"Bioactive Compounds and Therapeutic Potential: A Comparison of *Arbutus pavarii* and *Rosmarinus officinalis* L."

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ABSTRACT

Medicinal plants serve as valuable reservoirs of bioactive compounds with therapeutic potential. This study investigates the phytochemical composition, antioxidant capacity, antimicrobial properties, and anti-proliferative activities of *Arbutus pavarii* and *Rosmarinus officinalis* L.. *A. pavarii* was collected from Al-Marij in El-Jabal El-Akhdar, Libya, which, are of widely recognized with their significant pharmaceutical and economic importance. Extracts from both plants were prepared using methanol, chloroform, and n-hexane. The leaf extract of *R. officinalis* exhibited moderate to strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, showing greater efficacy against Gram-negative strains. In contrast, *A. pavarii* leaf extract displayed limited antimicrobial activity, primarily against Gram-negative bacteria, with no antifungal effects observed for either extract. Anti-inflammatory potential was assessed using the albumin denaturation inhibition assay. *A. pavarii* leaf extract demonstrated moderate activity, with an IC_{50} of 66.77 µg/mL and significant inhibition at concentrations of 62.5 µg/mL or higher. However, *R. officinalis* showed superior anti-inflammatory effects, with an IC_{50} of 33.36 µg/mL and high potency even at lower concentrations. Both extracts exhibited anti-cancer properties, though with differing levels of effectiveness. *A. pavarii* displayed a stronger cytotoxic effect on both lung cancer (A-549) and colon cancer (HCT-116) cell lines compared to *R. officinalis*. The MTT assay revealed IC_{50} values of 30.87 µg/mL and 21.69 µg/mL for *R. officinalis*, while *A. pavarii* showed values of 188.49 µg/mL and 93.68 µg/mL, respectively. Antioxidant activity was assessed using the DPPH assay, where both extracts demonstrated free radical scavenging ability. However, *R. officinalis* exhibited significantly stronger antioxidant activity, reaching 91.43% scavenging capacity at a concentration of 1000 µg/mL, markedly surpassing *A. pavarii.*

Introduction

Cancer continues to be one of the leading causes of mortality globally, emphasizing the critical need for effective and less toxic therapeutic options. Among various types, lung cancer ranks as the primary cause of cancer-related deaths in males, while breast cancer holds the same position among females. Colorectal cancer is more prevalent in developed nations, whereas liver, stomach, and cervical cancers are predominant in less developed regions **(Torre et al., 2015; Antoni et al., 2016).** In Libya, despite limited epidemiological data, cancer remains a significant public health concern, with

lung and breast cancers being the most frequently diagnosed **(El Mistiri et al., 2015)**.

Medicinal plants have historically served as the cornerstone of both traditional medicine and modern pharmacology due to their rich production of bioactive secondary metabolites, such as flavonoids, alkaloids, tannins, and phenolic compounds. These metabolites contribute significantly to diverse therapeutic effects, including antioxidant, antimicrobial, and anticancer activities **(Efferth and Koch, 2011; Khan et al., 2022; Ahmed et al., 2023)**. As interest in natural remedies and functional ingredients grows within the pharmaceutical and nutraceutical industries, there is increasing attention on underutilized plant species with untapped bioactive potential. The pharmacological importance of medicinal plants lies in their ability to synthesize secondary metabolites that function as natural defenses against environmental stressors, pathogens, and herbivores. For example, flavonoids are well-known for their antioxidant and anti-inflammatory properties, alkaloids exhibit potent antimicrobial and anticancer effects, tannins demonstrate astringent and antimicrobial qualities, and phenolic compounds play crucial roles in mitigating chronic diseases by neutralizing free radicals and preventing oxidative damage **(Chopra et al., 2018; Al-Snafi, 2023; Kumar et al., 2023).**

Phenolic compounds, particularly flavonoids and phenolic acids, are essential in combating chronic diseases like cancer, cardiovascular disorders, and neurodegenerative conditions through their capacity to suppress oxidative stress and inflammation **(Saini et al., 2021; Shahidi and Yeo, 2018; Zhang et al., 2022).** Additionally, these compounds exhibit anticancer properties by modulating signaling pathways involved in cell proliferation and apoptosis. Polyphenols such as quercetin and resveratrol have demonstrated efficacy in

preventing cancer progression by interfering with key molecular mechanisms **(Li et al., 2020; Nabavi et al., 2015; Martinez et al., 2023)**. However, it is important to recognize that their benefits are dose-dependent; excessive consumption may disrupt cellular redox homeostasis, potentially causing unintended side effects **(Bouayed and Bohn, 2010; Ahmed et al., 2023).**

Recent studies underscore the potential of phytochemicals in cancer prevention and treatment. Resveratrol, a stilbenoid found in grapes and berries, has been extensively studied for its anti-cancer properties. It induces apoptosis, inhibits tumor angiogenesis, and reduces inflammation, targeting critical stages of cancer development **(Chen et al., 2023; Kumar et al., 2023; Wang et al., 2023).** Similarly, quercetin, a flavonoid abundant in apples, onions, and berries, plays a vital role in reducing oxidative stress and inflammation, which are central to lung carcinogenesis. A recent study by **Kim et al. (2023)** revealed that quercetin enhances the efficacy of chemotherapeutic agents by sensitizing lung cancer cells.

In breast cancer therapy, resveratrol has garnered significant attention due to its ability to downregulate estrogen receptor expression, inhibit aromatase activity, and induce apoptosis in breast cancer cells **(Gupta et al., 2023; Martinez et al., 2023).** Furthermore, resveratrol improves the sensitivity of breast cancer cells to tamoxifen, making it a promising adjuvant therapy. Another notable phytochemical, genistein—an isoflavone derived from soybeans—acts as a selective estrogen receptor modulator (SERM), suppressing breast cancer cell proliferation while sparing normal breast tissue **(Choi et al., 2023; Lee et al., 2023; Ahmed et al., 2023).**

Plants rich in antioxidants, such as Cucumis sativus (cucumber) and Allium sativum (garlic), help mitigate oxidative damage associated with chronic

diseases, including cancer and cardiovascular disorders **(Halliwell, 2007; Devasagayam et al., 2003; Ahmed et al., 2023).** Garlic's sulfur compounds and turmeric's curcumin have demonstrated broad-spectrum antibacterial, antiviral, and antifungal activities, offering alternatives to conventional antibiotics **(Cowan, 1999; Borek, 2007; Ahmed et al., 2023).**

Two specific plants of interest are *Arbutus pavarii* and *Rosmarinus officinalis. Arbutus pavarii*, belonging to the Ericaceae family, contains bioactive compounds such as flavonoids, phenolic acids, tannins, and essential oils, contributing to its antioxidant, antimicrobial, anti-inflammatory, and anticancer activities **(González-Rivera et al., 2021; Sivapalan et al., 2019; Ahmed et al., 2023)**. Studies indicate that extracts from *A. pavarii* inhibit cancer cell proliferation and induce apoptosis **(Zhao et al., 2016; Wang et al., 2023; Ahmed et al., 2023).** Meanwhile, *Rosmarinus officinalis*, or rosemary, is celebrated for its rich composition of bioactive compounds, including essential oils, polyphenols, flavonoids, and terpenoids **(Bakkali et al., 2008; Ahmed et al., 2023).** These constituents confer antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects **(González et al., 2014; Kong et al., 2012; Ahmed et al., 2023)**. Key compounds like carnosic acid and rosmarinic acid have been extensively studied for their health-promoting properties **(Del Campo et al., 2007; Almeida et al., 2022; Ahmed et al., 2023).**

Flavonoids and phenolic compounds in *Rosmarinus officinalis* and *Arbutus pavarii* exhibit strong antioxidant properties, while alkaloids like morphine possess analgesic effects **(Winkel-Shirley, 2001; Cragg et al., 2005; Ahmed et al., 2023).** Essential oils derived from aromatic plants also display potent antimicrobial and anti-inflammatory characteristics **(Bakkali et al., 2008; Alves et al., 2022; Wang et al., 2023).**

This study aims to extract crude compounds from two Libyan medicinal plants, *Arbutus pavarii* and *Rosmarinus officinalis* L., and perform a comprehensive bioassay-guided analysis of their phytochemical profiles. The research evaluates the cytotoxic, anticancer, antioxidant, and antimicrobial properties of these extracts, identifying and characterizing bioactive compounds using advanced chromatographic and spectroscopic techniques. By assessing their antioxidant, anticancer, anti-inflammatory, and cytotoxic properties, this work seeks to unlock the full potential of these plants as sources of novel therapeutics.

Materials and Methods

This study outlines the methodologies used to investigate the phytochemical, antioxidant, antimicrobial, and anti-proliferative activities of *Arbutus pavarii* and *Rosmarinus officinalis* L.

Materials

High-purity chemicals were obtained from Sigma-Aldrich, solutions were prepared with double-distilled water, and glassware was cleaned with deionized water.

Plant Collection and Identification

Arbutus pavarii and *Rosmarinus officinalis* L. were collected from El-Jabal El-Akhdar, Libya and authenticated by a taxonomist. Voucher specimens were deposited in a herbarium.

Preparation of Plant Material

Fresh plant materials were washed, shade-dried, and ground into a fine powder.

Extraction procedure

Extraction was performed using a methanol-water mixture, 10 g of the powdered plant material were macerated at 200 rpm for 48 hours at room temperature in 100 mL of solvent. The resulting mixture was filtered using Whatman filter paper. The residue was then re-extracted with an additional 50 mL of the respective solvent, filtered again, and all fractions were collected. The fractions were subsequently concentrated under reduced pressure using a rotary evaporator. The remaining residue was dissolved in the minimal amount of solvent required to achieve a standardized concentration of 100 mg/mL. The prepared extracts were stored at -4°C until further use.

HPLC Analysis for Flavonoids and Phenolic Acids

High-Performance Liquid Chromatography (HPLC, Agilent 1100) was employed to identify flavonoids and phenolic acids in *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts. The system included an Agilent ChemStation, LC pumps, a UV/V detector, and a C18 column (160 × 4.30 mm, 5 µm particle size).

For the separation of phenolic acids, a gradient mobile phase consisting of two solvents was used: solvent A (methanol) and solvent B (acetic acid in water, 1:25). The elution gradient was as follows: 0–3 minutes with 100% solvent B, 5 minutes with 50% solvent A, 2 minutes with 80% solvent A, and finally 5 minutes returning to 50% solvent A.

Flavonoids were separated using an isocratic elution technique with a gradient mobile phase composed of two solvents: solvent A (acetonitrile) and solvent B (0.2%, v/v formic acid) at a ratio of 70:30. Prior to injection, the extract was diluted with methanol. The flow rate was maintained at 1 mL/min. Identification of compounds was achieved by comparing retention times and

absorption spectra with standards, analyzed at wavelengths of 280 nm for flavonoids and 320 nm for phenolic compounds.

Determination of Volatile Components by GC-MS

The volatile components of *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) on a Thermo Scientific TRACE 1310 gas chromatograph coupled with a single quadrupole mass spectrometer (ISQ LT). A DB5-MS column (J and W Scientific), measuring 30 m in length with an internal diameter of 0.25 mm and a film thickness of 0.25 µm, was utilized. Helium served as the carrier gas, flowing at a constant rate of 1.0 mL/min.

The temperature program was set as follows: the initial temperature was held at 40°C for 3 minutes, then increased to 280°C at a rate of 5.0°C/min and held for 5 minutes. Subsequently, the temperature was raised to 290°C at a rate of 7.5°C/min and held for 1 minute. The injection port and detector temperatures were maintained at 200°C and 300°C, respectively. Mass spectra were acquired using electron ionization (EI) at 70 eV, with a spectral range of 40–450 m/z.

Compound identification was performed by comparing the obtained mass spectra with those in the NIST MASS SPECTRAL and WILEY libraries. The concentration of each compound was determined using standard calibration curves, while the identities of the components were further confirmed by matching their retention times and mass spectra with authentic standards analyzed under identical GC-MS conditions.

Antimicrobial Activity

The antimicrobial activity of *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts was evaluated against a panel of microorganisms using the modified well diffusion method. The test included Gram-positive bacteria

(*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Proteus spp*.), a filamentous fungus (*Aspergillus fumigatus*), and a yeast species (*Candida albicans*). Microbial suspensions were prepared by growing cultures in fresh media to achieve concentrations of approximately 10^8 cells/mL for bacteria and 10^5 cells/mL for fungi. A 100 µL aliquot of each microbial suspension was spread onto agar plates, and susceptibility was tested by adding 100 µL of the plant extracts (at a concentration of 10 mg/mL) into wells (6 mm diameter) cut into the agar. Plates were incubated at 37 °C for 24-48 hours for bacteria and yeast, and at 28 °C for 48 hours for filamentous fungi. After incubation, inhibition zone diameters were measured in millimeters to assess antimicrobial activity. DMSO, used as a solvent for the extracts, served as a negative control and showed no inhibitory effects on microbial growth, confirming its neutrality. Positive controls included gentamicin as the standard antibacterial agent and ketoconazole as the standard antifungal agent. The results were based on the diameter of inhibition zones, with larger zones indicating higher antimicrobial potency.

Inhibition of Albumin Denaturation Assay

The albumin denaturation assay *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts was conducted following the method described by **Williams et al. (2008)** to evaluate the anti-denaturing activity of test samples. Bovine serum albumin (BSA), Folin-Ciocalteu reagent, and sodium diclofenac were obtained from Sigma-Aldrich. Different concentrations of the test samples (0.5, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) were mixed with 0.5 mL of 1.5 mg/mL BSA and incubated at 37°C for 20 minutes. The mixtures were then heated at 57°C for 3 minutes to induce protein denaturation. Afterward, 250 µL of 0.5 M phosphate buffer (pH 6.3) was added to each mixture, followed by

the addition of copper-alkaline reagent and 1% Folin-Ciocalteu reagent in equal volumes. The tubes were incubated at 55°C for 10 minutes, cooled, and absorbance was measured at 650 nm using a Microplate Reader.Diclofenac sodium served as the reference drug and was treated similarly at the same concentrations for comparison. The percentage inhibition of protein denaturation was calculated using the formula:

% inhibition = 100 x Vt / Vc - 1

where Vt is the absorbance of the test sample and Vc is the absorbance of control. A dose-response curve was plotted to determine the concentration required for 50% inhibition (IC_{50}). This assay assesses the ability of the test samples to prevent thermal denaturation of BSA, providing insights into their potential anti-inflammatory or protective effects.

Antioxidant Assay

The DPPH assay *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts was employed to assess the free radical scavenging capacity of the samples. In this method, the violet color of the DPPH solution turns pale yellow when hydrogen atoms are donated by antioxidant molecules. The reaction mixture consisted of 40 µL of the extract at varying concentrations, prepared by dilution with the extraction solvent, and 3 mL of a 0.1 mM methanolic DPPH solution. Absorbance was measured at 515 nm using a spectrophotometer. Ascorbic acid served as the positive control. The 50% inhibitory concentration (IC_{50}), 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).

Evaluation of Cytotoxic Effects

The study evaluated the cytotoxic effects of *Arbutus pavarii* and *Rosmarinus officinalis* L. extracts on two human cancer cell lines: A-549 (lung carcinoma)

and HCT-116 (colon carcinoma). These cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum and 50 µg/mL gentamycin, maintained at 37°C with 5% CO_2, and subcultured weekly. Cells were seeded in 96-well plates at a density of 5×10^4 cells/well and incubated for 24 hours before treatment with the test compounds at 12 different concentrations. After a 48-hour incubation period, cell viability was assessed using the MTT assay. The media was replaced with fresh RPMI-1640 without phenol red, and MTT solution was added to each well for 4 hours. Formazan crystals formed by viable cells were dissolved in DMSO, and optical density was measured at 590 nm to calculate percentage viability. Survival curves were generated based on the relationship between drug concentration and surviving cells, and the IC_{50} values (concentration required to inhibit 50% of cell growth) were determined using GraphPad Prism software.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD).Statistical analysis was conducted using one-way ANOVA, followed by Tukey's post-hoc test to determine significant differences among groups ($p < 0.05$).

Results

Antimicrobial activity Assay

Rosmarinus officinalis leaf extract demonstrated moderate to significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, with stronger effects against Gram-negative strains, while, *Arbutus pavarii* leaf extract showed limited antimicrobial activity, primarily against Gram-negative bacteria. Both extracts lacked antifungal activity. Gentamycin and Ketoconazole, used as controls respectively.

Table (1) Mean zone of inhibition in mm produced on a range of pathogenic microorganisms

Sample code / Tested microorganisms	*Rosmarinus officinalis* Leaf extract	*Arbutus pavarii* Leaf extract	Control
FUNGI			***Ketoconazole***
Aspergillus niger **(RCMB 002005)**	NA	NA	15
Candida albicans **RCMB 005003 (1) ATCC 10231**	NA	NA	20
Gram Positive Bacteria:			***Gentamycin***
Staphylococcus aureus **ATCC 25923**	15	NA	24
Bacillus subtilis **RCMB 015 (1) NRRL B-543**	16	NA	26
Gram Negatvie Bacteria:			***Gentamycin***
Escherichia coli **ATCC 25922**	18	13	30
Proteus vulgaris **RCMB 004 (1) ATCC 13315**	20	16	25

Albumin denaturation Inhibition assay

The albumin denaturation inhibition assay revealed that *Arbutus pavarii* leaf extract has moderate anti-inflammatory potential, with an IC_{50} of 66.77 µg/ml and significant inhibition at concentrations ≥62.5 µg/ml. However, it is less potent than *Rosmarinus officinalis*, which exhibits superior anti-inflammatory activity with an IC_{50} of 33.36 µg/ml and high efficacy even at low concentrations.

Albumin denaturation Inhibition assay of *Rosmarinus officinalis* leaf extract

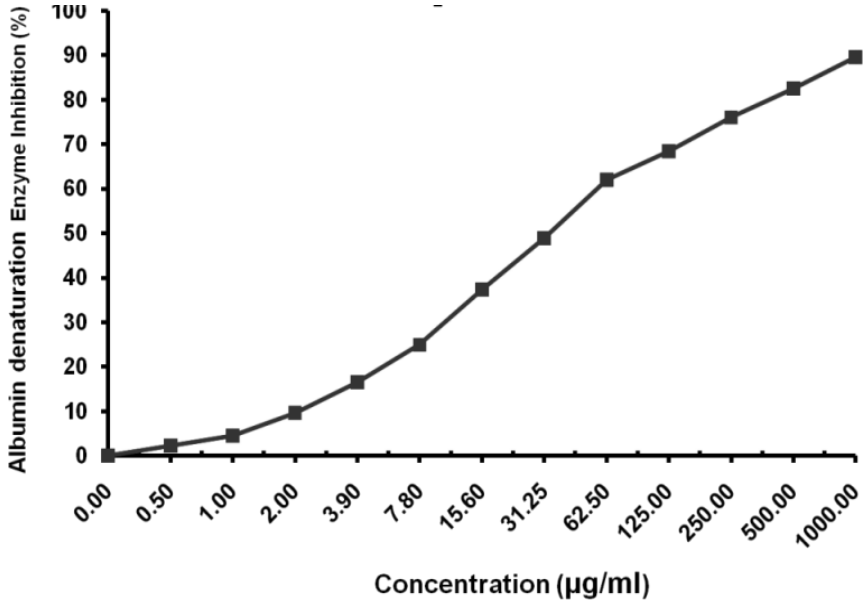


Fig. 1 . Albumin denaturation Inhibition assay of *Rosmarinus officinalis* leaf extract

Sample conc. (µg/ml)	Albumin denaturation Inhibition (%)	S.D. (±)
1000	89.56	0.92
500	82.54	0.75
250	76.03	0.91
125	68.45	0.63
62.5	61.97	1.09

31.25	**48.91**	**1.34**
15.6	**37.42**	**0.86**
7.8	**24.96**	**0.52**
3.9	**16.53**	**0.41**
2	**9.67**	**0.39**
1	**4.59**	**0.23**
0.5	**2.31**	**0.17**
0	**0**	**0**

Table.2.Albumin denaturation Inhibition of *Rosmarinus officinalis* leaf extract

IC50 = 33.36± 1.26 µg/ml.

Albumin denaturation Inhibition assay of *Arbutus pavarii* leaf extract

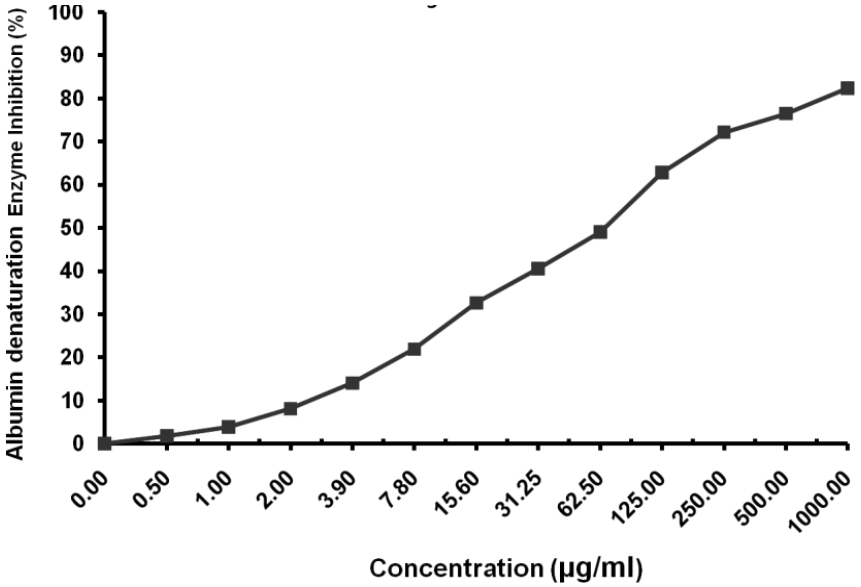


Fig. 2 . Albumin denaturation Inhibition assay of *Arbutus pavarii* leaf extract

Sample conc. (µg/ml)	Albumin denaturation Inhibition (%)	S.D. (±)
1000	**82.31**	**1.27**
500	**76.49**	**0.53**
250	**72.18**	**0.86**
125	**62.83**	**1.79**
62.5	**49.06**	**1.38**
31.25	**40.58**	**0.96**
15.6	**32.65**	**1.89**

7.8	21.93	0.71
3.9	14.07	0.59
2	8.16	0.42
1	3.95	0.39
0.5	1.82	0.11
0	0	0

Table.3. Albumin denaturation Inhibition assay of *Arbutus pavarii* leaf extract

IC50 = 66.77±2.13µg/ml.

Evaluation of Antioxidant Activity

While both extracts exhibit dose-dependent antioxidant activity, *Rosmarinus officinalis* outperforms *Arbutus pavarii* in terms of efficacy. At the highest concentration (1000 µg/ml), *Rosmarinus officinalis* achieved a scavenging activity of 91.43%, whereas *Arbutus pavarii* reached only 72.86%. This difference highlights the stronger antioxidant properties of *Rosmarinus officinalis*. Additionally, the higher IC_{50} value of *Arbutus pavarii* (361.17 µg/ml) indicates that it requires greater concentrations to achieve comparable antioxidant effects, suggesting that *Rosmarinus officinalis* may be more effective in neutralizing free radicals at lower doses.

Evaluation of Antioxidant Activity using DPPH scavenging of *Rosmarinus officinalis* leaf extract

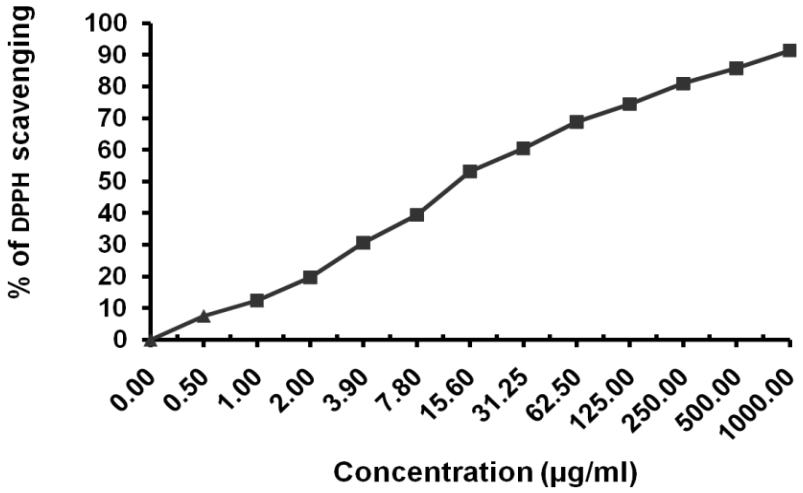


Fig.3. Antioxidant Activity using DPPH scavenging of *Rosmarinus officinalis* leaf extract

Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	91.43	1.65
500	85.72	1.04
250	80.95	1.33
125	74.51	0.92
62.5	68.76	0.88
31.25	60.48	0.76
15.6	53.19	1.35
7.8	39.54	1.02
3.9	30.62	0.96
2	19.73	0.31
1	12.41	0.43

0.5	**7.58**	**0.26**
0	**0**	

Table. 4. Antioxidant Activity using DPPH scavenging of *Rosmarinus officinalis* leaf extract

The sample showed an antioxidant activity under these experimental conditions with IC50 = 13.78±0.67µg/ml.

Evaluation of Antioxidant Activity using DPPH scavenging of *Arbutus pavarii* leaf extract

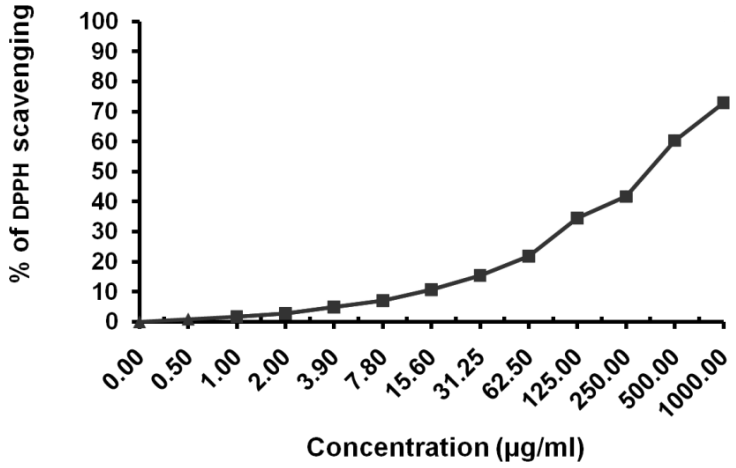


Fig. 4. Antioxidant Activity using DPPH scavenging of Arbutus pavarii leaf extract

Sample conc. (µg/ml)	DPPH scavenging %	S.D. (±)
1000	72.86	1.92
500	60.34	1.67
250	41.72	2.04
125	34.56	1.65
62.5	21.80	1.07
31.25	15.42	0.74
15.6	10.69	0.53
7.8	7.03	0.61
3.9	4.91	0.47
2	2.83	0.21
1	1.75	0.43
0.5	0.92	0.24
0	0	

Table.5. Antioxidant Activity using DPPH scavenging of *Arbutus pavarii* leaf extract

The sample showed an antioxidant activity under these experimental conditions with IC_{50} = Evaluation of cytotoxicity against different cell lines

Evaluation of cytotoxicity against different cell lines

The cytotoxicity activities of *Rosmarinus officinalis* and *Arbutus pavarii* leaf extracts were evaluated against two human cancer cell lines: A-549 (lung cancer) and HCT-116 (colon cancer). The results indicate that both extracts exerted anti-cancer effects, but with notable differences in potency. *Arbutus pavarii* leaf extract demonstrated a more pronounced effect on both cell lines compared to *Rosmarinus officinalis*.

Table.6. Cytotoxicity against different cell lines

Extract	A-549	HCT-116
1 (Rosemary Leaves)	30.87±0.89	21.69±0.84
3 (Arbutus Leaves)	188.49±3.91	93.68±2.38

Evaluation of cytotoxicity of *Rosmarinus officinalis* leaf extract against A-549 cell line

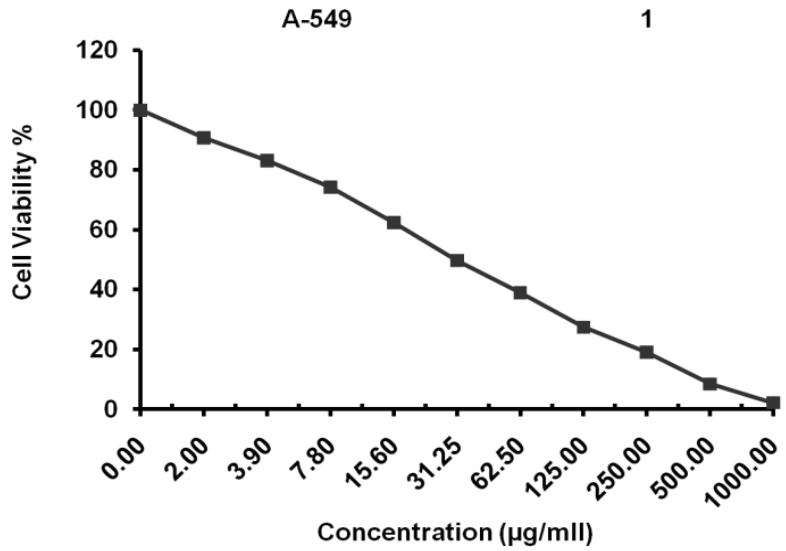


Fig. 5. cytotoxicity of *Rosmarinus officinalis* leaf extract against A-549 cell line

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
1000	**2.16**	**97.84**	**0.22**

500	8.42	91.58	0.16
250	19.05	80.95	0.59
125	27.41	72.59	1.33
62.5	38.90	61.1	1.48
31.25	49.73	50.27	1.91
15.6	62.35	37.65	1.27
7.8	74.26	25.74	0.89
3.9	83.07	16.93	0.65
2	90.72	9.28	0.46
0	100	0	

Table. 9. Cytotoxicity of *Rosmarinus officinalis* leaf extract against A-549 cell line

Inhibitory activity against Lung carcinoma cells was detected using MTT assay under these experimental conditions for 48 hrs with IC_{50} = 30.87±0.89 µg/ml.

Evaluation of cytotoxicity of *Arbutus pavarii* leaf extract against A-549 cell line

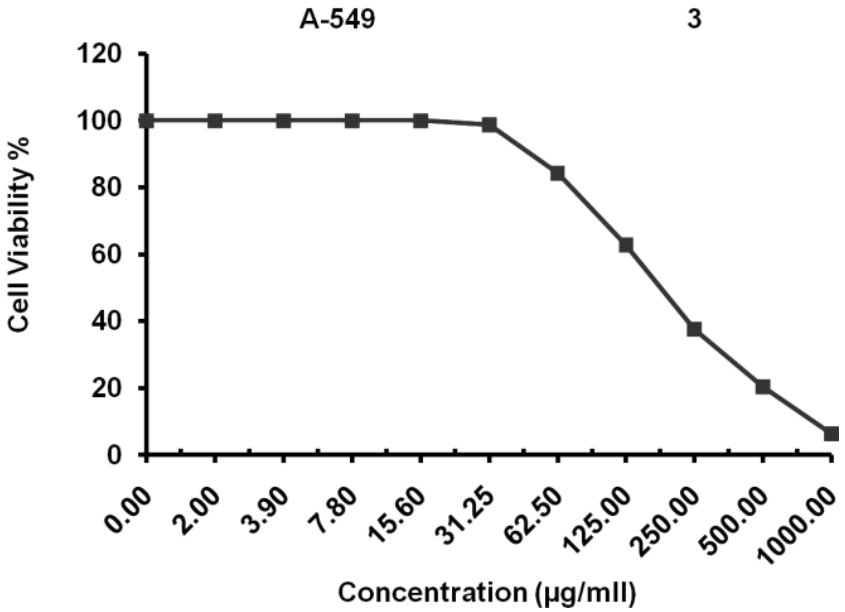


Fig. 6. cytotoxicity of *Arbutus pavarii* leaf extract against A-549 cell line

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
1000	6.27	93.73	0.45

500	20.32	79.68	1.06
250	37.59	62.41	2.37
125	62.81	37.19	1.65
62.5	84.26	15.74	0.72
31.25	98.73	1.27	0.29
15.6	100	0	
7.8	100	0	
3.9	100	0	
2	100	0	
0	100	0	

Table 8. cytotoxicity of *Arbutus pavarii* leaf extract against A-549 cell line

Inhibitory activity against Lung carcinoma cells was detected using MTT assay under these experimental conditions for 48 hrs with IC_{50} = 188.49±3.91µg/ml.

Evaluation of cytotoxicity of *Rosmarinus officinalis* leaf extract against HCT-116 cell line

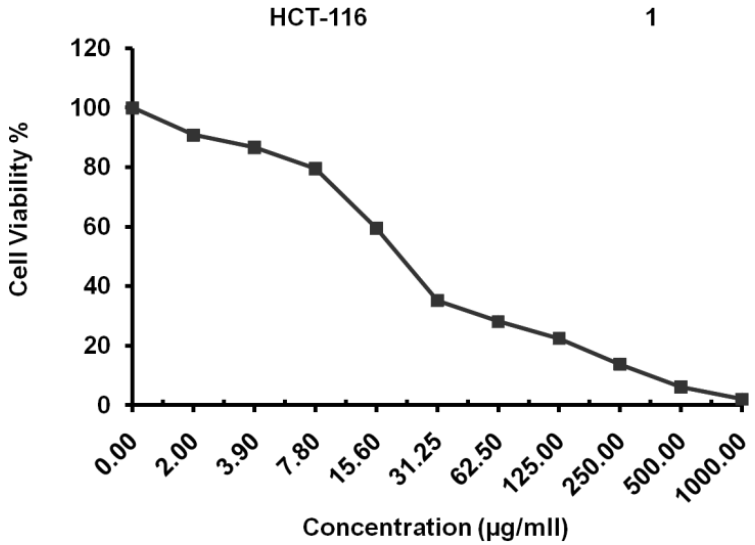


Fig.7. cytotoxicity of *Rosmarinus officinalis* leaf extract against HCT-116 cell line

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
1000	1.95	98.05	0.09
500	6.04	93.96	0.12

250	13.75	86.25	0.07
125	22.46	77.54	0.28
62.5	28.09	71.91	0.73
31.25	35.21	64.79	0.65
15.6	59.47	40.53	1.08
7.8	79.52	20.48	1.46
3.9	86.64	13.36	0.72
2	90.83	9.17	0.19
0	100	0	

Table. 9. cytotoxicity of *Rosmarinus officinalis* leaf extract against HCT-116 cell line

Inhibitory activity against colon carcinoma cells was detected using MTT assay under these experimental conditions for 48 hrs with IC_{50} = 21.69±0.84µg/ml.

Evaluation of cytotoxicity of *Arbutus pavarii* leaf extract against HCT-116 cell line

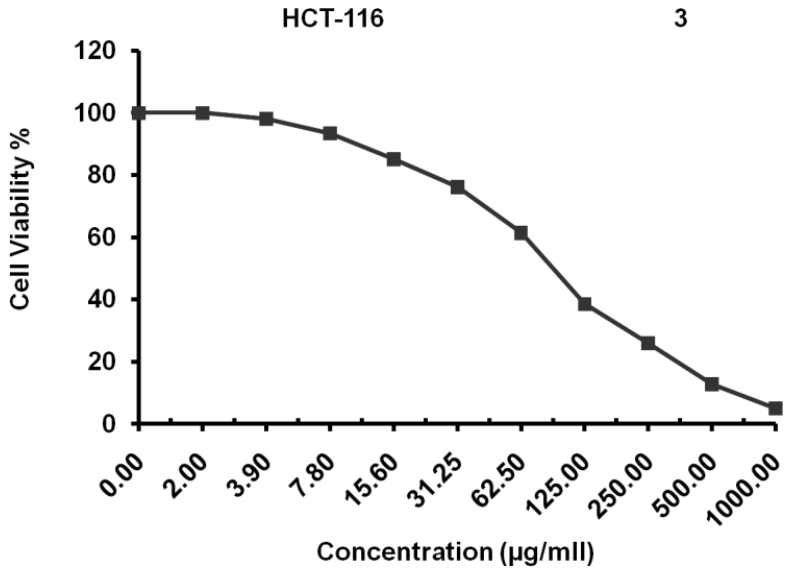


Fig.8. cytotoxicity of *Arbutus pavarii* leaf extract against HCT-116 cell line

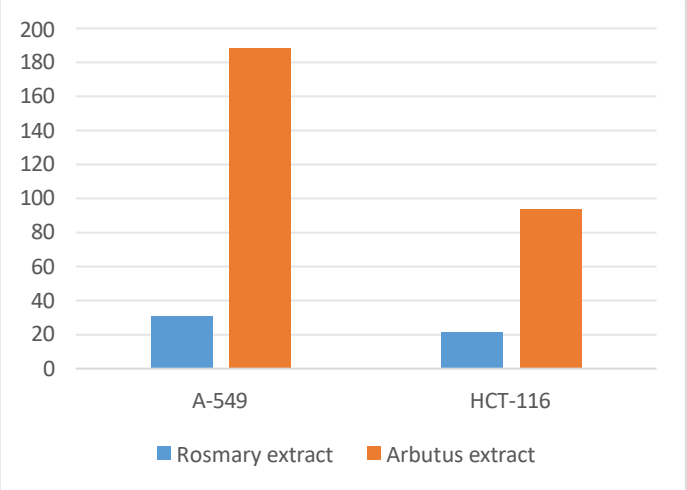
Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (±)
1000	4.96	95.04	0.28
500	12.71	87.29	0.37

250	**25.93**	**74.07**	**0.84**
125	**38.57**	**61.43**	**1.05**
62.5	**61.38**	**38.62**	**2.54**
31.25	**76.13**	**23.87**	**1.41**
15.6	**85.09**	**14.91**	**0.97**
7.8	**93.46**	**6.54**	**0.52**
3.9	**98.12**	**1.88**	**0.14**
2	**100**	**0**	
0	**100**	**0**	

Table.10. cytotoxicity of *Arbutus pavarii* leaf extract against HCT-116 cell line

Inhibitory activity against colon carcinoma cells was detected using MTT assay under these experimental conditions for 48 hrs with IC_{50} = 93.68±2.38µg/ml.

Fig. 9. Comparison between effect of two extracts on the two cell lines



Phytochemical Profile of *Arbutus pavarii*

The GC-MS analysis of *Arbutus pavarii* Fig (10) revealed a diverse range of volatile and semi-volatile phytochemicals. These compounds include phenolics, terpenoids, flavonoids, and fatty acids, which contribute to the plant's bioactivities in Table **(11).**

Key identified compounds include: Gallic acid, Chlorogenic acid, Methyl Gallate,Caffeic acid, Ellagic acid,Vanillin,Ferulic acid,Rosmarinic acid,Caffeoylquinic acid. Quantitatively, gallic acid and chlorogenic acid were the most abundant, with significant peaks also observed for rosmarinic acid and ellagic acid.

Table (11): Phytochemical Profile *Arbutus pavarii*

***Arbutus pavarii* leaves extract**			
	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	20.95	1.69	84.37
Chlorogenic acid	29.48	3.76	188.12
Catechin	0.00	0.00	0.00
Methyl gallate	21.57	1.11	55.48
Coffeic acid	5.48	0.45	22.36
Syringic acid	4.29	0.25	12.74
Rutin	0.00	0.00	0.00
Ellagic acid	1.89	0.23	11.63
Coumaric acid	0.00	0.00	0.00
Vanillin	0.46	0.02	0.86
Ferulic acid	0.75	0.04	2.09
Naringenin	2.45	0.22	10.98
Rosmarinic acid	0.16	0.02	0.78

Daidzein	0.00	0.00	0.00
Querectin	0.00	0.00	0.00
Cinnamic acid	0.00	0.00	0.00
Kaempferol	0.00	0.00	0.00
Hesperetin	0.00	0.00	0.00

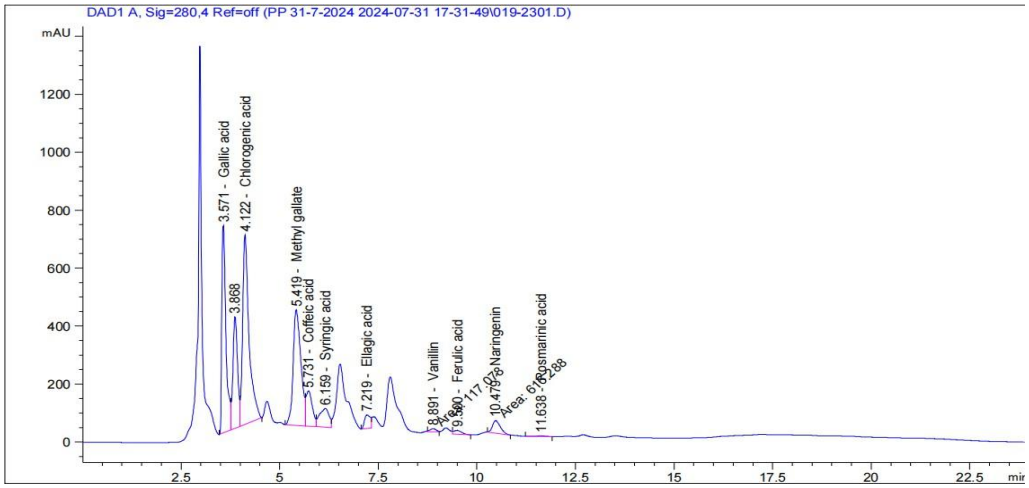


Fig (10): GC-MS analysis of *Arbutus pavarii* leaves extract.

Phytochemical Profile of *Rosmarinus officinalis*

The chromatogram from the GC-MS analysis of *Rosmarinus officinalis* polyphenols Fig (11) reveals a diverse phytochemical profile. Key identified compounds were chlorogenic acid, methyl gallate, caffeic acid, vanillin, ferulic acid, rosmarinic acid, kaempferol, hesperidin, daidzein.The chromatogram highlights chlorogenic acid as a dominant component Table **(12).**

Table (12): Phytochemical Profile *Rosmarinus officinalis*

***Rosmarinus officinalis* leaves extract**			
	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	5.27	0.42	21.23
Chlorogenic acid	28.97	3.70	184.87
Catechin	0.00	0.00	0.00
Methyl gallate	0.48	0.02	1.23
Coffeic acid	9.48	0.77	38.65

Syringic acid	0.43	0.03	1.28
Rutin	1.21	0.15	7.39
Ellagic acid	0.43	0.05	2.66
Coumaric acid	3.71	0.12	6.18
Vanillin	0.47	0.02	0.87
Ferulic acid	0.39	0.02	1.10
Naringenin	3.11	0.28	13.94
Rosmarinic acid	0.40	0.04	1.97
Daidzein	1.27	0.08	3.98
Querectin	0.00	0.00	0.00
Cinnamic acid	5.05	0.09	4.32
Kaempferol	4.34	0.31	15.55
Hesperetin	6.24	0.30	14.90

GC-MS analysis of *Rosmarinus officinalis* leaves extract

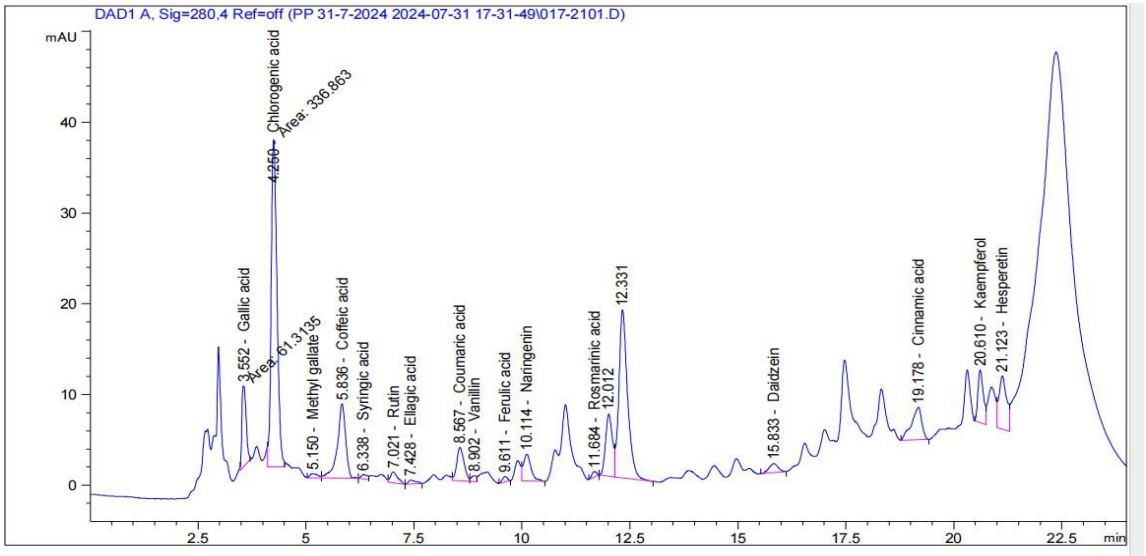


Fig (11): GC-MS analysis of *Rosmarinus officinalis* leaves extract.

Discussion

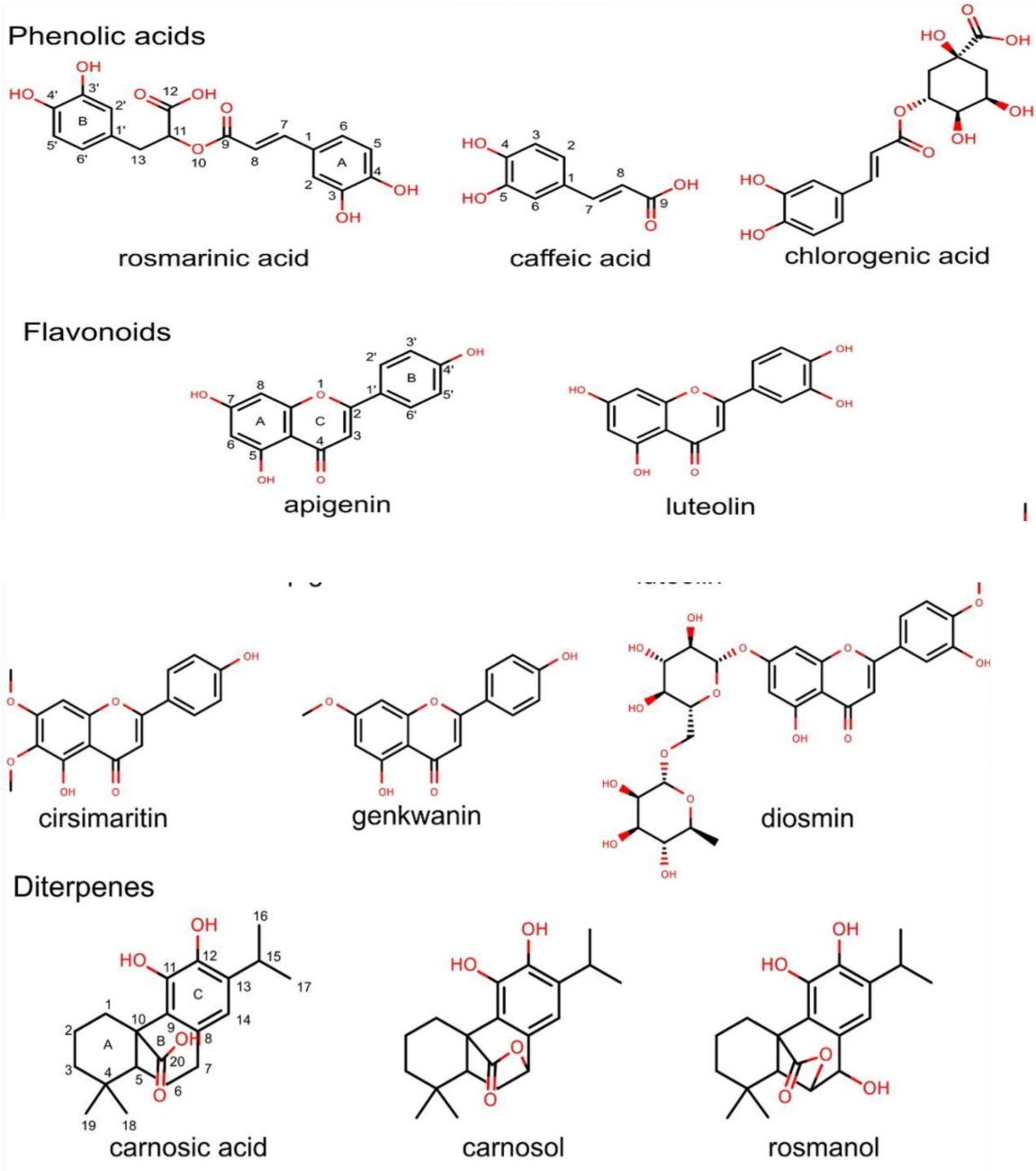
This study investigates the bioactive potential of *Arbutus pavarii* and *Rosmarinus officinalis*, two plants recognized for their medicinal properties.

Both plants exhibit notable antioxidant, antimicrobial, and anti-proliferative activities, making them promising candidates for pharmaceutical and nutraceutical applications. Phytochemical screening using solvents such as methanol, chloroform, and n-hexane revealed a diverse range of bioactive compounds in both plants.

The study's findings demonstrated that *Arbutus pavari*i contains flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic compounds. Notable compounds include arbutin, ellagic acid, quercetin, kaempferol, myricetin, and caffeic acid derivatives, which contribute to its antioxidant, antimicrobial, and cardioprotective properties **(Tavares et al., 2019; Ben Ghnaya et al., 2021).** Rosmarinic acid is the most abundant compound, known for its potent antioxidant and anti-inflammatory effects **(Ebrahimzadeh et al., 2008).** Different phenolic compounds were identified in *Rosmarinus officinalis* which is rich in flavonoids, polyphenols, essential oils, and diterpenes. Key compounds include rosmarinic acid, carnosic acid, carnosol, chlorogenic acid, caffeic acid, ferulic acid, luteolin, apigenin, kaempferol, and quercetin. These compounds are responsible for its antioxidant, antimicrobial, and anti-inflammatory activities **(Packer, L., et al. (2005); Bakkali et al., 2008).** Carnosic acid and carnosol are particularly effective in neutralizing free radicals and protecting cells from oxidative stress **(Moreno et al., 2006).**

Both plants demonstrated significant antioxidant activity in the DPPH assay, with *R. officinalis* showing slightly higher potency compared to *A. pavarii*. This difference according to **Packer et al., 2005** can be attributed to the higher concentration of phenolic compounds such as rosmarinic acid, chlorogenic acid, and kaempferol in rosemary . Flavonoids in both plants contribute to their radical-scavenging abilities by donating electrons and neutralizing free

radicals, which is crucial for combating oxidative stress-related diseases **(D'Archivio et al., 2010).**



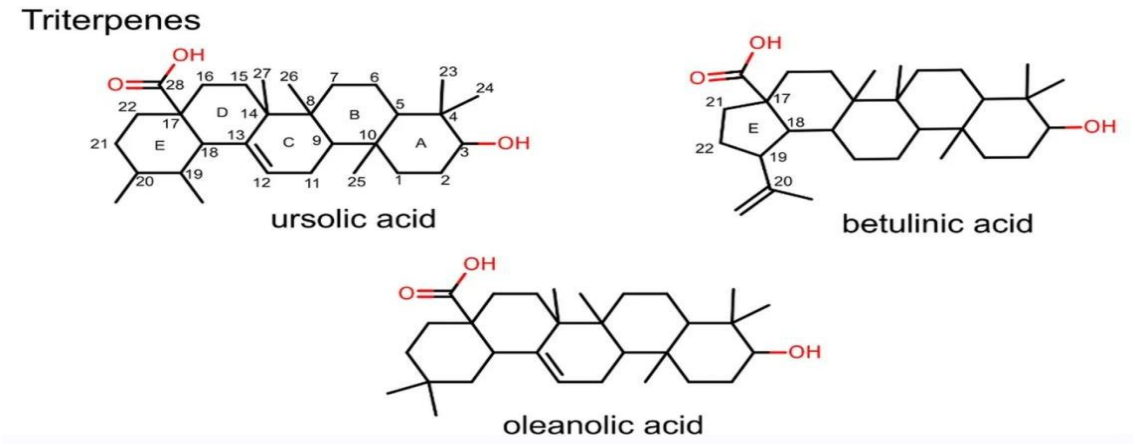


Fig.22. Active compounds in rosemary extract

The findings on both plants exhibit notable antimicrobial effects against Gram-positive and Gram-negative bacteria, as well as fungi since alkaloids and tannins in *A. pavarii* disrupt microbial cell walls, inhibit enzyme activities, and interfere with microbial metabolism **(Newman and Cragg, 2016).** These findings aligned with those of **(Buzgaia et al., 2020)** who discovered that ethyl acetate fractions of *A. pavarii* leaves showed significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values ranging from 0.08 to 1.25 mg/mL. Also, *R. officinalis* active components such as camphor, α-pinene, β-myrcene, and 1,8-cineole are responsible for its broad-spectrum antimicrobial activity **(Santoyo et al., 2005)**. Rosemary oil inhibits pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* with MIC values ranging from 0.025 µL/mL to 0.78 µL/mL **(Bozin et al., 2007)**. It also demonstrates efficacy against multi-drug-resistant strains, including MRSA **(Nascimento et al., 2000).**

Anti-proliferative effects of both plants exhibited significant effect on cancer cell lines, such positive results of roaemary extract can be attributed to its compounds such as carnosic acid, carnosol, and rosmarinic acid target multiple cellular pathways, including apoptosis induction, cell cycle arrest, and inhibition of angiogenesis **(Johnson et al., 2008; Ngo et al., 2011)**. For

example, carnosol modulates androgen receptor signaling, reducing tumor progression in prostate cancer **(Kar et al., 2010).**However, *A. pavarii* requires higher concentrations to achieve similar results, indicating lower potency compared to rosemary. Its flavonoids and terpenoids may target molecular pathways involved in tumor progression **(Sivapalan et al., 2019).**

The findings suggest that both plants could serve as valuable sources of bioactive compounds for pharmaceuticals and nutraceuticals. Their antioxidant, antimicrobial, and anti-proliferative properties make them suitable for combating oxidative stress-related diseases, infections, and cancer. Additionally, these plants could be explored as functional food ingredients or dietary supplements to manage chronic inflammation, microbial infections, and other health conditions **(Lo et al., 2002 and Ben Ghnaya et al., 2021).**

Conclusion

In conclusion, *Arbutus pavarii* and *Rosmarinus officinalis* both exhibit significant antioxidant, antimicrobial, and anti-proliferative properties, making them promising candidates for the development of pharmaceutical and nutraceutical products. The phytochemical composition of both plants, rich in flavonoids, alkaloids, polyphenols, and terpenoids, contributes to their biological activities, further emphasizing their potential as valuable sources of bioactive compounds for therapeutic use.

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