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Review On: Biological Activities and Extraction Methodology of Hesperidine

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Abstract
Citrus fruits contain significant quantities of hesperidin, a bioflavonoid. Numerous health advantages, including antioxidant, antibacterial, antimicrobial, anti-inflammatory, and anticarcinogenic characteristics have been linked to its use. Citrus fruit is extensively used in the culinary sector, particularly for the creation of juice. Huge quantities of byproducts, including peels, seeds, cell, and membrane remnants, which are also a good source of hesperidin, end up being accumulated as a result. In order to utilise them as natural antioxidants, its extraction from these byproducts has received significant scientific interest. The extraction techniques for quantifying hesperidin in fruits and by-products are provided in this review, along with a discussion of its biological functions. In this review article structure of hesperidein, extraction methodology and biological activities was discussed.

Keywords
Hesperidin, extraction methods, Soxhlet Extraction, Maceration, biological activities.

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1.INTRODUCTION
Hesperidin (3, 5,7-trihydroxyflavanone 7-rhamnoglucoside, hesperetin-7-*O*-rutino- side) belongs to flavanone compounds, one of the flavonoids subclasses (Fig.1). It is utilised as a radioprotector, a treatment for type 2 diabetes, cancer, cardiovascular diseases, neurological and psychiatric disorders, and has recently undergone significant testing for its health-promoting and pharmacological benefits. Hesperidin administrations can also improve a number of cutaneous functions in both healthy and diseased skin. **[1-7].**

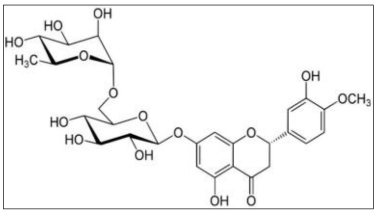

Fig.no.1: Structure of hesperidin

Citrus fruits (Rutaceae family) as orange (Citrus sinensis), grapefruit (Citrus paradise), tangerine (Citrus reticulata), lime (Citrus aurantifolia), and lemon (Citrus limon) are known for their unique components known as hesperidin and its derivatives. Their presence in citrus fruits varies depending on the fruit variety, the fruit's composition, the climate, and the fruit's level of maturity. 100 mL of a suitable juice has 20–60 mg of hesperidin for oranges, 8–46 mg for tangerines, 4-41 mg for lemons, and 2–17 mg for grapefruit, according to the review by Gattuso *et al*. Citrus flavedo (the coloured outer layer of the peel) and albedo (a white soft middle layer part) contain higher amounts of hesperidin in comparison to hand-squeezed juice. This flavanone is abundant in commercial juices that have also had peel components added to the squeeze. Hesperidin was also discovered in mint (Mentha), honeybush (Cyclopia mac ulata), and aromatized tea in addition to citrus fruits. It is important to note that hesperetin, the aglycone form of hesperidin, is not as prevalent as its glycosides in nature.

Following oral ingestion, hesperidin is hydrolyzed into aglycone form (hesperetin) by the gut bacteria rhamnosidases in the small intestine and mostly in the colon before being converted to glucuronides in the large intestine. Hesperetin first manifests in the plasma 3 hours after consumption as glucaronides (87%) and sulphaglucooronides (13%), peaking between 5 and 7 hours later. Later, they underwent ring fission and catabolization, resulting in the production of phenolic acids and their corresponding metabolites. However, hesperidin exhibits low water solubility and limited bioavailability. Various approaches such as the micronization and encapsulation of hesperidin have been proposed, particularly for drug productions, to improve its bioavailability, stability and controlled release **[8-21].**

2.EXTRACTION METHODOLOGY

Hesperidin is frequently utilised in the food, cosmetic, and pharmaceutical industries because to its biological properties. These applications imply the best extraction methods from high-quality, pure plant resources.

Using dipping, percolation, reflux, or continuous reflux are common extraction techniques. Several variables, including the solvent type, temperature, extraction time, and liquid-solid ratio, have an impact on the quality of an extract and the effectiveness of a method.

Hesperidin was isolated from sweet orange pulp (Citrus sinensis L.) using two sequential extractions with 90% methanol for 20 minutes of agitation at 55 °C; however, under the same conditions with 90% ethanol, only 17.9 mg/g of hesperidin was obtained (with significant differences at p 0.05) [23].

Gómez-Mejia *et al.*: proposed that extraction from orange peels with low concentration of ethanol (maximum 40%, *v*/*v*) run in an ultrasound bath for 10–15 min. [22]

Methanol and ethanol, or their mixture with water at different proportions, as well as dimethyl sulfoxide (DMSO), is usually used. To improve efficiency and selectivity, modern procedures are rapidly replacing maceration and Soxhlet extraction. They often have higher degrees of automation, are speedier, and are more ecologically friendly. On accelerated solvent extraction (ASE)-based methods [24], Hesperidin has been isolated from plant materials by microwave-assisted extraction (MAE), ultrasound-assisted extraction (USE), subcritical water extraction (SWE), pressurized liquid extraction (PLE), and high hydrostatic pressure (HHP).

It was claimed that a very quick and effective extraction process may make up for the use of a rather high temperature (90 °C). On the other hand, a DMSO:methanol mixture (1:1) proved to be a better medium for hesperidin extraction from mandarin (Citrus reticulata Blanco) rinds during a 10-minute ultrasound operation. The amount of hesperidin extracted from Citrus unshiu fruit peels using the MAE procedure (70% aqueous ethanol, heating 140 °C, 7 min) was comparable to the amount extracted using the DMSO:methanol (1:1) mixture at room temperature for 30 min. The maximum yield of hesperidin from *Citrus unshiu* peel using SWE method was obtained at 160 °C for an extraction time of only 10 min. It was 1.9-, 3.2- and 34.2-fold higher than those when 70% ethanol or methanol and hot water, respectively, were used. [25-32].

Deep eutectic solvents (DESs) and room temperature ionic liquids (ILs) have recently been introduced as new types of substitute solvents for the extraction and/or purification of bioactive chemicals. ILs are organic salts made entirely of ions, including small inorganic anions (Cl, Br, BF4, and PF6) and relatively large organic cations (imidazolium and pyridinium) with a variety of tailorable properties. Their melting point is often less than 100 °C. In the microwave-assisted extraction of flavonoids from plant material, a number of imidazolium-based ionic liquids with various alkyl positions and alkyl chain lengths were assessed. The 1, 3-dibutyl-2-methyl imidazolium bromide showed the best extraction efficiency (temperature of 80 °C, period of 60 min, microwave power of 300 W, and IL concentration of 1.0 mol/L). Using 1-decyl-3-methylimidazolium bromide as an addition (2.5 mg/mL) in 200 mL of methanol during Soxhlet extraction at 200 °C for 8 hours, Tang *et al*. identified the ideal extraction settings for the isolation of certain flavonoids from plant leaves. In vacuum microwave-assisted extraction, hesperidin, hyperoside, and rutin were separated using 1-hexyl-3-methylimidazolium tetrafluoborate. Although they have physicochemical characteristics that are very comparable to those of ILs, such as low volatility and strong thermal and chemical stabilities, deep eutectic solvents made from Lewis or Brönsted acids and bases are less hazardous and more biodegradable. By combining solid substances in the right ratios after slight heating, they are easily formed. The melting point of the resulting eutectic mixture is substantially lower than that of the constituent parts. In a study by Bajkacz and Adamek, the effectiveness of extracting flavonoids, including hesperidin, from fruits, vegetables, and spices was assessed using 17 different natural DES systems with 2 or 3 components based on choline chloride, acetylcholine chloride, choline tartrate, betaine, and carnitine with different compositions. Using a 30% water solution of acetylcholine chloride/lactic acid (molar ratio 2:1) and 30 min of extraction time at 60°C, the greatest extraction yield of the target compounds (>70%) was achieved. Xu *et al*. looked into the connection between the DESs' physicochemical characteristics and the yields of flavonoids extracted from citrus peels. [33-41]. In a molar ratio of 1:2, choline chloride (ChCl), sugars, amides, alcohols, and carboxylic acids were utilized as the second ingredient. The efficiency of hesperidin extraction for amide- and carboxylic acid-based DESs increased linearly in the following order: ChCl-urea, ChCl-N-methylurea, and ChCl-acetamide, which is consistent with the upward trend in log KO/W values.

Table 1: Recent examples of extraction conditions for the isolation of hesperidin from different plant materials.

Sample	Conditions	Hesperidin mg/g dw	Ref.
Mandarin (*Citrus reticulata*) rinds	USE, 35 °C, 10 min: DMSO:methanol (1:1) 80% ethanol	32.0 5.46	[27]
Mandarin (*Citrus reticulata*) rinds	USE, 35 °C, 10 min: DMSO: methanol (1:1) 80% ethanol	32.0 5.46	[27]
Navel orange peels (*Citrus sinensis*)	40% ethanol, USE, 90 °C, 15 min	498	[22]
Thinned *Citrus unshiu* fruits	70% ethanol, MAE, 140 °C, 7 min DMSO: methanol (1:1), room temperature, 30 min	58.6 64.3	[25]
Peels of mandarin (*Citrus reticulata*)	70% methanol, PLE, 160 °C, 20 min 100% methanol, HR, 80 °C, 60 min	58.4 58.6	[31]
Peels of mandarin (*Nobis tangerine*)	Choline chloride-acetamide, 45 °C, 25 min	38.0	[41]

Abs: HR—heat reflux, PLE—pressurized liquid extraction, MAE—microwave-assisted extraction, USE—ultrasound-assisted extraction, SWE—subcritical water extraction.

2.BIOLOGICAL ACTIVITY

Hesperidin's bioavailability is primarily responsible for the human benefits that can be obtained from ingesting it in foods, drinks, or medications. The amount of a substance released from food that passes through the intestinal barrier, enters the bloodstream, enters the systematic circulation, is distributed to organs and tissues, and is then converted into a biochemically active form that is efficiently utilised by the organism is known as the bioavailable fraction [42].

Hesperidin's limited water solubility, intestinal absorption, modification by microbes, and quick excretion all contribute to its low bioavailability [43]. The enzyme-rhamnosidase, which participates in these processes, is thought to be the limiting step in the hydrolysis and absorption of hesperidin. Hesperidin has a reasonable half-life of 6 hours despite being poorly absorbed and quickly removed. [44].

Hesperidin's biological and pharmacological characteristics have been thoroughly investigated to reveal, among other things, its antioxidant, anti-inflammatory, anti-cancer, antiviral, preventive cardiovascular diseases, and neurological capabilities.

Table 2: Lists of several instances of hesperidin's primary biological actions.

Biological Activities	Method	Hesperidin Dose	Results	Ref.
Antioxidative	Evaluation of marker enzymes and antioxidant status in blood, tissues, bronchoalveolar lavage cells and fluid after subcutaneous injection of nicotine	25m g/kg	Protection against the lung damage caused by nicotine, which induces the lipid peroxidation	[52]
	Examination the iron chelation activity on the brain tissue of iron-overloaded mice	50 mg/kg per day (4 weeks)	Strong chelation of excessive iron from the serum and deposit iron	[50]
Prevention of cardiovascular diseases	Analysis of biochemical, histopathological, ultrastructural and immunohistochemical studies of rat heart after isoproterenol induced cardiac hypertrophy	200 mg/kg/ per day (4 weeks)	Improved hemodynamic and cardiac function parameters with a reduction in the levels of cardiac injury markers	[57]
	Evaluation of the effect of orange peel extract on streptozotocin-induced diabetic nephropathy	200 mg/kg for 4 weeks	Improved renal functions, significant prevention of the increase of creatinine, urea and blood urea nitrogen levels	[14]
Anti-inflammatory	Evaluation of the effects on neutrophil recruitment, edema, colon lesions and cytokines production in a pre-clinical model of ulcerative colitis induced by acetic acid in mice	100 mg/kg in saline by oral gavage	Reduction of inflammation, increase in colon antioxidant status, inhibition of proinflammatory cytokines	[74]
	Determination of blood pressure, serum antioxidant capacity, tumor necrosis factor alpha and inflammatory markers	500 mg/day (6 weeks)	Hesperidin has antihypertensive and anti-inflammatory effects in type 2 diabetes	[73]
Anticancer	The effect of hesperidin on the proliferation and apoptosis of non-small cell lung cancer in mice	60 mg/kg per day	Modulation of antioxidative enzymes induced apoptosis, suppression of cancer cell proliferation and invasiveness	[59]

Several scientists have used several assays to look into the antioxidant capacity of hesperidin. Its antioxidant capabilities are mostly demonstrated by the direct scavenging of free radicals or indirectly through the inhibition of prooxidative enzymes involved in the production of these radicals, as well as through the chelation of transition metals involved in the production of reactive oxygen species. The findings demonstrated that hesperidin, a prominent chelator for treating chronic iron overload, has greater potential iron chelation activities than deferoxamine. Hesperidin's antioxidant action is also demonstrated by a decrease in the formation of ROS and an increase in the activity of the antioxidant enzymes catalase and superoxide dismutase.

Citrus peels showed higher antioxidant ability than pulp due to its high content of flavonoids, vitamin C and carotenoids. Hesperidin's cardioprotective properties are demonstrated by a reduction in diastolic blood pressure, glucose levels, and other lipid profile indices, a reduction in platelet aggregation, and an increase in the expression of antioxidative enzymes. Hesperidin administration increased the expression of miRNA-132, which in turn promoted apoptosis and inhibited the growth of non-small cell lung cancer cells in mice. ZEB2, a transcription factor that binds to particular regions of DNA, was decreased by this process. [45-59].

Citrus peel extracts have been demonstrated to be a promising treatment agent for diabetes mellitus, which is characterized by deficiencies in insulin metabolism that can affect the metabolism of carbohydrates, proteins, and fats. According to a review of the literature, pancreatic beta-cell malfunction and insulin resistance are both associated with obesity. Hesperidin has an inhibitory impact against the onset of neurodegenerative disorders including Alzheimer's and Parkinson's, according to growing data. It demonstrated how immunity plays a role in the onset and progression of neurodegenerative diseases. Several investigators have dedicated their effort to explore neuropharmacological mechanisms and the molecular target of citrus flavonoids, including hesperidin [60-70].

Hesperidin's potential anti-inflammatory benefits for prospective therapeutic use against a variety of illnesses have been assessed. According to the findings of Homayouni *et al*., type 2 diabetes patients may benefit from the anti-inflammatory and antihypertensive properties of hesperidin supplementation. Scientists are becoming more interested in using naturally occurring chemicals as powerful antibacterial agents as a result of the rise in germ resistance to synthetic antibiotics. Hesperidin has been shown in numerous papers to be effective against various harmful microorganisms. To lessen microbial pathogenicity, it can either directly suppress bacterial growth or operate indirectly by regulating the expression of virulence proteins. Supplemental hesperidin may be effective as a preventative measure against SARS-CoV-2 by obstructing several viral infection and replication pathways.

In addition to the above-mentioned positive health effects, hesperidin has also been linked to radioprotective protection against ionising radiation-induced damage, UV protection, wound healing, and cutaneous functioning. It is advised for treating acute hemorrhoidal attack symptoms as well as venous circulation abnormalities (swollen legs, discomfort, and nocturnal cramps) since it reduces capillary fragility when combined with the flavone diosmin, which is sold under the trade name Daflon® [71-82].

3.CONCLUSION

Hesperidin is one of the most intriguing and promising bioflavonoids due to the variety of pharmacological effects it exhibits in the human body. Citrus fruits and juices are readily available dietary sources for its consumption and are consumed widely around the world. Hesperidin is a citrus bioflavonoid that can be found in supplements, either by itself or in combination with other citrus bioflavonoids. Furthermore, due to the significant amount of peel generated during the processing of citrus by-products, it is a rich source of hesperidin. Their use can be put to use to create brand-new nutraceuticals or to make existing ones better. Future research into innovative hesperidin delivery systems should concentrate on potential interactions between the flavanone and the food matrix. It could also be very interesting to investigate the possible use of co-encapsulating two or more bioactive compounds to provide a synergistic impact. In this review article, we studied the extraction and biological activities of hesperidin. But in future aspect we should highly focus on recent biological activities of hesperidin in appropriate manner.

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