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# Screening of Phytochemical Constituents in Ethanolic Extract from *Astragalus spinosus* Roots and Assessment of Its Anticancer, Antioxidant, and Antibacterial Activities

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**Abstract**: The study aims to analyze the chemical components of *Astragalus spinosus* roots extract after the ethanolic extraction process using techniques such as gas chromatography-mass spectrometry, infrared, and UV-Vis spectroscopy to indicate the phytochemical content of the extract. The extract contained various compounds, including alkaloids, terpenoids, flavonoids, phenols, and carboxylic acids. Flavonoid and phenolic content were measured by the colorimetric method and found to be 511.19±35.75 and 24.64±0.07 mg, respectively. Subsequently, antibacterial, antioxidant, and anticancer activities were evaluated for the ethanolic extract. Antibacterial effectiveness was concentration-dependent against Staphylococcus aureus and Streptococcus mutans, showing optimal inhibition at 100 mg/mL with inhibition areas of 2.81±0.88 and 2.68±2.93 mm, respectively. Antioxidant activity was measured using DPPH with a 200 µg/mL of extract concentration, displaying maximum scavenging activity (71.85±6.43%) and FRAP activity (55.93±1.4%) at 0.64 µg/mL. The alcoholic extract exhibited decreasing vitality of (MCF-7) breast cancer cells as concentration increased, with viability reaching a minimum of 35.378 ± 5.072 at 400 µg/mL.

**Keywords:** *Astragalus spinosus*, Chemical components, GC-mass, Antioxidant activity, Antibacterial inhibition, Anticancer effect.

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## 1. INTRODUCTION

Lately, there has been a notable increase on a global scale in the utilization of medicinal plants or herbal remedies as complementary or adjunctive treatments alongside conventional medicine (1,2). Currently, medicinal plants and their active constituents are widely acknowledged as a promising alternative approach for addressing and treating a variety of human ailments (3-7). *Astragalus spinosus* is an annual herbaceous species of the genus *Astragalus*. The *Astragalus spinosus* plant abounds in the deserts of Iraq and neighboring countries. The genus *Astragalus* consists of around 3000 species. The *Astragalus spinosus* bush is a compact perennial that reaches a height of approximately 70 cm and forms a semi-circular shape (8). Its growth cycle begins afresh in the spring. Notably, it stands out with its white blossoms that appear in January (9-10). Moreover, the plant is characterized by robust, cylindrical, and lengthy thorns measuring up to 10 cm in length. Traditionally, these plants have been employed in native remedies to address conditions like hypertension, stomach ulcers, bronchitis, diabetes, and gynecological disorders (11). Additionally, various research efforts have explored their potential for anti-inflammatory, antiviral, immunomodulatory, analgesic, antioxidant, anticancer, and cardiotonic properties through diverse screening models (12-17). Therefore, *Astragalus spinosus* presented itself as a suitable candidate for investigating these therapeutic activities (18). Currently, cancer remains a widespread issue, with global projections indicating around 2 million new cancer cases diagnosed annually, resulting in an approximate 80% mortality rate, including cases of breast cancer (19). Breast cancer has the potential to metastasize beyond the breast through blood vessels and lymphatic vessels. To address breast cancer effectively, chemotherapy is frequently employed alongside other treatments like surgery, radiotherapy, or

hormonal therapy. The use of chemotherapy to treat breast cancer carries the possibility of inducing side effects that vary from short-lived and mild to more severe or potentially lasting. To address the challenges posed by this debilitating condition, there is a pressing necessity to explore novel medicines derived from natural sources, which can enhance the quality of life for patients (20). Contemporary drug discovery endeavors are increasingly highlighting the significance of natural origins. As a result, herbal therapy emerges as a potential option. According to several research sources, herbal extracts with antioxidant and anticancer properties have been identified as essential in managing disorders associated with inflammation (21). Healthcare specialists worldwide recognize the need to research medicinal plants to avoid these negative effects while maintaining optimal performance. As a result, it is critical to explore innovative medications derived from plants with fewer adverse effects.

## 2. EXPERIMENTAL SECTION

### 2.1. Chemicals and Analytical Devices

*2.1.1. Chemicals and Apparatus*

Analytical grade pure solvents, including ethanol, DMSO, and methanol (with a purity level of 99.5%), were obtained from the Central Drug House, India. The MTT assay kit consisted of MTT Solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and a solubilization solution, ferric reducing antioxidant power assay and chemicals such as sodium dihydrogen phosphate dihydrate, iron chloride, potassium ferricyanide, trichloroacetic acid, and vitamin E were purchased from Merck, Malaysia. In the DPPH radical scavenging activity test, 2,2-diphenyl-1-picrylhydrazyl and ascorbic acid were obtained from Himedia, India. To measure total flavonoids and phenols, sodium nitrate, sodium hydroxide, aluminum chloride, and sodium carbonate were procured from Sigma Aldrich, Malaysia. The instruments used to determine the phytochemical constituents were gas chroma-tography-mass spectrometry [GC-MS (Mass Spectrometer 5977, Gas Chromatography 7890, Agilent), USA], Fourier-transform infrared spectroscopy (FTIR), performed using a Shimadzu FTIR 8400S instrument from Japan, and UV-visible spectrophotometry (Shimadzu UV-1650PC, Japan).

### 2.2. Collecting Plants and Extraction Procedure

*Astragalus spinosus* samples (depicted in Figure 1A) were collected during the period spanning October to November 2023 from the Salman district in the southern region of Iraq (at coordinates 30°30′19″N, 44°32′18″E) (herbarium no. 4227). After the collection, the roots underwent meticulous cleansing with running tap water and were subsequently air-dried in a shaded environment (as shown in Figure 1B). After achieving dryness, the roots were processed into a powdered form (as illustrated in Figure 1C) utilizing an electric blender, and the resulting powder was stored securely in an airtight container. To initiate the extraction procedure, 30 grams of finely ground *Astragalus spinosus* roots were inserted into a thimble. The Soxhlet extraction technique was employed, using 250 mL of ethanol within a hot Soxhlet apparatus. The extraction process was carried out over a period of 4 hours, ensuring that the temperature remained below the boiling point of the solvent. Following completion, the obtained extract was filtered using Whatman No. 1 filter paper, followed by concentration under reduced pressure utilizing a rotary evaporator (ISOLAB, Germany) at 80 °C. The resulting extract's weight was measured to ascertain its yield (expressed as a weight percentage) and subse-quently stored at a temperature of 4 °C for potential future investigations. The extraction yield was calculated using Equation 1a and b (22) and expressed as a weight percentage, as referenced.

$$Extraction\ yield\,(\%) = \frac{Weight\ of\ extracted\ root}{Dry\ weight\ of\ the\ root} \times 100$$

(Eq. 1a).

$$Extraction\ yield\,(\%) = \frac{2.2\,g}{30\,g} \times 100 = 7.33\,wt\,\%$$

(Eq. 1b)

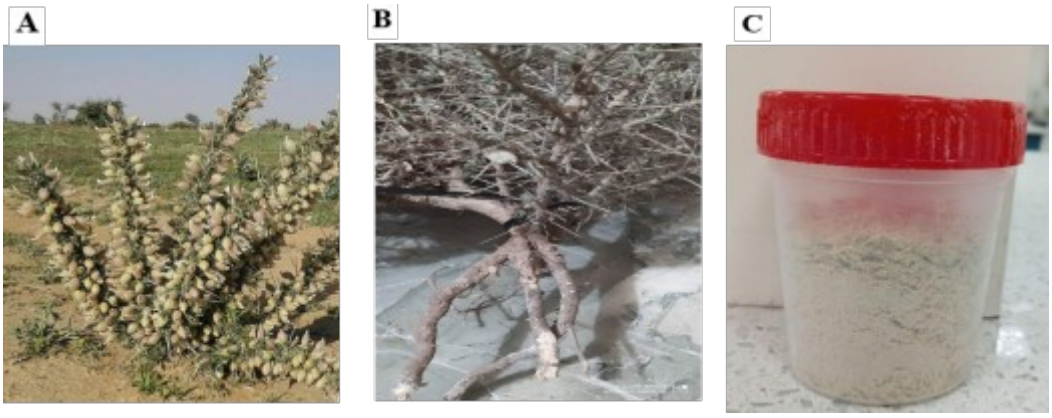


**Figure 1:** A: *Astragalus spinosus* plant, B: *Astragalus spinosus* roots, and C: Extracted *Astragalus spinosus* roots powder.

### 2.3. Identification and Quantification of Bioactive Compounds

*2.3.1. Double Beam UV-Vis Spectrophotometry*

The UV-Vis spectrum of *Astragalus* roots extract was measured using a UV-Vis (UV-1650 PC, Shimadzu, range 190 to 1100 nm) spectrophotometer using 100% ethanol as a standard reference in the Central Laboratory, Department of Chemistry, College of Science, Al-Muthanna University.

*2.3.2. Fourier Transform Infrared Spectroscopy (FTIR)*

The infrared spectrum was recorded in the solid state of the EtOH extract of *Astragalus spinosus* roots by using the

84005-FTIR Shimadzu device using KBr disks within the range 400–4000 $cm^{-1}$ of wavenumber at the Chemistry Laboratory within the Department of Chemistry, College of Science at Al-Muthanna University.

*2.3.3 Gas Chromatography-Mass Spectrometry (GC-MS)*
The GC-MS analysis took place within the laboratory of the Basra oil company, utilizing an Agilent Technologies 7890B GC system coupled with an Agilent Technologies 5977A MSD that included an electron ionization (EI) signal detector. The chromatography utilized HP-5ms with a 5% phenyl and 95% methyl siloxane composition, configured at dimensions of 30 m*250 mm*0.25m. The oven's starting temperature was set to 40 °C and sustained for a period of 5 minutes. Subsequently, the temperature was incrementally elevated at a rate of 8 °C per minute. Helium was employed as the carrier gas with a 1 mL/min flow rate, and a 3 mL/min purge flow was maintained. The total runtime for the GC procedure was 40 minutes. A pulsed split-less mode was engaged for the injection process, featuring an injection temperature of 290 °C, and the injected sample volume amounted to 0.5 microliters. The ion source temperature of the mass spectrometer was set at 230 °C, and scans were conducted at a speed of 1562 ($N_2$), encompassing a mass range of 44 to 750 m/z. In order to corroborate the identification of compounds, the data was cross-referenced with the NIST 2020 Library database.

**2.4. In Vitro Cytotoxicity MTT Assay**
The cells were cultivated at different densities, varying from $1\times10^4$ to $1x10^6$ cells/mL. A total of 100 microliters of MCF-7 cells were introduced into 96 wells of the microplate, repeating the process for each concentration as well as the control. Afterward, the microplate was covered and placed in a 37 °C environment for a duration of 2 to 4 hours, all while maintaining a 5% $CO_2$ atmosphere. Following this, the RPMI-1640 culture medium was eliminated. Over a 48-hour period at 37 °C, 100 µL of *Astragalus spinosus* EtOH roots extract, at different levels of concentration (25, 50, 100, 200, and 400 µg/mL) was administered to each well containing treated cells in the microplate. For every concentration, three replicates were prepared. Following the administration of the extract, ten microliters of MTT solution were introduced, and a 4-hour incubation at 37 °C followed. Afterward, each well received 100 microliters of solubilization solution and was allowed to stand for 5 minutes. The absorbance readings were taken using an ELISA reader (ASYS/Austria) with a wavelength set at 575 nanometers. The percentage of growth inhibition (GI) for each concentration of the extract was computed using formula 2 (23):

$$Viability\,(\%) = \frac{Optical\ Density\ of\ Sample}{Optical\ Density\ of\ Control} \times 100 \quad \text{(Eq. 2)}$$

Statistical significance was determined using ANOVA with Duncan's test at a confidence level of $p \leq 0.05$ (24). The outcomes were displayed as the average ± standard deviation. GraphPad Prism version 9 was utilized to conduct the statistical analyses. The evaluation of cytotoxicity using the human breast (MCF-7) cell line took place at the Department of Pharmacology, situated within the Center for Investigation of New Therapies at the University of Malaya in Malaysia.

**2.5. In vitro Antioxidant Assay**
To evaluate the antioxidant activity of the EtOH extract, the FRAP reductive ability test and the DPPH free radical scavenging test were performed.

*2.5.1. Ferric Reducing Power (FRAP) Assay*
To measure the reductive ability, the methodology used followed the procedure given in (25). A volume of 1 mL of EtOH roots extract (with concentrations ranging from 0.04 to 0.64 g/mL) was mixed with 1 mL of a phosphate buffer solution (pH of 6.6) at a concentration of 0.2 M, along with 1.5 mL of a potassium ferricyanide solution at a concentration of 1%. Subsequently, the mixture was introduced into an incubator at a temperature of 50 °C for a duration of 20 minutes. The process was stopped by introducing 1 mL of trichloroacetic acid (TCA) at a concentration of 10%. Following that, the resulting blend was subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes. Then, a mixture was prepared by combining 2.5 mL of the suspension with 2 mL of distilled water and 0.5 mL of a liquid solution containing 1% ferric chloride. Following that, the absorbance was measured at a wavelength of 700 nm. The same steps were then replicated for vitamin E, and the complete procedure was repeated three times for each concentration.

*2.5.2. DPPH Free Radical Scavenging Activity Assay*
The antioxidant activity of EtOH extract of *Astragalus spinosus* roots with standard (vitamin C) was evaluated based on the DPPH-stabilized free radical scavenging effect, and the experiment was carried out according to the working method (26). For every test tube, 0.1 mL of the EtOH roots extract containing vitamin C, across different concentrations (0.0625, 0.125, 0.250, 0.500, and 1.000 µg/mL), was mixed with 3.9 mL of DPPH solution. Subsequently, these test tubes were positioned within an incubator adjusted to 37 °C for a duration of 30 minutes. Using a spectrophoto-meter and a 517 nm of wavelength, the absorbance of each solution was determined, and each concentration was replicated three times. Equation 3 (27) was employed to establish the relationship between a decrease in absorbance and an enhancement in the activity of scavenging free radicals:

$$Scavenging\ Activity\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad \text{(Eq. 3)}$$

The measurements $A_{control}$ and $A_{sample}$ represent the absorbance readings of the control and the sample, respectively.

**2.6. Total Flavonoids and Total Phenols**
Total flavonoid content was assessed using the colorimetric method (28). Firstly, take 3.2 mg of the EtOH extract and dissolve it in 5 mL of a 50% methanolic solution. Next, add 1 mL of a solution comprising 5% (w/v) sodium nitrate. Following a duration of 6 minutes, add 1 mL of a solution containing 10% (w/v) aluminum chloride to the mixture and leave it for 5 minutes. Following this, blend 10 mL of a 10% (w/v) sodium hydroxide solution into the mixture. The next step is to use distilled water to adjust the volume to 50 mL,

ensuring complete mixing. After 15 minutes, use a spectrophotometer to measure the absorbance at 450 nm to determine the total flavonoid concentration. This method was used across six different concentrations (2.5, 5, 10, 20, 40, and 80 g/mL), and the standard curve was created using curve-fitting techniques. The complete phenolic content was determined by applying the Folin-Ciocalteu method, as referenced in (29). To evaluate the content present in the extract, a solution containing 1 mL of the extract within the range of 100 to 500 µg/mL concentrations was blended with 2.5 mL of a Folin-Ciocalteu reagent (Sigma Aldrich, Malaysia) at a concentration of 10% (w/v). After an interval of 5 minutes, 2.0 mL of $Na_2CO_3$ (at a concentration of 75%) was added to the mixture, followed by incubation at 50 °C for 10 minutes with intermittent agitation. After the sample had cooled down, the absorbance of the samples was measured utilizing a UV spectro-photometer (Shimazu, UV-1900i) at a wavelength of 765 nm compared to a reference blank solution without extract. The data assembled were expressed as (mg/g) of gallic acid equivalents in (mg GAE/g) of the dry extract. The procedure was conducted within the Department of Pharmacology, located within the Center for Investigation of New Therapies at the University of Malaya in Malaysia.

## 3. RESULTS AND DISCUSSION

### 3.1. Analysis of Phytochemicals

A phytochemical investigation derived from *Astragalus spinosus* roots revealed various phytochemical compounds. The essential phytochemical constituents, including alkaloids, terpenoids, flavonoids, phenols, carboxylic acids, furans, and alkene derivatives, were detected in the ethanol extract, as illustrated in Table 1.

### 3.2. UV-Vis Spectrum

The UV-Vis spectrum of the extracted *Astragalus* plant roots dissolved in ethanol compared to pure ethanol as a reference solution exhibited three peaks at specific wavelengths (243.76 nm, 251.66 nm, and 272.63 nm), as shown in Figure 2. These peaks are of significant interest as they provide valuable insights into the molecular composition and electronic structure of the Astragalus root extract. The appearance of these peaks can be attributed to specific electronic transitions within the molecules present in the extract. The first two peaks, at 243.76 nm and 251.66 nm, correspond to electronic transitions as π → π* transitions. In this context, the π → π* transitions are associated with the (C=O) group and the (C=C) group (30). The third peak, observed at 272.63 nm, corresponds to a different type of electronic transition known as the n → π* transition (31). This transition also involves the (C=O) group, indicating the presence of carbonyl compounds in the *Astragalus* roots extract.

**Table 1:** Phytoconstituents of the ethanol fraction of *Astragalus spinosus* roots.

| Test. no. | Phytochemical constituents | Test/ Reagents | Result |
|---|---|---|---|
| **1** | Flavonoids | $NH_4$ + Conc. $H_2SO_4$ | ++ |
| **2** | Saponins | Foam test | - |
| **3** | Phenols | Ferric chloride test | + |
| **4** | Alkaloids | Dragendorff's test | + |
| **5** | Coumarins | Alcoholic NaOH | - |
| **6** | Terpenoids | Salkowski test | + |
| **7** | Glycosides | Acetic acid + $FeCl_3$ | + |

Key indicators: ++: abundant, +: moderate, -: absent.

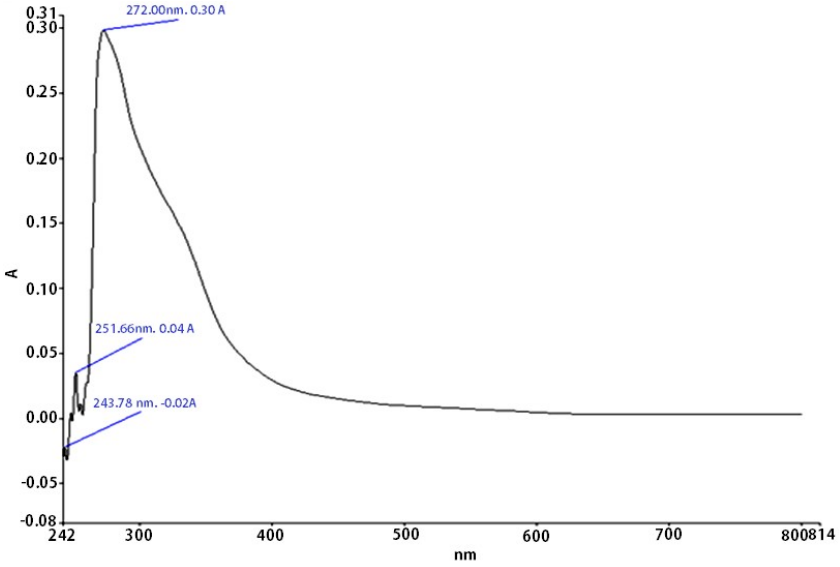


**Figure 2:** UV-Vis Spectrum of the EtOH roots extract.

### 3.3. FTIR Spectrum

Crude EtOH extract for *Astragalus spinosus* roots was assayed to determine the functional groups present, and the result is depicted in Figure 3. The spectrum showed broad bands at positions 3537.57 $cm^{-1}$ and 3201.99 $cm^{-1}$, which are attributed to the stretching vibrations of υ(OH) groups in phenols and flavonoids, respectively. The peaks at 2931.94 and 2872.10 $cm^{-1}$ correspond to asymmetric and symmetric stretching vibrations of υ(CH) in ($CH_2$) and ($CH_3$) groups, respectively (32). The spectral profile exhibits peaks at wave numbers 1728.28 $cm^{-1}$ and 1737.92 $cm^{-1}$, corresponding to carbonyl groups' stretching vibrations (33). The bands within the 1631.83 and 1514.17 cm-1 range are ascribed to the stretching vibrations of υ(C=C) groups. The bending of the hydroxyl group was observed at wavenumber 1452.45 $cm^{-1}$ (34).

### 3.4. GC-Mass Analysis

GC-MS analysis of the EtOH extract of *Astragalus spinosus* roots (Figure 4 and Table 2) has provided valuable insights into the chemical composition of this medicinal plant. The analysis revealed a total of 38 peaks, and through meticulous identification based on their retention time on the silica capillary column(35), the compounds present in the extract were characterized. The comprehensive chroma-togram can be observed in Figure 4, while the detailed information regarding the chemical constituents, including their retention time (RT), molecular formula, match score, and area (%), is presented in Table 2. Several prominent constituents have been identified among the diverse bioactive compounds detected in the GC-MS analysis of the ethanol fraction of Astragalus spinosus roots. Notably, 1,1-ethanediol diacetate is the main compound in this organ, constituting approximately 20.02% of the total identified compounds. Additionally, the following compounds were found in relatively significant amounts within the EtOH extract of *Astragalus spinosus* roots: pentadecanoic acid (17.39%), N-Methoxy-Formamide acid (13.10%), Benzyl Chloride (6.26%), 2-Methoxyphenol (4.55%), 1-Methyl-5-fluorouracil (3.98%), 5-Hydroxymethylfurfural (3.88%), 2-Methoxy-4-vinyl phenol (2.63%), 2,6-Dimethoxyphenol (2.40%), 3-Hydroxy-4-methoxy benzaldehyde (2.38%), Acetovanillone (2.08%), Butylated Hydroxytoluene (2.07%), 1-Hexadecene (1.77%), 2,4-Di-tert-butyl-6-nitrophenol (1.75%), 4-(1-Hydroxyallyl)-2-methoxy phenol (1.35%), 1-Hexadecanol (1.20%), Isophytol (1.17%), Pentadecanoic acid (1.16%) as the important compounds in the EtOH extract of *Astragalus spinosus*.

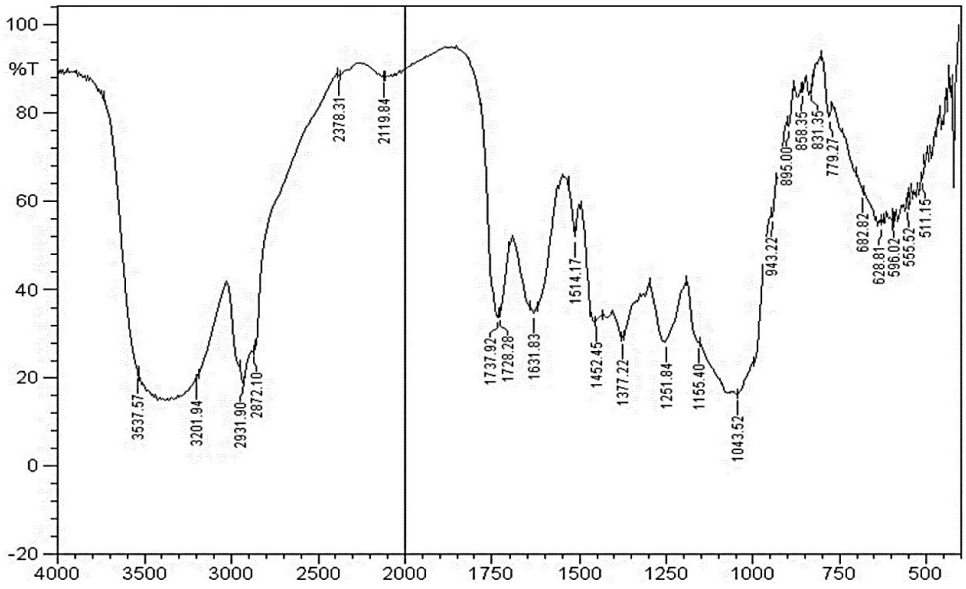


**Figure 3:** FTIR Spectrum of the EtOH roots extract.

It was found through the chromatogram and the table of GC-MS that the EtOH extract of the roots contains different chemical compounds belonging to different classes, which are alkaloids, terpenoids, flavonoids, phenols, esters, carboxylic acids, formamides, alkyl halides, furans, and alkene derivatives. Some alkaloids have shown promising potential in research as anticancer agents against human breast cancer (36). Research studies have investigated the specific anticancer properties of 1-methyl-5-fluorouracil (also known as 5-fluoro-1-methyluracil or 1-methyl-5-FU) (37). This compo-und serves as an analog of 5-fluorouracil (5-FU), a widely recognized chemotherapy medication emplo-yed in treating various cancer types, including breast cancer. 5-fluorouracil is classified as a pyrimidine analog that interferes with DNA synthesis and obstructs cell division in rapidly dividing cells, such as cancer cells. In clinical practice, 5-fluorouracil is frequently used in combination with other drugs to effectively treat breast cancer and other tumors. Terpenoids, commonly referred to as terpenes, function as antioxidants, defending cells against oxidative stress induced by free radicals (38). Various terpenoids showed inhibition by impeding the growth and multiplication of cancer cells. Overall, terpenoids have displayed a range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer attributes (39). Flavonoids are a diverse group of phenolic compounds that may be found in different parts of the plant. They have been extensively studied for their potential health benefits and medical applications. Some of the key medical applications of flavonoids 2-methoxyphenyl

(Guaiacol), 2-methoxy-4-vinyl phenol (4-Vinylguaiacol), and 4-(1-hydroxyallyl)-2-methoxyphenyl (Eugenol) are known for their antioxidant and antimicrobial pro-perties (40). It has been used in traditional medicine and has potential applications in treating respiratory disorders and as an antiseptic. As for phenolic and aromatic compounds such as 2,6-dimethoxy phenyl, butylated hydroxytoluene, and 2,4-di-tert-butyl-6-nitrophenol, they reported anti-oxidant and anti-inflammatory properties (41).

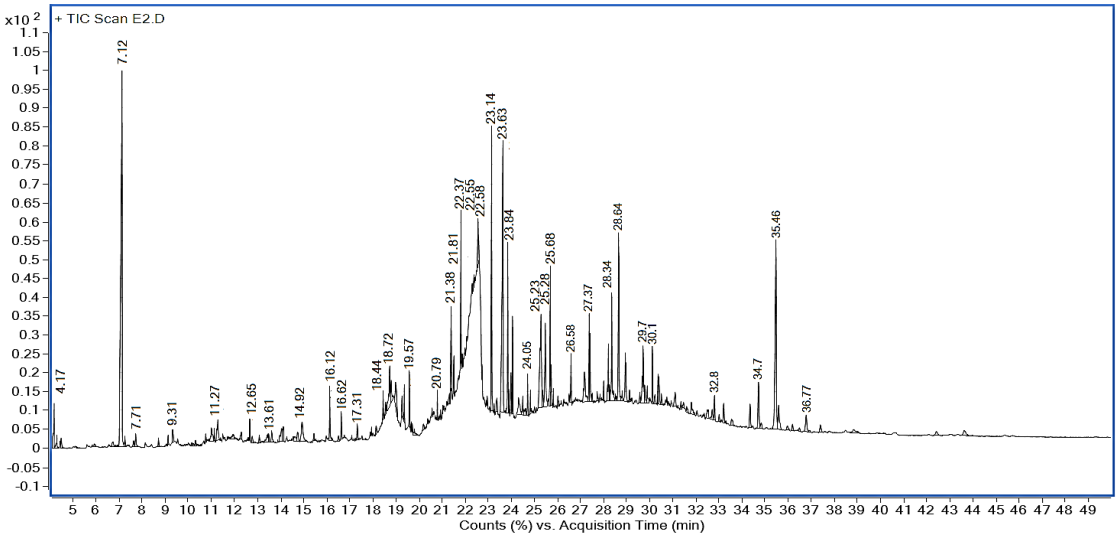


**Figure 4:** The comprehensive ion chromatogram for GC-MS examination of the *Astragalus spinosus* extracted roots.

**Table 2:** GC-MS peak identification for *Astragalus spinosus* roots.

| Peak No. | RT | Name | CAS No. | Formula | Area % | Similarity Index |
|---|---|---|---|---|---|---|
| **1** | 4.18 | Methyl Formate | 107-31-3 | $C_2H_4O_2$ | 0.66 | 98.6 |
| **2** | 7.12 | N-Methoxy-Formamide | 34005-41-9 | $C_2H_5NO_2$ | 13.107 | 79.5 |
| **3** | 7.72 | Trimethyl Orthoformate | 149-73-5 | $C_4H_{10}O_3$ | 0.306 | 71.1 |
| **4** | 9.31 | 2-(Dimethylamino) ethylacetate | 1421-89-2 | $C_6H_{13}NO_2$ | 0.68 | 82.2 |
| **5** | 11.3 | Benzyl chloride | 100-44-7 | $C_7H_7Cl$ | 0.65 | 97 |
| **6** | 12.7 | 2-Methoxyphenol | 90-05-1 | $C_7H_8O_2$ | 0.364 | 98.2 |
| **7** | 13.6 | 1-Methyl-5-fluorouracil | 28564-83-2 | $C_5H_5FN_2O_2$ | 0.298 | 93 |
| **8** | 14.9 | 5-Hydroxymethylfurfural | 67-47-0 | $C_6H_6O_3$ | 0.981 | 90.1 |
| **9** | 16.1 | 2-Methoxy-4-vinylphenol | 7786-61-0 | $C_9H_{10}O_2$ | 0.763 | 97.5 |
| **10** | 16.6 | 2,6-Dimethoxyphenol | 91-10-1 | $C_8H_{10}O_3$ | 0.448 | 96.9 |
| **11** | 17.3 | 3-Hydroxy-4-methoxybenzaldehyde | 121-33-5 | $C_8H_8O_3$ | 0.306 | 96.2 |
| **12** | 18.4 | Acetovanillone | 498-02-2 | $C_9H_{10}O_3$ | 0.474 | 94.4 |
| **13** | 18.7 | Butylated hydroxytoluene | 128-37-0 | $C_{15}H_{24}O$ | 3.884 | 70.1 |
| **14** | 19.6 | 1-Hexadecene | 629-73-2 | $C_{12}H_{26}O$ | 0.907 | 98.6 |
| **15** | 20.8 | 2,4-Di-tert-butyl-6-nitrophenol | 20039-94-5 | $C_{14}H_{21}NO_3$ | 0.341 | 85.1 |
| **16** | 21.4 | 4-(1-Hydroxyallyl)-2-methoxyphenol | 32811-40-8 | $C_{10}H_{12}O_3$ | 2.087 | 95.2 |
| **17** | 21.8 | 1-Hexadecanol | 1000130-97-9 | $C_{16}H_{34}O$ | 2.402 | 97.9 |
| **18** | 22.4 | Isophytol | 505-32-8 | $C_{20}H_{40}O$ | 0.403 | 67.7 |
| **19** | 22.6 | Pentadecanoic acid | 1002-84-2 | $C_{15}H_{30}O_2$ | 17.393 | 79 |
| **20** | 22.6 | 1,1-Ethanediol diacetate | 542-10-9 | $C_6H_{10}O_4$ | 20.02 | 75.6 |
| **21** | 23.1 | Lidocaine | 137-58-6 | $C_{15}H_{22}N_2O_3$ | 3.985 | 98.3 |
| **22** | 23.6 | 3-Eicosene, (E) | 57-10-3 | $C_{20}H_{40}$ | 4.559 | 87.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **24** | 23.8 | Ethyl palmitate | 628-97-7 | $C_{18}H_{36}O_2$ | 2.389 | 98.1 |
| **25** | 24.1 | Methyl 3-(3,5-di tert-butyl-4-hydroxyphenyl) propionate | 36294-24-3 | $C_{19}H_{30}O_3$ | 1.173 | 93.2 |
| **26** | 25.2 | 7-Methyl-3,4-octadiene | 37050-05-8 | $C_9H_{16}$ | 1.2 | 71.5 |
| **27** | 25.3 | 9(E)-Octadecenoic acid | 112-79-8 | $C_{18}H_{34}O_2$ | 2.639 | 85.4 |
| **28** | 25.7 | 1-Hexadecanol | 36653-82-4 | $C_{16}H_{34}O$ | 2.074 | 95.6 |
| **29** | 26.6 | Tetracosane | 646-31-1 | $C_{24}H_{50}$ | 0.646 | 94.5 |
| **30** | 27.4 | 2-Methyl-4-pentyltetrahydro-2H-thiopyran 1,1-dioxide | 67715-80-4 | $C_{11}H_{22}O_2S$ | 1.359 | 93 |
| **31** | 28.3 | 2-Palmitoylglycerol | 23470-00-0 | $C_{19}H_{38}O_4$ | 1.775 | 93.4 |
| **32** | 28.6 | Denatonium benzoate | 3734-33-6 | $C_{15}H_{23}NO_2$ | 1.756 | 83.2 |
| **33** | 29.7 | 9,12,15-Octadecatrienal | 26537-71-3 | $C_{18}H_{30}O$ | 0.871 | 77.3 |
| **34** | 30.1 | Heptacosane | 593-49-7 | $C_{24}H_{38}O_4$ | 0.776 | 96 |
| **35** | 32.8 | 4,6-cholestadienol | 14214-69-8 | $C_{27}H_{44}O$ | 0.462 | 67.6 |
| **36** | 34.7 | Stigmasterol | 83-48-7 | $C_{29}H_{48}O$ | 1.164 | 87.5 |
| **37** | 35.5 | Clionasterol | 83-47-6 | $C_{29}H_{50}O$ | 6.262 | 94.9 |
| **38** | 36.8 | 3,5-Stigmastadien-7-one | 2034-72-2 | $C_{29}H_{46}O$ | 0.436 | 74.5 |

### 3.5. Antibacterial Activity

The findings from Table 3 and Figure 5 present compelling evidence of the remarkable antibacterial potential exhibited by the EtOH extract of *Astragalus spinosus* roots. The results demonstrate a positive correlation between the extract's concentration and its antimicrobial activities against *S. aureus* and *S. mutans* bacteria. As the concentration of the EtOH roots extract increases, the inhibitory effects on bacterial growth become more effective, as indicated by the enlargement of the inhibition zone. A maximum zone of inhibition of 2.81 ± 0.88 mm and 2.68 ± 2.93 mm was recorded for *Astragalus spinosus* roots against *S. aureus* and *S. mutans*, respectively, at a 100 mg/mL concentration as compared to other tested concentrations. The outcomes detailed in Table 3 are presented in millimeters (mm), with three replicates so that statistical tests could be applied to evaluate the average ± standard deviation (S.D.).

**Table 3:** The ability of EtOH root extracts to inhibit the growth of the examined bacterial strains.

| Concentration of the EtOH Extract (µg/mL) | Diameter of Inhibition Zone (mm) | |
|---|---|---|
| | Bacterial Strains | |
| | ***S. aureus*** | ***S. mutans*** |
| **25** | 1.38 ± 0.67 | 1.54 ± 1.20 |
| **50** | 1.73 ± 0.33 | 2.09 ± 0.57 |
| **75** | 2.52 ± 0.58 | 2.56 ± 0.72 |
| **100** | 2.81 ± 0.88 | 2.68 ± 2.93 |

### 3.6. Cytotoxicity MTT assay

Cytotoxicity assessment was employed to evaluate the harmful impacts of the EtOH extract derived from the roots of *Astragalus spinosus* on the MCF-7 breast cancer cell line. The MTT assay was utilized to gauge cell viability and inhibition ratios for cancer cells, employing diverse concentrations of the alcoholic roots extract within the 12.5–400 µg/mL range. The influence of the EtOH roots extract on the MCF-7 breast cancer cell line demonstrated a reduction in cell viability that corresponded to the concentration, with cancer cell vitality diminishing as the extract concentration increased. Specifically, the viability of cancer cells revealed a minimum value of 35.378 ± 5.072 at 400 µg/mL while achieving a maximum value of 96.527 ± 0.722 at 25 µg/mL, and the assay is repeated three times as outlined in Table 4. The inhibition effectiveness of the root's alcoholic extract against the HdFn cell line yielded an IC50 of 62.39 µg/mL. In comparison, the inhibition efficiency against the MCF-7 breast cancer cell line resulted in an IC50 of 23.53 µg/mL (Figure 6).

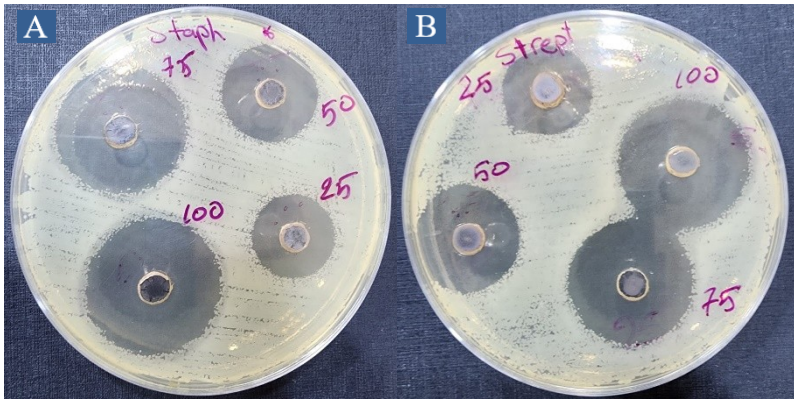


**Figure 5:** The inhibitory zone created by the EtOH extracts on the specified bacterial strains. (a) *S. aureus*. (b) *S. mutans*.

The EtOH extract demonstrates greater toxicity towards the MCF-7 cell line at elevated doses compared to lower doses. The outcomes indicate that the heightened dosage had a more pronounced toxic impact on cancer cells. This observation aligns with a referenced study that highlights how the suppression of cancer cells by plant extracts is contingent on the dosage (42). Research has substantiated that *Astragalus spinosus* exhibits anticancer properties through its possession of antioxidants, inducing a toxic effect on genes across all concentrations within MCF-7 cells (43). The toxicity of the EtOH extract towards cancer cells can be attributed to its elevated content of numerous anticancer components, including glycosides, flavonoids, phenols, and alkaloid compounds.

### 3.7. Antioxidant Study

The antioxidative characteristics of the EtOH extract were assessed using the DPPH radical scavenging assay. As illustrated in Figure 7A, the free radical scavenging capacities of the EtOH extract exhibited variability when compared to vitamin C. While ascorbic acid demonstrated higher potency at the initial concentration, the EtOH extract displayed greater scavenging abilities at other concentrations. Three tests were conducted in both DPPH and FRAP assays to assess differences, aiming to enhance precision by averaging the results obtained from these replicates. The extract's remarkable performance at a concentration of 1 µg/mL is particularly noteworthy, yielding a significantly high scavenging ability of (78.27±6.70), as detailed in Table 5 and Figure 7A. Furthermore, the EtOH extract outperformed vitamin E across all concentrations regarding free radical scavenging capabilities at the FRAP assay. At a concentration of 0.64 µg/mL, the extract showcased a higher scavenging ability of (0.55± 0.014), underscoring its robust antioxidative potential, with the lowest observed scavenging ability being (0.11±0.008) at a concentration of 0.04 µg/mL, as highlighted in Table 5 and Figure 7B. This substantiates the extract's capacity to neutralize free radicals effectively and suggests its promising role as a potent antioxidant agent.

**Table 4:** The impact of the EtOH extract from *Astragalus spinosus* roots on the (MCF-7) and (HdFn) cell lines.

| Conc.(µg/mL) | Viable cell count of HdFn±SD | Viable cell count of MCF-7±SD |
|---|---|---|
| **400** | $64.96\pm3.08^{D}$ | $35.37\pm5.07^{F}$ |
| **200** | $66.47\pm1.97^{D}$ | $42.20\pm2.72^{E}$ |
| **100** | $71.14\pm1.83^{C}$ | $54.01\pm3.23^{D}$ |
| **50** | $86.30\pm3.74^{B}$ | $64.69\pm4.71^{C}$ |
| **25** | $95.37\pm0.90^{A}$ | $75.03\pm4.98^{B}$ |
| **12.5** | $96.64\pm0.70^{A}$ | $96.52\pm0.72^{A}$ |

Distinct letters: significant variation ($P \leq 0.05$).

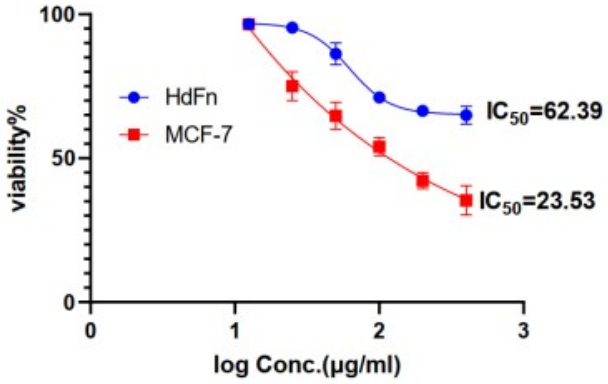


**Figure 6:** The effect of *Astragalus spinosus* roots EtOH extract in the MCF-7 and HdFn cell lines.

**Table 5:** Assessment of the antioxidant potential of the EtOH extract from *Astragalus spinosus* roots using the DPPH and FRAP assays.

| Conc. (µg/mL) | DPPH assay Mean± S.D. | | Conc. (µg/mL) | FRAP assay Mean± S.D. | |
|---|---|---|---|---|---|
| | Roots Extract | Vitamin C | | Roots Extract | Vitamin E |
| **0.062** | 29.57±4.47 | 39.66±2.25 | **0.04** | 0.11±0.008 | 0.101±0.001 |
| **0.125** | 53.36±4.33 | 41.33±10.01 | **0.08** | 0.31±0.010 | 0.108±0.001 |
| **0.250** | 65.69±1.23 | 48.33±8.50 | **0.16** | 0.41±0.005 | 0.114±0.004 |
| **0.500** | 71.85±6.43 | 53.00±10.53 | **0.32** | 0.45± 0.011 | 0.132±0.007 |
| **1.000** | 78.27±6.70 | 57.18±6.34 | **0.64** | 0.55± 0.014 | 0.211±0.015 |

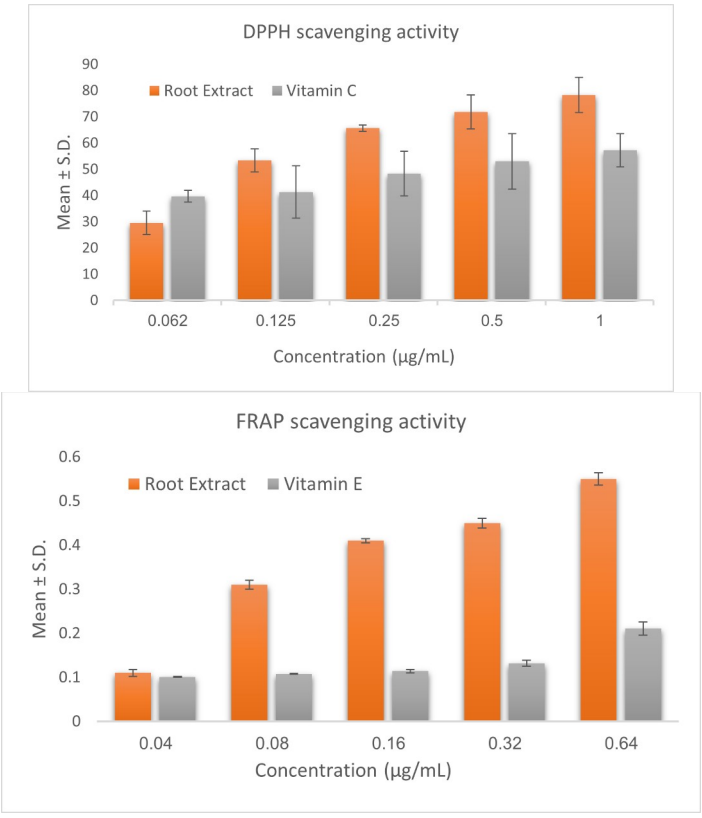


**Figure 7:** Evaluation of the antioxidant capacity of the extract derived from *Astragalus spinosus* roots, encompassing (A) the scavenging of DPPH radicals and (B) the FRAP scavenging activity.

## 4. CONCLUSION

This research yielded valuable insights into promising biological effects and phytochemical profiles, aiding in the identification and characterization of Iraqi *Astragalus* species. The present study employed GC-MS, FTIR, and UV-VIS analyses, as well as assessments of total flavonoids, total phenolics, antibacterial, antioxidant, and anticancer properties of the ethanol-extracted roots of *Astragalus spinosus*. The roots of *Astragalus spinosus* contain diverse phytochemical constituents that exhibit numerous pharmacological attributes. The GC-MS analysis identified 38 phytochemical components contributing to antimicrobial, antioxidant, anticancer, and related activities. Furthermore, this research provides compelling evidence of the roots extract's effectiveness against microbes (*S. aureus and S. mutans*), its antioxidant capabilities (measured through DPPH and FRAP assays), and its potential as an anticancer agent (evaluated against the MCF-7 cell line). Further research is required to identify and isolate specific bioactive chemicals, aiming to enhance our understanding of the intricate molecular processes of phytoconstituents' actions that contribute to biological activities, whether directly or indirectly.

## 5. CONFLICT OF INTEREST

There are no declarations of conflicts.

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