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Exploring the antioxidant, anticancer properties and phenolic composition of *Anchusa strigosa*

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Abstract: This study utilised *A. strigose*, a herbaceous plant widely used in folk medicine and commonly distributed in the Middle East. The antioxidant activity in the extracts of this plant has been underscored. It employed the High-Performance Liquid Chromatography (HPLC) technique to separate and identify phenolic compounds and evaluate their activities. Ex vivo biological activity was assessed through the utilization of the DPPH (2,2-diphenyl-1-picrylhydrazyl), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and HCs (healthy controls) tests. The DPPH test was employed to evaluate the antioxidant activity of the separated phenolic compounds in vitro. Phenolic compounds showed varying antioxidant activity on two cancer cell lines, sarcoma RSAR001 and melanoma A375. Based on cytometric indicators, the results demonstrated that the separated compounds induced apoptosis in cancer cells. The results of the MTT test revealed that the fraction (I) applied to sarcoma (RSAR001) exhibited inhibition percentages of 35.30%, 55.99%, 64.21%, 72.34%, 4.47% and 24.96% at concentrations of 12.5, 25, 50, 100, 200 and 400µg/ml, respectively. Similarly, the fraction (II) tested on RSAR001 showed inhibition percentages of 5.90%, 21.60%, 35.61%, 46.53%, 4.40% and 5.23% at concentrations of 12.5, 25, 50, 100, 200 and 400µg/ml, respectively. These findings suggest that *A. strigosa* possesses potential antioxidant and anticancer activities.

Keywords: *Anchusa strigose,* HPLC, extraction*,* phenolic*,* DPPH, MTT.

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INTRODUCTION

Cancer has emerged as one of the leading causes of death worldwide, with the number of fatalities attributed to this disease increasing each year. Although significant progress has been made in understanding the biology of cancer, it remains a major health challenge and remains the second leading cause of death worldwide (Newman *et al*., 2019; Chhikara and Parang. 2023). Various factors contribute to the development of cancer, including smoking, poor dietary habits, hormonal influences, prolonged infections, genetic mutations, exposure to free radicals and UV radiation (Chen *et al*., 2021). Efficient control, survival and eradication of cancer cells are important strategies in cancer management and treatment (Gomathi *et al*., 2020). Ideal anticancer drugs should selectively target and eliminate cancer cells and minimize any adverse effects on normal cells (Cheng *et al*., 2023). Recently, researchers have focused on the screening of natural products derived from medicinal plants, which have been shown to inhibit cancer cell proliferation, induce apoptosis, prevent metastasis and provide chemotherapeutic agents the disease works well (Sun and Shahrajabian. 2023; Alaboo and Mohammed. 2023). Through anticancer trials, these medicinal plants have demonstrated various mechanisms for exerting their anticancer actions. Natural products play an important role in synthesis and drug discovery, as they have long been used to treat cancer (Oves *et al*., 2018; Purnamasari *et al*., 2019). The revelation of normal growth of tumor-derived cells has significantly contributed to our current understanding of cancer and has laid the groundwork for groundbreaking efforts in the discovery and in vitro testing of anticancer drugs in the 19th century. Especially in the early 1990s, several human tumour cell lines were established, coinciding with the emergence of the 96-well high-throughput screening (HTS) system This convergence of events was one to discover and develop many drugs fighting cancer, leveraging the capabilities offered by this novel screening approach (Fenwick *et al*., 2023).

Antioxidants are key chemicals that play an important role in blocking the ageing effects of free radicals. Extensive research has shown that free radicals are highly reactive compounds that form covalent bonds with specific enzymes, causing tissue damage (Gulcin, 2020). However natural products and phytochemicals, such as flavonoids and polyphenolic compounds, have the ability to destroy these free radicals due to their unique redox properties this enables them to act as reducing agents, hydrogen donors, chelators and singlet oxygen scavengers and neutralize the negative effects of free radicals in the body (Trifan *et al*., 2021; Santos and Silva.2020; Alaboo and Mohammed. 2023; Al-Assaf *et al*., 2024).

Anchusa strigosa Labile, commonly known as Bugloss or Alkanet, is a species of plant in the Boraginaceae family.

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It is mainly found in tropical regions, especially the Mediterranean and tropical regions. The plant grows in Iraq, Jordan and Palestine, where it is called Lisan-el-Thor in Arabic. The family *Boraginaceae* has about 2000 members distributed mostly in Asia and Europe (Seifaddinipour *et al*., 2018; Al-Snafi. 2015; Asnaashari *et al*., 2018). *Anchusa strigosa* is known to contain a wide range of biologically active compounds, including alkaloids, polyphenols, oils, proteins, aliphatic hydrocarbons, flavonoids, terpenoids, and phenols. Throughout the history, *Anchusa strigosa* has been used in medicine, including its wound healing and wound healing agents. In addition, it has been used as a laxative, sedative, diuretic, and to treat stomach pain. Its pharmacological effects include anticancer, antioxidant, antiviral, central nervous system regulation, endocrine modulation, gastroprotection, antimicrobial activity, hypotensive and antidiabetic effects, as well as anti-inflammatory, antiviral and antibacterial properties. It is difficult, to show the activity of anticancer (Al-Khatib *et al*., 2021; Boskovic *et al*., 2018; Salayová *et al*., 2021).

The present study aims to extract and isolate phenolic compounds from *Anchusa strigosa*, followed by chromatographic methods such as column chromatography (CC), thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) Also, phenolic compounds extracts have been identified Their antioxidant potential and effectiveness against cancer cells will be reviewed.

MATERIALS AND METHODS

Plant collection and classification

The *A. strigosa* plant was collected in April and June of the year 2022, from the area located in northern Iraq (200 24.0000 north and 43° 70 48.0036 east) carefully, during the flowering season of the plant, taking care to select plants free of mechanical damage or disease. The geographical location was chosen very carefully based on advice from specialized experts as well as on a comprehensive survey of the literature. Plant parts were collected while wearing gloves to maintain sterility and before being transported to the laboratory and stored, they were placed in sterile plastic bags. The identification and verification of the plants were carried out by the Ministry of Agriculture in Mosul, Iraq, under the guidance of Dr. Talal Taha Ali Suleiman serving as the Director of the Medicinal Plants Project and as a member of the Genetic Resources Committee for the Ministry of Agriculture, ensured the accuracy and reliability of the plant specimens.

Extraction of A. strigosa by soxhlet apparatus

During the experimental procedure, 100mL of each solvent, namely hexane, ethyl acetate and ethanol, was obtained from Sigma Aldrich (Baghdad, Iraq), were added to a Soxhlet apparatus. Following this, 10 g of finely crushed plant powder or paste was introduced. To ensure a comprehensive extraction, this entire process was repeated three times for each solvent. The extraction phase took place over 72 hours, where a constant temperature of 60°C was maintained using a water bath. The organic solvent was then added by rotary evaporator and removed leaving the valuable extract. The extract was carefully filtered through a 0.22µm polytetrafluoroethylene (PTFE) filter to remove any impurities. All extracts were then accurately weighed and placed in sterile, opaque, airtight containers to preserve their integrity until further analysis (Wiktor *et al*., 2019).

Isolation and fractions of A. strigosa extract by column

Column chromatography (CC) was employed for the separation of the *A. strigosa* extract into distinct fractions. The chromatography column was packed with 300 g of silica gel, primarily 60-120 mesh, sourced from Sigma Aldrich in Baghdad, Iraq, serving as the adsorbent. A slurry was formed by combining hexane with silica gel, and this blend was meticulously introduced into the column. To ensure thorough mixing, the extract was then carefully introduced. Various solvent mixtures, including hexane, ethyl acetate, and ethanol, were utilized as the mobile phase within the column. Subsequent to obtaining the CC fractions, they underwent analysis via thin layer chromatography (TLC). (Ingle *et al*., 2017).

High-performance liquid chromatography (HPLC)

HPLC played an important role in evaluating the quality of the phenolic compounds we extracted. The mobile phase used was a mixture of acetonitrile, water and phosphoric acid at a certain concentration. The flow rate was 0.4mL/min, and the experiments were carried out at 25°C under constant conditions. Each survey lasted 8 minutes, followed by a 15-minute cleanup period. The isolated compounds were detected using an SPD-10A UV-Vis detector with a wavelength of 288 nm. By comparing the time to the values listed in table 1, we were able to determine the accuracy of each compound (Skendi *et al*., 2017).

DPPH radical scavenging activity assay

Using DPPH (2,2-diphenyl-1-picryl hydrazyl), a widely known organic compound, minor modifications were made to evaluate the potential for free radicals by spectrophotometric analysis with DPPH studied radical scavenging activity. Different fractions were prepared at different concentrations (10, 20, 40, 80 and 160g/mL) and dissolved in 1mL of ethanol. Then, 1mL of DPPH (20 mg/100mL ethanol) was added to the mixture. The solution was then shaken and incubated in the dark for 30 min at room temperature. For comparison, experiments were performed using only DPPH solution and water-soluble vitamin ascorbic acid. The antioxidant activity was measured using a UV-Visible spectrophotometer with a wavelength of 517 nm. The percentage of DPPH inhibition was determined using the following formulation:

DPPH Inhibition % = [(Ao − A1)/Ao] × 100
Where Ao represents the absorbance of the control test after 30 minutes, and A1 denotes the absorption of the sample extract after 30 minutes (Ai *et al*., 2024).

Cancer cell lines used
In this study, we used three cell lines: RSAR001, smooth muscle sarcoma cells; A375, melanoma cell line; and WRL68, normal hepatocyte line. These cell lines were maintained and cultivated at the Biotechnology Research Center of Al-Nahrain University, where various tests and experiments were conducted on them (Xhori *et al*., 2024).

STATISTICAL ANALYSIS

The results were prepared using Microsoft Excel (2007) and BioStat. The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS). The mean comparison was made using Tukey's test at a significant level (alpha = 0.05), a critical factor that underlines the importance of our findings. Multiple comparisons were carried out by analysis of variance (ANOVA). The results were expressed as mean value ± standard deviation (n = 3).

RESULTS

Composition of phenolic compounds in A. strigosa fractions
Table 2 provides a comprehensive breakdown of the results obtained from the High-Performance Liquid Chromatography (HPLC) analysis of phenolic compounds within two distinct fractions, labeled as Ia and IIb. Fraction Ia, originating from ethyl acetate extraction, reveals two prominent peaks with retention times (R.T) of 3.096 and 3.357 minutes, corresponding to concentrations of 17.0±0.6 ppm and 20.0±0.2 ppm, respectively. The theoretical retention times for these compounds are calculated to be 3.031 and 3.299 minutes, respectively. Identified compounds within this fraction include Vanillic acid and Thymol. Fraction IIb, derived from ethanol extraction, exhibits four distinct peaks with retention times of 2.719, 3.061, 3.891 and 1.989 minutes, associated with concentrations of 2.5±0.3 ppm, 2.4±0.2 ppm, 22±0.2 ppm and 24±0.2 ppm, respectively. The theoretical retention times for these compounds closely match their observed values. Compounds identified within this fraction include Catechol, Vanillic acid, Rutin, and Gallic acid. It is notable that the values provided represent the mean and standard deviation, with each measurement conducted in triplicate. The distinction between the fractions is clarified by the extraction solvents used, with ethyl acetate for Fraction Ia and ethanol for Fraction IIb. This detailed analysis offers valuable insights into the composition and concentration of phenolic compounds within the examined fractions, facilitating a deeper understanding of their potential biological activities and applications.

Antioxidant activity of A. strigose extraction
The results of the analysis of phenolic compounds from *A. strigosa* clearly indicate significant antioxidant properties, as summarized in table 3. The first fraction showed remarkable antioxidant activity, with 100 percent of 17.40% to 35.07% µg/mL at concentrations ranging from 12.5 to 200, respectively. The calculated IC_{50} value for these compounds was shown to be 20.47. Similarly, the second fraction showed greater antioxidant activity, with percentages ranging from 81.52% to 17.63% in the concentration range of 12.5-200µg/mL Notably, the IC_{50} value of fraction a the second concentration was 25.58µg/mL, as in fig. 4 and 5 was shown in the middle. These findings strongly suggest that *A. strigosa* phenolic compounds can be effective antioxidants, and reveal their potential applications in various applications, such as pharmaceutical applications, food additives to provide various forcing mechanisms under their antioxidant activities, their therapeutic possibilities are unclear - Further studies are needed to investigate the benefits.

Cytotoxic effect of A. strigosa extraction
The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltet-razolium bromide) cytotoxicity test was employed to assess the toxic impact of the active compounds extracted from the plant under investigation on two distinct types of cancer cells, namely RSAR001 soft tissue carcinoma cells (sarcoma) and A357 melanoma cells (melanoma). This test relies on the conversion of MTT into an insoluble violet formazan substance, catalyzed by the succinate dehydrogenase enzyme located within the mitochondria. By utilizing this method, researchers were able to evaluate the potential cytotoxic properties of the tested compounds against these cancer cell lines.

The results obtained from testing Fraction I on the soft tissue carcinoma cell line RSAR001 revealed its effectiveness in inhibiting cell growth, with inhibition rates ranging from 35.30% to 72.34% at concentrations of 12.5 to 400µg/mL. However, when evaluating its impact on WRL68 normal cells, the compounds displayed inhibition rates varying from 3.36% to 37.73% at the same concentration range (table 4). Statistical analysis demonstrated significant differences ($P<0.0001$) in the IC50 values for the tested compounds against both RSAR001 cancer cells (12.28µg/mL) and WRL68 normal cells (66.18µg/mL), as depicted in fig. 6. Additionally, when investigating the effect of Fraction I on the melanoma cell line A375, it exhibited an inhibition rate of 10.38%, 17.94%, 35.18%, 46.52%, 4.90% and 5.40% at concentrations of 12.5, 25, 50, 100, 200, and 400µg/mL, respectively. Similar to RSAR001, the compounds showed inhibition rates ranging from 4.05% to 34.61% on WRL68 normal cells at concentrations of 12.5 to 400µg/mL (table 5). The calculated IC50 values for A375 cancer cells (156.4µg/mL) and WRL68 normal cells (165µg/mL) were significantly different ($P<0.0001$), as depicted in fig. 7.

Table 1: Standards of phenolic compounds and their retention time.

Standards	Retention Time (min)	Concentration (ppm)	Area [1] (mAU*s)
Vanillic acid	3.031	25	445452319 (0.01)
Thymol	3.299	25	377069604 (0.01)
Catechol	2.715	25	775188e4 (0.01)
Vanillic acid	3.031	25	445452319(0.01)
Rutin	3.031	25	408127856 (0.01)
Gallic acid	2.019	25	677179e4 (0.01)

[1]Area represented as mean (n = 5) with coefficient of variation in brackets.

Table 2: HPLC of phenolic compounds in two fractions and their retention time.

Fractions	No. of Peak	R. T (min)	Conc. (ppm)[c]	Theoretical R. T (min)	Identified Compounds
I[a]	1	3.096	17.0 ± 0.6	3.031	Vanillic acid
	2	3.357	20.0± 0.2	3.299	Thymol
II [b]	1	2.719	2.5 ± 0.3	2.715	Catechol
	2	3.061	2.4 ± 0.2	3.031	Vanillic acid
	3	3.891	22± 0.2	3.911	Rutin
	4	1.989	24± 0.2	2.019	Gallic acid

[a]Fractions identified from ethyl acetate extraction; [b]Fractions identified from ethanol extraction, [c] Values represent mean and standard deviation (n = 3).

Table 3: Ascorbic acid reducing antioxidant power of different fractions.

Concentration (µg/mL)	Ascorbic acid%	Fraction I (%)	Fraction II (%)
12.5	22.90±1.836	17.40±4.207	17.63±7.196
25	39.00±1.732	17.67±5.208	40.43±7.081
50	57.60±2.207	19.75±2.695	53.32±1.571
100	74.07±1.007	30.99±3.291	68.71±4.753
200	86.03±4.028	35.07±6.991	81.52±0.9279
IC_{50} (µg/mL)	36.09	20.47	25.58

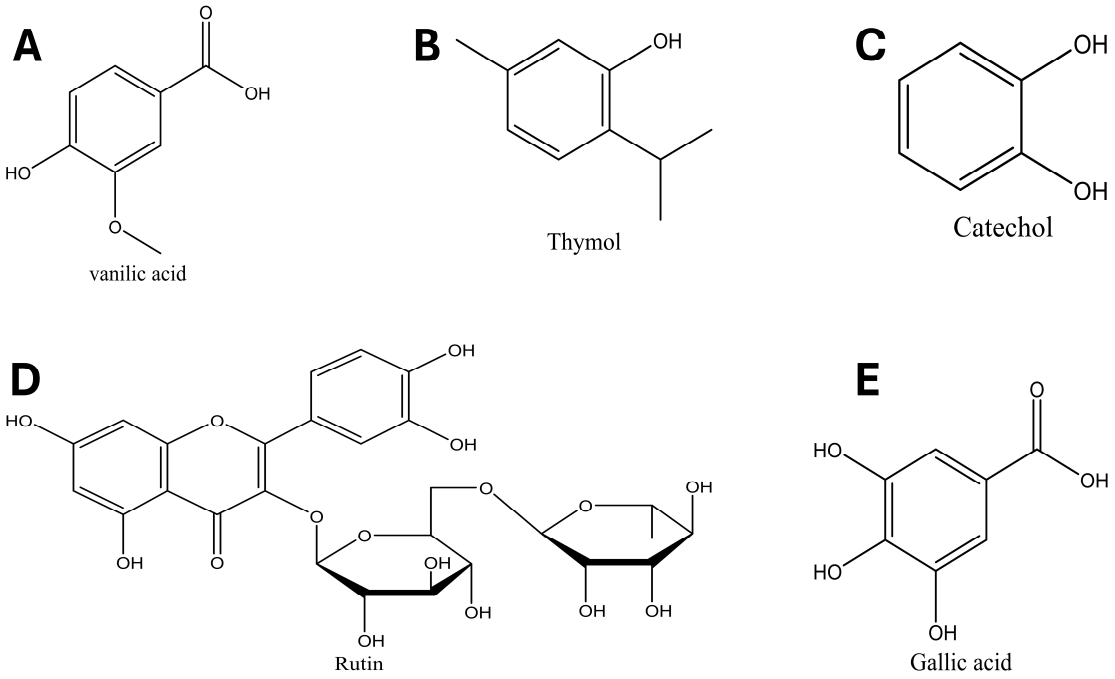


Fig. 1: 2D- chemical structure of phenolic compounds extraction from *A. strigosa*.

Table 3: Ascorbic acid reducing antioxidant power of different fractions.

Concentration (µg/mL)	Ascorbic acid%	Fraction I (%)	Fraction II (%)
12.5	22.90±1.836	17.40±4.207	17.63±7.196
25	39.00±1.732	17.67±5.208	40.43±7.081
50	57.60±2.207	19.75±2.695	53.32±1.571
100	74.07±1.007	30.99±3.291	68.71±4.753
200	86.03±4.028	35.07±6.991	81.52±0.9279
IC_{50} (µg/mL)	36.09	20.47	25.58

Table 4: The effect of fraction (I) on RSAR001 and WRL68 using MTT for 24 hours at 37°C.

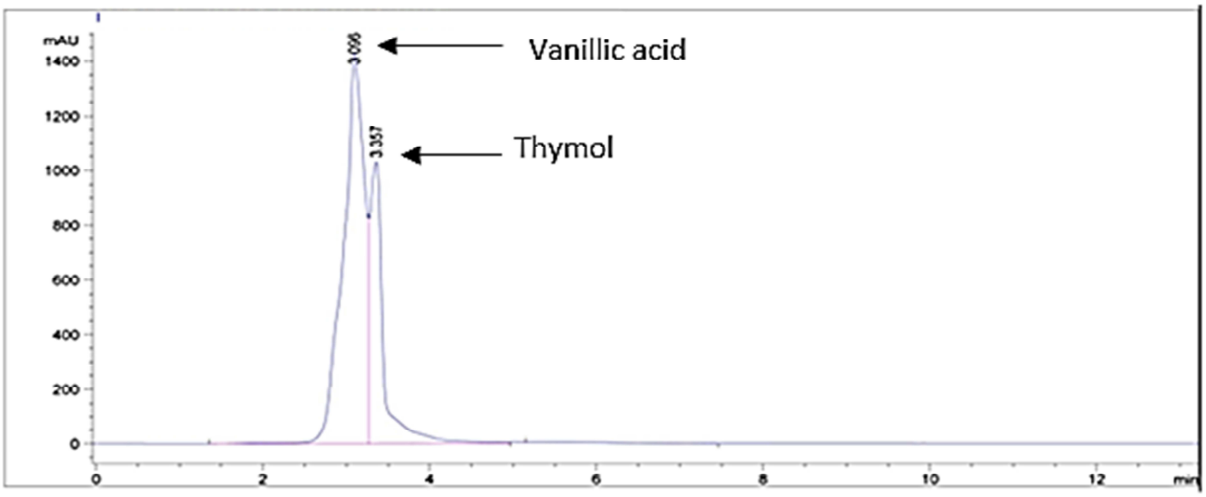
	Viability% Mean± SD Conc. µg \ ml fraction (I)					
	400	200	100	50	25	12.5
RSAR001	27.66±2.28	35.79±3.78	44.01±3.24	64.70±4.71	75.04±4.98	96.53±0.72
WRL68	62.27±4.63	65.97±1.10	71.14±1.83	86.30±3.75	95.37±0.90	96.64±0.70

Table 5: The effect of fraction I on A375 and WRL68 using MTT for 24 hours at 37°C.

Cell line	Viability% Mean± SD Conc. µg \ ml fraction (I)					
	400	200	100	50	25	12.5
A375	53.48±4.01	64.82±6.24	82.06±5.01	89.62±3.51	94.60±0.64	95.10±2.27
WRL68	65.39± 6.90	73.23 ±1.20	93.60 ±2.10	95.33±1.18	95.2±20.82	95.95±1.03

Table 6: The effect of fraction (II) on RSAR001 and WRL68 using MTT for 24 hours at 37°C

Cell line	Viability% Mean± SD Conc. µg \ ml fraction (II)					
	400	200	100	50	25	12.5
RSAR001	53.47±1.14	64.39±4.03	78.40±0.75	94.10±2.21	94.87±1.40	95.60±0.50
WRL68	71.95±0.81	76.70±2.70	87.67±3.77	86.07±1.92	95.22±0.82	95.95±1.03

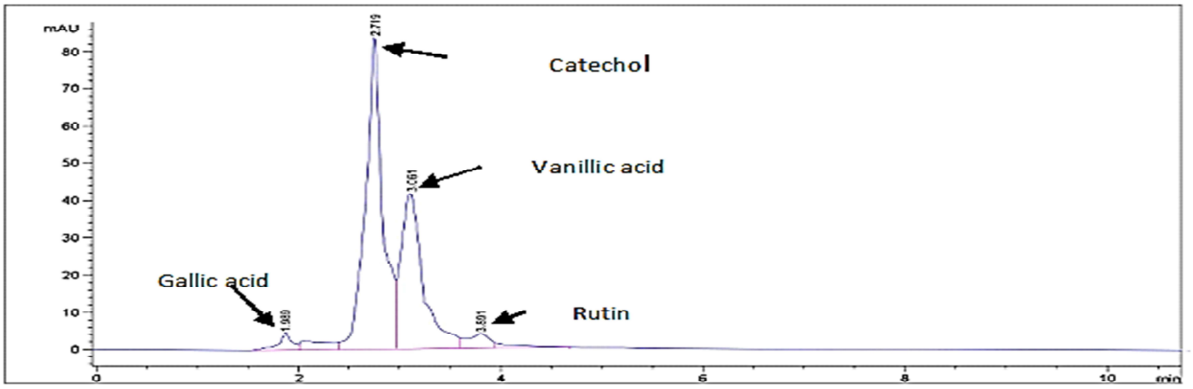


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.096	BV	0.2313	2.52380e4	1434.14709	68.0884
2	3.357	VB	0.1678	1.18285e4	1031.51709	31.9116
Totals :				3.70665e4	2465.66418	

Fig. 2: HPLC chromatogram of fraction I.

Table 7: The effect of fraction II on A375 and WRL68 using MTT for 24 hours at 37°C

Cell line	Viability% Mean± SD Conc. µg\ ml fraction (II)					
	400	200	100	50	25	12.5
A375	21.60±4.69	29.98±4.11	35.76±1.75	49.54±3.26	62.58±3.22	73.23±1.77
WRL68	63.48±2.75	65.31±9.20	88.97±1.04	92.28±3.68	95.25±1.03	93.29±1.82



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.989	BV	0.1092	40.44353	4.85433	2.2503
2	2.719	VV	0.1716	1030.78418	83.74816	57.3527
3	3.061	VV	0.2348	678.50610	41.93279	37.7529
4	3.891	VV	0.2415	47.52969	4.59784	2.6446
Totals :				1797.2635	135.13312	

Fig. 3: HPLC chromatogram of fraction II.

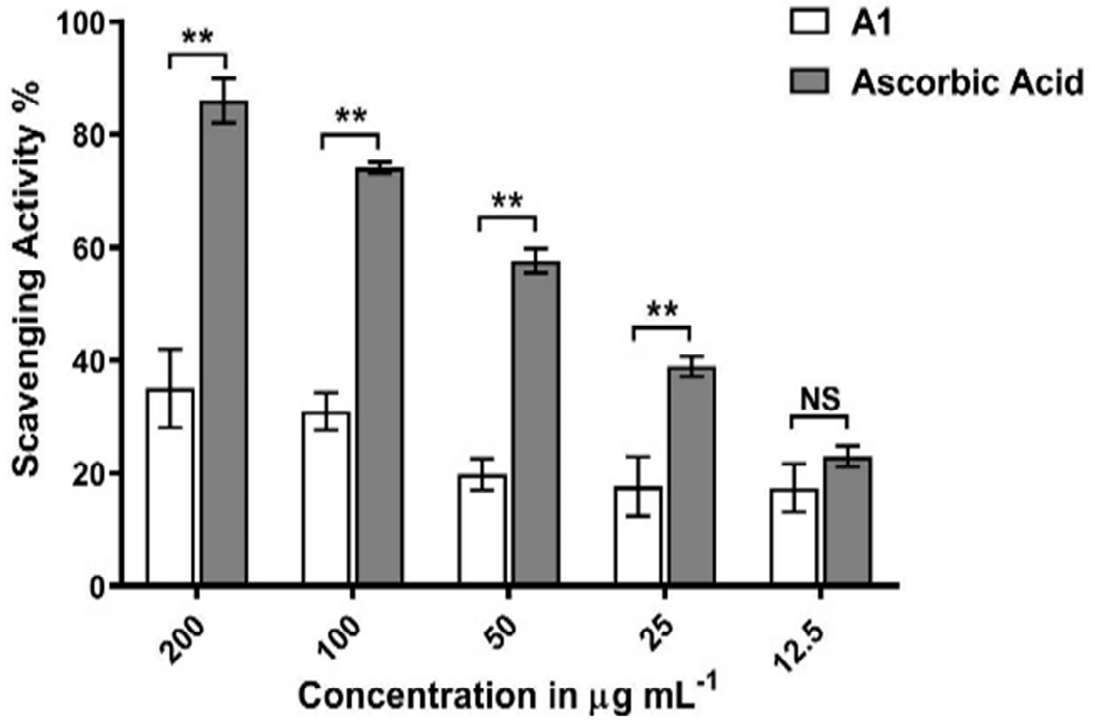


Fig. 4: The antioxidant activity of fraction I

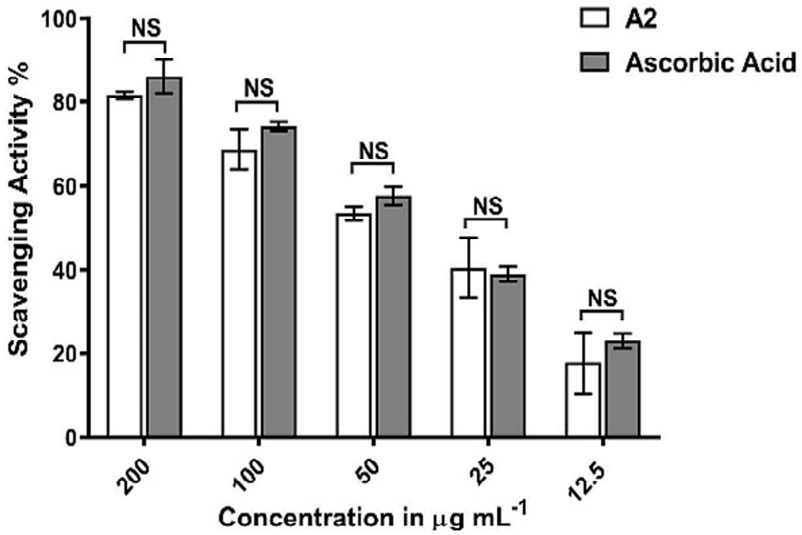


Fig. 5: The antioxidant activity of fraction II

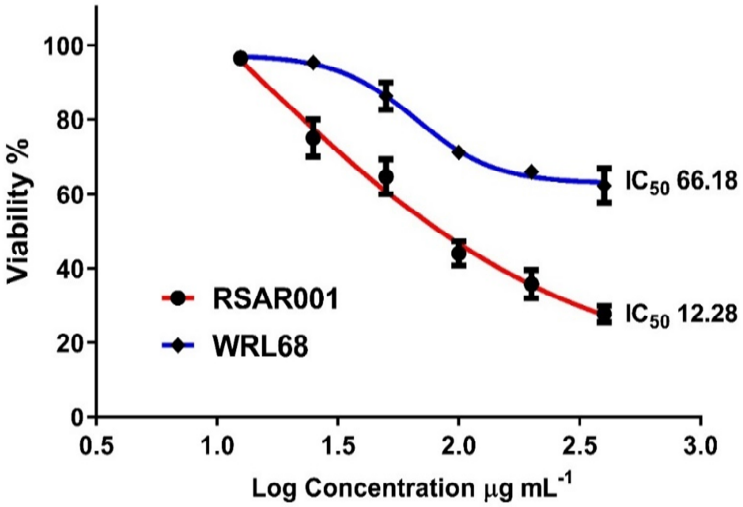


Fig. 6: MTT used to assess RSAR001 and WRL68 of fraction (I).

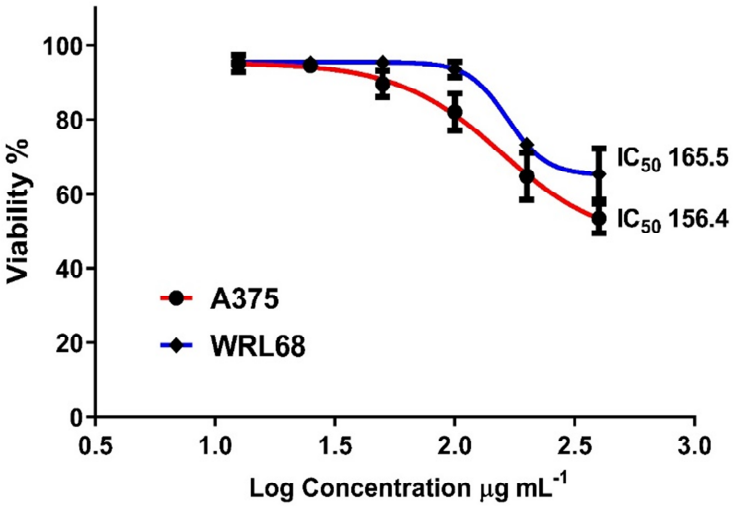


Fig. 7: MTT used to assess A375 and WRL68 of fraction (I)

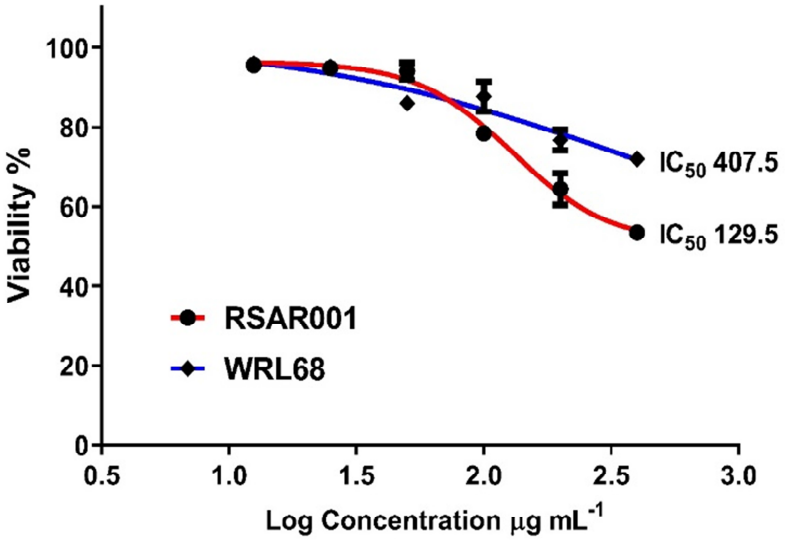


Fig. 8: MTT used to assess RSAR001 and WRL68 of fraction (II).

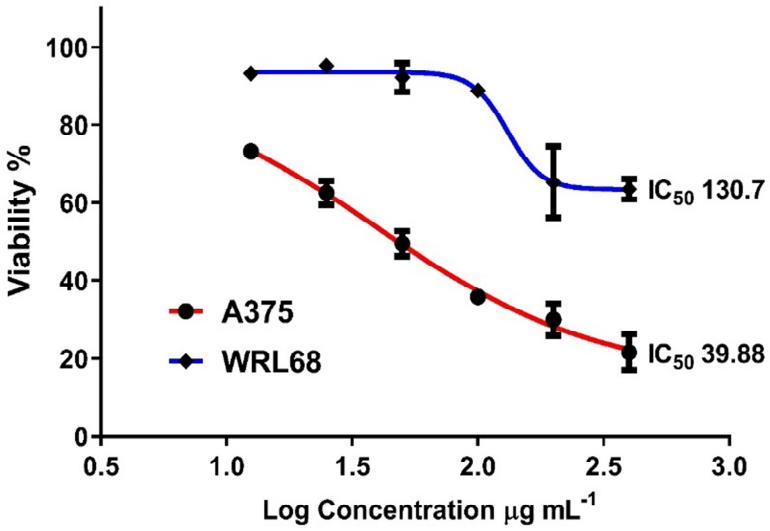


Fig. 9: MTT used to assess A375 and WRL68 of fraction (II).

In the present study, the efficacy of fraction II was evaluated on the soft tissue cancer cell line RSAR001 and the melanoma cell line A375. The results obtained for RSAR001 demonstrated a notable effectiveness, as evidenced by inhibition rates of 5.90%, 21.60%, 35.61%, 46.53%, 4.40% and 5.23% at concentrations of 12.5, 25, 50, 100, 200 and 400µg/mL, respectively. Interestingly, fraction II did not exhibit significant cytotoxicity on WRL68 normal cells, with the percentage of inhibition ranging from 4.05% to 28.05% at concentrations of 12.5 to 400µg/mL, as indicated in table 6. Furthermore, a significant difference ($P<0.0001$) was observed in the calculation of IC_{50} values between RSAR001 (129.5µg/mL) and WRL68 (407.5µg/mL) cells, as illustrated in fig. 8.

Similarly, the evaluation of fraction II on A375 cells revealed substantial effectiveness, with inhibition rates of 50.44%, 64.24%, 70.02% and 78.40% at concentrations of 12.5, 25, 50, 100, 200 and 400µg/mL, respectively. Notably, fraction II exhibited minimal cytotoxicity on WRL68 normal cells, with inhibition percentages ranging from 6.71% to 36.52% at concentrations of 12.5 to 400µg/mL, as shown in table 7. The IC_{50} values further supported the significant differences ($P<0.0001$) between A375 (39.88µg/mL) and WRL68 (130.7µg/mL) cells, as demonstrated in fig. 9.

DISCUSSION

A. strigosa was subjected to extensive phytochemical studies, which revealed the presence of various

metabolites. The chloroform extract contained four aliphatic hydrocarbons, whereas the methanolic extract showed more carbohydrates. Furthermore, the extracts were found to contain significant amounts of amino acids and free proteins. The bright pink-purple color of the flowers was attributed to the anthocyanidins malvidin and pelargonidin detected in the methanol-HCl extract .In addition to these findings, *A. strigosa* was found to contain oils, proteins, pyrrolizidine alkaloids and polyphenols. Notably, leaves of *A. strigosa* showed several phenolic compounds such as rosmarinic acid, caffeic acid, tormatic acid and gallic acid (Chebaro *et al*., 2023; Amat *et al*., 2012). Previous studies have shown that *A. stigosa*. In addition, phytochemical studies revealed the presence of various anti-inflammatory and anti-cancer compounds in *A. stigosa*, such as flavonoids, terpenes, sterols, altoids, phenolic acids and notably, rosmarinic acid, caffeine, genistein were identified and silybin in these plants.

These compounds were identified using techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and nuclear magnetic resonance (NMR) (Saleem *et al*., 2020; Ghalib and Kadhim. 2021). These findings provide valuable insights into the phytochemical composition of *A. strigosa*, highlighting its potential as a rich source of bioactive compounds with diverse biological applications. Further studies and studies are needed to explore therapeutic and pharmacological potential which these compounds have for the development of new drugs and pharmaceutical applications.

The present study provides compelling evidence that all phenolic compounds isolated from *A. strigosa* have remarkable antioxidant properties, as evidenced by their dose-dependent high activity in the DPPH assay. These data are consistent with previous studies that reported the antioxidant activity of the aqueous extract of *A. strigosa*, the aqueous extract showed an IC_{50} value of 66.7µg/mL and the methanolic extract showed an IC_{50} value of 43.6 µg/mL These results also support the hypothesis that *A. Strigosa* contains phenolic compounds that have a significant effect on free radicals (Chebaro *et al*., 2023). Several studies have identified phenolic compounds found in various plants such as catechol whose potency is due to the presence of hydroxyl (OH) groups and is therapeutically important due to its antioxidant and antioxidant properties (Lu *et al*., 2016; Ali and Mohammed. 2019). Vanillic acid, another phenolic compound commonly found in medicinal plants, not only acts as a flavoring agent but also exhibits antioxidant, antibacterial and anti-mutagenic activities revealed (Kaur *et al*., 2022; Punvittayagul *et al*., 2021, Zheng *et al*., 2022). The natural flavonoid rutin acts as an antioxidant and antioxidant by interacting with free radicals and counteracting their harmful effects Gallic acid is widely known for its antioxidant properties (Sahoo *et al*., 2021). Moreover, the claim of thymol as one of the most important anti-inflammatory and anti-inflammatory compounds has been confirmed in previous research (Calabrese *et al*., 2024). Plants have been recognized as a valuable source of anticancer agents, leading to an expanding field of research focused on exploring novel natural anticancer compounds derived from plants. Within this context, particular attention has been given to plants investigated for their total phenol and flavonoid contents as well as their anticancer effects (Kopustinskiene *et al*., 2020). Notably, the findings of this study align with the research conducted by Kumar *et al* (2022), which demonstrated significant inhibition of sarcoma tumors (82%, 63% and 96%) upon treatment with *A. strigosa* plant extracts. In addition, various authors have reported the antitoxic potential of plant extracts isolated from *Boraginaceae* family against various types of cancer cells (Bošković and Rando, 2018). Paun and others (2020) observed moderate cytotoxicity in melanoma cells when exposed to *A. strigosa* extract, while our study found that *A. strigosa* extract did not damage melanoma cells. Interestingly, previous studies on the methanolic extract of *A. strigosa* have reported similar levels of overall anticancer activity. HPLC analysis of *A. strigosa* extracts showed a predominance of phenolic compounds, which is consistent with similar analyzes of plants of the family *Boraginaceae* (Zengin and Aktumsek. 2015). These phenolic compounds are known to exhibit a variety of biological activities including antioxidant and anticancer properties (Sadeghi *et al*., 2024). Notably, among the tested compounds, the ethanolic extract of *A. strigosa* showed the most effective cytotoxic activity, probably due to strong antioxidant activity, various pharmacokinetic compounds for the availability of work, and synergistic effects, corroborating the findings of other studies (Srikanth and Chen, 2016).

CONCLUSION

The results of this study provided valuable insights into the composition, anticancer and antioxidant properties of various extracts from *Anchusa strigosa* Six phenolic compounds were identified by high-performance liquid chromatography (HPLC). compared to previous findings: vanillic acid, thymol, catechol Increased chemicals were found phenolics such as, rutin and gallic acid Fractions from *Anchusa strigosa* extract exhibited different antioxidant and anticancer activities, as determined by the experiments carried out in this study with the well-known organic chemical DPPH (2,2-diphenyl-1-picryl hydrazyl) was used to evaluate the free radical decomposition and potential toxicity of active compounds isolated from the studied plants In order to evaluate the kta effect , MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity tests were performed Cells:

RSAR001 soft tissue carcinoma (sarcoma) cells and melanoma cells A375.

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