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Research on the isolation and characterisation of 3-ramno-glycoside quercitine from *Rosa canina*

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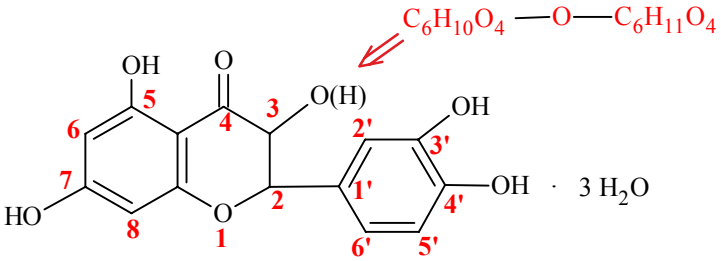
Abstract

In this paper we aim at valorising natural bioactive principles from the spontaneous flora belonging to the geographical areas in Western ***România (Timiş*** and ***Caraş-Severin Counties)*** by isolating and characterising ***3 – ramno – glycoside quercitine*** (rutin) from dog rose (*Rosa canina*). We accessed different solvent systems for the solid/liquid extraction (alcohols, acetone, benzene, etc.) and then we replicated re-crystallisations. We obtained yellow needle-like crystals that we characterised chemically and physically and chemically. Confirmed extraction yields and purity recommend dog rose (*Rosa canina*) as a source of rutin of alimentary and/or pharmaceutical quality.

Keywords: flavone, rutin, vitamin P, quercitine

1. Introduction

Natural flavones present, in general, in the flowers, roots, stems, and flowers of many plants supply over ***50*** derivatives as such or their glycosides.



(I)

$(C_{27}H_{30}O_{16} \cdot 3\ H_2O)$ **O)** M = 610,51 (CAS 153 - 18 - 4)

(3, 3', 4', 5, 7 – pentaiydroxy – flavon – 3 – rutinoside)

To note common buckwheat (*Fagopyrum esculentum*), Japanese pagoda tree (*Sophora japonica*), and dog rose (*Rosa canina*) widely spread over the plain areas in Western ***România***.

Rutin (rutoside, quercetin – 3 – rutinoside) (I) is a major component of the group of ***vitamins P (citrine)*** with vitamin components that also act on blood capillary permeability similar to those of hesperidine, esculine, quercitine, etc. It has a beneficial impact on taste and flavour, and it is potentially antioxidant in the preservation of the polyunsaturated lipid fractions in

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foodstuff whose nutritious value it also enhances.

Muszynsky achieved water extraction of rutin from different vegetal materials at ***100°C,*** with the same yield as in the variant based on ethylic alcohol ***[1]***.

Krewesen & Concle obtained rutin from fresh and dehydrated plants or exclusively from dehydrated plant powder in industrial conditions. As extraction solvents, they used methanol, ethanol, isopropanol, and acetone (at normal temperature or close to boiling point temperature) of different concentrations. In fresh plants, they recommended hot isopropanol ***85%*** extraction for about ***10 minutes***. Dehydrated powder can also be extracted with cold or warm isopropanol ***70 – 85%***. To purify ***5g*** of dehydrated raw extract, dissolve it in ***1 L*** hot H_2O, added ***2.5 g*** silica gel, we stirred energetically for ***1 – 2*** hours and we filled ***[1]***.

El Ridi & Strait extracted ***rutin*** with ***ethylic alcohol 90%***, that they later evaporated in void at ***60°C***. Rutin slowly precipitated in time (completed after ***2 days) [3]*** from the syrupy mass thus resulted.

Bognar obtained rutin from the sainfoin (*Onobrychis sativa*). The dried and divided vegetal matter was extracted with warm alcohol. Partially evaporated until syrupy, it was dissolved in water and recaptured with benzene to remove chlorophyll through replicated rinsing. After void concentration, the solution crystallised pre rutin (a few crystallisation germs were introduced). Recrystallisation from alcohol ***20%*** or from a larger ***100 times*** amount of water led to tri-hydrated crystalline rutin under the form of green – light yellowish "long needles" ***[4]***.

Valentin & Wagner recommended the following method of isolating and purifying rutin: ethylic alcohol extraction ad recrystallisation from acetic acids or from alcohols. Purification was done by transfer of the ether solution on layer (column) filled with Al_2O_3 and later elution with methanol. Besides rutin ***(2 – 3.5%)***, they also identified tannins ***3.8% [5]***.

Miller obtained rutin from vegetal raw matter by pressing cell destruction, ad advanced refrigeration of the "juice" thus obtained at ***pH = 7 – 7.5***. The precipitate thus obtained contained rutin, chlorophyll, carotene, and vitamin ***E*** that were separated from the solution by centrifugation and dried at temperatures that were not above ***200°C***. The raw product obtained after removing carotene and chlorophyll extraction with ethylic alcohol or hot water) was accessed to remove rutin (recrystallisation from ethylic alcohol or from acetic acid) ***[6]***.

Horhammer et al. separated rutin from fruits of *Betula humilis* with methanol (***24 hours***). The extract thus obtained and concentrated in void, treated with hot water crystallised pure rutin ***(1.4%) [7]***.

Saprometow recommended obtaining vitamin ***P*** from tea fruits. Catechines with activity enhanced by vitamin ***P*** were extracted in the chloroform system to which ***1.5 – 2%*** alcohol was added, and later was extracted with alcohol ***96%*** when caffeine, resin, and most of the vegetal pigments are removed. The extract evaporated up to ***10 – 25%*** dry matter has the aspect of a greenish powder ***[8]***.

Schimake & Westhoff extracted rutin by percolation of divided dog rose fruits with methanol ***98%*** at room temperature. The percolation product later energetically stirred with CCl_4 was added under water stirring for the mass ratio (CCl_4:H_2O=1:2). After separating the phases, the methanol layer containing rutin was filtered and distilled in void to remove methanol. After cooling the residue, rutin deposits under the form of crystals ***[9]***. According to ***[10]***, rutin was isolated from raw alcoholic extracts methanol, ethanol) by treating with aromatic or aliphatic hydro-carbons (xylene, benzene, CCl_4) with minimum addition of water for crystallisation.

Spada & Cameroni isolated rutin from the pollen of silver wattle (*Accacia dealbata*) by precipitation with neural lead acetate

and chromatographic separation of rutin myricetin – 3 – glycoside on open column. For the mutual separation of the pigments, they also used the combined paper and column chromatography method ***(Cellulose – Whatman & Amberlit IR – 4B) [11]***.

Fedorowitsch & Wedeneijew obtained rutin from buds of Japanese pagoda tree (*Sophora japonica*) by boiling in water ***(1 ½ hours)***, with later filtering of the hot solution in void through a device ***(NUCE)***, and the clear solution was refrigerated at ***5 - 8°C, 24 hours***. They separated rutin by crystallisation and centrifugation ***[12]***.

2. Experimental part

2.1 Materials, reactants

- ***dog rose leaves*** (different stages of vegetation) from two geographical areas, i.e. 6 harvesting points ***(Făget***, ***Lugoj***, ***Buziaş) (Timiş County)*** and ***(Berzovia, Bozovici, Caransebeş) (Caraş-Severin County)***;
- ***dog rose fruits*** (physiological maturity) (same geographical areas);
- ***organic solvents a.p. (Sigma Aldrich)***;
- ***rutin sample (Sigma Aldrich) (CAS 153 – 18 – 4)***.

2.2 Working method

Variant 1

Isolation of 3 – ramno – glycoside quercitine by solid/liquid extraction from vegetal matter (figure 1)

Freshly harvested vegetal matter (leaves, green fruits) was divided with a stainless steel knife and, in the case of the dehydrated product with a disc mill.

Seventy g of freshly dried vegetal matter was solid/liquid in a ***Soxhlet*** installation for ***3 hours*** at ***70°C*** with ***350 mL*** of methyl alcohol ***90%***. The extract thus obtained ***(340 mL)*** centrifuged for ***10 minute (65 mL*** clear fluid) was transferred (about ***85 mL***) into an ***Erlenmeyer*** recipient with rode cap ***(600 mL)*** and treated with ***412 mL*** of ethylic ether, while the other half was put into a similar recipient to which ***412 mL*** benzene was added. After ***2 – 3 minute***, we could note the appearance of the first crystallisation germs in both recipients, with the difference that in the ethylic ether system the crystals developed normally, while in the benzene system a fine crystal matter appeared. Crystal suspension was filtered and later washed with ethylic ether and benzene, respectively, until the rinsing solutions became clear. The crystals were transferred on a clock glass and dried quickly but in a managing way in the absence of light, and later stored in a cool place in close tight dark colour recipients.

Variant 2 (figure 2)

The fresh, moist vegetal matter (leaves, green fruits) was divided with a knife ad, in the case of dehydrated matter, with a disc mill.

Seventy g of fresh or dehydrated vegetal matter was extracted in warn atmosphere in a ***Soxhlet*** device for *about* ***3 hours***, at ***60°C***, with ***350 mL*** absolute methyl alcohol. The cooled extract thus obtained ***(341 mL)*** was transferred into a ***Berzelius*** recipient of ***1000 mL*** to which we added by energetically stirring ***114 mL*** of carbon tetrachloride and ***227 mL*** water for ***10 minutes***.

The suspension was decanted, the green chlorophyll extract separated, and the methanol phase transferred into a void distillation installation (water trunk 10^{-1} – 10^{-2} ***mm col. Hg***) for concentration. In the concentrated solution, after cooling at room temperature we introduced crystallisation germs and left the system ***about 24 hours***, after which it was filtered, the crystals were dried in a managing way over an air bath at ***30 - 40°C*** in a dynamic system (blower). Extraction yield was ***85%***, and quercitine 3 – ramno – glycoside purity ***95 – 98%***.

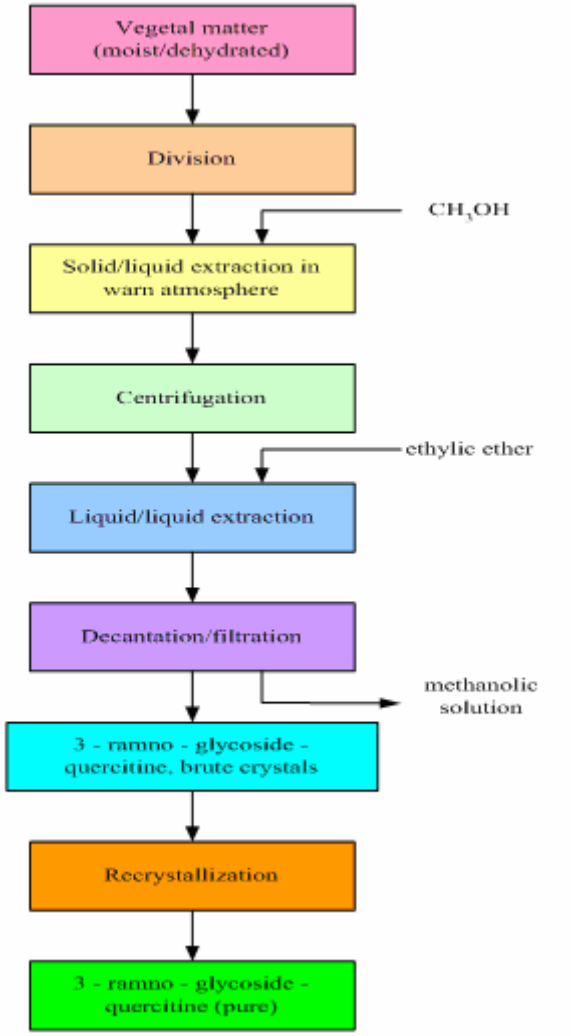


Figure 1 Operation block scheme in the solid/liquid extraction process (methanol/ethylic ether operation variant) of 3 – ramno – glycoside quercitine from dog rose (Rosa canina)

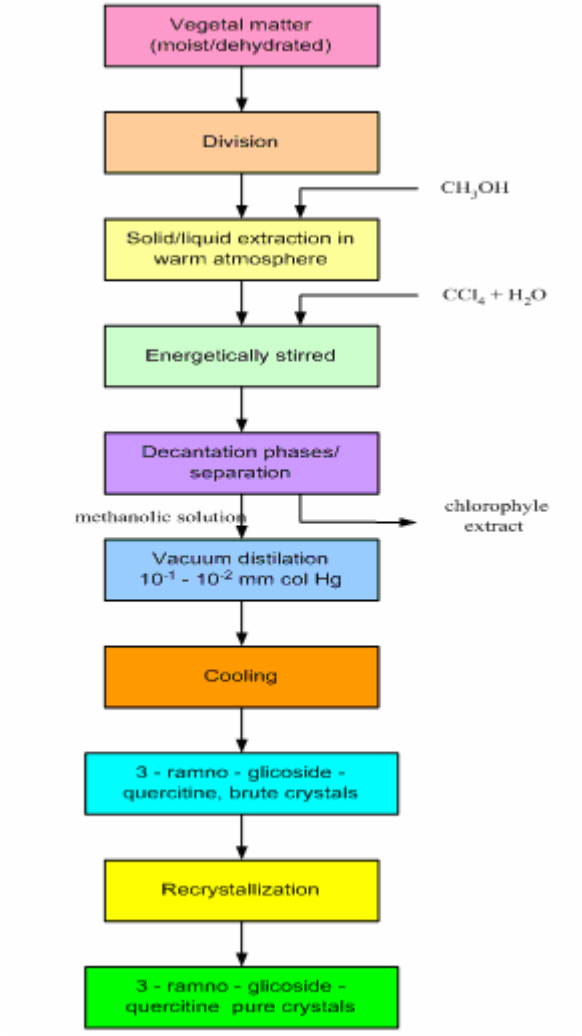


Figure 2 Operation block scheme in the solid/liquid extraction process (methanol/carbon tetrachloride operation variant) of 3 – ramno – glycoside quercitine from dog rose (Rosa canina)

Colourimetric determination of quercitine 3 – ramno – glycoside

Variant 1

Three mL filtered methanol solution of quercitine 3 – ramno (obtained by solid/liquid extraction) was transferred into a ***50 mL*** balloon and completed with absolute methanol. We left for ***10 minutes***, and then filtered through filter paper (removing the first portions). From the clear filtered solution we transferred ***50 mL*** in the ***25 mL*** balloon adding ***5 mL natrium acetate 10%*** and ***3 mL aluminium chloride 2.5%*** (stirring each time another additive is added) and completed with methanol. We assessed solution coloration extinction after ***15 minutes*** in a colorimeter ***(430 nm)*** compared to a control sample prepared from ***5 mL acetate solution 10%***, ***3 mL aluminium chloride 2,5%*** completed in a ***25 mL*** balloon with methanol.

Tracing the sampling curve (figure 4)

An amount of ***0.01 g of pure 3 – ramno – glycoside quercitine (CAS 153 – 18 – 4)*** weighed with analytic precision was transferred into a ***100 mL*** balloon and completed with methanol. In three ***25 mL*** balloons we dropped ***1, 2*** and ***3 mL***

solution. In each balloon we added ***5 mL natrium acetate 10%*** and ***3 mL aluminium chloride 2.5%*** stirring each time another reactant was introduced. We completed with methanol and energetically stirred the balloon. We assessed coloration intensity at ***430 nm (figure 3)*** compared to the control sample.

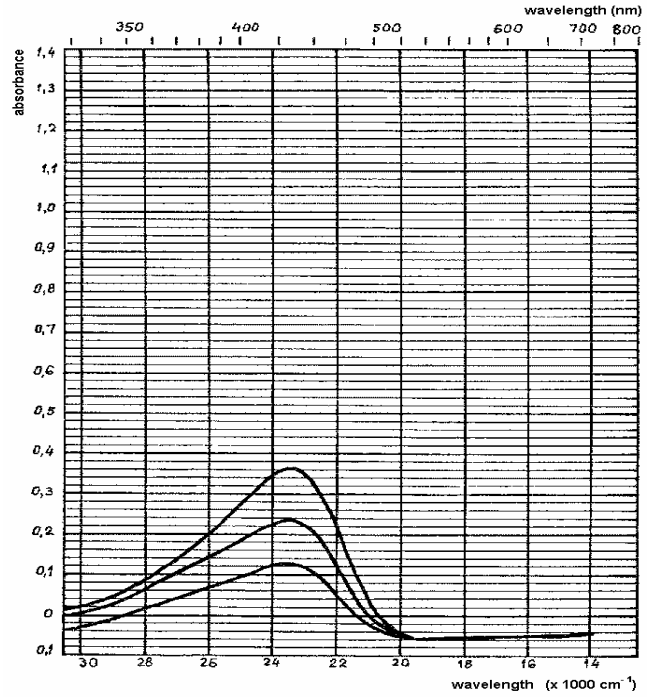


Figure 3 UV – VIS adsorption scheme of the solutions of different concentrations of 3 – ramno – glycoside quercitine

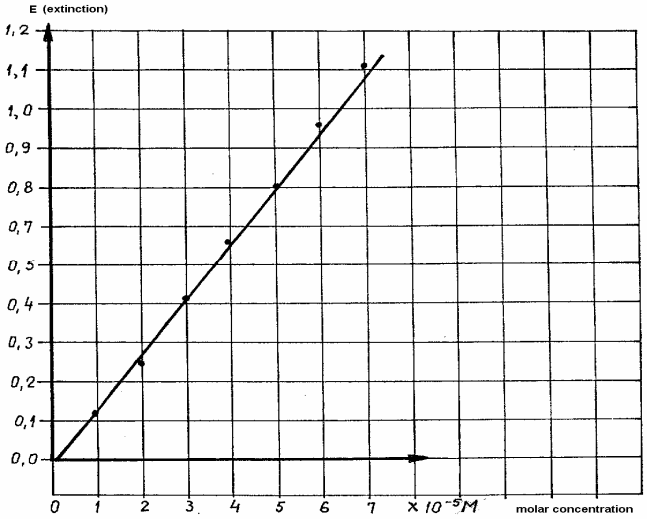


Figure 4 Sampling curve of 3 – ramno – glycoside quercitine

3. Results. Discussions

In this research, we aimed initially a choosing the most efficient system of rutin solid/liquid extraction solvents from fresh (moist) and dry (carefully dehydrated vegetal matter. We could see that extraction yield is higher when the matter is moist ***(92%)*** than when it is dehydrated ***(87%)***, probably due to the decomposition of the bio-active principle during the drying process (ethylic ether processing variant). Assessing comparatively the solid/liquid extraction efficiency in similar conditions

[temperature, mass ratio moist vegetal matter/extraction solvent (ethylic ether ***(1/1)*** number of solid/liquid extraction cycles ***(3)***] between the different organs of the dog rose (*Rosa canina*) (leaves, fruits, flowers) we could see there are significant additional differences between fruits and leaves at maturity (ripening) ***(90%)***, on one hand, and flowers ***(85%)***, on the other hand. Among different vegetation periods [budding, growth, maturity (ripening)] there wee optimal yields ***(91%)*** in the budding phase and at maturity phase maybe due to the role of the active as antioxidant and/or growth factor of other plant components.

Between the different geographical areas, the samples in different vegetation stages and in the different plant organs did not confirm representative differences under similar conditions, which could suggest the similarity of agro-pedo-climatic conditions of the areas under study.

The solid/liquid extraction system proved more efficient in the first separation variant (ethylic ether ***92 %***) than in the second one (methanol/carbon tetrachloride) ***(88%)***.

Similar high thermal sensitiveness of other poly-phenol structures s lower tan when isolating vitamin C.

The number of solid/liquid extraction cycles under identical operation conditions [temperature, mass ratio moist vegetal matter/ethylic ether ***(1/1)***, dog rose leaves] increases the isolation yield up to three recirculations, after which the amount of isolated product remains practically constant and does not justify economically the continuation of the process.

Among the factors that decisively influences the isolated content and content purity, light and atmospheric oxygen affects negatively final results qualitatively and quantitatively). To note the browning of the needle-like crystalline mass, which can be explained if we take into account the poly-phenol character of rutin which confers strong self-oxidation competence (similar to pyrogaol).

4. Conclusions

Experimental results confirm the importance of dog rose (*Rosa canina*) as a carrier of alimentary and pharmaceutical usefulness (3 – ramno – glycoside quercitine) and recommend systematic continuance of research on recipe valorisation of bio-active principles of the spontaneous flora in Western Romania.

In this research, we have also tested another method of determining rutin extracted from the vegetal matter, following these steps:

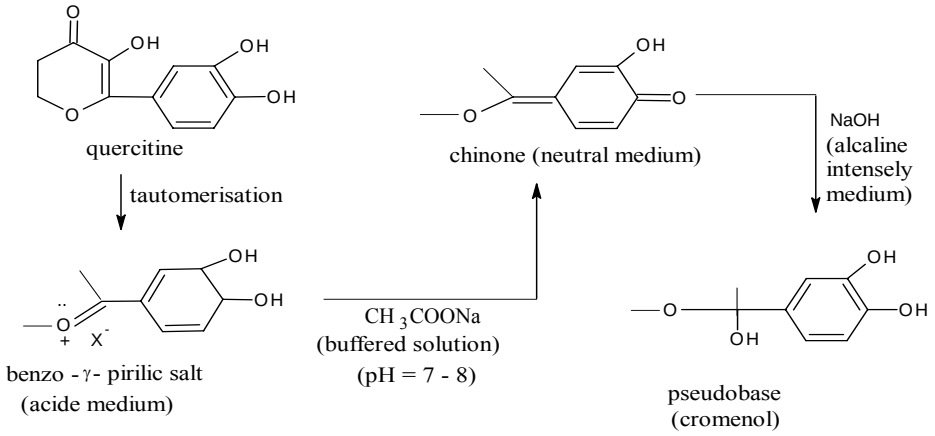
a) solid/liquid extraction with absolute methyl ether in a ***Soxhlet*** device and dilution of the extract with isoamyl alcohol;

b) extraction of the isoamyl phase with a water solution of ***aluminium chloride 0.1 M*** with the formation of a yellow complex (3 – ramno – glycoside – quercitine) in aqueous phase, separated by the centrifugation from the alcoholic phase;

c) colourimetric assessment of the maximum absorption ***(430 nm)*** compared to water.

When accessing dehydrated raw dog rose fruits, we could see that using isopropyl alcohol ***85%*** (aqueous) as solid/liquid extraction solvent de yielded more 3 – ramno – glycoside – quercitine than other extraction systems.

In the purification (recrystallisation) process, the variant with larger needle-like crystal formation (low formation speed) the purity of the final product was superior to the previous variant ***(97 – 99%)***.



In the colourimetric assessment presented above, we could see that the change in the complex colour made up by rutin with aluminium can be rigorously avoided if the assessment environment is buffered at a neutral pH.

As a natural flavone, 3 – ramno – glycoside – quercitine contains two hydroxyl phenol groups in ortho (peri) positions grafted on the lateral benzene nucleus of the benzo – γ – pyran (I) that makes up coloured chelate combinations established with aluminium cation (III). It is known that benzo – γ – pyrilin salts corresponding to benzo – γ – pyran are more stable.

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