



Research Article

Isolation and characterization of hesperidin from dried orange peel

Chaudhri VK, Hussain Z, Pandey A, Khan R, Srivastava AK


Department of Pharmacy, Mahatma Gandhi
Institute of Pharmacy, Lucknow-227101,
Uttar Pradesh, India

Address for Correspondence:
Vikash Kumar Chaudhri
E-mail: vikashk464@gmail.com

ABSTRACT

India is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. General phytochemical screening of *Citrus sinensis* (Orange) belonging *Rutaceae* family revealed the presence of flavones glycoside. The aim of this study is to identify and characterize the bioactive principle compound (Hesperidin) from the dried peel of *C. sinensis* fruit. It has wide folk medicinal uses. The isolation and characterization of phytoconstituents was done from the methanolic extract of dried peels.

Key words: *Citrus sinensis*, Hesperidin, Dried orange Peel.

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INTRODUCTION

There is great demand for herbal medicine in the developed as well as developing countries like India, because of their wide biological activities, higher safety of margin than the synthetic drugs and lesser costs¹. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as well as valuable components of seasonings, beverages, cosmetics, dyes, and medicines. Consumption of fruit and vegetables, as well as grains, has been strongly associated with reduced risk of diseases².

Natural products are organic compound that are formed by living system. The elucidation of their structure, chemistry, synthesis and biosynthesis are major areas of chemistry. Phytochemistry or the chemistry of natural products may be strategically placed somewhere in between natural product, organic chemistry and plant biochemistry. In fact it is intimately related to the above two discipline³. However, in a border sense phytochemistry essentially deals with the enormous different type of organic substances that not only elaborated but also accumulated by plant. It is also solely concerned with the following various aspects namely Natural distribution, Chemical structure, Biosynthetic structure, Biosynthesis (biogenesis), Metabolism and Biochemical function⁴. Hesperidin is a flavanone glycoside (flavonoid) (C₂₈H₃₄O₁₅) found abundantly in citrus fruits. Its aglycone form is called hesperetin. Hesperidin was first isolated by Leberton in 1828 from the albedo (the spongy inner portion of the peel) of oranges of the

family Hesperides, and was given the name hesperidin. Its presence was detected in lemons by Pfeiffer as early as 18743⁵. Hesperidin is believed to play a role in plant defense. It acts as an antioxidant according to *in vitro* studies. In humans it contributes to the integrity of the blood vessels. Hesperidin reduced cholesterol and blood pressure in rats. In a mouse study large doses of the glucoside hesperidin decreased bone density loss⁶. Another animal study showed protective effects against sepsis. Hesperidin has anti-inflammatory effects. A number of researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems. The current literature highlights that hesperidin exerts, efficiently, an attenuating effect on the progression of hyperglycemia and also on some diabetes-induced complications in rat brain⁷.

Importance of Hesperidin

Hesperidin, an abundant and inexpensive bioflavonoid in Pengan (*Citrus reticulata*) peel, has been reported to possess a wide range of pharmacological properties like antioxidant, anti-inflammatory, hypolipidemic, vasoprotective and anticarcinogenic and cholesterol lowering actions. Hesperidin is also an enzyme inhibitor and inhibits phospholipase A₂, lipoxygenase, HMG-CoA reductase and cyclo-oxygenase⁸.

Hesperidin improves the health of capillaries by reducing the capillary permeability. Hesperidin is used to reduce hay fever and other allergic conditions by inhibiting the release of histamine from mast cells. The possible anti-cancer activity of hesperidin could be explained by the inhibition of polyamine

synthesis. Sources of hesperidin include citrus fruits, berries, onions, parsley and green tea⁹.

Hesperidin has been extracted from a variety of sources using both analytical as well as preparative techniques. Waste orange peel from the citrus industry has been used as the raw material using styrene-divinylbenzene (SDVB) resin followed by desorption in much reduced volume of alkaline eluents. By this procedure good yield and high purity after acidification of the concentrated solutions, thus overcoming disadvantages due to the high dilution. Hesperidin was extracted from peel with an aqueous saturated $\text{Ca}(\text{OH})_2$ solution, allowing precipitation of calcium pectates from colloidal pectins that can interfere in the subsequent phases of adsorption and separation of hesperidin. The clear extracts were neutralized to optimize adsorption on resin. The most effective eluent was 0.5 N NaOH solution containing 10% ethanol¹⁰.

Another procedure used was ultrasonic assisted extraction of hesperidin from Citrus reticulata combined with parameters like extraction solvents, solvent volume, temperature, extraction time, ultrasonic power, ultrasonic frequency. It was observed that solvent, frequency and processing temperature were the most important factors for improving the extracting yields of hesperidin. The optimum ultrasonic conditions were determined as: methanol, frequency of 60 kHz, extraction time of 60 min, and temperature of 40°C¹¹.

Another procedure is by treating the orange peel with $\text{Ca}(\text{OH})_2$ and recycling of the extracting liquor led to an increase of the yield of both extracted hesperidin and naringin. The highest yield of hesperidin was 15.5 g/2 kg peel, and the highest yield of naringin was 12 g/2 kg peel. The effect of maturity of the peel and recycling of the extracting liquor upon the yield of glucoside were investigated. The highest yields of hesperidin were obtained from orange peel extracted at the early season, increase in maturity led to a decrease in yield of hesperidin extracted and a decrease of its purity¹².

MATERIAL AND METHOD

Collection of Plant Material

The fruits of *Citrus sinensis* (Orange) were purchased from local market of Junabganj, Lucknow and they were peeled off and peels were dried under shade.

Extraction of Crude Hesperidin

800 mL petroleum ether (40 – 60°C) is filled in a 250 mL round bottom flask with magnetic stir bar. 250g dried and powdered dried orange peel are placed in the extraction sleeve of a Soxhlet extractor and covered with a little glass wool. A reflux condenser is put on the Soxhlet extraction unit, and then the reaction mixture is stirred and heated for 4 hours under strong reflux. The petroleum ether extract is discarded. In order to remove the adherent petroleum ether, the content of the

extraction sleeve is laid out in an extensive crystallisation dish. Afterwards the substance is placed again in an extraction sleeve and, like before, but with 800 mL methanol, extracted unless the solvent leaving the extraction sleeve is colour less (1 to 2 hours). After complete Soxhlet extraction and maceration the filtrate was then acidifying (pH 3-4) with 6% acetic acid, Keep the concentrated residual liquid in refrigerator (4-6°C) over night when a solid crystalline substance appears. It was again filtered and the crude hesperidin was separated out on buchner funnel as amorphous powder. The hesperidin was further characterized and identified according to various Physical and analytical test.

Physical data for Hesperidin

Hesperidin should yield white needles upon recrystallization.

Melting point range : 242-244°C.

Color : Yellowish brown

Odor : Aromatic and characteristic

Yield : 1.75 gm

RESULT AND DISCUSSION

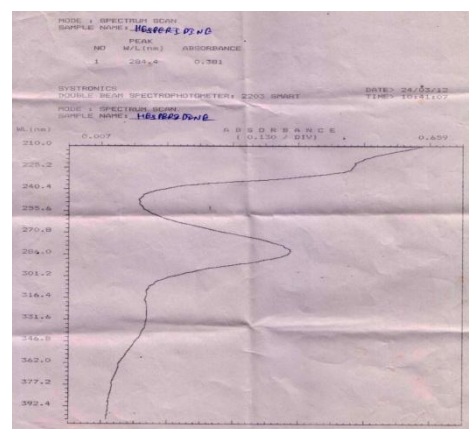
Analytical test observation result

1. Ferric chloride test : Wine red color
2. Shinoda test : Bright pinkish violet color
3. Thin layer chromatography : [n-butanol: Acetic Acid: Water (4:1:5)] one spot observed and R_f value found at 0.48.

UV Spectral analysis

To prepare 5µg/ml of methanol and water (1:1) solution of isolated compound hesperidin and UV spectra were recorded on a UV-Visible Spectrophotometer Pharma spec-1700 (SHIMADZU). The λ_{max} was found to be 284.4 nm. The UV spectra given in fig. 1.

Fig. 1. UV Spectra of isolated compound hesperidin



FTIR Spectral analysis

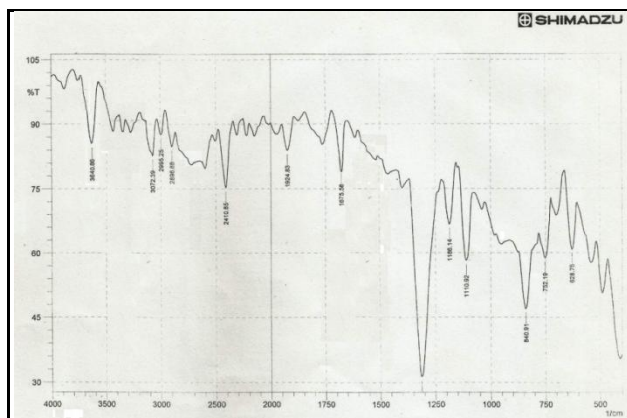
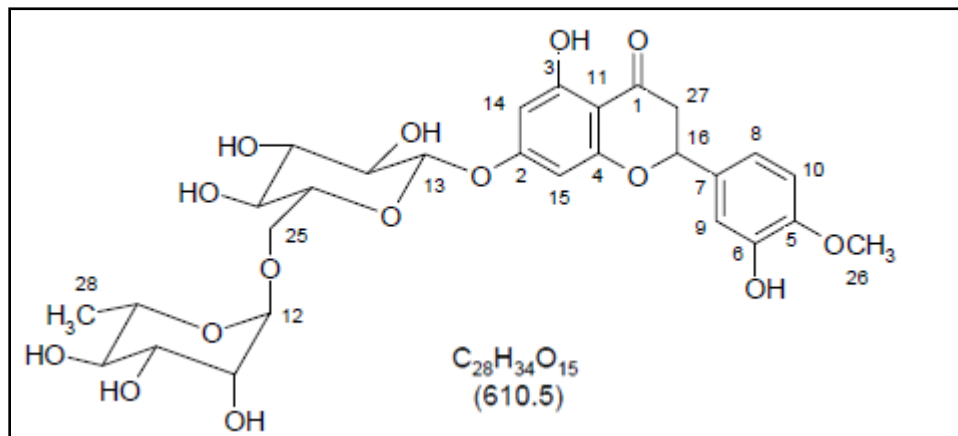
FTIR spectrum of compounds was recorded on a Perkin Elmer Spectrum RXI FTIR system by using potassium bromide pellets.

Preparation of KBr pellets of compounds

100 mg of anhydrous KBr (IR grade) was accurately weighed and 1.0 mg of compound was added to it and triturated well.

The mixture was placed in an evacuable die and subjected to a pressure of 5-6 tones for 5 minutes. A transparent disc was produced which was then placed in a pellet holder and IR spectra was recorded and given in fig. 2.

FTIR (KBr, ν_{\max} , cm^{-1}): 3640 (O-H str.), 3072 (C-H str. (arene)), 2995 (C-H str. (alkane)), 2896 (C-H str. (alkane -OCH₃)), 1675 (C=O Str.), 1625 (Aromatic C=C str.)

Fig. 2. IR Spectra of isolated compound Hesperidin**Fig. 3. Chemical Structure of Hesperidin**

The compound was isolated from maceration and Soxhlation shows a positive ferric chloride and Shinoda test for flavonoids, indicating that the compound may be a flavonoid. It is yellow brown in color, characteristic aromatic in odor, with melting range 242-244°C. There are two spots were observed in thin layer chromatography of crystal using n-Butanol: Acetic Acid: Water (4:1:5) as mobile phase at 0.48, physical and analytical characters determine by UV and FTIR methods. The yield of hesperidin is 1.75 gm respectively.

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