

Anticancer Activity of Rosella Flowers (*Hibiscus Sabdariffa* L.) In HepG2 cells

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Abstract: The natural ingredients of rosella flowers have the potential as natural anti-cancer and chemopreventive agents, contain secondary metabolite compounds with cancer therapeutic mechanisms with inhibiting and killing cancer cells and their development through inhibition of apoptosis (cell death program) and in vitro inhibited cell flow. The study aims to identification of secondary metabolite compounds and also determine the cytotoxic activity of ethanol extract from the rosemary plant against HepG2 cancer cells. This study used by identification of secondary metabolite compounds of ethanol extract from the rosemary plant (*Hibiscus sabdariffa* L.) and the cytotoxicity test used with MTT assay on HepG2 liver cancer cells with a test concentration series of 12,5; 25; 50; 100; 200; 250; 500 and 1000 µg/ml. : The results showed the secondary metabolite compounds of rosella flowers have flavonoids, alkaloids, saponins and tannins and data of 50% cell growth inhibition activity, which was showed that rosella flower had IC₅₀ values 629,919 µg/mL. The ethanol extract of rosella flowers have activity on HepG2 cells growth inhibition had considered moderately active anticancer activity.

Keywords: Anticancer Activity; Ethanol Extract; HepG2 cells; Rosella Flowers

Introduction

Cancer is one of the most dangerous diseases. Whether in an industrialized country or a developing country, there is a high mortality rate (Khazaei et al., 2020). One of the highest cancer is liver cancer, where it continues to be a diseases for world health. 90% of cases, hepatocellular carcinoma (HCC) of most prevalent of liver cancer (Llovet et al., 2021). Hepatitis B and C by infections are most common the primary risk factor for the development of HCC, despite the association of non-alcoholic steatohepatitis with risk factors for diabetes mellitus or the metabolic syndrome are increasingly common (Mayer & Engle, 2022). Many preventive efforts are being undertaken ranging from provide public awareness to rehabilitation efforts for cancer patients (Zheng et al., 2021). Factors that can cause cancer can be external: free radicals, infections, cigarettes, ultraviolet rays, radiation, viruses, and chemicals. But cancer can

also be caused by genetic, hormonal, psychological, and antibody factors (Martemucci et al., 2022). Currently, there are many treatments for cancer patients. Radioactive use and surgery are one way of treating cancer. In addition to the use of radioactive and surgical, chemotherapy methods can also be performed. For non-metastatic cancers, radiation and surgery are more effective. Unlike metastatic cancer, which affects many organs of the body, chemotherapy is more effective than surgery and radiation.

There have been attempts to develop chemotherapy medications that are successful, yet selectivity and toxicity issues persist. Resistance of cancer cells by drugs have toxicity of contemporary with chemotherapy and have prompted research into other forms of treatment and preventative measures (Anand et al., 2022). Natural plant items could provide a remedy (Khaghani et al., 2011). The rosella flower plant is a well-known plant today because the rosella leaf can be used as

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a healthy drink that can cure a variety of diseases such as hypertension, diabetes and diuretics (Mungole & Chaturvedi, 2011).

The most active ingredients in rosella leaves include gossypetin, anthocyanin, and hibiscus glucoside (Guardiola & Mach, 2014). The red color of rosella flowers have anthocyanin content (Djaeni et al., 2017). In Indonesia rosella can grow fertile, a member of the *Malvaceae* family of plants, *Hibiscus sabdariffa* L have a high polyphenol content as a anthocyanins have been employed in traditional medicine. Against fever, hypertension, and liver disorders. As possible anticancer or chemopreventive chemicals, the extracts made from the various (*Hibiscus Sabdariffa* L.) plants have attracted a lot of attention (Yasmin et al., 2023). Rosella plant also compound of belongs group flavonoids (Hayati et al., 2012; Laskar & Mazumder, 2020).

Anthocyanins act as an antioxidant that is believed to cure degenerative diseases. An antioxidant known as a scavenger is a molecule that can react with free radicals and function as a neutralizer (Sak, 2014). The role prevent to cancer and clinical trials in humans have shown to have important effects as an anti-cancer chemopreventive of antioxidants protect (Kopustinskiene & Savickas, n.d.). The antioxidant acts immune system protection against cancer cells by enhancing the primary function of the immune systems in B and T lymphocytes as well as macrophages, where B cells produce antibodies to block the activity of pathogens and T cells are divided into two cells, the cytotoxic T (T CD8⁺) and T cells (T CD4⁺) which secretes cytokines and mediators that regulate other cells such as B lymphocytes and macrophages, antioxidants also have immune response activity to antiproliferative in cancer cells with pathway inhibition PI3k-phosphatidylinositide target on the pathway of cancer cells, so with the blocking pathway PI3k/Akt/mTOR Effector differentiation on cells decreased (Hosseinzade et al., 2019). In this study, HepG2 cell cultures were used to examine the ethanol extract of rosella flower to determine as an anticancer agent.

Method

Chemicals and botanical materials

In March 2023, rosella flower (*Hibiscus Sabdariffa* L.) were collected in Palu Central Sulawesi, Indonesia. Absolute Ethanol was employed as a substance (Sigma-Aldrich, and Gibco BRL, Grand Island, New York, USA Non-essential (D-PBS, Gibco BRL, Grand Island, NY, USA) Foetal bovine serum (FBS; Gibco BRL; Grand Island; amino acid Eagle's Medium modified with Dulbecco (Invitrogen, NY, USA), Kanamycin (Sigma-Aldrich, St. Louis, MO, Carlsbad, CA, USA), St. UV-vis

spectrophotometers, Missouri, Dimethyl sulfoxide (DMSO), GloMax-Multi, Missouri, USA (Promega Corp., WI, USA), Microplate Multimode Reader Phosphate-buffered saline (PBS), Annexin-PI, HepG2 cell line, DMEM, BD Biosciences Dulbecco PBS, t-EDTA, USA 2,2-diphenyl-1-picrylhydrazyl (DPPH), MTT, and Quvetes Glass (3-(4,5-dimethylthiazol-2yl)-5-(3carboxymethoxyphenyl)-2-(4-solution of sulfophenyl)-2H-tetrazolium (Sigma-Aldrich, St. Louis.

Phytochemical test for detection

To determine the chemical makeup of plants based on their groups, a phytochemical screening test was performed. used as a starting point to identify the category of chemical compounds from a plant that have biological activity. Chemical substances were subjected to phytochemical screening assays, including examinations for alkaloids, flavonoids, saponins, and tannins.

Preparation of Rosella flower (Hibiscus Sabdariffa L.) ethanol extract

The rosella flower (*Hibiscus Sabdariffa* L.) were macerated with 500 mL of ethanol. This process was repeated three times over the course of an overnight period. The remaining ethanol was evaporated using a rotary evaporator at 50°C after the precipitates were collected. The extract was subsequently turned into a powder by lyophilization. To create the stock solution, each powder was dissolved in 1 mg of DMSO (10 µL).

Cell culture for HepG2

In Petri dishes, HepG2 cells were grown in DMEM with 10% FBS (Biowest, Nuaille, France), 150 µg/mL kanamycin (Sigma-Aldrich), and nonessential amino acids (Invitrogen) addition.

The cytotoxicity evaluation

The cytotoxicity of rosella flower (*Hibiscus Sabdariffa* L.) ethanol extract was tested colorimetrically using the MTT tetrazolium reduction assay method (El-Shiekh et al., 2020). HepG2 cells are planted on a 96-well microplate with a density of 1×10⁴ cells/well and incubated overnight (CO₂ 5%, 37°). Before being treated, the media is removed from the whole well and extracts of rosella flower (*Hibiscus Sabdariffa* L.) ethanol extract are given in a series of concentrations of 12,5 µg/ml; 25 µg/ml; 50 µg/ml; 100 µg/ml; 200 µg/ml; 250 µg/ml; 500 µg/ml; 1000 µg/ml. Then the cells were incubated with therapeutic time for 24, 42, and 72 hours at a temperature of 37°C in a 5% CO₂ incubator. Then 10 µL of MTT solution was administered and incubated for 4 hours (CO₂ 5%, 37°). The cell morphological condition was observed using an inverted microscope to see the formation of formative crystals. Next, 10 µL of DMSO

solvent is added and incubated in a dark room at room temperature for 10 minutes. Absorption of solute forming crystals measured at 600 nm wavelengths using the GloMax® Explorer Multimode Microplate Reader (Promega). MTT test absorption data calculated with the formula: The formula used to compute the percentage growth inhibition was %cell inhibition. % cell inhibition = $100 - \frac{(At - Ab)}{(Ac - Ab)} \times 100$. Where, At = Absorbance value of test compound Ab = Absorbance value of blank Ac = Absorbance value of control.

Results and Discussion

Table 1. Phytochemical Screening Test Results of rosella flower ethanol extract

Secondary metabolic compound	Reactor	Result
Flavonoids	5 ml Hydrochloric acid 2n + 3 drops dragendrof *brown-black precipitate)	+
Alkoloids	10 ml hot water+shake+hydrochloric acid 2N (form 1- 10 cm foam)	+
Saponins	20 ml hot water + 3 drops of 10% NaCl + FeCl ₃ (formed black blue or greenish-black)	+
Tannins	10 ml hot aqua dest + 3 drops FeCl ₃ (a bluish-green color is formed)	+

Table 2. Activity Results of Rosella flower (*Hibiscus Sabdariffa* L.) ethanol extract on HepG2 cells growth inhibition.

Concentrations (µg/ml)	% Inhibition	IC ₅₀ (µg/ml)
12.5	0.286	629.919
25	2.89	
50	2.68	
100	2.85	
200	16.63	
250	15.55	
500	32.05	
1000	84.75	

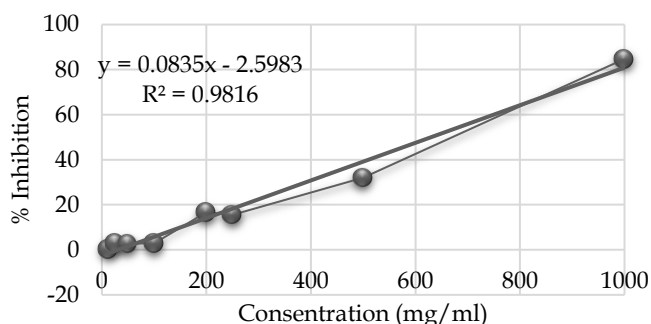


Figure 1. Relationship chart activity Results of Rosella flower (*Hibiscus Sabdariffa* L.) ethanol extract on HepG2 cells growth inhibition.

Discussion

Cancer is a collection of improperly growing cells. Many cancer treatments, such as chemotherapy, are currently extremely effective for both non-metastatic and metastatic cancer (Wang et al., 2022). However, the side effects are extremely severe because chemotherapy also attacks the body's natural cells. Herbal treatments are typically well tolerated by breast cancer patients. Many women with breast cancer use complementary treatment cycle, where popular of usage of herbal medicine, where it would reduce side effects of therapy and enhance quality of life. As a result, various studies are being conducted to develop a treatment that can lessen these adverse effects, such as replacing chemotherapy medications with anticancer substances derived from natural plants. *H. sabdariffa* has a lot of polyphenols (Bassong et al., 2022).

Roselle or karkade and is a member of the *malvaceae* family of tropical plants endemic to Southeast Asia (Sáyago-Ayerdi et al., 2007). It is a herbaceous plant that grows in subtropical mountain. It's fleshy, bright red calyces contain significant levels of l-ascorbic, arachidic, citric, stearic, and malic acids, as well as pectins, phytosterols (e.g., -sitosterol and ergosterol), and polyphenols (Malacrida et al., 2022). The antioxidant impact of rossela, which demonstrated effect (particularly cytotoxic and genotoxic) of the various soluble fractions of rossela by free radical elimination and suppression of unscheduled DNA synthesis (Ilyas et al., 2021). Anthocyanins have the potential to be employed as chemopreventive treatment. Doses can be met with a conventional diet and no supplements (Fithrotunnisa et al., 2020).

Anthocyanins found in floral extracts have been shown in vitro to reduce LDL oxidation and macrophage mortality. Recent evidence suggests that LDL oxidation plays a role on aetiology of atherosclerosis, however the in vivo influence of diet should be evaluated. HS extract inhibited lipid formation in the cytoplasm, especially at dosages of 2 mg/mL. *H. sabdariffa* prevented adipogenic transformation, morphology by reduction of lipid droplets within cells during adipogenesis. Rossela inhibit the MAPK pathway by blocking it, modulation of transcription factors MAPK-mediated signalling pathways during the development of adipocytes, Rossela extract where regulation includes inhibition (Kim et al., 2007). Adipogenic transcription expression C/EBP and PPAR-gamma transcription factors MPAK and PI3-K pathways. Flowers that have dried Ethanol extraction of *H. sabdariffa* has an antioxidant effect, where Rat hepatocytes 0.05 mg/mL PCA 0.10 mg/mL PCA 30 min treatment with t-BHP (1.5 mM), where of mechanism have Hepatocytes treated with t-BHP are protected against cytotoxicity and genotoxicity.

Rossela has the ability to absorb free radicals. In vitro studies have demonstrated that this plant has anticancer properties against gastric cancer (Da-Costa-Rocha et al., 2014; Nova et al., 2021), hepatocellular cancer, and leukaemia. Hibiscus protocatechuic acid has also been demonstrated to reduce the carcinogenic effect of several substances in rat tissues such as the liver, oral cavity, colon, glandular stomach tissue, bladder, and skin. The plant extract has a very low toxicity. In rats, the LD₅₀ of extract was determined to be greater than 5000 mg/kg. Phytochemical screening is performed with the addition of reagents aimed at identifying the secondary metabolites contained in a natural substance qualitatively (Table 1).

Phytochemical screening is an early step to explore the chemical content of a natural ingredient to find a new drug based on the results of the qualitative tests contained in the extract of Rosella flower. From the results of the research obtained results, where the secondary metabolite compounds of rosella flowers have flavonoids, alkaloids, saponins and tannins. Flavonoids' anticancer effects on blood cancer cells are dependent on whether they are myeloid, lymphoid, or erythroid; flavonoids' cytotoxic effects on breast and prostate cancer cells are largely tied to hormone receptor expression. There are various flavonoids and typically preferentially contained in certain food items, and understanding of the anticancer effects of on malignant tissue.

Flavonoids have the potential to be used in both cancer prevention and treatment (Riaz & Chopra, 2018). The cytotoxicity test used the MTT assay method. The IC₅₀ value of anticancer can be divided into four categories, where IC₅₀ < 20 g/mL is considered; where IC₅₀ 20-100 g/mL is considered moderately and IC₅₀ 100-1000 g/mL is considered slightly. Rosella as a potential sources of anticancer medicines. Rossela is a common medicinal herb used to treat a variety of diseases, including cancer. Polyphenols, flavonoid, and anthocyanin chemicals with oxidising activity were found in plant extracts, which have recently received interest as chemopreventive agents (Kao et al., 2009; Malacrida et al., 2022). Rossela demonstrated a wide spectrum of anti-cancer action, including selective cytotoxicity, apoptosis induction, and inhibition of metastasis and angiogenesis. Stress and an increase in cellular reactive oxygen species (ROS) levels may promote apoptosis triggered by Rossela extract. Polyphenols have antioxidant characteristics and are thought to play an essential role in cancer prevention (Anggi & Masyita, 2022). Flavonoids were discovered in one of the polyphenol compounds found in Rossela. Rossela have polyphenols were found to reduce the expression of Proliferating cell nuclear antigen, which is greatly higher in metastatic cancers and so works as a

marker of proliferation (Lin et al., 2005). Rossela inhibited connective tissue growth factor and downstream extracellular matrix (ECM) proteins protein accumulation while increasing tissue inhibitor of metalloproteinase-2 (TIMP-2) expression in proliferating vascular smooth muscle cells and colon cancer cells.

Conclusion

This study reveals that activity Results of Rosella flower ethanol extract have metabolite compounds of rosella flowers as a flavonoids, alkaloids, saponins and tannins and have activity on HepG2 cells growth inhibition had considered moderately active anticancer activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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