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Isolation and Bioactivities of Secondary Metabolites from Leaf Extracts of (*Rhodomyrtus Tomentosa* (Aiton) Hassk.)

Marwati Marwati[1,2], Yusnita Rifai[1,4*], Gemini Alam[3,4] and Risfah Yulianty[1]

1. Department of Pharmaceutical Science and Technology, Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
2. Department of Pharmacy, Faculty of Health Sciences, University Almarisah Madani, Makassar, Indonesia 90242, Indonesia
3. Department of Phytochemistry, Faculty of Pharmacy, Hasanuddin University, 90245, Indonesia
4. Drug Discovery and Development Centre, Institue of Research and Community Service, Hasanuddin University, Makassar 90245, Indonesia

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*Corresponding author
Yusnita Rifai

Email:
yusnitarifai@gmail.com

ABSTRACT

Cancer is characterized by abnormal cell development, frequently involving p53 gene mutation pathways. Approximately, 60% of cancer cases exhibit abnormalities in the p53 gene, which plays a pivotal role in normal cell division. This study explores the potential anticancer properties of *Rhodomyrtus tomentosa* leaves, known for their rich content of chemical compounds such as phenolics, flavonoids, tannins, saponins, and terpenoids. This study aimed to isolate, identify, and determine the bioactivity of secondary metabolite compounds extracted from *R. tomentosa* leaves against cancer cells. Finely powdered *R tomentosa* leaves were macerated with 70% ethanol. The extract obtained was partitioned and fractionated by the isolation of compounds carried out by thin-layer chromatography, The isolated compounds were characterized and tested for cytotoxic properties and selectivity against various cells. The analysis identified flavonoids, tannins, saponins, alkaloids, steroids, and phenolics in the extracts and fractions obtained from *R. tomentosa* leaves. The compound isolated from *R. tomentosa* leaves was identified as 2-(4-hydroxyphenyl)-acetic acid, confirmed through FTIR and NMR analyses. The bioactivity test shows that 2-(4-hydroxyphenyl)-acetic acid has anticancer activity in inhibiting cancer cell proliferation with IC_{50} values obtained in MCF-7 cells of 9.66 µg/mL (strong), T47D 59.47 µg/mL (medium), WiDr 39.21 µg/mL (strong), HeLa 199.60 µg/mL (medium), and on normal cells (Vero Cell) with an IC_{50} value of 1292.50 µg/mL. The presence of the active compound 2-(4-hydroxyphenyl)-acetic acid in *R. tomentosa* suggests a promising potential for the plant as an anticancer agent.

Keywords: Anticancer; cytotoxic; isolation; *Rhodomyrtus tomentosa* (Aiton) Hassk

INTRODUCTION

In 2020, the global prevalence of cancer exceeded 19 million cases worldwide, with breast cancer accounting for 51.6% of all cancer-related deaths, representing the highest percentage at 11.7%. According to Globocan in 2020, Indonesia was one of the countries with the highest number of cancer cases (396,914) and 59% of deaths from cancer. Breast cancer occupies the leading position, with a total of 65,858 cases (Globocan, 2020b). It is caused by a condition of abnormal cell development that can disrupt the biological functions of the human body, resulting in fatal consequences that can cause death (Hassanpour and Dehghani, 2017; Marwati *et al.*, 2021; Nur *et al.*, 2022). Cancer can occur, one of which is caused by mutations in the p53 gene that result in the formation of abnormal proteins. This p53 gene mutation event is molecularly and biologically abnormal, which can lead to the formation of cancer cells. Abnormalities in the p53 gene occur in 60% of cancer cases. Under normal conditions, p53

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has an important role in cell division, cell death, aging, angiogenesis, differentiation, and DNA metabolism (Chae *et al.*, 2011; Hassanpour and Dehghani, 2017).

Several treatment methods have been carried out for cancer patients, such as surgery, chemotherapy, radiotherapy, immunotherapy, hormone therapy, angiogenesis inhibition, and stem cell transplantation (Emens and Middleton, 2015; Fischer, 2022; Nur *et al.*, 2021). However, some of the current cancer therapies produce side effects from the use of synthetic drugs that can disturb cancer patients. In addition, the current disproportionate cost of chemotherapy and cancer treatment does not align with the success rates in cancer treatment (Devlin *et al.*, 2017; Fischer, 2022; Shah *et al.*, 2014). According to Devlin *et al.* (2017), there were several side effects that may occur as a result of cancer treatment, including hair loss, diarrhea, decreased sleep quality, fatigue, and several other side effects. Moreover, Kharani *et al.* (2019) reported similar side effects such as fatigue, nausea, vomiting, lack of appetite, fever, pain in the joint area, diarrhea, and several other side effects, as well as a decrease in the quality of life of patients on chemotherapy treatment. Hence, there is a critical need for supportive therapies or chemotherapy agents with minimal side effects to enhance the treatment of cancer patients. Previous studies have explored natural materials as anticancer candidates based on it active compounds.

R. tomentosa emerges as a promising candidate for anticancer development, supported by the findings of phytochemical screening in ethanol extracts of its leaves. Gayathri and Kiruba (2014) reported the presence of terpenoids, quinones, phenolics, flavonoids, proteins, carbohydrates, and cellulose in *R. tomentosa*. Furthermore, some of the secondary metabolites isolated from the *R. tomentosa* leaves are rhodomyrtosone A, rhodomyrtosone B, rhodomyrtosone C, rhodomyrtosone D, tomentose, tomentodione N-T and several other compounds. (Hamid *et al.*, 2016; Kusuma *et al.*, 2016; Liu *et al.*, 2016b; Vo and Ngo, 2019). Scientifically, those metabolites has been reported to have antibacterial activity (Liu *et al.*, 2016a; Saising and Voravuthikunchai, 2012; Salni and Marisa, 2020), immunomodulator activity (Na-Phatthalung *et al.*, 2018), anticancer activity, and antioxidant activity (Marwati *et al.*, 2020). *R. tomentosa* leaves present a potential avenue for the development of anticancer agents, as indicated by Marwati *et al.* (2020), showing its toxicity effects on shrimp larvae with an LC value of 24.451 µg/mL, classifying it as highly toxic. Building on these findings, our research aims to delve deeper into the anticancer activity, focusing on compound classification by polarity and evaluating the toxicity effects of *R. tomentosa* leaves fractions as anticancer.

MATERIALS AND METHODS

Chemicals and reagents

The materials used in this study were ethanol 70% (Merck, Germany), sterile distilled water (Merck, Germany), *Dulbecco's Modified Eagle Medium* (Sigma-Aldrich), Dimethyl Sulfoxide (Sigma-Aldrich), *fungizone, Fetal Bovine Serum (*Sigma-Aldrich), ethyl acetate (Merck, Germany), herpes, *Phosphate Buffer Saline* (Sigma-Aldrich), MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium *bromide*), Roswell *Park Memorial Institute* (RPMI) medium, n-hexane (Merck, Germany), $NaHCO_3$ (Merck, Germany), sodium dodecyl sulfate 10% in 0.1 N HCl (Sigma-Aldrich), penicillin (Sigma-Aldrich), streptomycin (Sigma-Aldrich), trypsin-EDTA (Sigma-Aldrich), and MCF-7, T47D, WiDr, HeLa, and Vero cancer cells were obtained from Cancer Chemopreventive Research Center, Pharmacy Faculty, Universitas Gadjah Mada.

Plant material

Plant material of *R. tomentosa* was obtained from Kanuruan village, Salu Sopai, North Toraja Regency, South Sulawesi Province, Indonesia (7º22'17 "N, 130º03'55 "E, ±674 m.asl.), in January 2020 (rainy season). Samples were identified based on flora specimens made in the form of herbarium at the Biology Research Centre, Institute of Natural Sciences, Makassar State University, Indonesia.

Experimental Methods

Sample Preparation

Preparation of R tomentosa Leaf Simplicia

The *R. tomentosa* that have been collected underwent initial wet sorting, then washed using running water to remove impurities and drained. Once drained, the samples were finely chopped and then dried by simplicia oven for two days at 40 °C. The dried simplicia are sorted and powdered (Marwati *et al.*, 2022).

Preparation of *R. tomentosa*

R. tomentosa leaf is a woody shrub with a height of up to 4m. This plant can grow at an

altitude of 300 m; it is rarely found in areas with an altitude of 1,300 m. The leaves are located crosswise and have three leaf bones from the base. They are oval in shape, tapered at the tip and base, and have flat margins. The upper surface is glossy, while the lower surface is rough because it has fine hairs that are 5-7 cm long and 23 cm wide.

Extraction Method

First, 200 g of powdered *R. tomentosa* were dissolved with two liters of ethanol 70% in a maceration vessel simplicia and allowed to stand for ± 30 minutes. Next, the remaining solvent is added to the maceration vessel, stirred until the mixture is evenly distributed, then stored at room temperature, protected from light for 3–4 days, and occasionally stirred. After the extraction process is complete, it was filtered, and the residue is remacerated using two liters of ethanol 70% for three days. The filtrate obtained was then evaporated using a rotary evaporator until a concentrated extract was obtained and the yield of the extraction was then calculated (Marwati *et al.*, 2022).

Fractionation Method

Fiveteen grams of concentrated extractwas dissolved using 150 mLof water-ethanol in a ratio of 1: 9, then the filtrate was put into a separating funnel. Next,150 mL of n-hexane was added, shaken for 15 minutes, and allowed to form two separate layers, namely the water-ethanol fraction and the n-hexane fraction. The shaking was repeated until the n-hexane layer was clear. The water-ethanol fraction was reintroduced into the separating funnel and 150 mL of ethyl acetate was added. The mixture then vigorously shaken for 15 minutes, and allowed to form two separate layers, namely the water-ethanol fraction and the ethyl acetate fraction. The addition of ethyl acetate and shaking were carried out until the ethyl acetate layer was clear. Then the ethyl acetate fraction obtained was evaporated too until dry. Three fractions were obtained, namely the n-hexane fraction, the ethyl acetate fraction, and the water-ethanol fraction. (Marwati *et al.*, 2022).

Chlorophyll Separation

50 grams of the concentrated extract was dissolved in 500 mL of distilled water and stirred for 1 hour at 40 °C. Subsequently, the hot extract solution underwent filtration, and the resulting water fraction was concentrated using a rotary evaporator.. After that, it was further fractionated using vacuum liquid chromatography (Salsabila *et al.*, 2022).

Fractionation with Vacuum Liquid Column Chromatography

Fractionation was carried out using vacuum liquid column chromatography using a column filled with silica gel 60 GF254 as the stationary phase. The silica gel was packed dry and filled into the column until the silica height reaching approximately 8 cm and then the top was covered with filter paper. The vacuum device was turned on to obtain maximum density. The sample it suspended first using silica gel before the separation process with a vacuum liquid chromatography column. Furthermore, the sample it inserted from the top of the column, spread evenly, and then placed on top of the filter paper. Before the separation process with a vacuum chromatography column, eluent orientation was carried out using thin-layer chromatography until an eluent with good stain separation obtained (Salsabila *et al.*, 2022).

Separation by Radial Chromatography

The eluent mixture used in this study were (a) n-hexane: ethyl acetate (8:2), (b) ethyl acetate: methanol:water (8:1.5:0.5), (c): Eluent n-hexane: ethyl acetate (6:4). Before radial chromatography separation, an eluent preliminary study was conducted to obtained the best separation. The first eluent used n-hexane: ethyl acetate (8:2), the second eluent used ethyl acetate: methanol: water (8:1.5:0.5), and the third eluent used n-hexane: ethyl acetate (6:4), so these eluents were used in the separation process using Radial Chromatography. The stationary phase was silica gel, while the mobile phase was a mixture of organic solvents (eluents). The separation of compounds was monitored using a UV 366 lamp, which separated them based on the color of the stain. Pure compounds were characterized by separation results appearing as single-spot observations with TLC (Salsabila *et al.*, 2022).

Anticancer Activity Study

The anticancer activity of *R. tomentosa* leaf extract was evaluated using the colorimetric method using MTT based on the research of Aisyah *et al.* 2022). Evaluation of the cytotoxic effect of *R. tomentosa* leaf extract was carried out using four cancer cell lines, namely MCF-7 cells, T47D cancer cells, WiDr cancer cells, and HeLa cancer cells, as well as by looking at the toxic effect on normal cells

(Vero cells) to find out which cells are affected. First, cancer cell lines were cultured in complete RPMI media (RPMI-1640 media supplemented with 10% fetal bovine serum), then penicillin 100 units/mL and streptomycin 100 µg/mL was added. Cell growth was observed by daily replacement of media using complete RPMI media. After that, cells filled the dish's surface or confluent; about 80% of cells were collected. Second, the cells were then distributed to the wells and incubated in a CO_2-fed incubator at 37°C for 24 hours to allow them to adapt and attach to the wells until they were ready to be treated. After incubation, serial concentrations of samples (1–500 µg/mL), doxorubicin as positive control, and media as negative control were treated and incubated for 24 hours in a CO_2 incubator at 37°C. After the incubation period, the cell medium was removed, and an MTT reagent with a concentration of 0.5 mg/mL was prepared. MTT solution was distributed into well-plate by 100 µL and incubated again for 4 hours in a CO_2 incubator at 37°C. At the end of incubation, 100 µl of 10% sodium dodecyl sulfate was added as stopper solution and allowed to stand overnight. The absorbance was measured with a microplate reader at a wavelength of 595 nm. The percentage of viable cells was calculated from the absorbance data obtained.

Characterization of Isolated Compound

The pure isolated compound was then characterized by fourier-transform infrared spectroscopy (FTIR) nuclear magnetic resonance (^{1}HNMR and ^{13}CNMR) to obtained the chemical structure (Nur *et al.*, 2020).

RESULTS AND DISCUSSION

In this study, the anticancer activity was explored *in vitro* with MTT assay method from extracts and fractions of *R. tomentosa* leaves. *R. tomentosa* leaf extract. The samples obtained are then continued in the extraction process, which aims to attract the components of the compounds contained in the samples that have previously been dried into simplicia. Based on the extraction results (Table I), the extraction yield was 11.21%. The obtained extracts were then fractionated using non-polar to polar solvents. Based on the fractionation results (Table I) in ethanol fraction (EF) the percent yield of the extract obtained was higher than that of n-hexane fraction (HF) and ethyl acetate fraction (EAF) with a percent yield of ethanol fraction of 45.5% from 15 g of ethanol extract of *R. tomentosa* leaves. The process is to identify the compounds' content in extracts and fractions of *R. tomentosa*. The purpose of identifying chemical compounds is to determine the compounds' content in extracts and fractions of *R. tomentosa*. Based on the results of the identification of compound content (Table II), the ethanol fraction shows that it has more compound content than ethanol extract (EE). Nevertheless, the EF did not exhibit any alkaloid content, suggesting that alkaloid compounds in the *R. tomentosa* leaf extract possess non-polar properties. This observation aligns with the identification results of the HF and EAF, both of which indicated the presence of alkaloid compounds.

Table I. Weight of the extracts and fractions of the leaves of *R tomentosa*

Sample	Weight (g)
Ethanol extract (EE)	56.05
N-hexane fraction (HF)	2.70
Ethyl acetate fraction (EAF)	4.54
Ethanol fraction (EF)	6.83

Table II. Phytochemicals screening of *R. tomentosa* leaf extracts

Compounds assay	EE	HF	EAF	EF
Alkaloid	+	+	+	-
Flavonoid	+	+	+	+
Saponin	+	-	-	+
Tannin	+	-	+	+
Terpenoid	+	-	+	+
Steroid	+	+	+	+

Furthermore, cytotoxic testing was performed on extracts and fractions. The findings presented in based on the anticancer activity using the MTT assay method from extracts and fractions of *R. tomentosa* leaves (Table III). In EF, there was moderate toxicity effect activity on three cancer cell lines, namely T47D, WiDr, and HeLa, with a range of IC_{50} value of 50–200 µg/mL and a very strong category on MCF-7 cancer cells with IC_{50} value >50 µg/mL. However, it did not have a toxicity effect on Vero cells as normal cells with an IC_{50} value >1000 µg/mL when compared to HF and EAF, the anticancer activity that showed toxic effect on normal Vero cells.

Table III. Anticancer activity of *R. tomentosa* leaf extracts, fractions and isolate against four cancer cell lines

Sample	IC_{50} (ppm)					Selectivity Index (SI)Value
	MCF-7	T47D	WiDr	HeLa	Vero	
Ethanol extract (EE)	189.749	164.72	39.21	96.27	350.97	1.85
N-hexane fraction (HF)	698.29	428.16	172.86	825.73	459.23	0.66
Ethyl acetate fraction (EAF)	133.95	68.67	78.58	201.8	196.51	1.47
Ethanol-water fraction (9:1) (EWF)	9.66	59.47	39.21	199.6	1292.50	133.80
Doxorubicin			3.27			

From these results, the selectivity value was then calculated to determine the excellent selectivity value of the cell used. Based on the results of the selectivity values (Table III), it was found that the extracts and EWF of *R. tomentosa* leaves had active selectivity. In contrast, a chemotherapy agent categorizeds as highly selective if the SI value ≥ 3 and less selective if the SI value < 3(Rahmawaty, 016). In this study, SI value of 0.95 was obtained, which indicate that the EE from *R. tomentosa* leaves was not selective against cancer cells. Furthermore, the results of activity tests on aqueous extracts with strong anticancer activity are carried out in the compound isolation process. The ethanol-water extract of *R. tomentosa* leaves was identified to contain chlorophyll, which can interfere with the separation of active compounds.

The pure isolate obtained is white-crystalline, as much as 50 mg, and was a compound of 2-(4-hydroxyphenyl)-acetic acid, which is then analyzed using FTIR (Shimadzu, Japan). The results of the IR spectrum shown in the presence of functional groups OH, C=O and C-O.

Compounds produced from separation and purification by radial chromatography are then characterized using nuclear magnetic resonance (NMR) Bruker 500 MH_Z to determine protons (^{1}H-NMR) and carbon (^{13}C-NMR). Analysis by NMR aims to determine the number and type of carbon atoms with hydrogen in the isolated molecule. ^{13}C NMR signals can provide clues about the number and type of carbon atoms in organic molecules, and ^{1}H NMR signals provide structural information about the characteristics of hydrogen atoms in an organic molecule.

Compound form White powder; $C_8H_9NO_2$; 1H-NMR (500 MHz, CDCl3) (ppm); 7.36 (1H, d, J=6.9 Hz, H-4 or H-6), 7.34 (1H, d, J=7.6 Hz, H-4 or H-6), 7.29 (1H, t, J=7.3 Hz, H-5), 7.27 (1H, s, H-2), 5.93 (1H, br s), 5.44 (1H, br s), 3.57 (2H, s, H-7). 13C-NMR (125 MHz, CDCl3) (ppm); 173.8 (C-8), 134.9 (C-3), 129.5 (2C), 129.2 (2C), 127.6 (C-1), 43.4 (C-7).

Furthermore, the bioactivity test of the isolated compounds was carried out against cancer cells MCF-7 breast cancer cells, T47D, WiDr colon cancer cells, HeLa uterine cancer cells, and Vero normal cells, showing that the compounds of *R. tomentosa* leaf isolates have moderate activity against MCF-7 breast cancer cells, namely with an IC_{50} value of 74.72 ppm. Meanwhile, the bioactivity test of isolated compounds against T47D 186.17 ppm breast cancer cells, WiDr colon cancer cells 235.9 ppm, HeLa 270.4 ppm and Vero 2286,6 µg/mL uterine cancer cells showed inactive results with IC_{50} values of more than 100 ppm for the three cells.

This study aims to isolate, identify, and determine the bioactivity of secondary metabolite compounds in extracts, fractions, and isolates of R. tomentosa leaves against cancer cells. The initial stage of this study is the extraction process carried out using the maceration method with 70% ethanol used as a solvent to draw the compound components contained in *R. tomentosa* leaves. The choice of extraction method with maceration in this study is one of the safest methods for compounds that are thermolabile due to increased temperature (Akinmoladun *et al.*, 2022; Luliana *et al.*, 2019). Based on the results obtained, the extraction process in this study was carried out perfectly, with a yield value of >10 % (Nur *et al.*, 2023). After the extract is obtained, compounds are separated based on their level of polarity from non-polar to polar using the liquid-liquid extract (LLE) method. Based on the results of the separation of compounds based on the level of polarity, the ethanol extract contained more compounds with more polarity properties, with a percent yield of the EWF of 45.5%. The EWF has a higher compound content compared to HF and EAF (Table II). However, in EF, there is no alkaloid content, which indicates that alkaloid compounds in *R. tomentosa* leaf extract have non-polar properties.

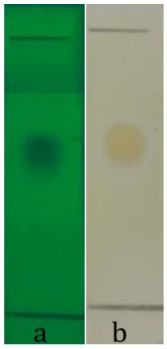

Figure 1. TLC profile of a 2-(4-aminophenyl) acetic acid compound using three different mobile phases, namely n-hexane eluent: ethyl acetate: acetone (5:2:3), chloroform: methanol (8.5:1.5), and ethyl acetate: methanol. Water (9.5:0.25:0.25) eluents were visualized under UV 366 (A) and (B) at UV 254.

Based on the results of anticancer activity testing of extracts and fractions of *R. tomentosa* leaves against four cancer cell lines, (Figure 1) shows that the cytotoxic effect produced depends on the concentration of the test sample solution used; the higher the concentration of the test sample solution, the fewer formazone crystals are formed, so that there is a decrease in cancer cell viability. The results of the decrease in cancer cell viability obtained then determine the IC_{50} value of each sample extract and fraction of *R. tomentosa* leaves against cancer cells. Samples of *R. tomentosa* leaf fractions that have been obtained are then evaluated for anticancer activity by looking at the toxicity effect of inhibiting the proliferation of cancer cells from four cancer cell lines, *R. tomentosa* leaves have anticancer activity on MCF-7, T47D, WiDr, and HeLa cancer cells and have a weak toxic effect on normal vero cells compared to the ethanol fraction, ethyl acetate fraction, n-hexane fraction, and isolates, namely MCF-7, T47D, WiDr, HeLa, and normal cells (Vero Cell), using the MTT assay method. The MTT assay method is a colorimetric test to measure cell metabolic activity based on the conversion of MTT tetrazolium salt, which will break down into purple formazan in active mitochondria in cells (Kuete *et al.*, 2017; Nur *et al.*, 2021). Cytotoxic testing using the MTT assay method aims to determine the toxicity of a compound contained in the extract of *R tomentosa* leaf fraction by evaluating the decrease in cell viability and toxic effects based on the IC_{50} value. The IC_{50} value is the concentration required for a sample to inhibit cell proliferation by 50 percent in cells categorized for strong cytotoxic effects <50 µg/mL, moderate cytotoxic effects 50–200 µg/mL, weak cytotoxic effects 200–1,000 µg/mL, and no cytotoxic effects >1,000 µg/mL (Kuete *et al.*, 2017; Nur *et al.*, 2021). Based on the results obtained, it is based on the content of compounds contained in *R tomentosa* leaves that have been reported (Marwati *et al.*, 2020, 2021) that the ethanol extract of *R. tomentosa* leaves contains flavanoid and phenolic compounds. In addition, Hamid *et al.* (2016; Kusuma *et al.* (2016) have explained the many compounds contained in *R. tomentosa* leaves. The content of flavonoid and phenolic compounds contained in *R. tomentosa* leaves as inhibitors of the apoptosis pathway, resulting in the release of proximal DNA chains by reactive oxygen compounds such as hydroxyl radical. Phenolic and flavonoid compounds can also inhibit cancer cell proliferation by activating protein kinases so as to inhibit the transduction pathway from the membrane to the cell nucleus (Dai & Mumper, 2010; Gupta and Pramanik, 2016; Kopustinskiene *et al.*, 2020; Li *et al.*, 2014).

From the cytotoxic activity test, extracts and fractions that have strong IC_{50} values are continued for the compound isolation process (Table III) results of the strong activity test are shown in the ethanol-water fraction. It has been isolated one pure compound from the ethanol-water fraction of *R. tomentosa* leaves, namely one simple phenolic benzoic acid derivative, 2-(4-aminophenyl) acetic acid, which was first reported from the ethanol-water fraction of *R. tomentosa* leaves. Determination of compound structure using FT-IR spectrum analysis and ^{1}D NMR (^{1}H-NMR and ^{13}C-NMR) (Figure 2). Finally, NMR data comparison between isolated compounds with the same or close reference compounds and bioactivity evaluation of isolates were also carried.

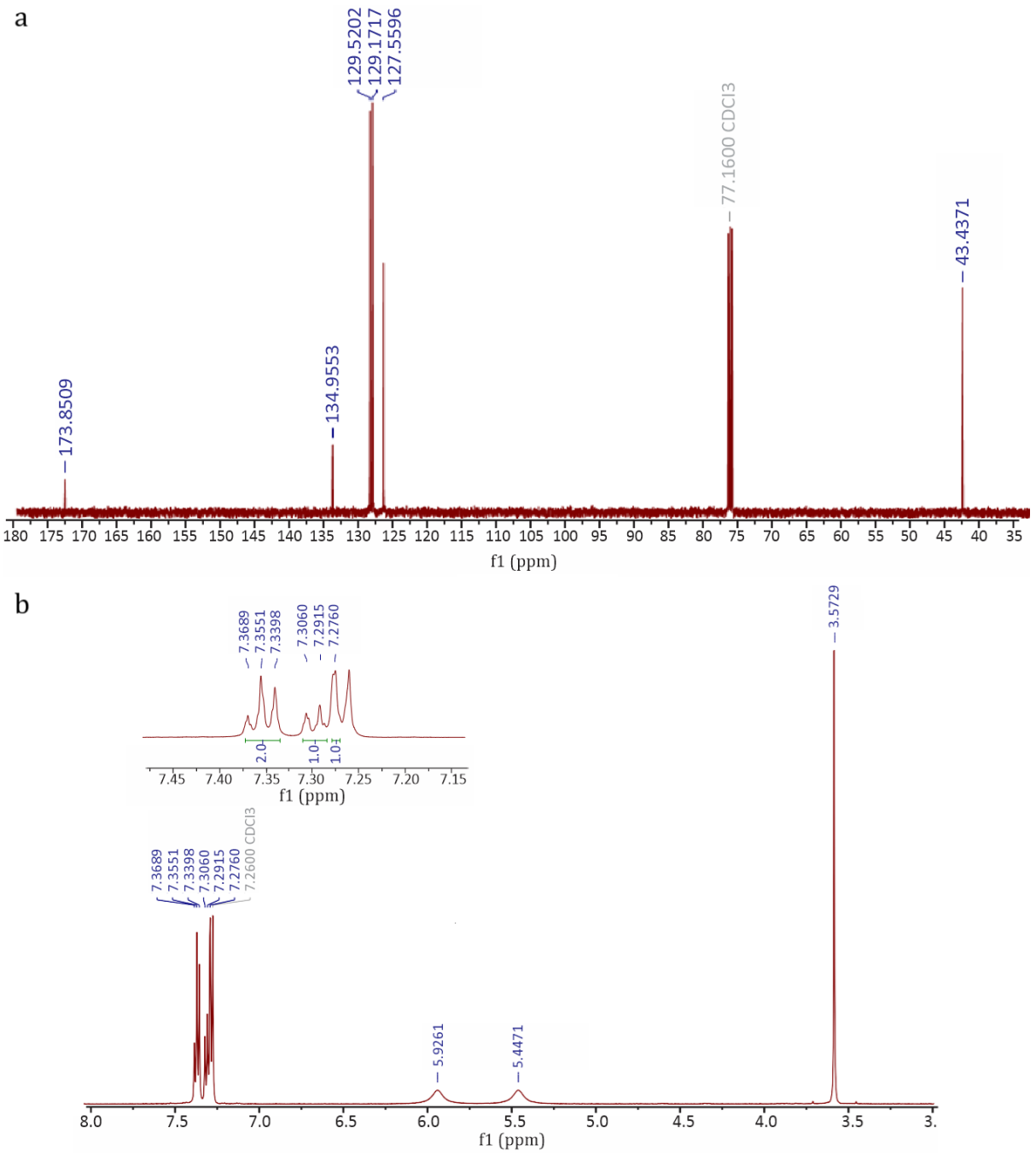

Figure 2. Spectrum a ^{13}C-NMR b. ^{1}H-NMR

The results of analysis using FT-IR showed that the functional groups, the compounds detected are flavonoid compounds with C-O, C=O, and O-H, to ensure the compounds obtained are then carried out elucidation of the compound structure using C-NMR and ^{1}H-NMR, it is known that the isolated compounds have one methylene group four olefin methine groups, three quaternary carbons, one carbonyl group, one hydroxy group and one amine group so that it has the molecular formula $C_8H_9NO_2$ with 5 DBEs corresponding to three double bonds, one cyclic (monocyclic) and one carbonyl group that leads to a benzene base skeleton with one carbonyl group on its aliphatic side chain.

Table IV. Comparison of NMR data of *R tomentosa* leaf isolate compounds with comparator compounds

No	Cluster	Compound 2-(4-hydroxyphenyl)-acetic acid	δC ppm	Compound 2-(4-hydroxyphenyl)-acetic acid ((CD3)2CO) (Ohtani *et al.*, 2011)	
		δH ppm (∑H, *m*, J (Hz))		δH ppm (∑H, *m*, J (Hz))	δC ppm
1	Cq	–	127.6	–	126.5
2	CH	7.27 (1H, *s*)	129.2 / 129.5	6.78 (2H, *dd, J*=8.7 & 1.5 Hz)	115.9
3	Cq	–	**134.9**	7.12 (2H, *dd, J*=8.7 & 1.5 Hz)	131.2
4	CH	7.34 (1H, *d, J*=7.6 Hz) / 7.36 (1H, *d, J*=6.9 Hz)	129.2 / 129.5	–	**157.1**
5	CH	7.29 (1H, *t, J*=7.3 Hz)	129.2 / 129.5	7.12 (2H, *dd, J*=8.7 & 1.5 Hz)	131.2
6	CH	7.34 (1H, *d, J*=7.6 Hz) / 7.36 (1H, *d, J*=6.9 Hz)	129.2 / 129.5	6.78 (2H, *dd, J*=8.7 & 1.5 Hz)	115.9
7	CH_2	3.57 (2H, *s*)	43.4	3.51 (2H, *s*)	40.4
8	C=O	–	173.8	–	173.2
		C-3 – **NH_2 (shielding)**		C-4 – **OH (de-shielding)**	

compounds have a basic framework of benzene-1,3-disubstituted or meta-substituted rings with side groups in the form of acetic acids and amines, which are simple phenolic compounds derived from benzoic acid, 2-(4-aminophenyl) acetic acid, and m-aminophenyl acetic acid. Therefore, other comparative compounds are used that are close to the hydroxy-substituted compounds in the para position of 2-(4-hydroxyphenyl)-acetic acid (Table IV).

The cytotoxic effect of a substance is assessed by the value of the half-maximal inhibitory concentration, better known as IC_{50}, which can kill 50% of cancer cells. The successfully isolated compounds were tested for bioactivity against cancer cells, namely MCF-7, T47D, WiDr, HeLa and normal cells (Vero Cell). The compound 2-(4-hydroxyphenyl) acetic acid has toxic activity against MCF-7, T47D, WiDr and HeLa cancer cells and is non-toxic to normal cells (Vero cells). The results are presented in Table III. The test data show that the compound has moderate activity against MCF-7 breast cancer cells with an IC_{50} value of 74.79 ppm. Because the compound 2-(4-hydroxyphenyl)-acetic acid is a pure compound, the levels are high compared to the EF. The levels of active compounds are still small because they are mixed with other impurity compounds, and pure isolate compounds have a direct target mechanism when compared to doxorubicin as a positive control with an IC_{50} value of 3.28 ppm . Based on these data, it can be concluded that compounds with positive optical rotation values have better activity than compounds with negative optical rotation values. In addition, the presence of carbonyl groups increases the bioactivity of isolated compounds, although this is not so significant.

CONCLUSION

Based on the results obtained from this study, it can prove that the compounds isolated from the ethanol-water fraction of *R. tomentosa* leaves are 2-(4-aminophenyl) acetic acid compounds and the water fraction of *R. tomentosa* leaves have activity as anticancer in MCF-7, T47D, WiDr, HeLa cancer cells and have a weak toxic effect on normal cells (Vero cells) compared to extracts, ethyl acetate fractions, n-hexane fractions, and isolates. As well as, the extract and aqueous fraction are selective against cancer cells. Therefore, the leaf extract has the potential to be developed as an anticancer drug candidate.

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CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

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