The Optimization Method for Synthesis of ^{99m}Tc-Rutin as Potential Radiotracer in The Development of Cancer Drugs from Flavonoid

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Abstract

Based on the Basic Health Research Data of Ministry of Health's of Indonesia in 2013, mortality rates from malignant and tumor malignancies in Indonesia are still high with prevalence of cancer is about 1.4%. Chemotherapy is still the primary choice in cancer modality that uses chemotherapeutic drugs to eradicate and inhibit the growth of cancer cells; however the cost this treatment is extremely high. Therefore, patient tends to seek alternative treatment such as consuming traditional herbal medicine for cancer treatment. Rutin is one of the attractive phytochemicals flavonoids because of its antioxidant activities. However, as traditional herbal medicine, its effectiveness is not yet been fully established due to the lack of scientific information. A radiotracer can be defined as a specific radiolabeled molecule that monitors the *in vivo* behaviour of a functional molecule, and can be used to provide biological information in a living system. Hence, to provide pharmacological information of rutin for cancer treatment, we synthesized radiolabeled flavonoid ^{99m}Tc-rutin as radiotracer. The aim of the present study is to develop ^{99m}Tc-rutin under varying conditions of rutin quantity, reducing agent concentration and incubation time. Labeling studies were performed by changing the selected parameters one by one and optimum labeling conditions were determined. After observing the conditions for maximum labeling efficiency, ^{99m}Tc-rutin was obtained with preparation of 700 µg of rutin with addition of 20 µg of $SnCl_2.2H_2O$ as reductor and 1-3 mCi $^{99m}TcO4^-$ without any incubation. Radiochemical yield of ^{99m}Tc-rutin was determined with radio thin layer chromatography which was found 99.28 ± 0.14% and stable up to 4 hour. From the result of this study, the successfully labeled ^{99m}Tc-rutin can be used as a reference for following preclinical study. Furthermore radiolabeled ^{99m}Tc-rutin is expected as tools in research and development of rutin as cancer drugs from natural product to obtain detailed information its efficacy.

Keywords: *radiotracer, ^{99m}Tc-rutin, cancer, labeled compounds*

INTRODUCTION

Based on the Global Cancer Statistic 2012 Data, mortality rates from malignant and tumour

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malignancies in the world are still high (8.2 million deaths) with the prevalence of cancer is about 14.1 million case (Torre, *et al.*, 2015). Chemotherapy is still the primary choice in cancer modality that uses chemotherapeutic drugs to eradicate and inhibit the growth of cancer cells, however the cost this treatment is extremely high. Therefore, the patient tends to seek alternative treatment such as consuming traditional herbal medicine for cancer treatment (Institute for Health Metrics and Evaluation University of Washington, 2017).

Flavonoids are a class of natural compounds which have abundant availability found in plants, these compounds play an important role in the treatment of various disorders because of its pharmacological properties therefore it was an excellent source of pharmaceutical products for phytotherapy. Rutin (3′,4′,5,7-tetrahydroxyflavone-3-rutinoside) (Figure 1) is one of the attractive phytochemicals flavonoids because of its pharmacological activities including anti-oxidation, anti-inflammation, anti-diabetic, anti-adipogenic, neuroprotective and hormone therapy (Lambrecht, *et al*., 2010; Sharma, *et al*., 2013; Chua, 2013). Through its antioxidants activity, rutin has received considerable interest due to their potential roles in the prevention and treatment of cancer by protecting cells against the effects of free radicals. Rutin was apparently inhibiting the process of angiogenesis in cancer cells where this process may stop cancer form new blood vessels which needed by cancer cells to grow and develop (Jain, *et al*., 2012; Dixit, 2014). Rutin is mostly found in edible plants such as onions, apples, berries, tea and wine (Atanassova & Bagdassarian, 2009). However, as traditional herbal medicine, its effectiveness is not yet been fully established due to the lack of scientific information, therefore its efficacy and safety should be evaluated scientifically.

In recent years, kind of researches had reported *in vivo* biological and pharmacological activities of rutin. Most of the pharmacological activities parameters of rutin have been investigated using conventional tools such as High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS). However, those methods require a complicated process, time consuming and costly. Nuclear technology provides several advantages over conventional methods in terms of detection and availability sensitivity. The labeling technique using radioisotopes on flavonoids as radiotracer has been done to determine the biodistribution and pharmacokinetic patterns in some flavonoid compounds (Hosseinimehr, *et al*., 2010; Jeon, *et al*., 2015; Seyitoglu, *et al.,* 2009; Sriyani, *et al*., 2015; Xie, *et al*., 2017; Choi, *et al*., 2016). A radiotracer can be defined as a specific radiolabeled molecule that monitors the *in vivo* behaviour of a functional molecule, and can be used to provide biological information in a living system. Therefore, the radiolabeled rutin as radiotracer will be useful to understanding its antioxidants activity and determine their efficacy and effectiveness as anti-cancer. Previous research on ^{99m}Tc-rutin has been carried out by Bernardo, *et al*., but does not explain the optimum conditions for labeled rutin with technetium-99m radioisotopes

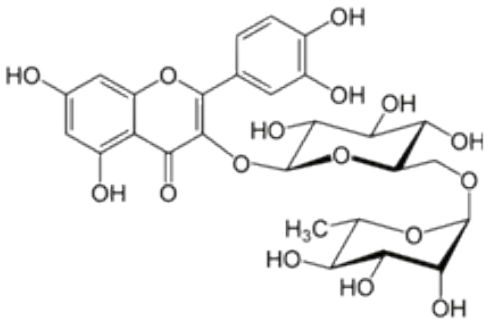

Figure 1. Chemical structure of rutin (Djelili, *et al.*, 2012)

(Bernardo, *et al.,* 2001). The present study was conducted to develop ^{99m}Tc-rutin under varying conditions of rutin quantity, reducing agent concentration and incubation time conditions for maximum labeling efficiency. This study will give the good preparation of ^{99m}Tc-rutin with high radiochemical yield for the development of ^{99m}Tc-rutin as radiotracer.

MATERIAL AND METHODS

The materials that used to this research were rutin (Sigma Aldrich, Steinheim Germany), tin(II) chloride/$SnCl_2$ (Sigma-Aldrich, St Louis USA), ethanol lichrosolv (Merck, Darmstadt Germany), ammonium hydroxide (Merck, Darmstadt Germany), sodium hydroxide (Merck, Darmstadt Germany), hydrocloric acid (Merck, Darmstadt Germany), sodium chloride 0.9 % (IPHA, Bandung Indonesia), sterile aquabidest (IPHA, Bandung Indonesia), pH indicator (Merck, Darmstadt Germany), generator ^{99}Mo/^{99m}Tc (Polatom, Otwock Polandia), ITLC-SG (Agilent, Folsom USA) and TLC-SG F_{254} (Merck, Darmstadt Germany).

The equipment that used in this experiment were dose calibrator (Victoreen, Melbourne USA), vortex mixer, single channel analyzer (Ortec, Canberra Australia), and paper chromatography apparatus.

Labeling of ^{99m}Tc-rutin

Labeling of rutin with radioisotope technetium-99m was performed using direct labeling method using $SnCl_2.2H_2O$ as reducing agent. Some parameters were varying to obtain the optimum conditions such as incubation time, amount of $SnCl_2$ and rutin. The labeling process was carried out by adding of $SnCl_2$ solution (1 mg/mL HCl 0.1 N) into the vial that contains rutin solution in ethanol, then incubated in room temperature for 10 minutes. After incubation time reached, phosphate buffer 0.2N with pH 7.5 was added into the vial. A saline solution of $^{99m}TcO_4$– with an activity of 1-3 mCi was injected into the vial and its volume was adjusted to 1.5 mL. All experiments were carried out in a volume of 1.5 mL and incubation at room temperature.

Determination of Labeling Efficiency of ^{99m}Tc-rutin

The determination of labeling efficiency/ radiochemical purity of ^{99m}Tc-rutin was done using ascending paper chromatography method with ITLC-SG (10 × 1 cm) as the stationary phase and mixture of ethanol:water:ammonia (2:5:1) as the mobile phase to separate the impurities of 99mTc-reduced ($^{99m}TcO_2$) at Rf = 0.0. While to separate the impurity of ^{99m}Tc-pertechnetate ($^{99m}TcO_4$-), using TLC-SG F254 (10 × 1 cm) as the stationary phase with NaCl 0.9 % as a mobile phase where Rf of $^{99m}TcO_2$ = 1.0. The chromatograms were dried in the oven at 80^{o}C, and then every 1 cm piece of paper was cut and measured using Single Channel Analyzer (SCA) with NaI(Tl) detector.

Optimization of Reducing Agent $SnCl_2.2H_2O$

Into five vials containing 500 µL rutin (1 mg/mL) were added varying amount (10, 20, 30, 40, and 50 µL) of $SnCl_2$ (1 mg/mL) and added water to adjust the final volume to 600 mL, then incubation for 10 minutes in room temperature. After incubation time reached, 500 mL phosphate buffer 0.2 N solutions with pH 7.5 were added into the vials. Then a solution $Na^{99m}TcO_4$ with an activity of 1-3 mCi was added. The mixture was shaken with a vortex mixer until homogenous and then incubated at room temperature for 30 minutes. The optimum amount of reducing agent was determined from the labeling efficiency of ^{99m}Tc-rutin using TLC method as described above.This test is repeated as many as three times.

Optimization of Rutin Amount

Into each vial, containing varying concentrations of rutin (500, 600, 700, 800, and 900 µg/500 µL) were added the optimum $SnCl_2$ amount (20 µg/20 µL) and added water to adjust the final

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volume to 600 mL, then incubation for 10 minutes in room temperature. After incubation time reached, 500 mL phosphate buffer 0.2 N solutions with pH 7.5 were added into the vials. Then a solution $Na^{99m}TcO_4$ with an activity of 1-3 mCi/ 400 µL was added. The mixture was shaken with a vortex mixer until homogenous and then incubated at room temperature for 30 minutes. The optimum amount of rutin was determined from the labeling efficiency of ^{99m}Tc-rutin using TLC method. This test is repeated as many as three times.

Optimization of Incubation Time

Labeling was done by adding 20 µL of $SnCl_2$ solution (1 mg/mL) into a vial containing 0.7 mg of rutin that was dissolved in 500 µL of ethanol. To adjusted the final volume to 600 mL was added 80 µL of water and then the solution was incubation for 10 minutes in room temperature. After the incubation time reached, 1-3 mCi/400 µL of $Na^{99m}TcO_4$ solution was added. The mixture was stirred gently until homogeneous and incubated at room temperature for varying periods of time *i.e.*, 0, 15, 30, 45, and 60 minutes. The optimum incubation time will be the time that gives a high labeling efficiency of ^{99m}Tc-rutin. The labeling efficiency was determined when the intended incubation interval was reached; the labeling efficiency was determined using TLC method as described above. This test is repeated as many as three times.

Stability of ^{99m}Tc-rutin in Room Temperature

The labelled compounds ^{99m}Tc-rutin that obtained from the optimum conditions are as follows: Into a vial containing 0.7 mg/500 µL of rutin solution was added 20 µL of $SnCl_2$ solution (1 mg/mL) as reductor and 80 µL of water. The mixture was incubation for 10 minutes in room temperature. After the incubation time reached, 1-3 mCi/400 µL of $Na^{99m}TcO_4$ solution was added. Rutin was successfully labelled using technetium-99m without any incubation time. Stability testing was performed at 0, 1, 2, 3, and 4 hours after adding $Na^{99m}TcO_4$ to see the stability of ^{99m}Tc-rutin. Stability data was then analyzed by using bidirectional ANOVA followed by Tukey analysis with two parameters of time and radiochemical purity.

RESULT AND DISCUSSION

^{99m}Tc-rutin (Figure 2) have been successfully prepared using direct labeling method with-

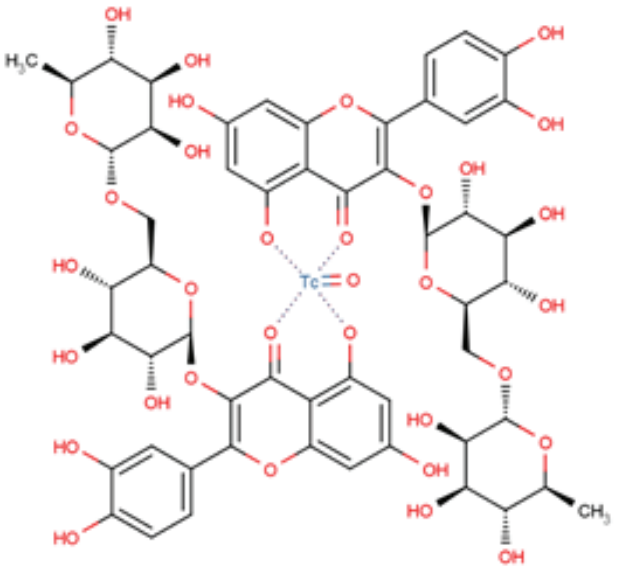

Figure 2. Complexes of ^{99m}Tc-rutin prediction (Tan, *et al.*, 2009; Zhou, *et al.*, 2001).

out addition bifungctional agent. Three parameters which influence the labeling process have been varied to find the optimum labeling condition (Widyasari, *et al*., 2019). In this study pH variations were not carried out like the previous study, because in this study using a phosphate buffer (pH 7.5) which would maintain the pH of the solution around 7. Rutin is a flavonoid antioxidant compound that is insoluble in water and the pH of the solution also greatly affects its solubility. At acidic pH the rutin solubility will decrease while at alkaline pH there will be degradation (Jurasekova, *et al.*, 2014). In addition, at neutral pH the chance of radiolysis will be very small.

The identification of ^{99m}Tc-rutin labeling efficiency was performed by ITLC-SG with mixture of ethanol, water, and ammonia (2:5:1) as the solvent to separate $^{99m}TcO_2$ impurities (Thrall, *et al.*, 1978). In this system, the colloid ($^{99m}TcO_2$) remained in the origin but the free $^{99m}TcO_4$- and ^{99m}Tc-rutin migrated to the top. This statement difference with Bernardo, *et al.*, 2001 which state that "The colloid migrates to the top of the strip due to its apolar characteristics" (Bernardo, *et al.*, 2001). While to separated free $^{99m}TcO_4$- impurities using system TLC-SG with salin as the solvent, free $^{99m}TcO_4$- impurities migrated to the top of the strip that the colloid and ^{99m}Tc-rutin remained in the origin.

To obtained the maximum yield, the first parameter was varied is the amount of $SnCl_2.2H_2O$ as reducing agent. In labeling reaction the presence of a reducing agent is important to decrease oxidation state of ^{99m}Tc (VII) in $^{99m}TcO_4$- to produce ^{99m}Tc complex with the ligand. The result of variation $SnCl_2.2H_2O$ showed in Figure 3 and the highest radiochemical purity of ^{99m}Tc-rutin was reached at 20 µg of $SnCl_2.2H_2O$ with the labeling efficiency 96.44 ± 2.07 %. Figure 3 shows that the higher level of $SnCl_2$ then 20 µg make the radiochemical purity decreased, this is caused by the higher level of $SnCl_2$ make the higher level of ^{99m}Tc-reduce impurities.

The second parameter was the amount of rutin as a ligand. Rutin is a yellow crystalline solid which is insoluble in water. In this study, rutin was dissolved in ethanol. The optimum rutin amount in the labeling process was shown in Figure 4. Figure 4 shows that the optimal rutin amount was 700 µg with the labeling efficiency 95.83 ± 0.90 %. Figure 4 illustrates that radiochemical purity of ^{99m}Tc-rutin was stable in rutin amount higher than 700 µg, this is caused by that almost all technetium-99m present

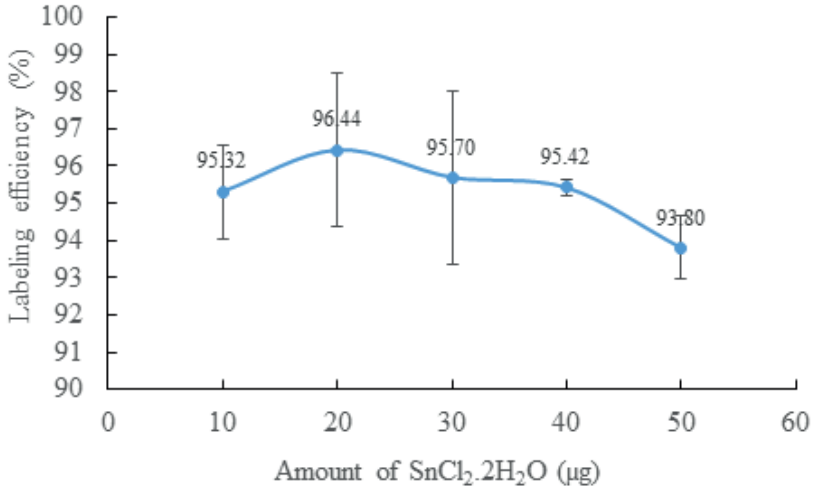

Figure 3. Optimization of $SnCl_2.2H_2O$ amount on the labeling efficiency of ^{99m}Tc-rutin. Labeling efficiency were determine using paper and thin layer chromatography with three times replications.

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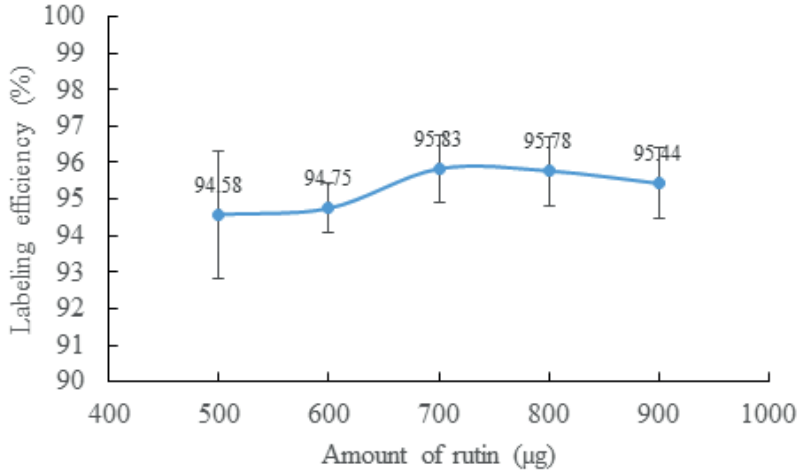

Figure 4. Optimization of rutin amount on the labeling efficiency of ^{99m}Tc-rutin. Labeling efficiency were determine using paper and thin layer chromatography with three times replications.

in the system has bonded with rutin so the addition of rutin has no effect.

The third parameter was the optimum incubation time. The optimum incubation time is the reaction time that results in the highest labeling efficiency. In this study, incubation was done at room temperature to avoid degradation of rutin as antioxidant compounds. The optimum incubation time was shown in Figure 5. From this figure with the three times repetitions data, we can see that the highest labeling efficiency was reached just after the addition of technetium-99m and the labeling efficiency was not much different for up to 60 minutes.

^{99m}Tc-rutin (Figure 2) is rutin compunds (Figure 1) that labeled with $^{99m}TcO_4$- radioisotope is called labeled compounds. The stability analysis

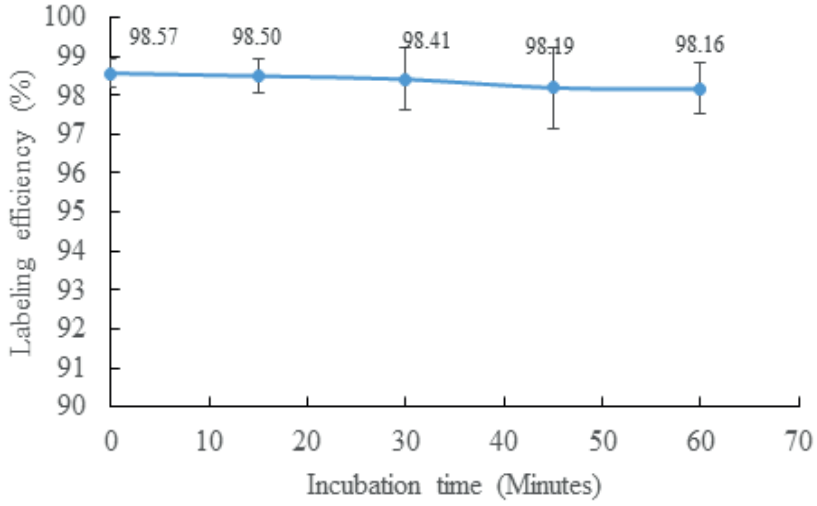

Figure 5. Optimization of incubation time on the labeling efficiency of ^{99m}Tc-rutin. Labeling efficiency were determine using paper and thin layer chromatography with three times replications.

of labeled compound aim to find out information about how long the labeled compound were still good in storage. The quality of labeled compound is determined by the radiochemical purity. The good radiopharmaceuticals are labeled compounds that have radiochemical purity greater than 90 % (Zolle, 2007). The half-life of labeled compound is influenced by the radioisotope that labeled that compound. The stability analysis of ^{99m}Tc-rutin in room temperature showed that until 4 hours storage the labeled compound still has high labeling efficiency (Figure 6). In this study the stability determination of ^{99m}Tc-rutin carried for up to 4 hours, because the half-life of Tc-99m is 6 hours and over 4 hours the radioactivity of ^{99m}Tc-rutin decreased so the radioactivity dose that must be given in preclinical/clinical testing will be different. From the statistical analysis using ANOVA and continued with the Tukey analysis showing the results that the radiochemical purity of ^{99m}Tc-rutin from 0 to 4 hours does not show significantly different results, it is shown that the labeled compounds ^{99m}Tc-rutin is stable up to 4 hours. The information of stability is required when applied in nuclear medicine.

Rutin labeled with technetium already published by Bernardo (Bernardo, *et al.*, 2001) but in the paper was not clearly informed the optimum formulation to produced ^{99m}Tc-rutin with high radiochemical purity. From this study, the optimum formulation to produce ^{99m}Tc-rutin with high radiochemical purity can be known. By obtaining ^{99m}Tc-rutin with high radiochemical purity, it can be used as a radiotracer to study the effectiveness of rutin as an anticancer from natural compound.

CONCLUSION

This study described the method and formulation for preparation ^{99m}Tc-rutin complex. ^{99m}Tc-rutin was successfully prepared with direct labeling method with labeling efficiency was 99.28 ± 0.15 %. The optimum labeled condition obtained from this study was 20 µg $SnCl_2.2H_2O$ as reductor, 700 µg rutin hydrate as a ligand and without incubation time at room temperature. The ^{99m}Tc-rutin complex was stable in room temperature until 4 h after labeling. With this successfully to preparation ^{99m}Tc-rutin there is an opportunity to continue to *in vitro* and *in vivo* study.

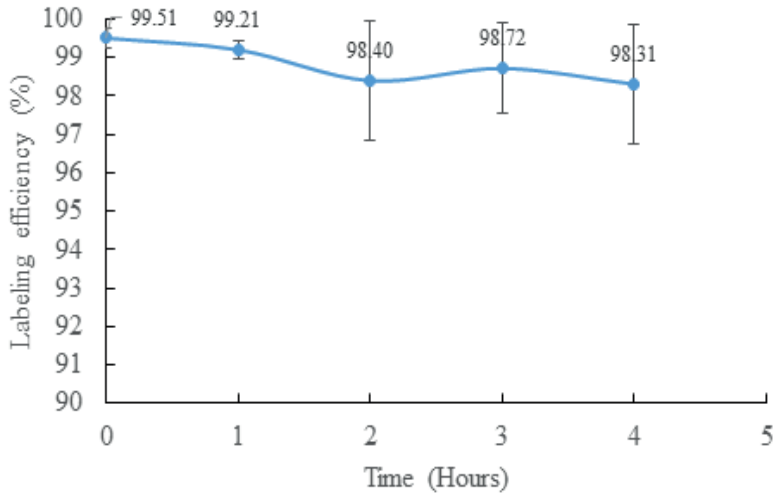

Figure 6. Stability of ^{99m}Tc-rutin at room temperature (24°C). Labeling efficiency were determine using paper and thin layer chromatography with three times replications.

REFERENCES

Atanassova, M. and Bagdassarian, V., 2009, Rutin Content in Plant Products, *Journal of the University of Chemical Technology and Metallurgy,* **44**(2), 201–203.

Bernardo, L.C. *et al.*, 2001, Rutin Labeled with Technetium-99m and its Biodistribution in Wistar Rats, J. *Labelled Cpd. Radiopharm*, **44**(1), 645–647.

Choi, M.H. *et al.*, 2016, Efficient radiolabeling of rutin with125I and biodistribution study of radiolabeled rutin, *Journal of Radioanalytical and Nuclear Chemistry*, **308**(2), 477–483.

Chua, L.S., 2013, A review on plant-based rutin extraction methods and its pharmacological activities, *Journal of Ethnopharmacology*, **150**(3), 805–817.

Dixit, S., 2014, Anticancer Effect of Rutin Isolated from the Methanolic Extract of Triticum aestivum Straw in Mice, *Medical Sciences*, **2**(4), 153–160.

Djelili, H. *et al.*, 2012, Relaxant Effects of Quercetin and Rutin on Human Isolated Bronchus, *Chinese Medicine*, **03**(02), 94–100.

Hosseinimehr, S.J., Ahmadi, A. and Taghvai, R., 2010, Preparation and biodistribution study of technetium-99m-labeled quercetin as a potential radical scavenging agent, *Journal of Radioanalytical and Nuclear Chemistry*, **284**(3), 563–566.

Institute for Health Metrics and Evaluation University of Washington, 2017, Indonesia Basic Health Research 2010.

Jain, S., Dhanotoya, C. and Malviya, N., 2012, Physicochemical Characterization and Determination of Free Radical Scavenging Activity of Rutin-Phospholipid Complex, *International Journal of Pharmaceutical Sciences and Research*, **3**(03), 909–913.

Jeon, J. *et al.*, 2015, Radiosynthesis of123I-labeled hesperetin for biodistribution study of orally administered hesperetin, *Journal of Radioanalytical and Nuclear Chemistry*, **306**(2), 437–443.

Jurasekova, Z. *et al.*, 2014, Effect of pH on the chemical modification of quercetin and structurally related flavonoids characterized by optical (UV-visible and Raman) spectroscopy, *Physical Chemistry Chemical Physics*, **16**(25), 12802.

Lambrecht, F.Y. *et al.*, 2010, Could be radiolabeled flavonoid used to evaluate infection?, *Journal of Radioanalytical and Nuclear Chemistry*, **283**(2), 503–506.

Seyitoglu, B., Yurt Lambrecht, F. and Durkan, K., 2009, Labeling of apigenin with 131I and bioactivity of 131I-apigenin in male and female rats, *Journal of Radioanalytical and Nuclear Chemistry*, **279**(3), 867–873.

Sharma, S. *et al.*, 2013, Rutin: therapeutic potential and recent advances in drug delivery, E*xpert Opin Investig Drugs*, **22**(8), 1063–1079.

Sriyani, M.E. *et al.*, 2015, Iodination Method of Quercetin for Synthesis of Anticancer Labelled Compound, *Procedia Chemistry*, 16(December 2015), 245–250.

Tan, J., Wang, B. and Zhu, L., 2009, DNA binding, cytotoxicity, apoptotic inducing activity, and molecular modeling study of quercetin zinc(II) complex, *Bioorganic and Medicinal Chemistry,* **17**(2), 614–620.

Thrall, Jamese H. Freitas, Jhon E; Swanson, Dennis, Rogers, W. Leslie, Clare, Jean M.; Brown, Manuel L.; Pitt, B. & Brown, M., 1978. Clinical Comparison of Cardiac Blood Pool Visualization with Technetium-99m Red Blood Cells Labeled in Vivo and with Technetium-99m Human Serum Albumin, *J Nucl Med,* 19, 796–803.

Torre, L. a. *et al.*, 2015, Global Cancer Statistics, 2012, C*A: a cancer journal of clinicians.*, **65**(2), 87–108.

Widyasari, E.M. *et al.*, 2019, Preparation of ^{99m}Tc-quercetin as cancer radiotracer in drug discovery, *Rasayan Journal of Chemistry*, **12**(1), 278–283.

Xie, Q. *et al.*, 2017, Preparation and evaluation of131I-quercetin as a novel radiotherapy agent against dedifferentiated thyroid cancer, *Journal of Radioanalytical and Nuclear Chemistry*, **311**(3), 1697–1708.

Zhou, J. *et al.*, 2001, Synthesis, characterization, antioxidative and antitumor activities of solid quercetin rare earth(III) complexes, *Journal of Inorganic Biochemistry*, **83**(1), 41–48.

Zolle, I., 2007, Technetium-99m Pharmaceuticals Dr Ute Heilmann, ed., Vienna: Springer.