SYNTHESIS AND CHARACTERIZATION OF IODINATED RUTIN THROUGH OXIDATION METHOD USING CHLORAMINE-T

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ABSTRACT

Aim: In this study, Iodine was used to react with Rutin (quercetin-3-O-rutinoside) and form iodorutin through a direct oxidation method at alkaline pH using chloramine-T as an oxidizing agent. The aim of this study is to synthesize and characterize physically and chemically the iodination product.

Methods: Iodination of Rutin through a direct oxidation method at alkaline pH using chloramine-T as an oxidizing agent and the product then analyzed for physical characteristics such as the shape, solubility and melting point, as well as the chemical characteristics using a UV-visible spectrophotometer, FT-IR, and ^{1}H-NMR.

Results: The results showed that iodorutin was different from its initial state (Rutin). The crystal color was brownish yellow with the melting point at 198-200°C. The product has a cubic shape, hygroscopic and soluble in HCl, NaOH, and water. A new peak appeared at a wavelength of 224 nm on the UV-Visible Spectra, an absorption at wavelength ± 500 nm using FT-IR spectra and there was the loss of 3 chemical shift at 6.20; 6.39 and 6.89 at ^{1}H-NMR spectra.

Conclusion: It can be concluded that iodorutin product of 5',6,8-triiodorutin sodium salt has a cubic shape, hygroscopic and soluble in HCl, NaOH and water. It is successfully synthesized using direct oxidation method with chloramine-T as an oxidizing agent.

Keywords: Iodination, Rutin, labeled compound, synthesis, natural compound.

INTRODUCTION

Cancer is one of the most deadly diseases in the world. In 2012 there were 8.2 million people who died caused by cancer [1]. Some organs which are often attacked by the cancers are lungs, liver, stomach, colorectal, and breast [2]. The majority of cancers are caused by extrinsic factors. These include environmental carcinogens (chemical carcinogen, radiation and virus) and modifying factors (e.g., hormonal imbalance and dietary deficiency) [3]. Foreign substances like cigarette smoke, radiation, drinking alcohol, air and water pollution or ingesting artificial products can lead to higher levels of free radicals in the body. Certain gases and even sunlight can affect the free radical levels in our bodies. Free radicals and ROS are found to be involved in a number of pathological processes such as cancer [3]. Active oxygen may be involved in carcinogenesis through two possible mechanisms: the induction

of gene mutations that result from cell injury and the effects on signal transduction and transcription factors. The mechanism of carcinogenesis depends on factors such as the type of active oxygen species involved and the intensity of stress [4].
They are mainly polyphenolic compounds which inhibit free radical reaction by stabilizing free radicals. One of them are phytochemical. Phytochemicals are phenolic compounds which inhibit free radical reaction by stabilizing free radicals i.e. flavonoids, tannins, terpenoids, catechin, carotenoids etc [5]. Rutin (3,3',4',5'7-pentahydroxiflavone-3-ramnoglukosida; quercetin-3-O-rutinoside) (Figure 1.) is one of the flavonoids that widely distributed in plants such as oranges, grapes, lemons, limes, mulberries, and cranberries. The most source of Rutin can be found in rye and *Fagopyrum esculentum* [6-7]. Previous studies showed that Rutin could be used in the handling of various kinds of chronic diseases such as cancer, diabetes, hypertension, and hypercholesterolemia [8].

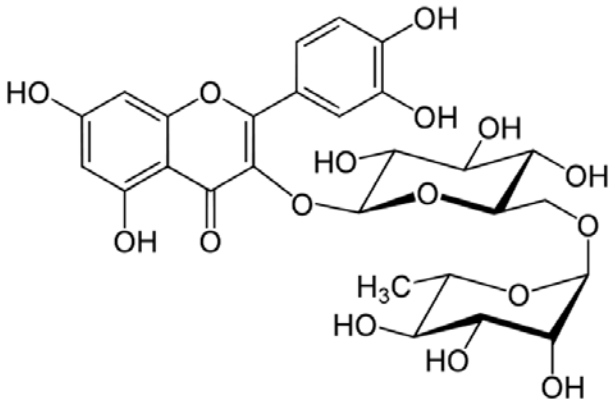

Figure 1: Structure of Rutin (quercetin-3-O-rutinoside)

The variety of Rutin benefits for health makes finding information about biodistribution and pharmacology more interesting. This research is done to determine the bioavalilibity and bioactivity as well as for the development of Rutin as a compound with potential as a natural ingredient-based medicine [9].The radiolabeling of molecules for clinical use has developed a significant benefit. The use of radionuclides in molecular imaging provides vital data on the characteristics of new drugs *in vivo* [10].
Several radioisotopes have been utilized for biodistribution studies of biologically natural product. One of the radioisotopes that most suitables for the labeling of flavonoid including Rutin is radioiodine. The iodine was used because it can be easily introduced to a phenol groups on the substrate with a minimal structural change from the original substrate [9].
The previous research, conducted by Mee Hee Choi et.al, proved that rutin could be reacted with iodine. The reaction was carried out through an oxidation method using chloramine-T with DMSO as a solvent under acidic condition. This condition is not allowed since it will be used for humans. The intravenous injection of DMSO can induce seizures. the responses including inflammatory, hemorrhagic, gelatinous, and edematous tissue reactions, but there is no abscess formation, necrosis, or sloughing [11]. Therefore, this research is conducted in the radioiodination process with a water-based solvent under alkaline conditions [12]. The iodination method was done through an oxidation reaction using chloramine-T as an oxidizing agent [13]. The Research on Iodorutin is still rarely found, especially to find out the groups that react in the iodination of rutin. The novelty in this study is the use of a nonradioactive iodine atom to identify and analyse the structure of the radioiodination product. As a result, the compound product of the reaction of iodination of rutin can be well known.

MATERIALS AND METHODS

Materials

The material used in this research is Rutin (Sigma Aldrich), Chloramine-T hydrate (Sigma-Aldrich®), sodium metabisulphyte (E. Merck®), potassium iodide (E. Merck), sodium hydroxide (E. Merck®), chloroform p.a. (E. Merck®), Saline solution (NaCl 0.9%), and sterile aquabidest. (IKA pharma).

The equipment used to support this research include FT-IR spectrophotometer (Shimadzu, model 02321), ^{1}H-NMR (Agilent, frequency of 500 MHz), melting point (Fisher Scientific), polarization microscope (Olympus, model 9E11152), shaker (Biotech) oven (Memmert), analytical balances (Mettler Toledo), vortex mixer (Retcsh), universal pH indicator (E. Merck), syringe (Terumo), separating funnel, adjustable micropipettes (Eppendorf), tip, and 10 mL vial.

Method of iodination

The solution of Rutin (1.83 mg/mL in NaOH 0.1N) and KI (0.5 mg/mL H_2O) were placed in a 10 mL vial, then added with a solution of chloramine-T (0.69 mg/mL H_2O). The color and pH of the mixture are recorded. The mixture then homogenized for 30 minutes at room temperature, then the color and pH of the mixed solution were recorded by using pH indicator test strip. Then $Na_2S_2O_5$ (1.15 mg/mL H_2O) was added into the mixture. The mixture was transferred into a separating funnel then the liquid-liquid extraction was conducted three times with chloroform. The water phase was taken and moved into a new vial, then dried in an oven (50ºC) overnight. The obtained powder was weighed and observed for the color, microscopic form, and the melting point. Furthermore, the characterization of the chemical structures obtained using UV-Visible spectrophotometry, FT-IR and ^{1}H-NMR.

RESULTS AND DISCUSSION

Flavonoids were labeled using iodine because of the phenolic groups from flavonol can be easily introduced with iodine with a minimal structural change from the original substrate [9]. The iodination generally conducted in the presence of catalytic or stoichiometric amounts. The formation of Iodorutin was carried out by reacting the Rutin, KI, and Chloramine-T with equimolar amounts. Based on the references, the comparison of each compound was 0.3 mmol [12]. Formation of the iodorutin took place in the alkaline solution due its solubility. Rutin act as salt at pH 10. Therefore, the solubility of Rutin has increased in the aqueous solution. The phenyl groups dissociated due to the strong alkaline medium and activating both rings A and B for iodination [12].

Chloramine-T oxidize I^- from KI to I^+ and bind strongly to rutin molecules [14]. The hypoiodous acid (HOI) that formed during the reaction decreasing the pH of the solution. The hydrated iodonium ion, H_2OI^+ and hypoiodous acid, HOI, are believed to be the iodinating species in the iodination process. Iodination occurs by electrophilic substitution of a hydrogen ion by an iodonium ion in the molecule of interest as the equation (1) as follows [15]:

$$R\text{-}I + H_2O^{131}I \rightleftarrows R\text{-}^{131}I + HI + H_2O \quad (1)$$

The free molecular iodine has the structure of $I^{+}–I^{-}$ in aqueous solution. The Species of I^{-} was not reacted with OH^{-} in the alkaline solution [16]. Therefore, to minimizing the amount of the impurities, the oxidation of I- must be stopped by the addition of reducing agent ($Na_2S_2O_5$). The impurities of I_2 was isolating through the extraction process using chloroform [17].

The physical characterization result of rutin iodination shown in Table 1. The results are compared to the rutin hydrate as the initial compound since there has never been any research conducted in the structure determination of iodinated rutin. Based on the result, that there are five different physical aspects. That is the color that turns brown, a slight increase in the melting point, the resulting crystal form, solubility, and hygroscopicity. From the Figure 2 and Figure 3, it can be seen through the polarization microscope that the crystal form of rutin hydrate and iodorutin crystal was totally different. The rutin hydrate showing the dark round spot in the microscope, while the iodorutin showing the cubical shape of the crystal.

Table 1. Physical characterization of Rutin Hydrate and Iodorutin

No	Physical characterization	Rutin Hydrate	Iodorutin
1	Color	Yellow	Brownish
2	Melting point	196 – 198°C	198 - 200°C
3	Shape	Round	Cubic
4	Solubility	DMSO, NaOH, ethanol	HCl, NaOH, H_2O
5	Hygroscopicity	-	+

Figure 2. Rutin hydrate appearance using a microscope polarization 10x magnification

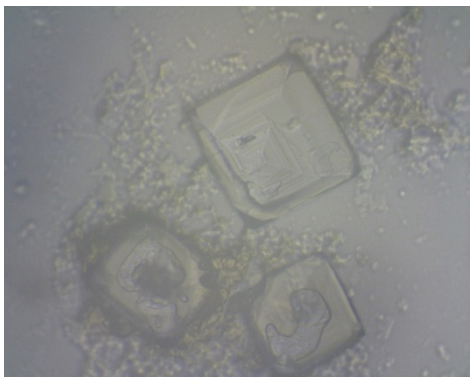

Figure 3. Iodorutin appearance using a microscope polarization 10x magnification

The spectrum of UV-VIS spectroscopy showed in Figure 4. The absorbance peak (Table 2.) in the visible region at 340.2 nm and 331.6 is for cinnamoyl system (B-ring). While the benzoyl system (A-ring) showing the lower absorbance at 265,8 and 266,2 nm respectively for rutin hydrate and iodinated rutin. The absorbance at 224 nm only showed at iodinated rutin. These absorbance caused by n-σ* transitions that are typical for compounds containing one heteroatom (O, N, halogen). The λ-max shifted to lower energies (bathochromic) by 40-50 nm [18].

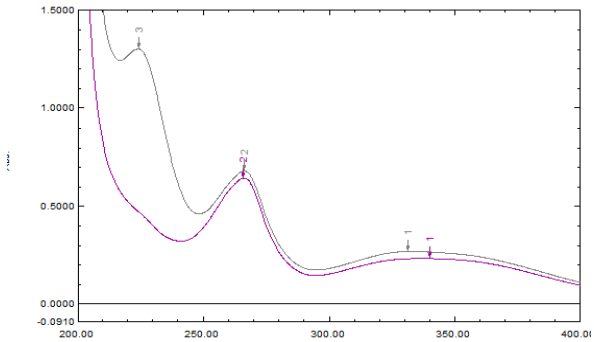

Figure 4. UV-Vis Spectrum comparison of Rutin hydrate and iodinate rutin

Table 2. Spectral Shifts of Band I, Band II and Band III of rutin hydrate and iodinated rutin

Molecules	Cinnamoyl (nm)	Benzoyl (nm)	Halogen (nm)
Rutin hydrate	340,2	265,8	
Iodinated rutin	331,6	266,2	224

Figure 5 shows the FTIR spectrum for rutin hydrate and iodinated rutin. There is a difference between spectra of rutin hydrate and iodinated rutin, especially in the fingerprint area (500-600 cm^{-1}) that refer to the iodinated compound, while the strong wagging band in the absorption area of 1300 -1150 cm^{-1} is for the CH_2I group [18]. The characteristic band at rutin hydrate spectrum at 1270 cm^{-1} is associated with phenolate ion formation, whereas not available in the iodinated rutin spectrum. The most well known broad peak at 3000-3500 cm^{-1} 1600 cm^{-1} refer to the bending vibration of H-O-H that correlated to the presence of the water molecules and O-H that contained in the rutin molecules [19].

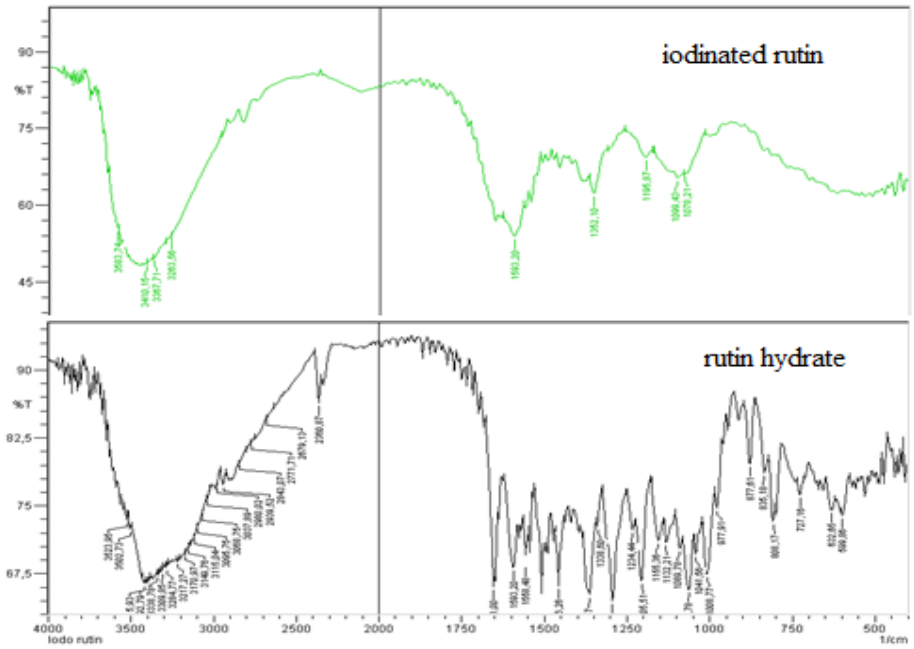

Figure 5. FTIR Spectrum of iodinated rutin and Rutin hydrate

^{1}H-NMR spectroscopy analysis proved to be the most valuable tool for determining the linkage property of the ^{127}I. Since, the functional groups such as ^{127}I can coordinate to the main structure with the more than three possible alternatives [20]. In figure 6, there are the ^{1}H-NMR spectral data assignment for rutin [21], while Table 3 presented the data for proton-NMR of rutin hydrate and iodinated rutin based on the data from figure 7 and figure 8. From the data obtained, there were three protons missed in the H-NMR spectrum of iodinated rutin. It indicates that there are three H atoms to be replaced by iodine at 6.20; 6.39 and 6.89. All three are the H peaks which are bound to each atom C no 8, 6 and 5 '. From all the data obtained via UV-Vis, FTIR and ^{1}H-NMR spectroscopy, Based on the data that has been obtained through UV-Vis, FTIR, and NMR Spectroscopies, possible structures formed from iodinated rutin is as shown in figure 9. A total of three atoms of iodine reacts with one rutin molecule to form sodium salt of 5 ', 6, 8 -triiodorutin.

Table 3. ^{1}H-NMR data for rutin hydrate and iodinated rutin

Rutin Hydrate (σ ppm)	Iodinated rutin (σ ppm)	Assignment
1,12	1,9	3H, CH_3
3,38- 3,56	3.34- 3,56	8H, rutinoside proton and CH_2O
3,62	3,61	1H,CH-CH_3 ramnosa
3,82	3,78- 3,90	1H, CH-CH_2O glukosa
4,51	4,7	1H, OCHO ramnosa
5,10	4,7	1H, OCHO glukosa
6,20	-	1H, Ar-H
6,39	-	1H, Ar-H
6,89	-	1H, Ar-H
7,61- 7,67	7,75-7,76	2H, Ar-H

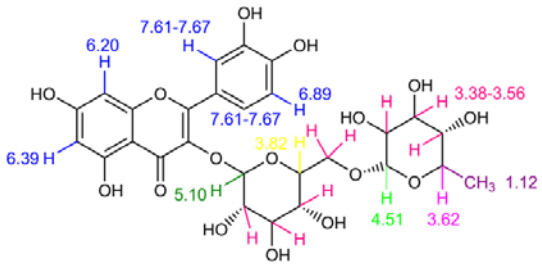

Figure 6. ^{1}H-NMR spectral data assignment for rutin [21]

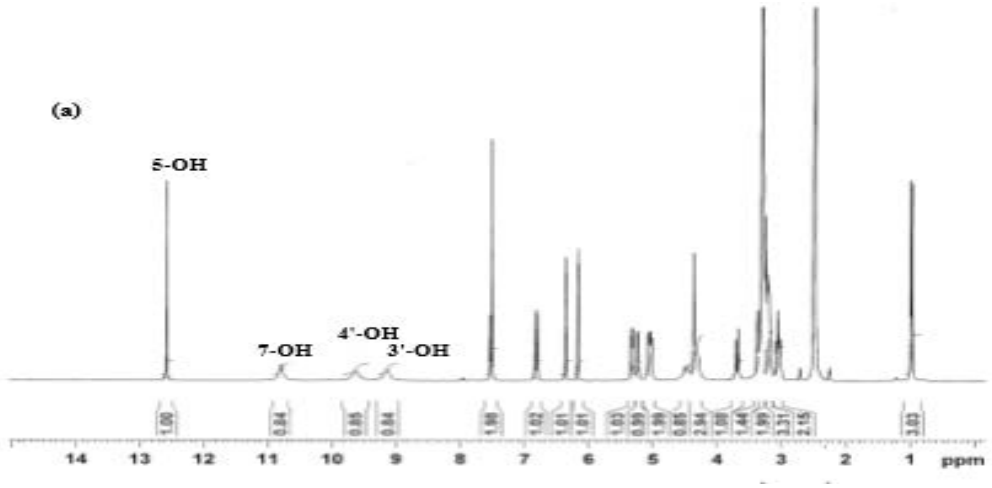

Figure 7. ^{1}H-NMR spectrum for rutin hydrate [19]

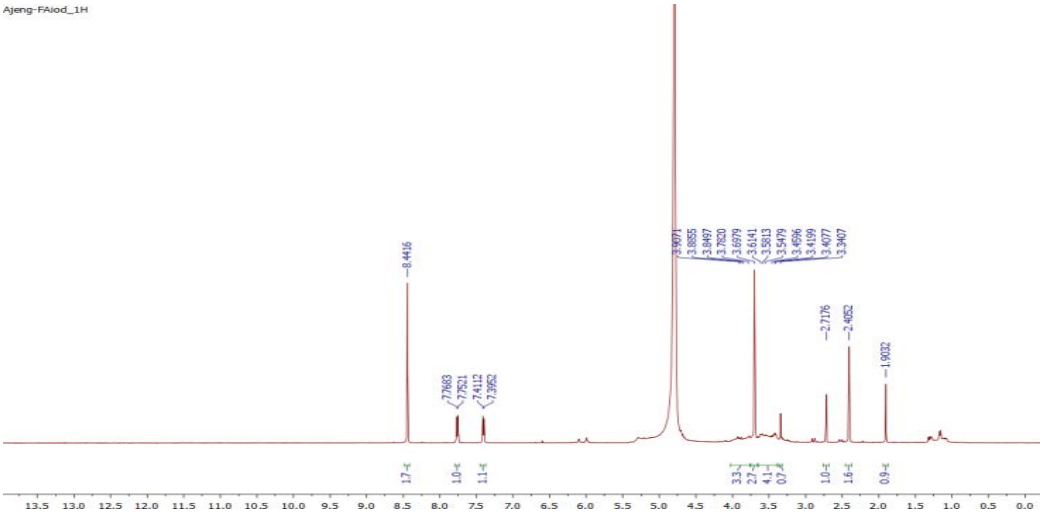

Figure 8. ^{1}H-NMR spectrum for iodinated rutin.

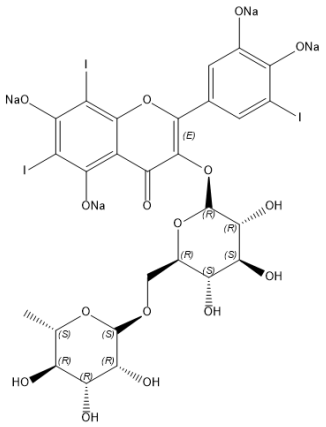

Figure 9. Proposed chemical structure of iodinated rutin.

CONCLUSION

This research corresponds to the preparation and characterization of iodinated rutin. It concluded that this iodination process has successfully synthesized using direct oxidation process using chloramine-T as an oxidizing agent and requires one molecule of rutin to react with three molecules of iodine to formed 5',6,8-triiodorutin sodium salt. The physical characteristics indicate that there are differences between rutin hydrate as the initial compound and the iodinated rutin as the product. The product has brownish crystal color with the melting point at 198-200°C. The cubic shape, hygroscopic and soluble in HCl, NaOH, and water. At UV-Visible spectra, a new peak appeared at a wavelength of 224 nm, an absorption at wavelength ± 500 nm using FT-IR spectroscopy and the loss of 3 chemical shift at 6.20; 6.39 and 6.89 at ^{1}H-NMR spectra.

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