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ANTICANCER ACTIVITIES AND METABOLITE FINGERPRINTING OF UPLC-QToF-MS/MS METHOD FROM *Chrysanthemum cinerariifolium* (Trev)

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ABSTRACT

Chrysanthemum cinerariifolium (*C. cinerariifolium*) is an empirically proven medicinal plant that has anticancer activities. This study aimed to profile metabolites, and cytotoxic activity of root, leaf, stem, and root extracts *C. cinerariifolium* on T47D cells and to determine the correlation of metabolite content with cytotoxic activity. The metabolite profile was carried out using UPLC-QToF-MS / MS, and cytotoxic activity was carried out using the MTT method. The results obtained in the form of a chromatogram were processed with the application *Masslynk* so that a metabolite profile data obtained. The data is then analyzed statistically using *Principal Component Analysis* (PCA). The results obtained on metabolite profiling showed that there were differences in metabolite profiles in the roots, stems, leaves, and chrysanthemums. The characteristic compounds in the flower section are *D - (-) - Morphine* and in the leaf part of *genistein* and *N - [(5-Chloro-1,2,3-thiadiazol-4-yl) methyl] - 1- (2-isopropyl-4 -methyl-1,3-thiazol-5-yl) -N-methyl ethanolamine*. The major compounds in the roots, stems, and leaves are *Orphenadrine* with successive percentages of 9.11%, 10.16%, and 3.24%, and the major compounds in the flower section are *D - (-) - Morphine* with a percentage of 10, 86%. Furthermore, the results of the cytotoxic activity test showed differences in anticancer cytotoxic activity in the parts of the flower, leaf, stem, and root of *C. cinerariifolium*. Besides, there is a relationship between the metabolite content and anticancer cytotoxic activity of each part of *C. cinerariifolium*. The higher the level of orphenadrine compounds in the plant, the higher the potential for anticancer.

Keywords: *C. cinerariifolium*, UPLC-QToF-MS/MS, T47D cells.

INTRODUCTION

Chemoprevention is the use of natural materials to prevent (stop the activation of carcinogens, blocking) at the stage of initiation of carcinogenesis pressing (suppressing) cancer growth and restoring (reversing) the normal function of cellular regulation so as to reduce the development of cancer or reduce the possibility of progressive to invasive cancer [1][2]. Some chemoprevention agents from natural materials show high potential to be developed when clinically tested so that it becomes a priority for further research, including flavonoid and polyphenol compounds such as quercetin, luteolin, biochanin A, genistein [3].

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One of the traditional medicinal plants which are rich in the content of quercetin flavonoids is chrysanthemum (*Chrysanthemum cinerariifolium*).

Chrysanthemum flower (*Chrysanthemum cinerariifolium*) is a plant of the family Asteraceae which has been used by the community as an ornamental plant because of its beautiful flowers. Empirical evidence (traditional use) shows that chrysanthemum plants have widely used as antibacterial, anti-inflammatory, hypo-allergenic, and anticancer drugs [4][3]. Previous studies reported that chrysanthemums have potent anticancer activity [5]. It is also said that the most dominant compounds in chrysanthemum plants are terpenoid compounds and flavonoid compounds [6]. These compounds can act as anticancer. In breast cancer, flavonoid compounds and terpenoid groups play a role in inhibiting the mutation of the p53 gene so that it prevents excessive cell proliferation and can increase cell apoptosis [7][8]. The results of the antitumor activity test extracts of red chrysanthemums *(Chrysanthemumkunlun)* in *vitro* stated that flower extract *C. kunlun* could inhibit cell growth of Eca-109, H22 cells and HeLa cells [9].

Ophthalmology of secondary metabolites in a plant or natural product is a method that has widely developed today. One of the advantages of this method is that the stages of finding active compounds are shorter and require fewer samples than the method *Bioassay-Guided Isolation.* Also, metabolite profiling can be done to maintain the quality and consistency of the raw materials of herbal products used as traditional standardized medicines. Secondary metabolites are used not only from one organ part of the plant such as leaves, but rarely used parts do not rule out the possibility of containing secondary metabolites that have the potential to be developed, such as roots, stems, and flowers. It can be quickly done using an UPLC-QToF-MS/MS instrument. Based on this description, in this study profiling of metabolites and their correlation with the cytotoxic activity of extracts of flowers, leaves, stems, and roots of *Chrysanthemum cinerariifolium* (Trev.) On T47D breast cancer cells.

MATERIALS AND METHODS

Cell line and Reagents

The cell line used was T47D cells obtained from the Parasitology Laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta. The materials used for the test are the radix, caulis, folium, and flos *C. cinerariifolium*, technical ethanol 96%, and distilled water, n-hexane, ethyl acetate, H_2SO_4 10%, Complete RPMI 1640 medium (Gibco , USA), MK

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MI99 (Gibco, USA), PBS, Trypsin-EDTA, DMSO (EMSURE ACS, Japan), SDS (Merck, Germany), ethanol extract 96% flower, ethanol extract 96% leaf, ethanol extract 96% stem , ethanol extract 96% of plant roots *C. cinerariifolium*, doxorubicin HCL 50 mg, and MTT solution (Bio Basic Canada Inc, Canada).

Plant Determination

Determination of Plants was *C. cinerariifolium* (Trev) carried out at UPT Materia Medika Batu, East Java, Indonesia by number: 074/374/10272017 Plant specimens stored in the Pharmacognosy Laboratory of the Pharmacy Department of the Medical Faculty of Maulana Malik Ibrahim Malang.

Ethical Clearance

This research has received ethical approval from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim the Islamic State University of Malang on April 23, 2018, with numbers 004 / EC / KEPK-FKIK / 2018.

Sample Preparation

Sample *C.cinerariifolium* harvested by cut each piece of flowers, leaves, stems, and roots. Then each part is sorted early, washed, dried under the sun, and finally sorted. The dry sample is fertilized with a grinding machine and then weighed the powder.

Extraction of *C. cinerariifolium The*

Powder of flowers, leaves, stems, and roots of *C. cinerariifolium* put into Erlenmeyer and ethanol 96% added with a ratio of 1:20. Mixture extracted using UAE (Ultrasonication Assisted Extraction) for 2 minutes with three replications. The filtrate for each part of *C. cinerariifolium* from UAE was evaporated using a rotary evaporator at a temperature of 50ºC to produce the crude extract. A crude extract of flowers, leaves, stems, and roots concentrated using an oven at a temperature of 40ºC until the texture of the extract becomes concentrated. Percentage of rendement calculated by the formula:

$$\%\ \text{rendement} = \frac{Extract Weight\ of\ extract}{Simplicia\ weight} \times 100\%$$

Thin Layer Chromatography (TLC)

In the identification of compounds with TLC silica gel plate, 60 F_{254} used as a stationary phase by optimizing mobile phase n-Hexane and ethyl acetate (5: 5); (7: 3); and (8: 2). While the appearance of the stain used is H_2SO_4 10%. Identify compound stains using the Thin Layer Chromatography (TLC) Visualizer.

Sample preparation for Analysis of *Metabolite Profiling*

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Determination of the metabolite types of ethanol extract of roots, stems, leaves, and chrysanthemums using the UPLC-QToF-MS/MS instrument with three replications. Carefully weighed 10.00 mg of ethanol extract of roots, stems, leaves, and chrysanthemum flowers, then dissolved in methanol into a 10 ml volumetric flask and added microsyringe was 5 µl. The applied liquid was a mixture of (A) Water (HPLC grade) / formic acid (Merck, Darmstadt, Germany) 99.9 / 0.1 [v / v]; (B) Acetonitrile (Merck, Darmstadt, Germany) / formic acid 99.9 / 0.1 [v / v] and the system of gradient elution. The comparison presented in Table 1. Data obtained in the form of a chromatogram processed using software Masslynk version 4.1, so the data is in the way of peak area and m / z spectra of each detected peak and database www.chemspider.com. The data profile is then analyzed statistically by Principal Component Analysis (PCA) using software Minitab version 17.0.

Statistical Analysis

The identification data from the extract component was classified based on the sample origin, and the percentages of the area were analyzed using the Principal Component Analysis (PCA) to get the loading plot and score plot. PCA was performed using Minitab 17 (Minitab Inc, Pennsylvania, USA)

Preparation of Samples for Cytotoxic Activity Test

Flower, leaf, stem, and root extracts weighed as much as 10 mg, dissolved with DMSO and made seven kinds of concentration series namely 1000; 800; 600; 400; 200; 100; 50 µg / mL. The positive control of doxorubicin is made with 7 types of concentration, namely 2000; 1000; 500; 250; 125; 62.5; 31.25 nM

Cytotoxicity Test

Cytotoxicity tests were carried out on T47D cell cultures with RPMI medium. T47D cell culture was grown on 96 *well plates* and then incubated for 24 hours. After 24 hours the media was removed and washed with PBS, then each series of extract concentrations was inserted into each well with three replications and incubated for 24 hours. After 24 hours the media was removed and washed with PBS, then added MTT reagent 100 µL to each well, including media control (without cells), then incubated again for 4 hours in a CO_2 incubator. After 4 hours the cell conditions under the inverted microscope were observed. Then a stopper of 100 µL SDS 10% was added and incubated at room temperature for one night. Then read the absorbance value using ELISA reader and calculated cell viability using the following formula:

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$$Cell\ viability\ = \frac{(treatment\ abs\ -\ media\ control)}{abs(cell\ control\ abs\ -\ media\ control\ abs)} \times\ 100\%$$

Results of cell viability obtained are carried out IC_{50} analysis using Microsoft Excel. Then the data were analyzed by one way ANOVA to determine the cytotoxicity differences of each sample.

Correlation Analysis of Metabolic Content and Cytotoxic Activity

Analysis of the correlation of metabolite content and cytotoxic activity was carried out using the Pearson correlation test. Pearson correlation test was used to test two variables, namely the percentage levels of orphenadrine compounds from parts of flowers, leaves, stems, roots with cytotoxicity activity in T47D cells. Correlation test results indicate whether there is a correlation between metabolite content and cytotoxic activity in that part.

RESULTS

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a physicochemical separation method based on two phases, namely the mobile phase in the form of liquid and the stationary phase in the way of solids [10].

The optimization results obtained showed the best mobile phase in n-hexane and ethyl acetate (8: 2) by comparing before and after derivatization with H_2SO_4 10%. The results of the identification of TLC visualizers with UV 366 light showed differences in the separation of compounds between before and after spraying. The TLC plate after spraying shows many stains with several colors. It can be seen in the results of the Rf obtained, the compound Rf on the TLC plate after spraying was more than the value of Rf on the TLC plate before spraying. The amount of TLC Rf plate after spraying on flower, leaf, stem, and successive extracts is 9; 8; 10; and 11. According to Harborne [11], the yellow color with wavelengths 341-389 after being sprayed showed flavonol compounds and red-purple indicating terpenoid compounds. Whereas according to Muti'ah et al., [10] purple stains are suspected as sesquiterpenes. Flavonoid compounds and terpenoid groups play a role in health, one of which is to have anticancer activities [7][12].

Based on Figure 1 shows that there are differences in the chromatogram profile on the flowers, leaves, stems, and roots of *C. cinerariifolium*. The difference in the chromatogram indicates that there are differences in the metabolite content in each part of the organ *C. cinerariifolium* .

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Analysis of *Metabolite Profiling* with UPLC-QToF-MS/MS

The results obtained from the UPLC-QToF-MS/MS instrument were in the form of a chromatogram. The compounds that appear early on the *peak* chromatogram are polar compounds, and their polarity decreases at the *peak* next. At this stage, repetition carried out until a constant peak chromatogram obtained. The chromatogram obtained was processed using the application *Masslynx* version 4.1, so that the m/z spectra of each can be displayed *peak* on the chromatogram. Each peak of the chromatogram indicates the presence of one compound. Figure 2 is the result of a chromatogram of plant extract *C. cinerariifolium*. Based on the results of the chromatogram interpretation obtained from each peak, the prediction data found on chrysanthemum plants presented in table 2. It shows that there are differences in the compound content of the roots, stems, leaves, and flowers of *C. cinerariifolium.* Of the four parts of the organ, there is one dominant compound where the percentage greater than the other compounds, the compound is Orphenadrine (Figure 3). Orphenadrine is a flavonoid group compound that has activity tumor-preventing liver [13]. Figure 4, 5 and 6 are spectra of m/z compounds and the structure of the characteristic compounds found in parts of the chrysanthemum plant.

Statistical Analysis *Principal Component Analysis*

Principal Component Analysis (PCA) is a mathematical method for reducing data, to reduce the dimensionality of a series of data and reveal a cluster [14]. The data used in the PCA analysis are data on the name of the compound found and the percentage of the area of the chrysanthemum plant (roots, stems, leaves, flowers). The results of the PCA analysis obtained are loading plots and plot scores (Figure 7). Loading plots show compounds that are thought to be characteristic compounds in parts of chrysanthemum plants. In this study marker compounds were specific compounds not found in other parts of the plant. The compound is D - (-) - Morphine (Figure 4) found in the flower section and in the leaf part N - [(5-Chloro-1,2,3-thiadiazol-4-yl) methyl] -1- (2-isopropyl-4-methyl-1,3 -thiazol-5-yl) -N-methylethanamine and genistein (Figures 5 & 6).

Based on Figure 5 shows that Loading plots can interpret the characteristic compounds in the flower section are D - (-) - Morphine and in the leaf part is N - [(5-Chloro-1,2,3-thiadiazol-4-yl) methyl] -1- (2-isopropyl-4- methyl-1,3-thiazol-5-yl) -N-methylhexanamine and genistein, whereas in the roots and stems there are no organ compounds found. The plot score shows that the contents of the root and stem compounds of C. Cinerariifolium have close physical and chemical properties.

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Cytotoxic test for t47D cells Cytotoxicity

The analysis aims to determine the anticancer cytotoxic differences of the parts of the flowers, leaves, stems, and roots of *C. cinerariifolium* by decreasing the percentage of living cells based on 50% Concentration (IC_{50}) [15]. Cytotoxicity tests were carried out on T47D cells. The results of the cytotoxicity test presented in Figure 8 and Table 4.

Based on the results of IC_{50} values in Table 3, extracts that have cytotoxic activity against T47D cells are extracts of leaves, stems, and roots. An extract otherwise has a high cytotoxic activity when the value of IC_{50} <500 mg / mL and is said to have a weak activity where the IC_{50} values>500 pg / mL [16]. The IC50 amount of positive control is 208.82 nM. The results obtained were close to the value of IC_{50} researcher which stated that the IC value of$_{50}$ doxorubicin for T47D cells was 250 nM. Doxorubicin hasIC_{50} a low value because it has high activity against breast cancer cells [17]18].

Correlation Analysis of Metabolite Content with Cytotoxic Activity

The results of the correlation analysis between metabolite profiles and cytotoxic activity shown in Table 4. Based on Table 4, the results obtained indicate that there is a significant correlation between orphenadrine levels and anticancer activity in the flower section, stem, and roots. Relationship of orphenadrine compounds with cytotoxic activity is a negative correlation. It shows a correlation in the opposite direction, where the higher the level of orphenadrine compounds, the smaller the IC_{50} value. Based on the results of these correlations, the higher the content of the compounds in the plant *C. cinerariifolium*, the higher the anticancer potential.

DISCUSSION

Metabolite profiling aims to determine differences in the content of compounds in the roots, stems, leaves, and flowers of *C. cinerariifolium* and determine the characteristic compounds and major compounds with statistical analysis Principal Component Analysis (PCA). Metabolite profiling is carried out using the UPLC-QTof-MS / MS instrument that offers high resolution, speed, and sensitivity, and is effective for identifying the structure of components of natural and mixed organic compounds [19]. The findings of the compounds in the roots, stems, leaves, and flowers of *C. cinerariifolium* predicted by UPLC-QTof-MS / MS showed differences in compound content. The difference in the compound content of a part of a plant can be influenced by differences in the process of synthesizing compounds at certain stages so that a complex production of compounds occurs. The results of the profile of

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the binding compounds in section *C. cinerariifolium* were then analyzed by *Principal Component Analysis* (PCA) to determine the characteristic compounds and major compounds. Identifying compounds are specific compounds that only found in parts of the organ of *C. cinerariifolium*. The compound is D - (-) - Morphine found in the flower section and N - [(5-Chloro-1,2,3-thiadiazol-4-yl) methyl] -1- (2-isopropyl-4-methyl- 1,3-thiazol-5-yl) -N-methylhexanamine and genistein in the leaf part. Further identification is a major compound which is the dominant compound with the largest percentage of area in each part. The major compounds in the flower section are D - (-) - Morphine at 10.86%, and in the roots, stems, leaves are Orphenadrine in the amount of 9.11%, 10.16%, and 3.24% respectively.

As a result of the metabolite profiling with UPLC-QToF-MS / MS, it is shown that in each part *C. cinerariifolium* contained a major compound, the compound Orphenadrine. Compound Orphenadrine is a flavonoid compound [13]. Flavonoid compounds have been reported to have antiproliferative effects on breast cancer cells and can induce cell apoptosis [17][12][19][20]. Each part of *C. cinerariifolium is* known to have different levels of compounds orphenadrine, while the compounds highest Orphenadrine found in stem part extracts of 10.16%.

The cytotoxic activity test aims to determine the differences in the anticancer cytotoxic activity of the flowers, leaves, stems, and roots of *C. cinerariifolium*. The value of anticancer cytotoxicity in each part shows different data. It is because the content of orphenadrine compounds in each section also shows different levels. In the flower section, there are 8% orphenadrine compounds, with IC_{50} T47D cells of 782.33 µg / mL. In the leaf contains orphenadrine compounds of 2%, with IC50 values of 362.58 µg / mL. In the stem contains 10.16% orphenadrine compounds with IC50 values of 168.46 µg / mL. Whereas at the root part contains orphenadrine compounds of 9.11% with IC_{50} values of 293.81 µg / mL.

Analysis of the relationship of orphenadrine compounds with anticancer cytotoxicity was carried out using Pearson correlation analysis. The results obtained showed that there was a significant correlation between the levels of orphenadrine compounds and anticancer activity in the flower, stem, and root parts, with $P < 0.05$. The higher the level of orphenadrine compounds, the smaller the IC_{50} value. It shows that the higher the compound content in the plant part, the greater the anticancer potential in a part of the plant.

CONCLUSION

Flower, leaf, stem, and root extracts of *C. cinerariifolium* have different chemical content and differences in T47D cell cytotoxic activity with IC_{50} values 682.27; 411.43; 170.94; and 286.58 µg/mL. There is a relationship between the levels of orphenadrine compounds and anticancer cytotoxic activity in each part of *C. cinerariifolium*. The higher the level of Orphenadrine compounds in the plant, the higher the potential for anticancer.

REFERENCES

[1] Doyle A, and Griffiths JB. (2000) Cell and Tissue Culture for Medical Research. New York: John Willey and Sons Ltd.

[2] Keputusan Menteri Kesehatan Republik Indonesia (Kemenkes RI). (2015) Situasi Penyakit Kanker. Jakarta: Pusat Data dan Informasi.

[3] Listiyana A, Annisa R, Suryadinata A. 2018. Efek Antikanker dan Induksi Apoptosis bunga krisan (*Chrsantemum indicum*) Herbal Tea terhadap Sel Kanker Payudara. Laporan Penelitian Lembaga Penelitian dan Pengabdian Kepada Masyarakat

[4] Alviana, N., Sidharta, B. R. dan Martini, T. 2016. Uji Efektivitas Antibakteri Ekstrak Etanol Daun Krisan (Chrysanthemum morifolium Syn. Dendrathema grandiflora) Terhadap Staphylococcus aureus dan Escherichia coli, Yogyakarta: Universitas Atma Jaya Yogyakarta.

[5] Boutaghane N, Voutquenne-Nazabadioko L, Simon A, *et al*. (2013) A New Triterpenic Diester from the Aerial Parts of *Chrysanthemum macrocarpum*. Phytochemistry Letters, 1-7.

[6] Ukiya M, Akihisa T, Tokuda H, Suziki H, *et al* (2002) Constituents of Compositae Plants III Anti-tumor Promoting Effects and Cytotoxic Activity against Human Cancer Cell Lines of Triterpenoid Diols and Triols from Edible Chrysanthemum Flowers. Cancer Letters, 177, 7-12.

[7] Bishayee A, Ahmed S, Brankov N, and Perloff M. (2011) Triterpenoids as Potential agents for the Chemoprevention and Therapy of Breast Cancer. NIH Public Acces, Vol. 12, 980-996.

[8] Kalia R, Katnoria JK, and Nagpal AK. (2016) Antitumor Activity of Aqueous Leaf Ekstracts of Different Cultivars of Chrysanthemum morifolium R. Using Potato Disc Tumor Assay. Journal of Pharmaceutical Science and Research, Vol 8 No. 11, 1262-1265.

[9] Jing S, Zhang X, dan Yan LJ. (2015) Antioxidant Activity, Antitumor Effect, and Antiaging Property of Proanthocyanidins Extracted from *Chrysanthemum kunlun* Flowers. Oxidative Medicine and Cellular Longevity, 1-10.

[10] Muti'ah R, Hayati EK, and Triastutik Y. (2013) Pemisahan dan identifikasi Ekstrak Daun Bunga Matahari (*Helianyhus annus* L.) dengan Kromatografi Lapis Tipis. Alchemy, Vol. 2 No. 3, 190-194.

[11] Harborne JB (1987) Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan. Bandung : ITB.

[12] Morita R, Yafune A, Shiraki A, Itahashi M, Akane H, Nakane F, Suzuki K, Shibutani M, Mitsumori A. (2013) Enhanced Liver Tumor Promotion Activity in Rats Subjected to Combined Administration of Phenobarbital and Orphenadrine. Journal Toxicol Science 38(3): 415-24

[13] Williams DH and Ian F (2013). Metode Spektroskopi Dalam Kimia Organik. Edisi 6. Jakarta: EGC.

[14] Rohman A. 2014. Statistika Dan Kemometrika Dasar Dalam Analisis Farmasi. Dalam "Uji Kebermaknaan" Yogyakarta: Pustaka Pelajar. 95-96.

[15] Mutiah R, Listiyana A, Indradmojo C, Griana TP, Dwi HH, Atmaja RR. (2017) Induction of Apoptosis and Phase-Cell Cycle Inhibition of G0-G1, S, G2-M of T47D Breast Cancer Cells on Treatment with Ethyl Acetate Fraction of Jackfruit Parasite Leaves (*Macrosolen cochinensis*). Journal of Applied Pharmaceutical Science, 07 (10): 138-143.

[16] Costa EVS, Brigido HPC, Silva JV, *et al*. (2017) Antileishmanial Activity of *Handroanthus serratifolius* (Vahl) S. Grose (Bignoniaceae). Evidence-Based Complementary and Alternative Medicine Hindawi. 1-6.

[17] Abdolmohammadi MH, Fouladdel Sh, Shafiee A, *et al* (2008) Anticancer Effects and Cell Cycle Analysis on Human Breast Cancer T47D Cells Treated with Extracts of *Astrodaucus persicus* (Boiss.) Drude in Comparison to Doxorubicin. Journal Daru. Vol. 16, No. 2, 112-118.

[18] Anjarsari EY, Kristiani N, Larasati YA, *et al*. (2013) Synergistic Effect of Cinnamon Essential Oil (*Cinnamomum burmannii*) and Doxorubicin on T47D Cells Correlated with Apoptosis Induction. Indonesian Journal of Cancer Chemoprevention, 450-456.

[19] Foster JS, Henley DC, Ahamed S, and Wimalasena J. (2001) Estrogens and Cell-cycle Regulation in Breast Cancer. Trends in Endocrinology & Metabolism, Vol. 12, 320-327.

[20] Mutiah R, Widyawaruyanti A, Sukardiman S. 2018. *Calotropis gigantea* Leaf Extract Increases the Efficacy of 5-Fluorouracil and Decreases the Efficacy of Doxorubicin in Widr Colon Cancer Cell Culture. Journal of Applied Pharmaceutical Science, 8(4): 51-56.

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Table 1. Comparison of the mobile phase system gradient

Time (second)	% Eluent A	% Eluent B
0.00	95.0	5.0
2.00	75.0	25.0
3.00	75.0	25.0
14.00	0.0	100.0
15.00	0.0	100.0
19.00	95.0	5.0
23.00	95.0	5.0

Table 2. Predicted compounds of *C.cinerariifolium*

Ethanol 96% Extract of *C.cinerariifolium* Root						
No.	**Rt (minute)**	**% Area**	**Measured M/Z**	**Calculated M/Z**	**Formula**	**Compound Name**
1	0,723	0,2283	538,1092	538,1093	$C_{24}H_{22}N_6O_5S_2$	3-[(2-{[(4,5-Diphenyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetyl}hydrazino)carbonyl]-4-methoxybenzenesulfonamide
2	0,952	0,2600	171,1114	Unknown	Unknown	Unknown
3	1,969	4,4400	202,1318	202,1317	$C_9H_{18}N_2O_3$	Isoleucine-Alanine dipeptide
4	3,398	0,7432	216,0905	216,0906	$C_5H_{12}N_8S$	N^5-[1-(5-Tetrazolidinyl)ethyl]-1,2,4-thiadiazole-3,5-diamine
5	3,513	0,1509	203,1158	203,1158	$C_9H_{17}NO_4$	L-Acetylcarnitine
6	3,799	0,1501	419,1785	419,178	$C_{23}H_{25}N_5OS$	2-[(5-Ethyl-5H-[1,2,4]triazino[5,6-b]indol-3-yl)sulfanyl]-N-(1-phenylethyl)butanamide
7	4,164	1,5326	437,2307	437,2306	$C_{20}H_{32}N_7O_2Cl$	1,3-Dimethyl-8-(1-piperidinyl)-7-[3-(3,4,5,6-tetrahydro-2-pyridinylamino)propyl]-3,7-dihydro-1H-purine-2,6-dioneHydrochloride
8	4,645	1,4967	498,1168	498,1171	$C_{26}H_{26}O_6S_2$	1,4-Phenylenebis(methylene) bis[2-methoxy-4-(methylsulfanyl)benzoate]
9	4,93	0,8488	516,1270	516,1268	$C_{25}H_{24}O_{12}$	Cynarine
10	5,113	0,0569	462,1164	462,1162	$C_{22}H_{22}O_{11}$	2,2'-[(3,4,5-

						Trimethoxyphenyl)methylene] bis[3-hydroxy-6-(hydroxymethyl)-4H-pyran-4-one]
11	5,296	0,0534	678,1586	678,1585	$C_{34}H_{30}O_{15}$	(3R,5R)-3,4,5-Tris{[(2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-1-hydroxycyclohexanecarboxylic acid
12	5,411	0,0143	505,2147	505,2148	$C_{27}H_{31}N_5O_3S$	2-{Benzyl[2,2-dimethyl-5-(4-morpholinyl)1,4-dihydro-2H-pyrano[4",3":4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl]amino}ethanol
13	5,628	0,0879	518,1054	518,1049	$C_{29}H_{18}N_4O_4S$	N'-[(2E)-5-[(3-Oxo-3H-benzo[f]chromen-2-yl)carbonyl]-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene]benzohydrazide
14	5,776	0,0297	358,1893	358,1893	$C_{20}H_{26}N_2O_4$	Itopride
15	5,891	0,0408	194,0943	194,0943	$C_{11}H_{14}O_3$	Butylparaben
16	6,142	0,0825	213,1524	Unknown	H15N13O	Unknown
17	6,257	0,0368	678,1592	Unknown	$C_{25}H_{18}N_{20}O_3S$	Unknown
18	6,36	0,0264	592,1793	592,1792	$C_{28}H_{32}O_{14}$	Acaciin
19	6,691	0,1132	460,1000	460,0999	$C_{14}H_{24}N_2O_{13}S$	(2S,3R,4R,5S)-3-[(2-Acetamido-2-deoxy-6-O-sulfo-α-D-glucopyranosyl)oxy]-4,5-dihydroxy-2-piperidinecarboxylic acid
20	6,76	0,0963	241,1167	241,1678	$C_{13}H_{23}NO_3$	2-Methyl-2-propanyl 2-isopropyl-4-oxo-1-piperidinecarboxylate
21	6,908	0,0758	296,2143	296,2140	$C_{21}H_{28}O$	Pregna-1,4,20-trien-3-one
22	7,171	0,1858	411,3136	412,3214	$C_{27}H_{41}NO_2$	Cyclopamine
23	7,72	0,6027	445,2108	445,2107	$C_{17}H_{31}N_7O_5S$	Alanylarginylcysteinylproline
24	7,903	0,6371	589,2517	589,2518	$C_{43}H_{31}N_3$	3,3',3"-Methanetriyltris(2-phenyl-1H-indole)
25	8,52	1,1771	230,0951	230,0950	$C_7H_{14}N_6OS$	3-{2-[(3-Amino-1,2,4-thiadiazol-5-yl)amino] ethyl}-1,1-dimethylurea
26	8,852	0,7089	439,3814	439,3814	$C_{30}H_{49}NO$	(1R,6aR,8S,10aS,12aR)-7,7,10a,12a-Tetramethyl-1-[(2R)-6-methyl-5-methylene-2-heptanyl] 1,2,3,5,6,6a,7,8,9,10,10a,11,12,12a-tetradecahydronaphtho[1,2-h]quinolin-8-ol
27	9,138	2,0262	326,1518	326,1518	$C_{20}H_{22}O_4$	Dentatin
28	9,367	1,5253	469,2615	469,2617	$C_{31}H_{35}NO_3$	Cyclohexyl 4-(4-ethylphenyl)-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydro-3-Quinolinecarboxylate
29	9,469	0,8146	267,1628	Unknown	$C_3H_{17}N_{13}O_2$	Unknown
30	9,835	0,8792	770,3297	Unknown	$C_{57}H_{42}N_2O$	Unknown
31	10,167	0,8157	293,2366	Unknown	$C_{11}H_{31}N_7S$	Unknown
32	10,418	1,4244	544,2116	544,2117	$C_{26}H_{28}N_{10}O_2S$	3-Methyl-8-(4-phenyl-1-piperazinyl)-7-{3-[(1-phenyl-1H-tetrazol-5-yl)sulfanyl]propyl}-3,7-dihydro-1H-purine-2,6-dione
33	10,933	9,1093	269,1783	269,1780	$C_{18}H_{23}NO$	Orphenadrine
34	11,413	0,7639	273,2099	Unknown	$C_3H_{23}N_{13}O_2$	Unknown

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35	11,596	0,9682	208,1108	Unknown	$C_5H_{16}N_6OS$	Unknown
36	11,848	0,3086	212,0837	212,0837	$C_{14}H_{12}O_2$	Benzoin
37	11,996	0,2354	276,2097	Unknown	$C_{11}H_{29}N_6S$	Unknown
38	12,179	6,6129	275,2253	275,2249	$C_{18}H_{29}NO$	1,3,5-Tris(2-methyl-2-propanyl)-2-nitrosobenzene
39	12,396	0,5288	251,2258	Unknown	Unknown	Unknown
40	12,545	0,0684	322,1210	322,1212	$C_{13}H_{18}N_6O_2S$	N-(2-Cyano-3-methyl-2-butanyl)-5,7-dimethyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide
41	12,694	0,4986	323,2252	323,2249	$C_{22}H_{29}NO$	(E)-1-(4-Butoxyphenyl)-N-(4 pentylphenyl)methanimine
42	12,945	7,0016	277,2413	Unknown	Unknown	Unknown
43	13,391	0,3227	291,2566	291,2562	$C_{19}H_{33}NO$	4-[(Diethylamino)methyl]-2,6-bis(2-methyl-2-propanyl)phenol
44	13,791	4,4868	279,2567	279,2562	$C_{18}H_{33}NO$	Linoleamide
45	14,157	3,9045	281,2720	281,2719	$C_{18}H_{35}NO$	(9Z)-9-Octadecenimidic Acid
Ethanol 96% Extract of *C.cinerariifolium* Stem						
1	0,62	0,1715	174,1121	190,1066	$C_6H_{14}N_4O_3$	4-Hydroxyarginine
2	0,837	0,0022	103,1000	103,0977	$C_5H_{13}NO$	L-(+)-Valinol
3	1,42	1,4998	119,9735	119,0735	C_8H_9N	Indoline
4	1,901	1,6853	202,1321	202,1317	$C_9H_{18}N_2O_3$	Isoleucine-Alanine dipeptide
5	2,266	0,0741	187,0634	187,0633	$C_{11}H_9NO_2$	Indoleacrylic acid
6	2,449	0,0478	315,1691	315,1691	$C_{16}H_{29}NOS_2$	N-[2-(1-Cyclohexen-1-yl)ethyl]-4,4 bis(ethylsulfanyl)butanamide
7	3,364	0,3949	217,0980	216,0899	$C_{12}H_{12}N_2O_2$	3',4'-Dihydro-1'H,2H,5H-spiro[imidazolidine-4,2'-naphthalene]-2,5-dione
8	3,696	0,3654	441,2626	441,2628	$C_{25}H_{35}N_3O_4$	N-{4-[(3-Methoxy-4-{2-[(2-methyl-2-propanyl)amino]-2-oxoethoxybenzyl) amino]phenyl}-3-methylbutanamide
9	3,947	0,7645	439,2471	439,2471	$C_{25}H_{33}N_3O_4$	N-Isobutyl-N^2-{2-[(4-methoxybenzoyl)amino] benzoyl}isoleucinamide
10	4,096	0,2378	439,2478	439,2481	$C_{19}H_{39}N_5O_2Cl_2$	N-(2-{4-[2-(2,5-Dimethyl-1-pyrrolidinyl)ethyl]-1-piperazinyl}-2-oxoethyl)-L-valinamide dihydrochloride
11	4,347	0,2550	462,0811	462,0812	$C_{22}H_{14}N_4O_8$	2,5,7-Trinitro-9-oxo-N-(1-phenylethyl)-9H-fluorene-4-carboxamide
12	4,862	0,9273	432,1064	432,1063	$C_{14}H_{20}N_6O_8S$	L-γ-Glutamyl-S-(1-methyl-4-nitro-1H-imidazol-5-yl)-L-cysteinylglycine
13	5,079	0,2746	462,1171	462,1171	$C_{23}H_{26}O_6S_2$	Bicyclo[2.2.1]hept-5-ene-2,3-diylbis(methylene) bis(4-methylbenzenesulfonate)
14	5,479	0,1150	337,2608	Unknown	$C_{15}H_{36}N_5OCl$	Unknown
15	5,811	0,0544	225,1368	225,1365	$C_{12}H_{19}NO_3$	Terbutaline
16	5,959	0,0349	286,0467	286,0464	$C_{12}H_2N_{10}$	2,2'-[1,2,3]Triazolo[4,5-f]benzotriazole-4,8(2H,6H)-diylidenedimalononitrile

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17	6,074	0,1311	287,0478	286,0477	$C_{15}H_{10}O_6$	Kaempferol
18	6,325	0,0370	592,1804	592,1805	$C_{29}H_{28}N_4O_{10}$	6-[(1,3-Dihydroxy-2-propanyl)amino]-12-(D-glucopyranosyl)-2-hydroxy-12,13-dihydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione
19	6,84	0,7766	270,0532	270,0503	$C_8H_{18}N_2O_2S_3$	Methyl {2-[(2-methyl-2-propanyl)sulfamoyl]ethyl}carbamodithioate
20	7,057	0,1229	300,0638	300,0639	$C_{12}H_9N_8Cl$	4-Chloro-2-[(6-hydrazino-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino]benzonitrile
21	7,24	0,3950	330,0737	330,0740	$C_{17}H_{14}O_7$	Rhamnazin
22	7,491	0,2355	192,0787	192,0786	$C_{11}H_{12}O_3$	Myristicin
23	7,972	0,4806	471,2268	471,2270	$C_{27}H_{29}N_5O_3$	Ethyl 4-[7-(4-ethoxyphenyl)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-1-piperazinecarboxylate
24	8,12	0,0976	487,2585	487,2583	$C_{28}H_{33}N_5O_3$	8-[2-(Adamantan-1-yl)ethyl]-7-(4-methoxyphenyl)-1,3-dimethyl-1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione
25	8,521	0,4004	521,2416	521,2414	$C_{30}H_{35}NO_7$	2-Phenoxyethyl 2,7,7-trimethyl-5-oxo-4-(2,3,4-trimethoxyphenyl)-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
26	8,635	0,0150	290,1885	290,1882	$C_{18}H_{26}O_3$	Octyl methoxycinnamate
27	9,184	2,5971	229,1470	229,1467	$C_{15}H_{19}NO$	pronetalol
28	9,401	0,1554	368,1257	368,1260	$C_{21}H_{20}O_6$	Curcumin
29	9,835	1,1838	770,3275	770,3275	$C_{40}H_{46}N_6O_{10}$	2-(1,3-Benzodioxol-5-yl)-N[(6S,10S,12S,15S,18S)-6-benzyl-15-[(1R)-1-hydroxyethyl]-18-methyl-4,7,13,16,19-pentaoxo-2-oxa-5,8,14,17,20-pentaazatricyclo[21.2.2.0^{8,12}] heptacosa-1(25),23,26-trien-10-yl] acetamide
30	10,098	0,4848	315,2780	Unknown	Unknown	Unknown
31	10,236	0,1737	303,2209	Unknown	Unknown	Unknown
32	10,899	10,1668	269,1784	269,1780	$C_{18}H_{23}NO$	Orphenadrine
33	11,379	1,2072	285,2093	285,2093	$C_{19}H_{27}NO$	(R)-Pentazocine
34	11,596	1,0216	208,1098	208,1099	$C_{12}H_{16}O_3$	Asarone
35	11,813	0,1758	297,2094	297,2093	$C_{20}H_{27}NO$	Butaminophen
36	11,996	0,2293	365,3268	Unknown	Unknown	Unknown
37	12,145	3,9746	275,2245	275,2249	$C_{18}H_{29}NO$	1,3,5-Tris(2-methyl-2-propanyl)-2-nitrosobenzene
38	12,476	0,5490	341,3300	Unknown	Unknown	Unknown
39	12,694	0,3503	323,2254	323,2249	$C_{22}H_{29}NO$	p-butoxybenzylidene p-pentylaniline
40	12,911	4,1215	277,2408	277,2406	$C_{18}H_{31}NO$	4-(Dodecyloxy)aniline
41	13,208	0,0798	392,2319	392,2320	$C_{22}H_{36}N_2S_2$	3-[(Tetradecylamino)methyl]-1,3-benzothiazole-2(3H)-thione

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42	13,494	0,1143	267,2566	267,2562	$C_{17}H_{33}NO$	Hexadecyl isocyanate
43	13,757	1,5396	279,2571	Unknown	Unknown	Unknown
44	14,157	3,3119	281,2723	281,2719	$C_{18}H_{35}NO$	Oleamide
45	14,374	1,458	293,2732	Unknown	Unknown	Unknown
46	14,706	0,1309	295,2874	295,2875	$C_{19}H_{37}NO$	1-Isocyanatooctadecane
47	15,106	0,1145	333,3027	333,3032	$C_{22}H_{39}NO$	p-Hexadecyloxyaniline
48	15,289	1,3600	309,3030	309,3032	$C_{20}H_{39}NO$	1-Hexadecanoylpyrrolidine
49	15,472	0,0141	610,4597	610,4597	$C_{39}H_{62}O_5$	[1-(2-{[(3β)-3-Hydroxylup-20(29)-en-28-yl]oxy}-2-oxoethyl)cyclopentyl]acetic acid
50	15,586	0,2357	311,3198	Unknown	Unknown	Unknown
51	16,204	0,2967	493,5588	493,5587	$C_{34}H_{71}N$	N-Hexadecyl-1-octadecanamine
52	16,604	0,7435	521,5911	Unknown	$CH_2N_2O_3S_5ClBr_3$	Unknown
Ethanol 96% Extract of *C.cinerariifolium* Leaf						
1	0,586	0,0837	150,0280	150,0277	$C_3H_6N_2O_5$	Urea ethanedioate
2	0,769	0,0398	292,0567	292,0565	$C_{11}H_{12}N_6S_2$	9-Methyl-5-(methylsulfanyl)-8,9,10,11-tetrahydropyrido[4',3':4,5]thieno[3,2-e]tetrazolo[1,5-c]pyrimidine
3	1,42	1,0781	119,0735	119,0735	C_8H_9N	Indoline
4	1,935	0,1100	202,1318	202,1317	$C_9H_{18}N_2O_3$	Isoleucyl-Alanine
5	2,266	0,7538	187,0634	187,0633	$C_{11}H_9NO_2$	Indoleacrylic acid
6	2,518	0,0526	185,1163	185,1164	$C_8H_{15}N_3O_2$	1-Acetyl-3-piperidinecarbohydrazide
7	3,364	0,2818	216,0902	216,0899	$C_{12}H_{12}N_2O_2$	3',4'-Dihydro-1'H,2H,5H-spiro[imidazolidine-4,2'-naphthalene]-2,5-dione
8	3,764	0,3684	243,1474	243,1471	$C_{12}H_{21}NO_4$	Tiglylcarnitine
9	3,947	0,3261	439,2475	439,2471	$C_{25}H_{33}N_3O_4$	N-Isobutyl-N^2-{2-[(4-methoxybenzoyl)amino]benzoyl} isoleucinamide
10	4,347	0,1707	462,0800	462,0798	$C_{17}H_{23}N_4O_5S_2Cl$	4-Chloro-2-({4-[(2,6-dimethyl-4-morpholinyl)sulfonyl]-1-piperazinyl}sulfonyl)benzonitrile
11	4,645	0,1249	578,1638	578,1636	$C_{27}H_{30}O_{14}$	Kaempferitrin
12	4,862	0,5915	446,0862	446,0862	$C_{22}H_{14}N_4O_7$	N-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)methyl]-3,5-dinitro-N-phenylbenzamide
13	5,262	0,0129	349,2245	Unknown	$C_{15}H_{32}N_5O_2Cl$	Unknown
14	5,662	0,0720	527,1920	527,1922	$C_{25}H_{34}NO_9Cl$	1-(Nitrooxy)-2-propanyl(5Z)-7-{(1R,2R,3R,5S)-2-[(1E,3R)-4-(3-chlorophenoxy)-3-hydroxy-1-buten-1-yl]-3,5-dihydroxycyclopentyl}-5-heptenoate
15	5,776	0,1556	459,2259	459,2257	$C_{25}H_{33}NO_7$	2-Methoxyethyl 2,7,7-trimethyl-5-oxo-4-(3,4,5-trimethoxyphenyl)-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
16	6,074	0,2245	286,0479	286,0477	$C_{15}H_{10}O_6$	Kaempferol
17	6,257	0,1001	316,0585	316,0583	$C_{16}H_{12}O_7$	Isorhamnetin
18	6,84	1,7179	270,0533	270,0528	$C_{15}H_{10}O_5$	Genistein
19	7,274	1,8113	330,0737	330,0740	$C_{13}H_{19}N_4S_2Cl$	N-[(5-Chloro-1,2,3-thiadiazol-4-yl)methyl]-1-

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						(2-isopropyl-4-methyl-1,3-thiazol-5-yl)-N-methylethanamine
20	7,572	0,0101	488,2162	488,2159	$C_{25}H_{32}N_2O_8$	(2-Methyl-1,4-piperazinediyl)bis[(3,4,5-trimethoxyphenyl)methanone]
21	7,674	0,0150	309,2302	309,2304	$C_{18}H_{31}NO_3$	N,N-Diisobutyl-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxamide
22	7,972	0,2336	471,2255	471,2257	$C_{26}H_{33}NO_7$	2-Methoxyethyl 4-(4-acetoxy-3-ethoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
23	8,223	0,0365	344,0906	344,0905	$C_{19}H_{20}O_2S_2$	2-(7,8-Dimethyl-1,5-dihydro-2,4-benzodithiepin-3-yl)phenyl acetate
24	8,406	0,0689	234,1627	Unknown	$C_8H_{22}N_6S$	Unknown
25	8,52	0,0225	521,2415	521,2414	$C_{30}H_{35}NO_7$	2-Phenoxyethyl 2,7,7-trimethyl-5-oxo-4-(2,3,4-trimethoxyphenyl)-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate
26	9,138	0,4170	229,1472	229,1467	$C_{15}H_{19}NO$	Pronetalol
27	9,652	0,0098	403,1164	403,1168	$C_{22}H_{17}N_3O_5$	Azoxystrobin
28	10,235	0,1533	218,1672	218,1671	$C_{15}H_{22}O$	(+)-Nootkatone
29	10,716	0,5352	267,1622	267,1623	$C_{18}H_{21}NO$	Azacyclonol
30	10,899	3,2354	269,1780	269,1780	$C_{18}H_{23}NO$	Orphenadrine
31	11,379	0,5113	387,0986	387,0986	$C_{19}H_{18}N_3O_4Cl$	Pyraclostrobin
32	11,596	0,1104	519,3324	519,3322	$C_{29}H_{41}N_7O_2$	3-[(4-Cyclohexyl-1-piperazinyl)(1-cyclohexyl-1H-tetrazol-5-yl)methyl]-6-ethoxy-2(1H)-quinolinone
33	12,145	1,0840	275,2257	Unknown	Unknown	Unknown
Ethanol 96% Extract of *C.cinerariifolium* Flower						
1	0,62	0,2307	174,1122	174,1117	$C_6H_{14}N_4O_2$	L-Arginine
2	1,42	1,5649	119,0736	119,0735	C_8H_9N	Indoline
3	1,901	1,9443	202,1319	202,1317	$C_9H_{18}N_2O_3$	Isoleucine-Alanine dipeptide
4	2,266	2,1291	187,0639	187,0633	$C_{11}H_9NO_2$	Indoleacrylic acid
5	3,364	0,5735	441,2623	441,2628	$C_{25}H_{35}N_3O_4$	N-{4-[(3-Methoxy-4-{2-[(2-methyl-2-propanyl)amino]-2-oxoethoxy}benzyl)amino]phenyl}-3-methylbutanamide
6	3,947	1,5082	439,2469	439,2471	$C_{25}H_{33}N_3O_4$	N-Isobutyl-N^2-{2-[(4-methoxybenzoyl)amino]benzoyl} isoleucinamide
7	4,862	3,7310	432,1057	432,1056	$C_{21}H_{20}O_{10}$	5-Hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl hexopyranoside
8	5,479	1,9686	640,3257	640,3254	$C_{29}H_{48}N_6O_8S$	L-Lysyl-L-leucyl-L-methionyl-L-seryl-L-tyrosine
9	5,959	2,1848	286,0490	286,0486	$C_{16}H_{14}OS_2$	(2Z,6E)-2,6-Bis(2-thienylmethylene)cyclohexanone
10	6,108	1,962	286,0480	286,0480	$C_8H_{18}N_2O_3S_3$	2-

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						{[3(Methylsulfinyl)propyl] sulfamoyl}butanethioamide
11	6,325	0,1416	592,1790	592,1792	$C_{28}H_{32}O_{14}$	Acaciin
12	6,394	0,0817	474,1152	474,1150	$C_{28}H_{18}N_4O_2S$	3-(4-Methylbenzoyl)-2-(4-phenyl-2-thioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1-benzofuran-5,6-dicarbonitrile
13	6,84	3,1285	270,0531	270,0530	$C_8H_{18}N_2O_2S_3$	Methyl {2-[(2-methyl-2-propanyl)sulfamoyl]ethyl} Carbamodithioate
14	7,24	1,5010	360,0846	360,0845	$C_{18}H_{16}O_8$	(R)-(+)-rosmarinic acid
15	7,572	0,1700	488,2156	488,2159	$C_{25}H_{32}N_2O_8$	(2-Methyl-1,4-piperazinediyl)bis[(3,4,5 trimethoxyphenyl)methanone]
16	7,972	1,0043	471,2262	471,2262	$C_{22}H_{30}N_9OCl$	N-{2-[(7-Chloro-4-quinolinyl)amino]ethyl}-N'-[2-(dimethylamino)ethyl]-6-(4-morpholinyl)-1,3,5-triazine-2,4-diamine
17	8,223	0,6838	344,0897	344,0896	$C_{18}H_{16}O_7$	(±)-Usnic acid
18	8,406	0,3363	271,1210	271,1208	$C_{16}H_{17}NO_3$	O-Benzyl-L-tyrosine
19	8,669	0,1494	284,0687	284,0687	$C_9H_{20}N_2O_2S_3$	(3-Methyl-1,1-dioxidotetrahydro-3-thiophenyl)carbamodithioic acid–N,N-dimethylmethanamine
20	9,401	10,8649	285,1367	285,1265	$C_{17}H_{19}NO_3$	D-(-)-Morphine
21	9,584	6,7201	285,1375	285,1375	$C_{11}H_{25}N_3OCl_2$	2-[4-(4-Piperidinyl)-1-piperazinyl] ethanol Dihydrochloride
22	9,835	0,3062	277,1473	Unknown	$C_4H_{15}N_{13}O_2$	Unknown
23	9,984	0,0280	291,2200	291,2198	$C_{18}H_{29}NO_2$	1-(2-Cyclopentylphenoxy)-3-[(2-methyl-2-propanyl)amino]-2-propanol
24	10,281	3,4908	311,1524	311,1521	$C_{19}H_{21}NO_3$	Nalorphine
25	10,75	2,8304	267,1624	267,1263	$C_{18}H_{21}NO$	Azacyclonol
26	10,899	8,6205	269,1785	269,1780	$C_{18}H_{23}NO$	Orphenadrine
27	11,264	1,8554	339,1833	339,1834	$C_{21}H_{25}NO_3$	Pipethanate
28	11,413	2,5239	285,2094	285,2093	$C_{19}H_{27}NO$	(R)-Pentazocine
29	11,596	0,7026	519,3323	519,3322	$C_{29}H_{41}N_7O_2$	3-[(4-Cyclohexyl-1-piperazinyl)(1-cyclohexyl-1H-tetrazol-5-yl)methyl]-6-ethoxy-2(1H)-quinolinone
30	11,962	3,1903	343,2147	343,2147	$C_{21}H_{29}NO_3$	Smenospongine
31	12,179	4,9042	275,2253	275,2259	$C_{18}H_{29}NO$	1,3,5-Tris(2-methyl-2-propanyl)-2-nitrosobenzene
32	12,396	0,5785	251,2252	251,2249	$C_{16}H_{29}NO$	(6Z,8S,8aS)-8-Methyl-6-[(2R)-2-methylhexylidene]octahydro-8-indolizinol
33	12,694	1,1111	323,2249	323,2249	$C_{22}H_{29}NO$	(E)-1-(4-Butoxyphenyl)-N-(4-pentylphenyl)methanimine
34	12,911	4,6764	277,2411	277,2406	$C_{18}H_{31}NO$	4-(Dodecyloxy)aniline
35	13,345	0,0842	602,5275	602,5274	$C_{39}H_{70}O_4$	(2S)-1-(Hexadecyloxy)-3-hydroxy-2-propanyl 5,8,11,14-icosatetraenoate
36	13,757	1,3846	279,2566	279,2562	$C_{18}H_{33}NO$	Linoleamide

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Table 3. IC_{50} value of T47D cell line after treatment with *C. cinerariifolium*

Sample	IC_{50} T47D cell line ± SD (%)*
Flower	782,33 µg/mL ± 13,98
Leave	362,58 µg/mL ± 19,07
Stem	168,46 µg/mL ± 5,83
Root	293,81 µg/mL ± 12,40
Doxorubicin	208,82 nM ± 22,95

Description:
*SD : standard deviation in treatment with 3x repetition

Table 4. Correlation test result of orphenadrine and cytotoxic activity

		Orpenadrine	T47D cell
Orphenadrine	Pearson correlation Sig. (2-tailed)	1	-.859** .003*
T47D cell	Pearson correlation Sig. (2-tailed)	-.859** .003*	1
Vero cell	Pearson correlation Sig. (2-tailed)	-.872** .002*	

Description:
* : $p < 0.05$
**: Correlations negative

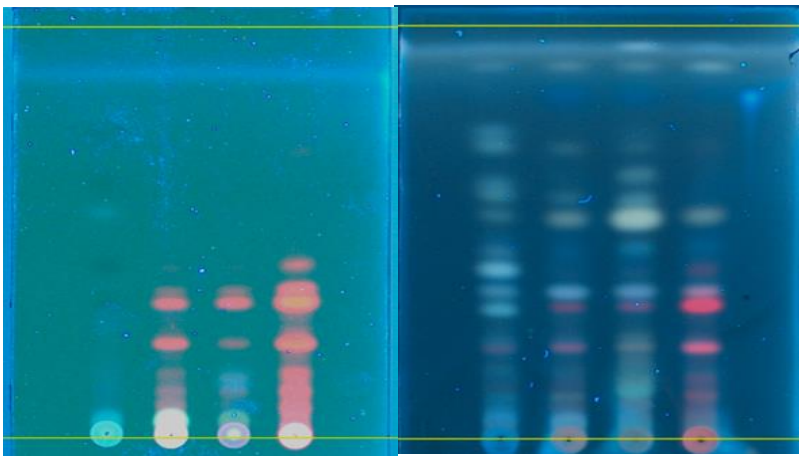

Figure 1. Resuts of identification *C. cinerariifolium* with *TLC Visualyzer*. Silica gel GF254 as Stationary phase, n-hexane: ethyl acetate 4:1 as mobile phase. Root extract (ER); stems extract (ES); flower extract (EF); and leave extract (EL). (A) TLC plates with UV 366 light not derivated, and (B) with derivated.

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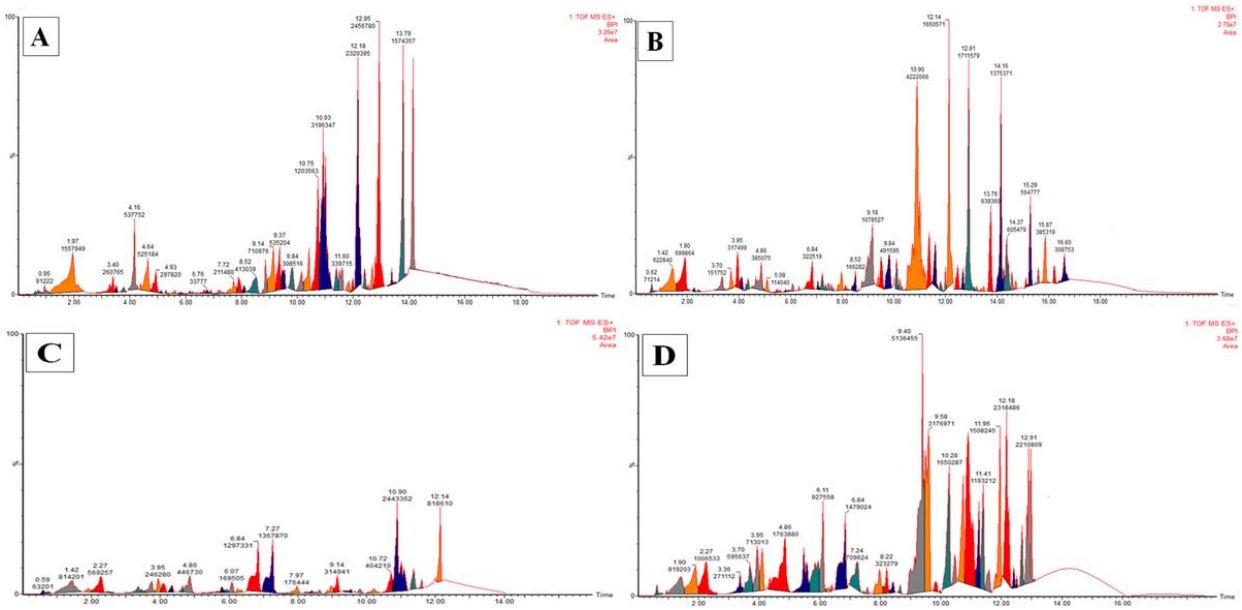

Figure 2. The results of chromatogram *C. cinerariifolium* extract. A) Chromatogram of roots extract; B) Chromatogram of stems extract; C) Chromatogram of leaves extract; D) Chromatogram of flowers extract.

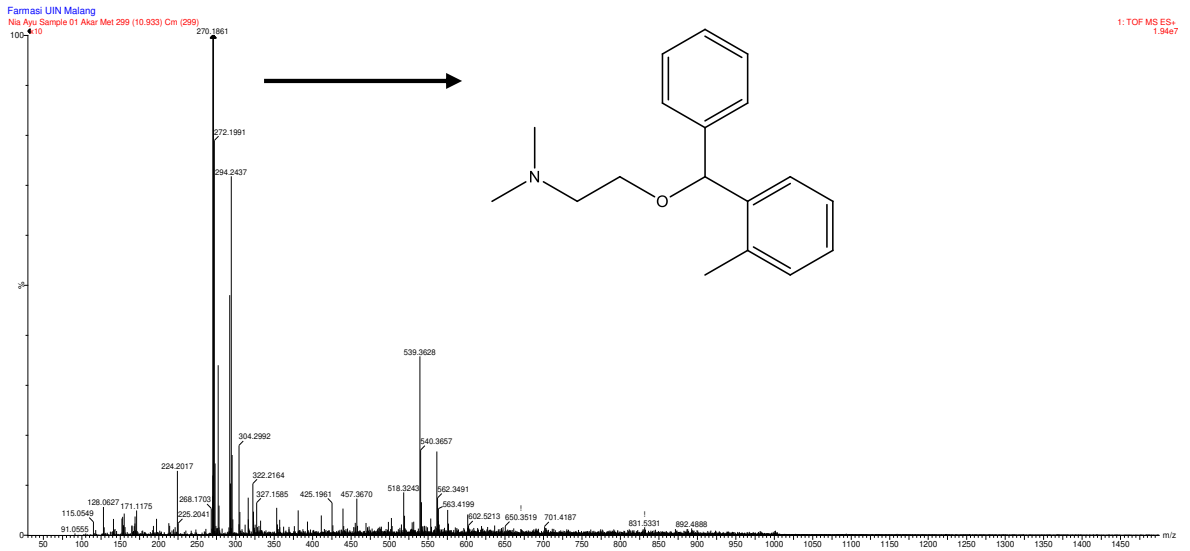

Figure 3. Spectra m/z and structure of *Orphenadrine*

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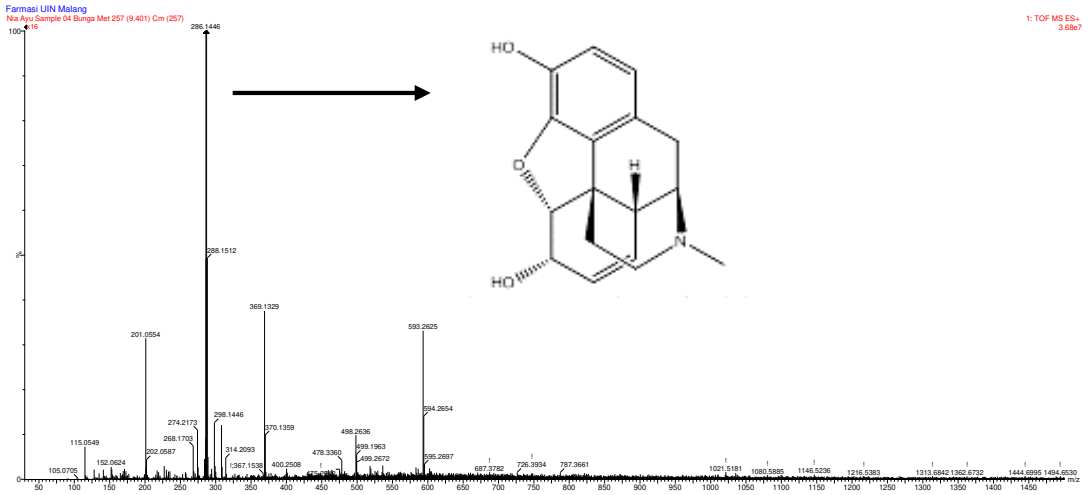

Figure 4. Spectra m/z and structure of *D-(-)-Morphine*

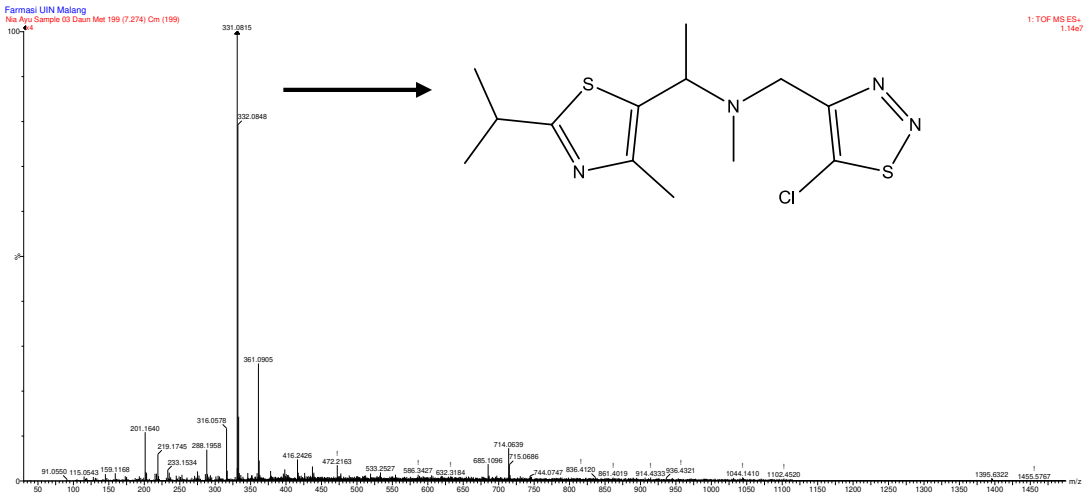

Figure 5. Spectra m/z and structure of *N-[(5-Chloro-1,2,3-thiadiazol-4-yl)methyl]-1-(2-isopropyl-4-methyl-1,3-thiazol-5-yl)-N-methylethanamine*

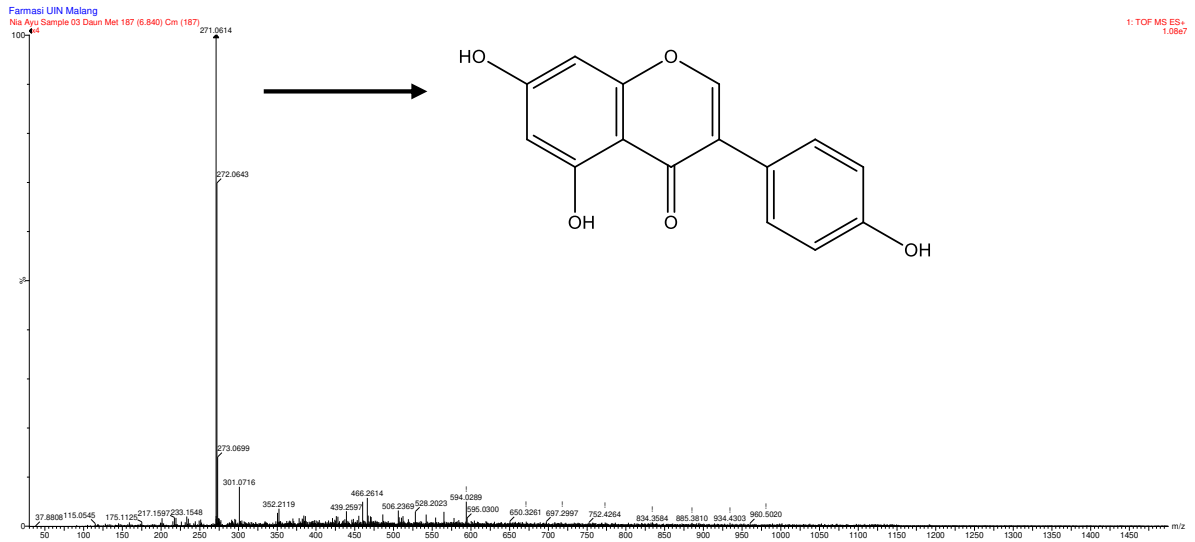

Figure 6. Spectra m/z and structure of *Genistein*

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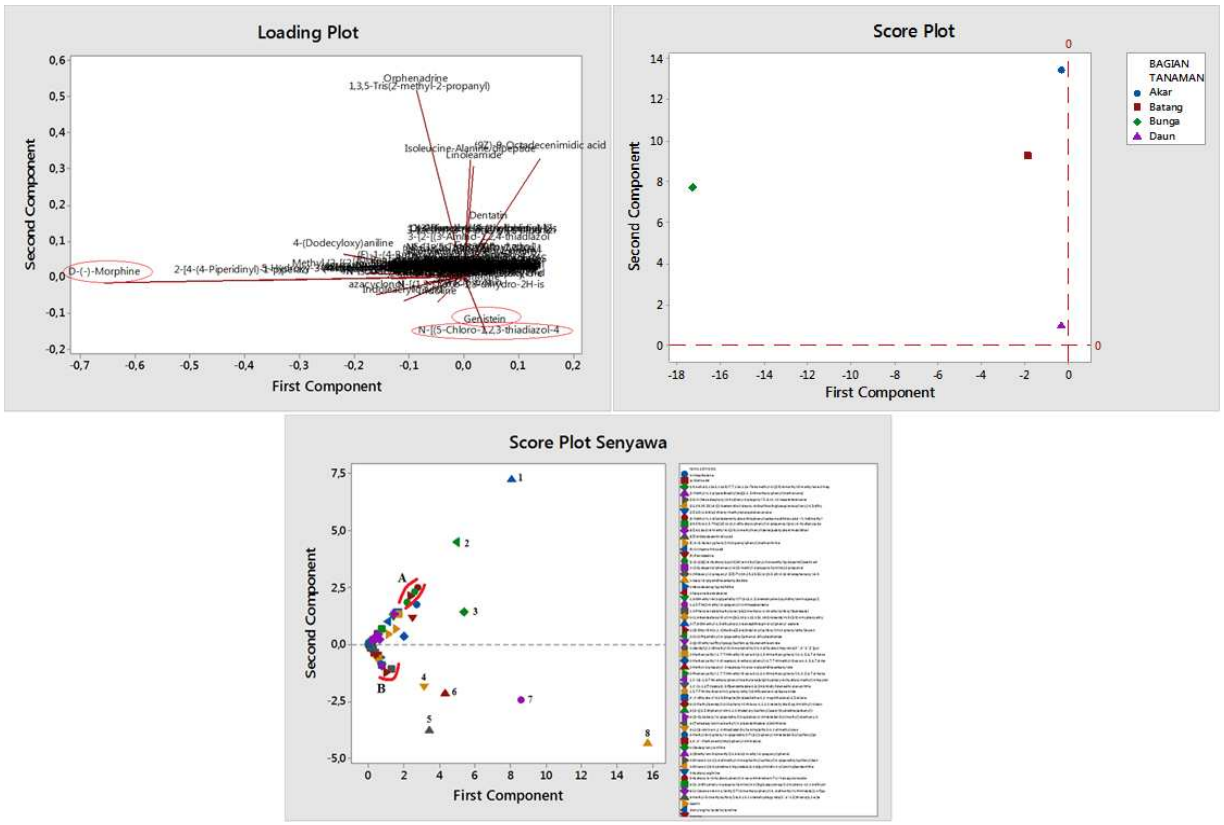

Figure 7. The results of statistical analysis using Principal Component Analysis (PCA). A) Loading plot shows marker compound; B) Show plot closeness compound score on the part of *C. cinerariifolium*; C) Score plot shows compound compounds which have similarity and difference physical and chemical characteristic.

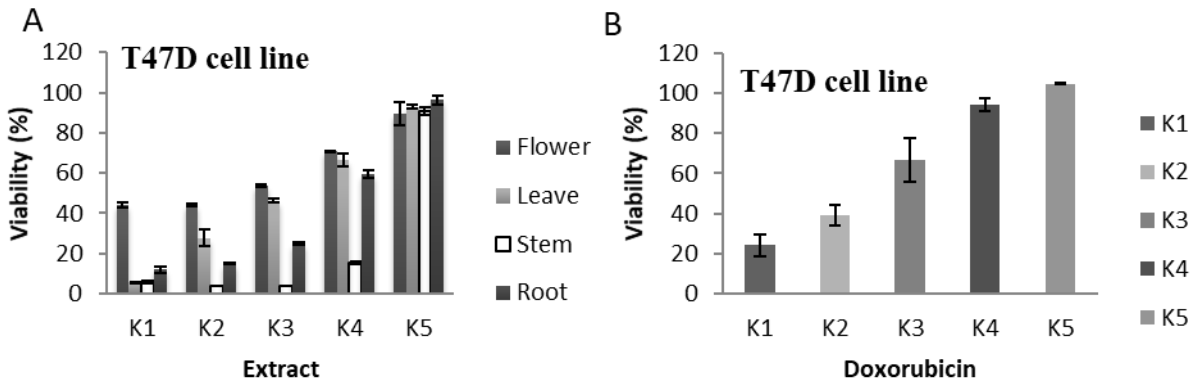

Figure 8. Viability of T47D cells due to the administration of the flowers, stem leaves and roots of extract *C. cinerarifolium* (A). Viability of T47D cells due to the administration of doxorubicin as a comparison.