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PREPARATION OF ^{99m}Tc-QUERCETIN AS CANCER RADIOTRACER IN DRUG DISCOVERY

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ABSTRACT

Quercetin is a natural compound that has many biological activities to treat various diseases especially as an antioxidant for cancer treatment. This research was conducted to prepare ^{99m}Tc labeled quercetin as a potential cancer radiotracer. The ^{99m}Tc-quercetin complex was prepared with varied parameters to find optimum labeling conditions. In this study, ^{99m}Tc-quercetin was prepared with the formulation of 0.5 mg quercetin hydrate and 30 µg $SnCl_2.2H_2O$ in pH7.5 with the addition of 1-3 mCi ^{99m}Tc. The reaction of complexation was quite rapid within a few minutes in room temperature. The radiochemical yield was confirmed by thin layer chromatography with labeling efficiency of 98.52 ± 0.96 %. These results would make ^{99m}Tc-quercetin continue for following in vitro and in vivo studies to discover its effectiveness as an anticancer from a natural compound.

Keywords: quercetin, technetium, cancer, radiotracer, drug discovery

INTRODUCTION

During the last decade, interest in antioxidants compounds from the plant which can protect the body from several degenerative diseases has increased in the community. This recognition is based on the believed that the substance isolated from edible plants are supposed to be less toxic to the human body. Quercetin (3,3′,4′,5,7-pentahydroxyl-flavone) is a polyphenol flavonoid, categorized as a flavonol (Fig. 1), one of the major dietary flavonoids which are commonly present in various plants [1-2]. The chemical structure of quercetin contains five hydroxyl groups which specify the biological activity of the flavonol compound. Antioxidant activity was due to the presence of double bonds between C2 and C3, as well as hydroxyl groups at positions C3 and C4'. Quercetin has importance purpose in pharmacology such as antioxidant[3-7], neurological effects[8-10], antiviral[11-12], anticancer[13-14], cardiovascular protection[15-17], antimicrobial[18-19], anti-inflammatory[20-21], and hepatoprotective[22-24]. Several studies show that quercetin has a significant role in the inhibition of cancer cells in breast, ovary, prostate, colon, endometrium and lung[14,25], its inhibitory effect on *tyrosine kinase* was supposed for its antitumor therapeutic potentials[26].

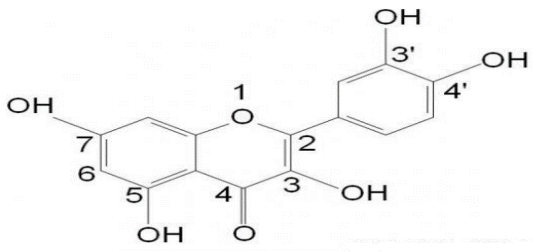

Fig.-1: Chemical Structure of Quercetin

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Nuclear medicine is one of the medical fields which harnesses the nuclear properties of radioactive for diagnostic of anatomic/physiologic conditions of the human body; it also provides therapy with unsealed radioactive sources. Nuclear medicine requires radiopharmaceuticals/labeled compounds in the implementation of patient examination to obtain the results of imaging. A labeled compound can be described as a chemical substance which contains radioactive atoms within its structure and compatible with human administration to diagnose or treat diseases[27]. Quercetin is a chelating agent that able to build complexes with metals[28-30]. This ability as a chelator gives the chance of quercetin being labeled with a technetium-99m radioisotope ($^{99m}TcO_4^-$). Labeled compound of quercetin (^{99m}Tc-quercetin) will interact with free radicals and help to recognize the accumulation of oxidative stress tissue. These free radicals and oxidative stress, both are the primary lead of cancer and other chronic diseases[31].The prior paper presumed the molecular structure of ^{99m}Tc-quercetin in Fig. 2, though the details of the chemistry of molecular structure are not available yet[32]. Previously, a study of technetium-99m labeled quercetin has been done by Sayed et al in 2010 with the radiochemical purity of ^{99m}Tc-quercetin > 99 %, but their study did not identify ^{99m}Tc-reduced ($^{99m}TcO_2$) as radiochemical impurities[28].

The objective of this study was to prepare a labeled compound of quercetin with technetium-99m. The reaction was conducted through the direct labeling method using tin(II) chloride ($SnCl_2$) as reducing agent. Some parameters were observed to find the optimum labeling conditions including a number of quercetin, reaction pH, incubation time, and amount of reducing agent.

This research was expected to obtain the optimum condition of ^{99m}Tc-quercetin preparation which is proficient to be used for the in-vivo study; therefore the effectiveness of quercetin as an anticancer natural compound could be revealed.

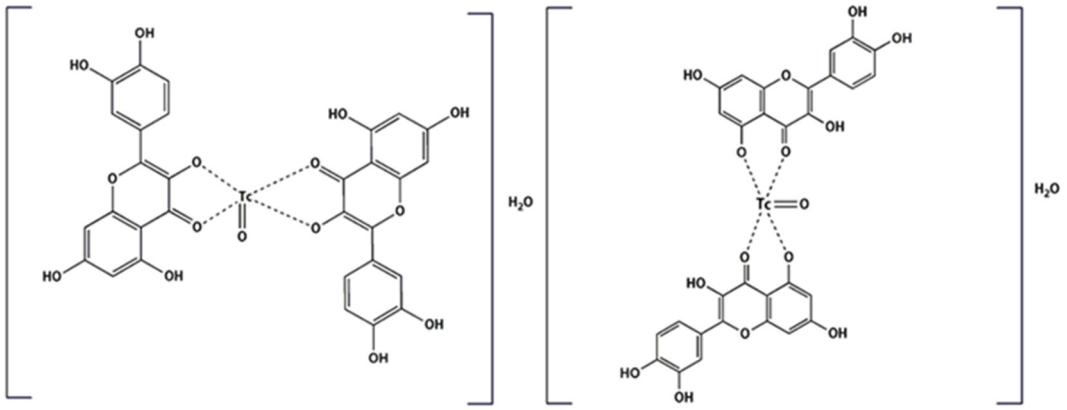

Fig.-2: Complexes of ^{99m}Tc-Quercetin [32]

EXPERIMENTAL

Materials

All of the chemicals in this research were used without any further purification. Quercetin hydrate and tin(II) chloride dehydrate were from Sigma-Aldrich. Acetone, sodium hydroxide, hydrochloric acid, pH indicator and TLC-SG F_{254} were purchased from Merck. Phosphate buffer pH 7.5 was produced in house. ^{99m}Tc/^{99}Mo generator to produce $^{99m}TcO_4^-$ was purchased from Polatom. Dose calibrator (Victoreen) and single channel analyzer (Ortec) were used to measured radioactivity.

Synthesis of ^{99m}Tc-Quercetin

Labeling of quercetin with ^{99m}Tc was performed using direct labeling method. To obtain optimum conditions, the experiment was carried out by varying some parameters such as the amount of reducing agent ($SnCl_2$), ligand, pH reaction, and time of reaction. Each step of the reaction only one parameter varied while the other parameters are fixed. The labeling process was carried out by adding 200 µL phosphate buffer 0.2 N pH 7.5 into five vials containing varying amount (0.2 - 0.6 mg/320 µL) of

quercetin in ethanol and added the varying amount (10-50 µL) of $SnCl_2$ (1 mg/mL HCl0,1 N). Then pH was varying from 6 to 8 by adding 0.1 N NaOH/HCl. After the addition of all reagents, $^{99m}TcO_4$ with an activity of 1-3 mCi was put into the vial. The final volume for all experiments was carried out into 1 mL and incubation at room temperature was done with varying time reaction for 0, 15, 30, 45, and 60 minutes.

Determination of Radiochemical Yield of ^{99m} Tc-Quercetin

Radiochemical yield of ^{99m}Tc-quercetin was determined using thin layer chromatography. To separate impurities ^{99m}Tc-reduced ($^{99m}TcO_2$) at Rf = 0.0 was used TLC-SG F_{254} (10 × 1 cm) as the stationary phase and acetone as the mobile phase. While, to separate the impurity of ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$), NaCl 0.9 % was used as a mobile phase where Rf of $^{99m}TcO_2$ = 1.0. To determine the distribution of radioactivity on chromatograms strips, every 1 cm segments of chromatograms strips were cut and measured using Single Channel Analyzer (SCA) with detector NaI(Tl).

RESULTS AND DISCUSSION

Quercetin is a yellow crystalline solid which is insoluble in water, therefore in this study quercetin was dissolved in ethanol absolute. As well as quercetin, $SnCl_2$ is metal salts that are not soluble in aqueous. $SnCl_2$ could be dissolved in water if the pH is quite low. Therefore in this study, preparation of $SnCl_2$ solution was using HCl 0.1 N to maintain the low pH solution. When $SnCl_2$ dissolved in HCl, a coordination complex of tin-chlorohexacoordinate was formed during the desolvation of $SnCl_2$ in HCl, and the tin kept soluble by the complex[33]. In every experiment, $SnCl_2$ solutions were freshly prepared and purged with nitrogen for 10 minutes.

Four parameters which influence the labeling process have been varied to find optimum labeling condition. The first studied parameter was the amount of the reducing agent. $SnCl_2$ as reducing agent will decrease the oxidation number of ^{99m}Tc-pertechnetate (Tc(VII)). ^{99m}Tc-complex with high yield and purity was only formed when ^{99m}Tc in the lower oxidation state. The result of the variation in the reducing agent showed in Fig. 3, the highest radiochemical purity (RCP) was reached at 30 µg, this $SnCl_2$ amount is equal to Sayed's experiment[28]. If the number of reducing agent is getting smaller or more than 30, it will decrease the RCP but not significantly different.

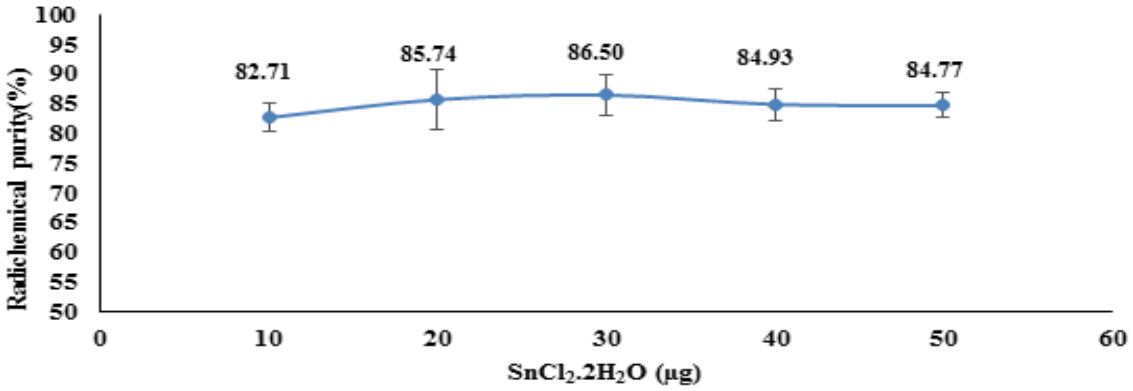

Fig.-3: The Effect of $SnCl_2.2H_2O$ Amount on the RCP of ^{99m}Tc-Quercetin

Variation of reaction pH was carried out from acid to alkaline condition to determine the optimal reaction. The optimum pH needs to be determined because pH does not only affect solubility but also affects the rate of reaction. The RCP reach the highest values at the neutral condition (pH 7.5) as 91.29 % (Fig 4). In this reaction phosphate buffer pH 7.5 was added to the stability of pH reaction and protected quercetin compounds from damage caused by alteration cause of pH. In acid condition, the solubility of the quercetin compound will decrease, while in alkaline condition may occur the degradation of quercetin compound[34].

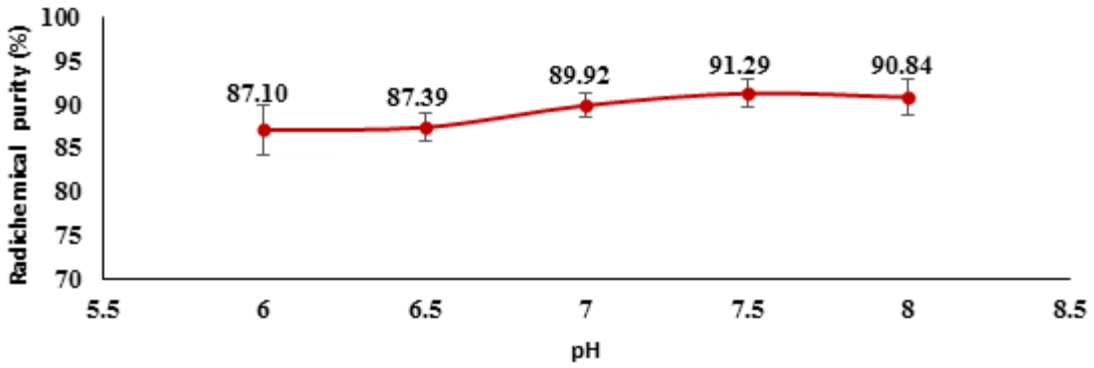

Fig.-4: The Effect of pH on the RCP of ^{99m}Tc-Quercetin

The optimal amount of quercetin in the labeling process was 500 µg with 93.20 ± 0.56 % of RCP (Fig 5). This result is similar to the optimum number of ligand from Sayed's experiment[28]. The optimal amount of quercetin must be determined to find the optimal concentration of ligand so the radiochemical impurities that occur due to deficiencies or excess ligand can be minimized.

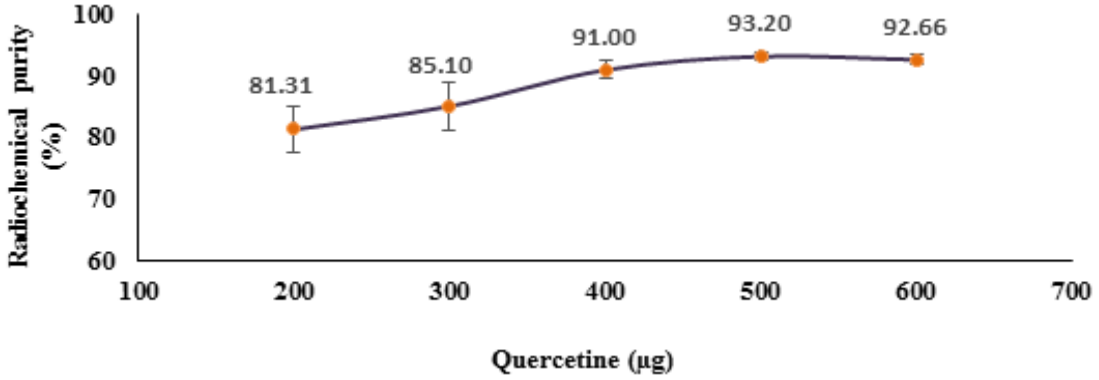

Fig.-5: The Effect of Quercetin Amount on the RCP of ^{99m}Tc-Quercetin

The optimum incubation time is reaction time that has the high RCP. Changing the temperature will affect the rate of a reaction, increase the temperature will increases the rate of reaction. But in this study incubation was done at room temperature, because quercetin is an antioxidant compound that is easy to be oxidized by heat. The optimum RCP was reaching right after adding ^{99m}Tc to the mixture. The complexation process was rapid (Fig.-6), only in 0 minutes the RCP was reached 98.52 ± 0.96 % and decreased continually until 97.36 % in 60 minutes incubation time.

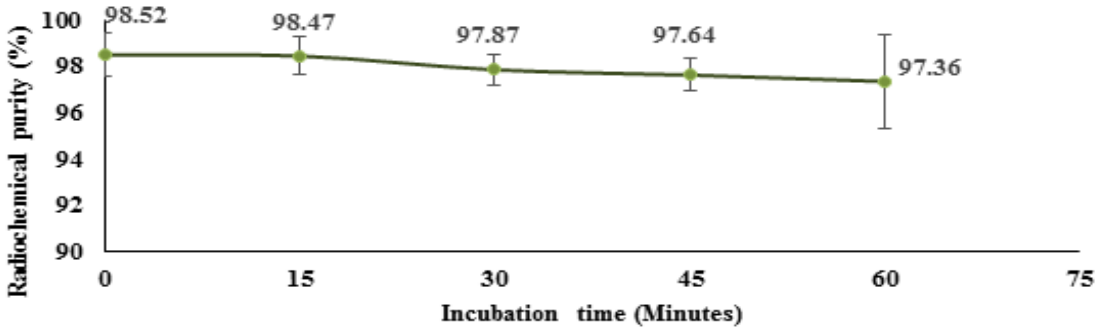

Fig.-6: The Effect of Incubation Time on the RCP of ^{99m}Tc-Quercetin

CONCLUSION

Quercetin was labeled successfully using ^{99m}Tc with a radiochemical purity of 98.52 ± 0.96 %. The optimum labeled conditions obtained with the formulation of 30 µg $SnCl_2.2H_2O$ as reducing agent, 0.5

mg quercetin hydrate in pH 7.5, with incubation for a few minutes at room temperature after addition of $^{99m}TcO_4$. ^{99m}Tc-quercetin could be labeled high efficiency; hence this complex could be continued to in-vitro and in-vivo study to investigate the effectiveness of quercetin as an anticancer natural compound.

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