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Therapeutic effect of hydroalcoholic extract of *Moringa oleifera* and sulfurphane on melanoma caused by B16f10 cell line in mouse model C57bl/6

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Abstract

Background: *Moringa oleifera*, an herbal medicine, has been found to possess anti-tumor effects against various cancers.

Objectives: The aim of this study was to investigate the anti-inflammatory and antioxidant properties of Moringa oleifera plant extract on metastasis.

Methods: Initially, the presence of phenolic compounds in Moringa oleifera was confirmed through HPLC and spectrophotometer methods. Following that, the anticancer effects of the phenolic chemicals sulforaphane and Moringa oleifera on B16F10 cancer cells were investigated. Following this, melanoma tumors were induced in C57BL6 mice using the B16F10 cell line, and the mice were treated with the extract. The extract was identified to contain quercetin, gallic acid, and caffeic acid.

Results: The rate of cell death observed in the sulforaphane-exposed cell group was lower compared to the group treated with Moringa oleifera. Furthermore, tumor volume decreased significantly after one and two weeks of therapy with all three doses of Moringa extract or phenolic chemicals ($P<0.001$). Notably, the tumor volume decreased the most with the 0.02 g dose compared to the other two doses ($P<0.001$).

Conclusion: This study demonstrates the positive effect of antioxidants present in Moringa oleifera extract on reducing the viability of cancer cells and tumor volume in mice. Further research is recommended to establish the potential of Moringa oleifera as an effective anticancer drug with greater certainty. Additionally, it is advisable to conduct studies on human subjects as well.

Keywords: *Moringa oleifera*, Sulforaphane, Melanoma, B16F10 melanoma cell line.

Introduction

At the basal level of the epidermis, there are melanocyte cells responsible for the production of UV-absorbing pigments known as melanin. Keratinocytes release melanocyte-stimulating hormone alpha (-MSH) when exposed to UV radiation, which binds to the melanocortin 1 receptor (MC1R) on the surface of melanocyte cells. This triggers a signaling pathway that stimulates melanin production.[1] The melanin is then distributed to surrounding sun-exposed keratinocytes, where it accumulates and shields the nucleus of keratinocytes from the damaging effects of UV radiation. As keratinocytes mature, they undergo keratinization and eventually lose their nucleus before dying. The melanin pigment in keratinocytes protects the skin's outer layer, as does a layer of dead keratinocytes that acts as a barrier to protect the living cells in the underlying layers.[2] It should be noted that melanoma is a malignant tumor that originates from melanocyte cells.

Based on 2021 statistics in the United States, melanoma

ranks as the fourth most common cancer, following breast, prostate, and lung cancers. The death rate associated with melanoma is alarmingly high, with an estimated 7,180 deaths among newly diagnosed cases.[3] In Iran, skin cancers have been reported as the most prevalent cancer overall, as well as in men, and the second most common cancer in women during the period of 2003–2008. Among the different provinces in Iran, East Azerbaijan had the highest incidence of skin cancer, while Gorgan had the lowest. Notably, a study conducted in Golestan province in 2009 by Marjani and Kabir found that the age range of 80–84 years had the highest age-specific incidence rate, reaching 161.90 out of 105 in men. This incidence was noted as the highest worldwide.[4] Considering these statistics, it becomes evident that melanoma holds significant importance in Iran.

Melanoma, a type of skin cancer, can be treated using various methods, such as surgery, radiotherapy, and medication (both herbal and chemical). Over the past 50 years, the FDA has approved numerous drugs for the treatment of melanoma. These include Dacarbazine (1974), Interferon α-2b (1996), Interleukin-2 (1998), Ontak (1999), Penginterferon α-2b (2011), Vemurafenib and Ipilimumab (2011), Dabrafenib and Trametinib (2013), Nivolumab (2014), Dabrafenib + Trametinib (2014), Talimogene laherparepvec (2015), and Cobimetinib + Vemurafenib (2015).[5] Despite significant progress in therapy, there is still no definitive drug for treating melanoma. However, researchers have become increasingly interested in using herbal and natural compounds for cancer treatment due to the harmful side effects of chemical drugs on the body's tissues.

The World Health Organization reports that over 80% of people use medicinal plants,[6] highlighting their importance in nutrition and health maintenance globally. Many herbs have been used to treat various diseases,[7] including cancer. These plants contain compounds that have been classified into three categories: apoptosis-inducing compounds, cell proliferation inhibitors, and metastasis inhibitors, all of which have shown anticancer effects.[8] One plant that has garnered a lot of attention from cancer researchers is Moringa oleifera, an herb from the Moringaceae family that grows naturally in Iran. It is a rich source of vitamins (A, B, C, E, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, and beta-carotene), iron, calcium, and alpha-tocopherol. This plant has been used for its medicinal properties for centuries and has shown potential in the treatment of various diseases, including cancer.[9] In addition, vitamins and elements that have antitumor effects are a source of many chemical compounds that have been shown to have antitumor effects. These chemical compounds include glucosinolate precursors, niacinimine, niacimycin, eugenol, isopropyl isothiocyanate, D-allose, and ethyl hexadecanoic acid.[10,11]

The investigation of Moringa oleifera has led to the identification of these compounds, which have been studied for their potential antitumor properties against a range of cancers, including breast and colorectal cancer,[12] ovarian and prostate cancer,[13] chronic and acute myeloid leukemia,[14] pancreatic cancer cells,[15] lung cancer cells, melanoma,[11] and ileocecal adenocarcinoma.[12]

Epidemiological data suggests that incorporating Brassica oleracea vegetables such as broccoli, lettuce, cauliflower, and kale into one's diet can help reduce the risk of cancer. Although the precise mechanism by which kale reduces cancer risk is not yet fully understood, these vegetables are known to be rich in glucosinolates. When these vegetables are chopped or ground, the enzymes myrosinase (also known as thioglucosidase glucohydrolase) and glucosinolates come into contact with each other. Myrosinase breaks the beta-thioglucosidase bond of glucosinolate molecules, yielding glucose, sulfate, and other aglycone derivatives. Through a non-enzymatic process, these aglycone products are further converted into isothiocyanates, thiocyanates, or nitriles. One such isothiocyanate is sulforaphane, an organosulfur compound belonging to the isothiocyanate family that has shown potential for reducing cancer risk.

This compound, known as sulforaphane, can be derived from glucoraphanin, a glucosinolate that is naturally present in cabbage vegetables. It is formed through the action of myrosinase. Sulforaphane is particularly intriguing due to its potent anti-carcinogenic and antimicrobial properties. Numerous studies have demonstrated its ability to effectively reduce the risk of various cancers, including lung, colorectal, breast, and

prostate cancer. Additionally, it has been found to be beneficial for managing diabetes, respiratory illnesses, neurodegenerative disorders, ovulation disorders, and cardiovascular disease.

Sulforaphane is an extremely potent natural chemical generated from glucoraphanin (4-methyl sulfine butyl glucosinolate). It is recognized for its ability to induce phase II enzymes, including quinone reductases and glutathione S-transferase. This profound effect makes sulforaphane an invaluable tool for enhancing the body's detoxification mechanisms.

Furthermore, sulforaphane exhibits significant anti-tumor capabilities in numerous experimental models by lowering the prevalence of specific tumor forms and blocking the cell cycle and apoptosis. Its significance lies in the fact that, even at nutritionally relevant doses, sulforaphane can modulate xenobiotic-metabolizing enzyme systems and promote the inactivation of carcinogenic metabolism. This is achieved by impeding the activation of specific enzymes derived from carcinogens within the body while simultaneously increasing the production of other enzymes that aid in the elimination of carcinogens before they can inflict damage to the body's cells.

These findings underscore the potential of sulforaphane as a preventive agent against carcinogenesis and highlight its promising role in maintaining overall cellular health.[16]

Objectives

The purpose of this study was to identify the phenolic and flavonoid compounds present in the extract obtained from Moringa oleifera leaves. Furthermore, it sought to explore the possible antitumor effects of the leaf extract and sulforaphane in a C57BL/6-induced male melanoma cancer model using the B16F10 cell line.

Methods

The first step involved the preparation of Moringa oleifera leaves and stems obtained from Giah Daroo Baresh Company of Jahrom Company in Shiraz province. These were then evaluated and approved by university experts to ensure they were the correct species. The leaves were then cleaned, dried, and transferred to a laboratory in a sealed glass container. The flavonoid compounds, including quercetin, gallic acid, and folic acid, were analyzed using spectrometry (Uv/vis, Shimadzu, Uv2550, Japan) and high-performance liquid chromatography (HPLC) (China SY8100 CMC).

Preparation of the extract

To begin with, Moringa oleifera leaves weighing 25 g were subjected to a drying process in an oven at a temperature of 40 °C for a duration of 24 hours. Subsequently, the dried leaves were finely ground using an electric grinder and then sifted through an 80-mm mesh sieve. The extraction process was carried out by utilizing 250 ml of 99% methanol in a ratio of 1 to 10. To accomplish this, the sample was poured onto a Whatman filter paper 42 and inserted into a Soxhlet apparatus. Simultaneously, the solvent was poured into a flask, and the temperature of the heating element was adjusted to a medium-to-high setting. In order to obtain an extract of high purity, the excess solvent (methanol) was separated through vacuum distillation (rotary) at a temperature of 50 °C. Finally, the resulting extract was carefully stored in a sealed bottle under low-temperature conditions.

Determination of the phenolic and flavonoid content of the whole extract

The modified method of Meda et al.[17] was utilized to determine the flavonoid content of the whole extract. To do this, 0.5 ml of the extract was mixed with 0.5 ml of a 2% aluminum chloride solution in methanol solvent and incubated at room temperature for 30 minutes. The absorbance of the sample was then measured using UV-Vis spectroscopy at 415 nm, and a standard curve was created using various doses of quercetin. The total flavonoid content of the extract was expressed as milligrams per gram of extract based on quercetin.

Equation 1; $Y=0.029x+0.105$, $R2=0.986$

The Wolf et al. technique, as well as the Folin Ciocalteu reagent, were used to determine the total level of phenolic compounds. This method relies on the reduction of the Folin reagent by phenolic compounds in an alkaline medium.[18] To carry out the procedure, 20 µl of the extract was combined with 1.16 ml of distilled water and 1 ml of Folin reagent. Following an 8-minute interval, 350 µl of a

20% sodium carbonate solution was added. The resulting mixture was then incubated at a temperature of 40 °C for a duration of 40 minutes. Subsequently, the absorption of the mixture was measured at a wavelength of 765 nm. A standard curve was constructed using various concentrations of gallic acid. The quantity of phenolic compounds present in the extract was determined using equation 2, which was derived from the gallic acid-based curve and expressed in milligrams per gram of extract.

Equation 2; Y=0.0061+0.0019, R2=0.9912

Determination of phenolic and flavonoid compounds in *Moringa* extract by HPLC

The standard quercetin, gallic acid, and caffeic acid were acquired from Sigma for the purpose of this study. A quantity of 0.001 g of these substances was combined with 5 ml of methanol solvent, thoroughly mixed, and then filtered using a 0.2µm filter. The resulting standard solution was then introduced into the device using a syringe. Additionally, a separate solution was prepared by passing 5 ml of Moringa oleifera leaf extract (10 mg/ml) through a 0.2-micron filter and injecting it into the device. The samples were subjected to analysis using a multi-solvent HPLC system equipped with a single pump and an Agilent 1100 detector (Agilent 1100 SY-8100 UV/VIS Detector). The mobile phase, consisting of 50% methanol and 50% acetonitrile, was passed through a C18 column with dimensions of 5 µm and 4.6×250 mm at a flow rate of 0.9 ml/min and at room temperature. The absorbance of the samples was measured at 370 nm. The flavonoid composition (quercetin, gallic acid, and caffeic acid) was identified in the chromatograms obtained from the methanolic extract of Moringa oleifera leaf by comparing the inhibition time with the chromatogram obtained from the standard sample. The amount of flavonoids was determined by plotting the calibration curve of quercetin, gallic acid, and caffeic acid using a specific equation.

Equation 3; Y=131825X-797554, R^2=0.999

Measuring the regenerative power of antioxidant compounds (FRAP)

Oyaizu's method was employed to assess the reducing power of the phenolic and flavonoid compounds present in the extract.[19] To carry out this method, 1 ml of Moringa oleifera extract was prepared at various concentrations ranging from 20 to 100 mg/ml. These concentrations were then combined with a solution containing 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of potassium ferric cyanide (1% weight/volume). The absorbance of the resulting mixture was measured at 700 nm using a UV-Vis spectrophotometer (Shimadzu Uv-1240).

Assessment of antioxidant capacity by the DPPH radical method (2,2 diphenyl 1-picryl hydrazyl)

To prepare the samples, the leaf extract was dissolved in a methanol solvent at a concentration of 10 mg per 25 ml. Following that, 1 ml of DPPH solution was added to 5 test tubes, followed by 4 ml of extract at different concentrations. The tubes were then kept in a dark environment for 15 minutes. A control tube was also prepared with only 1 ml of DPPH and 2 ml of methanol. Methanol was used to calibrate the spectrophotometer. The absorbance of all samples was measured at 517 nm, and the inhibition rate of adsorption was calculated using equation 4.

Equation 4; Y=Abc-Abs/Abc*100

Cell culture and 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

The B16F10 cells were obtained from the Pasteur Institute Cell Bank in Tehran, Iran. They were cultured in a flask with DMEM supplemented with glutamine, 10% fetal bovine serum (FBS), and 1% streptomycin-penicillin. The cells were incubated in a CO_2 incubator at 37 °C with 5% CO_2 and maintained at saturated humidity until they reached 80% density. Every two days, the cells were passaged. Some passages of these cells were preserved. Initially, the cells were subjected to MTT testing, and later they were used to induce cancer in C57BL/6 mice.

Cell survival and determination of IC50 were assessed using MTT (3-(4), (5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide). This method relies on the breakdown of tetrazolium salt by the mitochondrial enzyme succinate dehydrogenase in living cells, resulting in the formation of insoluble formazan crystals. These crystals are then dissolved by adding a solvent, and the amount of dye produced is directly proportional to the number of viable cells. Initially, the cells were counted and

distributed into a 96-well plate at a density of 15,000 cells per well. The plate was then placed in a CO2-incubator for 24 hours. Subsequently, the extract concentrations were prepared in a culture medium containing 6% FBS and added to the wells, replacing 100 µl of the medium. The plate was again incubated in a CO_2 incubator for 24 hours. 1. The wells were emptied after the designated time and then filled with 100 µl of MTT solution (0.5 mg/ml). Following a 4-hour incubation period in the CO_2 incubator, the formazan crystals were dissolved by adding 100 µl of DMSO to the wells. After 10 minutes, the ELISA reader was used to measure the color intensity produced at 570 nm. The experiment was repeated eight times, and wells containing cells without extracts were used as controls.

In vivo Studies

In order to conduct in vitro studies, a total of 24 male C57BL/6 mice were obtained from the Royan Institute of Isfahan. These mice were then divided into four distinct groups and housed in separate cages.

The first group, known as the control group, consisted of six cancer mice that did not receive any extract (1). The second group, referred to as the first treatment group, comprised six cancer mice that received 14 intra-tumor injections of the extract at a concentration of 0.08 g/kg body weight over a period of two weeks (2). Similarly, the third group, known as the second treatment group, consisted of six cancer mice that received 14 intra-tumor injections of the extract at a concentration of 0.04 g/kg body weight in two weeks (3). Lastly, the fourth group, referred to as the third treatment group, included six cancer mice that received 14 intra-tumor injections of the extract at a concentration of 0.02 g/kg body weight over a period of two weeks (4).

Development of a melanoma model in C57BL/6 mice

A suspension of one million B16F10 cells per 200 µL of sterile PBS solution was produced and injected subcutaneously into each mouse to induce cancer. The formation of tumors was observed through macroscopic examination. The size of the tumors was measured using a collis, and the mice were monitored daily for their physical well-being. Throughout the entire 28-day study period, the mice were euthanized in accordance with ethical guidelines for working with animals after the injection of the cell team and treatment. Subsequently, the necessary samples for further experiments were prepared.

Statistical analysis

All measurements were repeated three times, and all data were reported as Mean+SD. Data analysis was performed using a one-way analysis of variance (one-way ANOVA). Compare the means, the Tukey test was used at a 5% confidence level. Kruskal Wallis's nonparametric test was used to analyze in vitro and in vivo data. GraphPad Prism software version 8 was used to analyze data and draw graphs.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval was obtained.

Results

The content of total phenol and flavonoid in the extract

The dry extract contained an average of 60.65±1.75 mg of quercetin acid per gram, while the total phenol content was measured at 20.25±1.23 mg of gallic acid per gram.

Phenolic and flavonoid compounds of the extract

The HPLC method was utilized to conduct a chemical analysis of phenolic and flavonoid compounds. The extract of Moringa oleifera leaf was found to contain phenolic compounds such as quercetin, gallic acid, and caffeic acid, which were identified through the HPLC method. Table 1 provides a list of the polyphenolic compounds that were identified and their respective amounts in the leaf extract. Three phenolic compounds were identified by comparing the inhibition time in the chromatograms obtained from methanolic extracts of leaves and the standard sample, as shown in Figure 1. Additionally, Figure 2 displays the chromatogram of the methanolic extract of the leaf.

Determination of total flavonoid content

The phenolic compound content in the extract was calculated as milligrams of gallic acid per gram of dry

extract. The extract prepared by the soxhlet technique using methanol solvent contained the most phenolic compounds (20.251.23 mg gallic acid per gram of dry extract). The total flavonoid content, measured in milligrams equivalent to quercetin per gram of extract, was determined using the standard quercetin curve. The extraction with methanol solvent using the Soxhlet method resulted in a higher amount of flavonoids (60.65±1.75 mg quercetin acid per gram of dry extract).

Evaluation of the antioxidant effect of the extract by DPPH free radical scavenging method

By 420.73 degrees, the DPPH free radicals were inhibited. As the concentration of the extract increased, there was a significant increase in the scavenging of DPPH free radicals ($P<0.05$). The inhibitory power of free radicals was expressed using the IC50 index [Figure 3A].

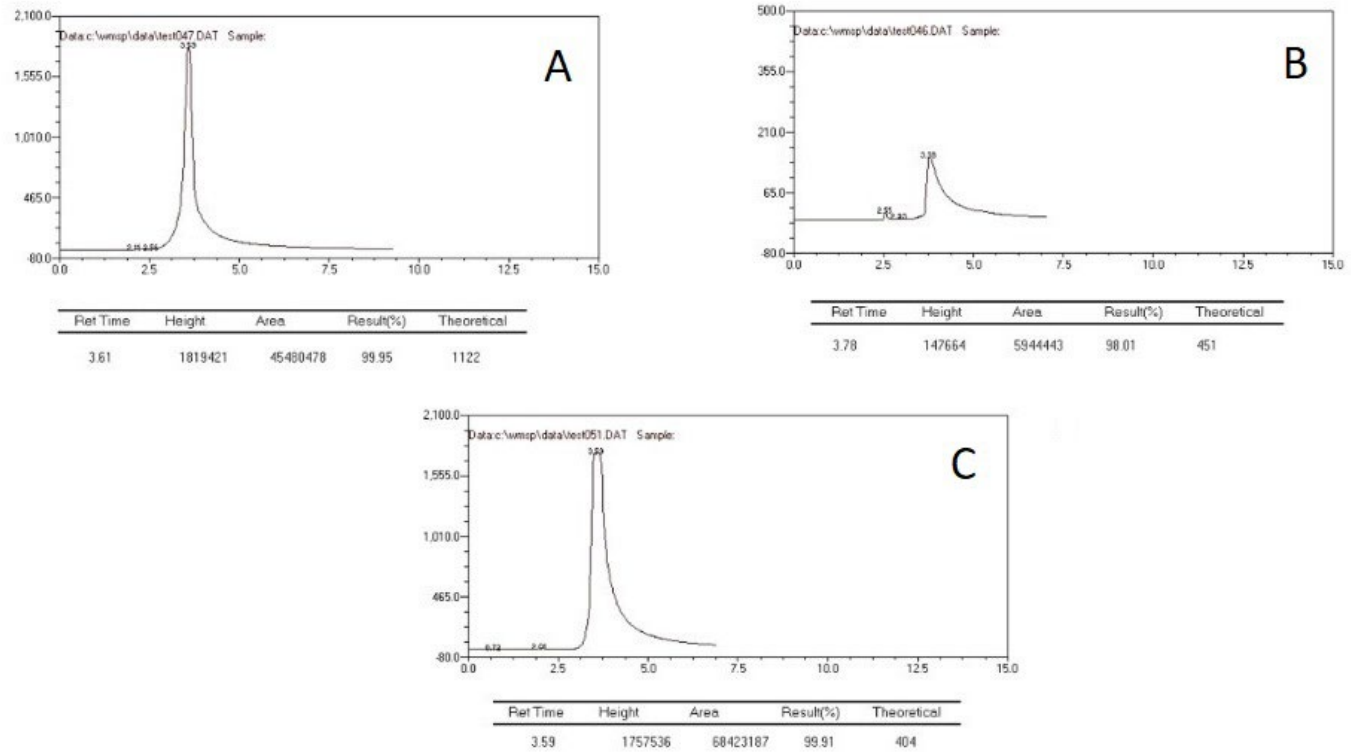

Figure 1. A: Chromatogram of a standard sample of caffeic acid (the standard sample is removed from the chromatographic column at the time of inhibition of 3.61). B: Chromatogram of a standard quercetin sample (the standard sample is removed from the chromatography column at the time of inhibition of 3.78).C: Chromatogram of a standard sample of gallic acid (the standard sample is removed from the chromatographic column at the time of inhibition 3.59).

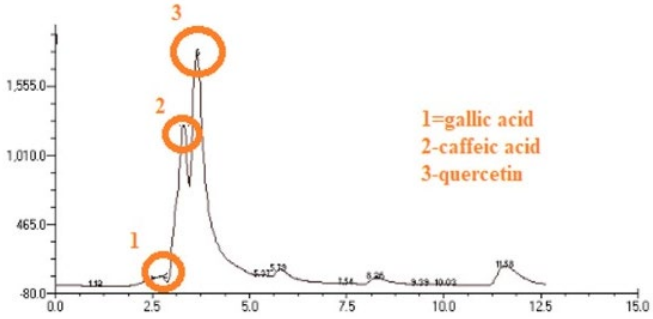

Figure 2. Chromatogram obtained by high-performance liquid chromatography (HPLC) of *Moringa oleifera* leaf extract (by comparing the inhibition time in the obtained chromatogram from methanolic leaf extract and obtained chromatogram from standard samples, three phenolic compounds were identified).

Table 1. Polyphenolic compounds identified by HPLC method and their amounts in Moringa oleifera leaf extract

Phenolic compounds	**Phenolic compounds** (leaf extract) (mg/100 g)
Quercetin	3.078±0.3636
Caffeic acid	2.210±0.7735
Gallic acid	2.107±0.8299

Reducing power of antioxidant compounds (FRAP)

The research revealed that as the concentration of extracts increased, the reducing power also exhibited an upward trend. Notably, a direct and linear correlation was observed between the regenerative power and the phenolic and flavonoid content, as depicted in Figure 3B.

MTT test

In vitro test results

The MTT test was employed to determine the cell survival percentage in this study. The MTT yellow salt was converted by the mitochondrial dehydrogenase enzymes of the active cells, resulting in the production of formazan, an insoluble purple compound. The optical absorption of this compound was then measured at 570 nm after being dissolved in DMSO solvent using an ELISA reader. Based on the findings presented in Figure 4A, the cancer cell survival rate was observed to be higher in the sulforaphane-treated cell group compared to the Moringa oleifera group. Consequently, Moringa oleifera was selected for further experiments to assess its anticancer effect on mice.

The results of in vivo test

The administration of Moringa extract at varying doses of 0.02, 0.04, and 0.08 g/kg body weight resulted in a significant decrease in tumor volume after one and two weeks (P<0.001). Notably, the tumor volume reduction was more pronounced at a dose of 0.02 g/kg body weight compared to the other two doses (P<0.001) [Figure 4B].

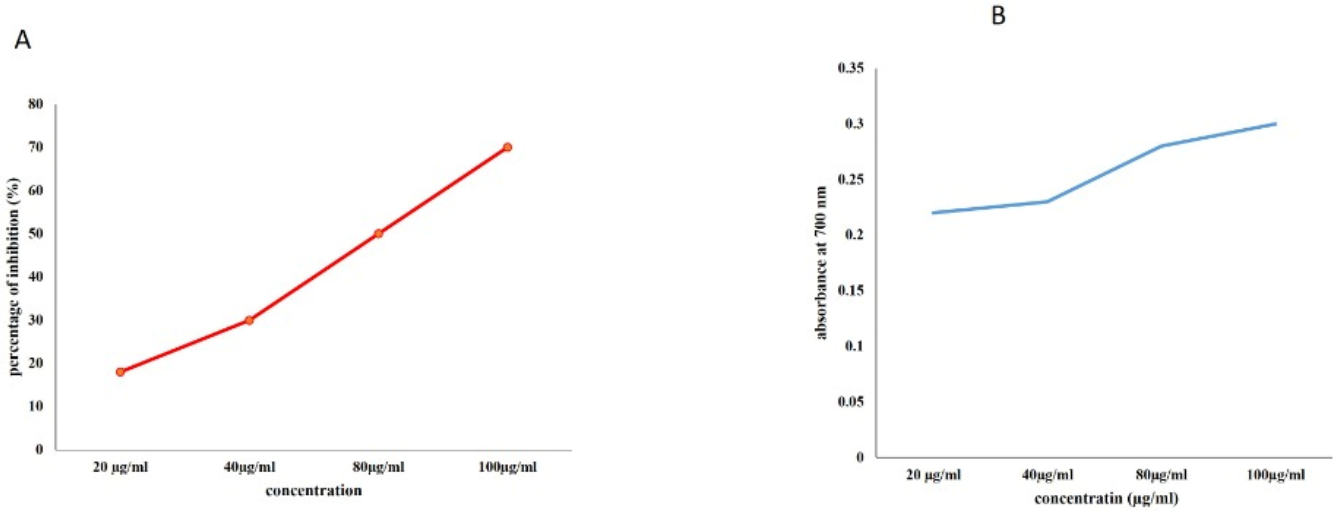

Figure 3. A: DPPH free radical scavenging activity of *Moringa oleifera* leaf extract at different concentrations. B: Reduction power of different concentrations of methanolic extracts of *Moringa oleifera* leaf (increased with increasing concentration of Fe^{3+} to Fe^{2+} conversion (reduction power of phenolic compounds) and solution absorption increased at 700 nm.

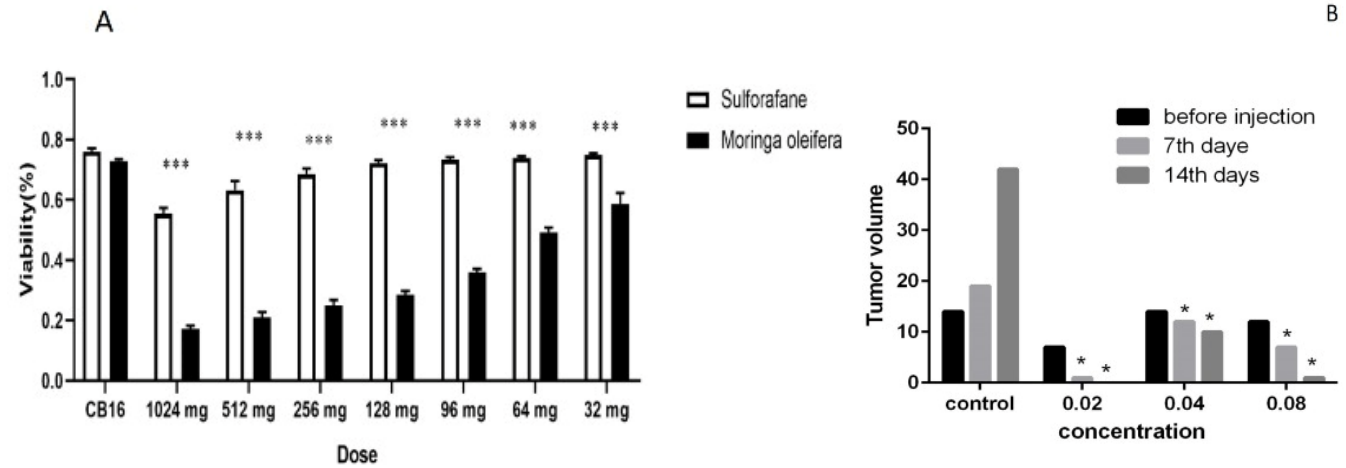

Figure 4. A: CB16 cell viability percentage is adjacent to different concentrations of sulforaphane and Moringa oleifera by MTT method. B: Comparison of tumor volume in different doses of *Moringa oleifera* in the animal model (*: P <0.001).

Discussion

Numerous studies have demonstrated that Moringa contains a wide range of beneficial compounds, including 46 antioxidant compounds and 36 natural anti-inflammatory agents.[20,21] Among these compounds are zeitin, a potent antioxidant; quercetin, a flavonoid known for its ability to neutralize free radicals; beta-sitosterol, which helps reduce inflammation in the body; caffeic acid, another powerful anti-inflammatory compound; and kaempferol.[22] The effectiveness of Moringa in combating various health conditions such as Alzheimer's disease, gastric ulcers, cholesterol control, and wound healing has been extensively researched and proven.[21-23] Researchers have also found that Moringa can enhance cellular resistance to chemotherapy and increase the tolerance threshold for chemicals.[23] Therefore, the primary objective of this study was to assess the impact of Moringa oleifera on melanoma cancer, both in vitro and in vivo.

Firstly, the initial step of this research involved determining the overall flavonoid and phenolic content of the prepared Moringa leaf extract. Subsequently, the dominant flavonoid compounds present in the extract were identified, and their concentration was measured. Within this investigation, three specific phenolic and flavonoid compounds (quercetin, gallic acid, and caffeic acid) were recognized for their notable antioxidant properties. The flavonoid content of the extract was determined to be 60.651.75 mg of quercetin acid per gram of dry extract when the extraction process was completed using a methanol solvent in a soxhlet system. In a separate investigation, Ishaqzadeh discovered that the total flavonoid content of Moringa hydroalcoholic extract, which was amplified in a glass, was 78.1890.01 mg/g.[24] The study's results revealed a greater concentration of total flavonoid content in Moringa, resulting from the expression of flavonoid concentration. Quercetin, the predominant flavonoid type, served as the basis for expression in this study, while Ishaqzadeh's study reported the total flavonoids. The findings highlight the significant total flavonoid content in Moringa extract, which can be attributed to the antioxidant properties of flavonoid compounds.

The antioxidant capacity of the extract was evaluated using two tests, namely the DPPH and FRAP tests. The Moringa leaf extract exhibited a DPPH free radical inhibition potency of 42±0.73. Interestingly, the study revealed that the free radical scavenging and regenerative abilities of the extract increased significantly with an increase in its concentration ($P<0.05$). Furthermore, the presence of phenolic and flavonoid compounds in the extract was discovered to have a direct and linear relationship with its antioxidant activity, which is similar to Jeyakumar et al.'s findings.[25] The DPPH inhibition values for ethanolic extracts of Moringa leaves were found to be 29.49, 43.72, and 54.59 at 500, 1000, and 1500 ppm, respectively, which were similar to the average values obtained in our research. In a different study, Abdolshahi et al.[26] used the DPPH method to examine the antioxidant activity of a methanolic extract of medicinal herbs and discovered that it has the potential to function as a natural antioxidant.[27-30]

In the current investigation, a dose-dependent decrease in the growth of melanoma cancer cells (B16F10) was observed when they were exposed to varying concentrations of Moringa extract ranging from 1024 to 32 µM. The IC50 value of Moringa extract was determined to be 73 µM/mol, indicating its strong efficacy against melanoma cancer cells. These findings highlight the potent anti-cancer activity of the extract obtained in this study.

In the study conducted by Tae Eun Guon, it was discovered that the utilization of Moringa extract in A2058 melanoma cells leads to the generation of hydroxyl radicals and hydrogen peroxide, ultimately resulting in apoptosis.[31] Despite the fact that previous research has consistently highlighted the antioxidant properties of Moringa extract and its components, as well as the detrimental impact of active oxygen species on the development of cancer and various diseases, Tae Eun Guon's findings may appear contradictory. However, in order to gain a clearer understanding, two crucial points should be taken into consideration. Firstly, the induction of apoptosis through the reactive oxygen species-dependent pathway has been observed exclusively in cancer cells, not in healthy cells. Secondly, reactive oxygen species, despite their destructive effects, can also function as intercellular signaling mediators.[32]

The experiment involved inducing tumors in mice and subsequently treating them with Moringa extract at varying doses of 0.02, 0.04, and 0.08 g/kg body weight. The findings revealed that the Moringa extract had a significant impact on reducing tumor growth within a span of one to two weeks. Notably, the dose of 0.02 g/kg body weight exhibited the highest reduction in tumor volume compared to the other two doses. Furthermore, this study discovered that Moringa extract, enriched with phenolic and flavonoid compounds, possesses the ability to induce cell death in melanoma cells in vitro. Additionally, injecting the extract into tumors after one week demonstrated a reduction in tumor volume.

Conclusions

This study examined the anticancer effects of phenolic sulforaphane and Moringa oleifera compounds on B16F10 cancer cells. The study discovered that the structure of phenolic compounds influences their anticancer efficacy, specifically the presence of functional groups such as aromatic rings and hydroxyl groups. Compounds with a higher number of hydroxyl groups demonstrated superior anticancer performance compared to those without hydroxyl groups or with the OCH_3 group. The sulforaphane-treated cell group exhibited a lower rate of cell death compared to the Moringa oleifera group. Tumor volume significantly decreased after one week and two weeks for all three doses of 0.04, 0.08, and 0.02 g Moringa oleifera ($P<0.001$). Among these doses, the 0.02 g dose showed a greater reduction in tumor volume ($P<0.001$). However, further research is necessary to determine the effectiveness and safety of these treatments for cancer. Both basic and clinical studies on polyphenolic compounds can serve as a guide for their potential use in preventing and treating various types of cancer. Based on studies investigating the anticancer properties of this compound, it can be more confidently considered an anticancer medication.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Ultraviolet: UV; Melanocyte-stimulating hormone alpha: α-MSH; Melanocortin 1 receptor: MC1R; High performance liquid chromatography: HPLC; Fetal bovine serum: FBS.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval was obtained.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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