S. nicaeense* show that methanolic extracts from flowers and leaves exhibit the highest antioxidant activity among the evaluated extracts (figs 2 A and B). At each tested concentration (50, 100, 250, 500 and 1000µg/mL), ascorbic acid consistently demonstrates the highest absorbance, indicating superior antioxidant activity. Methanolic extracts (ME) from flowers and leaves follow, showing significant antioxidant activity, albeit lower than ascorbic acid, with ethanolic extracts (EE) demonstrating moderate activity and aqueous extracts (AE) displaying the lowest activity. Statistical significance highlights the differences between the extracts and the control, with methanolic extracts consistently outperforming ethanolic and aqueous extracts but still significantly lower in activity than ascorbic acid. This underscores the potential of methanolic extracts of *S. nicaeense* flowers and leaves as potent antioxidants, though not as effective as pure ascorbic acid.

In vitro antiproliferative activity of S. nicaeense flowers and leaves crude extracts

The antiproliferative activity of crude extracts derived from the flowers and leaves of *S. nicaeense* was investigated with a panel of human cancer cell lines (MCF-7, T47D, Caco-2 and HCT-116) and a normal fibroblast cell line (MRC-5). The findings are illustrated in figs. 3 and 4. table 3 presents the IC_{50} (µg/mL) values for crude extracts derived from the flowers and leaves of *S. nicaeense* and the standard cytotoxic drug doxorubicin against the tested cell lines.

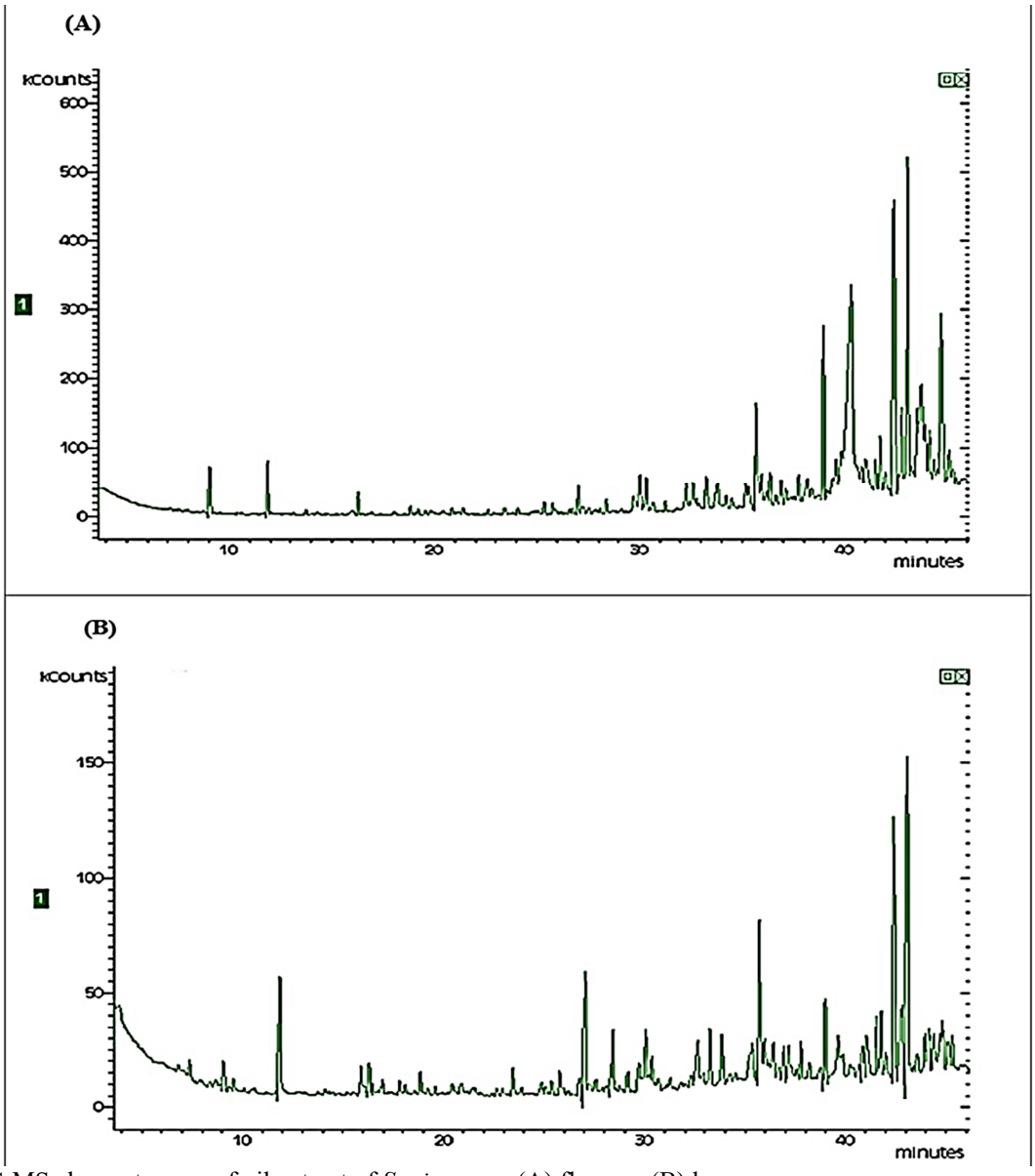

Fig. 1: GC-MS chromatogram of oil extract of *S. nicaeense* (A) flowers, (B) leaves.

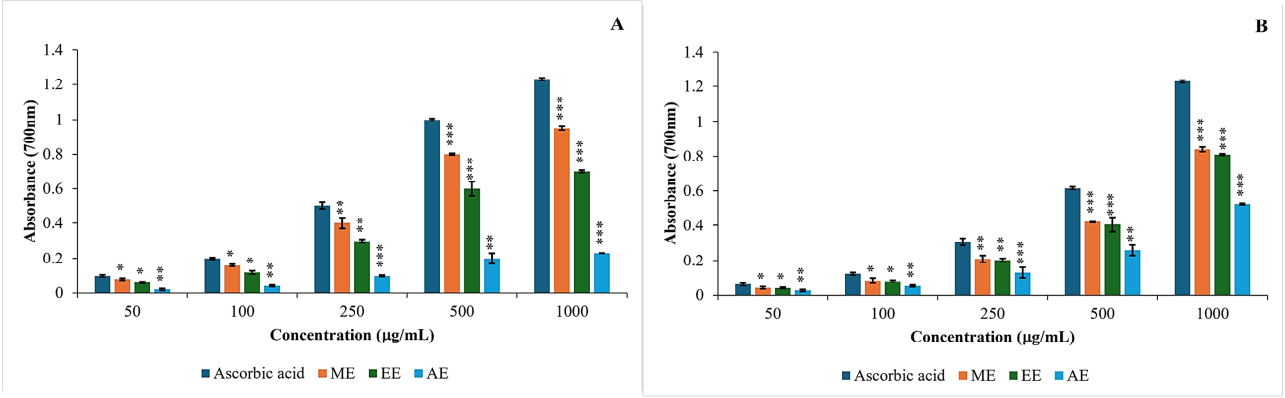

Fig. 2: Antioxidant activity of *S. nicaeense* extracts. (A) Flowers and (B) leaves tested using methanolic extract (ME), ethanolic extract (EE) and aqueous extract (AE) at concentrations ranging from 50 to 1000 µg/mL, measured by FRAP assay at 700 nm absorbance. Ascorbic acid served as the standard. Data are presented as mean ± SEM of three replicates. (*p<0.05, **p<0.01, ***p<0.001 compared to standard).

Table 2: Chemical composition of *S. nicaeense* leaves essential oil analyzed by GC-MS

KI ^{Exp}	KI^{Lit}	Compound	% Peak area	Identification method
889	894	5-Methyl-(3E)-hexene-Z-one	0.72	MS-KI
1033	1033	Eucalyptol	1.26	MS-KI
1106	1112	1-Undecyne	6.23	MS-KI
1208	1200	cis-4-Caranone	1.93	MS-KI
1369	1363	10-Undecanol	1.41	MS-KI
1452	1455	Geranyl acetone	7.94	MS-KI
1483	1488	β-Ionone	1.37	MS-KI
1522	1516	10-epi-Italicene ether	2.34	MS-KI
1530	1528	Lilial	1.18	MS-KI
1578	1576	Pentyl salicylate	0.85	MS-KI
1600	1600	n-Hexadecane	2.65	MS-KI
1616	1612	Tetradecanal	2.01	MS-KI
1655	1658	Patchouli alcohol	1.18	MS-KI
1664	1556	β-N methyl ionone	10.14	MS
1671	1666	Lyral	1.57	MS-KI
1682	1675	Salicylic acid, hexyl ester	0.81	MS-KI
1700	1700	n-Heptadecane	1.85	MS-KI
1718	1713	Cedroxyde	1.62	MS-KI
1751	1749	2-hexyl-(E)-Cinnamaldehyde	7.04	MS-KI
1768	1757	Ambroxide	1.04	MS-KI
1800	1800	n-Octadecane	2.69	MS-KI
1806	1803	14-hydroxy-δ-Cadinene	2.70	MS-KI
1820	1817	(2E,6E)-Farnesoic acid	2.72	MS-KI
1827	———	Musk ambrette (dinitro type)	3.05	MS
1845	1845	6,10,14-trimethyl-2-pentadecanone	18.09	MS-KI
1853	———	Musk xylene	1.19	MS
1856	———	Carboxylic acid derivative*	4.83	MS
1877	1860	Benzyl salicylate	1.54	MS-KI
1889	———	Unknown	1.54	-
1894	———	Unknown	2.29	-
1901	1900	n-Nonadecane	1.80	MS-KI
1914	1914	11,12-dihydroxy-Valencene	2.40	MS-KI
Monoterpenes (MT)				
Hydrocarbons MT: 0				
Oxygenated MT: 3.19%				
Sesquiterpenes (ST)				
Hydrocarbons ST: 0				
Oxygenated ST: 48.87%				
Diterpenes (DT)				
Hydrocarbons DT: 0				
Oxygenated DT: 1.04%				
Non-terpenoid non-aromatic compounds: 39.32%				
Non-terpenoid aromatic compounds: 5.49%				
Total identified %: 97.91%				

*N-(Tert-Butyloxycarbonyl)-1-amino-*cis*-2,3-dimethylcyclopropanecarboxylic acid.
KI ^{exp}: retention indices relative to (C8-C20) n-alkanes
KI ^{lit} retention indices from literature.
The identification method uses retention indices (KI) of authentic compounds on the DB-5 column; MS was identified by matching mass spectra with literature data and repositories maintained by NIST, Wiley and Adam's libraries.

Table 3: IC_{50} values (µg/mL) for three extracts of *Sedum nicaeense* flower, leaves, and doxorubicin.

	IC_{50} values Mean ± SEM (µg/mL) for three extracts of *Sedum nicaeense* flower.			IC_{50} values Mean ± SEM (µg/mL) for three extracts of *Sedum nicaeense* leaves.		Standard cytotoxic drug	
	Methanolic Extract	Ethanolic Extract	Aqueous Extract	Methanolic Extract	Ethanolic Extract	Aqueous Extract	Doxorubicin
MCF-7	77.96±9.18	77.60 ± 8.96	NI	79.62±6.08	120.50±3.02	NI	1.15±0.20
T47D	181.00±2.44	89.40 ± 9.4	NI	27.33±6.43	155.10±2.09	NI	2.10±1.30
CaCo-2	147.80±2.12	120.7 ± 2.01	202±2.11	152.50±3.72	143.50±3.65	NI	5.57±0.35
HCT-116	8.62±9.21	17.41 ± 9.54	NI	53.11±12.60	48.74±11.70	NI	1.30±0.54
MRC-5	NI	NI	NI	NI	NI	NI	28.82±3.23

NI: no inhibition.

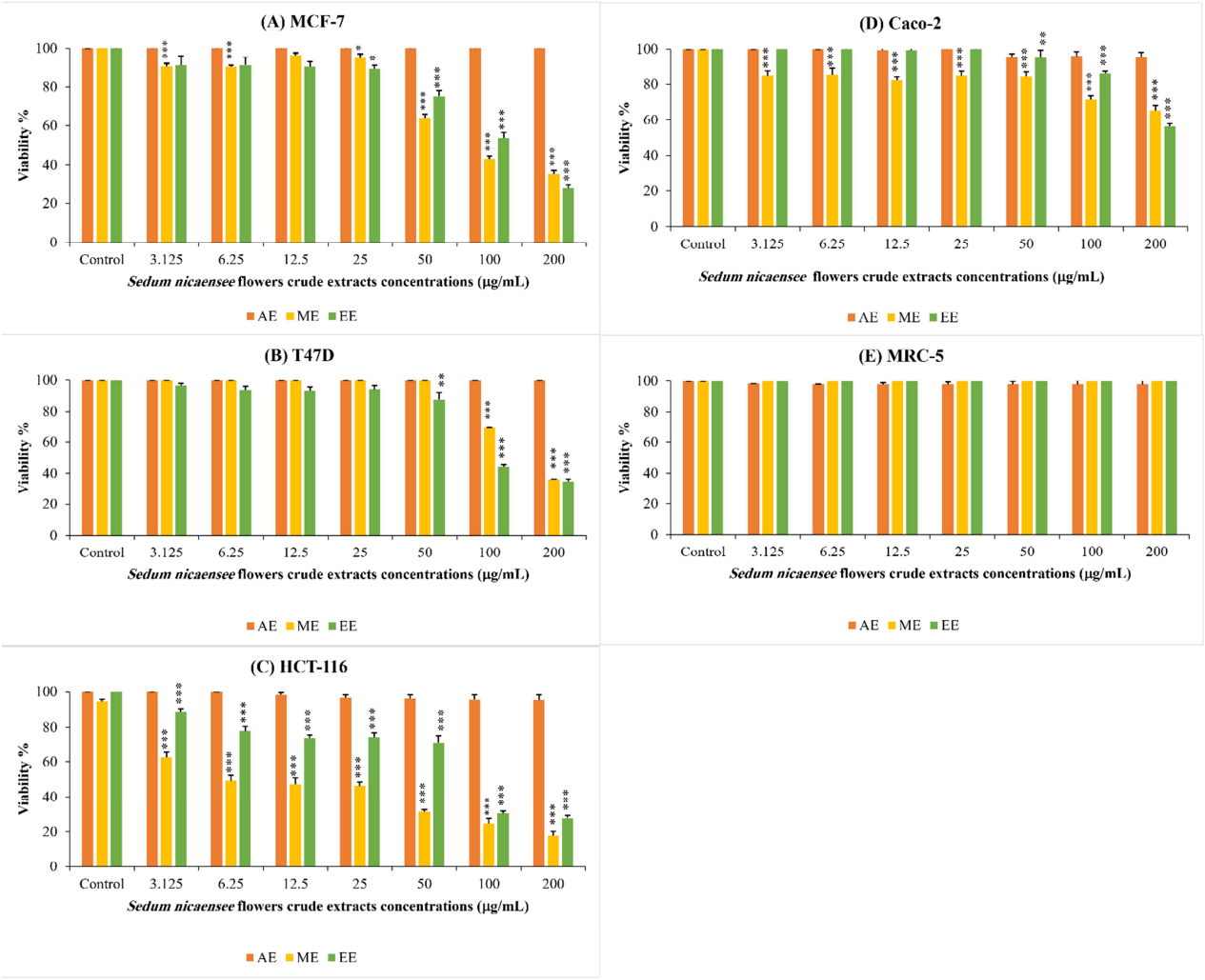

Fig. 3: The antiproliferative effect of aqueous (AE), methanolic (ME), and ethanolic (EE) extracts from *Sedum nicaeense* flowers against (A) MCF-7, (B)T47D, (C) HCT-116, (D) Caco-2 and (E) MRC-5. The data were represented as mean of three replicates ± SEM. (*p<0.05, **p<0.01, ***p<0.001 compared to the control (untreated cells)).

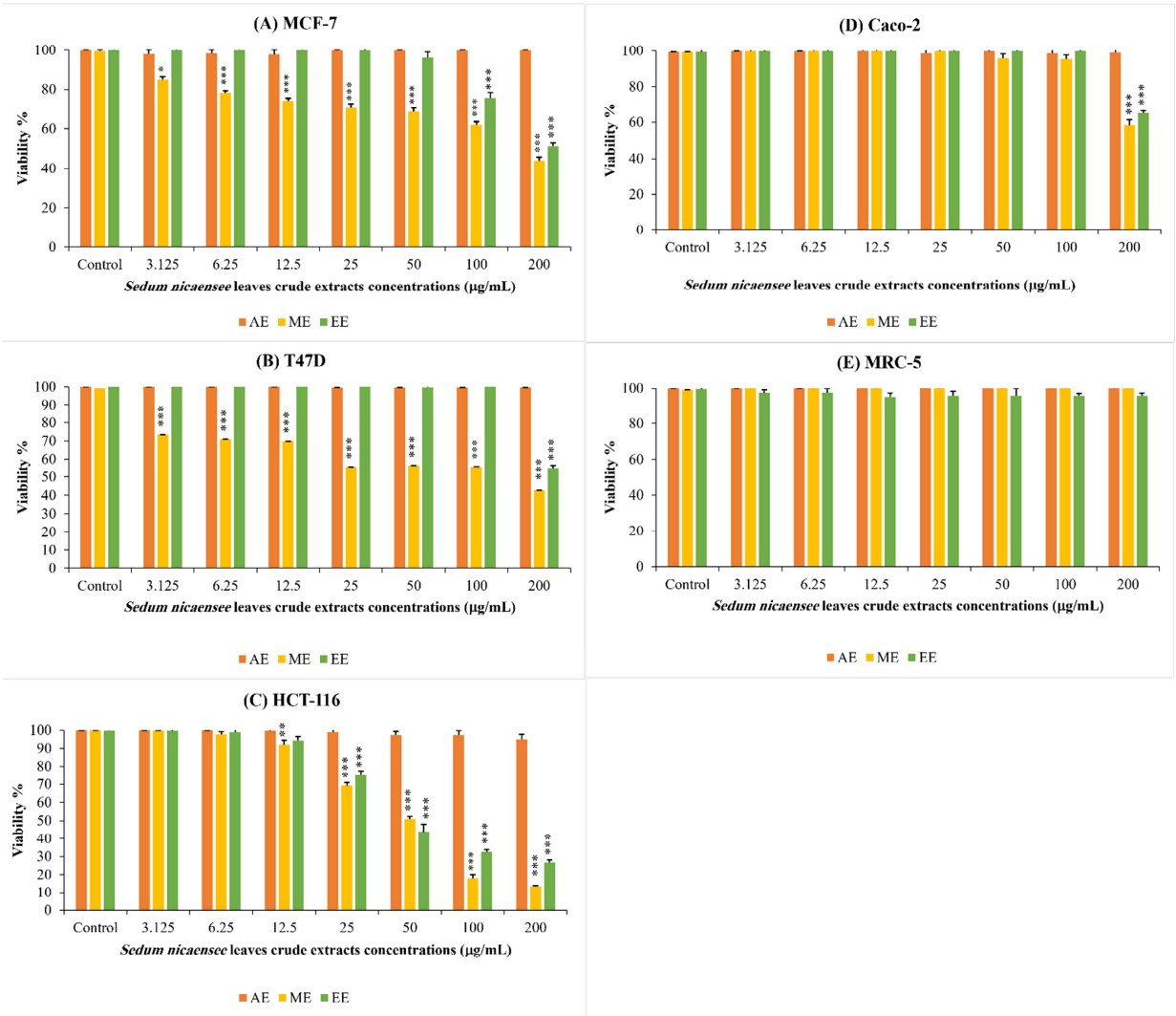

Fig. 4: The antiproliferative effect of aqueous (AE), methanolic (ME), and ethanolic (EE) extracts from *Sedum nicaeense* leaves against (A) MCF-7, (B)T47D, (C) HCT-116, (D) Caco-2, and (E) MRC-5. The data were represented as mean of three replicates ± SEM. (*p<0.05, **p<0.01, ***p<0.001 compared to the control (untreated)).

In general, the aqueous extract from the flowers and leaves of *S. nicaeense* has no significant effect on all tested cells (figs. 3 and 4). In contrast, the methanolic extract was observed to have a higher impact against cancerous cell lines than the ethanolic extract. It was selective towards the normal fibroblast cell lines (MRC-5), where both extracts had no effect.

In addition, fig. 3A demonstrated that methanolic and ethanolic extracts of *S. nicaeense* flowers had an influential effect against MCF-7 cells at concentrations of 3.125-200µg/mL compared to the control (untreated cells). Conversely, the methanolic and ethanolic extracts were found to have a significant effect against T47D at concentrations of 50-200µg/mL (fig. 3B). On the other hand, the effective concentrations of methanolic and ethanolic extracts on HCT-116 and Caco-2 were 3.125-200µg/mL (fig. 3C and 3D, respectively).

Table 3 shows that the methanolic extract derived from the flowers of *S. nicaeense* exhibited the highest antiproliferative activity against HCT-116 and MCF-7, with IC_{50} values of 8.62±9.21µg/mL and 77.96±9.18 µg/mL, respectively. Similarly, the ethanolic extract of *S. nicaeense* flowers also displayed excellent activity against HCT-116 and MCF-7, with IC_{50} values of 17.41± 9.54µg/mL and 77.60±8.96µg/mL, respectively.

On the other hand, figs. 4A and 4B demonstrated that methanolic and ethanolic extracts of *S. nicaeense* leaves had an influential effect against MCF-7 and T47D cells at concentrations (3.125-200µg/mL) compared to the control (untreated cells). On the other hand, the effective concentrations of methanolic and ethanolic extracts on HCT-116 were 12.5-200µg/mL (fig. 4C), while in Caco-2, the significant effect was only 200µg/mL (fig. 4D).

The methanolic extract of *S. nicaeense* leaves, on the other hand, was the most effective at stopping cell growth in T47D and HCT-116, with IC_{50}s of 27.33±6.43 and 53.11±12.60µg/mL, respectively. While the ethanolic extract of S. nicaeense leaves showed higher activity on HCT-116 and MCF-7 with IC_{50} values 48.74±11.70 and 120.50±3.02µg/mL, respectively, The standard cytotoxic drug (doxorubicin) demonstrates high cytotoxic activity against cancerous and non-cancerous cell lines without selectivity.

DISCUSSION

The study used GC-MS to analyze the essential oil composition of *S. nicaeense* flowers and leaves, identifying 22 compounds in flowers and 30 in leaves. Isopropyl myristate (21.63%) was the major component in flower oil, while 6,10,14-trimethyl-2-pentadecanone (18.09%) dominated leaf oil. Oxygenated sesquiterpenes were the most abundant compounds in both oils, comprising 36.26% and 48.87% of flower and leaf oils, respectively. Conversely, oxygenated monoterpenes (3.08%) and diterpenes (1.04%) were the least prevalent in flower and leaf oils, respectively.

Using GC-MS, researchers analyzed the essential oils of two *Sedum* species in Turkey: *S. pallidum* var. *bithynicum* and *S. spurium*. They found 38 and 35 components, respectively, with high percentages of sesquiterpenes (30.7% and 27.7%). This aligns with findings from *S. nicaeense*, which also had a high sesquiterpene content in its flowers and leaves. Significant components of *S. pallidum* var. *bithynicum* and *S. spurium* oils were caryophyllene oxide (12.8%) and hexahydrofarnesyl acetone (15.7%), respectively. Notably, geranyl acetone (3.6%) was a significant component of *S. spurium* (Yaylı *et al*., 2010), like geranyl acetone (7.94%) in *S. nicaeense* leaves.

The essential oil of *S. pallidum* flowers has 40 compounds, and the primary component is hexadecanoic acid (33.5%) (Dahpour *et al*., 2012). Moreover, the main constituent of essential oil from *Sedum sediforme* (Jacq.) Pau is α-selinene (20.4%) (Ertaş *et al*., 2014).

Other research done in Jordan discovered that the *S. microcarpum* (Sm.) species has a lot of oxygenated monoterpenes (36.39%), with myrtenol making up 21.7% of the species (Al-Qudah *et al*., 2012). Furthermore, during the pre-flowering (PF) and full-flowering (FF) phases, oxygenated sesquiterpenes (21.92%) and aliphatic hydrocarbons (45.71%) predominated in the essential oil of *S. rubens*. There are 28 compounds in the PF stage of Sedum rubens oil. 7-epi-α-eudesmol (7.81%) and (E)- β-damascenone (16.34%) were the primary ones. Whilst in the FF stage, 31 members were identified, with the undecanal being the main component (29.23%) (Odeh *et al*., 2023).

Additionally, this study rigorously examined the antioxidant and antiproliferative properties of crude extracts from *S. nicaeense* flowers and leaves. The antioxidant activity was meticulously measured using the ferric-reducing antioxidant power assay (FRAP), a method extensively used to assess the antioxidant power of plant extracts. This assay involves reducing a ferric-tripyridyl triazine complex to ferrous; the color change from yellow to green indicates the presence of antioxidant compounds (Namet *et al*., 2018). The study's rigorous approach revealed that the *S. nicaeense* methanolic flower extract exhibited the highest antioxidant potential, followed by the leaf extract. The ethanolic extracts from both parts showed moderate antioxidant activity, while the aqueous extract of *S. nicaeense* flowers and leaves demonstrated a lower antioxidant potential.

Many studies have assessed the antioxidant capacity of various extracts obtained from different parts and species of *Sedum*. The methanolic extract of *S. sarmentosum* leaves has potent antioxidant effects like butylated hydroxytoluene (BHT) (Mo *et al*., 2011). *Sedum praealtum* ethanolic extract from the aerial parts had a strong antioxidant effect because it contained phenolic compounds (del Carmen Beltrán-Orozco *et al*., 2013). Additionally, the antioxidant activity of *S. sediforme extracts* was assessed *in vitro*. The crude extract (CrE) showed high enzymatic and non-enzymatic scavenging activities, with IC_{50} values of 0.063±0.005mg/mL and 0.178±0.006mg/mL, respectively. The chloroform extract (ChE) exhibited excellent chelating activity with an EC_{50} of 0.397±0.001mg/mL (Trabsa *et al*., 2020). *Sedum dendroideum* lyophilized extract has significant antioxidant properties, with a total phenolic content of 33.67mg GAE/g and an antioxidant activity of 384.49 mM Trolox equivalents/mL (López-Montemayor *et al*., 2021). On the other hand, the full-flowering hydroalcoholic extract from *S. rubens* L. showed the highest total phenol content (136.9 mg gallic acid/g extract), total flavonoid content (234.7 mg quercetin/g extract), and vigorous DPPH radical scavenging activity (7.10 × 10-2 mg/mL) (Odeh *et al*., 2023).

These findings underscore the potential of *Sedum* plants as a rich source of natural antioxidants, offering a promising avenue for further research and potential health benefits.

The antiproliferative activity in the present study was evaluated *in vitro* using an MTT assay and the results exhibited that *S. nicaeense* flowers and leaves methanolic extracts have the highest antiproliferative effect on cancer cell lines, especially in the colon cancer HCT-116 cell line and breast cancer T47D and MCF-7, respectively. The ethanolic extracts from both parts also had cytotoxic effects on the colon cancer HCT-116 cell line and breast cancer MCF-7, respectively. In comparison, all aqueous

extracts have weak antiproliferative activity. All tested extracts of *S. nicaeense* flowers and leaves did not show significant activity against normal human fibroblast cells (MRC-5).

Doxorubicin, a well-established benchmark in cancer treatment, is chosen as a controlled drug in studies on natural extracts like those from S. nicaeense. It exhibits strong cytotoxic effects on cancerous and non-cancerous cell lines, albeit lacking selectivity. Its documented effectiveness against various cancer types makes it a reliable reference point. The comparison of natural extracts to doxorubicin is not just a step in the research process but a crucial one. It is this comparison that gauges the potency and potential therapeutic benefits of natural extracts, enhancing scientific rigor and providing a much-needed standard reference for interpretation in research and clinical contexts.

Compared to the present investigation, the aqueous extract derived from *S. sarmentosum* exhibited a cytotoxic impact on HepG2, a human hepatoma cell line (Huang *et al*., 2010). Additional research has shown that the *S. sarmentosum* Bunge extract has the potential to inhibit the growth of pancreatic cancer cells (PANC-1) and trigger apoptosis. The activation of Hedgehog signaling was found to be responsible for this effect (Bai *et al*., 2016). Furthermore, *S. dendroideum* was observed to decrease the proliferation of human pterygium fibroblasts (HPFs) (López-Montemayor *et al*., 2021). Additionally, the ethyl acetate extract of *S. emarginatum* was found to have a robust antiproliferative effect on human liver cancer HepG2 cells (Qiu *et al*., 2022). These outcomes suggest that *Sedum* species could have potential antiproliferative activity. Choi *et al*. (2023) investigated the effects of *S. middendorffianum* Maxim extract on human ovarian cancer cells. The researchers found that the extract induces apoptosis (programmed cell death) and inhibits the invasion capabilities of these cancer cells. The mechanism behind these effects is linked to regulating oxidative stress within the cells.

The extract effectively modulates oxidative stress, promoting cell death and reducing the metastatic potential of ovarian cancer cells. This implies its strong potential as a therapeutic agent in cancer treatment. The great tumor-fighting potential of limocitrin has been demonstrated by its discovery in *S. sarmentosum* Bunge. The MDA-MB-231 and MCF-7 cell lines, two distinct forms of breast cancer, were the subjects of this study, which focused on triple-negative breast cancer (TNBC). Limocitrin reduced cell viability with IC values of 29.33±0.010 μM and 28.70 ±0.030μM, respectively. It led to cell death through apoptosis, increased the number of cells undergoing apoptosis, and increased the levels of proteins related to apoptosis. It also reduced the levels of proteins that help cells survive, such as Akt, Bcl-2 and mTOR. The different responses in the two types of cells suggest that limocitrin affects their ability to survive in various ways. This supports the need for more research into limocitrin as a potential treatment for breast cancer, especially TNBC (Lim *et al*., 2023).

CONCLUSION

This study comprehensively explored the antioxidant and antiproliferative properties of extracts from *Sedum nicaeense* flowers and leaves. Methanolic and ethanolic extracts from *S. nicaeense* flowers demonstrated potent cytotoxic effects against HCT-116 colorectal cancer cells, with notable IC_{50} values. In contrast, the methanolic extract from *S. nicaeense* leaves significantly inhibited T47D breast cancer cell proliferation. Moreover, these extracts exhibited substantial antioxidant activity comparable to ascorbic acid. Analysis of essential oils from *S. nicaeense* revealed prominent constituents, shedding light on potential bioactive components.

These results underscore the potential of *S. nicaeense* in antioxidant therapy and cancer treatment. Future research should concentrate on uncovering the underlying mechanisms of these bioactivities through detailed mechanistic studies and confirming efficacy *in vivo*. Exploring synergistic effects with conventional cancer therapies could enhance treatment outcomes. Further chemical characterization of essential oils and identifying active compounds will provide deeper insights into their pharmacological potential.

Ultimately, harnessing the therapeutic benefits of *S. nicaeense* could lead to the development of novel treatments for cancer and oxidative stress-related disorders. This could open up new avenues in natural product-based medicine, potentially revolutionizing patient outcomes.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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