THE ISOLATION AND IDENTIFICATION OF RUTIN FROM PHARMACEUTICAL PRODUCTS

Şerban Georgeta*, Pop Iulia Ana*, Horvath Tünde*, Bota Sanda**

*University of Oradea, Faculty of Medicine and Pharmacy, Pharmacy Department, 29 Nicolae Jiga St., 410028, Oradea, Bihor, Romania, e-mail: getaserban_2000@yahoo.com
**University of Oradea, Faculty of Sciences, Chemistry Department, 1 Universităţii St., 410087, Oradea, Romania, e-mail: sbota@yahoo.com

Abstract

Recently, flavonoids are compounds of great interest for food and pharmaceutical industries due to their antioxidant effect. Rutin is one of the medicinally important flavonoids used in the treatment of capillary fragility, retinal hemorrhages and venous insufficiency. Rutin may be useful for the prevention and treatment of colorectal cancer and it can prevent the painful peripheral neuropathy induced by antitumor drugs due to its antioxidant property.

The efficiency of rutin extraction from pharmaceutical products using methanol as solvent was studied. Methanol is a very good solvent giving the rutin sample in an acceptable purity. Organoleptic study, chemical identification, thin layer chromatography TLC and ^{1}H-NMR spectroscopy were used in order to study the identity and purity of rutin sample. Organoleptic study and chemical identification were in agreement with Romanian Pharmacopoeia Xth edition techniques. The identity of rutin has been confirmed by ^{1}H-NMR spectroscopy. TLC study of rutin showed the best mixture of solvents as ethyl acetate-acetic acid 1:1.

Key words: flavonoids, rutin, venous insufficiency, thin layer chromatography TLC, ^{1}H-NMR spectroscopy

INTRODUCTION

Flavonoids are products of secondary metabolism in plants. The increased interest of food and pharmaceutical industries for these compounds is due to their antioxidant effect. Oxidative stress reflects an imbalance in the normal redox state of cells which can cause toxic effects through the production of free radicals. Flavonoids can interact with free radicals to form less reactive products. In this way, these compounds are able to prevent the damage of cell membranes and biomolecules (e.g. DNA, proteins, lipids) caused by free radicals (Paniwnyk et al., 2001; Griffiths et al., 1955).

Oxidative stress is suspected to play a key pathogenic role in the development of different types of age-related cancer as well as neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease, depression or multiple sclerosis (Halliwell, 2007; Patel, Chu, 2011). Even if the use of antioxidants to prevent some diseases is controversial, flavonoids are currently the components of some popular antioxidant supplements (Meyers et al., 1996; Bjelakovic et al., 2007).

Rutin (quercetin-3-O-rutinoside) is a flavonoid ubiquitously found in nature. Commonly known in the begining as a mixture of flavonoids extracted from plants under the name „vitamin P", rutin is used today as a single compound with chemical formula and biological activity very well studied. Rutin can reduce the fragility and the permeability of capillaries and it has been used in the treatment of capillary fragility, retinal hemorrhages and venous insufficiency (varicose veins, haemorrhoids, diabetic vascular disease, diabetic retinopathy) and for improving micro vascular blood flow (tired legs, night cramps) (Stroescu, 2001; Kalinova, Dadakova, 2009; Fathiazad et al., 2006). Rutin may be useful for the prevention and treatment of colorectal cancer and it can prevent the painful peripheral neuropathy induced by antitumor drugs due to its antioxidant property (Kalinova, Dadakova, 2009; Azevedo et al., 2013). In addition, recent researches report the semi-synthetic derivatives of rutin and quercetin and the pharmacological screening highlights their biological potential (Huang et al., 2009; Yuan et al., 2012; Wang et al., 2014).

MATERIAL AND METHOD

The literature reports different methods for the rutin extraction from vegetal products: conventional extraction using dilute aqueous alkali, methanol, 50% methanol in the presence of ascorbic acid as an antioxidant, 70% methanol or 95% ethanol as solvents (Paniwnyk et al., 2001; Kalinova, Dadakova, 2009; Fathiazad et al., 2006; Sando, Lloyd, 1924) and ultrasonic extraction using dilute aqueous alkali and methanol as solvents, respectively (Paniwnyk et al., 2001).

Rutin (Fig. 1) sample used in this study comes from Tarosin tablets produced by SC Zentiva SA (series AA/no 6030/18.10.2012). Taking into account that rutin is slightly soluble in ethanol and water and practically insoluble in chloroform and ether and considering the literature data concerning the rutin extraction from vegetal products, methanol was chosen as extraction solvent.

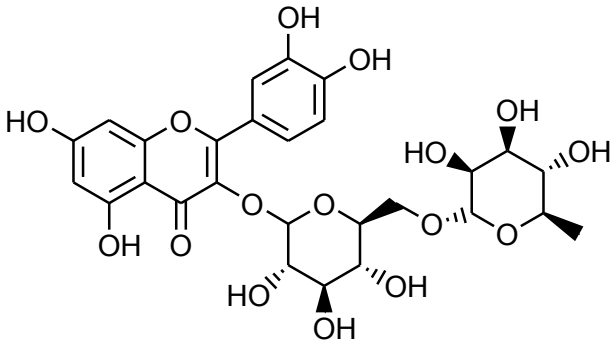

Fig. 1. Rutin chemical structure

Ascorbic acid extraction

50 tablets of Tarosin (20 mg rutin/50 mg ascorbic acid/tablet) after shredding to a fine powder were extracted at room temperature under stirring using 100 mL of water. The mixture was filtered under vacuum and the yellow residue was separated from the aqueous solution containing ascorbic acid.

Rutin extraction

The yellow residue was extracted three times with 90 mL of methanol and the mixture was filtered. The yellow filtrate was evaporated to dryness and the solvent was removed using a rotary evaporator to give approximately 2.1 g of rutin (out of 2 g rutin theoretically) on which the following tests were performed: organoleptic study, solubility, chemical identification, thin layer chromatography, ^{1}H-NMR spectral study (Pop, 2016).

RESULTS AND DISCUSSION

According to Romanian Pharmacopoeia Xth edition (RPh X), rutin is a crystalline powder, yellow or yellow-green, odorless and tasteless, soluble in 20 mL of methanol, soluble in 400 mL of water at 100ºC (Romanian Pharmacopoeia Xth edition, 1993). *Organoleptic study and solubility* were determined in accordance with working techniques provided by RPh X. Thus, rutin obtained after extraction is a yellow odorless crystalline powder, soluble in methanol and hot water, slightly soluble in ethyl acetate, freely soluble in dimethylformamide DMF (Pop, 2016).

Chemical identification was determined in accordance with RPh X (oxidation with Fehling's reagent and reduction with zinc and hydrochloric acid) and was in agreement with RPh X techniques (Pop, 2016).

Thin layer chromatography (TLC) used Merck chromatographic plates (20x20 cm) with silica gel 60 F254 applied on aluminum layer. The mixtures of solvents used are presented in table 1.

Table 1

Mixtures of solvents for TLC

MIXTURES OF SOLVENTS	COMPONENTS	RATE
S_1	dichloromethane-methanol	4:1
S_2	dichloromethane-methanol	2:1
S_3	acetic acid-water	1:1
S_4	ethyl acetate-acetic acid	4:1
S_5	ethyl acetate-acetic acid	1:1
S_6	acetic acid-water	2:1

A solution of rutin 1% in methanol was prepared. The samples were spotted at a distance of 1 cm from the edge of TLC plates using micro capillary. Migration was performed in a tightly closed flask saturated by the vapors of solvents. The development over a distance of 10 cm led to the separation of a single area in most cases. The identification of rutin in samples was based on R_f values after highlighting the spots in UV light at λ = 254 nm. The ultraviolet light has been selected as visualizing agent due to chromophore C=C-C=C-C=C-C=O included in flavones ring. Rutin shows a yellow spot in natural light and a yellow-brown fluorescence in UV light. The results of TLC study are presented in table 2.

Table 2

R_f values for rutin

MIXTURES OF SOLVENTS	**R_f**	
	Rutin	**Other compounds**
S_1	0.11	-
S_2	0.84	-
S_3	0.78	-
S_4	0.18	0.33
S_5	0.44	0.57
S_6	0.72	-

The purity condition of a compound studied by TLC requires a single spot on the chromatographic plate. The mixtures S_4 and S_5 showed a supplementary spot on TLC plates and thus a compound different of rutin was identified. We believe that this compound is a component of Tarosin tablets (e.g. excipients) which accompanied rutin during the extraction process. Among the developing mixtures, S_5 (ethyl acetate-acetic acid 1:1) is most recommended for the rutin study showing a round shaped spot and R_f value of 0.44. In addition, this mixture was useful for the supplementary compound separation. Anyway, the supplementary compound identified by TLC showed a spot much smaller compared to rutin's spot. S_6 mixture is also useful for rutin identification (R_f 0.72) but was unable to identify the supplementary compound (Pop, 2016).

^{1}H-NMR spectral study of rutin was performed by a Varian Mercury 300 MHz spectrometer using a sample of 10 mg rutin solved in deutherated methanol. The ^{1}H-NMR spectral data are reported in parts per million downfield from the internal standard (tetramethylsilane, δ 0.0).

^{1}H-NMR spectrum of rutin was compared to ^{1}H-NMR spectrum of rutin-standard. Thus, in the rutin spectrum could be seen some supplementary signals in the areas: 1.92-2.3 ppm, 2.89-3.05 ppm, 3.85-3.94 ppm corresponding to the supplementary compound identified also by TLC analysis. The supplementary signals are very small which corresponds to an

acceptable purity of the rutin obtained after extraction. The results of ^{1}H-NMR spectral study are presented in table 3 and figure 2.

Table 3

^{1}H-NMR spectral data for rutin

δ ppm	Assignment
1.12	d, 3H, CH_3
3.38-3.56	m, 8H, rutinoside protons and CH_2O
3.62	dd, 1H, CH-CH_3 ramnose
3.82	d, 1H, CH-CH_2O glucose
4.51	d, 1H, OCHO ramnose
5.10	d, 1H, OCHO glucose
6.20	d, 1H, Ar-H
6.39	d, 1H, Ar-H
6.89	d, 1H, Ar-H
7.61-7.67	m, 2H, Ar-H

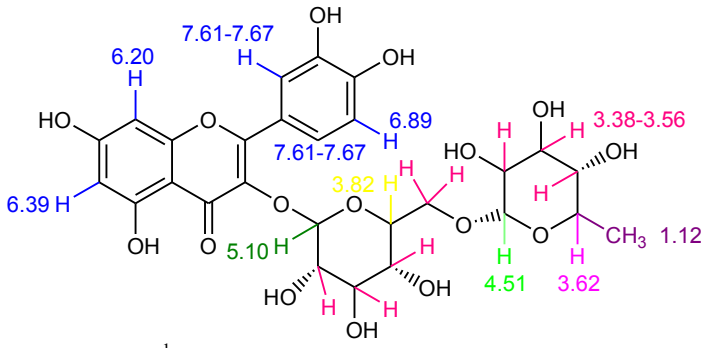

Fig. 2. ^{1}H-NMR spectral data assignment for rutin

CONCLUSIONS

The analyzed compound, rutin, was purchased from Tarosin tablets after separation of ascorbic acid and extraction with methanol and was characterized in accordance with working techniques provided by RPh X (appearance, colour, odour and solubility). Chemical identification was determined through RPh X techniques. All these tests were in accordance with the requirements of Romanian Pharmacopoeia Xth edition.

Identification of rutin was performed by TLC using six mixtures of solvents and UV light. TLC test served not only to identify rutin through R_f values, but also to determine the purity of the extracted substance. The mixture of solvents most recommended for rutin identification and purity determination is ethyl acetate-acetic acid 1:1 (R_f 0.44). This mixture was useful for a supplementary compound separation.

Rutin structure was studied through ^{1}H-NMR spectroscopy. ^{1}H-NMR spectrum was compared to ^{1}H-NMR spectrum of rutin-standard which

allowed us to assign the extracted compound as rutin. ^{1}H-NMR spectrum gave also information about the purity of the sample.

REFERENCES

1. Azevedo M.I., Pereira A.F., Nogueira R.B., Rolim F.E., Brito G.A.C., Wong D.V.T., Lima-Junior R.C.P., Ribeiro R.A., Vale M.L., 2013, The antioxidant effect of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. Molecular Pain, 9(53), pp.1-14
2. Bjelakovic G., Nikolova D., Gluud L.L., Simonetti R.G., Gluud C., 2007, Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA, 297(8), pp.842-857
3. Fathiazad F., Delazar A., Amiri R., Sarker S.D., 2006, Extraction of flavonoids and quantification of rutin from waste of Tobacco leaves. Iranian J. Pharm. Res., 3, pp.222-227
4. Griffiths J.Q., Krewson C.F., Naghski J., 1955, Rutin and Related Flavonoids: Chemistry, Pharmacology and Clinical Applications. Mack Publishing., Easton, Pennsylvania
5. Halliwell B., 2007, Oxidative stress and cancer: have we moved forward? Biochem. J., 401(1), pp.1-11
6. Huang H., Jia Q., Ma J., Qin G., Chen Y., Xi Y., Lin L., Zhu W., Ding J., Jiang H., Liu H., 2009, Discovering novel quercetin-3-O-amino acid esters as a new class of Src tyrosine kinase inhibitors. Eur. J. Med. Chem., 44, pp.1982-1988
7. Kalinova J., Dadakova E., 2009, Rutin and total quercetin content in Amaranth (Amaranths spp.). Plant Foods Hum. Nutr., 64, pp.68-74
8. Meyers D.G., Maloley P.A., Weeks D., 1996, Safety of antioxidant vitamins. Arch. Intern. Med., 156(9), pp.925-935
9. Paniwnyk L., Beaufoy E., Lorimer J.P., Mason T.J., 2001, The extraction of rutin from flower buds of *Sophora japonica*. Ultrasonics Sonochemistry, 8, pp.299-301
10. Patel V.P., Chu T.C., 2011, Nuclear transport, oxidative stress, and neurodegeneration. Int. J. Clin. Exp. Pathol., 4(3), pp.215-229
11. Pop I.A., 2016, Bioflavonoide cu implicaţii medicamentoase. Rutozidul şi quercetina. Lucrare de licenţă, Universitatea din Oradea, Facultatea de Medicină şi Farmacie, pp.40-59
12. Sando C.E., Lloyd J.U., 1924, The isolation and identification of rutin from the flowers of elder (*Sambucus canadensis* L.). J. Biol. Chem., 58, pp.737-745
13. Stroescu V., 2001, Bazele farmacologice ale practicii medicale. Ed. Medicală Bucureşti, ediţia a VII-a
14. Wang Z., Yang L., Cui S., Liang Y., Zhang X., 2014, Synthesis and anti-hypertensive effects of the twin drug of nicotinic acid and quercetin tetramethyl ether. Molecules, 19, pp.4791-4801
15. Yuan J., Wong I.L.K., Jiang T., Wang S.W., Liu T., Chow B.L.M.C., Sheng B.W., 2012, Synthesis of methylated quercetin derivatives and their reversal activities on P-gp- and BCRP-mediated multidrug resistance tumour cells. Eur. J. Med. Chem., 54, pp.413-422
16. * * *, 1993, Farmacopeea Română Ediţia a X-a. Ed. Medicală, Bucureşti, pp.830-832