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# Isolation and Confirmation of Quercetin-3-O-Glycosides from Rubber Cassava Leaves

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## Abstract

The core of the natural product chemistry laboratory is isolation secondary metabolites. One of the potential secondary metabolites for isolation in the natural product chemistry laboratory is routine (quercetin-3-O-glycosides). Routine (Quercetin-3-O-glycoside) has been isolated from ethanol extract of rubber cassava leaves (*Manihot glaziovii* MA). Isolation was done by maceration and recrystallization. The isolation method used in this study is complemented by the isolation method published. The isolated (Quercetin-3-O-glycoside) routine using this method obtained a yield of 0.118% of the total dried leaf extract. The routine (Quercetin-3-O-glycoside) was identified using a standard routine. Routine can be further utilized in the world of medicine as an amplifier of capillary structure, reducing the permeability and fragility of blood vessels.

## Keywords

Isolation, Routine (Quercetin-3-O-Glycosides), Cassava Leaves

## 1. Introduction

Secondary metabolites are compounds produced through pathways outside the biosynthesis of carbohydrates and proteins (primary metabolism) [1]. Secondary metabolites are organic compounds produced by plants that do not have a direct function in the process of growth and development of these plants. Often these compounds are considered as by products or intermediates of primary metabolism that have functions as plant defenses, resistance, attracting seed-spreading insects, competing devices, and making valuable contributions to interplants and the environment [2]. Isolation of secondary metabolites from medicinal plants

was examined in a laboratory of natural products [3].<sup>5</sup>

In general, secondary metabolite isolation consists of extraction, fractionation, purification, and explanation structure of secondary metabolites. The same secondary metabolites of plant species can be isolated in various ways, so there is no standard procedure for isolating secondary metabolites from plant species. This isolation activity provides an opportunity for students to design their own experiments [4]. The discovery of a simpler and cheaper secondary metabolite isolation procedure will provide a significant opportunity for the availability of secondary metabolites. One of the potential secondary metabolites for isolation in the natural product chemistry laboratory is routine (quercetin-3-O-glycosides) from rubber cassava leaves (*Manihot glaziovii* MA) (Figure 1).

### Rubber Cassava Leaves

*Cassava* is a type of agricultural plant that is commonly found in rural areas as garden plants or fields which are upright plants in the form of shrubs or small trees whose roots can thicken to form tubers that contain a lot of starch. This cassava has physical characteristics, namely the length of the tree around 50 - 80 cm with a diameter of 2 - 3 cm, the tubers are yellowish, the size of the tubers and the leaves are bigger and wider. Utilization of cassava is usually as an alternative feed by farmers because it is a source of carbohydrates, besides that cassava can grow easily in all types of soil, able to withstand pests or plant diseases, and is rarely consumed by humans because it has a bitter taste and is considered poisonous, so the availability is very much [5].

Rubber cassava (*Manihot glaziovii*) comes from Latin American countries, or rather from Brazil, spread almost all over the world, including Africa, Madagascar, India and China [6]. Currently, many cassava leaves are studied to be developed as raw materials for modern medicine. Rubber cassava leaves can be used as a source of antioxidants to inhibit DPPH free radicals, as an antimicrobial in tuna, help the body's metabolic processes, can facilitate digestion and are good for consumption on a diet in [7]. Phytochemical investigations of the basic ingredients of the drug contained in the rubber yam leaves are quercetin-3-O-glycosides (Routine), other components in the form of blue compounds or cyanide acid compounds (HCN), water, starch and crude protein [8].

Routine (quercetin-3-O-glycosides) can be further utilized in the world of medicine as an amplifier of capillary structure, reducing permeability and fragility of blood vessels [9]. Besides routine can cure various diseases including blood

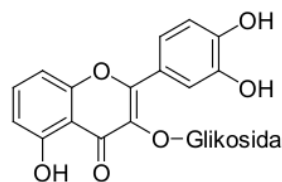


Figure 1. Routine (quercetin-3-O-glycosides) structure.

vessel bleeding (retinal haemorrhage), hypertension caused by increased capillary fragility, bleeding that is hereditary such as Hemophilia, headaches and bleeding gums [8].

## 2. Research Methods

Materials: *Rubber cassava leaves* and all chemicals obtained from the college laboratory

Methods: routine extraction and purification methods (quercetin-3-O-glycoside)

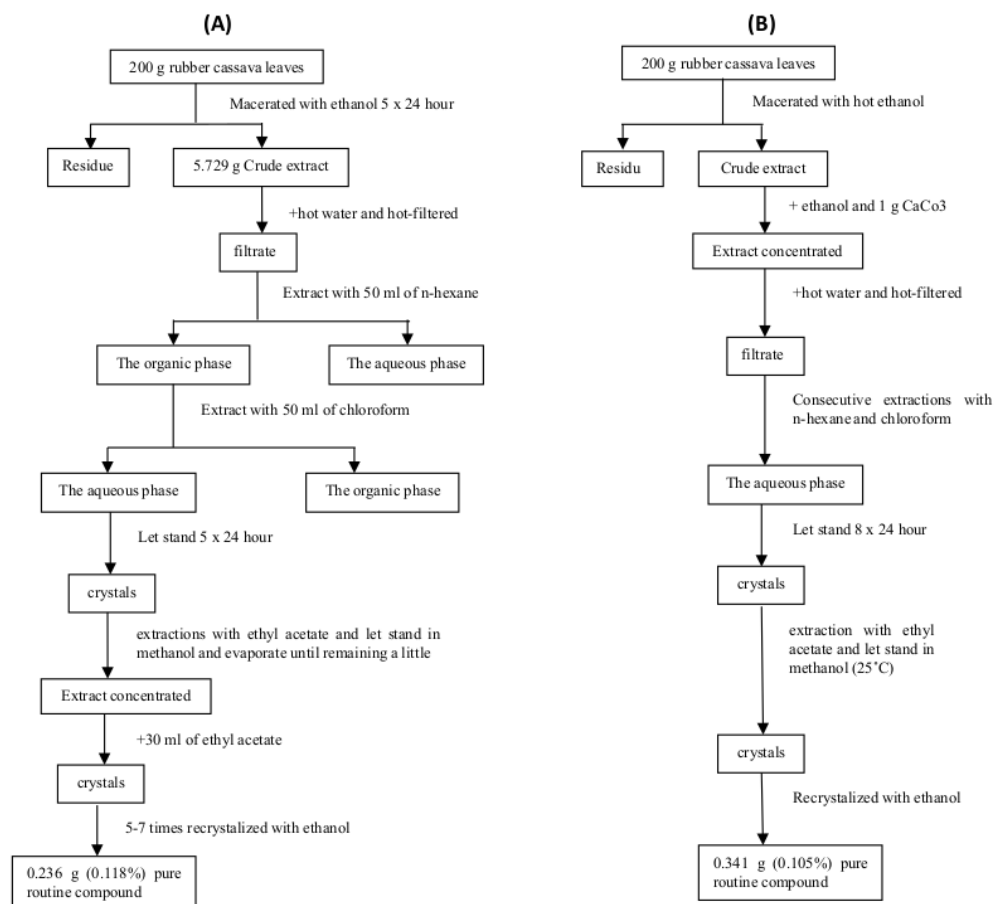
Extraction with ethanol: 200 grams of *Rubber cassava leaves* macerated for 5 days. The solution was filtered and concentrated with an evaporator at 60°C. Added ethanol and 1 gram of CaCO<sub>3</sub> and then re-concentrated. Then added hot water and filtered hot. Extracted with n-hexane, (organic phase) again extracted with chloroform, (the water phase) allowed to stand for 5 days. The formed crystals are extracted with ethyl acetate and allowed to stand in a methanol (concentrated) solvent then ethyl acetate (crystalline) was added. Purification with ethanol: the resulting crystals were recrystallized using ethanol for 5 - 7 times to obtain a pure routine compound. IR spectroscopy was used to identify the isolated compound.

## 3. Discussion

The used materials in this study consisted of routine standards, cassava leaves, ethanol, methanol, n-hexane, chloroform, ethyl acetate, aquades, TLC plates. The tools used in this study consisted of a set of maceration tools, filter paper, separating funnels, and rotary evaporators.

A total of 200 grams of powder of *Rubber cassava leaves* was macerated with ethanol for 5 × 24 hours. The extract obtained was collected and evaporated with a rotary evaporator until it was condensed (5729 g). The Crude extract added to hot water and filtered hot. The filtrate was extracted with n-heksana (the water phase) was extracted again with chloroform (the organic phase) left to stand for 5 × 24 hours. The crystals are extracted with ethyl acetate and allowed to stand in a methanol (concentrated) solvent then the crystalline ethyl acetate is added. The resulting crystals are recrystallized using ethanol for 5 - 7 times to obtain a purely pale yellow routine. The crystals are tested for purity with ethanol: n-hexane (8:2) eluent with R<sub>f</sub> = 0.62. Pure isolated structures were confirmed using routine standards. The results of the standard TLC routine and isolation routine on the TLC plate R<sub>f</sub> values are both the same. Routine standards have spectroscopic data such as IR. The results of the standard R<sub>f</sub> compound showed that the compound isolated was routine (quercetin-3-O-glycoside).

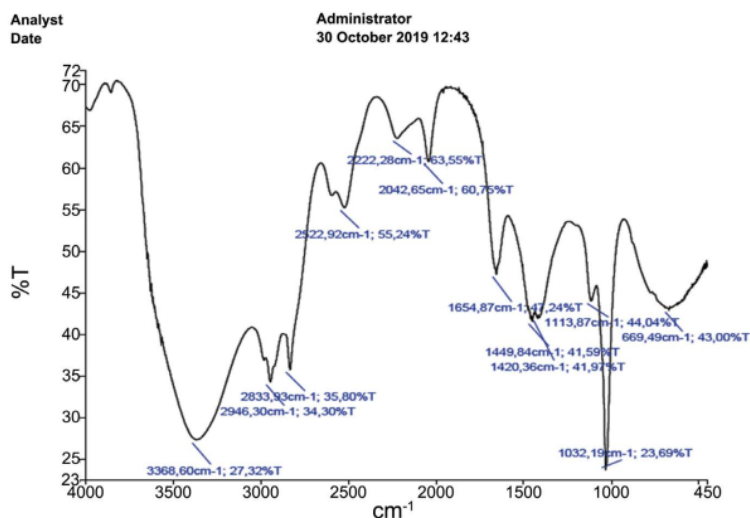
Routine isolation procedure from *Rubber cassava leaves* previously published [8]. Comparison of routine isolation procedures (quercetin-3-O-glycoside) was carried out in this study and reference [8] were shown in Figure 2. It is seen that the isolation procedure yield of reference [8] 0.105% of 325 grams of rubber sweet potato leaves, while the isolation procedure carried out in this study



**Figure 2.** Comparison of isolation procedures. (A) = This research, (B) = Reference [8].

yielded 0.118% yield of 200 grams of *Rubber cassava leaves*. Reference [8] did maceration with hot ethanol while this research did maceration using ethanol at room temperature. In addition to the isolation procedure of reference [8] the time for maceration is not clearly explained, and for the formation of crystals from the water phase the time required is  $8 \times 24$  hours, whereas in this study only requires  $5 \times 24$  hours. Then in the crystal process extracted using ethyl acetate and allowed to stand in the methanol solvent is allowed to stand until crystallized turns out not to be formed. So that in this study the solution to that problem is given by evaporating routine extracts in methanol until concentrated then adding ethyl acetate, forming routine crystals. The crystals obtained were purified using recrystallization with ethanol for 5 - 7 times. The isolated crystalline analysis was carried out using IR spectroscopy. IR spectrum of isolated compound was shown in Figure 3.

The IR spectrum (KBr) shows the absorption for the main functional groups of a flavone, namely the absorption at  $1655 \text{ cm}^{-1}$   $\nu_{\text{max}}$  for conjugated C=O



**Figure 3.** Spectrum IR rutin (quercetin-3-O-glycoside).

stretching vibrations, and the absorption at 1620, 1547, 1449, and 1420  $\text{cm}^{-1}$  stretching vibrations for aromatic CH stretching vibrations. The presence of aliphatic OH and CH groups of the isolated compound was seen at the absorption of 3368  $\text{cm}^{-1}$  and 2946  $\text{cm}^{-1}$ , respectively. The presence of COC groups from the glycosides was seen at 1032  $\text{cm}^{-1}$  absorption. The IR spectrum shows that the isolate is rutin (quercetin-3-O-glycoside). Based on the explanation above the rutin isolation procedure (quercetin-3-O-glycoside) carried out in this study is simpler than the rutin isolation procedure (quercetin-3-O-glycoside) which has been previously published [8]. Rutin (quercetin-3-O-glycoside) is isolated using a procedure carried out in this study which has a high amount (0.118% of the total extract). Rutin can be further utilized in the world of medicine as an amplifier of capillary structure, reducing the permeability and fragility of blood vessels [3].

#### 4. Conclusion

The rutin isolation procedure (quercetin-3-O-glycoside) in this study is simpler than the procedure previously published, which in this case had a slight error in one of the isolation steps and through this research it was found the solution of the problem. Rutin (quercetin-3-O-glycoside) is isolated using a procedure carried out in this study which has a high amount (0.118% of the total extract).

#### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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