Hesperidin: A Growing Nutraceutical Ingredient

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Abstract: Nutraceuticals, defined as substances providing medical or health benefits through food or supplements, have gained significance in promoting well-being. This paper explores the nutraceutical potential of hesperidin, a flavonoid abundant in citrus fruits. The classification of nutraceuticals, based on function and bioactive components, includes vitamins, minerals, amino acids, and antioxidants, with hesperidin falling under the flavonoid category. Secondary metabolites, derived from plant biosynthetic reactions, play a crucial role in plant structure and function. Hesperidin, a secondary metabolite, is synthesized through the shikimic acid pathway and classified under flavonoids. Its isolation from citrus fruits involves conventional and modern methods, such as maceration and high-pressure extraction. The chemical properties of hesperidin, including its molecular formula, solubility, and toxicity, etc... are discussed. Analytical techniques, such as high-performance liquid chromatography and spectrophotometry, are employed for its identification and separation. The extraction process and yield are influenced by factors like solvent choice and extraction method. Hesperidin's nutraceutical uses encompass potential benefits in cardiovascular health, anti-inflammatory effects, and neuroprotection. Studies suggest its efficacy in conditions like high blood pressure, diabetes, and neurodegenerative diseases. However, further research is required to establish its wider therapeutic applications definitively. In conclusion, hesperidin emerges as a promising nutraceutical with potential health benefits. Its extraction, analysis, and biological properties contribute to understanding its role in promoting well-being. The multifaceted applications of hesperidin make it a valuable compound, yet ongoing research is essential to substantiate its applications further.

Keywords: Antioxidants, Nutraceuticals, Hesperidin, Flavonoids, Citrus fruits, Secondary metabolites, Biosynthesis, Extraction, Analysis, Biological properties.

INTRODUCTION TO NUTRACEUTICALS

A Nutraceutical is well defined as any substance that is a food or part of a food and furnishes medical or health benefits, including the prevention and treatment of disease. The term nutraceutical was defined by Dr. Stephen L. Defelice, the Founder and Chairman of the Foundation for Innovation in Medicine in the year 1989. Nutraceutical is a term that combines nutrients as well as pharmaceuticals and is defined as a substance that provides nutrition when taken as food or food supplement and also possesses the ability to prevent diseases or to improve health. However, the term nutraceuticals has no governing definition. These products are available as isolated nutrients, dietary supplements and specific diets to genetically engineered foods, herbal products and processed food stuffs (soups, cereals and beverages.)

Nutraceuticals are generally classified based on their function, food source, and bioactive components. Most of them fall under two general groups:

1. Dietary supplements
2. Functional food. [1]

Types of nutraceuticals include:

- Vitamins and minerals
- Amino acids
- Fatty acids
- Herbal and botanical products
- Antioxidants
- Probiotics
- Prebiotics
- Dietary fiber [2]

Secondary metabolites are substances generated as byproducts in the course of biosynthetic reaction. Most of these byproducts serve no vital purpose for plants and are stored in different parts of the plant's structure. These secondary metabolites are not essential to the plant's basic functions; rather, they are incidental outcomes of metabolic processes. The production of these secondary metabolites is primarily influenced by the plant's genetic composition, which selectively enhances specific processes necessary for their production.

The genetically regulated essential processes can be referred to as fundamental metabolic pathways. Secondary metabolites, stemming from either a single or a combination of these pathways, give rise to a wide range of compounds, including carbohydrates, proteins, fixed oils and fats, alkaloids, glycosides, volatile oils, gums and mucilage, tannins, resins, and more. These plant-derived byproducts hold tremendous significance for humanity, serving as essential food materials, colorants, pesticides, and most notably, medicinal agents. In this context, we'll explore the primary biogenetic groups responsible for the origin of these medicinally valuable secondary metabolites. [3]

Biosynthesis is a complex, enzyme-driven series of steps in which basic substances are transformed into more intricate products within living organisms. During biosynthesis, straightforward compounds undergo alterations, conversions into different compounds, or combinations into large macromolecules. This intricate procedure frequently comprises metabolic pathways. Some of these biosynthetic pathways are confined within a single cellular organelle, while others rely on enzymes found in various cellular organelles. Instances of such biosynthetic pathways encompass the creation of lipid membrane constituents and nucleotides. Generally, biosynthesis is synonymous with anabolism. [4]

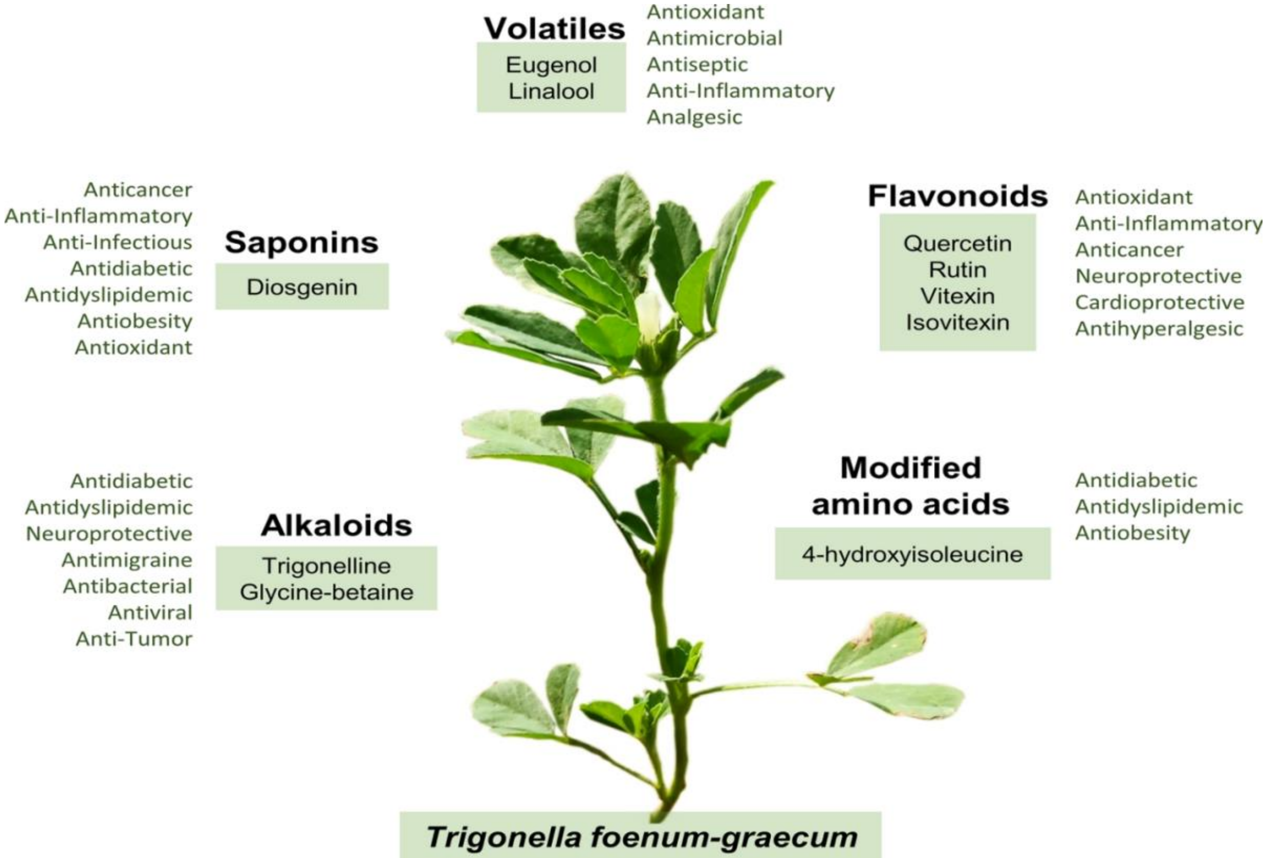

Fig No: 1- Components of the plant.

Examples of secondary metabolites:

Plant Toxins, gibberellins, alkaloids, antibiotics, and biopolymers are examples of secondary metabolites.

- The shikimic acid pathway is the primary source for the creation of numerous glycosides.
- With the exception of cardiac glycosides and steroids, glycosides are derived from amino acids.

CLASSIFICATION:

BASED ON THE CHEMICAL NATURE OF NON-SUGAR MOIETY: [5]

Anthraquinone glycoside	• Ex: Senna
Sterol or cardiac glycoside	• Ex: Digitalis
Saponin glycoside	• Ex: Liquorice
Cyanogenetic glycoside	• Ex: Bitter almond
Isothiocyanate glycoside	• Ex: Black mustard
Flavonoid glycoside	• Ex: Rutra graveolens
Coumarin glycoside	• Ex: Celery fruit
Aldehyde glycoside	• Ex: Vanilla pods
Phenol glycoside	• Ex: Salcive
Steroidal glycoside	• Ex: Salix species
Lactone glycoside	• Ex: Cantharide

FLAVONOID GLYCOSIDES

Flavonoids are a group of naturally occurring compounds that are frequently present in foods of plant origin. Flavonoids have a variety of biological effect in numerous mammalian cell systems, in vitro as well as in vivo. They have been shown to exert anti-inflammatory, antiallergic, antiviral, antibacterial and antitumor activities (Formica and Regelson, 1995). In fact, the pharmacological effects of many flavonoid compounds are due to their inhibiting ability on certain enzymes and also, their antioxidant activity (Pietta, 2000). Hesperidin (HES) is one of the most overflowing natural flavonoids, present in a huge number of fruits and vegetables (Garg et al., 2001. [6] Some authors reported that HES prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions (Fraga et al., 1987; Korkina and Afanas'ev, 1997; Miller and Rice-Evans, 1997; Jung et al., 2003). No preceding studies are available on the impact of HES against ACN-induced toxicity in rat brains. Therefore, the aim of this study was to explore the effect of HES on the lipid peroxidation status and the activities of enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) and on the non-enzymatic antioxidant; reduced glutathione (GSH) in the brains of rats treated with CAN. [7]

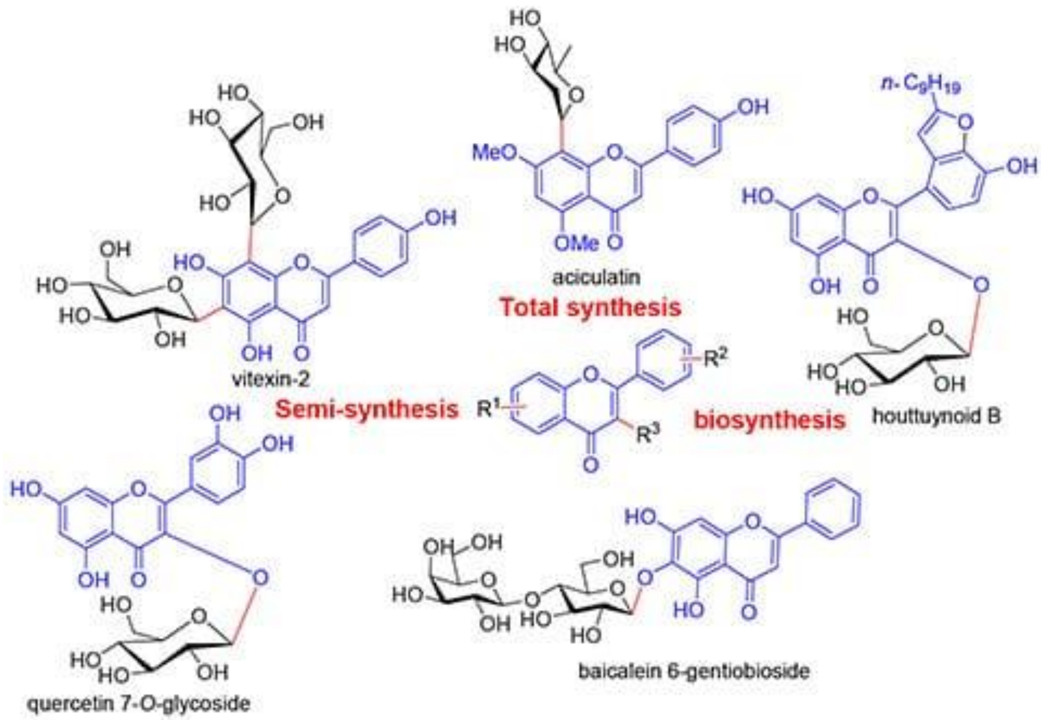

Fig No: 2- Synthesis.

Biological Source

Hesperidin is a flavanone glycoside that can be found in citrus fruits. Its aglycone form is known as hesperidin. The name "hesperidin" is derived from "hesperidiums," which refers to the fruits produced by citrus trees. This compound was initially isolated in 1828 by the French chemist M. Lebreton from the white inner layer of citrus peels. [8]

Fig. No:3- Citrus Fruit

Chemical constituents:

Hesperidin and its related compounds are distinctive components found in citrus fruits like oranges (Citrus sinensis), grapefruits (Citrus paradise), tangerines (Citrus reticulata), limes (Citrus aurantifolia), and lemons (Citrus limon), which belong to the Rutaceae family. The presence of these compounds in citrus fruits varies based on factors such as fruit variety, specific fruit part, environmental conditions, and the fruit's ripeness. According to Gattuso et al.'s review, a 100 ml portion of suitable juice typically contains 20–60 mg of hesperidin for oranges, 8–46 mg for tangerines, 4–41 mg for lemons, and 2–17 mg for grapefruits. The colored outer layer of citrus peel (flavedo) and the soft, white middle layer (albedo) [9]

Isolation:

Materials and Methods:

1. Plant Material Collection: Acquired Citrus sinensis (Orange) fruits from the local Jabalpur market in Madhya Pradesh. Peeled the fruits, and dried the peels in the shade. [10]

2. Isolation of Hesperidin:

A. Conventional Method: Macerated 250g dried orange peels with 800ml 10% KOH solution (pH 8-9) overnight. Filtered the mixture, and evaporated the filtrate to obtain a syrupy mass. [11]
B. Modern Method: Filled a 250ml round bottom flask with 800ml petroleum ether (40 – 60°C). Placed 250g dried and powdered orange peel in a Soxhlet extractor, extracting with petroleum ether for 4 hours. Discarded the extract, then extracted again with 800ml methanol until colorless.

Acidify the filtrate (pH 3-4) with 6% acetic acid. Refrigerated the concentrated liquid overnight, filtered the resulting crystalline substance, obtaining crude hesperidin as amorphous powder. Characterized and identified hesperidin based on various physicochemical parameters. Analyzed hesperidin yield graphically. [12]

Fig.No:4- Isolation equipment

Extraction:

The process involved taking shade-dried leaves, which were then powdered (250 g). These powdered leaves were subjected to extraction using a Soxhlet apparatus with 70% (v/v) ethanol. The resulting extract was vacuum-concentrated under reduced pressure at a temperature of 60° ± 1°C. After this, the extract was dried further in a hot air oven at temperatures ranging from 40 to 45°C. Finally, the dried extract was stored in an airtight container within a refrigerator set at 5°C. This resulting material is referred to as the hydro-ethanolic extract of E. neriifolia (HEEN). [13]

The dried leaves of E. neriifolia (250 g) were sequentially extracted using various solvents, starting from pet-ether and progressing through benzene, chloroform, ethyl acetate, and ethanol. Subsequently, they were macerated with distilled water, transitioning from non-polar to polar extraction. The presence and quantity of flavonoids were determined using Harborne's method from 1998, with quercetin as the standard. The extracts underwent analysis through TLC. Hesperidin, known for its biological activities, finds applications in the food, cosmetic, and pharmaceutical industries. To meet the high-quality and purity requirements for these uses, optimal extraction methods from plant materials are essential. Various environmentally-friendly procedures for extracting flavonoids, including hesperidin, have been explored for these purposes. [14]

Typical extraction techniques encompass methods like dipping, percolation, reflux, or continuous reflux. Several factors, including the choice of solvent, temperature, extraction duration, and liquid-to-solid ratio, influence the quality and efficiency of the extraction process. Commonly used solvents include methanol and ethanol, either alone or in various water proportions, along with dimethyl sulfoxide (DMSO). Traditional approaches like maceration and Soxhlet extraction are gradually being supplanted by advanced methods to boost efficiency and selectivity. These modern techniques are generally faster, more environmentally sustainable, and offer a higher level of automation. [15]

Fig No: 5- Maceration of orange peel

Hesperidin has been isolated from plant materials using various methods, including accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (USE), subcritical water extraction (SWE), pressurized liquid extraction (PLE) and high hydrostatic pressure (HHP). Various techniques, such as accelerated solvent extraction (ASE), microwave-assisted extraction (MAE) ultrasound-assisted extraction (USE) subcritical water extraction (SWE) pressurized liquid extraction (PLE), and high hydrostatic pressure (HHP), have been employed to extract hesperidin from plant sources. [16]

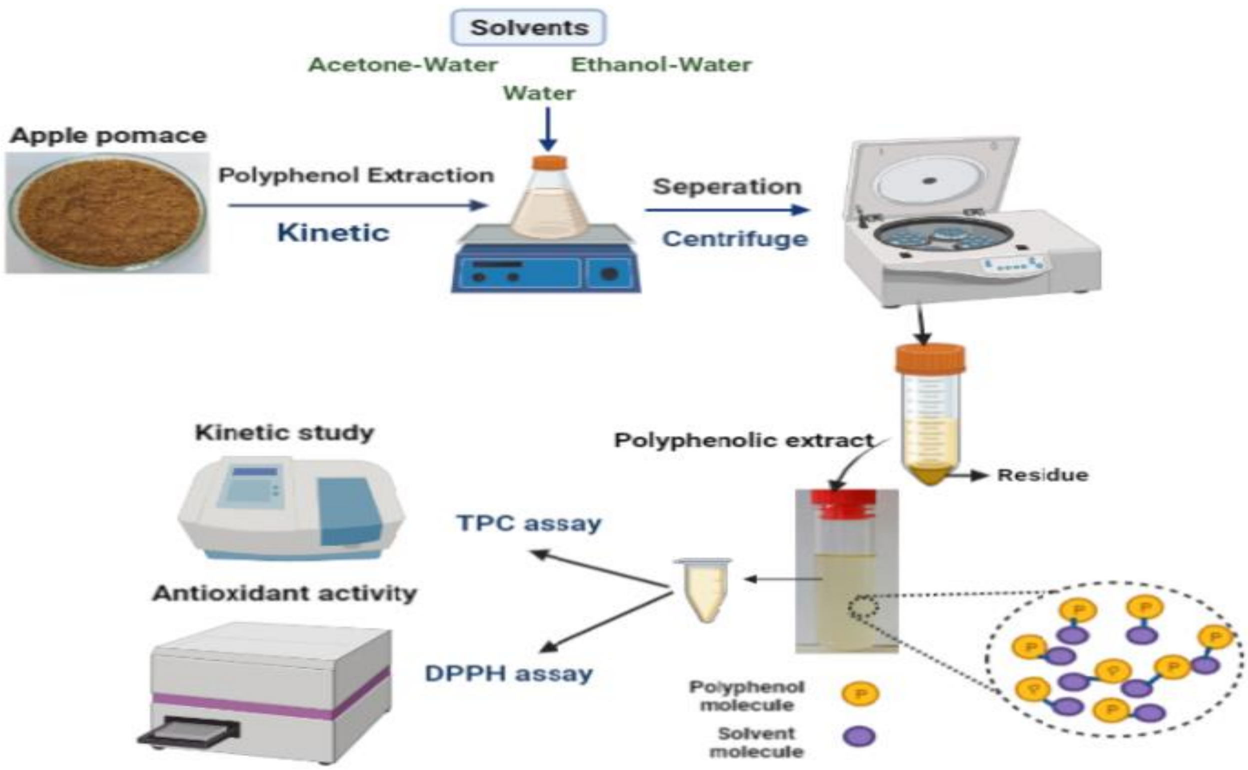

Fig No: 6- Extraction procedures

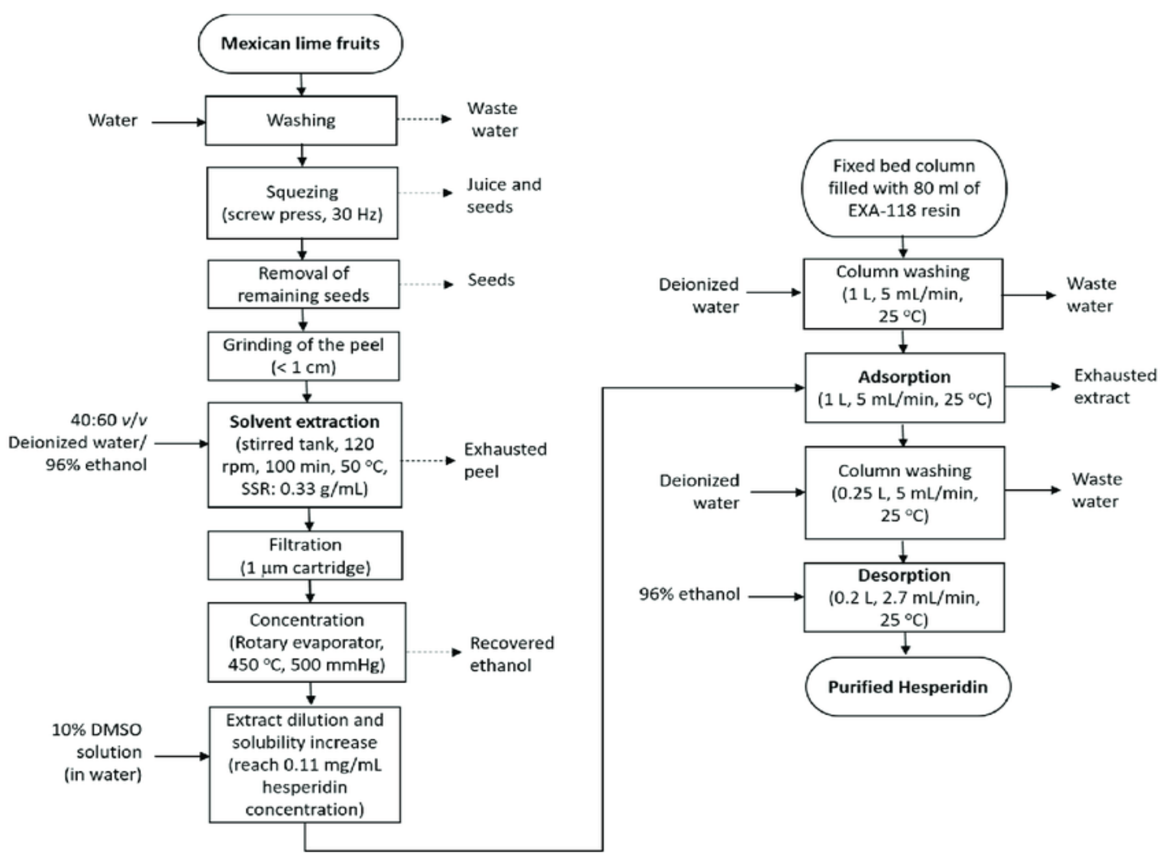

Fig No: 7 - Flow of Extraction process

Analysis:

Numerous techniques have been developed for analyzing hesperidin, either on its own or in conjunction with other flavonoids, across diverse sample types. Among these, the most commonly utilized methods involve reversed-phase high-performance liquid chromatography (RP-HPLC) in conjunction with diode array detection (DAD) and/or mass spectrometry (MS), utilizing a gradient of methanol/acetonitrile/water. Additionally, the introduction of an ionic liquid, didecyl dimethylammonium lactate, into the methanol/water eluent has been proposed to reduce analysis time and enhance peak symmetry in the assessment of pharmaceutical formulations.

In addition to liquid chromatography, other methods such as thin-layer chromatography capillary electrophoresis and electrochemical techniques have been utilized for detecting hesperidin in citrus juices and peels. Moreover, electro analytical sensors can be employed in a compact, portable format for this purpose.

Spectrophotometric techniques used to analyze hesperidin offer swiftness, cost-efficiency, and suitability for less costly equipment. Bennani et al. employed two spectrophotometric methods to concurrently assess the diosmin and hesperidin levels within their combined mixture. In the initial method, derivative spectrophotometry was employed by determining the zero-crossing points through the construction of linear calibration curves for first derivative values, specifically at 269 nm for hesperidin and 262.5 nm for diosmin. In the second method, the drug percentage was ascertained by calculating the peak absorbance ratio at the respective λmax for both compounds. Another spectrophotometric approach relied on the formation of a complex between hesperidin and Zn (II) in a 70% (v/v) methanol solution at pH 3.1, with a λmax of 283 nm and a $\log\beta 2$ value of 17.01. This developed method found application in quantifying hesperidin content in tablets and various samples of orange juices. [17]

Identification tests of Hesperidin:

- The Ferric chloride test involves introducing an alcoholic ferric chloride solution to hesperidin, resulting in the formation of a wine-red color.
- In the Magnesium-hydrochloric acid reduction test, also known as the Shinoda test, concentrated HCl is gradually added drop by drop to an ethanolic hesperidin solution containing magnesium, leading to the development of a vivid violet color (Ikan, 1991). [18]

Physical and chemical properties of Hesperidin:

- Pure hesperidin takes places as long hair-like spikes, tan or faint yellow in colour.
- Melting point-258° to 262°C.
- Molecular formula - C_1H4015.
- Molecular weight-610.57 Daltons.
- Miscible in dilute alkali and in pyridine providing a clear yellow solution.
- Slightly soluble in methanol, hot glacial acetic acid.
- Insoluble in acetone benzene and chloroform.
- Storage conditions - Hesperidin concentration in orange juice steadily decreases during storage, even when the juice was preserved at low temperature.
- Toxicity - Acute oral toxicity studies have shown that LD of hesperidin is more than 2000 mg/kg, while sub-acute and sub chronic toxicity studies have shown that the no-observed adverse effect level is more than 2000 mg/kg, showing the safety of hesperidin in herbal formulations.
- It has a possession of forming complicated crystals with other comparable glucosides, which greatly affects its solubility and other physical properties, making it difficult to get in a pure state.
- It can, however, be purified by washing with hot water and extraction with 95% methyl alcohol, followed by crystallization.
- It is tasteless and odorless. [19]

Nutraceutical Uses of Hesperidin:

Additives use should be customized and vetted by a healthcare professional, such as a registered dietitian, pharmacist, or healthcare provider. No supplement is designed to treat, cure, or prevent disease.

Hesperidin has been studied for the following conditions:

- Cardio vascular disease – stroke, high blood pressure and heart attack.
- Blood vessel disorders – hemorrhoids and venous ulcers.

- Metabolic disorders – type 2 diabetics and metabolic dysfunction like associated steatotic liver disease.
- Neurodegenerative disease.

Other Uses: In addition to the possible health benefits listed above, animal and/or test tube studies recommend the following potential properties of hesperidin:

- Anti-inflammatory properties
- Antioxidant properties
- Anticancer properties
- Antimicrobial properties
- Anti-glaucoma (group of eye diseases) properties. [20]

Conclusion: Hesperidin a flavonoid found in citrus fruits is a beneficial nutraceutical, Nutraceuticals like vitamins and antioxidants found in food can be good for our health and offer benefits like supporting our heart, brain and immune system. Research suggests that hesperidin have antioxidant, anti-inflammatory, and neuroprotective properties. Additionally, hesperidin shows promise in improving cardiovascular health, reducing cholesterol levels, and potentially aiding in cancer prevention. It to some extent lowers bp and other disorders. While more studies are needed to confirm its efficacy and safety, hesperidin a nutraceutical holds promise as a natural compound with potential therapeutic applications.

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