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Identification and Anticancer Evaluation of Paliasa Leaves (Kleinhovia hospital Linn) Extracts Obtained by Subcritical Extraction

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ABSTRACT

Many plants have been used as co-therapy for cancer drugs to minimize the side effects of existing cancer drugs. Cancer drug research is needed to test the potential properties of plants as cancer drugs. Currently, the use of drugs from natural ingredients has begun to develop. However, it is necessary to have a method of extracting raw materials from nature that is practical, cheap, and high-quality. One method that can be used is the extraction of natural materials by subtraction. This research aimed to see how successful this subcritical extraction method is on Paliasa leaves (Kleinhovia hospital Linn). Secondary metabolites extraction, fractionation, and subcritical extraction were then tested on cancer cell lines and determined metabolite profiling using GC/MS and LC/MS/MS. The fractionation process obtained a yield of 33.6%, while the extraction with ethanol yielded 8.84%, and the subcritical extraction yield value was 0.18%. Based on the GC/MS test, the compounds that are predicted to have anti-cancer properties are Isorhamnetin and Carbamic acid derivates group. Meanwhile, from the results of the LSMS-MS test, the 7-Hydroxy-5-methoxycoumarin compound is a coumarin compound derivative and Biatractylolide that has the potential to be an anti-cancer. Subcritical extract of Paliasa leaves has the best IC_{50} results among the three kinds of extracts tested by MTT assay on MCF-7 cells.

Keywords: Paliasa leaves, identification, anticancer, subcritical extraction, MTT assay

I. INTRODUCTION

Cancer is a disease whose morbidity and mortality rates continue to increase from time to time and become a severe health problem. Cancer can cause a decrease in the patient's quality of life, productivity, and economic status [1][2]. The Indonesian Ministry of Health calls cancer a catastrophic disease. This term represents the nature of the disease, which is equivalent to disaster or calamity. Data from the Ministry of Health of the Republic of Indonesia also shows that cancer patients need to pay more than other diseases for therapy, mainly if the treatment is carried out at an advanced stage of the disease. This has caused many policymakers to worry about increasing the disease cost so that the implementation of health insurance does not include this disease in the benefits package [2].

Surgery, chemotherapy, and radiotherapy are considered the most common methods of treating cancer, but the side effects are enormous. The side effects of chemotherapy vary depending on the chemotherapy regimen, including nausea, vomiting, diarrhoea, stomatitis, alopecia, susceptibility to infection, thrombocytopenia, neuropathy, and myalgia [3][4]. A common strategy in cancer treatment is to inhibit cell growth and kill cancer cells. Anticancer must kill cancer cells with minimal side effects on normal cells, possibly through induction of apoptosis. Several natural compounds, including plants, induce blocked apoptotic pathways through various mechanisms in cancer cells by interfering with the function of microtubules which halts the cell cycle and causes cell death through the induction of apoptosis [5]. Natural antioxidants have also been introduced as adjuvant anticancer therapy because of their antiproliferative and proapoptotic properties such as curcumin, berberine, and quercetin [6][7]. The FDA has approved herbal compounds derived from vinca alkaloids to treat several neoplasms. Vinblastine is widely used to treat breast cancer, Hodgkin's Lymphoma (ABVD), and Kaposi's sarcoma. Vincristine is given to treat non-Hodgkin leukemia, Hodgkin leukemia, and tumours. These drugs, such as vinca alkaloids, work through interference with microtubule function [8].

Taxanes (Paclitaxel, Docetaxel) is a cancer drug derived from the Taxus species; the Taxus plant is also found in Indonesia. Taxanes prevent microtubule depolymerization, and in other words, taxanes inhibit cell proliferation by growing mitosis in metaphase and anaphase resulting in apoptosis. Podophyllotoxin (topothecan, irinothecan) is a non-alkaloid lignan toxin extracted from the roots and rhizomes of the Podophyllum species. Lignans are a large group of polyphenols found in plants that are also used as cancer drugs. Camptothecin is a natural product for anticancer drugs isolated from the bark and stems of Camptotheca acuminate (Shankar) herbal medicine by controlling the performance of topoisomerase, which has a vital role in cancer treatment. Camptothecins inhibit topoisomerase I, and epipodophyllotoxin inhibit topoisomerase II [9][10][11].

Several surveys have reported that cancer patients usually use herbs or herbal products. Some of these drugs have gone through the clinical trial stage as cancer drugs. Indonesia has several plants that have the potential to be developed into cancer drugs. Many plants have been used as co-therapy for cancer drugs to minimize the side effects of existing cancer drugs. Cancer drug research is needed to test the potential properties of plants as cancer drugs [6].

From several studies, paliasa leaves have been shown to protect the liver and kidneys from the side effects of the cancer drug doxorubicin. However, it is necessary to explore the extraction of the paliasa plant. These studies use three different extraction methods: ethanol extraction, ethyl acetate fractionation, and subcritical extraction. Conventional extraction methods use many solvents, and it takes a long time to get a thick extract. Thus, various alternative extraction methods, such as subcritical extraction, can be carried out [12]. Subcritical extraction is a highly selective method using pressurized fluids as solvents.

A supercritical fluid originates from a fluid being forced to pressure and temperature above its critical point, causing the liquid and gas phases to become indistinguishable. Supercritical fluids have solvent properties similar to organic solvents but have lower viscosity and diffusivity rates. Supercritical fluids have better transport properties than conventional organic solvents. As a result, they can readily diffuse through materials, thereby improving the extraction efficiency and yield of the desired molecules [13][14].

Subcritical extraction is not a standard method compared to ordinary solvent extraction methods. The benefit of subcritical extraction is that organic solvents are not used, and the yield is free from solvent residue that may harm the human body. This proves the efficacy of the extraction results. In addition, an anti-breast cancer test was carried out on cell line MCF-7. Much research shows the effectiveness of paliasa leaves in curing cancer.

II. METHODS AND MATERIAL

A. Plant Material

The dried simplicia of Paliasa leaves was obtained from the Bogor Institute for Medicinal and Aromatic Plants (BALITRO) and has been determined at LIPI (Indonesian Institute of Sciences) Cibinong as much as 3 kg.

B. Extraction and Sample Preparation

Paliasa leaf simplicia was weighed as much as 125 grams then put into a 1000 ml beaker glass, add 375 ml 96% ethanol, and let stand for ± 1 hour. Percolate the soaked simplicia, and do it until the extract that comes out of the tube is clear. Concentrate the extract into a water bath until it thickens, weigh it, and calculate the yield [15].

C. Fractionation of Paliasa Leaves Ethanolic Extract

Dissolve 102 grams of paliasa leaf Ethanol viscous extract using ± 310 ml ethyl acetate. The ethyl acetate fraction produced in the steam cup is placed in a water bath to be concentrated, then weigh, and calculate the yield [15].

D. Subcritical Extraction of Paliasa Leaves Ethanolic Extract

Subcritical extraction of paliasa leaves using an HFC R134a extractor using: weighing 500 g of dry simplicia, put it in the extractor. Extraction is carried out for 3 hours at each extractor chamber with 3 cycles with a Freon recycling system (mutual transfer of freon between extractor chambers). The pressure is 10/9 Barr. Gass uses were 2.5 kg [12].

E. Characterization of Extraction Products

The chromatography method was conducted prior to the characterizations of products obtained from HFC-134a subcritical extraction, ethanolic extract, and ethyl acetate fraction of Paliasa leaves. GC-MS and LC-MS analysis was conducted [12]. Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institue of Standards and Technology (NSIT).

F. Anticancer Evaluation with MTT Assay

Cytotoxicity testing of cells with the staining method by adding MTT [3- (4,5-dimethyl-2-thiazolyl) -2, 5-diphenyl-2H-tetrazolium bromide] aims to determine the cytotoxic activity of the sample by observing cell viability. MTT will react with the enzyme succinate dehydrogenase in the mitochondria of living cells and form blue formazan crystals that can be observed at a wavelength of 570 nm using the Elisa Reader [15].

III.RESULTS AND DISCUSSION

A. Determination of Plant Material

The initial plant material determination certificate results stated that the sample used was correct, namely Paliasa leaves (Klinhovia hospital Linn) with certificate number 642/IPH.1.01/If.07/VI/2020.

B. Extraction Method

In the extraction process, 3 kg of paliasa leaves yields + 269 grams of extract, requiring 27 liters of alcohol, The paliasa powder (500 grams) uses 2,5 kg of freon gasses each. The extraction process lasts for 3

hours in the pressure 10/9 Barr. The triple experiment has three different yields 889.3mg, 817.5mg, and 1014.9 mg. The higher yield value produced indicates that the value of the extract produced is more than other methods. This can be seen by the fractionation process in the yield of 33.6%, while with the percolation method, the yield is 8.84%, and subcritical extraction is 0.18%.

Extraction by the subcritical method is an environmentally friendly extraction method because it only uses high-pressure gaseous solvents at optimal temperatures. This method has many advantages compared to conventional extraction methods such as Soxhlet extraction, maceration, kinetic maceration, and others. Conventional methods generally use large amounts of organic solvents such as methanol, ethanol, benzene, hexane, and other solvents, which are organic solvents that are harmful to health. For example, methanol can cause blindness and death if ingested, hexane is carcinogenic, and others [14][16].

C. Characterization of Extraction Products

The results of the GC-MS analysis showed that as many as 27 compounds were identified from the ethanol extract of the paliasa leaves, 10 compounds from the ethyl acetate fraction of paliasa leaves, and seven compounds from paliasa leaves extract by subcritical extraction method. The chemical constituents, along with their retention time (RT) and area (%) are shown in the following table 1.

Table 1. Bioactive Compounds Found In Ethanolic Extract Of Klinhovia Hospital

No.	Chemical structure	RT	Area (%)
1	Carbamic acid, butylmethyl-, methyl ester	8,25	1,41
	Methyl isopropylcarbamate		
	3,4-Dihydroxyproline		
2	5,6,7,8-Tetramethylbicyclo[4.1.0]hept-4-en-3-one	12	1,53
	4-Acetyl-2-hydroxy-2,4,6-cyclohept atrien-1-one		
	cis-anti-trans-Tricyclo[7.3.0.0(2,6)]dodecane		
3	1,3,4,5,6,9-Hexahydro-benzocyclohepten-2-one	12,4	2,76
	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one		
	2(1H)-Quinolinone, 6-amino-3,4-dihydro-		
4	6-Methyl-7,8-dihydro-2(1H)-pteridinone	13,3	0,96
	[1,2,4]triazolo[1,5-a]pyrimidine,5-methoxy-7-methyl-		
	1H-1,2,3-Triazole, 4-methyl-5-(3-methyl-5-isoxazolyl)-		
5	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	13,8	1,05
	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-		
	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-		
6	1,3,4,5,6,9-Hexahydro-benzocyclohepten-2-one	14,1	5,81
	1-Methoxybicyclo[2,2,2]oct-5-en-2-yl methyl ketone		
	Ethanol, 2-(2,4-dichlorophenoxy)-		
7	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	14,3	1,76
	2-Pyridinamine, 3-methyl-N-nitro-		
	11-Methylene-tricyclo[4.3.1.1(2,5)]undecane		
8	diethyl methyl phosphate	14,5	0,93
	1,3,5-Triazine-2,4(1H,3H)-dione		
	4-Fluoro-6-aminopyrimidine		
9	3,4-Dimethoxyphenyl isothiocyanate	14,6	8,45

	13-Oxadispiro[5.0.5.1]tridecane		
	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-		
10	Benzenamine, 2-(trifluoromethoxy)-	14, 7	22,78
	1-Pentalenol, 1,2,3,3a,4,6a-hexahydro-		
	Bicyclo[3.2.1]octan-3-one, 6-hydroxy-, exo-(.+-.)-		
11	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	14, 9	2,57
	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-		
	1-Methylcarbazole		
12	1,4-Benzenediol, 2-methoxy-	15	4,6
	Benzenethiol, 4-methoxy-		
	1-(2-Thienyl)-1-propanone		
13	4-[2-(4-Chloro-phenyl)-ethylamino]-1-oxa-spiro[4.5]dec-3-en-2-one	15, 3	2
	1-Penten-3-one, 4-methyl-1-(1-piperidinyl)-		
	Propylparaben, acetate		
14	7-Hydroxy-3,4-dihydronaphthalen-1(2H)-one	15, 4	1,47
	5-Hydroxy-1-tetralone		
	7(8H)-Pteridinone, 6-methyl-		
15	2,3,4,5,6-Pentamethyl benzyl alcohol	15, 5	2,04
	[1,2,4]Triazolo[1,5-a]pyrimidine-6carbonitrile, 5-amino-		
	2,3,5,6-Tetramethylbenzoic acid		
16	Benzaldehyde, 2-hydroxy-3-methoxy-	15, 6	3,44
	Bicyclo[3.3.1]nonane-2,9-diol, exo-anti-		
	Benzaldehyde, 2-hydroxy-3-methoxy-		
17	2-Imino-3-ethylbenzothiazoline	15,	22,6
	4H-1-Benzothiopyran-4-one, 2,3-dihydro-8-methyl-	7	
	2,4,6-Trimethyl-1,3-phenylenediamine		
18	1,2-Cyclohexanediol, 3-methyl-6-(1-methylethyl)-, (1.alpha.,2.beta., 3.beta.,6.alpha.)-	15, 9	1,34
	Cyclohexanol, 2-(1-piperidinyl)-, trans-		
	Galactitol, cyclic 2,3:4,5-bis(ethylboronate) 1,6-bis(diethylborinate)		
19	trans-p-Mentha-2,8-dienol	15, 9	1,02
	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-		
	13-Oxadispiro[5.0.5.1]tridecane		
20	Ethanol, 2-(2,4-dichlorophenoxy)-	16	1,38
	2-methyl-3-azabicyclo[3.2.1]octan-[3H]-pyrrol		
	Tricyclo[6.2.1.0(2,6)]undeca-2(6),3-diene, 11-methyl-5,11-diaza		
21	Spiro[5.5]undecan-3-one	16, 1	1,87
	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-		
	Spiro[5.5]undecane		
22	5-Methylthiophen-3-ylamine	16, 2	1,26
	4-Methylamino-2(5H)-furanone		
	diethyl methyl phosphate		
23	2-Amino-3-(2-pyridin-2-yl-ethylsulfanyl)-propionic acid	16, 5	1,67
	2-(2-Pyridylthio)propionic acid		
	4,7-Methano-5H-inden-5-one, octahydro-		
24	Bicyclo[4.1.0]heptan-2-ol, 3,7,7-trimethyl-, (1.alpha.,2.alpha.,3.beta.,6.alpha.)	16, 8	1,47

	-		
	4,2,7-Ethanylylidenecyclopenta[b]pyran, octahydro-7a-methyl-		
	Methane, bromo-		
25	Bicyclo[4.1.0]heptane-7-carboxylic acid, 3-methyl-, ethyl ester	17	1,57
	2-Oxaadamantane		
	Dihydro iso-jasmone		
26	1,4-Cyclododecanedione	17,1	1,73
	Tetrachloroethylene		
	1-Methyl-octahydro-2H-quinolin-4-amine		
27	Acetamide, N-[(4-hydroxy-3-methoxy phenyl)methyl]-	17,3	0,55
	3,4-Dimethoxyphenyl isothiocyanate		
	Phenol, 2-amino-5-methyl-		

Table 2. Bioactive Compounds Found In Ethyl Acetate Fraction Of Klinhovia Hospital

No.	Chemical structure	RT	Area (%)
1	Methyl isopropylcarbamate	8,25	16,18
	3,4-Dihydroxyproline		
	Carbamic acid, butylmethyl-, methyl ester		
2	5-Methylamino-1,3,4-thiadiazole-2- one	18,5	12,76
	(1'R,2S,3R)-1',3-Dimethyl-2,2'-spi robiindan-1-one		
	D-Ribo-Hexose, 2,6-dideoxy-3-O-methyl		
3	Naphthalene, 2-[(N-dimethyl)aminoacetyl}	20	3,64
	Urea, N,N-dimethyl-N'-propyl-N'-(3-methylbutyl)-		
	Propionamide, N-propyl-N-(3-methylbutyl)-		
4	Bicyclo[3.2.1]octan-2-one	20,2	6,44
	Bicyclo[3.2.1]octan-2-one		
	E,Z-1,3,12-Nonadecatriene		
5	d-Proline, n-propargyloxycarbonyl-, pentyl ester	23,6	6,15
	d-Proline, n-propargyloxycarbonyl-, decyl ester		
	N-(4-Fluorobenzyl)-N-methylhexadecan-1-amine		
6	Heptanal	25,6	6,05
	Heptane, 3-methylene-		
	Cyclohexane, (1,1-dimethylpropyl)-		
7	2-(3,4-Dimethoxyphenyl)-6-methyl-3-[2-(trifluoromethyl)benzyl]imidazo[1,2-a]pyridine	27,2	4,41
	5,12-Naphthacenedione, 1,6,10,11etrahydroxy-8-(.alpha.-methylbenzyl)-, tetraacetate		
	Nadolol dibutylboronate		
8	Silane, diethylheptyloxyoctadecyloxy	27,7	5,11
	Silane, diethyl(2-heptyloxy)octadecyloxy-		
	Silane, dimethyloctyloxyoctadecyloxy		
9	2H-pyrrol-2-one, 1-[4-(dimethylamino)phenyl]-1,3-dihydro-3,4-bis(4-methoxyphenyl)-3,5-diphenyl-	29,4	11,24
	2H-1-Benzopyran-2-one, 7-(5-butoxy-6-methyl-2H-benzotriazol-2-yl)-3-phenyl-		
	Silane, methylvinyl(2-ethylhexyloxy)hexadecyloxy-		
10	Silane, [[(3β.,5α.,20S)-pregnane-	29,	16,5

	3,20 diyl]bis(oxy)]bis[tripropyl-	6	9
	Isorhamnetin (4TMS)		
	Bisphenol G, bis(pentafluoropropionate)		

Table 3. Bioactive Compounds Found In Extract Klinhovia Hospital From Subcritical Extraction

No.	Chemical structure	RT	Area (%)
1	Phenol, 3-(1-methylethyl)-, methyl carbamate	4,92	12,19
	Ethanone, 1-(3-hydroxyphenyl)-		
	Phenol, 2,3,6-trimethyl-		
2	Carbamic acid, butyl-, ethyl ester	8,25	26,56
	Methyl isopropylcarbamate		
	Carbamic acid, butylmethyl-, methyl ester		
3	Methyl 4,6-di-O-acetyl-2,3,7-tri-O-methyl-.beta.-glycero-D-glucohept opyranoside	18,2	14,4
	Methyl 4-O-acetyl-2,3,6-tri-O-methyl-.alpha.-D-galactopyranoside		
	Methyl 6-O-acetyl-2,3,4,7-tetra-O-methyl-.beta.-glycero-D-glucohepto pyranoside		
4	2-(4-Methylphenyl)-4H-imidazo(2,1-c)(1,4)benzoxazine	18,5	19,11
	Methyl 5-(2,5-dimethoxyphenyl)-2H-pyrazole-3-carboxylate		
	dibenzo[b,g][1,8]naphthyridine-11,12(10aH,11aH)-dione		
5	13-Oxabicyclo[10.1.0]tridecane	19,8	4,97
	3-Dodecen-1-ol, (Z)-		
	Ecgonine methyl ester		
6	8-Azabicyclo[3.2.1]oct-2-ene	19,8	11,12
	9-Oxabicyclo[6.1.0]non-4-ene		
	Bicyclo[2.2.2]oct-5-ene-2-carbonitrile		
7	endo-(2s,7R)-Tricyclo[6.2.2.0(2,7)]-4,9-dodecadiene-3,6-dione	20,2	11,65
	Bicyclo[3.2.1]octan-2-one		
	(cyclohex-2-en-1-yloxy)benzene		

Based on the GCMS test results, the chromatogram peaks have different ion fragmentation each of these peaks. The resulting ion fragmentation is information to determine the type of compound in the peaks by comparing the ion fragmentation produced from the sample peaks with ion fragmentation from the library database. Detailed data are presented in Tables 1, 2, and 3. The results of the GC-MS analysis of the ethanolic extract of paliasa leaves showed that the major components of the extract were Benzenamine, 2-(trifluoromethoxy); 1-pentalol, 1,2,3,3a,4,6a-hexahydro; Bicyclo[3,2,1]octane-3-one,6-hydroxy-,exo-(.+-.)-; 2-imino-3-ethylbenzothiazoline; 4H-1-Benzothiopyran-4-one,2,3-dihydro-8-methyl-; and 2,4,6-trimethyl-1,3-phenylenediamine. The major components of the ethyl acetate fraction of paliasa leaves were Silane, [[3beta.,5alfa.,20S)-pregnane-3,20diyl]bis(oxy)]bis[tripropyl-; Isorhamnetin (4TMNS), Bisphenol G,bis(pentafluoropropionate); Methyl isopropylcarbamate; 3,4-Dihydroxyproline; Carbamic acid, butylmethyl-, methyl ester. The major components of extract paliasa leaves from the subcritical extraction method were Carbamic acid, buthyl, ethyl ester; Methyl isopropylcarbamate; and Carbamic acid, butylmethyl-, methyl ester.

From the results of the GC-MS analysis of the main compounds found, several compounds have been scientifically proven to have an anticancer activity such as Isorhamnetin and Carbamic acid derivates group. Isorhamnetin is a compound scientifically proven to have antioxidant and anticancer activity [17][18]. This compound has a broad antitumor activity, inhibiting cervical cancer cells in humans,

lung cancer cells, colon cancer cells, nasopharyngeal cancer cells, liver cancer cells, and gastric cancer cells. These compounds proliferate tumor cells and induce apoptosis. Isorhamnetin can also induce apoptosis by downregulating carcinogenic genes and upregulating apoptotic genes [19]. Isorhamnetin can also inhibit the growth of HeLa cells by inhibiting telomerase activity [17].

Carbamates and their derivatives have good chemical and proteolytic resistance, can penetrate cell membranes, and play an essential role in developing new drugs. Carbamates can function as structural and functional groups that have been widely marketed as therapeutic drugs for various diseases, such as cancer, epilepsy, hepatitis C, HIV infection, and Alzheimer's [20][21]. Recent studies have shown that compounds containing carbamate groups in their molecules can increase the biological activity of active pharmacophores of natural and synthetic compounds. For example, the exchange of unsaturated ester chains in the C-6 Fumagilin compound with the carbamoyl O-(chloroacetyl) group can increase antitumor activity as much as 50 times stronger [22].

Determination of the main components of extracts and fractions of paliasa leaves was also done using the LC-MS method. LC-MS is one of the high-resolution analytical methods to perform quantitative and structural analysis so that it helps determine secondary metabolites. The results of the LC-MS analysis of extracts and fractions can be seen in tables 4, 5, and 6 below.

Table 4. Bioactive Compounds Found In Ethanolic Extract Klinhovia Hospital Linn From Lc-Ms Analysis

No.	Chemical structure	Molecular formulas	M/Z	RT
1	Biatractylolide	C30H38O4	4.632.858	9.38
2	Epianhydrobelachinal	C30H44O4	4.693.323	9.18
3	Kaempferol-7-O-α-L-rhamnoside	C21H20O10	4.331.128	4.57
4	Melianone	C30H46O4	4.713.472	9.26
5	Quercetin	C15H10O7	3.030.501	4.47
6	Stigmastan-3,6-dione	C29H48O2	4.293.729	10.29
7	Candidate Mass C45H84O14	C45H84O14	8.715.728	10.85
8	Candidate Mass C25H46O14	C25H46O14	5.932.777	9.49
9	Candidate Mass C21H28N2O	C21H28N2O	5.932.777	6.40
10	Candidate Mass C35H47NO5	C35H47NO5	5.623.542	9.41

Table 5. Bioactive Compounds Found In Ethyl Acetate Fraction Of Klinhovia Hospital Linn From Lc-Ms Analysis

No.	Chemical structure	Rumus Molekul	M/Z	RT
1	Biatractylolide	C30H38O4	4632851	9.38
2	Epianhydrobelachinal	C30H44O4	4693313	9.21
3	Kaempferol-7-O-α-L-rhamnoside	C21H20O10	4.331.130	4.58
4	Melianone	C30H46O4	4.713.466	9.26
5	Quercetin	C15H10O7	3.030.502	4.48
6	Stigmastan-3,6-dione	C29H48O2	4.293.729	10.29
7	Candidate Mass C45H84O14	C45H84O14	8.715.755	10.85
8	Candidate Mass C25H46O14	C25H46O14	5.932.769	9.49

9	Candidate Mass C45H82O15	C45H82O15	8.855.533	10.46
10	Candidate Mass C21H28N2O	C21H28N2O	3.252.276	6.42

Table 6. Bioactive Compounds Found In Extract Klinhovia Hospital From Subcritical Extraction Method From LCMS Analysis

No.	Chemical structure	Rumus Molekul	M/Z	RT
1	3-Tert-butyl-4-methoxyphenol	C11H16O2	1.811.224	7.96
2	7-Hydroxy-5-methoxycoumarin	C10H8O4	1.930.496	4.94
3	Biatractylolide	C30H38O4	4.632.857	9.38
4	Coclaurine	C17H19NO3	2.861.445	8.87
5	Digiprolactone	C11H16O3	1.971.172	5.50
6	Pipernonaline	C21H27NO3	3.422.075	9.31
7	Stearidonic acid	C18H28O2	2.772.164	9.25
8	Candidate Mass C45H84O14	C45H84O14	8.715.743	10.84
9	Candidate Mass C45H84O15	C45H84O15	8.875.696	10.72
10	Candidate Mass C30H40O4	C30H40O4	4.653.011	9.47

Based on the results of the examination of LCMS-MS compounds in the three kinds of extracts and fractions of paliasa leaves, the predicted results of the active compounds contained in the extract were obtained. The ethanolic extract and ethyl acetate fractions identified the same type of compound. Whereas in the extract from subcritical extraction method, the identified compounds were of different types. Biatractylolide compounds were found in the three identified extracts and fractions. The isolated biatractylolide exhibits various pharmacological activities, such as antitumour and antioxidant activity. The protective effect of biatractylolide on glutamate-induced rat adrenal pheochromocytoma cells (PC12) and human bone marrow neuroblastoma (SH-SY5Y) cells injured and the first to explore the mechanism. Preincubation with biatractylolide (10, 15, and 20 μM) markedly increased cell viability, inhibited glutamate-induced cell apoptosis, and reduced LDH activity.Furthermore, AO staining showed that cell apoptosis was decreased. Besides, the western blotting results showed that pretreatment with biatractylolide could decrease GSK3β protein expression and increase p-Akt protein expression, thereby protecting PC12 and SH-SY5Y cells from injury. Biatractylolide has a neuroprotective effect on glutamate-induced injury to PC12 and SH-SY5Y cells via a PI3K-Akt-GSK3β-dependent pathway mechanism [23]. Another study also showed that biatractylolide, a double sesquiterpene ester isolated from the ethyl acetate extract of *Atractylodes macrocephala*, could effectively attenuate glutamate-induced ROS in PC12 and SH-SY5Y cell lines and showed an anti-cancer effect [24].

D. Anticancer Evaluation with MTT Assay

A cytotoxic assay was carried out to determine the cytotoxic effect of the extract and the fraction of paliasa leaves on MCF-7 cells. The positive control used was doxorubicin. The parameter used to express the cytotoxic potential is the IC_{50} value (median inhibitory concentration), which manifests the potential for toxicity. The IC_{50} value is obtained from the linear regression equation between the concentration log vs. % viability produced, and the probit five value is entered into a linear regretion equation so that the log content is obtained, which causes 50% inhibition of the cell population [25][26].

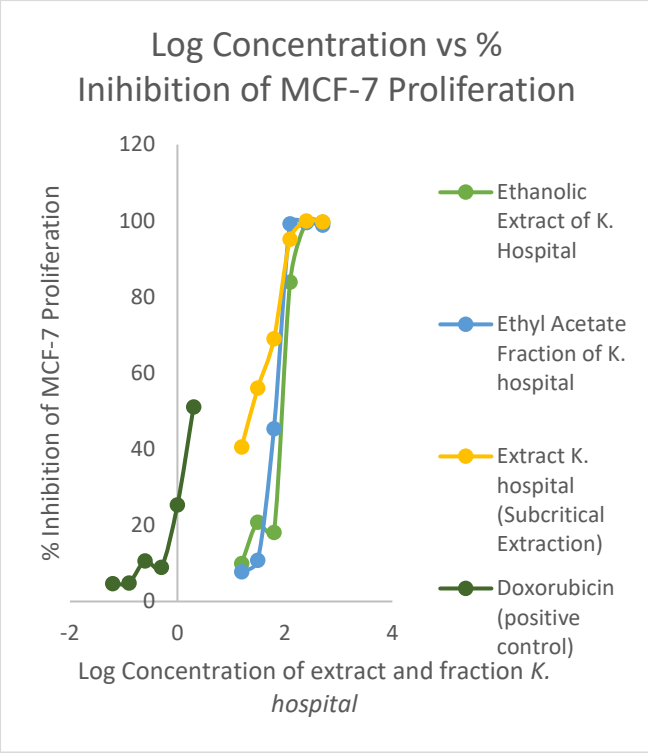

Figure 1. The relationship between the log concentration of extract and fraction of *Kleinhovia hospital* and the percentage inhibition of MCF-7 cell proliferation.

Table 7. MTT Test Results Against Mcf-7 Cell

No.	Process	IC 50 (ppm)
1.	Percolation	74,27
2.	Fractionation	64,07
3.	Subcritical extraction	21,14
4.	Positive control (Doxorubicin)	5,34

Compounds with the IC_{50} smaller, more potential to be developed as an anticancer, in addition to the American National Cancer Institute (NCI) too stated that the toxicity criteria for a compound against cancer cells where IC_{50} value 20 g/ml is very strong, IC_{50} 21-200 g/ml is moderately strong, and IC_{50} 201-500 g/ml is weak [27]. The cytotoxic test results of paliasa leaf extract (*Kleinhovia hospital* Linn.) against Breast cancer cells (MCF-7) showed that it could kill cancer cells specifically. An extract is considered active if it can inhibit the growth of 50% of the cancer cell population/tumour at a concentration below 30 ppm (IC_{50} <30 ppm). The results of the MTT Assay test showed that the extract from subcritical extraction method had the best IC_{50} results among the three kinds of extracts tested. The substance in extract *K. hospital* from the subcritical extraction method that does not exist in ethanol and ethyl acetate were: 3-tert-butyl-4-methoxyphenol, 7-Hydroxy-5-methoxycoumarin, Coclaurine, Pipernonaline, Pipernonaline, Stearidonic acid. Studies reveal that kaemferol has a role in cancer cure by inhibiting cell growth by involving signal transduction in apoptosis and angiogenesis [28]. Substance in subcritical extraction method has more potential to inhibit MCF cancer cells compared to ethanol extract or ethyl acetate fractionation. Coclaurine increases the sensitivity of cell contractile.

The MTT assay showed that the paliasa leaves extract obtained by subcritical extraction, fractionation, and percolation had a higher IC_{50} value than doxorubicin. This shows that doxorubicin has a more toxic effect on MCF-7 cells than the extract and fraction of paliasa leaves. Doxorubicin works non-selectively because it is toxic to cancer and normal cells, especially to normal cells with high proliferation rates. The mechanism of doxorubicin toxicity has been investigated and widely known [29]. Chronic toxicity of doxorubicin may be mediated by the metabolic conversion of doxorubicin to doxorubicin which involves various enzymes, including carbonyl reductase. The primary mechanism of toxicity of doxorubicin occurs because of its interaction with iron and reactive. Formation of destructive oxygen species (ROS) cell macromolecules. Doxorubicin, in the presence of a quinone group, is also capable of producing free radicals that are suitable for normal cells and cancer cells [30][31].

Identification of paliasa leaf simplicia extract (*Kleinhovia hospital Linn*) using the subcritical extraction method resulted in a better MTT assay with a very strong category IC_{50} value of 21.14 ppm.

There is still a need for further testing to analyze the toxicity of each compound produced both in silico and in vitro and in vivo to state that the extract meets the requirements for consumption for anti-cancer therapy.

IV. CONCLUSION

Identification of paliasa leaf simplicia extract (*Kleinhovia hospital* Linn) using the freonation subcritical method resulted in a better MTT assay with a very strong category IC_{50} value of 21.14 ppm. There is still a need for further testing to analyze the toxicity of each compound produced both in silico and in vitro and in vivo to state that the extract meets the requirements for consumption for anti-cancer therapy.

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