REVIEW ON NARINGIN: METHOD OF ISOLATION, ANALYTICAL DEVELOPMENT AND ITS RECENT PHARMACOLOGICAL ACTIVITIES

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ABSTRACT-

Naringin is a flavonoid which can be isolated from different citrus fruits like Grape fruit, Orange, Pomelo, Lemon etc. It can be analysed by using various analytical techniques: HPLC, TLC, UV, HPTLC, mass spectroscopy, Liquid chromatography, Chiral chromatography, LC/mass. Naringin can act as an antioxidant and scavenge free radicals. Naringin mainly focuses on the *invitro* and *invivo* animal studies showing its beneficial effects on cardioprotective, antioxidant anti-inflammatory, antimicrobial, hypolipemiant, neurological, thermogenic, pulmonary disorders and antidiabetic. In this pandemic Naringin is treated as a most promising treatment strategy against Covid-19 due to its antiviral and anti-inflammatory effects. Recently Naringin proven its activity towards various molecular docking studies. Naringin itself keeps the body healthy active against various illness and it is highly active against various major life style disorders and even as an antineoplastic agent**.**

KEYWORD-

Naringin, Flavonoid, Citrus fruit, Antioxidant.

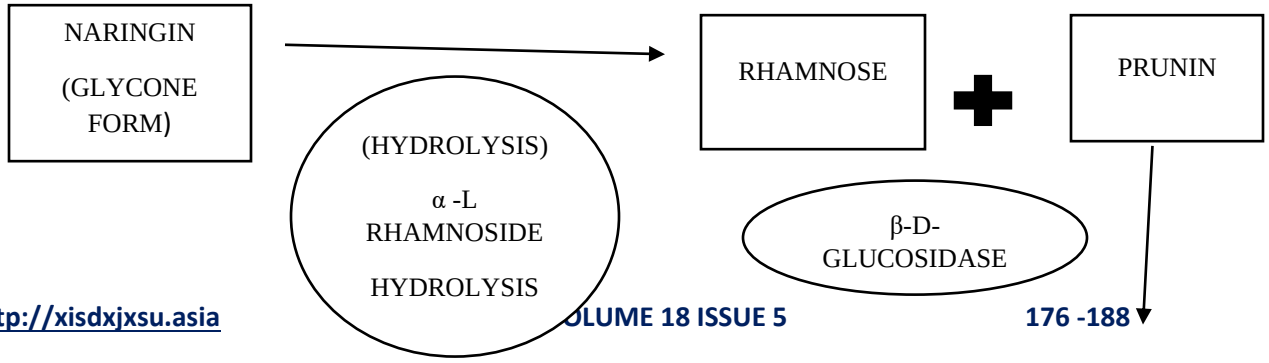
1. INTRODUCTION

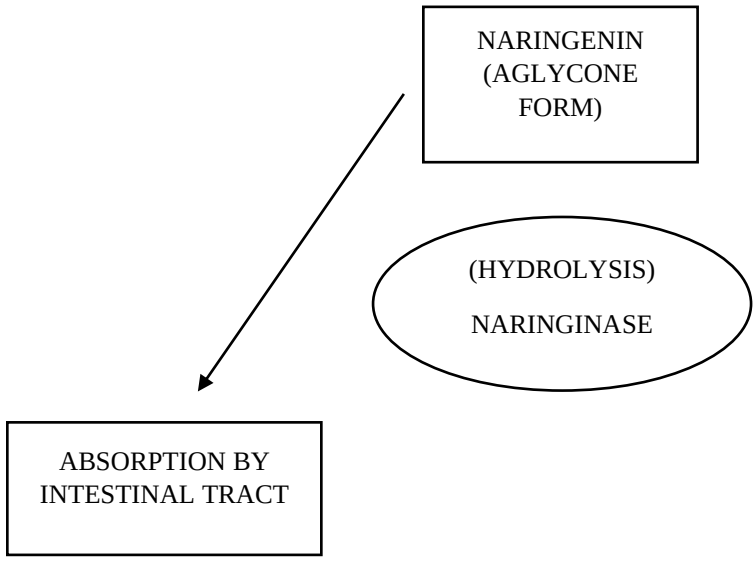
Citrus fruits are good source of flavonoids. Naringin is a common flavonoid which is present in citrus fruits like Grapefruit, orange, Pomelo, Lemon etc. All these fruits are easily available in India; called as local fruit or seasonal fruit. The proportion of Naringin is found in each citrus depending on variety of fruit, state of ripening and the climatic conditions to which it has been exposed. Citrus fruits are good source of antioxidant specially flavonoids which are mainly two types: flavonone glycoside and polymethoxylated glycoside. [1,2,3,4] Citrus is the most important cultivated

fruit in the world with reported production about 89 million tons in 2014(USDA, 2014). Estimated, 26% of Citrus fruits are industrially processed into juice. The amount of industrial Citrus coproducts is estimated at 15 $\times 10^6$ tons and it consists essentially in seeds, peels and pulp residue. Indeed, Citrus co-products are rich in bioactive molecules (pigments, fibres, essential oils, flavonoids) which can constitute a high added value for the industrialists. [11,12] It contains mainly bitter principle which was isolated in 1866 by De Vry in Java from grape fruit blossoms. It exerts various pharmacological effects such as antioxidant activity, blood lipid lowering, anticarcinogenic activity & anti diabetic activity. It also inhibits the selected cytochrome P450 enzymes including CYP3A4 & CYP1A2, which may result in several drug interaction in-vitro. In human Naringin is metabolised to the flavanone Naringenin. In this pandemic Naringin is treated as a most promising treatment strategy against Covid-19 due to its antiviral and anti-inflammatory effects. It is proven that the consumption of either grape fruit, orange or as Naringin itself keeps the body healthy active against various illness and it is highly active against various major life style disorders and even as an antineoplastic agent. [6] The class of flavanones is specific to citrus products (fruit, juice). They largely contribute to the total daily flavonoid intake range is 150-600 mg/day. Among flavanones found in Citrus co-products, Naringin has interesting biologic activities like antioxidant and antimutagenic activities. In fact, Naringin can reduce the level of cholesterol in the plasma, reduce the risk of atherosclerosis, protect the level of vitamin E in the plasma, enhance flavours for sweets, drinks and bakery products and stabilized oils. Naringin is found in the white spongy portion of citrus peel. Its content varies from 0.65 mg g-1 in mandarin peel to 14.40 mg g-1 in grapefruit peel. [4,9]

1.1 METABOLISM OF NARINGIN IN THE BODY

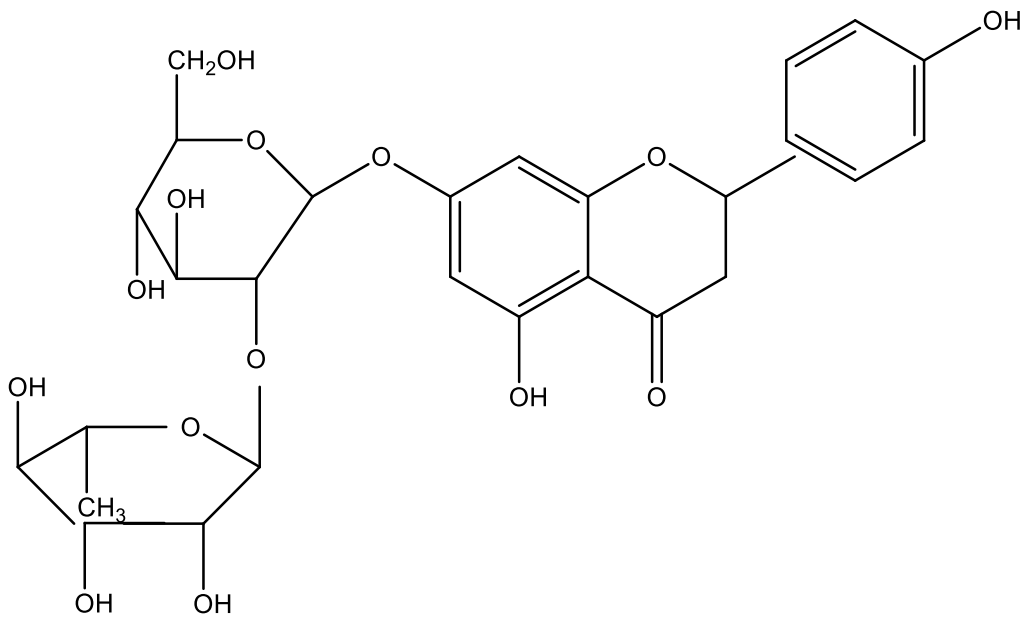
In humans, Naringinase is available in the liver and it rapidly metabolizes Naringin into Naringenin. It is occured in mainly two steps- first, Naringin is hydrolyzed by α-L-rhamnosidase activity of naringinase to rhamnose and prunin. The prunin formed is then hydrolyzed by β-d-glucosidase activity of Naringinase into Naringenin and glucose. Naringinase is an enzyme that has a wide occurrence in nature; in plants, yeasts, and fungi. It is commercially attractive due to its bitterness removal properties. [1,2, 3,5, 6,7,8]





1.2 CHEMISTRY OF NARINGIN

Flavonoids are composed of two aromatic rings linked through three carbon atoms that form an oxygenated heterocycle. Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-pyrone structure. Over 4,000 different flavonoids have been described and categorized into flavonols, flavones, flavanones, isoflavones, catechins, and anthocyanidins. Diverse biochemical properties of flavonoids including naringin, hesperidin, diosmin, and rutin have provoked interest in biology and medicinal chemistry. Naringin is a flavanone-7-O-glycoside between the flavanone Naringenin and the Disaccharide neohespiridose. Naringin, the bitter principle of grapefruit (Citrus paradisi), is found in the juice, flower, and rind of the fruit and constitutes up to 10% of the dry weight. Naringin and other Naringenin glycosides can be found in a variety of other sources. The flavonoid Naringin occurs naturally in citrus fruit, especially in grapefruit where Naringin is responsible for the fruits bitter taste. The chemical formula for Naringin is $C_{27}H_{32}O_{14}$ and molecular weight is 580.4 g/mol. The taste of NAR is bitter and color is beige. Its melting point ranges from 165°C to 170°C. Naringin is highly soluble in organic solvents; Ethanol, Methanol and Dimethyl Sulfoxide and sparingly soluble in aqueous buffer It is stable upto 2 years if stored at 20^0C. [60]



Naringin (4" ,5,7, -trihydroxy flavanone 7- rhamnoglucoside

2.ISOLATION AND EXTRACTION OF NARINGIN FROM VARIOUS SOURCES

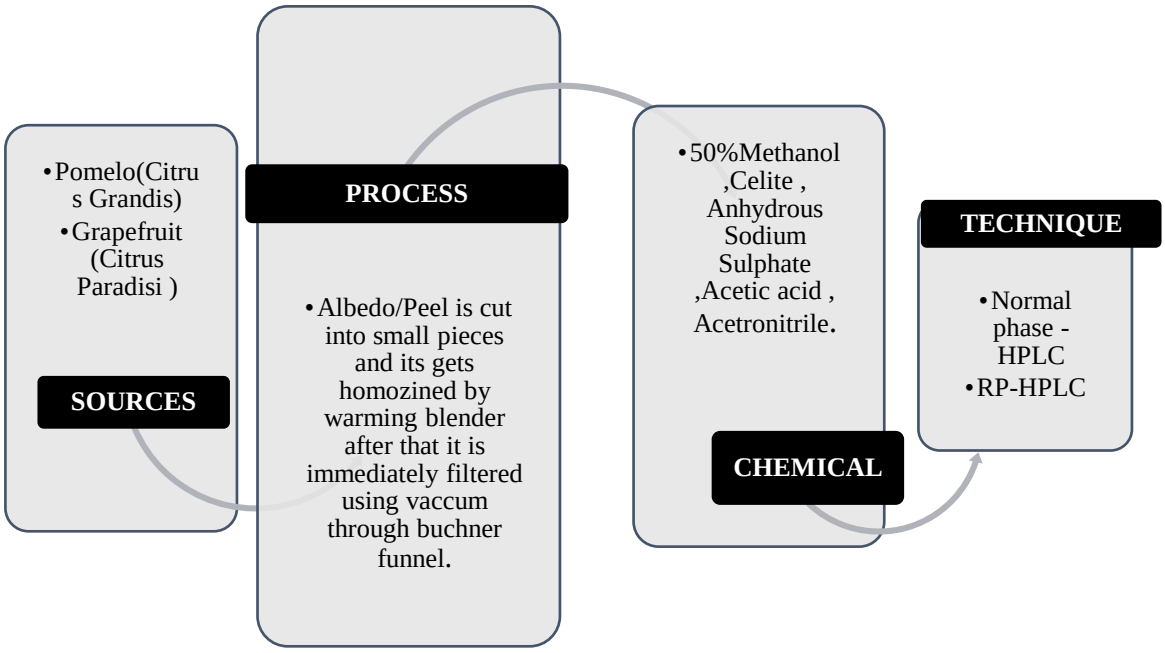
Isolation is a process from where we can obtain a purified compound and extraction process were moving one or more analytes from the sample to a physically separate location where further processing and analysis occurs. Mainly in extraction it separates the compound from mixture and in isolation process purification of a compound is occurring. [13]

2.1 Naringin extraction

TABLE -1

METHOD OF EXTRACTION	CHEMICAL	REFERENCE
1.Maceration, Reflux, Supercritical fluid extraction	Ethanol (AR), carbon dioxide and Nitrogen gas	[14,15]
2.Dry albedo/room temperature methanolic extraction (60-70^{0}C for 30 min)	Methanol, Dichloromethane	[18]
3.Dry albedo/hot methanolic extraction (55 ^{0}C for 3 hours)	Methanol, Dichloromethane	[18]
4.Wet albedo/hot methanolic extraction (55 ^{0}C for 3hours)	Methanol, Dichloromethane	[18]
5.Lquid phase extraction	Isopropanol, Methanol, n-Hexane	[21]

2.2 Isolation of Naringin from different sources [17,19,20]



During the extraction analysis effect of the temperature, light and oxygen shows that Naringin is a molecule which is very sensitive to its environment. Its degradation begins when temperatures are superior to 100 °C or in presence of light. The antioxidant activity of the Naringin solutions varies during their degradation, so biological activities of the Naringin can be modified during its extraction. The extraction methods there are different solvents are used. The use of accelerators for solvent extraction is interesting because it makes the procedure less time consuming. While Naringin, is a polar flavonoid, because of that extraction temperature or pressure must be increased to obtain a high Naringin content. So, this increase in temperature or pressure can cause the degradation of Naringin if its superior to 100 °C. During the Naringin extraction, two reactions occur parallelly with increasing extraction temperature or pressure. These two reactions are: (i) an increase of the Naringin released and (ii) degradation of Naringin. The study of the effects is monitored; temperature, light and oxygen, showed that Naringin does not degrade with an oxygen content of 85% and for temperatures lower than 100 °C. For preservation of Naringin, direct light needs to avoid. If, an extraction temperature is 80 °C it leads to an increase of the Naringin content since naringin is not affected by temperatures under 100 °C. when a temperature is above 100 °C is applied (microwave power of 400 W), then decreasing of the Naringin content is observed due to a degradation of the Naringin. [16]

3.ANALYTICAL METHODS FOR NARINGIN

Naringin is available as a powder and capsule. Analytical methods were developed for the determination of Naringin using RP-HPLC, LC, Mass, HPTLC, LC-mass and Spectrophotometry.

TABLE 2: Review on analytical methods for the assay of Naringin

Method	Mobile phase (v/v) / Reagent	Column	Reference
High-pressure Liquid Chromatographic (HPLC)	The mobile phase consisted of acetonitrile /water Water: Acetronitrile (80:20)	C18 reversed phase column	[21,23]
Improved High-pressure Liquid Chromatographic (HPLC)	Mobile phase consisting of methanol and water (38: 62, v/v, pH 3) at a flow rate of 1 ml/min	C18 reversed-phase column (4.6 mm x 250 mm; 10 µm)	[22,51]
RP-HPLC	The mobile phase consisted of tetrahydrofuran/ water/acetic acid (21:77:2, v/v/v) and was filtered through a 0.45-mm pore size nylon filter (Alltech, Deerfield, IL, USA) and degassed by ultrasonic treatment before use	Macherey Nagel Nucleosil C8 analytical column (250×4.6 mm, 5µm particle size)	[24]
HPLC	0.05% Formic aqueous solution and 20% Acetonitrile)	C18 column (3.9 mm × 150 mm, 5µm	[50]
HPLC	The mobile phase is acetonitrile/0.1 M ammonium acetate/glacial acetic acid (18:81:0.5, v/v)	Inertsil ODS-2 (Particle size 5 µm) column (250 × 4.6 mm)	[25]
HPLC	Formic acid: Methanol	C18 reverse phase Luna column 4.6 X250 mm	[49]
LC/ESI-MS	The mobile phase was methanol/10 mM ammonium acetate (60:40, v/v)	Nova-Pak C18 column (150 × 3.9 mm	[25]
Tandem mass spectrometry (LC/MS/MS	The mobile phase consisted of methanol (70%) and water (30%)	Beta basic C18 ODS column (100 mm × 2.0 mm 5 µm)	[26]
Liquid Chromatographic	The mobile phase consisted of water-acetonitrile-glacial acetic acid (79.5 + 20 + 0.5, v/v)	RP-C18 column (4.6 mm. x 50mm)	[27]
LC–MS/MS	Acetonitrile and water	Nova Pak C18 column	[28]
Liquid Chromatographic Method	Mobile phase consisted of acetonitrile and potassium phosphate buffer (25.0 mM;	Grace Smart RP C18 (250.0 × 4.6 mm, 5 μm) column	[29]

	pH 3.5 ± 0.1		
Liquid chromatography tandem mass spectrometry (LC–MS/MS) method	The mobile phase consisted of 0.1% formic acid water and acetonitrile	Zorbax SB-C18 analytical column (2.1 mm × 150 mm, 5 µm) (XDB-C18 column (50 x2.1 mm, 1.8 mm)	[30,48]
Colorimetric Method	30/5/60 methanol/acetic acid/water.	μBondapak C, column eluted at a flow rate of 1 ml/min	[31]
Simultaneous Quantification by HPLC	Mobile phase composed of ultra-pure water and acetonitrile	Symmetry C18 reversed-phase column (5-µm particle size, 3×250 mm) and Sep-Pak C18 Plus Short Cartridges	[32]
HPTLC	Ethyl acetate (EA) – EA: Methanol (MeOH) (60:40 v/v)	-	[35]
Chiral high-performance liquid chromatography	n-hexane/ethanol with 0.5% TFA as mobile phase	Chiralpak IB column, (250 mm × 4.6 mm	[33]
HPLC	water-acetonitrile (80:20, v/v)	A Waters Associates 30 cm X 4 mm i.d. reverse phase µBondapak C-18 column	[34]

4.PHARMACOLOGICAL ACTIVITY

TABLE-3

PHARMACOLOGICAL ACTIVITY	REFERENCE
Anti-inflammatory, anti-cancer activities, as well as effects on bone regeneration, metabolic syndrome, oxidative stress, genetic damage and central nervous system (CNS) diseases.	[37]
Metal chelating effect, antioxidant, anti-microbial, anti-viral, anti-allergic, anti-estrogenic, antidiabetic, adipolytic activity, ischemic heart disease anti-inflammatory, anti-obesity, Hypoxia, hepatoprotective activity and anti-cancer activity.	[38]
Cardiovascular diseases, Type 2 Diabetes Mellitus (T2DM), metabolic syndrome, pulmonary disorders, neurodegenerative diseases, cancer, and gastrointestinal pathologies.	[39]
Atherosclerosis, cardiovascular disorders, diabetes mellitus, neurodegenerative disorders, osteoporosis, and rheumatological disorders.	[40]
CYP3A4 inhibitor	[41]
Hyperlipidemia, Hypertension, Anti-oxidant, antineoplastic agent, DNA repair, Hepatitis C, Wound healing, Obesity, alcohol effect, Antiulcer, Antiatherogenic Bioenhancer, Gastroprotective, Bone marrow protective.	[42]
Neurogenerative illness	[43]
Antidiabetic Effect	[44]
Obesity, Diabetes, Hypertension, and Metabolic syndrome	[45]
Antioxidant, Anti-Inflammatory, Hepatoprotective, Nephroprotective, Immunomodulatory and Antidiabetic	[46]
Anti-Hyperglycemic, Anti-Hyperlipidemic, Anti-Oxidant	[47]

4.1 Naringin Recent Activity

TABLE -4

ACTIVITY	RESULT	REFERENCE (YEAR)
1.Evaluation of interaction between citrus flavonoid, naringenin, and pepsin using spectroscopic analysis and docking simulation	The naringenin-pepsin complex uncovered an average RMSD (1.34 nm) more than that of the free pepsin system (1.33 nm), which agreed with the finding of thermal stability. RMSF results show that the protein structure gains more rigidity. Kinetic studies showed that the activity of the enzyme was decreased.	[52]2021
2. Docking study of naringin binding with COVID-19 main protease enzyme	It is shown that residues His163, Glu166, Asn142, His41and Gln189 participate in the hydrogen bonding interactions, the same as happened with decahydroisoquinoline as a novel structure as a protease inhibitor for SARS 3CL.On the other hand, some of the known protease inhibitors and anti-influenza drugs docked with COVID-19main protease, it has a low binding affinity than naringin.	[53]2021
3. Evaluation of Anti-inflammatory and Regenerative Efficiency of Naringin and Naringenin in Degenerated Human Nucleus Pulposus Cells: Biological and Molecular Modeling Studies	Molecular docking showed that both NAR and NG bind to the selected genes of interest. Semi-quantitative RT-PCR analysis reveals differential gene expression of collagen (COL)9A1, COL9A2, COL9A3, COL11A2, COMT (catechol-O-methyltransferase), and THBS2 (thrombospondin 2); up regulation of ACAN (aggrecan), COL1A1, COL11A1, interleukin (IL)6, IL10, IL18R1, IL18RAP, metalloprotease (MMP)2, MMP3, MMP9, ADAMTS5 (a disintegrin and metalloproteinase with thrombospondin motifs 5), IGF1R (insulin-like growth factor type 1 receptor), SPARC (secreted protein acidic and cysteine rich), PARK2 (parkin), VDR (vitamin D receptor), and BCL2 (B-cell lymphoma 2); down regulation of IL1A, CASP3 (caspase 3), and nine genes with predetermined concentrations of NAR and NG.	[54]2019
4. Evaluation of the interaction between naringenin and human serum albumin: Insights from fluorescence spectroscopy, electrochemical measurement and molecular docking	The quenching mechanism of NAR with HSA has been evidenced to be static quenching, the reaction is spontaneous and electrostatic interactions altogether with the hydrogen bonds are the mainly forces. Nar binding to HSA was confirmed at both site I (subdomainIIA) and site II (subdomain IIIA), although with higher affinity to site II. In addition, the effects of metal ions and the binding distance were also investigated.	[55]2015
5. Molecular docking studies of natural compounds of naringin on enzymes involved in the urea cycle pathway in hyperammonemia	The results of the molecular docking study show that naringin interacts with urea cycle enzymes with more hydrogen bonds and higher bonding energy than the standard drug, sodium benzoate. This supports the hypothesis that naringin can prevent experimental hyperammonemia.	[56]2020
6. Investigation of the Interaction of Naringin Palmitate with Bovine Serum Albumin: Spectroscopic Analysis and Molecular Docking	Naringin palmitate was transported by BSA and was easily removed afterwards. As a consequence, an extension of Naringin applications for use in food, cosmetic and medicinal preparations may be clinically and practically significant, especially in the design of new Naringin.	[57]2013

7.Evaluation of Anti-inflammatory and Regenerative Efficiency of Naringin and Naringenin in Degenerated Human Nucleus Pulposus Cells: Biological and Molecular Modeling Studies	The molecular docking (in silico) studies showed the effective binding of these native ligands (Nar and NG) with genes have been identified as a potent inhibitor for inflammation. Hence, these natural flavonoids could serve as anti-inflammatory in treating low pain and sciatica.	[58]2019
8.Network Pharmacology Integrated with Molecular Docking Explores the Mechanisms of Naringin against Osteoporotic Fracture by Regulating Oxidative Stress	Naringin may treat osteoporosis fracture possibly by regulating numerous signaling pathways and targets related to oxidative stress and osteoclast differentiation. These results will provide a theoretical basis for the treatment of osteoporosis fracture. However, these predicted altered signaling pathways or target genes still need to be further verified in the future study.	[59]2021

5. FUTURE PROSPECTIVE

In different studies, Naringin is showing its various prevention to reduce the modern-day diseases; diabetes, cancer, inflammation, etc. in various animal model systems. Mainly Naringin modulates its antioxidative property, which leads to the reduction in the oxidative stress mediated pathogenesis. However, in context to the effect of Naringin on human, no epidemiological study has been performed except showing a linkage between the citrus fruits and lower rate in breast cancer patients. In many instances cancer is commonly observed in diabetic patients. Naringin has shown to reduce the diabetes and also help in the prevention of the cancer in diabetic patients. Based on the experienced gain from different studies, it could be safely concluded that Naringin may potentiate the outcome of radiotherapy by overcoming the radio-diminished immune response and give a better clearance of tumor by activating the host cytotoxic immune response. Beside it is also possible that Naringin may reduce the damage to the normal cells during radiotherapy due to its differential effects on the normal and cancer cells.

6. CONCLUSION

Naringin is a citrus Flavonoids it can be extracted from a grapefruit peel, Pomelo, Orange, Lemon etc. It can be extracted from powder not from juice due to expected higher flavonoid concentrations and because, as a common waste product, its usage for future work would likely be economical and help to reduce food waste. In conclusion, the HPTLC, LC/MS, HPLC procedures are simple, rapid, accurate, reproducible and applicable for determination of naringin in grape fruit. From various pre-clinical reports, there is self-evident strength of Naringin in the field of applications that deal with bone diseases or with the stem cells for osteogenic differentiation. Undeniably, Naringin can rectify various disorders with having lots of pharmaceutical approaches.

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