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Hesperidin and hesperitin preparation and purification from *Citrus sinensis* peels

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ABSTRACT

This study describe the procedure of extraction and spectroscopic analysis of Hesperidin, a naturally occurring citrus flavanone glycoside from leaves peel of three different citrus species, mandarin (C.reticulata),Clementine(C.clementina), sweet orange (C. sinensis). Hesperidin was isolated from orange peel by extracting the dry peel with petroleum ether. Spectroscopic analysis (UV, IR and NMR) confirms the structure and extent of purity of extracted Hesperidin. An improved procedure for the conversion of Hesperidin into high-purity, crystalline Hesperetin is reported.

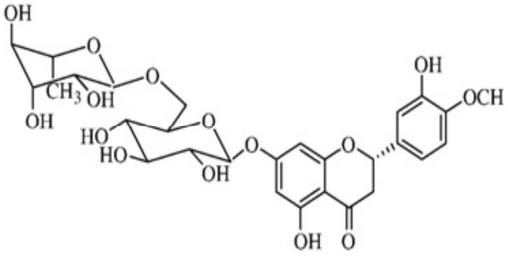
Key words: Hesperidin, Hesperitin, flavanone glycoside, orange peels.

INTRODUCTION

During fruit consumption's, large quantities of wastes accumulate, however these materials may have some constituents of great significance generate substantial quantities of phenolics-rich sub products, which could be valuable natural sources of polyphenols. Hesperidin, 3′, 5, 7-trihydroxy-4′-methoxy - flavanone-7- o - ß -rutinoside, is one of the bioflavonoids which is greatly found in Citrus species and is the major active constituent of tangerine *(Citrus reticulata)* and sweet orange *(citrus sinensis)* peel [1,2].

The beneficial health effects of a diet supplemented with fruit and vegetables have enhanced interest in their bioactive compounds. It has been shown that the positive effect of these natural products is usually connected with their antioxidant compounds. Naringin and Hesperidin are the main citrus flavonoids with physiological properties present orange juice. These flavonoids have been detected in human plasma after orange and grapefruit diets [3] Hesperidin may be associated with potential benefits in the prevention of many diseases, such as decreasing capillary permeability, anti inflammatory, antimicrobial and anticarcinogenic effects. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase [4-7]. Hesperidin is effectively used as a supplemental agent in the treatment protocols of complementary settings. Its deficiency has been linked to abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Supplemental Hesperidin also helps in reducing oedema or excess swelling in the legs due to fluid accumulation. A number of researchers have examined the radical scavenging properties of Hesperidin using a variety of assay systems [8- 11].

This study will explore the isolation of Hesperidin, from orange peel and the chemical characterization by spectroscopic methods.



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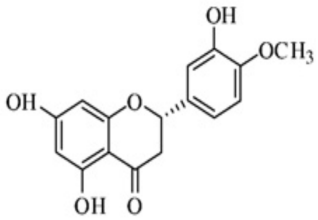

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Fig. 1: chemical structures of Hesperidin (1) and Hesperitin (2)

MATERIALS AND METHODS

Plant material: A peels of three citrus species has obtained from the university restaurant and from the wastes of home consumption.

Chemicals: All chemicals were of reagent grade. Acetic acid, methanol, dimethylformamid, petroleum ether, and chloroform were obtained from Merck (Germany). Distilled water was used throughout.

Extraction of Hesperidin:
Air dried sweet orange peels were grinded into powder and Extracted successively amount 12 cycle (total mass of powder is 960 g), 80 grams of this powder was placed in a reflux condenser. 600 ml of petroleum ether was added and refluxed for 1.5 h. after filtration of hot mixture through a Buchner funnel, the powder was allowed to dry at room temperature. The dry powder was returned to the flask and 600 mL of methanol was added. The contents were heated under reflux for 2h again and then hot mixture was filtered. The filtrate was concentrated with distillation column, leaving a syrup residue crystallized from dilute acetic acid (6%), and yielding orange needles (crude Hesperidin) melting point was 268°C.

Purification of Hesperidin:
Procedure A:
The crude Hesperidin (only a sample) was added to dimethylformamide (7 mL g^{-1} of syrup) before added acetic acid solution, prepared by warming to about 60 °C a little acetic acid was added. The solution was then filtered through a Buchner funnel, diluted with an equal volume of water and was allowed to stand for 4 hours in order to crystallize. The crystals of Hesperidin were filtered off.

Procedure B:
The crude Hesperidin was added to chloroform. The white crystalline hesperidin was then filtered through a Buchner funnel. Pure Hesperidin has melting point 240-253 °C.

Conversion of Hesperidin into Hesperitin:
A mixture of hesperidin (9 g) and methanol (250 mL) and concentrated sulfuric acid (9 mL) was stirred and heated at reflux amount 8 hours. The resulting homogeneous solution was cooled and concentrated and then diluted with ethyl acetate (500 mL). Every 100 ml of The organic solution is washed with water (4 x 100 mL), and dried with magnesium sulfate. Hesperetin was purified by the following procedure: Dissolve the crude product in minimum of acetone, and the resulting solution was added to a vigorously stirred mixture of water (200 mL) and acetic acid (3 mL). In an ice bath, precipitated hesperetin was washed and cooled with water. Pure yellow pouder of hesperetin obtained has melting point of 220-221 °C.

Identification of hesperidin and hesperitin:
The products were analyzed with chromatography and spectroscopy methods (UV, IR and NMR).

UV spectra were obtained in MeOH solvent with UNICAM UV300 spectrophotometer. IR spectra were obtained with a AVATAR 320 FT –IR spectrophotometer. The NMR spectra were taken on a bruker GP 250 (^{1} H,250MHZ; ^{13}C,125 MHZ) spectrometer.

RESULTS AND DISCUSSION

Crude hesperidin extracted from tangerine has weighing 1.75%, from Clementine and sweet orange 2.4%. The production were continued with this later because is abundantly consume.

Chromatographic analysis results:
Hesperidin extracted from orange peel and commercial hesperidin were chromatographed over Silica gel, eluting with butanol /acetic acid/ water (4:1:5) R_f = 0.6. hesperitin and commercial hesperitin also were analyzed over Silica gel, eluting with ethyl acetate R_f = 0.71, with methanol/ chloroform (9.5:0.5) R_f = 0.72 . The same result for the tow sampels.

Spectroscopic analysis results:
Structure of hesperidin is shown in Fig.1. Hesperidin extracted from orange peel was identified by spectroscopic data. The UV spectrum of the methanolic extract in methanol showed maximum absorption at 290, 315, and 345 nm.

The IR spectrum as KBr disk showed a strong band of OH at 3541 and 3470 cm^{-1}, CH (aliphatic) at 3077, 2973, 2935 and 2913 cm^{-1}, C=C (aromatic) at 1601, 1514, 1465 and 1443 cm^{-1} and of C=O at 1651 cm^{-1}, C-O at 1280, 1203 cm^{-1}. The pattern of the spectrum was the same as standard.

The second compound obtained from hesperidin hydrolysis (Fig.1), again was identified by spectroscopic data. The UV spectrum showed maximum absorption at 290 nm.

The IR spectrum as KBr disk showed a strong band of OH at 3497 cm^{-1}, CH (aliphatic) at 3120, 3039, and 2836 cm^{-1}, CH (aromatic) at 2890 – 2957 cm^{-1} C=C (aromatic) at 1579, 1498 cm^{-1} and of C=O at 1635 cm^{-1}, C-O at 1170cm^{-1}. Also the pattern of the spectrum was the same as standard.

^{1}HNMR and ^{13}CNMR spectra of both hesperidin and hesperitin:
Hesperitin : ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.89 (1H, br s, 5-OH), 8.04 (2H, dd, J = 9.0, 2.0 Hz, H-6′ and H-2′), 7.15 (2H, dd, J = 9.0, 2.0 Hz, H-3′ and H-5′), 6.92 (1H, s, H-3), 6.80 (1H, d, J = 2.0 Hz, H-8), 6.47 (1H, d, J = 2.0 Hz, H-6), 5.07 (1H, J = 7.2 Hz, H-1″), 4.58 (1H, br s, H-1), 3.88 (3H, s, 7-OCH_3), 3.20–3.60 (6H, m, H-2″ to H-6″), 3.20–3.60 (3H, m, H-2 to H-6), 2.51 (1H, d, J = 6.0 Hz, H-5), 1.10 (3H, d, J = 6.0 Hz, H-6) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.3 (s, C-4), 164.2 (s, C-7), 163.2 (s, C-2), 162.7 (s, C-9), 161.4 (s, C-5), 157.2 (s, C-4′), 128.7 (d, C-2′ and C-6′), 123.0 (s, C-1′), 115.0 (d, C-5′ and C-3′), 105.7 (s, C-10), 104.1 (d, C-3), 100.8 (d, C-1″), 100.2 (d, C-1), 99.9 (d, C-6), 95.1 (d, C-8), 76.5 (d, C-5″), 75.9 (d, C-3″), 73.4 (d, C-4), 72.3 (d, C-2″), 71.0 (d, C-4″), 70.6 (d, C-3), 69.9 (d, C-2), 68.6 (d, C-5), 66.4 (t, C-6″), 55.9 (q, 7-OCH_3), 18.1 (q, C-6).

Hesperidin : ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.01 (1H, br s, 5-OH), 6.97 (1H, d, J = 2.0 Hz, H-2′), 6.88 (1H, J = 8.0 Hz, H-5′), 6.83 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.14 (1H, d, J = 2.0 Hz, H-8), 6.13 (1H, d, J = 2.0 Hz, H-6), 5.50 (1H, dd, J = 11.0, 5.0 Hz, H-2), 4.97 (1H, d, J = 7.2 Hz, H-1″), 4.54 (1H, br s, H-1), 3.78 (3H, s, 4-OCH_3), 3.20–3.60 (6H, m, H-2″ to H-6″), 3.20–3.60 (3H, m, H-2 to H-6), 3.11 (1H, dd, J = 17.0, 11.0 Hz, H-3a), 2.78 (1H, dd, J = 17.0, 5.0 Hz, H-3b), 2.51 (1H, d, J = 6.0 Hz, H-5), 1.09 (3H, d, J = 6.0 Hz, H-6); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 197.2 (s, C-4), 165.5 (s, C-7), 163.2 (s, C-5), 162.7 (s, C-9), 148.1 (s, C-4′), 146.6 (s, C-3′), 131.1 (s, C-1′), 118.1 (s, C-6′), 114.3 (d, C-2′), 112.2 (d, C-5′), 103.5 (s, C-10), 100.8 (d, C-1), 99.6 (d, C-1″), 96.5 (d, C-6), 95.7 (d, C-8), 78.6 (d, C-2), 76.5 (d, C-5″), 75.7 (d, C-3″), 73.2 (d, C-4), 72.2 (d, C-2″), 70.9 (d, C-4″), 70.5 (d, C-3), 69.7 (d, C-2), 68.5 (d, C-5), 66.2 (t, C-6″), 55.8 (q, 4-OCH_3), 42.2 (t, C-3), 18.1 (q, C-6).

CONCLUSION

It can be concluded that the big wast results of menager from the larg consemption of oranges and the important yeld of hesperidin in orange peel can guide us to think about this economic procedure of production of pure hesperidin very useful in pharmaceutical formulation.

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