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Hesperidin from *Citrus sinensis* Cultivated in Dezful, Iran

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Abstract: This study described procedures for extracting and quantitation of hesperidin in the waste orange peel of Dezful. Two extracting procedure were used. In procedure A hesperidin was isolated from orange peel by extracting the dry peel first with petroleum ether, removing the essential oil and then with methanol. In procedure B alkaline extraction followed by acidification of the extract was used. It was purified by treatment with formamide-activated charcoal. Detailed analysis of UV, IR, ^{1H}NMR, ^{13C}NMR and Mass spectroscopic data confirm the structure and extent of purity of extracted hesperidin. The spectroscopic results of two extract showed that procedure A produced high extraction yield and more purified hesperidin. Pure hesperidin in gram quantity (11.7% for procedure A and 7.39% for procedure B) was obtained in one purification cycle.

Key words: Hesperidin, orange peel, extraction, purification

INTRODUCTION

Flavonoids are an important group of secondary metabolites, which are synthesized by plants as a result of plant adaptation to biotic and a biotic stress conditions (infection, wounding, water stress, cold stress, high visible light). Protective phenylpropanoid metabolism in plants has been well documented (Shetty, 2004; Harborne and Williams, 2000; Korkina, 2007). In recent years flavonoids have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals for their hydrogen radical donating abilities (Jeong *et al*., 2007; Lemanska *et al*., 2001). Many epidemiological studies have shown that consumption of edible plants rich in phenolic compounds is associated with a lowered risk of degenerative diseases such cancers (Harris *et al*., 2007), cardiovascular diseases (Naruszewicz *et al*., 2007) and immune dysfunctions (Kale *et al*., 2008). These epidemiological results are corroborated by many *in vitro* and *in vivo* studies demonstrating the impact of flavonoids on mammalian biology (Turner *et al*., 2004) and displaying the remarkable scope of biochemical and pharmacological actions of these compounds, among others their antiviral (Materska and Perucka, 2005), antiinflammatory (Wu *et al*., 2006) and antiallergic (Sökmen *et al*., 2004) properties.

Hesperidin is a flavanone glycoside abundantly found in sweet orange and lemon and is an inexpensive by-product of citrus cultivation (Garg *et al*., 2001). Hesperidin may be associated with potential benefits in the prevention of diseases, such as decreasing capillary permeability, anti inflammatory, antimicrobial and anti carcinogenic effects. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase (Uehara, 2006). 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 antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems (Orallo *et al*., 2004; Cho 2006; Hirata *et al*., 2005; Fujisaw *et al*., 2002).

This study will explore the isolation of the natural product, hesperidin, from orange peel and will also chemically identified and then characterized it by several spectroscopic methods.

MATERIALS AND METHODS

Plant material: Mature orange fruit, harvested in November 2005, was obtained from Safieabad Research Center, Dezful, Southwest of Iran and identified as *Citrus sinensis* (Rutaceae). Chopped green peels were sun dried for 36 h.

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Chemicals: All chemicals were of reagent grade. Hesperidin was obtained from Sigma (USA). Acetic acid, ferric chloride, calcium hydroxide, isopropanol, methanol, formamide, hydrochloric acid, Mg metal, petroleum ether, celite, activated charcoal, ethanol and dimethyl sulfoxide were obtained from Merck (Germany). Distilled water was used through out.

Extraction of hesperidin: Sun-dried green peel of orange was grinded into powder with two procedures (Ikan, 1991).

Procedure A: Two hundred grams of this powder was placed in a reflux condenser. One liter of petroleum ether was added and refluxed on a water bath for 1 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 1 L of methanol was added. The contents were heated under reflux for 3 h again and then hot mixture was filtered and washed with 200 mL hot methanol. The filtrate was concentrated under reduced pressure, leaving a syrupy residue crystallized from dilute acetic acid, yielding white needles, mp 252°C.

Procedure B: Two hundred grams of powder was transferred in an Erlenmeyer flask, 750 mL of calcium hydroxide was added and mixed thoroughly. The mixture was stayed at room temperature overnight. The mixture was filtered through Buchner funnel containing a thin layer of celie. The pH of the filtrate was adjusted to 4. An amorph powder was separated which was again filtered and washed with distilled water and kept for recrystallization by formamide.

Purification of hesperidin: The crude hesperidin was added to dimethylformamide (7 mL g^{-1} of syrup), prepared by warming to about 60°C and then was treated for 30 min with activated charcoal previously boiled with dilute hydrochloric acid. The formamide solution should be slightly acidic; a little glacial acetic acid was added. The solution was then filtered through celite, diluted with an equal volume of water and was allowed to stand for a few hours in order to crystallize. The crystals of hesperidin were filtered off and washed, first with hot water and then with isopropanol. Two such crystallizations were given a white crystalline product melting at 260-262°C.

Identification of hesperidin

Ferric chloride test: Addition of alcoholic ferric chloride solution to hesperidin produce a wine red color.

Magnesium-hydrochloric acid reduction test (Shinoda test): Dropwise addition of concentrated HCl to an ethanolic solution of hesperidin containing magnesium developed a bright violet color (Ikan, 1991).

Spectroscopic studies of hesperidin: (a) NMR spectra were recorded at 500 MHz for ^{1}H and ^{13}C by Bruker NMR spectrometer using DMSO-d_6 and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as internal standard, (b) Mass spectra were recorded on Finnganmat TSQ 70 USA spectrometer (c) UV-Visible recording spectrometer 7850, Jasco (Japan) was used for UV spectrum and (d) IR spectra were recorded on IR 700, Jasco (Japan).

RESULTS AND DISCUSSION

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 DMSO showed maximum absorption at 290 nm. The pattern of the spectrum was the same as standard for both extraction procedures.

The IR spectrum as KBr disk showed a strong band of OH_{str} at 3544 and 3470 cm^{-1}, CH (aliphatic) at 2976, 2916 and 2848 cm^{-1}, C=C (aromatic) at 1606, 1519, 1467 and 1443 cm^{-1} and of $C=O_{str}$ at 1648 cm^{-1}, $C-O_{str}$ at 1298, 1276, 1240, 1203, 1182, 1154, 1131, 1094, 1050, 1033 and 1009 cm^{-1}.

^{1H}NMR and ^{13C}NMR of extracted hesperidin by procedure A showed the following chemical shifts in ppm which are as those of standard. Those of procedure B showed a few more different chemical shifts.

^{1H}NMR: δ 12.00 (OH-5), δ 6.91 (H-6′, 5′), δ6.12 (H6, 8), δ 5.49 (H2), δ 3.79 (OCH_3).

^{13C}NMR: δ 78.4 (C-2), δ 42.0 (C-3), δ 196.7 (C-4), δ 163.3 (C-5), δ 96.7 (C-6), δ 165.2 (C-7), δ 95.8 (C-8),

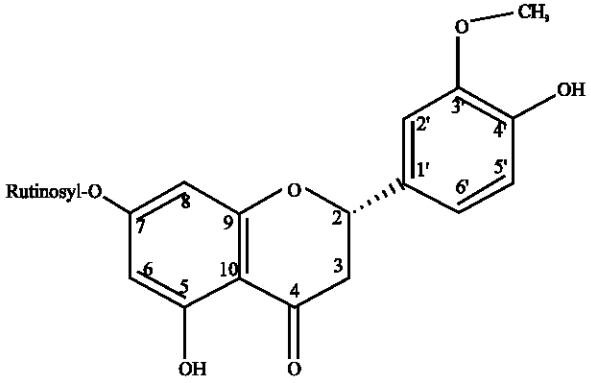

Fig. 1: Chemical structure of Hesperidin

δ 162.5 (C-9), δ 103.5 (C-10), δ 131.0 (C-1′), δ 114.3 (C-2′), δ 146.7 (C-3′), δ 148.1 (C-4′), δ 112.7 (C-5′), δ 117.8 (C-6′) and δ 56.0 (Ome).

Mass spectrum showed m/e of 483.0 (100%), 485.1 (45%), 349.0 (50%), 238.0 (26%).

No additional peaks were seen in this spectroscopic spectrum of procedure A. So, it can be concluded that the extracted hesperidin is almost pure. However the percent of hesperidin in orange peel of Dezful was determined by weighing. 11.7 g hesperidin was extracted from 100 g of Dezful orange peel by procedure A.

These data also showed that hesperidin can easily be extracted and purified from orange peel by procedure A.

So, it is economic to use orange peel as a source of hesperidin for pharmaceutical formulation.

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