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Full Length Article

Bioactive Content and Anticancer Bioactivity of MCF-7 Cell in Smadan Root Extract from Bitung City Forest North Sulawesi

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Abstract

Cancer is a disease with the second largest death rate in the world. Cancer Prevalence has continued to increase in the last decades. The research was conducted from May to August 2023. The aim of this research was to determine the bioactive content and anticancer bioactivity of michigan cancer foundation-7 (MCF-7) in Smadan root extract from the Bitung city forest, North Sulawesi. The bioactive content was analyzed using the high performance liquid chromatography (HPLC) method, total flavonoids were determined using ultraviolet and visible light spectrophotometry, while the anticancer activity was tested *in vitro* on MCF-7 cell cancer (breast cancer cells). The results of the high performance liquid chromatography analysis research showed that 26 compounds were at a wavelength of 254 nanometer, while 37 compounds were at a wavelength of 310 nanometer. The total flavonoid content of dry Smadan root extract was 62.66 mg QE/g, while the wet extract was 14 mg QE/g and the cytotoxic activity of Smadan root extract had IC_{50} = 50.12 µg/mL. These results indicate that Smadan root extract has strong cytotoxicity. Thus, the ethanol extract of Smadan roots has the potential to be developed as a source bioactives and drug of anticancer that can help people to cure cancer. © 2024 Friends Science Publishers

Keywords: Anticancer; Bioactive content; MCF-7; Smadan root

Introduction

Indonesia is a megabiodiversity country. There are various endemic species of flora and fauna. Indonesia is also known as a country that has an ethnomedical and ethnobotanical culture (Kaunang and Mokosuli 2017; Rahmawaty *et al*. 2019). The use of plants and animals as bioactive sources for the treatment of various types of diseases is carried out by many tribes in Indonesia and is passed down from generation to generation (Mokosuli *et al*. 2019). Indonesia has an abundance of medicinal plants (Ansori *et al*. 2021). Medicinal plants originating from forests have a very important role globally as well for society (Kaunang and Mokosuli 2017; Rahmawaty *et al.* 2019). Medicinal plants can lead to the discovery of new drugs that have the potential to treat diseases that are considered uncurable (Ansori *et al*. 2021; Roy *et al*. 2022).

Cancer is a chronic disease with the second highest prevalence of death in the world (Rakhmanovna 2022). World Health Organization (WHO) states that cancer is one of the main cause of death throughout the world. Around 8.2 million people die from cancer (Joya *et al*. 2020). Based on the number of cases and deaths due to cancer until 2018, 18.1 million cases and 9.6 million deaths in 2018 are data from the Global Burden of Cancer released by WHO (Pangribowo 2019). Breast cancer is the most common cancer in women (Rabiee *et al.* 2023). The incidence rate is still increasing in Asia (Choi *et al*. 2023). Breast cancer is a serious disease faced by the world and Indonesia (Jumaryatno *et al*. 2022; Shaluhiyah and Surjoputro 2023). Breast cancer is one of the types of cancer that has the highest prevalence in the world (Coelho *et al*. 2023; Hasnita and Meiriza 2023; Kurniawati 2023). Based on data from the World Health Organization in 2020, 2.1 million women were diagnosed with breast cancer and this cancer cannot be underestimated (Qodria and Nurrachma 2020).

Cancer treatment also currently has major obstacles because sideeffects have a big impact on cancer patients, so that treatment based on ethnomedical culture is one of the treatments preferred by cancer patients, specifically medicinal plants that have the potential to be anticancer or cytotoxic to cancer cells. In developing countries, plants from nature are used as natural sources to maintain public health. Traditional medicine is now used as alternative medicine because it is considered safer and has fewer side effects (Wahid and Raudah 2022). The bioactive content in plants has antioxidant properties that can prevent cancer. Bioactive compounds can prevent cells from mutating into cancer cells (Nurtiana and Budijanto 2017). Available therapies for cancer treatment have major hurdles (Jagdale

et al. 2023). Currently the treatment that cancer patients can undergo is chemotherapy, radiology, immunotherapy and in general surgery, however, this treatment can have side effects on cancer patients, namely hair loss, neurological disorders, bone marrow suppression and so on (Zein and Hazar 2022), so there is a need for more treatment strategies and drugs for the survival and quality of life of cancer patients (Malacrida *et al*. 2023; Li *et al*. 2023). For this reason, the use of natural ingredients will be beneficial (Roy *et al*. 2022), namely plants that have the potential to kill/suppress cancer cells.

Bitung City has medicinal plants that the community uses as traditional medicine, namely those known as Smadan roots. When the Smadan root is cut first, it will produce clear water. It is used by the agraris community of Bitung City as a substitute for drinking water and is believed to have the property of increasing the body's immune system. Smadan root decoction water is dark red. Local people generally consume water boiled from Smadan roots to keep the body healthy and to cure various diseases. Smadan roots originating from the city of Bitung have been used by the community as an anticancer and antitumor medicine, but have never been studied scientifically.

Smadan roots have some similarities with Bajakah in Kalimantan or in Latin *Spatholobus littoralis* Hassk (Sianipar *et al*. 2023) but are different. Based on the shape of the leaves and stems, are very different from Bajakah, but the shape of the roots has several similarities. It is found widely in the forests of Bitung city, and also in several places in the North Sulawesi region. However, with the acceleration of development and population growth, many forest areas that was home of Smadan's roots have changed due to plantations and/or housing areas, so their sustainability needs to be maintained. It is hoped that the research carried out can have a positive impact on the community in protecting forests and not over-exploiting medicinal plants. Therefore, the aim of this study was to determine the bioactive content and anticancer activity of MCF-7 in smadan root extract from the Bitung city forest, North Sulawesi.

Materials and Methods

Sample

Smadan root samples were taken in the forest of Apela Dua urban village, Ranowulu sub-district, Bitung city, North Sulawesi (Fig. 1).

Characteristics of Smadan roots: Smadan roots have brown and rough outer root skin, light green stems that grow from creeping roots. It has small leaves; approximately 1 cm. Smadan roots grow in damp forests and live on trees with their central roots embedded in the ground. Smadan roots can grow very long, up to approximately 50 m (Fig. 2).

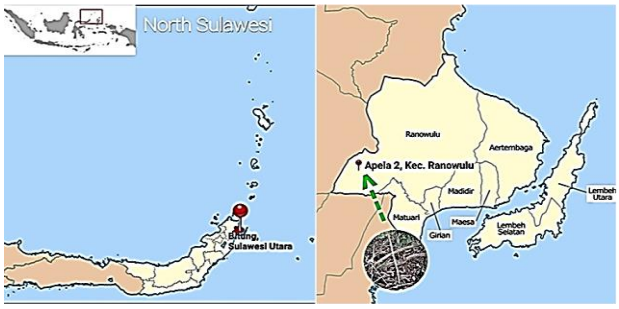

Fig. 1: Smadan root sampling location in Apela Dua urban village, Ranowulu district, Bitung city, North Sulawesi

Fig. 2: Roots, stem and leaves of Smadan from Apela Dua sub-district, Ranowulu sub-district, Bitung city, North Sulawesi (Personal Documentation)

Research procedure

This research was carried out in several stages: Making simplicia and extraction, testing the bioactive content using the HPLC method, testing the total flavonoid content using UV-Vis Spectrophotometry and testing the anticancer cytotoxic activity of MCF-7. This research was conducted from May to August 2023. Extraction and testing of total flavonoid content of Smadan root was carried out at the Biology Laboratory, Manado State University and the cytotoxic test on MCF-7 cancer cells as well as the bioactive content test using the HPLC method was carried out at the Central Laboratory of Padjadjaran University.

Making simplicia and extraction

Smadan root samples (plants aged ± 30 years) were taken by cutting the hanging roots (not the core roots) as samples. Smadan roots were taken from tropical forests in summer with environmental conditions temperature, 26–27°C, humidity 83%, and wind 3,5 km/h (BMKG 2023). Then the Smadan root samples were cleaned first, then finely cut into dry samples and wet samples. The dry samples are oven-treated for 60 min. The wet samples are not oven dried.

Both samples were blended separately until smooth. When the samples were smooth, the two samples were weighed at 100 g each and put into different jars to enter the maceration stage and 400 mL of 95% ethanol was added to both. Ratio 1:4 (Semuel *et al*. 2019) was used to macerate for 3 days. Samples that had been macerated for 3 days were filtered using filter paper. After that, it goes to the evaporation stage (changing the solvent into steam) to get a thick extract from Smadan root extract.

Bioactive content testing HPLC method

The use of the HPLC (High Performance Liquid Chromatography) method on Smadan root extract (dry samples) which was tested at the Central Laboratory of Padjadjaran University to analyze its bioactive content. The working principle of HPLC is to separate analyte components based on their cappolarity and any mixture that comes out will be detected with an existing detector and recorded in the form of a chromatogram. The steps involved weighing 100 mL of the Smadan root ethanol extract sample, then dissolving it with 2 mL of and 8 mL of methanol, then sonicating it for 10 min. After that, filtered with a 0.45 μm millipore and put 1 mL into HPLC vial (Semuel *et al*. 2019; Farida *et al*. 2020).

Flavonoid content testing using UV-Vis spectrophotometry

Determination of the maximum wavelength of quarcetin: To determine the maximum wavelength of kearcetin, the quercetin solution was analyzed in the wavelength range of 400–500 nm (Rogahang *et al*. 2023). The analysis results show that the maximum wavelength of the quercetin standard is 435 nm, which is used to measure the absorbance of the ethanol extract sample.

Making of a quercetin standard curve: In making standard curve, 25 mg of standard quercetin was used and dissolved in 25 mL of ethanol. The stock solution was pipetted at 1 mL and then added 10 mL of ethanol to reach a concentration of 100 mg. kg^{-1}. Using a standard 100 mg. kg^{-1} quercetin solution, several concentrations of quercetin were prepared: 6, 8, 10, 12 and 14 mg. kg^{-1}. To each of these concentrations, 1 mL of 2% $AICI_3$ was added and 1 mL of potassium acetate of 120 m*M*. Samples were incubated for 60 min at room temperature. The UV-Vis spectrophotometry method with a maximum wavelength of 435 nm was used to measure absorbance (Rogahang *et al*. 2023).

Measurement of total flavonoid levels: Total flavonoid determined by taking 15 mg of Smadan root extract dissolved in 10 mL of ethanol to reach a concentration of 1500 mg. kg^{-1}. Pipette 1 mL of solution then add 1 mL of 2% $AICI_3$ solution and 120 m*M* potassium acetate. Samples were incubated for 60 min at room temperature. The UV-Vis spectrophotometry method with a maximum wavelength of 435 nm was used to measure absorbance (Rogahang *et al*. 2023).

Cytotoxic (anticancer) testing

Smadan root samples that had been evaporated were tested for cytotoxic activity (dry samples) on MCF-7 cancer cells. The equipment used in cytotoxic testing: Biosafety cabinet (BSC) (Thermo scientific 1300 series a2), CO_2 incubator (Thermo scientific 8000DH series), Centrifuge (Thermo scientific micro CL17), Multimode reader (Tecan Infinite M200 PRO), Microscope (Thermo scientific EVOS XL Core). Prepared anti-proliferation assay working solution. The working solution to be used is Presto Blue™ cell viability reagent (Gusungi *et al.* 2020).

Cell preparation: The cells to be used are at least 70% confluent, then discard the media on a dish, then rinse the cells twice with 1 mL of PBS, add 1 mL of Trypsin-EDTA solution then incubate for 5 min so is dispersed (under an inverted microscope the cells will appear to float) transferred the cells into a tube containing media, centrifuged the cells at a speed of 3000 rpm for 5 min and discarded the supernatant, then the pellet was dissolved into a tube containing the media (Gusungi *et al.* 2020).

Seeding cells into 96 well plate: Determining the number and viability of cells by trypan blue exclusion and resuspend cells with a final cell density of 170,000 cells/mL in media (17,000 cells/well) where 10 μL of trypan blue was prepared in a sterile microtube, 10 μL of cell suspension was added to the trypan blue solution and then homogenized and clean the hemacytometer. Cover the slip using 70% ethanol then dry, slowly insert 10 μL of trypan blue cell solution into one side of the chamber using a pipette, count the number of healthy cells and determine the number of (viable) cells per mL. Seeding/culture of cells into 96 well plates is then incubated for 24 h or until the cells are at least 70% confluent at a temperature of 37°C and 5% CO_2 gas (Gusungi *et al.* 2020).

Treatment of cells with sample/positive control/negative control: Prepared 8 microtubes 1.5 mL and each microtube is labeled with the appropriate dilution concentration, then the stock sample is diluted into 8 concentration variants using media solvent, 96 well plates containing cells are removed from the incubator. Labeled on the plate along the left margin are which rows will be treated by the standard and which rows will be sample. Then remove the media from each well. Using a micropipette, transfer 100 µL of each sample and positive control from the microtube into each appropriate well on a 96 well plate containing cells, then incubate again for 48 h (Gusungi *et al.* 2020).

Administration of presto blue reagent and measurement of absorbance: At this stage, discard the media in each well, then prepare 9 mL of media in a tube to which 1 mL of "Presto Blue™ cell viability reagent" is added (10 μL of reagent for 90 μL of media). Put 100 μL of the solution mixture into each well of the microplate and then incubate for 1–2 h until a color change is visible (When entering living cells, the presto blue reagent will be reduced from the blue compound resazurin with no intrinsic fluorescent value, to the red and highly colored resorufin compound fluorescent).

The conversion value is proportional to the number of metabolically active cells and can be measured quantitatively (to measure absorbance, the absorbance spectrum for resazurin and resorufin is used), then the absorbance is measured at a wavelength of 570 nm (reference: 600 nm) using a multimode reader (Gusungi *et al.* 2020).

Data analysis

HPLC data analysis was carried out descriptively. Data analysis of total flavonoids was carried out using linear regression equations, anticancer content analysis was analyzed descriptively based on the IC_{50} value and the results obtained.

Results

Sample extraction

The extraction method used in this research is the maceration method. Sample that had been macerated for 3 days was dark red in color and had a fragrant smell like wood mixed with the smell of ethanol. The type of solvent used during maceration, namely 95% ethanol and a solvent volume of 400 mL, the weight of the sample used is 100 g. The final extract weight obtained from solvent evaporation was 4.75 g for the dry Smadan root sample and the wet Smadan root sample was 4.61 g. The soaking yield of Smadan root extract for dry samples was 4.75% and for wet samples 4.61% (Table 1). Using the formula% Soaking = Extract weight/ sample weight x 100%. Thus, the dry Smadan root extract was higher than the wet Smadan root extract in the yield results carried out.

Testing bioactive content HPLC method

In the test results, 26 active compounds in Smadan root extract were detected at a wavelength of 310 nm with a retention time (RT) of 30 min (Fig. 3). Table 2 clearly portrays retention time, area, % area and height of the 27 bioactive compounds detected and active compounds with a very high percentage, *i.e.*, 13,118 detected at a retention time of 2.853 min, Area 95,261 and area 17.29%.

At a wavelength of 254 nm, 30 active compounds were detected with a retention time (RT) of 30 min (Fig. 4). In (Table 3) the results of retention time, area, % area and height for 30 bioactive compounds are found. Active compounds with a very high percentage, *i.e.*, 20.1531, were detected at a retention time of 2.861 min, area 1,543,462 and area 31.26%.

Analysis of total flavonoid compound content of smadan root extract

The quercetin standards with concentrations of 6, 8, 10, 12 and 14 mg. kg^{-1} with the absorbance values obtained for

Table 1: Results of soaking Smadan root extract

Sample	Type of solvent	Solvent volume	Sample weight	Extract weight	Rendemen
Dry extract	95% ethanol	400 mL	100 g	4.75 g	4.75%
Wet extract	95% ethanol	400 mL	100 g	4.61 g	4.61%

Table 2: HPLC results of Smadan root extract, at a wavelength 310 nm

No.	RT	Area	Area (%)	Height
1	2.269	3582	0.65	283
2	2.444	5907	1.07	555
3	2.853	95261	17.29	13118
4	3.707	6585	1.20	836
5	4.358	2163	0.39	267
6	5.913	3749	0.68	305
7	6.844	8354	1.52	1048
8	7.071	58261	10.57	3112
9	9.859	16457	2.99	1712
10	10.941	1560	0.28	210
11	11.743	1478	0.27	201
12	12.301	32753	5.94	3091
13	14.079	2726	0.49	300
14	14.325	3222	0.58	361
15	15.720	10282	1.87	891
16	16.628	27039	4.91	2689
17	16.815	16028	2.91	1460
18	17.730	2565	0.47	296
19	18.882	41883	7.60	3196
20	20.385	33082	6.00	3150
21	21.134	21494	3.90	1959
22	21.614	2845	0.52	323
23	21.877	57213	10.38	4881
24	22.606	43974	7.98	3000
25	23.585	17240	3.13	1734
26	28.600	35253	6.40	719

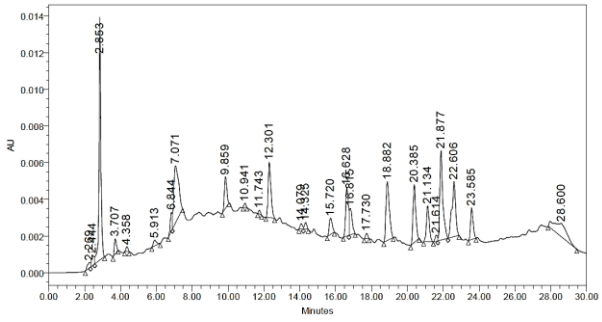

Fig. 3: Retention time of 310 nm wavelength extract

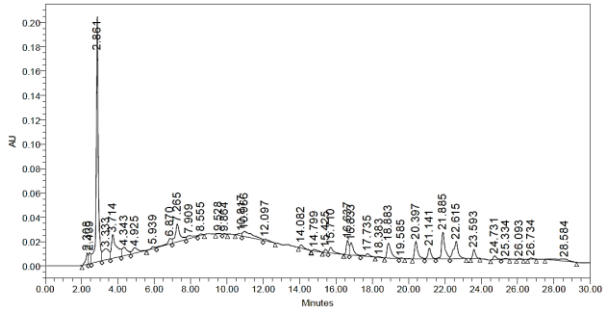

Fig. 4: Retention time of 254 nm wavelength extract

Table 3: HPLC results of Smadan root extract, wavelength 254 nm

No.	RT	Area	% Area	Height
1.	2.308	82636	1.67	9460
2.	2.439	81741	1.66	9161
3.	2.861	1543462	31.26	201531
4.	3.333	223011	4.52	9162
5.	3.714	366637	7.43	19452
6.	4.343	143739	2.91	6832
7.	4.925	97735	1.98	4509
8.	5.939	20625	0.42	1616
9.	6.870	73304	1.48	4515
10.	7.265	259136	5.25	14674
11.	7.909	56673	1.15	2382
12.	8.555	14900	0.30	1320
13.	9.528	6394	0.13	521
14.	9.864	4605	0.09	490
15.	10.817	28561	0.58	2378
16.	10.966	182283	3.69	4328
17.	12.097	29041	0.59	1533
18.	14.082	36996	0.75	2672
19.	14.799	24404	0.49	1349
20.	15.425	26713	0.54	2682
21.	15.710	75659	1.53	4816
22.	16.637	110266	2.23	11789
23.	16.833	148260	3.00	10205
24.	17.735	44089	0.89	2418
25.	18.383	9220	0.19	579
26.	18.883	188503	3.82	12225
27.	19.585	5339	0.11	527
28.	20.397	180532	3.66	14381
29.	21.141	106416	2.16	8891
30.	21.885	283545	5.74	21908
31.	22.615	243384	4.93	14031
32.	23.593	73411	1.49	7427
33.	24.731	38394	0.78	3065
34.	25.334	9897	0.20	737
35.	26.093	8339	0.17	836
36.	26.734	13301	0.27	1081
37.	28.584	95633	1.94	1788

each concentration (mg. kg^{-1}). This value shows that the higher the concentration of the solution used, the higher the absorbance value obtained (Table 4). The quercetin standard results obtained are plotted between the levels and absorbance to obtain a linear regression equation, namely y = 0.014x + 0.498 with a value of R^2 = 0.924. The quercetin calibration curve equation can be used as a comparison to determine the concentration of flavonoid compounds in the total extract of Smadan root samples (Fig. 5). The calculation results show that dry Smadan root extract (dry extract) has a total flavonoid content of 62.66 mg QE/g extract and the total flavonoid content of wet Smadan root extract (wet extract) is 14 mg QE/g extract (Table 5).

MCF-7 anticancer cytotoxicity activity assay

The curve of the results of testing Smadan root extract on MCF-7 cells shows IC_{50} value = 50.12 µg/mL, which means that Smadan root extract has strong cytotoxic properties in killing MCF-7 cancer cells or breast cancer cells (Fig. 6). The average viable cancer cells were = 104.45% and the

Table 4: Quercetin absorbance

Concentration ($mg.kg^{-1}$)	Absorbance (y)
6	0.580
8	0.608
10	0.663
12	0.682
14	0.689

Table 5: Total flavonoid content of Smadan root extract

Sample	Absorbance (y)	Total flavonoid content (mg QE/g)
Dry extract	1.895	62.66
Wet extract	0.882	14.00

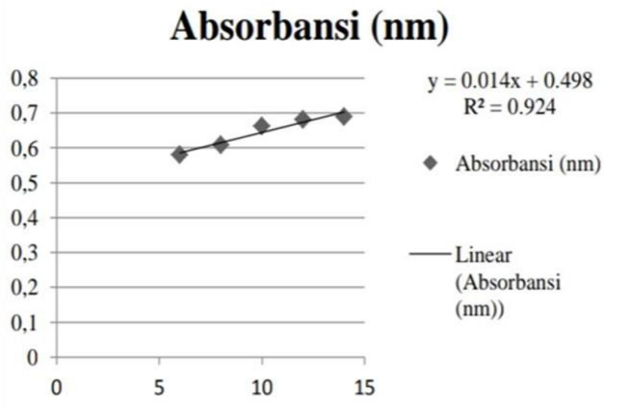

Fig. 5: Quercetin absorbance curve

average viable MCF-7 cancer cells after being given Smadan root extract at a sample concentration of 7.81 µg/mL = 104.73%, 15.63 µg/mL = 99.80%, 31.25 µg/mL= 61.06%, 62.50 µg/mL= 33.17%, 125.00 µg/mL = 31.24%, 250.00 µg/mL = 29.33%, 500.00 µg/mL = 5.43%, 1000.00 µg/mL = 0.66%. Based on these results, Smadan root extract with higher concentration can potentially kill cancer cells or had stronger cytotoxicity effect on cancer cells (Fig. 7). A concentration of 1000.00 µg/mL had the strongest cytotoxicity activity (Table 6). (Note: The cisplatin concentration used in the assay was 53 *µM*).

Discussion

The bioactive content and anticancer activity test of MCF-7 Smadan root extract from Bitung city forest, North Sulawesi was carried out extracting Smadan root samples. The extraction stage aims to extract chemical components or secondary metabolites contained in the Smadan root samples. Extraction is a method used to extract compounds from plants using certain solvents (Senewe *et al*. 2023). Factors that influence the extraction process include the extraction method, type of solvent, particle size, and duration of extraction time (Putri *et al*. 2021). The extraction process has 2 stages, namely sample maceration and solvent

Table 6: Absorbance of extract Smadan root results on MCF-7 cells

	Media	Media+ Cell	Ciplatin	Solvent	Sample concentration (μg/mL)							
					7.81	15.63	31.25	62.50	125.00	250.00	500.00	1000.00
Absorbance 570 nm	0.4786	0.7932	0.5217	0.7813	0.8204	0.7947	0.6685	0.6136	0.6015	0.6004	0.5138	0.5209
	0.4889	0.8024	0.5303	0.7827	0.8090	0.8027	0.7135	0.6117	0.6045	0.5973	0.5330	0.5338
Absorbance 600 nm	0.6127	0.2080	0.5923	0.2228	0.2226	0.2403	0.4199	0.5130	0.5214	0.5427	0.6147	0.6518
	0.6261	0.2087	0.6035	0.2241	0.2240	0.2428	0.3856	0.5231	0.5222	0.5191	0.6280	0.6650
Difference absorbance	-0.1341	0.5852	-0.0706	0.5585	0.5978	0.5544	0.2486	0.1006	0.0801	0.0577	-0.1009	-0.1309
	-0.1372	0.5937	-0.0732	0.5586	0.5850	0.5599	0.3279	0.0886	0.0823	0.0782	-0.0950	-0.1312
% living cells		103.84	9.37	99.99	105.65	99.40	55.35	34.03	31.08	27.85	5.01	0.68
		105.06	9.00	100.01	103.81	100.19	66.77	32.30	31.40	30.81	5.86	0.64
Average living cell		104.45	9.18	100.00	104.73	99.80	61.06	33.17	31.24	29.33	5.43	0.66
SEM		0.61	0.19	0.01	0.92	0.40	5.71	0.86	0.16	1.48	0.42	0.02
Data normalization												
Living cells (%)		104.45	9.18	100.00	104.73	99.80	61.06	33.17	31.24	29.33	5.43	0.66

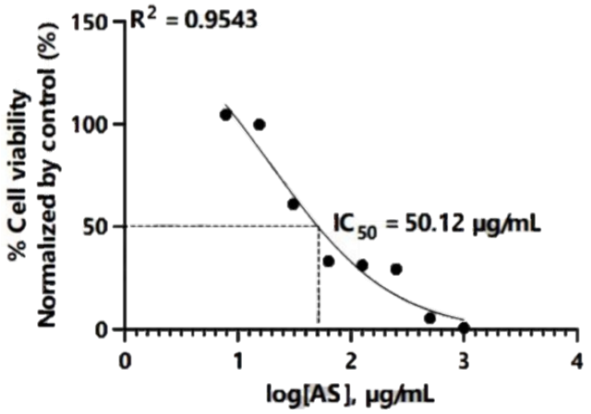

Fig. 6: Smadan test results curve for MCF-7 cells

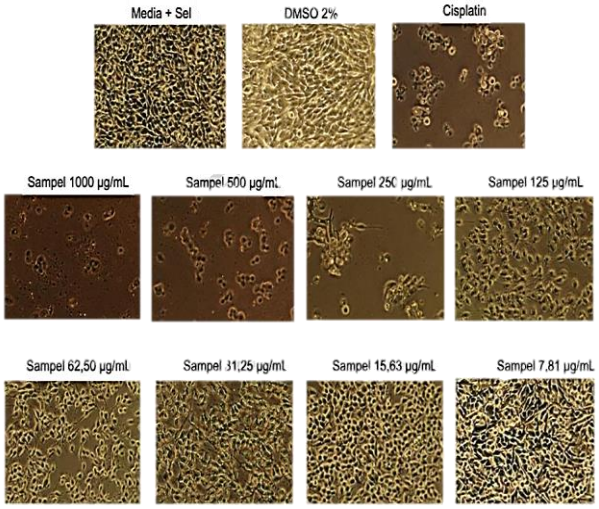

Fig 7: Documentation of MCF-7 cell morphology from extract Smadan root test results

evaporation. The maceration process is carried out by soaking the crushed Smadan root powder into a jar and then adding 95% ethanol. Based on previous research, ethanol solvent was used as an extraction solvent because ethanol has selective properties and is able to extract compounds contained in the sample (Chen *et al*. 2020). So in this study the researchers used ethanol solvent for use in the sample maceration process. In the maceration process, Smadan roots were soaked in a jar for 3 days at room temperature, because based on previous research the maceration process was carried out at room temperature to protect the bioactive content which is not heat resistant from being damaged (Nur *et al*. 2020). Maceration method was chosen because it can avoid the destruction of thermolabile compounds, which may have very important antioxidants (Setyawardhani and Saputri 2020). Apart from that, the advantage of the maceration method is that the procedure and equipment are simple and affordable. After maceration, the sample is filtered using filter paper to enter the solvent evaporation process. Evaporation of the solvent uses a rotary evaporator to convert the solvent into steam and the active compound content of the Smadan root extract (thick extract) remains. The thick extract resulting from evaporation of the solvent shows the distinctive aroma of the Smadan root plant, namely the smell of wood mixed with the smell of ethanol. Then the thick extract of Smadan roots (dry sample) was tested for bioactive content.

In testing the bioactive content of Smadan root extract using the HPLC method, 26 and 37 active compounds were detected in Smadan root extract. The use of the HPLC method shows good compound separation. This method is suitable for determining the content of active compounds (Seal 2016). Active compounds are very important as antioxidants (Gazali *et al*. 2018). Antioxidants prevent oxidation of compounds which can cause cancer (abnormal cells). The main cause of cell damage is the formation of ROS or Reactive oxygen species. Antioxidant compounds can control ROS (Andarina and Djauhari 2017). Antioxidants function as a defense system against free radicals (Aditya and Ariyanti 2016). Antioxidants outside the body can be obtained in synthetic and natural form (Aditya and Ariyanti 2016). The active compounds (antioxidants) found from the results of this research are very important in helping prevent cancer. In previous research, the active compounds obtained could prevent cancer (Ali *et al*. 2022;

Putra *et al*. 2023; Permatasari *et al*. 2023).

In the total flavonoid content test using UV-VIS spectrophotometry, the total flavonoid content of dry Smadan root extract was 62.66 mg QE/g extract and wet Smadan root extract was 14 mg QE/g extract. When compared with dry Smadan root extract, the flavonoid content is greater than wet Smadan root extract. In the results of research (Dalming 2021) to determine flavonoid levels, it was found that dry guava leaf extract was higher than wet guava leaf extract. However, in research (Ristanti 2019), the total flavonoid content of wet binahong leaves was higher than dry binahong leaves. This is because wet binahong leaves do not undergo a drying process. Flavonoid compounds can be reduced due to the drying process. However, if the drying process is carried out correctly you will definitely get good results (Santoso and Egra 2018). Flavonoids are natural phenolic compounds found in almost all plants in the flowers, roots, skin, leaves and even seeds. Flavonoids play an important role as medicine because they have antioxidant, antibacterial, antiviral and anticancer activities (Fahira 2023).

The cytotoxic test results of Smadan root extract against MCF-7 breast cancer cells obtained an IC_{50} value = 50.12 μg/mL, which means that Smadan root extract has strong cytotoxic properties to prevent and kill MCF-7 breast cancer cells. A very strong cytotoxicity test has an IC_{50} of less than 10 μg/mL, strong cytotoxicity has an IC_{50} value between 10-100 μg/mL and moderate cytotoxicity has an IC_{50} between 100–500 μg/mL (Tunjung and Sayekti 2019). Based on previous research, Bajakah from Kalimantan tested 4T1 breast cancer cells and found the IC_{50} value for the hexane fraction was 20.0 mcg/mL and the ethyl acetate fraction was 7.4 mcg/mL. Based on these results, it means that Bajakah has strong cytotoxicity and is very strong in killing 4T1 cancer cells (Iskandar *et al*. 2022). However, Bajakah research conducted by Yuniarti *et al.* 2021 showed weak anticancer activity against MCF-7 breast cancer (Yuniarti *et al*. 2021). In this study, Smadan roots were proven to be strong in killing MCF-7 cancer cells. MCF-7 cancer cells are cancer cells taken from the breast tissue of a 69 year old woman (Chusniasih and Tutik 2020), while 4T1 breast cancer cells are a cancer cell line originating from tissue in the mammary glands of the BAlB/c mouse strain (Schrors *et al*. 2020).

Conclusion

The Smadan root extract has the potential to prevent and kill MCF-7 breast cancer cells and can be developed as a cancer drug because it has been proven to be strong. Further research is needed to identify the DNA and side effects of Smadan root.

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Author Contributions

AGK and MYS were involved planning the research and AGK performed the data acquisition/collection. AGK, RM and MYS aided in interpreting the results. AGK performed data analysis. MYS, RM revised the manuscript. All authors were involved in this research.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this research will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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