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Study of Flavonoids presente in Pomelo (*Citrus máxima*) by DSC, UV-VIS, IR, ^{1}H AND ^{13}C NMR AND MS

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Flavonoids are among the most important plant metabolites. Due to their potential benefits, there is a considerable interest in this natural product. In genus *Citrus*, some plants have not yet been much exploited in Brazil, as in the case of grapefruit (*Citrus maxima*), whose main flavonoids are naringin and their aglycone naringenin. The physico-chemical characteristics are important pre-requisites of reference chemical in future studies. In this context, the objective of this study was to determine the characterization of naringin and naringenin by melting point, DSC, UV-VIS, ^{1}H and ^{13}C NMR, IR and MS. Results revealed that, naringin and naringenin after characterization, can be used in future studies and contribute to seeking possible technological applications.

***Keywords*:** Naringin; naringenin; flavonoids; C*itrus maxima*

Introduction

Medicinal plants are a major source for the production of new drugs. Although only approximately 10% of the world biodiversity has been explored, 140 thousand intermediate metabolites have been isolated and characterized, but not yet biologically evaluated (1,2). The study of flavonoids arose as a search for new compounds to develop new raw materials and active substances as the prototype of new drugs (3).

A few plants of the *Citrus* genus have not yet been much explored, as in the case of grapefruit (*Citrus maxima*). The main grapefruit flavonoids are naringin ((+/-) 4'5,7-trihydroxyflavanone 7-rhamnoglucoside) ($C_{27}H_{32}O_{14}$), with a molecular mass of 580.53 g mol^{-1}; and its aglycone, naringenin ((+/-) 4'5,7-trihydroxyflavanone) ($C_{15}H_{12}O_5$), with a molecular mass of 272.25 g mol^{-1} (4,5).

Structurally the flavonoids have a diphenylpropane skeleton (C6-C3-C6), containing 15 atoms of carbon and substitution in one or more hydroxyls, including derivates linked to sugars. Their chemical structure comprises two aromatic rings (A and B) that are joined by a heterocyclic ring, ring C (6-8).

Naringin and naringenin belong to the subclass of flavanones, with a carbonyl in position 4 of ring C and a saturated link in position 3. In ring B there is a hydroxyl in position 4'. What distinguishes them structurally is that naringin in position 7 of ring A presents a disaccharide constituted by a glucose molecule and another of ramnose, and in naringenin there is a hydroxyl (Figure 1) (6-8).

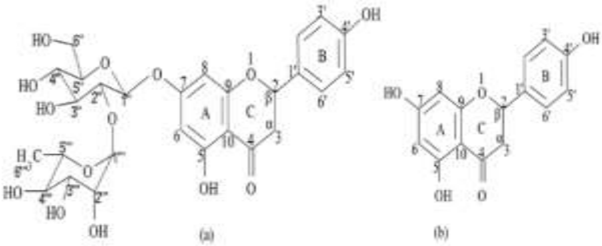

Figure 1 Chemical structure of naringin (a) and naringenin (b).

In the literature, several studies show important biological/pharmacological effects of these two flavonoids, such as their vasorelaxant, antioxidant, phosphodiesterase inhibitor, antiteratogenic, hepatoprotective, anti-inflammatory properties, lipid peroxidation inhibiting, and platelet aggregating, protective effect against cytochrome P450 3A4 activity (9-14).

The biochemical activities of flavonoids and their metabolites are related to their chemical structure which can change according to the substitutions in the three rings, including hydrogenation, hydroxylations, methylations, sulfatations and glycosylations (5,7,8).

The characterization of a pharmaceutical input using an adequate analytic methodology is an essential condition for the development of new pharmaceutical forms, both from the scientific and regulatory point of view (8). The International Conference on Harmonization (ICH) (15) affirms that the purity of the substances used is extremely important for the development and validation of an analytic methodology.

In this context, considering the relevance of the study, the objective of this study was to characterize naringin and naringenin using the melting point, differential scanning calorimetry (DSC) and spectroscopic techniques, such as: mass spectrometry (MS), nuclear magnetic resonance (NMR) of hydrogen (^{1}H) and carbon (^{13}C) and spectrometry in the ultraviolet (UV) and infrared (IR) region.

Experimental

Materials

Naringin (96.6%, Sigma-Aldrich, Buchs, Switzerland), naringenin (99.2%, Sigma-Aldrich, Buchs, Switzerland), ethanol, deuterated methanol, acetonitrile and water. All the reagents and solvents used were grade p.a., supplied by Tedia® and UV Tech®.

Physical characteristics

The aspect and color of the naringin and naringenin samples were evaluated.

Determination of the melting point

In order to determine the range of melt of naringin and naringenin using the capillary method, the Mettler Toledo®, model FP90 equipment was used. It determines the melting point by light transmission, preventing the operator from interfering in the procedure. Before performing the analysis the equipment was calibrated and immediately after this the samples were compacted into capillary tubes each (6 mm long and 1 mm diameter). The capillaries were introduced vertically into the equipment, with heating at 10 °C minute^{-1}. The reading was performed in triplicate.

Differencial scanning calorimetry

The naringin and naringenin were analyzed in DSC equipment with heat flow, of Shimadzu (Shimadzu, Kyoto, Japan), model DSC-60, with FC-60 flow controller, 60 WS integrator and TA 60 (version 2.0) control and analysis software. Calibration was performed previously with indium (156.6 °C, with transition energy of 28.45 J g^{-1}). About 1.00 mg of each of the samples was weighed, separately, and they were transferred to aluminum sample crucible, sealed and submitted to heating up to 300 °C, with a ratio of 10 °C minute$^{-\mathbf{1}}$, under a nitrogen atmosphere.

Ultraviolet spectroscopy

Naringin and naringenin were analyzed in a UV-1800, Shimadzu® Spectrophotometer. Spectra were traced on the 200-400 nm wavelength, using quartz cuvettes with a 1 cm optical path. Ethanol with a final concentration of 10.0 µg ml^{-1} was used to prepare the solutions.

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Infrared spectroscopy

The spectra were obtained with the IR Shimadzu® model 8001 spectrophotometer. Approximately 1.50 mg of naringin and naringenin were weighed separately. The reading was performed in the region of the 4,000- 600 cm cm^{-1} spectrum.

Nuclear magnetic resonance spectroscopy

The ^{1}H and ^{13}C NMR of naringin and naringenin were performed on 300 MHz, Varian equipment, model VNMRS-300, using deuterated methanol as a solvent. The chemical displacements were reported in ppm and the substance structures were defined based on the analysis of the spectroscopic data and on comparing the data obtained in the literature.

Liquid chromatography coupled to mass spectrometry (UPLC-Q-TOF-MS/MS)

The analyses were performed by liquid chromatography coupled to mass spectrometry (UPLC-Q-TOF-MS/MS). The equipment used was Waters Acquity Ultra Performance LC (Waters Corp., Milford, MA). The direct injection technique was employed for detection with a ionization interface by positive *electrospray* (ESI$^+$), using a Zorbax RRHD Eclipse Plus C118 column (2.1 mm x 50 mm; 1.8 µm). The conditions used were cone voltage 10 V; source temperature 110 °C; desolvation temperature 350 °C. Ultrapure water with a pH adjusted to 4.0 with hydrochloric acid (A) and acetonitrile (B) was used to prepare the mobile phase. Separation was done in three minutes using gradient: 0-1.33 min 10%-70% B; 1.33-2.00 min 70% B; 2.00-3.00 min 70%-10% B. The injection volume was 6.0 µl, with a flow of 0.4 ml minute^{-1}. The stock solution was prepared with ethanol and dilution was performed with water at pH 4.0 and acetonitrile (1:1) (*v/v*). The sample were filtered with a 0.22 µm nylon filter and analyzed.

Results and Discussion

Physical characteristics

Naringin was presented in powder form and slightly yellow. Naringenin was presented in powder form in a light brown color. The descriptions of the two substances are appropriate to the specifications informed by the manufacturer.

Determination of the melting point

The range of melt found for naringin was broad, from 220.1 to 249.7 °C, and for naringenin it was 253.0 °C. For the two flavonoids there was a change of color, both of them began to present a brown color. The flavonoids containing sugar molecules, such as naringin, present lower melting points and greater molecular masses than the aglycones, such as naringenin (16). The results found were compared to those obtained with the DSC technique.

Differential scanning calorimetry

DSC is a thermoanalytic method based on the determination of heat that involves transitions classified as endothermal or exothermal. This technique has great applications, especially in the field of quality control, to characterize drugs and adjuvants, evaluate the finished product and perform process control (17,18). The onset temperature (temperature at which the base line is extrapolated) is the temperature at which a thermal event begins to be observed and it is more reliable than the maximum temperature which can be influenced by experimental factors, such as warming velocity (18). Figure 2 shows the heating curve of naringin and naringenin.

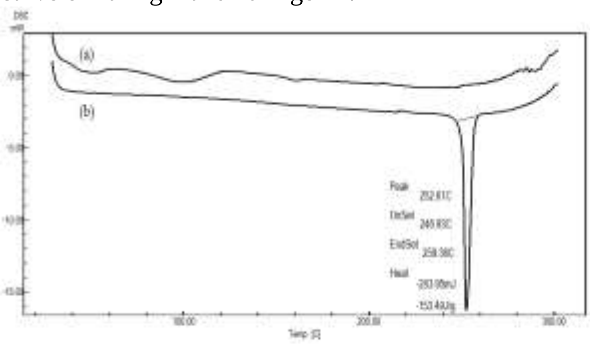

Figure 2 Heating curve obtained by DSC from naringin (a) and naringenin (b).

The DSC profile of naringin did not show a defined melting point. This may be occurring because substances with an amorphous structure do not have a melting point since there is no crystal lattice (19). In the literature there are reports that naringin presents an endothermal melting peak at 253 °C and the peaks that occur after 250 °C are due to its decomposition.[20] Binello and collaborators (21) mention that naringin presented two endothermal peaks, one at 83 °C and one at 171 °C, corresponding to the successive loss of two water molecules.

For naringenin the fusion temperature was 252.61 °C. The fusion enthalpy (ΔH) was – 153.49 $J.g^{-1}$. This shows an endothermal event which is represented by descending signals and the result is expressed through a negative value of the differential signal.

The results found for naringenin corroborate those found in the literature. According to Lauro (20), Semalty (22), Sansone (23) and Yang (24) naringenin presents a marked endothermal peak around 253 º C. The peak presented as fine and without deformations, characteristic of a pure product (18,25). It could be seen that the melting point found in the analysis of DSC is similar to the melting range obtained by the capillary method for naringenin.

Ultraviolet spectroscopy

UV radiation absorption occurs by exciting electrons inside the molecule to a state of greater energy (26). Ethanol was the most adequate of the solvents tested because both naringin and naringenin are soluble. Since both substances have distinct characteristics, naringin is polar, because in its structure it has the glycoside group and naringenin has apolar characteristics (27).

The spectrum of the flavonoids is generally characterized by presenting two maximums with distinct absorptions, a band II in the 240-295 nm range and a band I in the 300-400 nm range, referring to ring A and B, respectively (28). The naringin and naringenin spectra are shown in Figure 3. The maximum absorption found for naringin was 285 nm and for naringenin 288 nm. These absorptions are ascribed to the absorbance of ring A in its molecular structures (29,30).

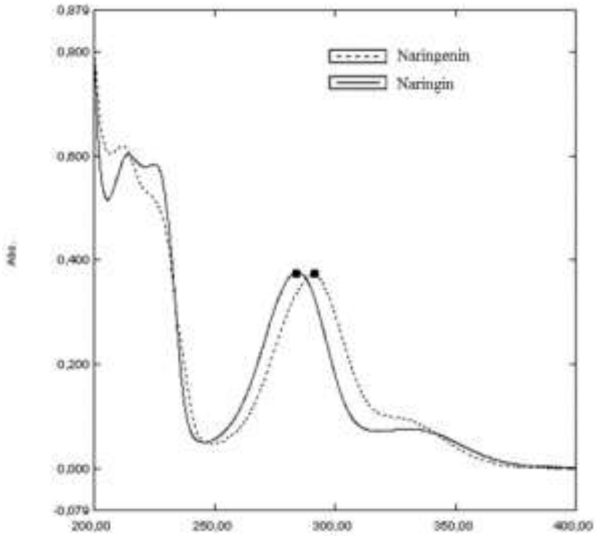

Figure 3 Spectrophotometry in the ultraviolet region of naringin and naringenin at the concentration of 10.0 μg ml^{-1} in ethanol.

Infrared spectroscopy

Spectrophotometry in the IR region is based on the principle of interaction of the electromagnetic radiation that passes through the sample and is absorbed by the links, leading to stretching or folding of these links (26). The absorption spectra obtained from naringin and naringenin in the IR region are shown in Figure 4.

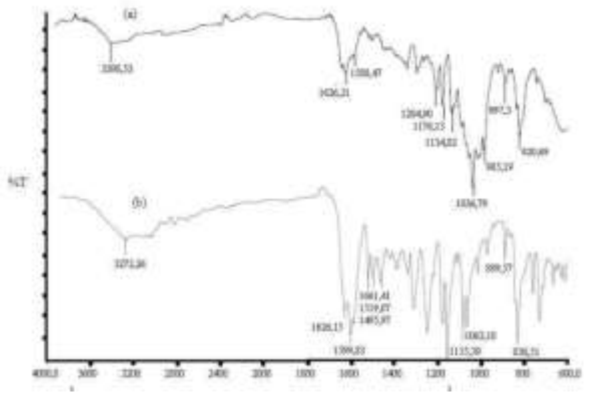

Figure 4 Spectrum in the infrared region of naringin (a) and naringenin (b).

In order to interpret the spectra of the two flavonoids that are being studied, the spectrum was divided into two regions: between 4,000-1,300 cm^{-1} which is the region called of functional groups and between 1,300-600 cm^{-1} called region of the molecule fingerprint. The attributions of the characteristic bands of the two molecules were established based on the literature and are described in Table 1 (27,31-34). Spectroscopy in the IR region enabled the confirmation of the naringin and naringenin flavonoid structure, since it was possible to confirm the presence of all characteristic bands.

Table 1 Attributions of the main bands of the naringin and naringenin spectrum.

	Naringin	**Naringenin**
Attribution	**Frequency (cm^{-1})**	
OH (axial deformation)	3398.53 1036.79	3272.26
C=O	1626.21	1626.15
C=C	1588.47	1599.83
Aromatics	~ 1200	~ 1500
Axial deformation of C-O-C	985.19	1155.39 1081.90
Angular deformation C-H	~800	~800

Nuclear magnetic resonance spectroscopy

Figures 5, 6 and Table 2 show the spectra and the characteristic signals of ^{1}H and ^{13}C of naringin and naringenin. According to Agrawal (35), the data on ^{13}C NMR are important to establish the structure of a glycosylated flavonoid. Based on these data it is possible to establish the number of sugars linked, nature, position and conformation.

Naringenin presents fifteen carbons, seven quaternaries, seven are CH and one is CH_2. The same occurs with naringin and there are another twelve carbons, one signal is attributed to methylenic carbon (CH_2) in 60.7 ppm (C-6''), one CH_3 in 16.90 ppm (C-6''') and 10 are CH. Characterizing the skeleton of a flavanone, the two molecules presented an oxymethynic group between carbons C-2 and C-3a-b (36-38).

The signal of deuterated methanol is represented in the region of 47 ppm in the spectra, corroborating the literature. Analyzing the ^{1}H NMR spectrum, the deuterated methanol is located at 3.39 and at 4.9 ppm (s) corroborating the literature (39).

In ring A of the two flavanones, there are two signals characteristic of hydrogen atoms, with a *meta* substitution corresponding to hydrogens H6 and H8. In the naringenin molecule displacements at 9.41 and 10.83 ppm are attributed to the hydrogen of the hydroxyl of carbon C-5 and C-7, respectively. In naringin, the hydroxyl of carbon C-5 is at 9.41 ppm (40-42).

Ring B revealed the presence of a substituted aromatic *para* system, represented by two doublets 7.33 ppm (H2' and H6') and 6.84 ppm (H3' and H5') in the naringin molecule and also two doublets at 7.31 ppm (H2' and H6') and 6.83 ppm (H3' and H5') in the naringenin molecule. The hydroxyl of carbon C-4' is at 12.08 ppm in both flavonoids (40-42).

The hydrogens present in the two sugar molecules of naringin show signals at 1.31 (d) ppm referring to CH_3, between 3.0 and 4.0 ppm, besides two signals attributed to the hydrogens linked to the anomeric carbons at 5.27 and 5.35 ppm (43,44).

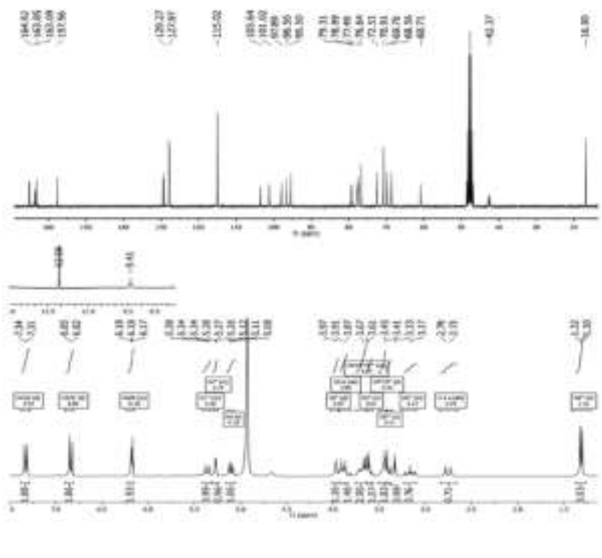

Figure 5 ^{1}H and ^{13}C NMR spectrum of naringin.

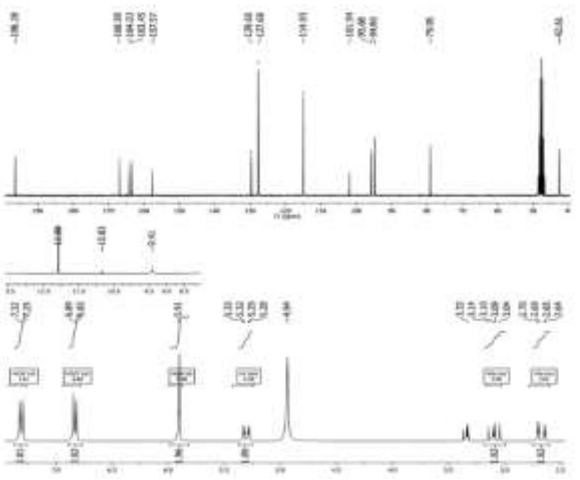

Figure 6 ^{1}H and ^{13}C NMR spectrum of naringenin.

Table 2 ^{1}H and ^{13}C NMR chemical shift values (ppm) of naringin and naringenin.

	Naringin		Naringenin	
Position	^{13}C	^{1}H	^{13}C	^{1}H
2	79.31	5.31	79.05	5.30
3a	42.37	3.89	42.61	3.09
3b	42.37	2.75	42.61	2.67
4	197.23	-	196.38	-
5	163.85	-	164.03	-
6	96.50	6.18	95.68	5.90
7	164.92	-	166.90	-
8	95.50	6.18	94.80	5.90
9	163.09	-	163.45	-
10	103.64	-	101.94	-
1’	127.47	-	129.66	-
2’	129.27	7.33	127.68	7.31
3’	115.02	6.84	114.93	6.83
4’	157.56	-	157.57	-
5’	115.02	6.84	114.93	6.83
6’	129.27	7.33	127.68	7.31
1’’	92.89	5.27	-	-
2’’	76.64	3.17	-	-
3’’	77.77	3.44	-	-
4’’	68.56	3.61	-	-
5’’	72.51	3.97	-	-
6’’a-b	60.71	3.67	-	-
1’’’	101.02	5.35	-	-
2’’’	70.91	3.67	-	-
3’’’	77.49	3.41	-	-
4’’’	79.31	3.44	-	-
5’’’	69.76	3.67	-	-
6’’’	16.90	1.31	-	-

Liquid chromatography coupled to mass spectrometry (UPLC-Q-TOF-MS/MS)

Figure 7 shows the mass spectra of naringin and naringenin, respectively. Under the conditions described, the analytes produced protoned molecules at 581 m/z for naringin and 273 m/z for naringenin. The results found corroborate what is found in the literature (45-47).

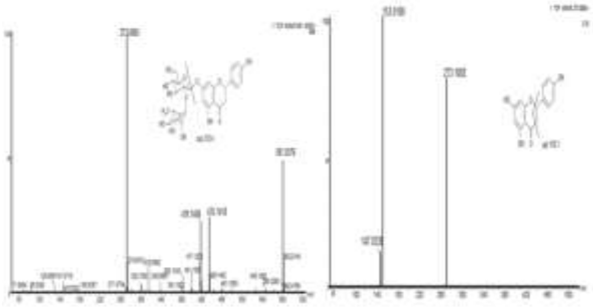

Figure 7 Mass spectrum of naringin and naringenin.

Conclusions

By chemical analyses, results found in the identity of the two flavanones, naringin and naringenin, could be confirmed, corroborating what is described in the literature. Thus, after they have been appropriately characterized, they can be used as a chemical of reference in future studies and contribute to seeking possible technological applications.

Conflict of Interest

The authors there are no conflicts of interest.

References

1. Verpoorte, R. Exploration of nature’s chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discover Today. 1998; 3:232-238.

2. Balunas, M.J., Kinghorn, A.D. Drug discovery from medicinal plants. Life Sciences. 2005; 78:431-441.

3. Middleton, E.J.R., Kandaswami, C., Theoharides, C.T. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. Pharmacol. Rev. 2000; 52:673-751.

4. Sudto, K., Pornpalakul, S., Wanichwecharungruang, S. An efficient method for the large scale isolation of naringin from pomelo (*Citrus grandis*) peel. Int. J. Food Sci. Technol. 2009; 44:1737-1742.

5. Sayre, C.L., Gerde, K.D., Yáñez, J.A., Davies, N.M., Yáñez, J.A. Clinical Pharmacokinetics of

Flavonoids, in Flavonoid Pharmacokinetics: Methods of Analysis, Preclinical and Clinical Pharmacokinetics, Safety, and Toxicology. 1st Ed, John Wiley & Sons, 2012

6. Yokozawa, T., Dong, E., Liu, Z.W., Shimizu, M. Antioxidative Activity of Flavones and Flavonols. Phytother. Res. 1997; 11:446-449.

7. Aherne, A.S., O'Brien, N.M. Dietary Flavonols: Chemistry, Food Content, and Metabolism. Nutrition. 2002; 18:75-81.

8. Gandhi, R., Pillai, O., Thilagavathi, R., Gopalakrishnan, B., Lal Kaul, C., Panchagnula, R. Characterization of Azithromycin hydrates. Eur. J. Pharm. Sci. 2002; 16:175-184.

9. Guengerich, F.P., Kim, H.D. In vitro inhibition of dihydropyridine oxidation and aflatoxin B1 activation in human liver microsomes by naringenin and other flavonoids. Carcinogenesis. 1990; 11:2275-2279.

10. Cook, N.C., Samman, S. Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem. 1996; 7:66-76

11. Wilcox, L.J., Borradaile, N.M., Huff, M.W. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. Cardiovasc Drug Rev. 1999; 42:725-734.

12. Badarya, O.A., Abdel-Maksoudb, S., Ahmedd, W.A., Owieda, G.H. Naringenin attenuates cisplatin nephrotoxicity in rats. Life Sciences. 2005; 76:2125-2135.

13. Ali, G., Hawa, Z.E.J. Synthesis of Phenolics and Flavonoids in Ginger (Zingiber officinale Roscoe) and Their Effects on Photosynthesis Rate. Asmah R., Int J Mol Sci. 2010; 11:4539-4555.

14. Liu, Q., Lu, L., Xião M. Cell surface engineering ofa-L-rhamnosidase for naringin hydrolysis. Bioresour Technol, 2012; 123:144-149.

15. ICH - International Conference on Harmonization. Q2R1- validation of Analytical procedure: Text and Methodology, 2005.

16. Ko, M.A., Cheigh, C., Chung M. Relationship analysis between flavonoids structure and subcritical water extraction (SWE). Food Chem. 2014; 143:147-155.

17. Giron, D. Applications of thermal analysis and coupled techniques in pharmaceutical industry. J. Therm. Anal. Calorim. 2002; 68:335-357.

18. Armijo, C.J.V., Cristian, J., Costa, I.M., Longhini, R., Petzhold, C.L., Petrovick, P.R. Métodos termo-analíticos e suas aplicações nas ciências farmacêuticas. Caderno de Farmácia. 2004; 20:29-47.

19. Yu, L. Amorphous pharmaceutical solids: preparation, characterization and stabilization. Adv. Drug Delivery Rev. 2001; 48:27-42.

20. Lauro, M.R., Simone, F., Sansone, F., Iannelli, P., Aquino, R.P. Preparations and release characteristics of naringin and naringenin gastro-resistant microparticles by spray-drying. J. Drug Delivery Sci. Technol. 2007 17:119-124.

21. Binello, A., Robaldo, B., Barge, A., Cavalli, R., Cravotto, G. Synthesis of Cyclodextrin Based Polymers and Their Use as Debittering Agents. J. Appl. Polym. Sci. 2008; 107:2549-2557.

22. Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M. Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery. Inclusion Phenom. Mol. Recognit. Chem. 2010; 67:253-260.

23. Sansone, F., Picerno, P., Mencherini, T., Villecco, F., D'ursi, A.M., Aquino, R.P., Lauro, M.R. Flavonoid microparticles by spray-drying: Influence of enhancers of the dissolution rate on properties and stability. J. Food Eng. 2011; 103:188-196.

24. Yang, L., Ma, S., Zhou, S., Chen, W., Yuan, M., Yin, Y., Yang, X. Preparation and characterization of inclusion complexes of naringenin with cyclodextrin or its derivative. Carbohydr. Polym. 2013; 98:861-869.

25. Ford, J.L., Tinmins, P. Pharmaceutical thermal analysis: technique and application. 1st Ed, New York: Willey-Interscience, 1986.

26. Silverstein, R.M., Webster, F.X., Kiemle, D.J. Identificação Espectrométrica de Compostos Orgânicos. 6th Ed, LTC: Rio de Janeiro, 2000.

27. Suetsugu, T., Iwai, H., Tanaka, M., Hoshino, M., Quitain, A., Sasaki, M., Goto, M. Extraction of Citrus Flavonoids from Peel of Citrus Junos Using Supercritical Carbon Dioxide with Polar Solvent. Adv. Chem. Eng. Sci. 2013; 1:87-90.

28. Farajtabar, A., Ghari, F. Spectral analysis of naringenin deprotonation in aqueous ethanol solutions. Chem. Pap. 2013; 67:538-545.

29. Harborne, J.B., Williams, C.A. Advances in flavonoid research since. Phytochemistry. 2000; 55:481-504.

30. Pereira, R.M.S., Andrades, N.E.D., Paulino, N., Sawaya, A.C.H.F., Eberlin, M.N., Marcucci, M.C., Favero, G.M., Novak, E.M., Bydlowski, S.P. Synthesis and Characterization of a Metal Complex Containing Naringin and Cu, and its Antioxidant,

Antimicrobial, Antiinflammatory and Tumor Cell Cytotoxicity. Molecules. 2007; 12:1352-1366

31. Lopes, W.A., Fascio, M. Esquema para interpretação de espectros de substancias organicas na região do infravermelho. Quim. Nova. 2004; 27:670-673.

32. Ma, X., Chen, R., Zheng, X., Youn, H., Chen, C. Preparation of molecularly imprinted CS membrane for recognizing naringin in aqueous media. Polym. Bull. 2011; 66:853-863.

33. Pavia, D.L., Lampman, G.M., Kriz, G.S. Introduction to Spectroscopy: a guide for students of organic chemistry. 3rd Ed, South Melbourme: Brooks/Cole, 2011.

34. Unsalan, O., Erdogdub, Y., Gulluoglub, M.T. FT-Raman and FT-IR spectral and quantum chemical studies on some flavonoid derivatives: Baicalein and Naringenin. J. Raman Spectrosc. 2009; 40:562-570.

35. Agrawal, P.K. Carbon-13 NMR of Flavonoids. 1st Ed, Amsterdam: Elsevier, 1989.

36. Moreira, F.P.M., Coutinho, V., Montanher, A.B.P., Caro, M.S.B., Brighente, I.M.C., et al. Flavonoides e triterpenos de Baccharis pseudotenuifolia– Bioatividade sobre Artemia salina. Quim. Nova, 2003.

37. Moccelini, S.K., Silva, V.C., Ndiaye, E.A., Sousa, P.T., Vieira, P.C. Estudo fitoquímico das cascas das raízes de Zanthoxylum rigidum Humb. & Bonpl. ex Willd (rutaceae). Quim. Nova. 2009 32:131-133.

38. Tang, D., Zhu, C., Zhong, S., Zhou, M. Extraction of naringin from pomelo peels as dihydrochalcone’s precursor. J. Sep. Sci. 2001; 34:113-117.

39. Pretsch, E., Buhlmann, P., Affolter, C. Structure determination of organic compounds. 3rd Ed, London: Springer, 2000.

40. Silva, T.M.S., Carvalho, M.G., Braz-Filho, R. Estudo espectroscópico em elucidação estrutural de flavonoides de Solanum jabrense agraa & nee S. paludosum moric. Quim. Nova. 2009; 32:1119-1128.

41. Maltese, F., Erkelens, C., Kooy, F., Choi,Y.H., Verpoorte, R. Identification of natural epimeric flavanone glycosides by NMR spectroscopy. Food Chemistry. 2009; 116:575-579.

42. Queiroz, L.H.K., Queiroz, D.P.K., Dhooghe, L., Ferreira, A.G., Giraudeau, P. Real-time separation of natural products by ultrafast 2D NMR coupled to on-line HPLC. Analyst. 2012; 137:2357-2361.

43. Costa, D.A., Silva, D.A., Costa, D.F., Silva, M.F.V., Souza, M.F., Agra, I.A., et al. Flavonóides glicosilados de Herissantia tiubae(K. Schum) Brizicky (Malvaceae) e testes farmacológicos preliminares do canferol 3,7-di-O-α-L-ramnopiranosídeo. Rev. Bras. Farmacogn. 2005; 15:23-29.

44. Bagno, A., Rastrelli, F., Saielli, G. Prediction of the 1H and 13C NMR Spectra of r-D-Glucose in Water by DFT Methods and MD Simulations. J. Org. Chem. 2007; 72:7373-7381

45. Fang, T., Wang, T., Ma, Y., Su, W., Bai, Y., Zhao, P. A rapid LC/MS/MS quantitation assay for naringin and its two metabolites in rats plasma. J. Pharm. Biomed. Anal. 2006; 40:454-459.

46. Ma, Y., Li, P., Chen, D., Fang, T., Li, H. Su, W. LC/MS/MS quantitation assay for pharmacokinetics of naringenin and double peaks phenomenon in rats plasm. Int. J. Pharm. 2006; 307:292-299.

47. Xiong, X., Jiang, J., Duan, J., Xie, Y., Wang, J., Zhai, S. Development and Validation of a Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for the Determination of Naringin and Its Metabolite, Naringenin, in Human Plasma J. Chromatogr. Sci. 2013.