

**STANDARDIZATION OF A PROCESS TO EXTRACT A  
BIOFLAVONOID -HESPERIDIN**

**A**

**Thesis submitted  
in partial fulfillment of the  
requirement of the degree of  
Master of Science  
In  
Chemistry**

**Under the supervision of  
Dr. Sanjai Saxena  
Assistant Professor**

**By  
Kavita Sood  
Regn No. 30702005**

**School of Chemistry & Biochemistry  
Thapar University, Patiala  
June 2009**

### Candidate's Declaration

I hereby declare that the work being presented in the thesis entitled "Standardization of a process to extract Bioflavonoids- Hesperidin" in partial fulfillment of the requirements for the award of degree of Masters in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala is my own laboratory work during the period of Jan 2009 to May 2009, under the supervision and conception of Dr. Sanjai Saxena, Assistant Professor, Department of Biotechnology and Environmental Sciences (DBTES), Thapar University, Patiala. I have not submitted the matter embodied in this thesis for the award of any other degree.

Patiala

Date: 02.07.09

Kavita Sood

Kavita Sood

(Roll No. 30702005)

This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge



Dr. Sanjai Saxena

Assistant Professor / Supervisor

Department of Biotech. & Environ. Sciences



Dr. Susheel Mittal

Professor & Head

School of Chemistry & Biochemistry

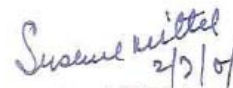
## Certificate

This is to certify that the thesis entitled "Standardization of a process to extract Bioflavonoids-Hesperidin" being submitted by Ms. Kavita Sood ( Roll No.30702005) in partial fulfillment of the requirements for the award of degree of Master of Science in the School of Chemistry & Biochemistry, Thapar University, Patiala is a bonafide work carried out under the supervision and conception of Dr. Sanjai Saxena and that no part of this thesis has been submitted for the award of any other degree.



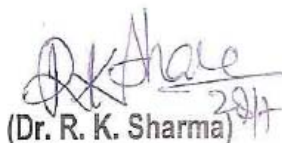
**Dr. Sanjai Saxena**

Assistant Professor,  
Deptt. of Biotech, & Environ. Sciences  
Thapar University



**Dr. Susheel Mittal**

Professor and Head,  
School of Chemistry & Biochemistry  
Thapar University



**(Dr. R. K. Sharma)**  
Dean, Academic Affairs  
Thapar University

## TABLE OF CONTENTS

1.	ACKNOWLEDGEMENT
2.	INTRODUCTION
3.	REVIEW OF LITERATURE
4.	AIM AND OBJECTIVES
5.	MATERIALS AND METHODS
6.	RESULTS AND CONCLUSION
7.	BIBLIOGRAPHY

## ACKNOWLEDGEMENT

Apart from the personal efforts, help and encouragement from number of individuals which served as the driving force to enable me to submit my thesis in the present form.

First of all I take this opportunity to express my deep sense of thankfulness to my guide Dr. Sanjai Saxena, Assistant Professor, Department of Biotechnology and Environmental Sciences, Thapar University, for his constant help and guidance.

My sincere thanks to Dr. Susheel Mittal, Head, School of Chemistry and Biochemistry for his sincere guidance, suggestions and thoughts. He has always been an inspiration to me for this hard work and efforts to excel.

I would be failing in my duty if I don't acknowledge the kind help and cooperation of all the faculty members and staff of School of Chemistry and Biochemistry. I am also all my family members have contributed significantly to bring this day in my life.

Dated 18<sup>th</sup> June, 2009

Kavita Sood

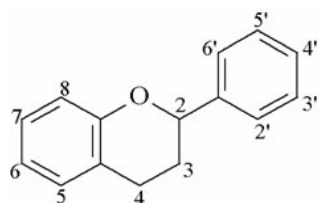
Place: Thapar University, Patiala

# **CHAPTER 1**

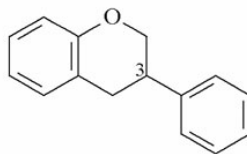
## ***INTRODUCTION***

## 1. INTRODUCTION

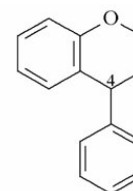
Of the scores of natural products produced by plants as a metabolic outcome under different environments, flavonoids play a central role in plant metabolism. Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom (Anonymous, 1996). The study of flavonoids chemistry has emerged, like that of most natural products, from the search for new compounds with useful physiological properties. The term “flavonoid” is generally used to describe a broad collection of natural products that include a C6-C3-C6 carbon framework, or more specifically phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products can be classified as the flavonoids (2-phenylbenzopyrans), isoflavonoids (3-benzopyrans), and the neoflavonoids (4-benzopyrans) (Marais *et al.* 2005).



FLAVONOIDS



Iso-FLAVONOIDS



Neo-FLAVONOIDS

The importance of natural products, and particularly of plant-derived natural products, as a source of molecular diversity for drug discovery research and development may appear to be self-evident. Flavonoids more so have been found to provide a large amount of biomedical/pharmaceutical applications as interdisciplinary applications were explored in developing new medicines or therapies (Buckshee *et al.* 1997; Allegra *et al.* 1995; Daftary *et al.* 1995; Cospite, 1994; Balas & Pagratis, 1989; Cospite & Dominici, 1989). Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions (Nijveldt *et al.* 2001). They also inhibit enzymes such as aldose reductase and xanthine oxidase (Narayana *et al.* 2001). They are potent antioxidants and have free radical scavenging abilities. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality.

They have been shown to inhibit the growth of various cancer cell lines *in vitro*, and reduce tumour development in experimental animals.

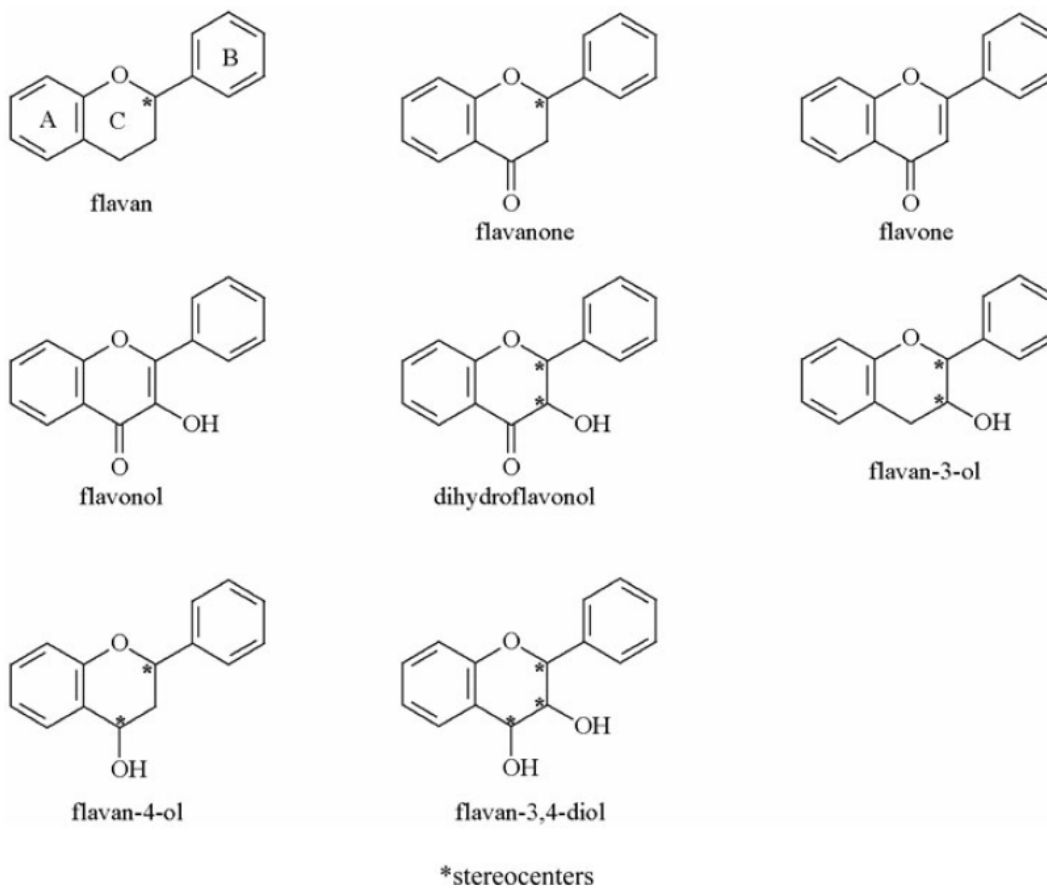
### 1.1 Structures and classification of flavonoids

Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid glycone consists of a benzene ring condensed with a six-membered ring, which in the 2-position carries

a phenyl ring as a substituent. Six-member ring condensed with the benzene ring is either a pyrone (flavonols and flavonones) or its dihydroderivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids and isoflavonoids. Flavonols differ from flavonones by hydroxyl group the 3-position and C2-C3 double bonds. Flavonoids are often hydroxylated in position 3, 5, 7, 2', 3', 4', 5'. Methyl ethers and acetylenes of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucor-hamnose, galactose or arabinose.

## 1.2 Types of flavonoids

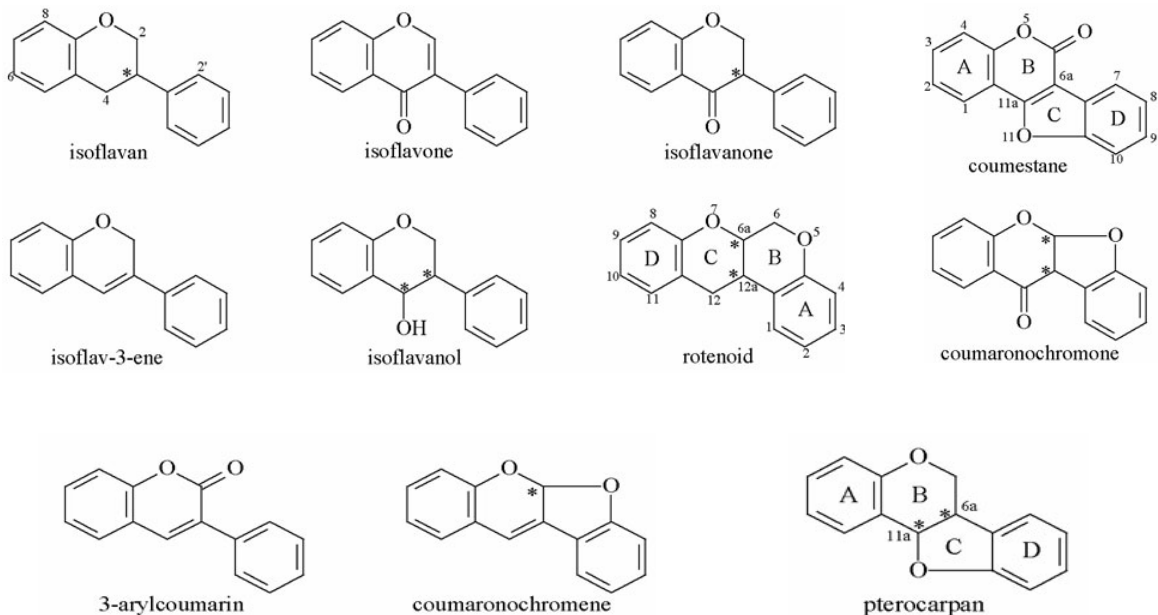
### 2-Phenylbenzopyrans (C6-C3-C6 Backbone)



These flavonoids can be of many types based on the degree of saturation and oxidation of the heterocyclic C- ring.

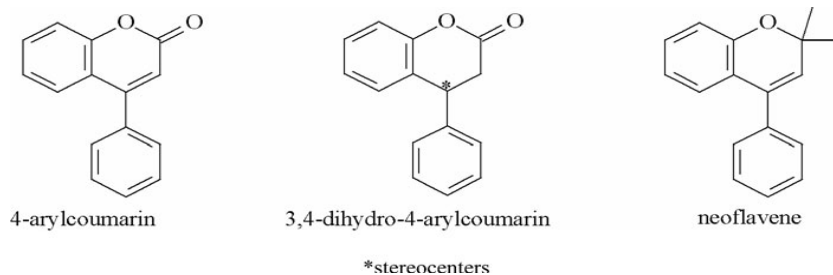
## Isoflavonoids

The isoflavonoids are a distinctive subclass which possess a 3-phenylchroman skeleton that is biogenetically derived by 1,2-aryl migration in a 2-phenylchroman precursor. These are divided into 11 sub groups.



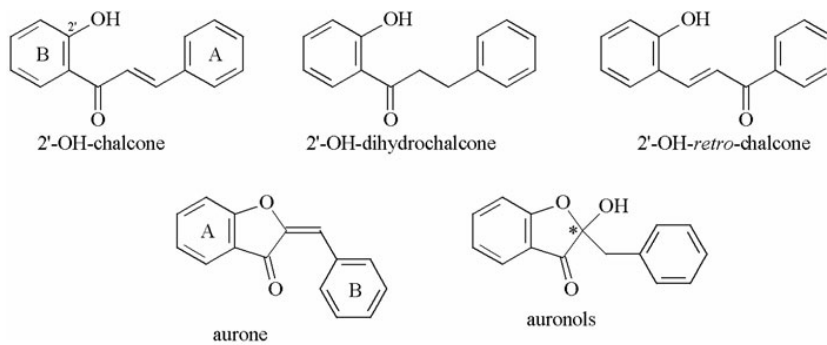
## Neoflavonoids

The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2H-1-benzopyran-2-ones), 3,4-dihydro-4-arylcoumarins, and neoflavenes.



## Minor flavonoids

Natural products such as chalcones and aurones also contain a C6-C3-C6 backbone

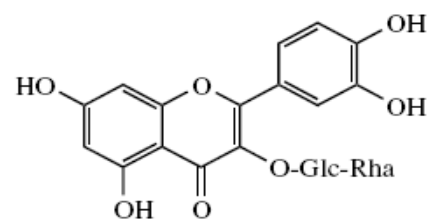


and are considered to be minor flavonoids.

### 1.3 Flavonoids & drug discovery

Flavonoids occur naturally in fruit, vegetables, and beverages such as tea and wine. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic (Toshio *et al.* 2002), anti-inflammatory (Hamalainen *et al.* 2007), anti-microbial (Cottiglia *et al.* 2001) and anti-cancer activity (Androutsopoulos *et al.* 2009). An important effect of flavonoids is the scavenging of oxygen-derived free radicals (Asgary *et al.* 1999). *In vitro* experimental systems also showed that flavonoids possess anti-inflammatory, anti-allergic, antiviral, and anti-carcinogenic properties.

It is the best-described property of almost every group of flavonoids as they act as antioxidants. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage.



RUTIN

The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species antioxidant-defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but also non-enzymatic counterparts such as glutathione, ascorbic acid, and  $\alpha$ -tocopherol (Hanasaki *et al.* 1994). Flavonoids in red wine (quercetin, kaempferol, and anthocyanidins) and in tea (catechins and catechin gallate esters) are also demonstrated to be effective antioxidants. The flavonol glycoside rutin from buckwheat (*Fagopyrum esculentum*; Polygonaceae) and rue (*Ruta graveolens*; Rutaceae), and the flavanone glycoside hesperidin from *Citrus* peels have been included in dietary supplements as vitamin P, conditions characterized by capillary bleeding, but their therapeutic efficacy is far from conclusive. Flavonoids extracted from the leaves of Indian holy basil, *Ocimum sanctum* showed promising results as radioprotection agent in rodents (Reshma *et al.* 2005).

### 1.4 HESPERIDIN – medicinal properties

**Hesperidin** is a flavanone glycoside (flavonoid) (C<sub>28</sub>H<sub>34</sub>O<sub>15</sub>) found abundantly in citrus fruits. Its aglycone form is called hesperetin. 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 (Monforte *et al.* 1995) and blood pressure

(Ohtsuki *et al.* 2003) in rats. In a mouse study large doses of the glucoside hesperidin decreased bone density loss (Chiba *et al.* 2003).

Another animal study showed protective effects against sepsis (Kawakguchi *et al.* 2004). Hesperidin has anti-inflammatory effects (Emim *et al.* 1994). 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 brain.

Pharmaceutical Industries in India as well as abroad are importing hesperidin for developing compounds which play a very important role as anti-osteoporotic, wounding healing agents. Apart from that it is also been given as an agent to improve capillary insufficiency in treatment of varicose veins. Hence hesperidin serves as a very important active pharmaceutical ingredient (API) in many therapeutic OTC and non-OTC drug formulations

## **CHAPTER 2**

### ***REVIEW OF LITERATURE***

## **2. REVIEW OF LITERATURE**

### **2.1 Bioflavonoids**

Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. They are widely distributed in the leaves, seeds, bark and flowers of plants. Approximately 4,000 flavonoids have been identified to date and have been in great detail studied for their therapeutic effects. Interest in the bioflavonoids is related to their diversity (Harbone, 1994) biological significance as secondary plant metabolites and ecological role, use as chemotaxonomic markers (Forkmann, 1992) physiological effects and industrial applications.

The term bioflavonoid was adopted to differentiate between the health-promoting flavonoids and the thousands of other flavonoids that do not have a proven biological role. Bioflavonoids contain a minimum of two phenol rings in their structure. The different subgroups of the bioflavonoids include the catechins, anthocyanidins, leucoanthocyanidins, flavonones, flavones, flavanonols, flavonols, isoflavones, flavans, dihydrochalcones, aurones, and chalcones. In turn, specific types of bioflavonoids belong to one of these classes. For example, the bioflavonoid quercetin belongs to the flavonol class; genistein belongs to the isoflavone class; catechin belongs to the catechins class; and cyanidin belongs to the anthocyanidins class.

Bioflavonoids are reported to have numerous health benefits. They are the natural pigments in fruits and vegetables. Researchers have reported over eight hundred different bioflavonoids. Most of these are the yellow pigments found in citrus fruit as well as other fruits and vegetables; these are referred to as flavonoids. Bioflavonoids have also been referred to as vitamin P, a name that nutrition scientists object to because it has not been proved that they are essential to human nutrition and health.

Recent studies have indicated the potential of bioflavonoid in removal of blood clots and in treating phlebitis. The mechanism which allows prevention of cellular damage is reducing the reactive oxygen species. Bioflavonoids also have a potential role as food preservatives due to their anti-oxidant action. Bioflavonoids apart from the above also exhibit anti-cancer, antibiotic properties. Foods that are high in bioflavonoids include apricots, blackberries, black currants, broccoli, cantaloupes, cherries, grapefruits, grapes, oranges and lemons.

## ***2.2 Role of Bioflavonoids in Plants***

In plants bioflavonoids provide different colors which are involved in attracting insects to flowers which are vectors for pollination. They also serve as plant anti—oxidants due to over exposure to UV irradiations.

One function of is to give plants their various colors. Many of these colors cannot be seen by humans or can be seen only under the illumination of ultraviolet light. Bioflavonoids are involved in attracting insects to flowers, in which they tend to occur in high concentrations.

## ***2.3 Medicinal Uses of Bioflavonoids***

The main health benefits of bioflavonoid' s fall into two categories: **health-promoting effects** and **therapeutic effects**. The health-promoting effects include better eyesight; improved cardiovascular health; increased capillary strength; improved structure of connective tissues and appearance of skin; and a stronger immune system. Bioflavonoids have a vitamin C and vitamin E enhancing effect, meaning that they can boost the function of these powerful antioxidant vitamins.

The therapeutic applications of bioflavonoids include treating a variety of diseases and disorders. Several of these are coronary heart disease, allergies, inflammation, hemorrhoids, respiratory diseases, viral infections, some types of cancer, and peptic ulcers-ulcerations of the mucous membranes of the stomach, esophagus and intestines.

**Rutin** can be used to treat chronic venous insufficiency , glaucoma, hay fever, hemorrhoids, varicose veins, poor circulation, oral herpes, cirrhosis, stress, low serum calcium, and for cataracts. It is helpful in reducing weakness in the blood vessels and the resultant hemorrhages. Rutin is also helpful in reducing serum cholesterol and in treating rheumatic diseases such as gout, arthritis, systemic lupus erythematosus. Rutin is most abundant in apricots, buckwheat, cherries, prunes, rose hips, the whitish rind of citrus fruits, and the core of green peppers.

**Anthocyanins** and proanthocyanidins can be used to treat a number of eye conditions such as cataracts, night blindness, diabetic retinopathy and macular degeneration. They are also useful for strengthening the walls of the blood vessels, and may help prevent bruising, hemorrhoids, varicose veins, and spider veins. Bioflavonoids can help to prevent osteoporosis by stabilizing collagen, the major protein in bone. Proanthocyanidins are able to cross the

blood-brain barrier to protect the brain from damage by free radicals and infection. Good sources of anthocyanins and proanthocyanidins include blackberries, cranberries, black and green tea, raspberries, grapes, eggplant, red cabbage, elderberries, and red wine.

**Ellagic acid** helps to inhibit cancer by neutralizing the effect of certain carcinogens. It is particularly helpful in reducing the effects of nitrosamines, which are found in tobacco and processed meat products such as bacon and hot dogs. Ellagic acid reduces the effects of the toxic and carcinogenic factors (aflatoxins) produced by *Aspergillus flavus* molds on food. Ellagic acid diminishes the effects of polycyclic hydrocarbons produced by tobacco smoke and air pollution, as well. Sources of ellagic acid include strawberries, grapes, apples, cranberries, blackberries, and walnuts.

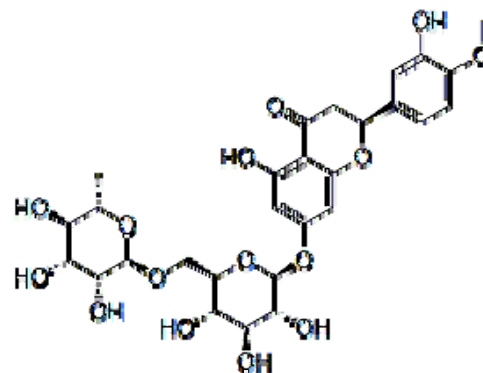
**Quercetin** is a good antihistamine and reduces the inflammation that results from hay fever, allergies, bursitis, gout, arthritis, and asthma. Quercetin stimulates detoxification in the liver, inhibition of tumor formation and treatment of complications due to diabetes. Quercetin inhibits the growth of *Helicobacter pylori*, which has been implicated in the development of peptic ulcers. Quercetin is found in green tea, onionskins, kale, red cabbage, green beans, tomatoes, potatoes, lettuce, strawberries, cherries, and grapes. It is also found in smaller amounts in many other foods.

**Catechins** and tannins can be used to stimulate detoxification by the liver and to strengthen the blood vessels. They also help reduce the inflammatory response. Catechins and tannins may help inhibit the formation of tumors. Catechins can be used to inhibit the breakdown of collagen and to treat hepatitis and arthritis. Catechins and tannins are both found in green and black teas.

#### **2.4 Hesperidin: Importance and Use**

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-oxygenase (Lee *et al.* 2004).



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 (Kamaraj *et al.* 2008). Sources of hesperidin include Citrus fruits, Berries, Onions, Parsley and Green Tea.

### **2.5 Extraction & Isolation of Flavonoids**

There are a variety of methods and strategies employed for the extraction of a particular class of class of flavonoid studied. Flavonoids (particularly glycosides) can be degraded by enzyme action when collected plant material is fresh or non-dried. It is thus advisable to use dry, lyophilized, or frozen samples. The plant material is ground into a powder when dry material is used. Another important consideration is polarity. Isoflavones, flavanones, methylated flavones and flavonols are less polar flavonoids and are extracted with organic compounds like chloroform, dichloromethane, diethyl ether, or ethyl acetate. Flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol–water mixtures. The bulk of extractions of flavonoid-containing material are still performed by simple direct solvent extraction.

Powdered plant material can also be extracted in a Soxhlet apparatus, first with hexane, to remove lipids and then with ethyl acetate or ethanol to obtain phenolics. This approach is not suitable for heat-sensitive compounds. However a more convenient and frequently used procedure is sequential solvent extraction in which the powdered raw material is first extracted with dichloromethane, for isolation of flavonoid aglycones and less polar material. A subsequent step with an alcohol will extract flavonoid glycosides and polar constituents. Flavanone solubility depends on the pH of water-containing solutions. Flavan-3-ols (catechins, proanthocyanidins, and condensed tannins) can often be extracted directly with water. However, the composition of the extract does vary with the solvent — whether water, methanol, ethanol, acetone, or ethyl acetate.

### **2.6 Extraction of Hesperidin**

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% ethanol (Mauro *et al.* 1999).

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 °C (Yaqin *et al.* 2008).

Another procedure is by treating the orange peel with  $\text{Ca (OH)}_2$  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 purity (El- Nawawi, 1995).

## **CHAPTER 3**

### ***AIMS AND OBJECTIVES***

**AIM**

The aim of the present study is process development of isolation of hesperidin from some members of Rutaceae family and assays its purity for use as an Active Pharmaceutical Agent (API).

**OBJECTIVES**

The extraction would be based on changes in pH using alkaline slurry and crystallization of the bioflavonoid from the slurry using a solvent. The Hesperidin extraction would be carried out from

- i. Pulp of orange
- ii. Peel of orange
- iii. Pulp of Bael

## **CHAPTER 4**

### ***MATERIALS AND METHODS***

#### **4.1 RAW MATERIAL USED**

The raw materials used for developing the process and evaluating the yield of crude hesperidin involved the use of peels of citrus fruits like oranges, mausumi and kinnnows being deposited at local fresh fruit juices shop. Apart from this pulp of Bael (*Aegle marmelos*) was also tested for the presence of Hesperidin and its yield in attempt to use it as a future commercially available raw material.

#### **4.2 PROCESSES FOR EXTRACTION OF CRUDE HESPERIDIN**

The methodology of extraction would be based on changes in pH using alkaline slurry and crystallization of the bioflavonoid from the slurry using a solvent.

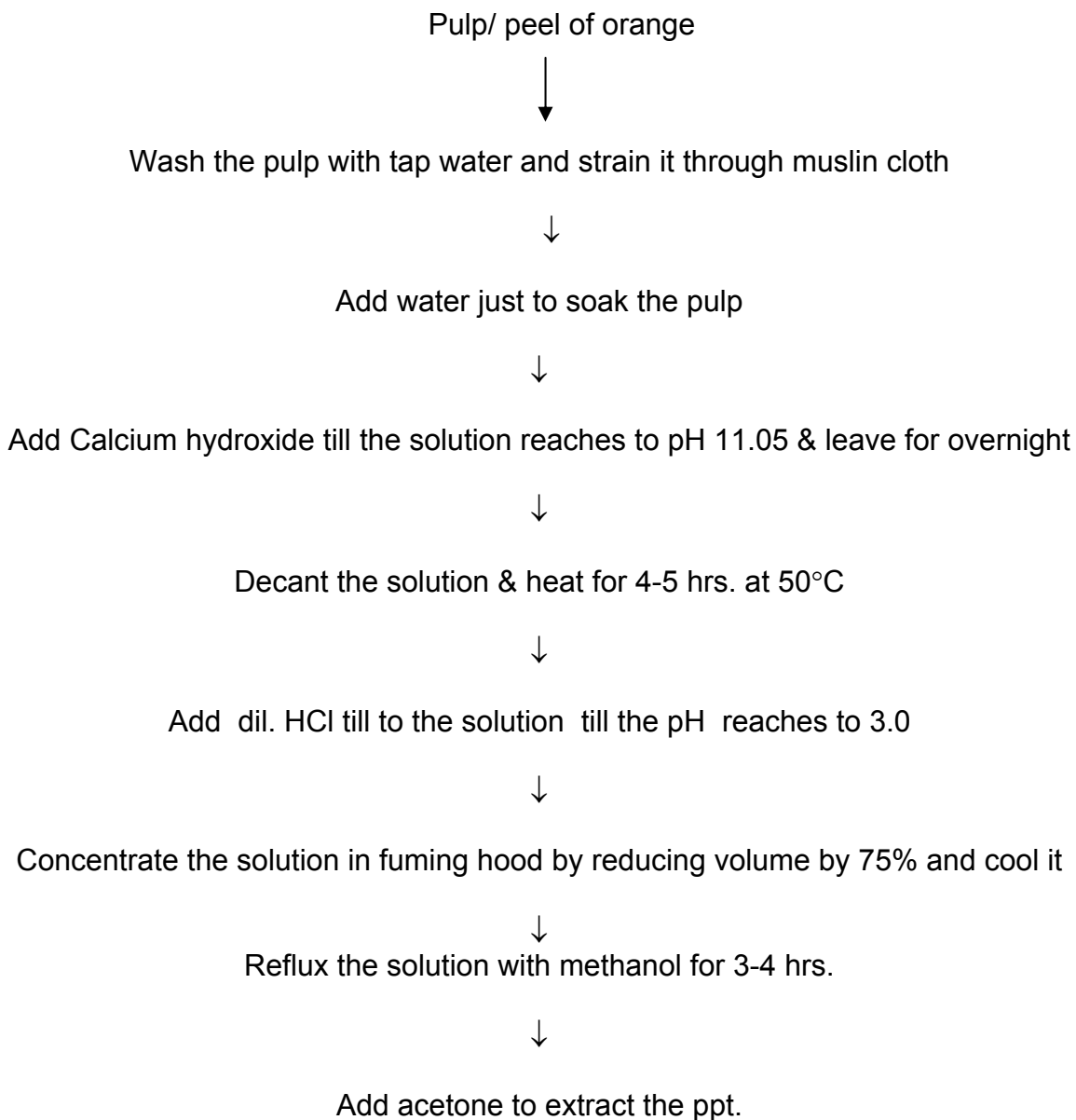
##### ***4.2.1 Extraction of Hesperidin from orange pulp/peel (Refer flowchart no.1)***

Briefly the orange pulp/ peel were washed with tap water and the juice was removed. More water was added to soak the pulp. Calcium hydroxide [ $\text{Ca}(\text{OH})_2$ ] till the pH reached at 11. The solution was kept overnight and next with muslin cloth and after which the solution was heated for 3-4 hours. The solution was cooled and further pH adjusted between 3-4 using HCl and kept for concentration at 50°C for 8-10 hours to bring it to 25% of the original volume. The solution was cooled and the precipitates were allowed to settle down. Subsequently the acetone was added extract the precipitates and centrifuged at 10,000rpm. The precipitates were removed and dried the precipitates at room temperature. Melting point of the precipitates was noted.

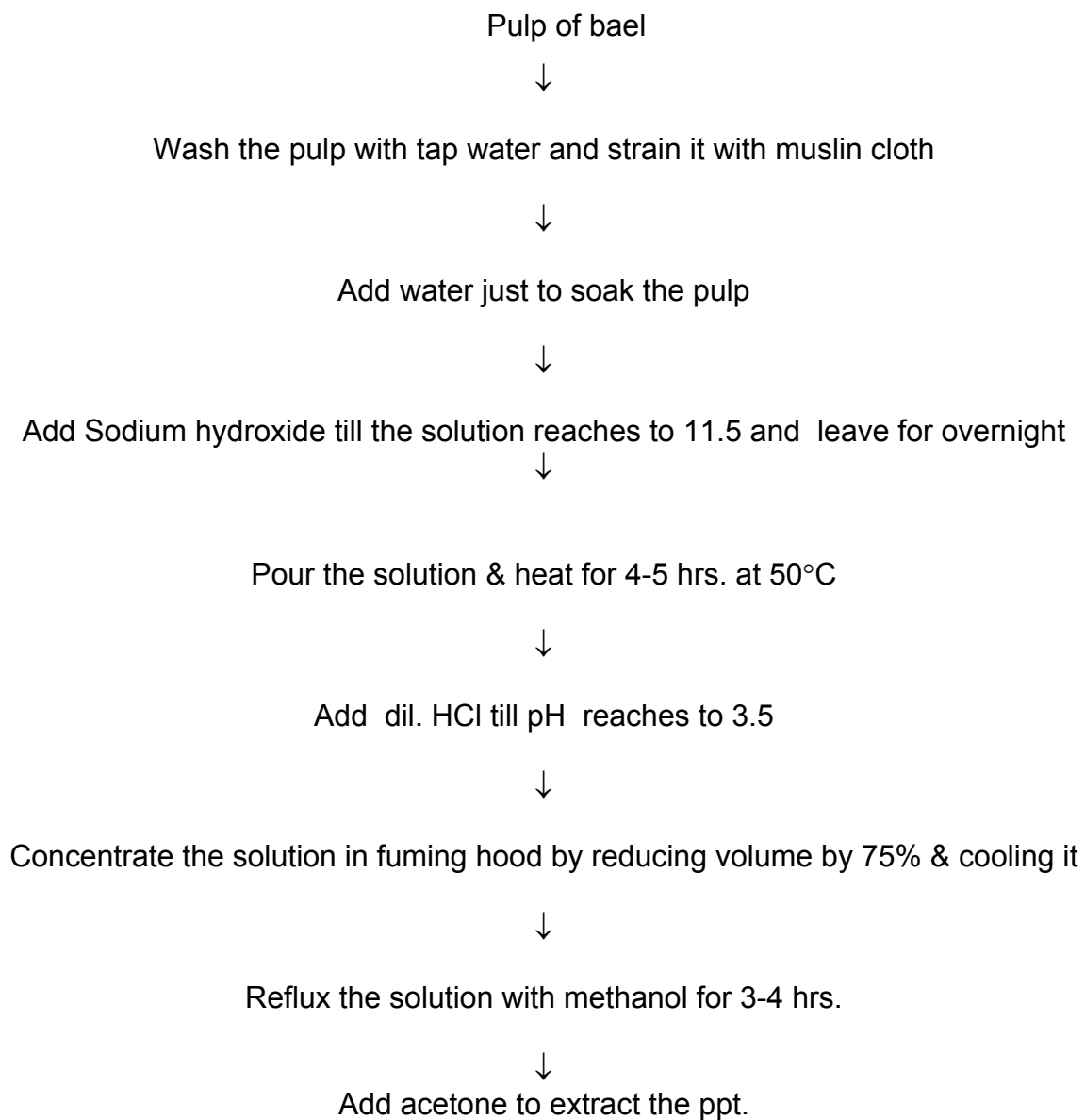
##### ***4.2.2 Extraction of Hesperidin from Aegle maemelos (BEL) pulp (Refer flowchart no.2)***

The Bel pulp was washed with tap water to remove the sweetness of the fruit thoroughly to remove maximum soluble carbohydrates. The pulp was then soaked in the water and Sodium hydroxide (NaOH) was added till the pH reached at 12.5. The solution was kept overnight and the solution recovered through muslin cloth. The solution was heated for 3-4 hours to contain any microbial growth. Further the pulp solution was cooled and pH brought between 2-3 using HCl. The resulting solution was concentrated to 30% of original volume at 65°C. This concentrated solution was refluxed with methanol for 3-4 hours. After this the solution was cooled down and the precipitates settled at the bottom. Acetone was used to extract the precipitates.

## Chart No. 1: Extraction of Hesperidin from Pulp/ Peel of Orange/ Kinnow



## Chart No. 2: Extraction of Hesperidin from Pulp of Bael



## **CHAPTER 5**

### ***RESULTS & CONCLUSION***

## **Results**

Hesperidin collected as precipitates from all the three sources resembled in physical appearance i.e was light brown in colour. The yield was highest in the case of Orange pulp followed by peel and bael pulp i.e. 0.085%, 0.04% and 0.01% respectively per liter of the leachate obtained. The melting point of the precipitates in all the three cases was same i.e. 258.2 °C. This corroborates to the appearance of the hesperidin pure chemical as well as melting point.

## **Conclusion**

Hesperidin can be extracted from Bael on commercial scale as is being done from the citrus fruits. However this method needs further critical detailed optimization of extraction procedure to enhance the recovery of Hesperidin in order to minimize the import from China and other countries.

## **CHAPTER 6**

### ***BIBLIOGRAPHY***

## REFERENCES

1. Allegra C, Bartolo M Jr, Carioti B et al (1995): An original microhaemorheological approach to the pharmacological effects of Daflon 500 mg in severe chronic venous insufficiency. *International Journal of Microcirculation & Clinical Experimentation*; 15(1): 50-54.
2. Androutsopoulos VP, Mahale S, Arroo RR & Potter G (2009): Anticancer effects of the flavonoid diosmetin on cell cycle progression and proliferation of MDA-MB 468 breast cancer cells due to CYP1 activation. *Oncology Reports*; 21 (6): 1525- 1528.
3. Anonymous. 1996. Wealth of India, publications and information directorate, CSIR, New Delhi, Vol. I, 1988, 217 Vol II. Ministry of Health & Family Welfare. Govt. of India, Controller of Publications, New Delhi, 53 - 54
4. Asgary S, Naderi GH, Sarrafzadegan N, Ghassemi N, Boshtam N, Rafie M & Arefian A (1999): Anti-oxidant effect of flavonoids on hemoglobin glycosylation. *Pharmaceutica Acta Helvetiae*; 73 (5): 223- 226.
5. Balas P & Pagratis N (1989): Vital capillaroscopy on microcirculation: pharmacodynamic activity of Daflon 500 mg in venous insufficiency. *Int Angiol*; 8(4): 51-52.
6. Buckshee K, Takkar D & Aggarwal N (1997): Micronized flavonoid therapy in internal hemorrhoids of pregnancy. *International Journal of Gynecology & Obstetrics*; 57(2): 145-151.
7. Chiba H, Uehara M, Wu J, Wang X, Masuyama R, Suzuki K, Kanazawa K, Ishimi Y (2003). Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J Nutr*, 133(6):1892-7.
8. Cospite M & Dominici A (1989): Double blind study of the pharmacodynamic and clinical activities of 5682 SE in venous insufficiency: advantages of the new micronized form. *Int Angiol*; 8(4): 61-65.
9. Cospite M (1994): Double-blind, placebo-controlled evaluation of clinical activity and safety of Daflon 500 mg in the treatment of acute hemorrhoids. *Angiology*; 45(6.2): 566-573.
10. Cottiglia F, Loya G, Garaua D, Florisb C, Causb M, Pompeic R & Bonsignorea L (2001): Antimicrobial evaluation of coumarins and flavonoids from the stems of *Daphne gnidium* L. *Phytomedicine*; 8 (4): 302- 305.
11. Daftary SN, Irani JS & Tsouderos Y (1995): The therapeutic activity of micronized flavonoid fraction (Daflon 500 mg(R)) in IUCD-induced bleeding. *Drugs Today*; 31(E):41-45.
12. El-Nawawi SA (1995): Extraction of citrus glucosides. *Carbohydrate polymers*; 27 (1): 1- 4.

13. Emim JA, Oliveira AB & Lapa AJ (1994): Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, dauricin and chrysin, in rats and mice. *Journal of Pharmacy & Pharmacology*; 46: 118–122.
14. Forkmann G (1992): In *Proceedings of the 16th International Conference of Groupe Polyphenols*, Lisbon; 16: 19–27.
15. Hamalainen M, Nieminen R, Vuorela P, Heinonen M & Moilanen E. (2007): Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappa B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of Inflammation*; 2007: 456- 473.
16. Hanasaki Y, Ogawa S & Fukui S (1994): The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radical in Biology & Medicine*; 16: 845–850.
17. Harborne JB (1994): Do natural plant phenols play a role in ecology. *ISHS Acta Horticulturae 381: International Symposium on Natural Phenols in Plant Resistance*; 381: 36.
18. Kamaraj S, Ramakrishnan G, Anandakumar P, Jagan S & Devaki T (2009): Antioxidant and anticancer efficacy of hesperidin in benzo(a)pyrene induced lung carcinogenesis in mice. *Investigational New Drugs*. 27 (3): 214- 222.
19. Kawaguchi K, Kikuchi S, Hasunuma R, Maruyama H, Yoshikawa T, Kumazawa Y(2004). A citrus flavonoid hesperidin suppresses infection-induced endotoxin shock in mice. *Biol Pharm Bull*. 27(5):679-83.
20. Lacaille-Dubois MA & Wagner H (1992): In *Proceedings of the 16<sup>th</sup> International Conference of Groupe Polyphenols*, Lisbon; 16: 217–228.
21. Lee NL, Choi SH, Park SH, Park EK, Kimb DH (2004): Antiallergic Activity of Hesperidin Is Activated by Intestinal Microflora. *Pharmacology*; 71 (4): 174- 181.
22. Marais JPJ, Deavours B, Dixon RA & Ferreira D (2005): The stereochemistry of flavanoids. In *The Science of Flavanoids (2005)*: Ed Grotewold E, Springer USA: 1- 47.
23. Mauro AD, Fallico B, Passerini A, Rapisarda P & Maccarone E (1999): Recovery of hesperidin from orange peel by concentration of extracts on styrene- divinylbenzene resin. *Journal of Agriculture & Food Chemistry*; 47 (10): 4391- 4397.
24. Monforte MT, Trovato A, Kirjavainen S, Forestieri AM, Galati EM & Lo Curto RB (1995): Biological effects of hesperidin, a Citrus flavonoid. (note II): hypolipidemic activity on experimental hypercholesterolemia in rat. *Farmaco*; 50(9): 595-9.

25. Narayana KR, Reddy MS, Chaluvadi MR & Krishna DR (2001): Bioflavanoids, classification, pharmacological , biochemical effects and therapeutic potential. Indian Journal of Pharmacology; 33: 2- 16.
26. Nijveldt RJ, Nood EV, van Hoorn DEC, Boelens PG, van Norren K & van Leeuwen PAM (2001): Flavonoids: a review of probable mechanisms of action and potential applications. American Journal of Clinical Nutrition; 74 (4): 418-425.
27. Ohtsuki K, Abe A, Mitsuzumi H, Kondo M, Uemura K, Iwasaki Y, Kondo Y. (2003): Glucosyl hesperidin improves serum cholesterol composition and inhibits hypertrophy in vasculature. J Nutr Sci Vitaminol (Tokyo). 49(6):447-50
28. Reshma K, Ashalatha VR, Dinesh RM & Vasudeven DM (2005): Effect of Ocimum flavonoids as a radioprotector on the erythrocyte antioxidants in oral cancer. Indian Journal of Clinical Biochemistry; 20 (1): 160-164.
29. Toshio I, Yukio S, Hideki M & Chiaki K (2002): Antiallergic effect of flavonoid glycosides obtained from *Mentha piperita* L. Biological & Pharmaceutical Bulletin; 25(2): 256-9.
30. Yaqin M, Xingqian Y, Yunbin H, Guoneng X, Guinhua X & Donghong L (2008): Ultrasound-assisted extraction of hesperidin from Penggan (*Citrus reticulata*) peel. Ultrasonics Sonochemistry; 15 (3): 227- 232.