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ANDROGRAPHOLIDE AND HESPERIDIN - A BRIEF OVERVIEW

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ABSTRACT: Andrographolide is a labdane diterpenoid isolated from the leaves and roots of *Andrographis paniculata* Nees (Acanthaceae). Andrographolide is isolated by various processes and the simple method is the maceration process. It is bitter in taste and used as hepatoprotective, cholinergic, antispasmodic, stomachic, anthelmentic, alterative, blood purifier, and febrifuge. It promotes the secretion of bile in the liver and is used in jaundice, torpid liver, cold and upper respiratory tract infection. It is also used in the treatment of snake bites and is found to be more effective than the anti-snake venom generally used. Hesperidin is a flavanone glycoside isolated from the peels of *Citrus sinensis* (Rutaceae). Hesperidin plays an important role in plant defense mechanisms. Hesperidin is commonly isolated by two laboratory processes maceration and continuous percolation method. The major pharmacological actions of the hesperidin reported are anti-hyperlipidemic, cardioprotective, antihypertensive, antidiabetic, antioxidant, anti-inflammatory, and antiartherogenic activity. However, what is noteworthy about this paper is highlighting the evidence-based overview on the chemical and physical nature of Andrographolide and Hesperidin along with a brief account on the plants from which they are isolated, the extraction and isolation procedures of the above chemical constituents, their physical properties and structure along with their pharmacological properties are also discussed.

INTRODUCTION: From the earliest times, herbs have been prized for their pain-relieving and healing abilities, and today we still rely on the curative properties of plants in about 75% of our medicines. Herbal medicine often complements conventional treatments, providing safe, well-tolerated remedies for chronicillness [1]. Andrographolide is a Labdane Diterpenoid which is the major chemical constituent of *Andrographis paniculata* Nees of Acanthaceae family and is called as Maha-Tita literally known as "King of Bitter" in Northeastern India [2].

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They are also called as Bhumi-Neem, meaning "Neem of the Ground" since it has a strong bitter taste than that of the Neem tree. In Malaysia, it is known as Hempedu Bumi, which means "Bile of Earth" [3]. The constituent Andrographolide is used for liver sluggishness and as an antidote for colic dysentery and dyspepsia [4].

Andrographis paniculata is also given in the case of snakebite asanti-venom agent [5]. They show various pharmacological actions such as anti-hyperglycemic, anti-inflammatory, and suppression of various cancer cells [6]. Hesperidin is a flavanone glycoside isolated from the peels of *Citrus sinensis* of the Rutaceae family, which is known as Sweet Orange. Hesperidin was first isolated by Chemist Lebreton in 1828 from the peels of the Citrus fruit [7]. Hesperidin has a wide range of biological activities. It possesses an Inhibitory effect against the development of neurodegenerative diseases

such as Parkinson's, Alzheimer's, and Multiple Sclerosis [8]. It also has an inhibitory effect against obesity diseases and regulates lipid metabolism and glucose metabolism. Hesperidin enriched dietary supplements can significantly improve symptoms such as postprandial hyperglycemia and hyperlipidemia [9]. Hesperidin is generally used as antihypertensive, antidiabetic, cardioprotective, anti-inflammatory, and antiartherogenic activity [10].

Plant Details:

Habitat: *Andrographis paniculata* Nees is found throughout India from Himachal Pradesh to Assam, Mizoram, and all over Southern India [11]. It is also distributed in tropical countries such as USA, Jamaica, Pakistan, Sri Lanka, Thailand, and West Indies. It is found in a variety of habitats such as Hill slopes, Dry and Wetlands, Forest areas, Farms and Wastelands [12,] and also in phytogeographical and edaphic zones of China, America, West Indies, and Christmas Island [13].

Citrus sinensis are found throughout Southeast Asia, Southern United States, and Mediterranean Region [11].

Vernacular Names:

***Andrographis paniculata*:** [11]

English	:	Creat
Ayurveda	:	Kaalmegha, Bhuunimba, Bhuuminimbaka, Vishwambhara Yavtikta, Kalpanaatha, Kiraata-tikt
Unani	:	Kiryaat
Siddha	:	Nilavembu
Chinese	:	Senshinren
Japanese	:	Senshinren
Thai	:	Fah Talai Jone

***Citrus sinensis*:** [14]

English	:	Sweet Orange
French	:	Oranger doux
Indonesia	:	Jeruk manis
Malaysia	:	Limau Manis, Chula, Choreng
Philippines	:	Kahel
Burma	:	Tungchin-thi
Cambodia	:	Krooch poosat
Thailand	:	Somkliang, Somtra, Makhun
Vietnam	:	Cam

Plant Description: *Andrographis Paniculata* is an annual, branched, herbaceous plant. The inflorescence of the plant is terminal and axillary in panicle, 10-30 mm long. The bract is small, and the pedicel is short. *Citrus sinensis*, commonly known as Orange or Sweet Orange, is a small evergreen tree, and its branch has large spines originally domesticated in Subtropical Asia. The fruit consists of two distinct regions pericarp and endocarp.

TABLE 1: DESCRIPTION OF PLANTS [15-21]

Parts	*Andrographis paniculata*	*Citrus sinensis*
Height	30-110 cm.	9-10 m.
Leaves	Simple, Opposite, Lanceolate, Glabrous and 2-12cm long	Alternate, Narrowly Winged Petioles,3-5 mm wide, 6.5-15 cm long
Stem	Much branched, acutely Quandrangular and dark green.	Branched, they develop a single tap root system from the branch.
Flowers	White with rose – purple spots on the petals. Calyx (5) and corolla tubes narrow about 6 mm long.	Axillary borne singly or in whorls of 6 with 5 white petals and 20-25 yellow stamens, creamy white flower.
Ovary	Superior ovary, 2 celled, style far exserted.	Orange is produced from single ovary. Fruit globose to oval and ripens to orange or yellow in colour.
Seeds	Yellowish brown, small, sub quadrate.	Numerous seeds within the sweet pulp.
Bloom Time	December to April.	March to April.

Phytochemistry:

***Andrographis paniculata*:** The main constituent is Andrographolide. The plant leaves possess the highest amount of Andrographolide, while the seeds contain the lowest amount [22]. The other chemical constituent generally includes Deoxyandrographolide-19β-D-glucoside and Neo-andrographolide which are bitter constituents. The roots have Andrographan, Andrographosterin, Andrographon, Homoandrographolide, and Stigmasterol [23]. They also contain Flavanoids, Diterpenoids, and Polyphenols [24].

***Citrus sinensis*:** The main constituent present is Hesperidin which is a Flavanone Glycoside, and its Aglycone form is called Hesperetin. They are usually present in higher amounts in the peels of the Citrus Rinds. They are rich in Vitamin C, Fiber,

Calcium, and Vitamin D. Orange juice contains β-cryptoxanthin, a natural Carotenoid Pigment which is converted to Vitamin A (Retinol) and known as Provitamin A [25-26]. They also contain other phytoactive compounds such as Potassium, Carotenoids, Flavanoids, and other volatile Organic compounds like Alcohols, Ketones, Terpenes, and Esters *etc.* [27]. Limonene, Linalool and Citral are the main volatile material present in *Citrus sinensis* [28].

Extraction:

Plant Material:

***Andrographis paniculata*:** The leaves are air-dried under shade for 15 days and then in a hot air oven below 60°. It is powdered to 40 mesh and stored in an airtight container at 15–20° until further use [29].

Citrus sinensis: The peels of the Citrus fruit are obtained and they are dried and grinded and stored in an airtight container until further use [30].

Procedure:

***Andrographis paniculata*:** Andrographolide is usually extracted by two processes. They are Solid-Liquid Extraction and Ultra Sound Assisted Extraction.

Solid-liquid Extractions: It is extracted with different solvents such as methanol, water, 10% water-methanol, 5% water-methanol.

Cold Extraction: In this process, the sample is dissolved in the solvent in the ratio of 1:20 in round bottom flasks overnight at room temperature. The supernatant obtained is filtered by the Vacuum Filtration process and concentrated at 40° at reduced pressure.

Hot Extraction: In this process, the sample is extracted by using the Soxhlet apparatus on a water bath for 5 hours, and then they are cooled, filtered, and concentrated on getting the extracts which are further tested for their activity [31].

Ultrasound-Assisted Extraction: It is done by using a rectangular Branson Ultrasonic cleaning bath device. The working frequency is 40 kHz, and Input Power is 230W and has digital operating control. The sample is transferred to a Screw-capped glass tube which is mixed with different ratio of Ethanol and solvent-to-solid- ratio. Then they are immersed in water, and the extraction process is carried out with the above-said frequency and power. Replicate Experiments were also carried out at room temperature. Then the extract was filtered by Whatman No.1 filter paper and centrifuged at 6000 rpm for 15 minutes at 4 °. Then the supernatant liquid is further filtered and stored at 4° for further use [32].

***Citrus sinensis*:** The extraction process involves the Supercritical Fluid extraction method and Organic Solvent extraction method, which result in the formation of Crude extract from which further studies are done.

Supercritical Fluid Extraction: The orange peel is dried under hot air, and they are ground to small particles. Extraction is carried out by using an extractor with SC-CO_2 as solvent. Optimization of Super Critical Extraction is determined by Surface Response Methodology [33].

Organic Solvent Extraction method: Organic solvent extraction methods are usually one of the conventional systems of extraction method. They involve extraction with methanol: dimethyl sulphoxide in the ratio 1:1 [34].

Isolation Procedure:

Isolation of Andrographolide: Andrographolide is usually isolated by cold maceration, hot maceration and microwave-assisted extraction.

Cold Maceration: The sample is taken in a round bottom flask and extracted exhaustively with 1:1 ratio of dichloromethane: ethanol. They are filtered, and the solvent is removed by vacuum. A green crystalline mass is obtained, which is separated and washed repeatedly with Toluene to remove the colouring matter present in them. Toluene should be completely removed from the residue [35].

Hot Maceration: Two samples are taken; one is dissolved in Methanol and another in 95% alcohol, and they are macerated overnight. Then the marc obtained is packed in a Soxhlet apparatus and the extraction process is carried out. The extract is obtained separately for both the process and the solvent is removed by Vacuum. A green crystalline mass will be obtained, which are separated and they are washed repeatedly with Toluene to remove

the colouring matter present. Then the Toluene should be completely removed from the residue [35].

Crystallization Procedure: The product obtained is dissolved in Hot Methanol and cooled for Crystallization process. The procedure is done several times to obtain the chemical constituents Andrographolide [36].

Purification Process: Purification process involves washing the green mass obtained with Toluene to remove the green colour matter present. The Toluene was completely removed by evaporation. Then the residue is dissolved in Hot Ethanol, and the filtrate was cooled and filtered while hot, and they are cooled in the refrigerator. The process is repeated several times until a small yellow colour crystal of Andrographolide is obtained [37].

Microwave-Assisted Extraction: The novel method for the isolation of Andrographolide is the Microwave-Assisted extraction process. In this process, the sample is powdered and mixed with the solvents such as methanol for polar compounds and hexane for non-polar compounds. The extraction was usually carried out in the Pyrex beaker, and they are placed inside the microwave oven and exposed to microwave radiation for 5 min under the power level of 20%. After the irradiation process, the samples were cooled, and they were centrifuged and made concentrated [38-39].

The extraction intensity, amount of solvent and the time required should be optimized prior to process [40]. Two parameters such as microwave power – 75 to 175 W and ethanol concentration – 20 to 85% are generally important in this process. Higher yield is obtained by initial screening on the mesh size and solid-solvent ratio.

The optimum condition was found at a microwave power of 140 W with 85% ethanol concentration, which generally gave the highest yield of Andrographolide. Hence, the microwave-assisted extraction process gave more yield of Andrographolide than the conventional methods [41].

Isolation of Hesperidin: Hesperidin can be isolated by the following methods from orange peels. They include the conventional method (maceration) and modern method (Soxhlet extraction process).

Conventional Methods (Maceration): The dried orange peels or powder (250 g) was macerated with 800 ml of aqueous alkaline solution (10% KOH) and kept overnight. The pH of KOH should be 8-9. After maceration the obtained mixture is filtered by Buchner funnel and the filtrate was evaporated to obtain the syrupy mass which is further treated with 6% acetic acid. They are refrigerated overnight to obtain solid crystalline substance and taken out and again filtered to obtain the crude hesperidin [42-43].

Modern Method (Soxhlet Extraction Process): Petroleum Ether of 150 ml (40 – 60°) is filled in a 250 ml round bottom flask with a magnetic stir bar, 50g of dried and powdered orange peel are placed in the extraction sleeve of a Soxhlet extractor and covered with a little glass wool. A reflux condenser is put on the Soxhlet extraction unit, and then the reaction mixture is stirred and heated for 4hours under strong reflux. The petroleum ether extract is discarded. In order to remove the adherent petroleum ether, the content of the extraction sleeve is laid out in an extensive crystallization dish. Afterward the substance is placed again in an extraction sleeve like before, but with 150 ml methanol, extracted till the solvent leaving the extraction sleeve is colourless (1 to 2 h).

The extract is evaporated at the rotary evaporator until a syrupy consistency is reached. The residue is mixed with 50 ml of 6% acetic acid; the precipitated solid is the crude Hesperidin. It is sucked off with a Buchner funnel, washed with 6% acetic acid, and dried at 60° until it is constant in weight. For recrystallization, 5% solution of the crude product in Dimethyl Sulfoxide is added, stirred, and heated to 60–80°. Afterward the same amount of water is added slowly while stirring. When cooling to room temperature, the Hesperidin precipitates. It is sucked off, first washed with little warm water and then with Isopropanol, and dried in the desiccators until it is constant in weight [44-45].

Rapid Extraction of Hesperidin: A new and novel method was identified in which the sample is extracted with methanol and crystallization is done by using water with the addition of dichloromethane, which resulted in rapid extraction of the flavonoid compound Hesperidin. In this process, the volume of solvent used and the extraction time is shorter compared to various other methods and

hence resulted in the production of a higher amount of Hesperidin [46].

Purification of Hesperidin: Hesperidin can be purified by two ways, they are

Procedure 1: The crude Hesperidin is added to dimethylformamide and warmed to about 60°, and little acetic acid is added. The solution was then filtered through a Buchner Funnel, diluted with an equal volume of water, and was allowed to stand for 4 h in order to crystallize. After crystallization, the crystal of Hesperidin is filtered.

Procedure 2: The crude Hesperidin obtained is added to chloroform. The white crystalline Hesperidin is obtained, and they are filtered through a Buchner Funnel [47].

TABLE 2: PHYSICAL PROPERTIES OF ANDROGRAPHOLIDE AND HESPERIDIN [48-51]

Physical Characterization	Andrographolide	Hesperidin
Molecular Formula	$C_{20}H_{30}O_5$	$C_{28}H_{34}O_{15}$
Molecular Weight	350.4 g/mol	610.6 g/mol
Appearance & Form	Rhombic Prism, Powder	Crystalline Powder
Colour	Yellow Colour	Light Brown
Solubility	Sparingly Soluble in water	Insoluble in water and soluble in organic solvents
Density	1.21g/cm^3 , 1.23g/cm^3	1.32 g/cm^3
Melting Point	229-232 °, 230-231 °	250-255 °
Boiling Point	557.3 °	576.16 °
Refractive Index	1.567	1.595
Storage Condition	They Should be Tightly closed and stored in a cool and dry place	Hygroscopic. Stored at temperature of 2-8 °

Identification and Characterization:

Andrographolide: The Identification and Characterization can be done by a variety of methods. The most commonly employed laboratory method generally include the following

Chromatographic Techniques: TLC Method (Thin Layer Chromatography)

Spectral Analyses: UV (Ultra Violet Spectroscopy)

Chemical Identification

Chromatographic Methods:

Thin Layer Chromatography: In this method precoated plates of Silica Gel 60F254 were used. Different Mobile phase used for the process are

Chloroform: Methanol: Ethylacetate (7:2:1)

Chloroform: Methanol (9:1)

Chloroform: Ethylacetate (6:4)

Chloroform: Acetone: Formic acid (7.5:1.65:0.85)

Spraying agent: 3, 5 -Dinitrobenzoic acid (2% w/v)

Visualizing agent: KOH in Ethanol (6% w/v).

The spotting was done by CAMAG LINOMAT IV Automatic TLC Spotter. The Purity was identified by using CAMAG TLC Scanner. The Mobile Phase Chloroform: Methanol: Ethylacetate (7:2:1) was effective for both the Extract used and Standard Andrographolide used [52-54].

Spectral Analysis:

Ultraviolet Spectroscopy: The UV Absorption was recorded in UV/VIS Spectrophotometer (JASCO Model 7850). The best solvent for the UV Spectroscopy experiment of Andrographolide is Methanol: Water (50:50 v/v).

In determining the UV Spectrum of the Andrographolide, they were scanned in UV Spectrophotometer between the ranges of 400 – 200 nm. They showed Maximum Absorbance at a wavelength at 312 nm [55].

In Nano Emulsion preparations, Detection and Quantification were done by UV/VIS Spectrophotometry and read at maximum with Picric Acid reagent and NaOH (8:2) in Methanol solvents. They are read at a maximum wavelength of 479 nm with 22 min of Incubation time [56].

Validation of UV spectroscopy: They are validated for Selectivity, Specificity, Linearity, Range, Limit of Quantification, Limit of Detection, Precision, Robustness, Ruggedness, and Solution Stability [57].

Chemical Identification: The small amount of the sample was dissolved in 5ml of Methanol and treated with 1 ml of 2,4 – Dinitrophenyl hydrazine, and 100 ml of 2M HCl was added. Yellow Orange colour is obtained, which indicates the presence of Andrographolide [58].

Alcoholic Potassium hydroxide, 8 drops were added to the sample powder, and Red colour is obtained which is kept for 14 min. The Red colour changed to Yellow, which indicates the presence of Andrographolide [59].

Hesperidin: The Identification and Characterization can be done by a variety of methods. The most commonly employed Laboratory method generally includes,

Chromatographic Techniques: TLC Method (Thin Layer Chromatography)

Spectral Analyses: UV (Ultra Violet Spectroscopy)

Chemical Identification:

Chromatographic Techniques:
Thin Layer Chromatography: The Thin Layer Chromatography can be carried out by using the solvent nButanol: Acetic acid: Water in (3:1:1) Ratio.

Two spots were obtained for the Hesperidin determination one at R_f 0.62 and Rf 0.20. Orange colour spots were obtained [60].

Spectroscopy Analysis:
Ultraviolet Spectroscopy: In this process UV/Visible Double Beam Spectrophotometer was used. The Hesperidin showed good absorbance when dissolved in 0.2N NaOH. The Maximum Wavelength of Hesperidin was 285 nm and they showed good absorbance at this Nanometer [61].

Validation of UV spectroscopy: They are validated for Selectivity, Specificity, Linearity, Range, Limit of Quantification, Limit of Detection, Precision, Robustness, Ruggedness and Solution Stability [62].

Chemical Identification: The Powder sample was treated with Ferric Chloride solution. A bright Red colour is obtained which indicates the presence of

Hesperidin Shinoda Test: A bright pinkish violet colour is obtained which indicates the presence of Hesperidin [63].

Pharmacological Actions:
Andrographolide:
Anticancer Properties: The cytotoxic potential of Andrographolide on HT- 29 cell was determined by MTT Assay, Tryphan Blue Exclusion Assay, Colon Formation Assay, Morphological Analysis Apoptotic Property by DAPI, Hoechst Staining, FITC Annexin Assay, DNA Fragmentation Assay and Caspase-3-Activity.

Therefore from the above study, it was identified that Andrographolide exhibited antiproliferative and apoptotic properties against Colon cancer HT-29 cells [64].

Recent work demonstrates that Tumour Necrosis Factor – α (TNF – α) related Apoptosis inducing ligand (TRAIL – An important member of extrinsic apoptosis pathway) was significantly enhanced in various Human cancer [65].

Andrographolide is also effective in combination therapy. They increased the Apoptosis rate in Multidrug-Resistant cancer cells when used in combination treatment with other anticancer agents like 5-Fluorouracil, Adriamycin, and Cisplatin [66].

The *in-vivo* antitumor efficacy of Andrographolide was examined in Xenografts nude mice, and the results indicated that Andrographolide could significantly inhibit the proliferation of Human Breast Cancers and inhibit COX-2 Mediated Angiogenesis in human Endothelial Cells [67]. Andrographolide inhibited the growth of Huh-7 cells independent of Apoptosis [68].

Antivenom Properties: Anti-Snake venom is the specific antidote to Snake venom actions. Experiments were carried out to investigate the Anti-Cobra Venom effect of alcoholic extract of *Andrographis paniculata*. Male Swiss Albino Mice was used for the experiment. The ethanolic extract of the plant increased the mean survival time and the protection fold but could not protect animals from death. So, therefore, Anti-Snake Venom was found more effective than the plant extract, but when given along with plant extract, it potentiates its effects [69].

Anti-malarial Properties: Malaria is a very serious hazard which causes major Mortality and Morbidity in the Endemic countries. The most affected region is Asia, Central and Latin America and Sub-Saharan Africa [70].

Andrographolide was found to have potent antiplasmodial activity when tested in isolation and in combination with Curcumin and Artesunate against the Erythrocytic stages of Plasmodium falciparum *in-vitro* and Plasmodium berghei *in-vivo*. Andrographolide activity exhibited better Antimalarial activity, not only by reducing Parasitemia but also by extending the life span by 2-3 folds [71].

Andrographolide could protect liver and renal injuries induced by Plasmodium berghei infection in mice. Blood glucose level control was also observed in the mice [72].

Respiratory Properties: Cigarette smoke is the major cause for Chronic Obstructive Pulmonary Disease (COPD). Andrographolide possesses Antioxidative properties against cigarette smoke-induced lung injury by augmentation of Nrf2 activity and has potential to treat COPD [73].

Andrographolide is used for Prophylaxis and treatment of Upper Respiratory infections, such as the Common Cold, Uncomplicated Sinusitis, Bronchitities and Pharayngotonsillitis and also used for Urinary tract infection and acute diarrhea [74].

Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) were the diseases caused by severe Bacterial Pneumonia, Burns and Trauma. So hence study was conducted on mice and its result indicate that Andrographolide dose-dependently suppressed the severity of LPS-induced acute lung injury by NF-KB inhibition. So hence Andrographolide is considered as effective and safe drug [75].

Antifertility Activity: The Antifertility activity of *Andrographis paniculata* were conducted on stored grain pest *Tribolium confusum* in 5th instar and 6th instar Larvae and Pupae with Andrographolide. Andrographolide caused interruption of insect reproduction and prevented normal growth and development of the ovaries of *Tribolium confusum*. So hence they caused sterility in *Tribolium confusum*. Therefore Andrographolide can be used for the development of safe and specific antifertility agent [76]. In another study, Andrographolide was injected intramuscularly into Male Albino rats to test the anti-fertility activity. They cause a decrease in the protein content with a significant increase in Cholesterol, Acid Phosphatase, and Alkaline Phosphatase levels with the appearance of Fructose in the reproductive system of rats. Hence all the above reason suggests the general inhibitory effects of Andrographolide in the Testis and Epidymis [77].

Hesperidin:

Neuroprotective Properties: Neurodegenerative diseases are known as a group of chronic disorders characterized by loss of brain and spinal cord cells [78]. These diseases include Alzheimer's disease, Parkinsonism, and Huntington disease [79].

A human study was conducted by Placebo-Controlled, Randomized and Double-Blinded clinical study by chronic administration of orange juice on 37 healthy adults (60-81 years) was examined. The study concluded that Hesperidin possesses an Inhibitory effect against the development of Neurodegenerative diseases. Neuroprotective effect is highly dependent on antioxidant and anti-inflammatory activities. Hesperidin also showed Antidepressant activity [8].

Antiobesity Properties: Obesity is a pathological state which causes excessive body fat and makes the bodyweight more than 20% of the standard bodyweight. Fat accumulation causes Cardio-vascular Hypertension, Hyperlipidaemia, Insulin Resistance, and Inflammation [80].

Hesperidin improves Hypercholesterolemia and Fatty Liver by inhibiting Cholesterol synthesis and absorption. Hesperidin has a therapeutic effect on Obesity by mediating AMPK and PPAR pathways to regulate inflammatory signalling pathways. So Hesperidin can help expand the range of weight loss and reduce the rate of obesity in the body [81].

Anticancer Properties: Liver cancer is caused by various factors such as Alcoholic Cirrhosis, Hepatitis B and C infections. In the given study, HepG2 cells were used and treated with crude Ethanolic extract from citrus seeds, and they cause Human Hepatocellular Carcinoma Apoptosis [82].

Breast cancer is the first-ranked case of cancer in women worldwide. The study was conducted to determine the effect of Hesperidin performed on MCF-7 and MCF-7/Dox cells. The results obtained were that Hesperidin has cytotoxic effect on MCF-7/Dox cells. The application of doxorubicin and hesperidin on MCF-7/Dox cells showed a synergism effect through inhibition of cancer cells [83]. Hesperidin can also induce both Apoptotic and Autophagic cell death in colon carcinogenesis [84].

Antidiabetic Properties: The study was designed to investigate the effect of Hesperidin on Serum Glucose, Blood Glycosylated Haemoglobin and Serum Insulin levels in high fat fed or Streptozotocin (STZ)-Induced type 2 diabetic rats. They cause elevated levels of Glucose, Glycosylated Haemoglobin, AST, LDH and CK-MB and Lowered Serum Insulin levels. So hence from the above result it is found that Hesperidin shows Antihyperglycemic and Antidyslipidemic efficacy as well as improve cardiac function in HFD/STZ – induced type 2 diabetic rats [85]. Another study was conducted on STZ-Induced marginal Type I diabetic rats. The Hesperidin normalizes blood glucose by altering the activity of glucose regulating enzymes and lower serum and liver lipid levels. So hence Hesperidin showed both Hypoglycaemic and Hypolipidemic effects but did not affect bone marginal type I diabetic rat [86].

Varicose Vein Treatment: Hesperidin is the most effective medication against Varicose Vein as it acts also as an Anti-inflammatory. They have greater activity when they are combined with Disomin along with other natural medication against varicose vein. Hesperidin also used for swelling sensations, Haemorrhoids, Cramps, Pain and Capillary Bruising. Disomin: Hesperidin (9:1) combination is used for Haemorrhoids and Varicose Vein [87].

CONCLUSION: This review summarizes the extraction, isolation and various pharmacological activities or properties of the chemical constituents Andrographolide and Hesperidin. They can be used to cure various ailments. In recent times the constituents Andrographolide has been proven effective against COVID-19 which acts as a immunostimulant. Further studies can be done by isolating the constituents and can be formulated into any form of novel drug delivery system which has nowadays created a huge impact in modern medicine. So, in conclusion, Andrographolide and Hesperidin have a huge impact on the well-being of individuals.

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