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BIOACTIVE COMPOUNDS OF RASAMALA (*ALTINGIA EXCELSA* NORNHA) LEAVES AS C-MYC PROTO ONCOGENE EXPRESSION SUPPRESSOR OF HUMAN TONGUE CANCER CELL *IN VITRO*

Risyandi Anwar[1], Arlette Setiawan[2], Supriatno[3], Unang Supratman[4]
[1]Department of Pediatric Dentristry, Faculty of Dentistry, University of Muhammadiyah Semarang, Jalan Kedungmundu raya no 22 Semarang
[2]Department of Pediatric Dentistry, Faculty of Dentistry, University of Padjadjaran, Jalan Sekeloa Selatan no 1 Bandung
[3]Department of Oral Medicine, Faculty of Dentistry, University of Gadjah Mada, Jalan Sekip Utara Yogyakarta.
[4]Department of Chemistry, Faculty of Mathematics and Sciences, University of Padjadjaran, Jalan Raya Bandung - Sumedang Km 21, Jatinangor, 45363, Sumedang

ABSTRACT

Background: *Tongue cancer is a common neoplasm found in oral cavity. It is characterized by aggressive cell growth, poor prognosis and being the cause of mortality.* ***Objectives***: *to discover bioactive compounds of Rasamala leaves which possess an activity to inhibit SP-C1 human tongue cancer cell proliferation by reducing the expression of c-Myc proto oncogene.* ***Methods***: *This is an experimental laboratory study using SP-C1 human tongue cancer cell. Separation of bioactive compounds from Rasamala leaves ethyl acetate extract was using various chromatography techniques guided by antiproliferative assay.* ***Results***: *Two compounds were produced consisting of kaempferol (1) and quercetin (2). Compound 1 and 2 were tested to assess antiproliferative activity of kaempferol and quercetin upon SP-C1tongue cancer cell.* IC_{50} *values obtained from antiproliferative assay of each compound were 0.72 and 0.70 ug/ml respectively. Data analysis using ANCOVA test attained a significant value of* $\alpha=0.05$ *and proceeded for probit analysis. The activity of compound 1 and 2 was tested on c-Myc proto oncogene and it was acquired that compound 1 and 2 can suppress c-myc proto oncogene expression.* ***Conclusion***: *Rasamala compounds consist of kaempferol (1) and quercetin (2) which possess an activity as tongue cancer cell proliferation inhibitor by reducing c-myc proto oncogene expression.*

Keywords: *Altingia excelsa, c-myc, tongue cancer cell SP-C1, kaempferol, quercetin.*

Correspondence : Risyandi Anwar, *Department of Pediatric Dentistry, Faculty of Denntistry, Universitas Muhammadiyah Semarang, Jalan Kedungmundu*

INTRODUCTION

Cancer is one of non-communicable diseases that afflicts the world population including Indonesia. Every year, 12.7 million cases worldwide illustrate the incidence of cancer and 7.6 million people among these cases are reported to die.[1] Basic Health Research survey affirmed that cancer has been the sixth leading cause of mortality in Indonesia with estimating prevalence around 478 per 100000 cases.[2] Oral cancer is one of neoplasm found in the body with 3-5% incidence rate of all cancers occurred in the human body.[3] Six percent of cancers diagnosed in United State every year were oral cancer[4], while the prevalence of oral cancer in Indonesia attained 3-4% from overall cancer incidence. The mortality rate is up to 2-3% among the cases found in Indonesia.[5]

Current cancer therapies are comprised of surgical approach, radiotherapy, chemotherapy and the combination of these remedies.[6,7] Location, type of cancer, stage of cancer, and patient status are several factors used to determine the choice of

therapy. Recently, the use of chemotherapy has frequently became the choice of cancer therapy, including oral cancer which commonly has a small size (<2cm) and an inaccessible location. It is a type of treatment which utilize chemical compounds to act directly on cancer cell but shows equivalent side effect as other treatments. There is an evidence from prehistoric era where humans already used plant extract as the treatment of diseases. As much as 60% of world population depend on traditional medicine and 80% of them are found in developing countries.[8] Herbal medicine plays significantly in the development of synthetic drugs. It is reported that 60% of anticancer drugs and 75% of infectious disease drugs released in 1981-2002 were obtained from natural products.[8] This attracts the interest of many researchers to study about natural products, including those derived from plants.[8]

One of plant families which often studied for anticancer therapy is *Hamamelidaceae*. Several plants from *Hamamelidaceae* family had been studied and showed anticancer activity such as *Liquidambar styraciflua*[9], *L. formosana*[10], and *Corylopsis coreana* Uyeki. [11] Rasamala (*Altingia excels* Nornha) is a plant classified in the family of *Hamamelidacae* and the genus of *Altingia*. It is found only in Asia and grows in tropical plateau areas especially in West Java mountains.[12] Rasamala leaves has been used by Indian community as antipiretic drug, vitality drug, antiinflammatory drug and cough medicine.[12] Meanwhile, Rasamala leaves are commonly used as cough medicine and abdominal treatment amongst West Java community.[12] Kanjilal *et al.* conducted a study using volatile compounds which isolated from Rasamala leaves. It is obtained that Rasamala leaves compounds were comprised of monoterpen as the main compound and sesquiterpen as minor component.[12] Another study conducted by Sharma and Joshi proclaims that Rasamala sap can be used as leukoderma medicine on skin.[12] Close taxonomy in plants such as being classified in one family or one genus can result in the similarity of bioactive compounds and activities.[13] Thus, it is expected for Rasamala leaves (*Altingia excelsa* Nornha) to obtain the same compounds which possess anticancer activity like other plants from *Hamamelidacae* family. Based on scientific review, there has been no evidence reporting about anticancer activity of Ramasala plant compounds. Hence, this becomes the basis for the researchers to conduct this study.

The study of drug derived from natural products which contain chemical compounds and biological properties pharmacologically is an important basic research to discover the effect and its functions. One of the methods used for the development of this study is through the isolation of compounds which is guided by biological testing. Biological testing-guided isolation is a common methodological approach conducted to isolate chemical compound which chastity are monitored through chromatography method and its biological activity is defined by various suitable tests. The discovery of anticancer drug is directed not only to increase selectivity and safety but also to decrease side effect on normal cell. Identification of anticancer agent other than using ethno botany and phytochemical review can be based on cytotoxic investigation in vitro. Antiproliferative assay in vitro can be advanced to observe cellular and molecular level of antiproliferative activity upon molecular target.

Cell proliferation activity is controlled by signal transduction and amplification from receipted environment of cell membrane receptor.[14] It is initiated by the binding between ligands and growth factor receptors on cell surface. One of growth factors contributed in cell proliferation is Fibroblast Growth Factor (FGF). The activation of FGF signal transduction occurred when FGF ligands bind FGF receptors (FGFRs) on cell surface. FGF binding initiate FGFRs dimerization and autophosphorylation from tyrosine residues in intracellular area.[15,16] FGF signal can proceed through Retrovirus-Associated DNA Sequence (RAS/MAPK) which started when RAS binds GTP as a catalyzed reaction by guanine nucleotide exchange factors (GEFs). RAS will activate c-Raf proto oncogene serine/threonine protein kinase to induce the phosphorylation and activation of mitogen activated protein kinase (MAPK) pathway.[16,17] Extracellular signal regulated kinase (ERK) 1/2 is a serine/threonine kinase protein which activated through MAPK pathway. Its activation occurred in the presence of serine and threonine amino acid phosphorylation residues on ERK 1/2 protein by upstream protein, namely Raf. After activated by Raf, ERK 1/2 protein will be

translocated into nucleus to continue signal transduction process and results in the change of specific protein transcription activity.[14] ERK 1/2 plays an important role in the regulation of protein-coding gene expression which functions in cell proliferation, life defense and cell differentiation.[18] It also induces the phosphorylation and the activation of c-Myc, c-Jun and c-Fos which play their roles in the activation of several protein coding gene related to proliferation of the cell. c-Myc protein promotes cell growth by increasing protein-coding transcription, such as cyclin D.[19] It is a protein-coding gene which has a role in increasing G1-CDK activity through the formation of G1-CDK protein complex. G1-CDK protein complex is a protein produced from the binding of Cyclin D1 to CDK4 which functions as a checkpoint in cell cycle so that cell in G1 stage can go to S stage and cell proliferation can occured.[14] This study aims to discover the chemical compounds of Rasamala (*Altingia excelsa* Nornha) plant which can inhibit SP-C1 cancer cell proliferation through the reduction of c-myc proto oncogene expression in vitro.

MATERIAL AND METHOD

Materials and Tools

This study had obtained ethical clearance letter from KEPK FK UNPAD NO 170/UN6.C3.3.2/KEPK/PN/2013. The sample used was Rasamala leaves from Wayang Windu mountains, Pangalengan, Bandung and determined at Plant Taxonomy Laboratorium, Department of Biology, Mathematics and Science Faculty, University of Padjadjaran. Study subject was Supri's Clone 1 (SP-C1) human tongue cancer cell. It is a cancer cell isolated from the lymph nodes of tongue cancer patient, originated from intermediate squamous cell carcinoma which not yet invade muscular tissue.[20] Chemical materials and tools include various solvents, silica gel and common glass tools used in laboratory of organic chemical material. Materials and tools used for activity assay were DMEM, DMSO, trypsine-EDTA, c-Myc assay kit (R&D Systems-USA), ELISA microplate reader (BioRed-Jepang), 96-wells plate (Iwaki-Jepang), 15 mL and 50 mL volumetric flask (Eppendoff-Germany), waterbath (Eyele-Japan), multi-channel micropipette (Effendorf-Germany), incubator 37°C-5% CO^2 (Sanyo-Japan), electronic digital scale (Metler-Swiss), Laminary Flow (Sanyo-Japan), spiritus lamp, microscope (Nikkon Eclipse-Japan), and Camera (Sony-Japan).

Rasamala leaves Extraction

Dried Rasamala leaves (2.5 kg) were macerated using ethanol in room temperature for 24 hours and the macerate was refilled for three times. Macerate was then concentrated by rotary evaporator to produce thick methanolic concentrate. Thick methanolic macerate was then dissolved in water and partitioned using n-hexane to produce n-hexane extract and water. n-hexane extract was separated and concentrated using rotary evaporator to produce thick n-hexane extract. Water layer then partitioned using ethyl acetate to produce ethyl acetate extract and water. Ethyl acetate extract was separated and concentrated using rotary evaporator to produce thick ethyl acetate extract (120 g). Each extract was examined using antiproliferative assay upon SP-C1 tongue cancer cell to discover the most active extract used as anticancer to be isolated for further investigation;

The isolation of ethyl acetate extract as the most active concentrate

Ethyl acetate extract (120 g) was separated using vacuum liquid chromatography (VLC) between stationary phase of G60 silica gel and mobile phase of n-hexane, ethyl acetate and methanol in 10% gradient (v/v). Five fractions (A-E) were obtained after solvents mixture based on thin layer chromatography (TLC) analysis. Antiproliferative activity assay was then conducted to the fractions. From IC_{50} value of antiproliferative assay and statistical analysis, concentrate which shows potential antiproliferative activity was found in D_2 fraction. D_2 fraction (800 mg) was then separated using vacuum liquid chromatography between stationary phase of G60 silica gel and mobile phase of n-hexane, ethyl acetate and methanol in 10% gradient (v/v) after combined using thin layer chromatography (TLC) analysis to obtain five different fractions (D_1-D_5). The five fractions were then investigated using antiproliferative activity assay. IC_{50} value of antiproliferative assay and statistical analysis showed that D_2 fractions possessed potential as

antiproliferative agent. D2 Fractions (800 mg) was further separated using gradient column chromatography between stationary phase of silica gel (70-230 mesh) and mobile phase of n-hexane, ethyl acetate, and methanol to obtain five fractions of D_{21} to D_{25} and then tested using antiproliferative activity analysis. From IC_{50} value of antiproliferative assay analysis, it was obtained that Fraction D_{22} and D_{24} has antiproliferative potential. D_{22} fraction (600 mg) was further separated using column cromatography (CC) between stationary phase of silica gel (70-230 mesh) and mobile phase of n-hexane:acetone (7:3) to obtain an amorphous solid and further recrystallized using the mixture of benzene:methanol (8:2) to obtain compound 1 in white crystal form (7 mg). D_{24} fraction (250 mg) was further separated using column chromatography (CC) between stationary phase of silica gel (70-230 mesh) and mobile phase of n-hexane:acetone (7:3) to obtain three fractions of D_{241} to D_{243} which then tested using antiproliferative activity assay. IC_{50} value of antiproliferative activity analysis showed that D_{243} fraction has potential as antiproliferative agent. D_{243} fraction was further separated using column chromatography (CC) between stationary phase of silica gel (70-230 mesh) and mobile phase of n-hexane:acetone (7:3) to produce amorphous solid and recrystallized using the mixture of benzene:methanol (8:2) to obtain compound 2 in white crystal form (6 mg).

Structure Determination of Compound 1 and 2

Physical structures comprised of colour and melting point of isolated compound were determined. Compound chemical structures were determined using spectroscopy data represented in UV, infared (IR), core magnetic resonance (CMR) and spectra data comparison based on literature.[21]

SP-C1 Tongue Cancer Cell Propagation

SP-C1 tongue cancer cell tissue was inserted into Flash (Falcon, USA) contained DMEM. The flash was then added with 10% Fetal Bovine Serum (FBS, morregate, Biotech, Australia) and 5 ml Fungizone liquid (Gibco, USA). Cancer cell was propagated inside the flash and incubated in 95% humidity and 5% CO^2 at 37°C temperature for 24 hours. DMEM should be renewed every 4-5 days until the number of cancer cells could be harvested. When the flash was overfilled by numerous numbers of cancer cell (can be observed under microscope), medium was then aspirated and washed using 5 ml sterile PBS for 1 minute and then aspirated once again before the following procedure. Trypsin-EDTA 0.25% liquid was inserted for 1 ml in each petri disc and then incubated at 37°C temperature for 5 minutes. Cells were given RPMI to stop EDTA. All cells were harvested and put into 20 ml conical tube. Cells were taken using pipette and inserted inside haemocytometer to count the number of cell per plate.

Antiproliferative Activity Assay

MTT Assay

5 plates with 96 wells were prepared for MTT assay after 24 hours. Each plate was propagated with 1×10^4 cells/wells of SP-C1 tongue cancer cell and then added with 100 uL DMEM (Dulbelco's Modified Eagle Medium) in accordance with compound concentration. All cells were then incubated at 37°C temperature for 24 hours. Each 96 wells were added with 15 µL MTT after 24 hours. Medium was incubated at 37°C temperature for 4 hours. The solvent was disposed and then filled with 100 µL isopropanol liquid. The medium was shaken for 10-15 minutes until the liquid mixed homogenously. 96-wells plate was measured with Bio-Rad Microplate Reader with 540 nm wavelength. Compound with $IC_{50} < 4$ µg/mL is identified as a potential anticancer agent.

c-Myc Expression Assay

c-Myc expression assay was conducted using western blotting method. It is a transferring process of resulted protein from electrophoresis-originated gel to membrane and used for protein detection on tissue sample. The result of electrophoresis antigen was then transferred to nitrocellulose membrane with electrical current assistance. Antigen attached on the membrane will be detected by antibody from the sample. Separated bands can be detected by the presence of color on the membrane. Brighter result showed the protein expression, while darker result showed the presence of suppression in c-myc expression. Western blot was widely used to determine and identify the size of the investigated antigen and antibody. The sample could obtain positive result

because it was a test used to detect protein of specific tested cell only. The sample would obtain a positive result if specific band appeared in accordance to its antigen.

RESULT

The antiproliferative assay showed ethyl acetate extract as the most active concentrate (Table 1), which then proceeded for the isolation process. Guided by antiproliferative assay for each extract and fraction, the isolation procedure was conducted using separating and refining method until two bioactive compounds were obtained.

Compound 1

White crystal, t.l. 277-279 °C, UV (MeOH) λ_{maks} nm: 257 dan 377 nm, (MeOH/$AlCl_3$) λ_{maks} nm 268 dan 349 nm; IR (KBr) v_{maks} 3427, 1640, 1066 dan 800-700 cm^{-1}; ^{1}H-NMR (aseton-d_6, 500 MHz) δ_H (ppm):6,26 (1H; *d*; 2,6 Hz, H-6), 6,52 (1H; *d*; 1,95 Hz, H-8), 8,16 (1H; *d*;9,1 Hz, H-2'), 7,02 (1H; *d*;9,05 Hz, H-3'), 7,02 (1H; *d*;9,05 Hz, H-5'), 8,16 (1H; *d*;9,1 Hz, H-6'). ^{13}C-NMR (aseton-d_6, 125 MHz) δ_C ppm: 147,0 (C-2), 176,6 (C-4), 160,2 (C-5), 99,1 (C-6), 165,1 (C7), 94,5 (C-8), 123,3 (C-1'), 129,1 (C-2'), 115,1 (C-3'), 158,9 (C-4'), 115,1 (C-5'), dan 130,5 (C-6'). TOF MS ES^+ *m/z* $[M-H]^-$ 285,29, $[M]^+$ 286,29, $C_{15}H_{10}O_6$ count was, *m/z* 286,29.

Structure of Compound 1

Compound 1 obtained as white crystal with melting point at 277-179°C. UV spectrum showed particular band absorption for flavonoid compound at λ 363,5 nm (band I) from sinamoil band. Other band absorption appeared at λ 266,5 nm (band II) which showed the presence of π-π* transition from benzoyl band. IR spectrum using KBr plate showed the presence of -OH group which observed at υ_{max}3426,7 cm^{-1}. It is strengthened by the presence of C-O range at υ_{max} 1019,6 cm^{-1}. Compound 1 also contained carbonyl group which manifested by the absorption at υ_{max}1656,5 cm^{-1}. ^{13}C NMR spectrum showed the presence of 15 carbons signal. Based on DEPT 135 data, these 15 groups comprised of six sp2 metins which resonated at the area of δ_C 94,5 – 145 ppm, six oxygenated sp2 quartener carbons which resonated at weak area δ_C 165 ppm,, one carbonyl group resonated at δ_C 176,6 ppm, and two sp2 quartener carbons. This resembles the flavon frame. The presence of flavon frame is also supported by ^{1}H NMR spectrum. ^{1}H NMR spectrum showed the presence of two equivalent protons H-2', H-6' (δ_H 8,16 ppm, d, *J* = 9,1 Hz) which ortho bind with two protons H-3', H-5' (δ_H 7,02 ppm, d,*J* = 9,1 Hz) at B ring. This showed phenyl group with para substitution at B ring. Furthermore, there is also the presence of two protons in meta coupling ((δ_H 6,26 ppm, d, *J* = 2,6 Hz, H-6) and ($_H$ 6,52 ppm, d, *J* = 2,6 Hz, H-8) on A ring. -OH grup position was arranged based on proton coupling pattern of two benzenes group. Carbonyl group resonance at C-4 depend on hydrogen bonding formed between this group and OH group. The presence of OH group at C-5 cause chemical shifting for deshielded C-4, range at δ_C182 ppm. If there is OH group at C-3 next to OH group at C-5, the resonance for C-4 will range between δ_C 171 – 173 ppm which indicate the presence of OH group at C-3. Thus, it can be observed in compound 1 that OH group was bound at C-3, C-5, C-7, and C-4' (Guvenalp *and* Demirezer, 2005). Based on above spectroscopy data, compound 1 was identified as 3,5,7-trihidroxy-2-2(4hydroxyphenyl)-4*H*-chromen-4-on or 3,4',5,7 tertrahydroxyflavon or mostly known as kaempferol (Figure 1).

Compound 2

White krystal. t.l. 315-317 °C, UV (MeOH) λ_{maks} nm 243, 327 dan 424; IR (KBr) v_{maks} 3413, 1606, 1494, 1082 dan 1051 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H (ppm): 6,26 (1H; *d*; 1,95Hz, H-6), 6,52 (1H; *d*; 1,95Hz, H-8), 7,83 (1H; *d*; 1,95Hz, H-2'), 7,00 (1H; *d*; 8,45Hz, H-5'), 7,70 (1H; *dd*; 1,95; 8,45Hz, H-6'). ^{13}C-NMR (aseton-d_6, 125 MHz) δ_C ppm: 146,9 (C-2), 136,8 (C-3), 176,6 (C-4), 162,4 (C-5), 99,3 (C-6), 165,0 (C-7), 94,5 (C-8), 157,8 (C-9), 104,2 (C-10), 123,8 (C-1'), 116,4 (C-2'), 145,9 (C-3'), 148,4 (C-4') dan 115,8 (C-5') . TOF MS ES^+*m/z* $[M-H]^-$300,9638, $C_{15}H_{10}O_7$ count was, *m/z* 302.

Structure of Compound 2

Compound 2 obtained as white crystal with melting point at 315-317°C. UV spectrum of this compound showed three particular bands

absorption at λ 243, 327, and 424 nm. Absorption at λ 266,5 nm showed the presence of n-π* transition from carbonyl band (band R). IR spectrum of these compounds showed the presence of -OH group, C=C double bonding and carbonyl group. ^{13}C NMR spectrum showed the presence of 15 carbons signal. Based on DEPT 135 data, these 15 groups comprised of five sp2 metins which resonated at the area of δ_C 94,5 – 120 ppm, seven oxygenated sp2 quartener carbons which resonated at weak area δ_C 165 ppm, one carbonyl group resonated at δ_C 177,3 ppm and two sp2 quartener carbons. This resembles the flavon frame. Based on ^{1}HNMR spectrum, there were three aromatic protons signal which showed the presence of ABX system at δ_H7,83 ppm (^{1}H, d, J = 1,95 Hz); δ_H7,70ppm (^{1}H, dd, J = 1,95; 8,45 Hz), dan δ_H7,00 ppm (^{1}H, d, J = 8,45 Hz). Proton at δ_H7,70 and 7,00 ppm have J value equals to 8,45 Hz by ortho coupling, while proton at δ_H7,83 and 7,70 ppm has J value = 1,95 Hz by meta coupling. These three protons form trisubstitution benzene which represented B ring in the flavonoid frame (Gurial et al, 2013). Based on spectroscopy data, compound 2 was identified as 3.3',4',5,6 pentahydroxyflavon or quercetin (figure 2).

Compound 1 and 2 were new bioactive compounds identified in Rasamala plant. Compound 1 and 2 were tested for antiproliferative activity (Table 2) and it was obtained that the kaempferol (1) and quercetin (2) had potential as anticancer agent (IC_{50} 0,72 and 0,70 ug/mL). Kaempferol and quercetine as potential compounds for anticancer agent were then tested upon c-myc oncogene. It was showed that there was a reduction of c-myc expression in SP-C1 tongue cancer cell which given kaempferol and quercetion treatments compared to control. Complete results can be seen at figure 3.

Table 1. IC_{50} value of various Rasamala leaves extract.

Ekstrak	IC_{50} (µg/mL)
Methanol	75,41
n-hexane	44,85
ethyl acetate	12,85
Water	18,02

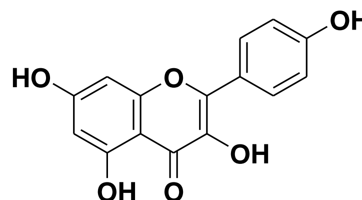

Figure 1. The structure of Kaempferol compound

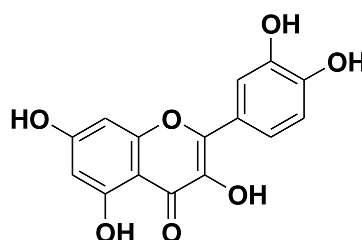

Figure 2. The structure of Quercetin compound

Table 2. IC_{50} value of Compound **1-2** on SP-C1tongue cancer cell

Compound	IC_{50} (µg/mL)
1	0,72
2	0,70

Figure 3. *Western blotting* result of SP-C1 cancer cell

1 : Control
5 : Quercetin 0,39 µg/mL
2 : Kaempferol 0,39µg/mL
6 : Quercetin 0,78 µg/mL
3 : Kaempferol 0,78 µg/mL
7 : Quercetin 1,56 µg/mL
4 : Kaempferol 1,56 µg/mL

DISCUSSION

Kaempferol and quercetin are compounds which classified in flavonoid group found in various plants. Several anticancer activities of kaempferol and quercetin has been reported. A

study conducted on EJ human bladder cancer cell showed antiproliferative activity up to 50% in the concentration of 38,1 µM and 20 µM.[22] Other study on PC-12 cancer cell showed that kaempferol can reduce cell growth up to 50% at 20 µM concentration.[23] A study about quercetin on HCT-116 cell showed antiproliferative activity of quercetin to inhibit up to 50% cell growth at 40 µM concentration.[21] Other study conducted on HeLa cancer cell showed quercetin can inhibit cancer cell growth with IC_{50} value at 13,2 µM concentration and NIH-13 cancer cell at 15,5 µM concentration.[24]

Kaempferol and quercetin can inhibit the proliferation of SP-C1 tongue cancer cell through the presence of hydrogen binding between carbonyl group at C4 atom and hydroxyl group at C5 atom which bind with ser 112 at c-Raf protein. It is known that c-Raf has phosphorylating activity or the ability to contribute in MEK1/2 activation. The inhibition of c-Raf induced by the presence of kaempferol/quercetin will result in MEK1/2 pathway inactivation. The inhibition of ERK1/2 activation will lead to the supression of c-myc expression. This will induce the inactivation of cyclin D-CDK4 binding which is important in cell cycle and resulted in the reduction of cell proliferation. This is supported by a study which stated that hydrogen bind occurred between carbonyl group at C4 atom and hydroxyl group at C5 atom to bind 112 amino acid series presented in Raf protein. Other study concluded that hydroxyl group at C5 atom and carbonyl group at C4 atom affecting cancer cell proliferation via cyclin-CDK inhibition.[25]

Quercetin cytotoxic activity is higher than kaempferol based on IC_{50} value. It is caused by the property of quercetin which is more hydrophobic compared to kaempferol. RAS binding with ATP will activate MAPK cell proliferation pathway. RAS-ATP binding occurred in ATP hydrophobic side. Other conducted study stated that hydrophobic interaction in the occurrence of ATP and RAS binding can be intercepted by quercetin which has hydrophobic properties.[17] This increase an assumption that quercetin was firstly bind ATP which lead to RAS inactivation so that MAPK cell proliferation pathway become inactivated.

In this study, it can be obtained that kaempferol and quercetin can suppress the expression of c-myc compared to control group. Kaempferol/quercetin is hypothesized to be able to bind with FGFR2 on SP-C1cancer cell. FGFR2 receptor on SP-C1 cancer cell is fathomed to experience mutation. FGFR2 mutation is known to cause the loss of FGFR2 binding specificity upon FGF2 ligand and increase FGFR2 binding sensitivity upon non-FGF2 compound. The loss of specificity and the increase of sensitivity of FGFR2 on SP-C1 cell upon non-FGF2 compound is suspected to cause FGFR2 to bind with kaempferol and quercetin so FGF ligand cannot bind to its receptor. The inhibition of FGF and FGFR2 binding cause the inhibition of receptor signal to be received by Ras monomeric G protein which is one of the most activated protein in the presence of external stimulus from the cell. Ras GTP unable to directly interact with several of effectors protein, including Raf protein isoform. Inactivated Raf protein unable to phosphorylate MEK1 and or MEK2. MEK1/2 protein is further inhibited for ERK1/2 activation specifically. The inhibition of ERK1/2 activation by MEK1/2 also can affect transcription factors presented in nucleus to regulate genes expression which contribute in cell proliferation. The inhibition of MAPK pathway by kaempferol/quercetin is suspected to cause phosphorylation of ERK1/2 inhibition so that c-myc expression can be suppressed. c-Myc transcription factor is known to have a role in the increase of cyclin D and cyclin E transcription. These transcription factors will increase G1-CDK (cyclin D1-CDK4) activity and G1/S-CDK (cyclin E-CDK6) in inducing cell to enter S phase in cell cycle. If c-Myc is suppressed by kaempferol and quercetin, the elevation of G1-CDK activity will not occurred. Cell cycle can be stopped in this phase so that the cell can continue to differentiate. The suppression of c-myc expression resulted in cell proliferation reduction.[17] It can be concluded from this study that bioactive compounds from Rasamala leaves are comprised of kaempferol (1) and quercetin (2) which possess an activity as SP-C1 tongue cancer cell proliferation inhibitor by reducing c-myc proto oncogene expression.

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