e-ISSN 2805-4356
p-ISSN 2805-4364

10.5281/zenodo.13933130 Vol. 07 Issue 09 Oct - 2024 Manuscript ID: #01586

COMPARISON STUDY OF PHYTOCHEMICAL PROFILE, CYTOTOXICITY AND ANTIOXIDANT ACTIVITIES OF *ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS* L., PLANTS

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Abstract:

Background: In Libya, arbutus and rosemary are used in traditional medicine to relieve pain and inflammation; however, the biological basis for these actions has not been fully studied.

Objective of the study: This study set out to characterize and examine the pharmacological characteristics of the phenolic and flavonoid chemicals found in the aerial sections of rosemary and Arbutus.

Material and methods: ***Arbutus pavarii and rosmarinus officinalis* l., plants** aerial parts were tested for antioxidant activity (DPPH), cell viability and cytotoxic effects. Phenolic and flavonoid contents (HPLC), and volatile constituents (GC-MS) were also characterized.

Results: The methanol extract of rosemary aerial parts had the highest antioxidant activity, while the methanolic extract of Arbutus aerial parts had the lowest. The antioxidant activity of the methanol extract of Rosemary increased from (32.12±1..74 %) at a concentration of 10 µg/mL to (98.47±0.81%) at a concentration of 1280 µg/mL. While the methanol extract Arbutus aerial parts increased from (30.12±1.75%) at a concentration of 10 µg/mL to (98.17±0.81%) at a concentration of 1280 µg/mL. MTT assay revealed that the methanol extract of Extract of Rosemary and Arbutus aerial parts had significant cytotoxic effects on the A549 and WI38 cell lines, respectively. MTT assay of methanol extract of Rosemery has cytotoxic effects higher than methanolic extract of Arbutus aerial parts. Rosmarinic and Caffeic were the most abundant phenolic acid, followed by p-coumaric, ferulic, syringic, and gallic acids, respectively in methanolic extract of rosemary, on the other hand, the Quercetin and Myricetin were the most abundant phenolic acid. GC-mass analysis showed that aerial parts of Rosemary were rich in Rosmarinic acid and the methanolic extract of Arbutus aerial parts was rich in Quercetin phynolic acid.

Conclusion: Based on the observed data, it can be stated that the antioxidant and cytotoxic activities of rosemary are greater than those of arbutus aerial parts. One potential source of readily available natural bioactive ingredients with potential health and therapeutic advantages is the methanolic extract of rosmarinus officinalis l and *arbutus pavarii*.

Keywords: ***ARBUTUS PAVARII* AND *ROSMARINUS OFFICINALIS L* antioxidant; cytotoxicity; cell lines, phenolics; flavonoids**

Introduction

A significant source of life-saving medications for most people worldwide is medicinal plants, such as Vinca major, *Zingiber officinale,* and *Aloe vera* [1]. The World Health Organization (WHO) reports that over 75% of people worldwide treat illnesses with herbs and other traditional medicines. There are also several instances of novel pharmaceuticals made from wild plant species [2]. Because of their extensive biological and pharmacological properties, medicinal plants have long been used as the main source of medications to treat a wide range of illnesses. Additionally, they are essential in the identification of possible medicinal drugs. [3]. According to Gennari et al. [4], one of the most deadly illnesses, cancer is caused by aberrant cells proliferating out of control, which allows them to infiltrate and disturb surrounding tissues. According to Kelloff [5], cancer is one of the most fatal illnesses, having over 100 distinct forms that arise from molecular alterations in cells. It ranks third globally in terms of cause of mortality, behind infectious diseases and cardiovascular disorders. Breast cancer is the most prevalent type of cancer in women, according to Soliman et al. [6]. Pakistan has the highest rate of breast cancer incidence among South Central Asian nations [6]. It makes about 38.5% of all female cancer cases and is the most common cancer in women [5]. Prostate cancer is the most prevalent disease diagnosed in men in the US, according to the American Cancer Society, which also reported that colorectal cancer was the second most common cause of cancer-related deaths in the country [7]. The idea that a large number of diseases in the modern world are caused by "oxidative stress," which arises from an imbalance between the production and neutralization of prooxidants, is beginning to take hold. Free radicals are the cause of oxidative stress because they bind to biological macromolecules including proteins, lipids, and DNA in healthy human cells in an attempt to find stability. This leads to damage to proteins and DNA as well as lipid peroxidation [8]. Aging, inflammatory diseases, cancer, atherosclerosis, and cardiovascular problems are associated with these changes [9]. Since oxidative stress is a condition that all cells encounter, free radicals and oxidation may play a significant role in the development of cancer at various tumor locations. The primary enzymes involved in recruiting inflammation are the cyclooxygenase isoenzymes COX-1 and COX-2 [10]. Thus, there is a strong relationship between inflammation, carcinogenesis, and free radicals. As anticancer medicines, pharmacological candidates with anti-inflammatory and free radical scavenging properties are highly valued. Cancer can be fatal because of the lack of effective treatments, the high cost of chemotherapeutic agents, and the adverse effects of anticancer medications. Consequently, ongoing research is being done to find potent naturally occurring anticarcinogens that could stop, slow down, or even reverse the development of cancer. There is a unique role for medicinal plants in the treatment of cancer [11]. It is believed that over 50% of anticancer medicines are derived from plants in one form or another [12]. Numerous naturally occurring substances derived from medicinal plants, or secondary metabolites, have been found to exhibit significant antioxidant and other activities [13, 14]. These include terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids. These compounds have also been found to be important in the treatment of cancer [15]. Numerous antioxidant chemicals exhibit anti-inflammatory, anticancer, anti-mutagenic, and anti-carcinogenic properties, according to studies by Kaur et al. [16]. ***Arbutus*** is a genus of 12 accepted species [17] of flowering plants in the family Ericaceae [18) native to temperate regions of the Mediterranean, western Europe, the Canary Island and North America, and commonly called madrones [19] **or strawberry trees**. The name *Arbutus* was taken by taxonomists from Latin, where it referred to the species now designated Arbutus Unedo [20]. The present study was designed to evaluate the anticancer, and antioxidant activities of extracts Arbutus and Rosemary aerial parts, as well as to analysis of volatile constituents and phenolic acids by GC-Mass and HPLC.

3. Materials and Methods

3.1. Chemicals

High analytical quality chemicals and HPLC standards were employed, and they were all acquired from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO), MTT and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

3.2. Plant Materials and Sample Preparation

Arbutus and Rosemary used in this study were obtained from El-Jabal El-Akhdar Province –Libya during years 2023-2024. After washing, the aerial parts were air dried in the shade. The temperature ranged between 20–25 ℃, with a relative humidity of 30–70% during the drying period (2 weeks). The content of water gradually decreased and became practically constant (about 8–9%), and then the leaf material was ground into a fine powder, and packed in screwed brown bottles until utilized in the following experiments.

3.3. Maceration Process

According to the method described by **Romani et al. [21]** 100 g of the powdered E. cuneata (aerial parts) was extracted by shaking at 150 rpm for 24 h at RT with 1 L of solvent (methanol, In a Buchner funnel, extracts were filtered using Whatman No. 1 filter paper. The residue was re-extracted with 500 mL of solvent, filtered, and the filtrates were pooled and evaporated in a rotary evaporator at decreased pressure (Heidolph VV 2000). It was then dried in a desiccators under vacuum until it reached a consistent weight [**22**]. The weights of the final extracts were recorded, and the residue was re-suspended in the smallest amount of solvent possible to achieve a concentration of 10 mg/mL. The extracts were maintained at 4 ℃ until use. Triplicate determinations (n = 3) were applied in the study.

3.4. Antioxidant Capacity Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [23] was used to determine the free radical cavenging activity. The violet color of DPPH was reduced to a pale-yellow tint in this assay due to the removal of hydrogen atoms from the antioxidant molecule. The DPPH reduction increase by increasing antioxidant activity was observed. The assay reaction mixture consisted of 40 _L of E. cuneata extract at various concentrations (1280–10 mg/mL) generated by diluting the extract with the extraction solvent and 3 mL of methanolic solution of 0.1 mM (0.004%, w/v) DPPH radical. After vigorous shaking, the mixture was incubated at 37 ℃ for 30 min. A UV-visible spectrophotometer was used to detect the absorbance at 515 nm (Milton Roy, Spectronic 1201). The positive control was ascorbic acid. The following equation was used to calculate the relationship between decreasing absorbance of the reaction mixture and higher free radical scavenging activity.

DPPH scavenging activity (%) = 100 x(A0 - A1) / (A0) (1)

The absorbances of the control and sample, respectively, are A0 and A1. The results are presented as the average of three replicate analyses, with the major values as well as the standard deviation (SD) provided. The 50% inhibitory concentration (IC50), the concentration required to 50% DPPH radical scavenging activity was estimated from graphic plots of the dose response curve using Graphpad Prism software (San Diego, CA, USA).

2.5. HPLC-PDA-MS/MS

The extract was analyzed by HPLC-PDA-MS/MS using a Thermo Finnigan LC system (Thermo Electron Corporation, Austin, TX, USA). A Zorbax Eclipse XDB-C18, Rapid resolution, 4.6 × 150mm, 3.5µm column was used (Agilent, Santa Clara, CA, USA). A gradient consists of water and acetonitrile (ACN), each having 0.1% formic acid, was applied and acetonitrile was increased from 5 to 30% within 60min in 1mL/min flow rate and a 1:1 split before the ESI source (**24**).

2.6. Determination of phenolics in *rosmarinus officinalis* and *arbutus pavarii* plant extracts

One milliliter of rosemary extract solution (2 mg/mL) was transferred to an HPLC vial and determined using a Waters 2690 high-performance liquid chromatography system (Milford, MA, USA) with a photodiode array detector and a C18 column (id 250 × 4.60 mm 5 micron) (Phenomenex, Torrance, CA, USA). The phenolics were quantified by using the calibration curves of their corresponding standards.

2.7. Cytotoxic Activity:

2.7.1. Cell lines

Lung carcinoma cells "A-549", and the normal human lung fibroblast cell line (WI38).

2.7.1.1. Cell culture and MTT assay

To evaluate the cytotoxicity of the rosemary plant extract, the (3-(4,5- dimethylthiazol-2-yl– 2,5-diphenyl tetrazolium bromide) MTT colorimetric assay was performed in 96-well plates. This assay relies on the mitochondrial reduction of the yellow MTT to form purple formazan,reflecting the normal mitochondrial function and cell viability **(25)**. The whole procedure was maintained under sterile conditions *via* the use of a laminar air-flow cabinet, following culturing and sub-culturing technique adopted by **Abd-El-Aziz *et al*., (25).**

The cells (1 X 10^4 / ml) were inoculated (100 µl/well) in the 96-well tissue culture plate and incubated for 24 h in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After the formation of confluent monolayer of cells, growth medium was decanted from 96 well microplate and cells were washed twice with washing media. Then cells were treated with a series of two fold dilutions of rosemary plant extract. Serial dilutions were prepared by dissolving the tested samples in DMSO followed by dilution with RPMI–1640 medium to give a final concentration (from 1000 to 7.812 µg/ml). Each dilution was tested in triplicate (100µl/well) leaving 3 wells as control receiving only maintenance medium (RPMI-1640) with 2% serum. The treated cells was incubated at 37°C for 24 h and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, shrinkage, rounding, or cell granulation, membrane blabbing, nucleus fragmentation or chromatin condensation. Later, MTT solution (5 mg/ml) was dissolved in PBS and 20 µl was added to each of the 96 wells. The plate was placed on a shaker at 150 rpm for 5 minutes to mix the MTT into the media thoroughly and incubated at 37°C (95% air and 5 % CO_2) for 1-5 h to allow the MTT to be metabolized. The media was carefully dumped off and the formazan (MTT metabolic product) was resuspended in 200 µl DMSO and placed on a shaker at 150 rpm for 5 min. to thoroughly mix formazan into the solvent. The amount of formazan produced is directly proportional to the number of viable cells. The optical density (O.D.) was recorded at 560 nm using the mindray micro plate reader. The viability percentages were calculated using the following equation **Mosmann, (1983**

$$\text{Viability (\%)} = \frac{\text{O.D. value of experimental sample}}{\text{O.D. value of experimental control}} \times 100$$

O.D. should be directly correlated with cell quantity.

2.7.1.2. Evaluation of Cytotoxic Effects of certain Chemical compound

2.7.1.2. 1. Mammalian cell lines: A549 cells (human lung cancer cell line) were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

2.7.1.2. 2. Cell line Propagation

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

2.7.1.2. 3.Cytotoxicity evaluation using viability assay

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve ten concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. 5-flourouracil was used as reference standard drug (positive control) for comaparison. After incubating for 48 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.The relation between surviving cells and drug concentration isplotted to get the survival curve of each tumor cell line after treatment with thespecified compound.The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) (**26).**

2.8. Statistical Analysis

An analysis of variance was performed on all of the data (ANOVA). The main values, and the standard deviation (SD), were calculated from three samples of each item. Duncan's multiple range tests (p _ 0.05) were used to examine the significance of the variable mean differences. SPSS 16 was used to conduct all of the analyses [27].

3. Results and Discussion

3.1. DPPH Radical Scavenging Activity of Arbutus aerial parts Extracts.

We investigated the antioxidant potential of various Arbutus aerial parts extracts in order to learn more about the potential health advantages of Arbutus aerial parts. All Arbutus aerial components extract the DPPH radical in a dose-dependent manner, as Table 1 demonstrates. Methanol extract

showed the highest scavenging potency (79.33%), followed by acetone (78.36%) and ethanol (75.76%) in terms of the extraction solvent's impact on antioxidant activity. On the other hand, ether had the lowest scavenging potency (42.31%) of all the extraction solvents. It was observed that when the concentration of Arbutus rose, so did the scavenging potency. Ascorbic acid was used as the standard in the DPPH method to assess the antioxidant activity. The range of ascorbic acid values was 1280 to 2.5 µg/mL. Ascorbic acid exhibited a 34.57% inhibition percentage at 2.5 _g/mL and a 99.23% inhibition percentage at 1280 µg/mL. Using a concentration of 1280 _g/mL produced the highest scavenging percentage (95.53%), followed by 640 µg/mL (91.16%) and 320 µg/mL (84.17%), respectively, according to statistical analysis. Conversely, the concentration of 10 µg/mL had the lowest average value of 21.43 ±1.90% for scavenging potency. Ascorbic acid's IC50 value was 10.6 µg/mL. The methanolic extract's IC50 value was 21.5 µg/mL, the acetone extract's was 22.14 µg/mL, and the ethanol extract's was 25.80 ± 2.13 µg/mL (Table 2). The IC50 values for extracts of Arbutus aerial parts were determined using the San Diego, California-based Prism program. A non-linear model was used to calculate the IC50. Ultimately, statistical analysis revealed no significant ($p < 0.05$) differences between the extracts of methanol, acetone, ethanol, water, ethyl acetate, and chloroform. At all concentrations examined using the DPPH assay, Munro et al. [28] found that the methanolic extract of Euphorbia tirucalli exhibited superior radical scavenging efficacy than the aqueous extract. Shaker et al. [29] discovered that E. schimperiana and its fractions (petroleum ether, ethyl acetate, and n-butanol/water) had the highest antioxidant activity when compared to *E. peplus* and *E. cuneata*. Our results, however, were different from those of Awaad et al. [30], who discovered that the ethyl acetate extract had the maximum activity and varied from 26.51 to 83.50%, whereas the reference standard "ascorbic acid" showed 87.8% at a dosage of 100 mM. All of us did agree, though, that the ether extract had the lowest percentage of activity out of all the extracts. In this study, phytochemical screening of A. Pavarii indicated the presence of simple and poly phenolic active compounds. These compounds are documented as disease preventive with antimicrobial activity and to reduce the risk of cancer [16, 17]. The methanolic and chloroform extracts showed a good antioxidant activity but n-hexan showed negative results. These results may be due to the polarity of the solvent. n-hexane is a non-polar solvent and therefore it may be did not extract the active constituent in contrast to methanol and chloroform. The results support the previously reported finding for this solvent [8].

3.2. DPPH Radical Scavenging Activity of rosemary aerial parts extracts

We screened the antioxidant capacity of several rosemary aerial part extracts in order to look into the possible health benefits of rosemary aerial parts. Table 3 shows that methanol extract had the maximum scavenging potency at 79.36%, followed by ethanol at 66.17% and acetone at 61.77%, with respect to the influence of the extraction solvent on antioxidant activity. Chloroform had the lowest scavenging potency at 40.34%. Concerning the effect of extract concentration, the scavenging potency rose as the concentration of rosemary aerial components increased. The concentration of 1280 µg/mL yielded the highest scavenging percentage of 94.37%, which was followed by 840 µg/mL yielding 89.72% and 320 µg/mL yielding 79.31%, respectively. Conversely, the lowest scavenging potency was seen at 10 µg/mL, with an average of 12.16%. The methanolic extract had the lowest IC50 value of 16.8 µg/mL, while the water extract had the highest value of 129.6 µg/mL (Table 4). The obtained outcomes supported the findings of Kebbab-Massime et al. [31], who discovered that at all doses assessed using the DPPH assay, the methanolic extract of Solenostemma argel exhibited more radical scavenging activity than the aqueous extract. The radical scavenging activity of Solenostemma argel rose from 32% at a concentration of 250 µg/mL to 84% at a concentration of 1000 µg/mL, according to Tajt al. [32]. Al-Juhaimi et al. [32] stated that the antioxidant activity of argel extract was due to the presence of phenolic acids, flavones, glycosylated flavonoids, polyphenols, b-carotene, b-

sitosterol, monoterpenes, pregnenes, and pregnan Benmaarouf et al. [33] and lsanhoty et al., [34] stated that the high content of flavonoids such as rutin, kaempferol-3-o-rutinoside, kaempferol-3-o-diglucoside-7-o-glucoside, astragalin, and kaempfero in *Solenostemma argel* imparted its antioxidant activity. Numerous studies indicated that strong antioxidant properties of rosemary extracts (**Ozcan, 2003; Gimenez et al. 2004; Moreno et al., 2006; Cadun et al.2008; Tironi et al., 2010**35, 36, 37, 38).

3.3. Major phenolic compounds of *Arbutus Pavarii* aerial parts methanolic extract

The main phenolic compounds found in *Arbutus pavarii* and rosemary plants were displayed in Tables 5, 6, Figures 1 and 2. Utilizing a gradient mobile phase comprising of solvents A (methanol) and B (acetic acid in ultrapure water, 1:25), phenolic acids were isolated. The following gradients were used to accomplish elution from the column: Solvent B for 0–3 minutes. After five minutes, 50% eluent A was added. Then, for the next two minutes, the concentration of A was raised to 80%, and for the next five minutes, it was once more lowered to 50%. The chemicals with the highest concentrations were rosmarinic (5280.57 µg/g), followed by caffeic (2442.60), ferulic (469.38), apigenin-7-glucoside (302.43), and sinapic compound (253.44). At 99.73, potocatechuic was then. The value of the p-hydroxybenzoic compounds was 65.46. Apigenin then measured 50.42 µg/g. The remaining seventeen compounds, according to data in Table 5 and Figure 1, ranged from 1-45 to 19.33 gallic, Gentisic, Cateachin, and other compounds. Table 5 and Figure 1 presented results indicating the inability to determine the presence of three phenolic compounds: rutin, cinnamic, and chlorogenic. Data in table 6 and figure 2 indicated the major phenolic compounds of *ARBUTUS PAVAR*II methanolic plant**.** Data showed that the major phenolic compound were ten compounds. the main compound was quercetin and the concentration was 456.893 followed myricetin, was then Kaempferol was 122.482 µg/g, then *p*-hydroxybenzoic acid was 61.735, then Cateachin was 51.47 µg/g. the most quercetin and Myricetin acid were the most abundant phenolic compounds. Caffeic acid (3,4-dihydroxycinnamic acid) has been known as an important source of natural antioxidants in different agricultural products [39, 40]. It has immense use in cancer treatment [286,287], and it could be known as an important natural antioxidant [41]. Caffeic acid can induce apoptosis in cancer cells through increasing ROS levels and impairing mitochondrial function, and it also benefits from reducing aggressive behavior of tumors via suppressing metastasis [42]. Caffeic acid has anti-inflammatory and antioxidant properties against 6-propyl-thiouracil (PTU)-induced hypothyroidism [43]. Meinhart et al. [44] reported that higher sums of mono-caffeoylquinic acids were found in mulberry, quince, and bilberry, and the dicaffeoylquinic acids sum was higher in granadilla. Hanan M. El-Basir [45] discovered similar results, indicating that the plant extracts contained flavonoids, tannins, glycosides, simple phenolics, free reducing sugars, triterpenes, and sterols. Comparing the methanolic and chloroform extracts to quercetin, the IC50 values were 4.55±1.90µg/ml and 21.55±1.1 µg/ml, respectively, suggesting the presence of potential antioxidant action. It has been proven that the most significant bioactive components found in plants include flavonoids, alkaloids, tannins, and phenolic chemicals [46]. Numerous biological activities as well as antibacterial properties have been described for plant tannins and flavonoids [47, 48, 65].

Table 1. Extracts from Arbutus aerial parts exhibit radical scavenging action against DPPH at varying doses.

	Radical scavenging activities of extracts[a]								
Conc. (µg/mL)	**Water**	**Acetone**	**Chloroform**	***Methylene chloride***	**Ether**	**Methanol**	**Ethanol**	**Ethyl acetate**	***Conc. mean±SE***
1280	**98.54±0.92**	**97.98±0.64**	**93.76±0.62**	**91.06±1.42**	**88.96±1.42**	**98.17±0.81**	**96.91±1.35**	**97.09±0.82**	**94.53±03.66**
640	**97.09±0.74**	**97.19±0.59**	**91.82±0.74**	**79.47±1.85**	**77.35±1.93**	**95.76±0.52**	**94.82±0.94**	**93.42±1.34**	**91.16±07.60**
320	**94.64±1.35**	**95.76±0.92**	**78.06±1.93**	**68.18±1.94**	**61.18±1.26**	**94.18±0.74**	**93.06±0.72**	**87.03±0.78**	**83.17±12.81**
160	**89.48±1.09**	**91.39±0.83**	**66.88±2.14**	**57.35±3.17**	**47.94±3.98**	**92.88±0.68**	**91.53±0.65**	**79.12±0.56**	**76.06±16.71**
80	**78.69±2.47**	**87.57±1.75**	**51.47±2.95**	**48.76±2.82**	**28.18±1.74**	**90.39±0.53**	**86.71±1.83**	**72.06±1.32**	**67.21±21.00**
40	**59.89±2.94**	**82.04±1.62**	**38.84±3.62**	**38.65±1.91**	**19.29±0.63**	**87.62±1.84**	**73.59±2.73**	**54.35±1.93**	**55.16±21.03**
20	**39.76±2.32**	**47.35±2.19**	**28.12±1.76**	**31.47±1.35**	**14.41±0.57**	**48.56±2.98**	**38.88±2.94**	**34.94±1.82**	**34.44±10.32**
10	**21.35±1.75**	**29.76±1.35**	**21.94±0.82**	**19.92±1.46**	**04.18±0.26**	**30.12±1.75**	**26.63±1.75**	**18.06±2.95**	**22.11±08.30**
Group mean±SE	**73.29±27.50**	**79.38±24.23**	**59.61±25.00**	**53.36±23.17**	**40.31±29.14**	**78.33±23.67**	**75.76± 25.98**	**67.81±26.86**	

[a]Percentage inhibition of radical scavenging activity
At a concentration of 1280 µg/mL, the standard chemical ascorbic acid had a percentage inhibition value of 99.23%.

Table 3. Extracts from Rosemary aerial parts **exhibit radical scavenging action against DPPH at varying doses.**

	Radical scavenging activities of extracts[a]								
Conc. (µg/mL)	**Water**	**Acetone**	**Chloroform**	***Methylene chloride***	**Ether**	**Methanol**	**Ethanol**	**Ethyl acetate**	***Conc. mean±SE***
1280	**98.56±0.92**	**98.78±0.64**	**93.76±0.62**	**92.06±1.42**	**87.96±1.42**	**98.47±0.81**	**97.91±1.35**	**97.06±0.82**	**95.53±03.66**
640	**97.08±0.74**	**97.15±0.59**	**90.82±0.74**	**78.47±1.85**	**78.35±1.93**	**96.76±0.52**	**95.82±0.94**	**94.82±1.34**	**91.16±07.60**
320	**94.63±1.35**	**95.06±0.92**	**77.06±1.93**	**69.18±1.94**	**60.18±1.26**	**95.18±0.74**	**94.06±0.72**	**88.04±0.78**	**84.17±12.81**
160	**89.47±1.09**	**91.29±0.83**	**65.88±2.14**	**58.35±3.17**	**46.94±3.98**	**93.88±0.68**	**91.53±0.65**	**79.12±0.56**	**77.06±16.71**
80	**78.59±2.47**	**87.53±1.75**	**52.47±2.95**	**47.76±2.82**	**29.18±1.74**	**90.39±0.53**	**86.71±1.83**	**73.06±1.32**	**68.21±21.00**
40	**59.88±2.94**	**81.04±1.62**	**39.84±3.62**	**37.65±1.91**	**19.29±0.63**	**80.62±1.84**	**74.59±2.73**	**56.35±1.97**	**56.16±21.03**
20	**39.76±2.32**	**46.35±2.19**	**28.12±1.76**	**32.47±1.35**	**13.41±0.57**	**47.56±2.98**	**39.88±2.94**	**35.94±1.82**	**35.44±10.32**
10	**20.34±1.75**	**29.71±1.35**	**20.94±0.82**	**18.92±1.46**	**03.18±0.26**	**32.12±1.74**	**25.63±1.75**	**18.06±2.95**	**21.11±08.30**
Group mean±SE	**72.29±27.50**	**78.36±24.23**	**58.61±25.00**	**54.36±23.17**	**42.31±29.14**	**79.33±23.67**	**75.76± 25.98**	**67.81±26.86**	

[a]Percentage inhibition of radical scavenging activity
At a concentration of 1280 µg/mL, the standard chemical ascorbic acid had a percentage inhibition value of 99.23%.

Table 2. IC50 values of Arbutus aerial parts extracts toward DPPH.

Type of Extract	**IC50 (ug/mL)**
Water	31.22 ±2.86
Acetone	22.14 ±1.99
Chloroform	72.26 ± 5.44
Methylene cholide	96.91 ±5.27
Ether	194.1 ± 13.3
Methanol	21.43 ±1.90
Ethanol	25.80 ± 2.13
Ethyl acetate	33.80 ± 2.84

Table 4. IC50 values of Rosemary aerial parts extracts toward DPPH.

Type of Extract	**IC50 (ug/mL)**
Water	127.61 ± 5.48
Acetone	61.10 ± 3.1
Chloroform	71.22 ± 5.44
Methylene cholide	26.10 ± 17.4
Ether	112.60 ± 4.6
Methanol	17.81 ± 0.62
Ethanol	25.80 ± 2.13
Ethyl acetate	124.20 ± 6.4

Table 5. Major phenolic compounds of rosemary plant extract

Compound	Concentration(µg/g)
Gallic	1.45
Protocatechuic	99.73
p-hydroxybenzoic	65.46
Gentisic	19.33
Cateachin	12.47
Chlorogenic	ND
Caffeic	2442.60
Syringic	69.44
Vanillic	3.03
Ferulic	469.38
Sinapic	253.44
Rutin	ND
p-coumaric	2.24
Apigenin-7-glucoside	320.43
Rosmarinic	5280.57
Cinnamic	ND
Qurecetin	12.46
Apigenin	50.42
Kaempferol	6.95
Chrysin	2.39

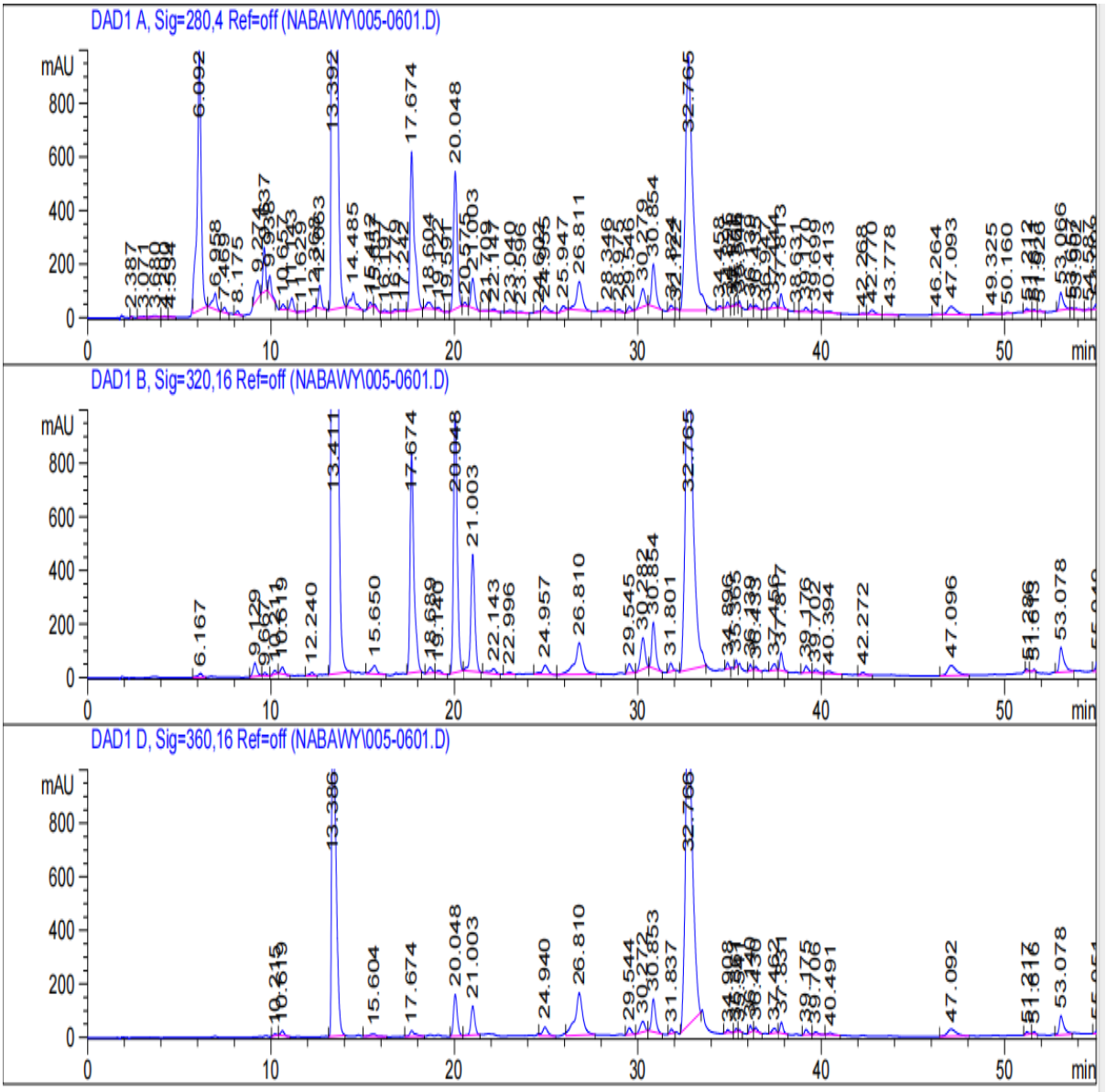


Fig 1: HPLC chromatogram of *ROSMARINUS OFFICINALIS* methanolic extract .

Table 6: Major phenolic compounds of *ARBUTUS PAVARII* methanolic plant

compound	**sample conc (ug/g)**
Gallic acid	5.200
arbutin	1.009
p-hydroxybenzoic acid	61.735
Gentisic acid	ND
Cateachin	51.471

Chlorogenic acid	ND
Caffeic acid	ND
Syringic acid	ND
Vanillic acid	55.759
Ferulic acid	ND
Sinapic acid	ND
p-coumaric acid	ND
Rutin	0.591
naringin	ND
Cinnamic acid	ND
quercetin	456.893
Kaempferol	122.482
Chrysin	ND
Myricetin	365.947
Quinic acid	7.152

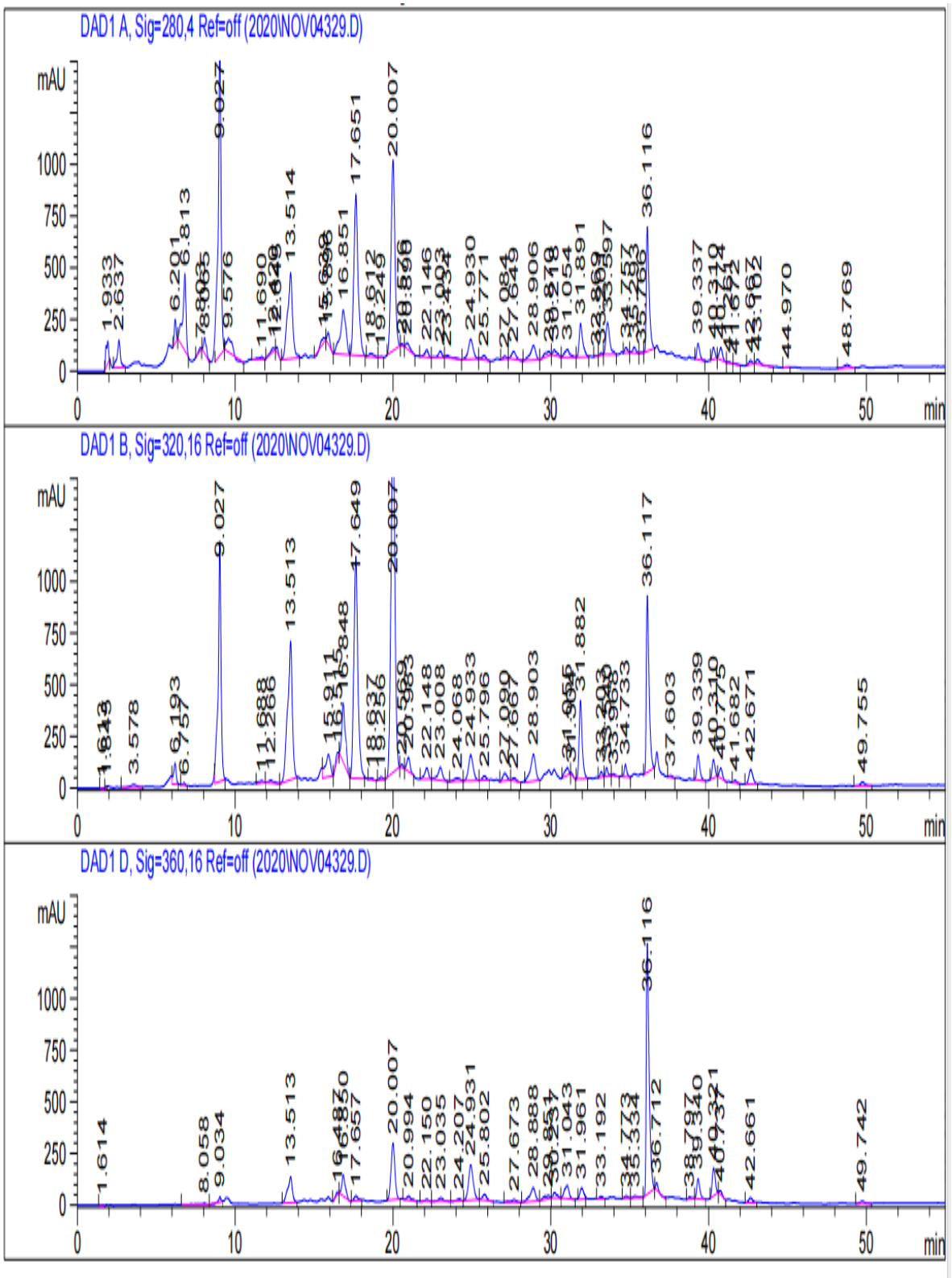


Figure 2: Fig : HPLC chromatogram of *ARBUTUS PAVARII* plant.

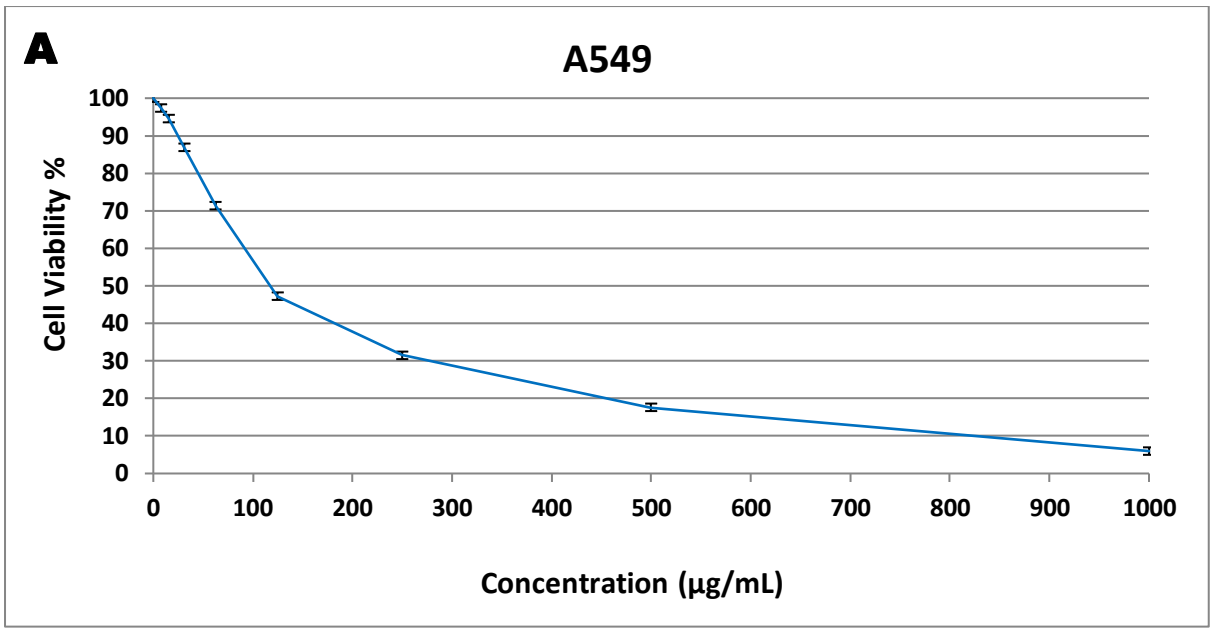
3.7. Antiproliferative Effects of Rosemary aerial parts on A549, , and WI38 Cells

Only the methanolic extract of rosemary aerial parts was tested for cytotoxicity against two distinct cancer cell lines (A549 and W38). The degree to which the methanolic extract was cytotoxic to the cell lines was determined using the micro-culture tetrazolium (MTT) test. Cytotoxic activity in A549 and W38 cell lines was expressed as a percentage of cell viability relative to the control. The dose-response curve was utilized to determine the effective doses after a variety of methanolic extract concentrations from Rosemary aerial parts extract were employed. Effective doses were determined utilizing the dose-response curve and a variety of methanolic extract concentrations from the extract of rosemary aerial parts. Figures 3A and B display the outcomes of a cytotoxicity test conducted on a variety of cell lines using an extract from rosemary aerial parts. The A549 cell line was used to test the cytotoxicity of a methanolic extract made from rosemary aerial parts. The IC50 of doxorubicin (standard reference materials) is 0.96±0.21 µg/mL, whereas the IC50 of the methanol extract extracted from rosemary aerial parts is 119±9.8 g/mL (Figure 3A and B). It was looked into whether the methanolic extract of rosemary aerial parts was cytotoxic to the W38 cell line. Doxorubicin's IC50 value was 192 ±0.24 µg/mL, while the methanol extract of rosemary aerial parts had an IC50 value of 144 ±17.2 µg/mL (Figure 1B). Conversely, the W38 cell line was tested for the cytotoxicity of a methanolic extract of rosemary aerial parts. Doxorubicin's IC50 value was 0.63 ± 0.02 µg/mL, while the methanol extract of rosemary aerial parts had an IC50 value of 172.1 ±16.3 µg/mL (Figure 1B). The A549 cells exhibited a higher rate of cell death following treatment with a methanol extract of rosemary aerial parts extract, relative to the W38 cell lines at the same dose of the extract utilized. There is evidence to suggest that the active ingredients of Euphorbia species may have anticancer characteristics [50], and previous research have established a relationship between the antioxidant activity and anticancer capabilities of plant extracts [49]. Ben Jannet et al. [51] reported that the polar and polar fractions of E. terracina and the polar fraction of E. paralias were particularly active against human acute myeloid leukaemia (THP1) cells, with no cytotoxicity observed against normal cells (CD14+ monocytes). *G. acutidentatum* methanolic and aqueous extracts were found to have low toxicity toward cancer-originating HT-29 (human colorectal adenocarcinoma) and HeLa (human cervical adenocarcinoma) cell lines, as well as no cytotoxicity toward non-cancerous PC12 cells (CRL-1721; derived from a transplantable rat pheochromocytoma) in Kocanci et al.'s study [52]. After incubating PC12 cells for 24 hours with either methanolic or aqueous extracts, the CC50 values were 979 µg/mL and 1383 µg/mL, respectively. Regretfully, the CC50 values could not be determined because the authors only considered the percentage of cellular viability at two concentrations—500 and 1000 µg/mL—while evaluating the anticancer potential. In our investigations, we chose the RKO cells because we found that these cells had increased cytotoxicity toward colorectal cancer cells. According to the American National Cancer Institute (NCI), the criteria for significant anticancer activity for crude extracts is CC50 < 20 µg/mL after 48 h or 72 h incubation [53]. Other reports [80–54] consider CC50 of up to 30 µg/mL as a promising crude extract for further research. FurthermoreIt has been observed that certain extracts include chemicals with noteworthy anticancer action. Boldine, for instance, exhibited cytotoxic effects on human colorectal cancer (CRC) and osteosarcoma cell lines in a concentration-ependent manner [55]. By inducing mitochondrial failure and cutting off cellular energy, isocorydine demonstrated a strong anticancer impact against oral squamous cell carcinoma (OSCC) and also prevented the growth of oral tongue squamous cells (Cal-27) [56]. A number of mouse tumor cell lines, including leukemia P388 and L1210, melanoma B16, bladder cancer MBC2, and colon cancer Colon 26, were demonstrated to be inhibited by the aporphine alkaloids glaucine and corydine in culture [57]. Protopine has been demonstrated to be effective against a variety of cancer cell types, including lung cancer (A549), pancreatic cancer (MIA Paca-2 and PANC-1), breast cancer (MDA-MB-231), prostate cancer (HRPC), pancreatic cancer (HCT116 and SW480), and colon cancer (HCT116 and SW480) [58]. In addition to these alkaloids, rutin

(flavonoid) has been shown to inhibit multiple cancers through a variety of mechanisms, including inflammation, cell cycle arrest, inhibition of malignant cell growth, oxidative stress, induction of apoptosis, and modulation of angiogenesis. All of these effects are mediated through the regulation of cellular signaling pathways [58]. It has been demonstrated that phenolic acids, especially derivatives of ferrulic acid, have a significant regularity effect on tumor resistance [59] and. Subsequent investigations ought to center on recognizing these substances and clarifying their distinct functions in relation to the examined cell lines.

3.8. Antiproliferative Effects of *Pavarii* aerial parts methanolic extract on A549 and WI38 Cells

The methanolic extract of *Pavarii* aerial parts showed strong antiproliferative potential against the tested cell lines, and the IC50 for extracts on A549 and WI38 cell lines was determined. The anti-proliferative activity of A. Pavarii extracts was tested against A549 and WI38 cell lines. The percentage viability of the plant extracts was measured by the MTT assay. IC50 of *Pavarii* aerial parts methanolic extract extract against the lung carcinoma cell line (A549), and WI cell line cell line were (179, 243 and 731), [µg/mL, respectively. While IC50 of Doxorubicin (reference standard) against the lung carcinoma cell line (A549), WI cell line were (0.97, 1.93 and 07), µg/mL, respectively (Figure 4 A and B). The methanolic extract of Solenostemma argel was reported by Abouzaid et al. [60] to have strong cytotoxic effects on lung cancer in male Wistar rats that had been generated by dimethylbenzanthracene (DMBA). The antitumor efficacy of Solenostemma argel's aqueous extract against Ehrlich carcinoma-bearing mice was studied by Hanafi and Mansour [61]. The aqueous extract of *Solenostemma argel* was found to both stimulate tumor cell death and reduce tumor volume. A high and extensive zone of apoptotic tumor cells was also detected Elsanhoty et al., [62] and Fouad et al.,[63].



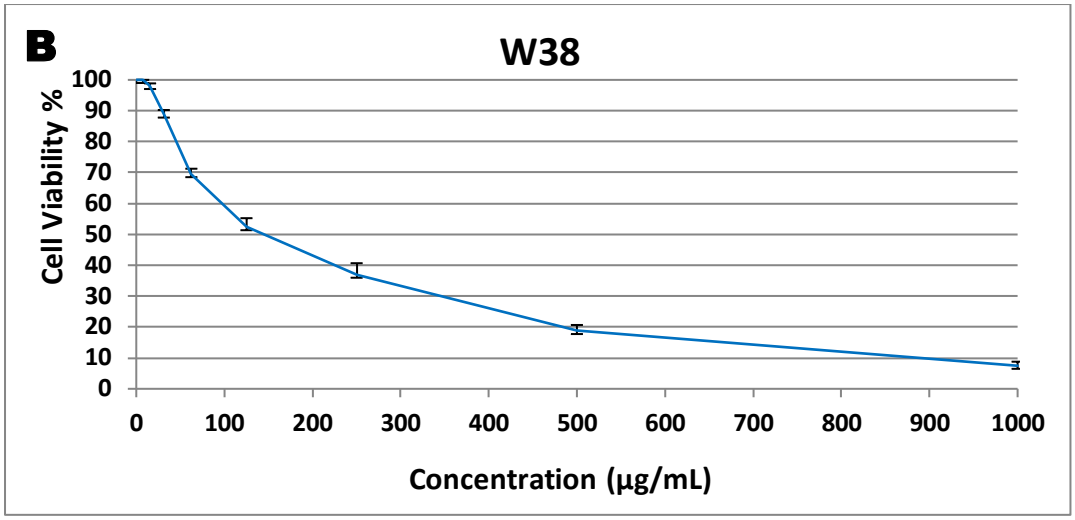
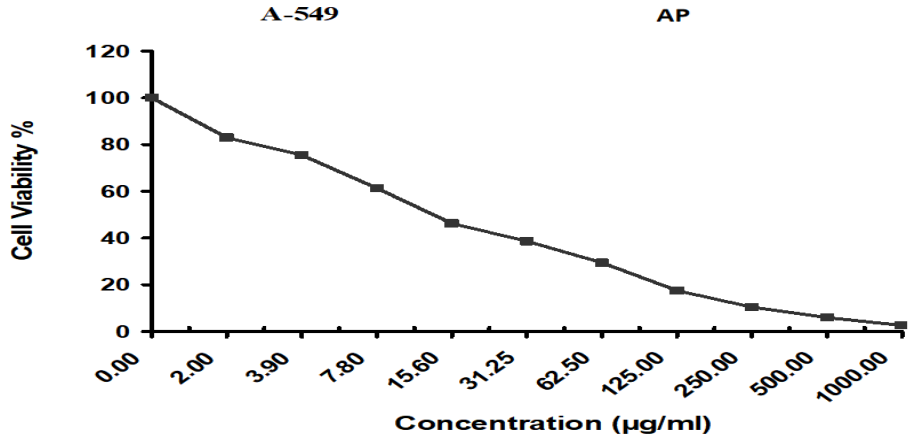


Figure (3 A and B). Toxicity effects of the Rosemary aerial parts methanol extract against cancer cell line: (**A**) A549, (**B**) W38 after 24 h of incubation

Evaluation of cytotoxicity against A-549 cell line

Sample Code: (AP)



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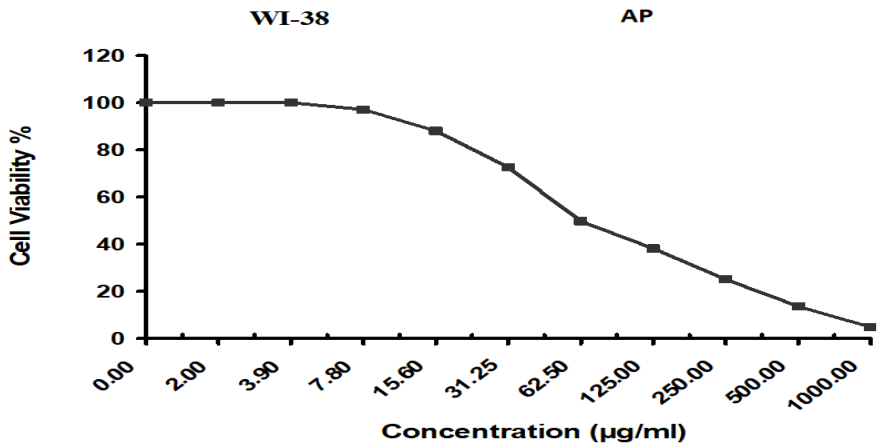

Figure 4. A, B: Evaluation of cytotoxicity of of *Pavarii* aerial parts methanolic extract on A549 and WI38 Cells *these experimental conditions with* IC_{50}

4. Conclusions

The aerial portions of ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L. PLANTS are rich in bioactive chemicals, according to the assays conducted for this study. The highest scavenging potency was found in the methanol extracts of ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L., PLANTS (32.12-1.75%) and (30.12±1.75%) for ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L., PLANTS, respectively, and at a concentration of 10 µg/ml; in the methanolic extracts of both ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L., PLANTS, respectively, at a concentration of 1280 µg/ml, and (98.17±0.81%) and 98.47±0.81%). The methanol extract showed strong anticancer activity in multiple cancer cell lines, according to in vitro models. The cytotoxic effects of rosemary methanol extract in the MTT experiment are greater than those of arbutus aerial parts methanolic extract. In the methanolic extract of rosemary, the most prevalent phenolic acids were found to be rosmarinic and caffeic, followed in order by p-coumaric, ferulic, syringic, and gallic acids, respectively. On the other hand, the most prevalent phenolic acids were found to be Quercetin and Myricetin. GC-mass analysis showed that aerial parts of Rosemary were rich in rosmarinic acid and the methanolic extract of Arbutus aerial parts was rich in quercetin phynolic acid. Based on the results, it can be said that rosemary has more antioxidant activity, cytotoxicity, and antioxidant activity than the aerial portions of arbutus. Arbutus pavarii and rosemary methanolic extracts are readily available sources of naturally occurring bioactive components, which have positive effects on health. We conclude that ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L., PLANTS, methanolic extract, could be used as an easily accessible source of naturally occurring bioactive chemicals with therapeutic effects, especially for the pharmaceutical industry. Potential directions for future research include investigating ARBUTUS PAVARII AND ROSMARINUS OFFICINALIS L., PLANTS as a natural pharmacological agent to replace pharmaceutical medications in vivo.

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