The Study of the Anthocyanin Extract Effect from *The Hibiscus sabdariffa* Plant on Skin Cancer

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Abstract

Background: Anthocyanins are water-soluble phytochemicals that are essential pigments in fruits and vegetables. Anthocyanins represent an important subclass of flavonoids known for their biological and pharmacological activities. Anthocyanins have been reported to exhibit significant anticancer activities through multiple biological mechanisms. Here, we examined whether anthocyanins could specifically inhibit the proliferation of skin cancer cells.

Methods: After extracting the anthocyanin pigment from *the Hibiscus sabdariffa* plant using a solvent extraction method, the anticancer effect of the pigment at several doses was assessed by measuring the cell viability.

Results: The effect of the anthocyanin extract showed highly significant inhibition in the A375 cell line ($p \leq 0.05$, $p \leq 0.01$). The results were 73.99 ±5.04, 65.12 ±1.68, 55.59 ±0.99, 49.19 ±0.92 at concentrations of 50, 100, 200, 400 µg/ml, correspondingly. When compared to WRL-68, that were 91.08 ±0.87, 91.08 ±0.87, 77.55 ±1.93 and 71.14 ±0.47 µg/ml respectively. The 25 µg/ml anthocyanin extract showed significant inhibition in the A375 cell line, with an IC50 of 86.34 ±3.11 µg/ml, compared to 96.76 ±0.53 µg/ml in WRL-68. It was also noted that the decrease in cell viability is inversely proportional to anthocyanin concentration, with computed IC50s of 150.8 and 97.9 for the WRL-68 and A375 cell lines, respectively.

Conclusion: The study's findings enhanced knowledge of roselle anthocyanins' selected anti-cancer action on the skin cancer A375 cell line.

Keywords: Anthocyanin; Plant extract; Ethanol solvents; Roselle; Anticancer; *Hibiscus sabdariffa;* Skin Cancer Cell Line.

1. Introduction

Skin cancer is considered one of the most prevalent malignancies worldwide, with a continuously rising incidence over recent years. It represents a major public health concern due to its increasing burden in both developed and developing countries [1]. According to estimates, one in five Americans will have skin cancer by the time they are 70 years old, with Caucasian populations having the greatest incidence. Non-melanoma skin cancer (NMSC), which includes a variety of forms, is the most prevalent type of skin cancer. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common types of non-melanoma skin cancers, with SCC being the most frequently diagnosed. In contrast, Merkel cell carcinoma (MCC) is rarer. While malignant melanoma carries a higher risk of mortality, MCC is less frequently seen. Skin cancer can develop when DNA damage triggers normal skin cells to become cancerous. Exposure to ultraviolet radiation is a major environmental factor that induces DNA damage and plays a critical role in the initiation and progression of skin cancer. UV exposure not only damages DNA integrity but also fosters an immunosuppressive and inflammatory milieu, which can accelerate the development of precancerous alterations into malignant tumors [2]. Melanocytes, which are skin cells that produce pigment, are the source of malignant melanoma, a type of skin cancer. One of the deadliest types of skin cancer is malignant melanoma. Its incidence has risen more quickly than any other cancer in the US during the last few decades [3]. Unlike other skin cancers, malignant melanoma can develop from preexisting nevi or occur in multiple locations on the body, including areas with little to no sun exposure. UV exposure, immunosuppression, atypical moles, pale skin, a history of skin cancer, and a genetic predisposition to the disease are known risk factors for malignant melanoma [4]). A rare and extremely malignant neuroendocrine tumor that develops in the skin is called Merkel cell carcinoma. Increased age, fair skin, long-term sun exposure, a history of numerous skin malignancies, and immunosuppression such as in HIV infection and following solid organ transplantation are risk factors. Merkel cell polyomavirus accounts for 80% of MCC cases, with UV-induced skin damage accounting for the remaining 20%. The potential role of nutrition in

controlling and preventing skin cancer has drawn greater attention in recent years. There is growing interest in the potential use of natural substances found in food as supplemental treatments and/or for cancer prevention. Flavonoids are naturally occurring bioactive compounds widely distributed in plant-based foods, particularly fruits and vegetables, are phytochemicals with potential anticancer effects, particularly against skin cancer. Chemo-prevention research has been greatly influenced by the renewed global interest in diets rich in phytochemicals, with a current focus on enhancing the therapeutic activity of these compounds.

Fruits and vegetables are rich in flavonoids, a broad class of chemicals originating from plants. Among their subclasses, anthocyanins water-soluble pigments that give vascular plants their red, blue, and purple hues have drawn a lot of scientific attention because of their possible health advantages. Regular consumption of foods high in anthocyanins, like roselle, has been associated in Epidemiological evidence suggests that regular consumption of anthocyanin-rich foods is associated with a reduced risk of several types of cancer, including skin cancer [5,6]. More than 700 anthocyanin compounds have been identified in nature, with a limited number of major forms accounting for the majority of naturally occurring anthocyanins [7]. Extensive research has demonstrated that anthocyanins exert strong anti-cancer effects through various molecular mechanisms. They are known for their high reactivity and are particularly sensitive to environmental changes such as temperature and pH, due to the electron-deficient nature of their phenolic structure [8]. Anthocyanins have been closely associated with anti-cancer activities, including their ability to protect cells from oxidative stress and to induce apoptosis in cancer cells [9].

This study aimed to: First investigate the anticancer activity of anthocyanin extract derived from Hibiscus sabdariffa against the human skin cancer cell line (A375). Second, to evaluate the dose-dependent effect of anthocyanin extract on cell viability. Third to compare the cytotoxic effects of anthocyanin extract on the cancerous A375 cell line and the normal WRL-68 cell line, and finally to determine the IC_{50} values of anthocyanin extract for both A375 and WRL-68 cell lines.

2. Materials and Methods

The Biotechnology Research Center at Al-Nahrain University in Baghdad, Iraq, conducted this study in 2025. Asita and Salehhuddin's [10] solvent extraction method was used to extract anthocyanin pigments.

2.1 Cell Line

2.1.1 Cell Line WRL68

This cell line was obtained from healthy fetal or embryonic liver tissues. This cell line has been shown to display morphological characteristics similar to those found in primary cultures of liver cells or hepatocytes. This is according to a study done by [10].

2.1.2 Cell Line A375

This is a cell line obtained from a human melanoma sample. This cell line is used in the study of cancer. It was first isolated from the skin of a female patient who was 54 years old and suffered from malignant melanoma.

2.2 Collection of Plant Material

Fresh Roselle plant material was obtained from a local market, and the plant was identified based on its morphological characteristics. The fresh roselle leaves were rinsed with distilled water at room temperature (25 ± 2 °C), and any residual moisture was wiped away using tissue paper. A vegetable peeler with a thickness of two to three millimeters was used to remove the colored outer layer of epidermis manually. After peeling, the layers were divided into pieces of consistent size. These pieces were pounded into a fine powder using a mortar and pestle after being allowed to air dry at room temperature for about two weeks. For later use, the resultant roselle powder was kept in sealed, dark containers [11]; (Basal Cell Skin Cancer Presented with Support from the Foundation Guiding Treatment). transforming lives. National Comprehensive Cancer Network ® (available online at NCCN.Org/Patientguidelines NCCN Guidelines for Patients ® Cancer Care Recommendations from Leadin, n.d.).

2.2.1 Preparation of Plant Extract

The plant extract was prepared using a modified solvent extraction method based on previously described protocols by [12] with some modifications. It was conducted with two concentrations of the solvent: acidified with 0.1% HCl water-ethanol solution at concentrations 95% ethanol. At a 1:5 solid-to-solvent ratio, a 20 g pretreated roselle powder was added to beakers containing 100 ml solutions of ethanol, distilled water, and HCl (95.2ml, 4.5ml, 0.3ml; 95% ethanol, 0.1% HCl). The prepared mixture was incubated in a dark, sealed container under controlled conditions to ensure optimal extraction efficiency. The magnetic stirrer was used for 1 hr to help the active ingredients dissolve in the mixture at 700 rpm/minute at 30 ◦C. The extract was then dried in the oven for 24 hours and re-diluted with distilled water to prepare a stock solution at 100mg/ml. A

series of concentrations of 400 µg/ml, 200 µg/ml,100 µg/ml, 50 µg/ml, and 25 µg/ml was prepared from the stock solution [12].

2.3 Statistical Analysis

SPSS (2019) was used to assess the impact of cell lines and concentration variations on study parameters (Inhibition%). The study's means were compared using the T-test and LSD test [13].

3. Results

The anthocyanin plant extract was prepared previously for the solvent extraction method [12]. The Cell Viability of A375 Cell Line after treatment with Athocyanin extract. The effect of the anthocyanin extract showed highly significant inhibition in the A375 cell line ($p \leq 0.05$, $p \leq 0.01$). The results were 73.99 ±5.04, 65.12 ±1.68, 55.59 ±0.99, 49.19 ±0.92 at concentrations of 50, 100, 200, 400 µg/ml, respectively. When compared to WRL-68, that were 91.08 ±0.87, 91.08 ±0.87, 77.55 ±1.93 and 71.14 ±0.47 µg/ml respectively. While the 25 µg/ml anthocyanin extract concentration showed significant inhibition on A375 cell line was 86.34 ±3.11 µg/ml compared to96.76 ±0.53 µg/ml of WRL-68 as shown in Table 1. Figure 1 shows that the decrease in cell viability is precisely inversely proportional to anthocyanin concentrations, with computed IC50s of 150.8 and 97.9 for WRL-68 and A375 cell line, respectively.

Table 1. Effect of Roselle Concentration on Inhibition in WRL-68 and A375 cell line

Roselle Conc. (µg/ml)	Mean ±SD of Inhibition (%)		Sig.	P-value
	WRL-68	A375		
400	71.14 ±0.47	49.19 ±0.92	**	≤0.0001
200	77.55 ±1.93	55.59 ±0.99	**	≤0.0001
100	91.08 ±0.87	65.12 ±1.68	**	≤0.0001
50	95.29 ±0.59	73.99 ±5.04	**	≤0.0044
25	96.76 ±0.53	86.34 ±3.11	*	<0.03952
P-value	≤0.0001	≤0.0001	---	---

* (P≤0.05), ** (P≤0.01), SD: Stander deviation.

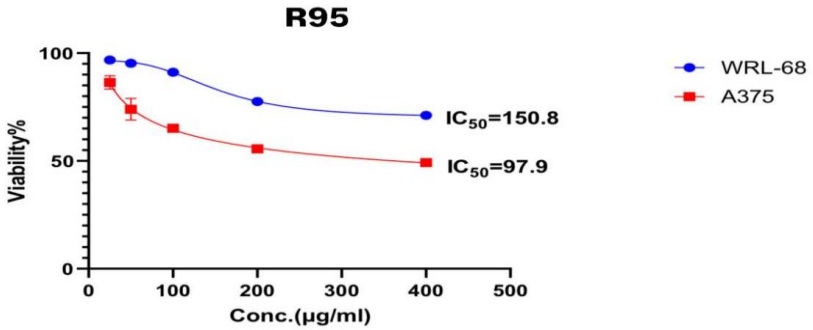


Figure 1. Cell survival curve of WRL-86 cells and A375 after treatment with different concentrations of 95% ethanol *Hibiscus sabdariffa* anthocyanin extract. R95: *Hibiscus sabdariffa* 95, IC50: Half-maximal inhibitory concentration

4. Discussion

Previous studies have demonstrated that anthocyanins possess the ability to inhibit the proliferation of cancer cells through various biological mechanisms. The findings of the present study are consistent with earlier reports highlighting the anticancer potential of anthocyanin-rich compounds of [13] and show that anthocyanin-enriched fruits and vegetables have anti-tumorigenic effects via a variety of pathways. Additionally, the study examined the cytotoxic capability of specific anthocyanin extracts from chokeberries and red grapes (C-ARE and RG-ARE) against A375 melanoma cell lines. Both C-ARE and RG-ARE anthocyanins decreased mitochondrial membrane potential, elevated oxidative stress biomarkers, and decreased cell proliferation following extract administration without endangering healthy cells [14].

In a study conducted recently [15], the B16-F10 melanoma cell line was utilized to study the chemopreventive potential of the extracts of blueberry anthocyanin. The study showed that the anthocyanin and anthocyanidin extracts inhibited the viability and proliferation of melanoma cells after treatment with the extracts. In the study on murine melanoma cells, the anthocyanin extracts from the same berries as the study above showed greater cytotoxicity compared to other anthocyanin extracts. At concentrations below 400 µg/mL and 200 µg/mL, the extracts inhibited the cell cycle at the G0/G1 phase. Flow cytometry was used to study the induction of apoptosis. The results suggest that the anthocyanin and anthocyanidin extracts can be applied topically to treat skin cancer. In the present investigation, anthocyanin extract considerably reduced the viability of A375 cells in a dose-dependent manner, with A375 cells being more sensitive than WRL-68 cells. The IC_{50} values were 97.9 µg/ml for A375 and 150.8 µg/ml for WRL-68. This result is quite in agreement with [16] where both anthocyanin and anthocyanidin blueberry fruit

extracts significantly inhibited the viability and proliferation of B16-F10 cells (melanoma cells) in a dose-dependent manner. The result is also relatively in agreement with [17] where anthocyanin inhibited the development and progression of lung cancer. In diverse colon cancer cell types and animal models, anthocyanin-rich whole foods, anthocyanin extract, anthocyanin single compounds, and anthocyanin metabolites have all demonstrated apoptosis-inducing properties [18]. Purple-shoot tea's anthocyanin extract encouraged cancer cells to undergo apoptosis [19]. By diminishing the production of anti-apoptotic proteins, anthocyanin extracts and individual chemicals trigger apoptosis [20]. According to [21]. Anthocyanins have been shown to promote apoptosis and regulate key signaling pathways involved in cancer cell survival.

According to a clinical trial, anthocyanins and glutathione were found to be effective in reducing radiation-induced skin erythema in patients with breast cancer [22]. Mulberry anthocyanin extract suppresses melanoma metastasis and protects atherosclerosis, according to another study [23]. The anti-proliferative effect of extracts from cell suspensions (strawberry, strawberry tree, blackberry, and red raspberry) on murine melanoma cells was reported by the other authors in a recent work. It was discovered that the anthocyanin-containing extracts decreased cell proliferation by 30% to 38% when compared to the control [24]. The possible antimetastatic activity of mulberry anthocyanins was studied. According to the research, mulberry anthocyanins displayed potent anticancer effects and prevented B16-F1 cells from spreading. These substances also demonstrated an antimetastatic action in a model of C57BL/6 mice [25].

5. Conclusions

This study focused on the anticancer potential of the isolated anthocyanins from the leaves of the roselle plant. The anthocyanins isolated in the study demonstrated a strong anticancer effect. Scientific studies and publications have proven that anthocyanins exhibit a number of biological activities that may be helpful in the chemoprevention of cancer. The recent study served to further affirm the occurrence of the process, and further research in the subject is needed to improve the outcome.

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Conflicts of Interest

The Authors Has no conflict of interest

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