Phytochemical analysis and Pharmacological properties of methanolic extract of Libyan *Arbutus pavarii* Pampan fruit

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ABSTRACT

Arbutus pavarii Pamp (Ericaceae) is one of the endemic species in Libya. Samples of the non-ripened and ripened fruits of Arbutus pavarii Pamp. Although the biological basis of these effects has not been well studied, traditional medicine has used the *Arbutus pavarii* Pampan fruit (Fam. Ericaceae) to cure a variety of human maladies, including gastritis renal infections, cancer and kidney diseases. Investigating the pharmacological properties and categorization of flavonoid and phenolic components present in the *Arbutus pavarii Pampan* fruit portions was the aim of this study. The cytotoxic effects, antioxidant activity, and cell viability of *Arbutus pavarii Pampan* fruit were investigated. The terpenes are flavonoids, phenolics, tannins, total saponins, and volatile components were conducted into *Arbutus pavarii* Pampan fruit methanolic extract (ME). The results that obtained indicated that the phenolic content in the methanolic extract was 25.33 mg GAE/g and the total alkaloid content of the methanolic extract was 9.86 E (µg/g). The total terpene content of the methanolic extract was 7.49 LE (mg/g). The total tannin content in the methanolic extract was 8.99 TAE (mg/g). The total saponin content in the methanolic extract was 11.33 AE (mg/g). The methanol extract exhibited 89.26±0.82% scavenging efficacy. The IC_{50} value of diclofenac (reference standard) was 4.66±0.39 µg/ml. The IC_{50} value of the methanolic extract of *Arbutus pavarii Pamp* was 55.59±1.96 µg/ml. the methanolic extract of *Arbutus pavarii* fruits was no active against Gram negative strain (*E. coli, P. vulgaris*), and Gram positive (*B. subtilis*) bacteria; except against *S. aureus* which had inhibitory effect at concentration of 10 mg/ml compared with gentamycin at concentration 4 µg/ml. Cytotoxicity of methanolic extract of *A. pavarii* Pampan fruits against HepG-2, A-549, HCT-116, MCF-7, and HL-60 cell lines were evaluated and IC_{50} values were 264.36±5.24, 373.31±7.86, 274.52±9.67, 217.50±5.06, and 193.65±3.91 µg/ml, respectively. It can be concluded that methanolic extract of *Arbutus pavarii* Pampan could be used as an available source of natural bioactive constituents with consequent health benefits. An excellent place to start would be to investigate the fruit of *Arbutus pavarii* Pampan as a natural pharmacological agent that could take the place of chemical medications through an in vivo investigation.

KEYWORDS: *Arbutus pavarii* Pampan fruit, phytochemical analysis, antimicrobial antioxidant; cytotoxicity; volatile components

1. INTRODUCTION

Bioactive chemicals found in plants are extremely numerous and have a variety of uses in both food and medicine. The proliferation of bacterial diseases and drug-resistant microorganisms is one of the most important problems the world is now dealing with. Due to the lack of adequate therapies and the adverse effects of synthetic antibiotics, microbiological infections claim the lives of millions of people every year. [**1, 2**]. The bioactive substances found in medicinal plants are made up of a wide variety of phytochemicals with different mechanisms of action. Numerous phytochemicals have been found to have potent antibacterial properties. [**3, 4]**. On the other hand, cancer is a major global cause of death and a serious health concern. Cancer is a multistage disease that begins with genetic changes and progresses to the production of aberrant cells. [**3**]. Globally, lung cancer accounts for 11.6% of all cancer cases. Colorectal cancer comes in at 6.1 percent, prostate cancer at 7.1 percent, and breast cancer at 11.6 percent. [**5**].

There are still problems with toxicity and selectivity in chemotherapy drugs, despite a lot of work to create successful ones. Due to the harshness of existing chemotherapy and the fact that cancer cells are resistant to anticancer medications, scientists are searching for novel treatments and ways to prevent cancer. Products derived from natural plants could be beneficial. 80,000 species and more than 300 genera [**6**].

According to Mwine and Van Damme [**7**], a number of plant species are prone to produce a broad variety of secondary metabolites to help them respond to different stimuli in their specific environment.

Arbutus pavarii Pamp. (fam. Ericaceae), commonly known as “Shmeri” or “Libyan Strawberry”, is an endemic Libyan medicinal plant, currently considered as an endangered species of shrub or tree [**7, 8**], and has attracted public attention in relation to its conservation.

The distribution of this important forage species of plant for honeybees to produce specific honey type is very much confined to the Al-Akhdar mountainous region in Libya. In addition to its ecological importance in honey production, *A. pavarii* has been used in the Libyan traditional medicine for the treatment of various human ailments including gastritis and kidney diseases [**9, 10**].

Berries of this plant are a good source of minerals, nutrients, carbohydrates and most importantly, vitamin C. The uses of its leaves, fruits and bark in the tanning process, and branches as a fuel are among other economic importance of this plant ***[11]***.

Limited phytochemical studies on this plant revealed predominantly the presence of simple phenolics e.g., arbutin, gallic acid and polyphenolics, including flavonoids and tannins e.g., apigenin, epicatechin, hesperidin, kaempferol, naringin, quercetin and rutin, and some triterpenes and sterols ***[*11, 12, 7 and 13*]***.. Rutin was found to be the most abundant flavonoid, while kaempferol was the least in the aerial parts of A. pavarii. Arbutin (**Fig. 1**) appears to be the chemotaxonomic marker compound of the genus Arbutus L., as this compound is present in other species of this genus.

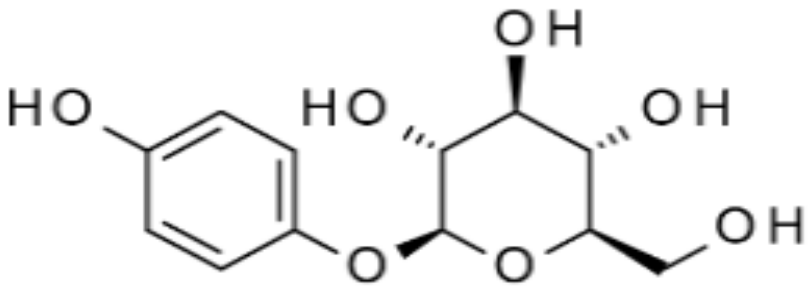

Fig. 1. Structure of arbutin

Although the high concentration of simple phenolic and polyphenolic compounds in this plant may contribute to its antioxidant activity, it is yet unknown whether the secondary metabolites this species produces have antimicrobial activity, primarily against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa. But according to a recent study, A. pavarii has anti-methicillin-resistant (anti-MRSA) activity, and by profiling the antimicrobial fractions using LC-MS/MS, 28 compounds—mostly phenolic—were tentatively identified ***[13].***

Because of its antioxidant properties, *Arbutus pavarii* Pamp. been identified as a potential anticancer medication. Indeed, it has the ability to combat free radicals and protect against their oxidative damage to proteins, lipids, and DNA **[*12*].** However, as was later noted, rosemary derivatives can also cause cytotoxicity under specific circumstances by releasing reactive oxygen species (ROS).

Only crude extracts were used in the initial cytotoxicity investigation against a small number of human cancer cell lines; separated chemicals were not used. Actually, no attempt has been documented to date to isolate active compounds using bioassay guidance and then evaluate the bioactivity of the purified molecules. To find out how this plant affected the performance and coliform count of 1260 male Cobb broiler chickens that were one day old, an intriguing study was conducted [**13**].

The medicinal properties of only crude extracts were used in the initial cytotoxicity investigation against a small number of human cancer cell lines; separated chemicals were not used. Actually, no attempt has been documented to date to isolate active compounds using bioassay guidance and then evaluate the bioactivity of the purified molecules. To find out how this plant affected the performance and coliform count of 1260 male Cobb broiler chickens that

were one day old, an intriguing study was conducted [**13**], which are used in traditional medicine to improve memory and reduce pain as well as for its anti-inflammatory, anti-cancer, and anti-infective properties, are currently gaining attention [**11**].

The active components of this plant may be are phenolic diterpenes and triterpenes. Phenolic elements are characterized by a chemical configuration of hydroxylated aromatic rings. They are generally present as secondary metabolites, and are the elements provided in the greatest quantities by food **[12].**

The Libyan strawberry, *A. pavarii* Pampan (ARB), is an evergreen shrub endemic to the Libyan Green Mountain in Gebal Al-Akhdar. The fruit of ARB is used in honey production and food supplements and possesses potent antioxidant activity. Moreover, the ripe fruit contains appreciably high amounts of the two fat-soluble antioxidant vitamins A and E, exceeding the amount in the unripening stage, while a high concentration of vitamin C characterises the unripe one. Furthermore, several phenolic acids and flavonoids have been identified from the plant, such as arbutin, gallic acid, apigenin, epicatechin, hesperidin, kaempferol, naringin, quercetin, and rutin [**14**].

An analysis of all these substances suggests that diterpenes are the most effective molecules against neoplastic growth, followed by triterpenoids, which have demonstrated minor effectiveness. Numerous methods have been employed to attain an increased quantity of bioactive elements from rosemary, including liquid and supercritical fluid extractions (SFEs). Between them, SFE has been suggested as one of the most effective methods for obtaining the bioactive elements of rosemary [15]. A modern approach uses new technology to identify, separate, and apply biologically active compounds to specific illnesses, drawing on the knowledge base of traditional medicine. Pathologies that cause pain and inflammation have

recently sparked a lot of interest in potential treatments based on physiologically active compounds extracted from medicinal plants [16, 17].Evaluation of the antioxidant, antibacterial, anti-inflammatory, and anticancer properties of extracts from *Arbutus pavarii* Pampan fruit was the aim of this study. Additionally, GC-mass and HPLC were to be used for the analysis of volatile compounds, phenolic acids, flavanoids, saponines, and tannines. This study's goal was to determine the chemical makeup of Arbutus pavarii extract (ME) by analyzing phenolic acids and volatile compounds using HPLC and GC-mass. Additionally, the antioxidant, antimicrobial, anti-inflammatory and anticancer properties of different Arbutus pavarii Pampan fruit extracts were examined against five cell lines: HepG-2, HCT-116, MCF-7, HL-60, and A-549.

2. MATERIALS AND METHODS

2.1 Chemicals

All high-quality analytical grade chemicals and HPLC standards utilized were acquired from Sigma Chemical Co. Germany.

Labiya's Al-Gabel Al-Akhdar regions provided the Arbutus pavarii Pampan fruit used in this study.

2.2. Preparation of sample

2.3. Extraction procedure

Separately, 10 g of the fruit were shaken at 200 rpm for 24 h at RT in 100 mL of solvent (methanol) according to the polarity index. Using Whatman filter paper, the mixture was filtered. After re-extraction with 50 mL of solvent, the residue was filtered, and the all fractions were collected and concentrated at low pressure using a rotary evaporator. In order to reach a standard

concentration of 100 mg/mL, the resultant residue was dissolved in the least amount of solvent feasible. Before being used, extracts were kept at -4˚C [18].

2.4.Antioxidant activity assay

The ability of the free radical to scavenge was assessed using the DPPH assay [**19**]. When hydrogen atoms are taken out of the antioxidant molecule in this test, the violet hue of DPPH turns pale-yellow. The reaction mixture included 3 mL of a methanol solution of 0.1 mM DPPH solution and 40 µL of extract at various strengths that had been diluted with extraction solvent. The mixture was shaken thoroughly before being incubated for 30 minutes at 37°C. The absorbance was measured at 515 nm using a spectrophotometer. A positive control was ascorbic acid. The following formula was used to calculate the relationship between reduced absorbance of the reaction mixture and increased free radical scavenging activity:the percent of DPPH scavenging activity = 100 x $(A_0 - A_1) / (A_0)$.

The absorbances of the sample and control are denoted by A0 and A1, respectively. The average of three duplicate studies is shown together with the key parameters and the standard deviation (SD). The concentration required for 50% DPPH radical scavenging activity, known as the 50% inhibitory concentration (IC50), was estimated using the Graphpad Prism software.

2.5. Cytotoxic effects and Cell Viability

2.5.1. Cell lines

Colon cancer (HCT-116), and breast cancer (MCF7) human Lung cancer (H1299), Liver cancer (HepG-2) and A549 lung cancer cell lines from the American Type Culture Collection (ATCC), USA, were utilized.

2.5.2. Cell culture

DMEM (Dulbecco's modified Eagle's media) supplemented with 1% L-glutamine, 10% heat-inactivated foetal bovine serum, 50 µg/mL gentamycin, and HEPES buffer was used to cultivate the cells. Two times a week, each cell line was grown at 37˚C in a humidified environment with 5% CO2.

2.5.3. Cytotoxicity assay

The cytotoxicity test was estimated according to the method described by Mosmann [**20**]. Cells were seeded at a concentration of 1×104 cells per well in a 96-well plate using 100 µL of growth medium. Fresh media with different EE concentrations was added after the seeds had been seeded for 24 hours. Serial twofold dilutions of EEs were applied to confluent cell monolayers scattered throughout a 96-well microtiter plate. The microtiter plate was incubated for 24 hours at 37˚C in a humidified incubator with 5% CO2.

Three wells were used for each test sample concentration. Control cells were subjected to extract as well as DMSO (dimethyl sulfoxide) treatment. The small amount of DMSO (0.1%) in the wells was found to have no effect on the experiment.

Following a 24-hour incubation period at 37°C, the number of living cells was determined using the colorimetric method. Following the conclusion of the incubation period, the medium was aspirated, and 1% crystal violet solution was added to each well for at least 30 minutes.

The dishes were rinsed with water after the stain was removed. A microplate reader was used to determine the absorbance of the plates at 490 nm after each well had been thoroughly mixed and 30% glacial acetic acid had been added. The relative viability ratio was calculated as (ODT/ODC) x 100, where ODT stands for the absorbance of the treated cells and ODC for the absorbance of the control cells. The number of viable cells was determined by measuring

absorbance. The half maximum inhibitory concentration (IC50) was calculated using the dose-response curve equation.

2.6. Identification of phenolic and flavonoid compounds

HPLC (Agilent 1100) was used to identify the flavonoids and phenolic acids in the methanolic extract (ME); necessary equipment comprised a C18 column (160 x 4.30 mm, 5 µm particle diameter), LC pumps, a UV/V detector, and an Agilent ChemStation.

The phenolic acids were separated using a grade mobile phase that included two solvents: solvent A (methanol) and solvent B (acetic acid in water, 1:25). The column was eluted with gradients of solvent B for 0–3 minutes, 50% solvent A for 5 minutes, 80% solvent A for 2 minutes, and 50% solvent A for 5 minutes. Flavonoids were extracted using a gradient mobile phase, an isocratic elution method (70:30), and two solvents: solvent A (acetonitrile) and solvent B (0.2%, v/v formic acid) [**21, 22**].

Prior to injection, methanol was used to dilute the extract. A flow rate of mL/min was established. Retention times and absorption spectra were compared to standards examined at 280 and 320 nm for flavonoids and phenolic chemicals, respectively, in order to identify the substances.

2.7. Tannins determination Method

Tannic was used as a reference component in the procedure to determine the tannin contents. Three milliliters of a vanillin (4% in methanol) solution and 1.5 milliliters of strong hydrochloric acid are mixed with 400 microliters of extract. The absorbance at 500 nm was measured following a 15-minute incubation period [23].

2.8. Total Saponins Content (TSC) Assay (Modified Vanillin-Sulphuric Acid)

2.8.1 Preparation of the Standard Curve

A standard curve was created using aescin, a naturally occurring blend of saponins. 150.0 mg of aescin were dissolved in 10 mL of methanol to create a 15 mg/ml aescin stock solution. Then, using methanol as the solvent blank for the standards, a serial dilution was carried out in triplicate [**24**].

2.8.2. Preparation of Extract

In a 100 mL conical flask, 30 mL of ethanol and 1 gram of powder were combined. The suspension was pre-leached at room temperature for 30 minutes. After being quickly chilled to room temperature in an ice water bath, the suspension was filtered using a 0.45 membrane filter. After gathering the clear extract, its TSC was established.

2.8.3. Methodology

Every tube was submerged in a 65°C water bath until the methanol evaporated completely (around five minutes). Next, 2.5 mL of 72% H2SO4 (v/v) and 0.5 mL of 4% vanillin in ethanol (w/v) were added to each tube. Following a 15-minute incubation period at 60°C in a water bath, the tubes were covered, vortexed, and cooled for five minutes in room temperature water. Once the Biosystem 310 spectrophotometer was zeroed with the blank, the absorbance of the solutions was measured at 560 nm. A standard curve was created by plotting the measured absorbance values against the concentrations.

Total saponin content = (weight o f saponins in the extract (mg))/((weight of powder (g))x(1-(moisture %/100). The TSC was expressed as mg aescin equivalents per gram dry weight of powder (mg AE/ gm).

2.9. Determination of total terpens

The following procedures were used to calculate the total terpens, per Ghorai [25].

1. 10mg of plant extract was dissolved in 95% methanol.

2. Add 1.5 ml Chloroform in each 2 ml centrifuge tube & then add 200µl sample. For the standard curve 200µl of Limonene solution in methanol was added to 1.5 ml Chloroform & serial dilution must be done[dilution level100mg/ ml to 5mg/ml (12.965 µM1.296 µM) Linalool Conc. In case of serial dilution total volume of 200µl will be make up by addition of 95% (Vol/Vol) Methanol.

3. Vortex the sample mixture thoroughly & take the time 3 min to rest.

4. Add 100µl Conc. Sulfuric acid (H2SO4) to each 2 ml centrifuge tube.

If heat generation occurs then the entire system must be cooled by using ice pad but not more than 15 min.. Then the assay tube must be incubated at room temperature for 1.5h-2h in dark.

For standard solution (Linalool) incubate not more than 5 minutes and during

incubation time centrifuge tube must not be disturbed.

6. At the end of incubation time a reddish brown precipitation will be formed in each assay centrifuge tube. Then carefully & gently decant all supernatant reaction mixture liquid without disturbing the precipitation.

7. The reddish brown precipitation is partially soluble in reaction mixture solution so must gently decant the supernatant fluid.

8. Add 1.5 ml of 95% (Vol/Vol) Methanol & vortex thoroughly until all the precipitation dissolve in Methanol completely.

11. Transfer the sample from assay tube to colorimetric cuvette [95% (Vol/Vol) Methanol will be used as blank] to read the absorbance at 546 nm using Biosystem 310 plus spectrophotometer

Total Terpenoid Calculation

12. Using the blank corrected at wave length at 546 nm of the Limonene standard, compute a standard curve (Fig. Utilizing the regression equation of the Limonene standard curve, determine the total terpenoids concentration of an unknown plant sample in terms of Limonene equivalents.

2.10. Volatile components determination

The NIST MASS SPECTRAL database and WILEY were used to identify the compounds. Rosmarinus officinalis L (ME) was analyzed by GC-MS using a single quadrupole mass spectrometer (ISQ LT) and a Thermo Scientific TRACE 1310 gas chromatograph. J&W Scientific used a 30-meter-long DB5-MS column with an internal diameter of 0.25 mm. Helium, the carrier gas, traveled at a rate of 1.0 mL/min. Hold for three minutes at 40°C. After that, raise the temperature to 280°C at a rate of 5.0°C per minute and hold it there for five minutes. After that, raise the temperature to 290°C at a rate of 7.5°C per minute and hold it there for one minute. Injection and detection were carried out at 200°C and 300°C, respectively. Using a spectral range of 40-450 m/z, mass spectra were obtained at 70 V using electron ionization. The WILEY & NIST mass spectrometry database was used to find compounds. The concentration of the compounds was determined using the standard calibration curve, and the identities of the constituents were verified by mass spectrometry and retention of the genuine compounds obtained under the same GC-MS circumstances [**27**].

2.11. Statistical analysis

Analysis of variance (ANOVA) was performed on all of the data. The standard deviation (SD) and main values for each item were computed using three samples. Duncan's multiple range tests were used to determine whether the meaningful differences variables were significant ($p \leq 0.05$). SPSS 16 was used for all analyses [**26**].

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical analysis of the crude methanolic extract of *Arbutus pavarii* Pampan fruits are reported in **Table 1**.

Table (1)

Phytochemical constituents of methanolic *A. pavarii* extract

Phytochemical constituents (mg/g)	Methanolic extract of *A. pavarii* fruits	S.D. (±)
Phenols	**25.33**	**0.67**
Flavonoids	**21.00**	**1.42**
Ascorbic acid	**2.20**	**0.04**
Tannins	**8.99**	**0.33**
Alkaloids	**0.00986**	**0.56**

Saponins	**11.33**	**0.33**
Terpenoids	**7.49**	**0.24**

Total phenolic content

The total phenolic content of the methanolic extract of *A. pavarii* fruits was calculated using the gallic acid standard curve and the formula y = 396.85x - 16.172, with R^2 = 0.9978. Gallic acid equivalents (GAE) per gram of dry weight were used to represent the phenolic content. The phenolic content in the methanolic extract was 25.33 mg GAE/g.

Total flavonoid content

The estimated flavonoid content in the methanolic extract was represented in terms of quercetin equivalent (QE) mg/g. The linear equation of the quercetin standard curve, y = 220.71× - 17.875 with R^2 = 0.9972, was used to determine the total flavonoids. The total flavonoid content of the methanolic extract was 21.00 QE (mg/g).

Total alkaloids content

The estimated alkaloid content of the methanolic extract was expressed in terms of atropine equivalent (AE) µg/g. The linear equation of the atropine standard curve, y = 133.0× - 30.62 with R^2 = 0.994, was used to determine the total alkaloids. The total alkaloid content of the methanolic extract was 9.86 E (µg/g).

Total terpenoids content

The estimated terpenoid content in the methanolic extract was expressed in terms of limonene equivalent (LE) mg/g. The limonene standard curve's linear equation, y = 0.013× - 0.199 with R^2 = 0.991, was used to determine the total terpens. The total terpene content of the methanolic extract was 7.49 LE (mg/g).

Total tannins content

The estimated tannins content of the methanolic extract was expresseed in terms of tannic acid equivalent (TAE) mg/g. The tannin standard curve's linear equation, y = 61.326× - 0.8511 with R^2 = 0.8511, was used to determine the total tannins. The total tannin content in the methanolic extract was 8.99 TAE (mg/g).

Total saponin content

The estimated saponin content in the methanolic extract was expressed in terms of aescin equivalent (AE) in mg/g. The linear equation of the standard aescin curve, y = 5.4971× + 0.2084 with R^2 = 0.9981, was used to determine the total saponins. The total saponin content in the methanolic extract was 11.33 AE (mg/g).

The vitamin C content of the plant extract was also estimated and reached 2.20 mg/g as ascorbic acid equivalent.

The methanolic extract of *A. pavarii* fruits was found to contain significance amounts of phenols and flavonoids. These groups of phytochemicals present in the extract may be responsible for its pharmacological activity. The results also recommend that *A. pavarii* fruits are a potential source of natural compounds with valuable bioactive properties that could be explored by pharmaceutical, chemical and food industries.

Phytochemical screening of *A. pavarii* revealed the presence of simple phenolic and polyphenolic compounds, including flavonoids, tannins, glycosides, free reducing sugars, triterpenes, and sterols, according to **Asheg et al. [27]**. According to **El Hawary et al. [28]**, apigenin, epicatechin, hespiridin, querecetin, naringin, and rutin are the six favonoid compounds isolated from the aerial parts of *A. pavarii*. Other simple phenolic compounds that have been reported include arbutin, gallic acid, and pyrogallol. Pyrogallol was the most prevalent phenolic

component in the methanolic extract of *A. pavarii*, according to HPLC examination of the extract's phenolic contents, and cinnamic acid was the least prevalent phenolic.

DPPH radical scavenging activity of *methanolic* extract *of* Arbutus pavarii Pampan fruits

We examined the antioxidant activity of a methanolic extract of *A. pavarii* Pampan fruits to learn more about the possible health advantages of these leaves. The methanolic extract of *A. pavarii* Pampan fruits effectively scavenged the DPPH radical in a dose-dependent manner, as shown in **Table (2)** and **Figure (2)**. The methanol extract exhibited 89.26±0.82% scavenging efficacy.

Increasing the concentration of the fruit extract was found to enhance the scavenging efficacy. Using ascorbic acid as a standard, the DPPH technique was used to determine the antioxidant activity. The range of ascorbic acid values was 1000–0.5 µg/ml. Inhibition ratios of ascorbic acid at 0.5 and 1000 µg/ml were 9.34±0.46 and 98.65±0.10%, respectively.

The highest scavenging percentage was observed using a concentration of 1000 µg/ml that, followed by 500 µg/ml (84.93±0.59%), and 250 µg/ml (78.19±0.43%), respectively. On the other hand, the concentration of 0.5 µg/ml gave the lowest scavenging potency with an average value of 5.03±0.41%.

The IC_{50} value of ascorbic acid was 10.21±0.77µg/ml. The IC_{50} value of the methanolic extract was 26.45±0.69 µg/ml.

Vitamin C and carotenoids, which have been found in the fruits of the plant, may also contribute to some of the antioxidant activity of *A. pavarii* fruits. The plant antioxidant activity was significantly influenced by the phenolic acids and flavonoids found in the fruit extract of *A. pavarii* **[30, 31]**.

Table (2)

Radical scavenging activity of methanolic extract of *A. pavarii* Pampan fruit at different concentrations toward DPPH

Radical scavenging activities of extracts[#]		
	Methanolic extract	Ascorbic acid (standard)
Conc. (µg/mL)	DPPH scavenging %	
1000	89.26±0.82	98.65±0.10
500	84.93±0.59	96.34±0.65
250	78.19±0.43	94.25±0.92
125	70.65±0.73	90.89±0.17
62.5	63.94±1.25	86.42±0.71
31.25	55.81±0.97	79.35±1.22
15.6	36.74±1.62	65.05±0.21
7.8	24.29±1.33	43.01±2.87
3.9	17.53±0.79	31.04±1.04
2	11.28±0.64	22.18±1.37
1	6.74±0.28	14.48±0.62
0.5	5.03±0.41	9.34±0.46
0	0	0
IC_{50} value[@]	26.45±0.69	10.21±0.77

[#] *Radical scavenging activity given as percentage inhibition*

[@] *The IC_{50} value was calculated using Graphpad Prism software (San Diego, CA. USA)*

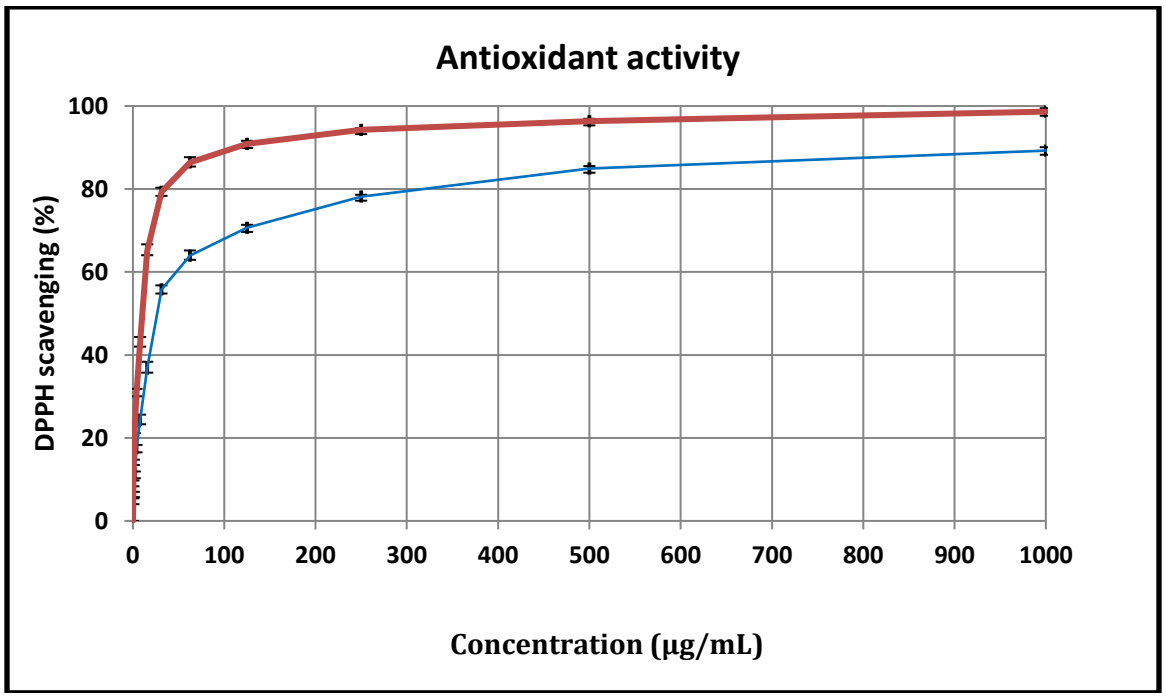

Figure (2)
Radical scavenging activity of methanolic extract of *A. pavarii* Pampan fruit at different concentrations toward DPPH

In vitro anti-inflammatory activity

The anti-inflammatory activity of methanolic extract of *A. pavarii* Pampan fruits was assessed using the albumin denaturation assay. The extract was tested at various concentrations and compared to reference standard values. The results revealed a percentage of inhibition of 87.80±0.69 at a concentration of 1000 µg/ml, 80.17±0.54% at 500 µg/ml, 74.89±0.73% at 250 µg/ml, 65.32±0.66% at 125 µg/ml, and 53.26±0.92% at 62.5 µg/ml. These findings demonstrate that the methanolic extract exhibits significant anti-inflammatory activity in the albumin denaturation assay. Furthermore, the anti-inflammatory properties of the methanolic extract of *A. pavarii* Pampan fruits were found to be comparable to the standard diclofenac at all tested concentrations.

The IC_{50} value of diclofenac (reference standard) was 4.66±0.39 µg/ml. The IC_{50} value of the methanolic extract was 55.59±1.96 µg/ml. Therefore, the extract shows promising anti-inflammatory activity in the context of the albumin denaturation assay **(Table 3 and Figure 3)**.

Analgesic medications are prescribed to treat pain, and it seems that both central (such as morphine) and peripheral (such as aspirin) pathways influence their effects **[**32]. The plant *A. pavarii* contains one or more active compounds that have both central analgesic and peripheral antinociceptive effects, which may be connected to the activation of opioid receptors. Methanolic extract of *A. pavarii* leaf shown strong anti-inflammatory properties. Rats' paw oedema brought on by ether formalin was reduced by the extract. It is commonly recognized that flavonoids and phenolic constituens have strong anti-inflammatory properties **[33, 34].**

Table (3)*In vitro* anti-inflammatory activity of methanolic extract of *A. pavarii* Pampan fruit at different concentrations

	Methanolic extract	Diclofence (standard)
Conc. (µg/mL)	Albumin inhibition#	

1000	87.80 ± 0.69	97.95 ± 0.17
500	80.17 ± 0.54	96.87 ± 0.39
250	74.89 ± 0.73	94.38 ± 0.62
125	65.32 ± 0.66	92.49 ± 0.75
62.5	53.26 ± 0.92	89.34 ± 0.24
31.25	38.51 ± 1.73	78.72 ± 0.64
15.6	27.64 ± 1.02	65.31 ± 0.75
7.8	20.59 ± 0.97	57.68 ± 1.44
3.9	13.24 ± 0.68	48.13 ± 0.65
2	7.53 ± 0.31	41.37 ± 0.21
1	4.12 ± 0.46	28.76 ± 0.48
0.5	1.96 ± 0.32	21.38 ± 0.67
0	0	0
IC_{50} value[@]	55.59 ± 1.96	4.66 ± 0.39

[#] *anti-inflammatory activity given as percentage albumin inhibition*

[@] *The IC_{50} value was calculated using Graphpad Prism software (San Diego, CA. USA)*

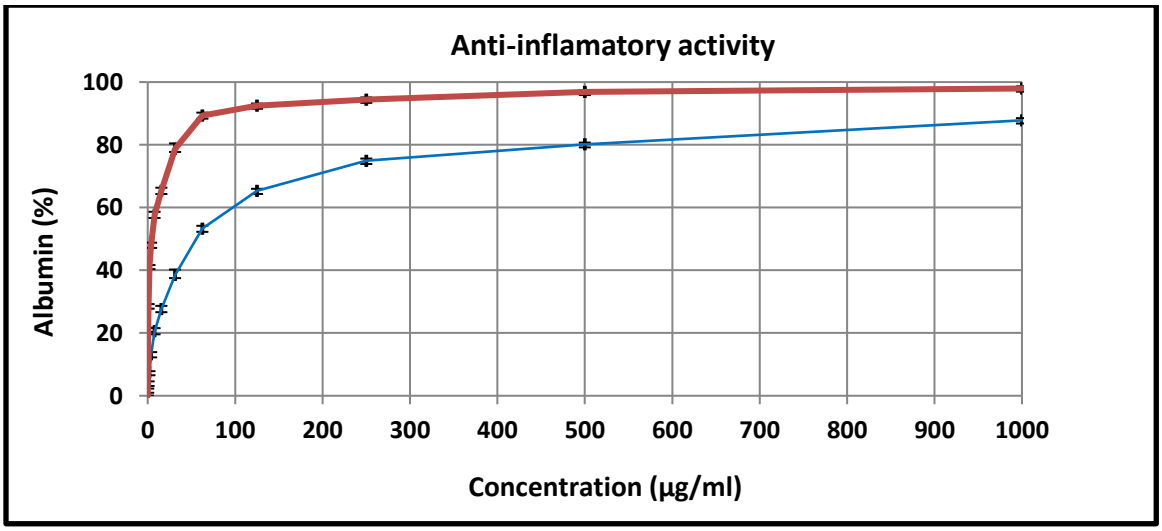

Figure (3)Anti-inflammatory activity of methanolic extract of *A. pavarii* Pampan fruit at different concentrations

Anti-cancer effects on HepG-2, A-549, HCT-116, MCF-7, and HL-60 cell lines

Five different cancer cell lines (HepG-2, A-549, HCT-116, MCF-7, and HL-60) were used to test the cytotoxicity of the methanolic extract of *A. pavarii* fruits. The micro-culture

tetrazolium (MTT) assay was used to measure the degree of cytotoxicity of the methanolic extract towards the cell lines; cytotoxic activity was expressed as a percentage of cell viability in HepG-2, A-549, HCT-116, MCF-7, and HL-60 cell lines. The multiple concentrations of methanolic extract from *A. pavarii* fruits were used and effective doses were calculated from dose-response curve. The dose-response curve was used to calculate effective doses using several concentrations of methanolic extract from *A. pavarii* Pampan fruits.

The results of a cytotoxicity test of the methanolic extract against numerous cell lines are shown in table (4). Cytotoxicity of methanolic extract of *A. pavarii* Pampan fruits against HepG-2, A-549, HCT-116, MCF-7, and HL-60 cell lines were evaluated and IC_{50} values were 264.36±5.24, 373.31±7.86, 274.52±9.67, 217.50±5.06, and 193.65±3.91 µg/ml, respectively.

Upon treatment with methanol extract of *A. pavarii* fruits, the HL-60 cells showed an increased rate of cell death when compared to that in the HepG-2, A-549, HCT-116, and MCF-7 cell lines at the same concentration of the extract used.

The fruit of the *A. pavarii* Pampan plant has been utilized in Libyan traditional medicine, but there are currently only few published publications that mostly describe its cytotoxic, antioxidant, and antibacterial properties [35, 29]. Only crude extracts were used in the first cytotoxicity investigation against a small number of human cancer cell lines; separated chemicals were not used. Actually, no attempt has been documented to date to isolate active compounds using bioassay guidance and then evaluate the bioactivity of the purified molecules.

Simple and polyphenolic active constituents that have been shown to reduce the risk of cancer were found during the phytochemical screening of *A. pavarii*. MTT test was used to evaluate the anti-proliferative activity of the aerial parts of *A. pavarii* extracts on breast adenocarcinoma (MCF7) and lung carcinoma (A549) cancer cells [35]. El Hawary et al. [29]

conducted another toxicological investigation using methanol extract of the aerial part of *A. pavarii* against the T47D and HEPG2 cancer cell lines. Both cell types were susceptible to cytotoxicity from *A. pavarii* extract [29]. In the aerial sections of *A. pavarii*, rutin was found to be the most prevalent flavonoid, while kaempferol was the least prevalent. Since arbutin is found in other species of the genus, it seems to be the chemotaxonomic marker chemical of Arbutus, and may play a role in fighting cancer cell lines.

Table (4)

Toxicity effects of methanolic extract of *A. pavarii* Pampan fruits against different cancer cell lines

Samples Conc. (µg/ml)	methanolic extract of *A. pavarii*														
	cancer cell lines														
	HepG-2			A-549			HCT-116			MCF-7			HL-60		
	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)	Viability (%)	Inhibitory (%)	S.D. (±)
1000	10.74	89.26	0.82	15.39	84.61	1.23	14.97	85.03	1.08	11.29	88.71	0.83	9.72	90.28	1.24
500	28.03	71.97	0.91	40.62	59.38	1.47	38.69	61.31	1.57	30.65	69.35	0.71	24.16	75.84	0.92
250	49.21	50.79	1.47	59.13	40.87	1.69	51.23	48.77	1.49	43.18	56.82	2.34	37.45	62.55	1.73
125	76.34	23.66	2.06	87.45	12.55	0.91	78.06	21.94	0.97	69.41	30.59	1.95	65.29	34.71	1.97
62.5	90.23	9.77	1.24	98.61	1.39	0.35	93.14	6.86	0.48	88.02	11.98	0.86	84.61	15.39	1.43
31.25	96.75	3.25	0.63	100	0		99.52	0.48	0.16	96.73	3.27	0.51	98.20	1.8	0.62
15.6	99.81	0.19	0.12	100	0		100	0		99.64	0.36	0.27	100	0	
7.8	100	0		100	0		100	0		100	0		100	0	
3.9	100	0		100	0		100	0		100	0		100	0	
2	100	0		100	0		100	0		100	0		100	0	
0	100	0		100	0		100	0		100	0		100	0	
IC_{50} value	264.36±5.24			373.31±7.86			274.52±9.67			217.50±5.06			193.65±3.91		

Mammalian cell lines:
HepG-2: *hepatocellular carcinoma cell line*
A-549: *lung carcinoma cell line*
HCT-116: *colon carcinoma cell line*
MCF-7: *breast cancer cell line*
HL-60: *promyelocytic leukemia carcinoma cell line*

Antibacterial activity

Test microorganisms were used to examine the methanolic extract's antibacterial properties. All of the microbial strains were obtained from the Regional Centre for Mycology and Biotechnology (RCMB) culture collection at Al-Azhar University in Cairo, Egypt. Using the well diffusion method, the antimicrobial profile was tested against fungi, including one filamentous fungus (*Aspergillus niger*) and one yeast species (*Candida albicans*), as well as Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*). Positive controls were performed using gentamycin as standard antibacterial drug and ketoconazole as standard antifungal drug.

The results are summarized in **Table 5** which showed that the methanolic extract of *Arbutus pavarii* fruits was no active against Gram negative strain (*E. coli*, *P. vulgaris*), and Gram positive (*B. subtilis*) bacteria; except against *S. aureus* which had inhibitory effect at concentration of 10 mg/ml compared with gentamycin at concentration 4 μg/ml.

An antimicrobial study was carried out by **Alsabri et al. [35],** on the aerial parts of *A. pavarii* by using the agar well diffusion method. The methanol extract showed an antimicrobial effect against *S. aureus*, *E. coli* and *C. albicans*, using ciprofloxacin and amphotericin B as positive controls. Another study by **Alghazeer et al. [39**] showed the high growth inhibition capacity towards five bacterial strains: *S. aureus*, *B. subtilis*, *Streptococcus faecalis*, *E. coli*, and *Salmonella typhi*. Also, **[29]** reported that *A. pavarii* did not exhibit any antimicrobial activity against *P. vulgaris*.

Table (5)
Antibacterial activity of methanolic extract of *A. pavarii* fruits against bacterial pathogens strains measured as zone of inhibition diameter (ZOI, mm)

Microorganisms	Methanolic extract	Control
A. niger (RCMB 002005)	**NI**	**15**
C. albicans RCMB 005003 (1) ATCC 10231	**NI**[@]	**20**
S. aureus ATCC 25923	**9**[#]	**24**
B. subtilis RCMB 015 (1) NRRL B-543	**NI**	**26**
E. coli ATCC 25922	**NI**	**30**
P. vulgaris RCMB 004 (1) ATCC 13315	**NI**	**25**

[#]*Zone of inhibition (ZOI) diameters included the diameter of paper disc (6 mm)*
[@]*NI: no inhibitory effect*

GC-MS analysis of A. pavarii methanolic extract

The volatile components of the methanolic extract of *A. pavarii* fruits were determined using GC-MS based on their retention time and peak area **(Figure 4 and 5)**. Twenty four bioactive substances were identified and the most abundant compounds were categorized based on their chemical structures using GC-MS analysis. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (4.54%), 1,5-dihydroxynaphthalene (2.11%), 5-hydroxymethylfurfural (9.05%), 2-furancarboxaldehyde, 5-(hydroxymethyl)- (13.33%), 1,3,4,5-tetrahydroxycyclohexa necarboxylic acid (3.08%), 17-octadecynoic acid (2.65), hexadecanoic acid (2.34), 9-octadecenoic acid (Z)- (1.92), glycerol 1-palmitate (1.97), 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (2.38), ç-sitosterol (2.39), á-amyrin (4.28), and lupeol (33.74) were the main volatile components **(Table 6)**.

According to **Nawal Buzgaia et al. [37]**, 76 compounds were identified from the leaf and stem bark of *A. pavarii* using LC–ESI–MS/MS analysis. These compounds included a significant amount of plant phenolics (flavonoids and phenolic acids), terpenoids, and fatty acid derivatives. A. pavarii's anti-methicillin-resistant activity was demonstrated in a recent study, and 28 chemicals, mostly of phenolic nature, were tentatively identified using LC-MS/MS-based profiling of the antimicrobial fractions **[38]**.

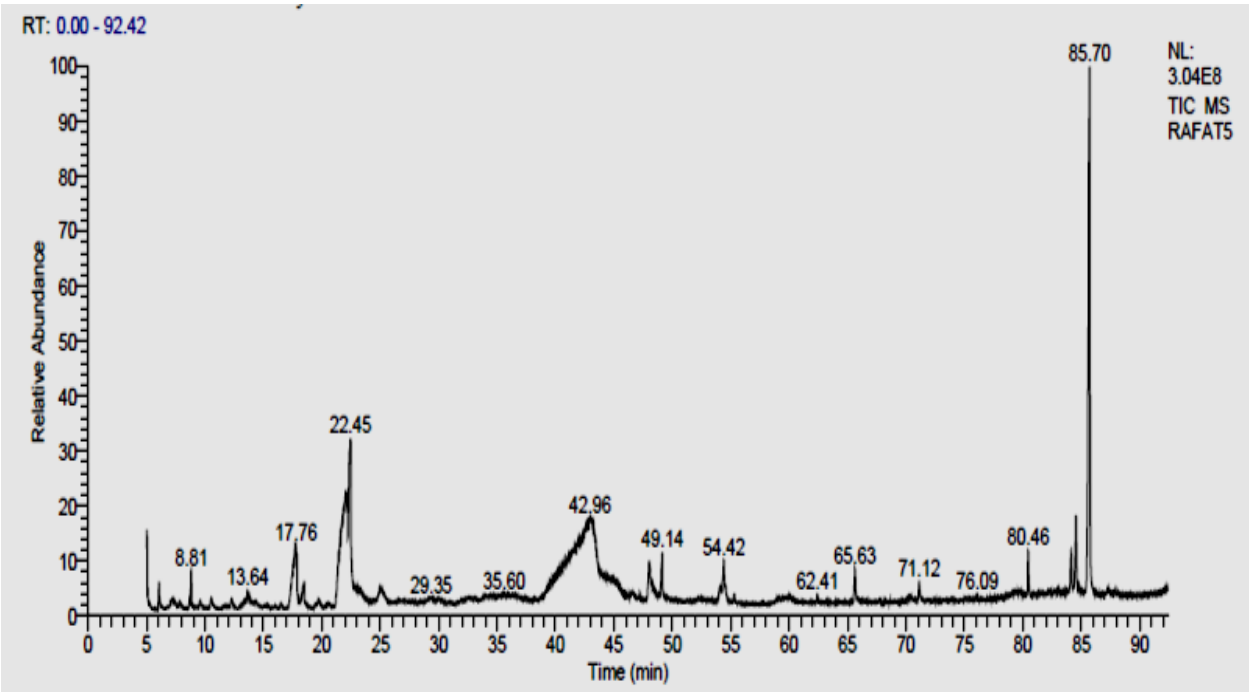

Figure (4)
Volatiles composition of methanolic extract of *A. pavarii* Pampan fruits

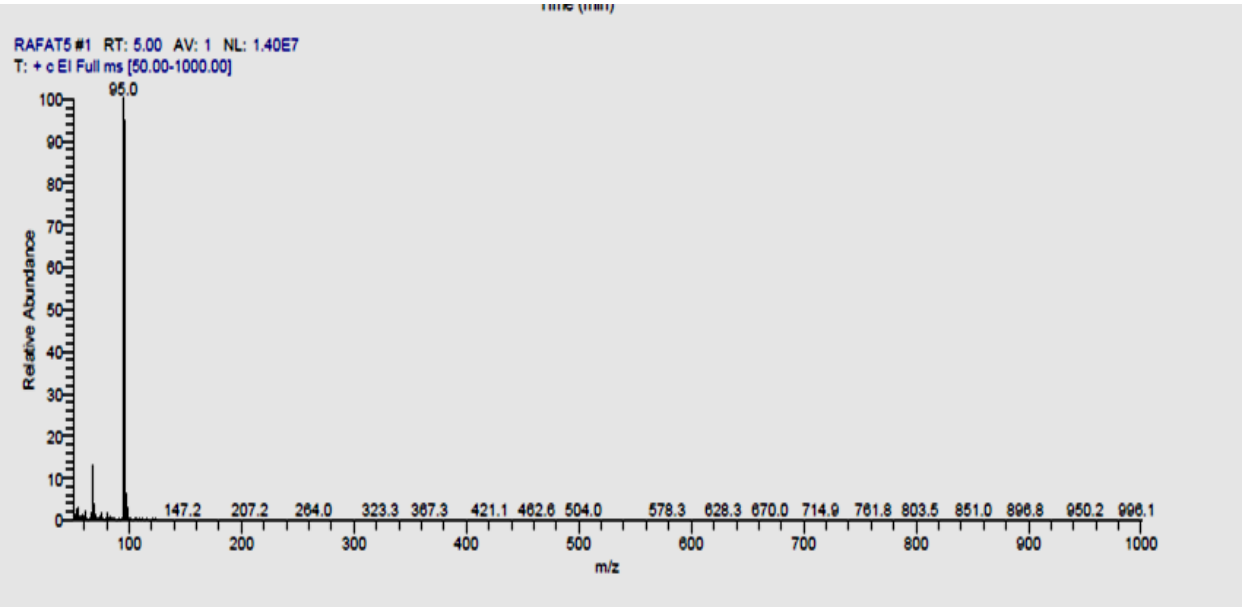

Figure (5)
GC-Mass chromatographic for identification of compounds in methanolic extract of *A. pavarii* Pampan fruits

Volatiles composition of *A. pavarii* fruits

Table (6)
Volatiles composition of methanolic extract of *A. pavarii* fruits

Compound name	Retention time (min)	Molecular formula	Peak Area percentage*
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	17.81	C6H8O4	**4.54**
1,5-dihydroxynaphthalene	18.50	C10H8O2	**2.11**
5-hydroxymethylfurfural	21.75	C6H6O3	**9.05**
2-furancarboxaldehyde, 5-(hydroxymethyl)-	22.47	C6H6O3	**13.33**
1,3,4,5-tetrahydroxycyclohexa necarboxylic acid	43.13	C7H12O6	**3.08**
17-octadecynoic acid	48.04	C18H32O2	**2.65**
hexadecanoic acid	49.13	C16H32O2	**2.34**
9-octadecenoic acid (Z)-	54.42	C18H34O2	**1.92**
glycerol 1-palmitate	65.63	C19H38O4	**1.97**
2,5,7,8-tetramethyl-2-(4,8,12-trim ethyltridecyl)-6-chromanol	80.46	C29H50O2	**2.38**
ç-sitosterol	84.13	C29H50O	**2.39**
á-amyrin	84.54	C30H50O	**4.28**
lupeol	85.70	C30H50O	**33.74**

**The most intense ion is indicated by the base peak (tallest peak)*

CONCLUSION

Arbutus pavarii Pampan fruit (ME) contains high levels of flavonoids, phenolic, total terpenes, total saponins, tannins, and volatile compounds, according to the current study. Additionally, it is a very promising source of novel antioxidant and nontoxic components. These findings also offer compelling evidence for its application in the treatment of cancer. To identify the precise active ingredient in Arbutus pavarii Pampan fruit (ME) and discover its mode of action, more investigation is needed. The use of Arbutus pavarii Pampan fruit methanolic extract, a readily

accessible source of naturally occurring bioactive compounds with therapeutic properties, would be particularly advantageous to the pharmaceutical industry. The possibility of performing an in vivo study and looking at the fruit of *Arbutus pavarii Pampan* as a natural pharmacological agent to replace chemical drugs could be a good place to start.

Conflict of interest

The authors have no conflict of interest.

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