Anthocyanins and Hepatoprotection

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Anthocyanins are water-soluble, colored compounds of the flavonoid class, abundantly found in the fruits, leaves, roots, and other parts of the plants. The fruit berries are prime sources and exhibit different colors. The anthocyanins utility as traditional medicament for liver protection and cure, and importance as strongest plants-based anti-oxidants have conferred these plants products different biological activities. These activities include anti-inflammation, liver protective, analgesic, and anti-cancers, which have provided the anthocyanins an immense commercial value, and has impelled their chemistry, biological activity, isolation, and quality investigations as prime focus.

Keywords: anthocyanins ; traditional medicine ; liver protection ; hepatocellular longevity ; hepatic carcinoma ; anti-oxidant ; anti-inflammation ; NrF2 ; TNF-α

1. Introduction

The interest and information on anthocyanins, and its aglycone precursors, anthocyanidins, as being part of the phenolics-rich and flavonoid class of products, with exhibitions of a wide spectrum of different biological activity and broadly considered health benefits is of immense value. The strong anti-oxidant potential together with their effectiveness as anti-inflammatory, analgesic, anti-cancers, and liver protective, and diseased liver therapy activities of the flavonoids in general, and to certain extent, the anthocyanins in particular with certain biological activity, especially the antioxidant, liver protective, and anti-liver cancer have propelled the anthocyanins and the anthocyanins-rich extracts into limelight. The continued interest in structurally advanced flavonoids, and their perpetual contributions and discoveries of newer roles in plants and animal kingdoms of their biology, pharmacological actions, metabolism, physiology, plants' protectives roles, and the intrinsic inter-relationship in plants' survival as well as their constituents' medicinal values have caught the attention of the scientific community.

2. Anthocyanins' Aesthetics, and Plant Kingdom's Distribution

The colorful world of the plant kingdom owes its beauty, attractiveness, attention, and diligence primarily to the charged and colorful flavonoid-based structures which are identified as anthocyanins. The anthocyanins are regarded as the largest, most interesting, as well as intriguing group of plants-based pigments under use by humans from very early times as colorants for foods, beverages and clothes, baits, armors, phytopharmaceuticals, colors for drawings, cave-arts, and for festivities. The anthocyanin stands for two Greek words, i.e., *anthos* for flower, and *kyaneos* for dark blue color. They are located in plant cell vacuoles, and owing to multi-colored appearances in different visible parts of the plants, including flowers, fruits, leaves, tubers, and roots, they have been in focus in various human activities including medicinal uses. The anthocyanins are appealing to humans, and attracted insects and animals in utility toward the pollination, seeds and fruits dispersal, as well as indirect carriers of plants' species spread, conservation and natural balances [1]. The anthocyanins constitute nearly one-third of the flavonoids and are water-soluble, structurally polyphenolic in nature. They are specifically distributed in plums, cherries, and berries of several plants, have acquired different colors of purple, red, violet, pink, and blue, which indicated the apparent presence of this class of compounds in nature. The plant families of Berberidaceae, Eleaocarpaceae, Myrtaceae, Solanaceae, and Rosaceae are among the major contributors [2][3]. Several crops, including the fruits of acai, cherry, black currants, black crowberry, blueberries, blackberries, bilberry, Andean black berries, cranberry, cowberry, gojiberry, Chilean berries, European bilberry, American cranberry, mulberry, red raspberries, black raspberries, choke berries (aronia berry), boysenberry, strawberry, sourberry, bosberry, jostaberry, rabbit-eye-berry, low-bushberry, high-bushberry, half-high-bushberry, buffaloberry, skunkberry, oval-leaf-huckleberry, Canadaberry, olallieberry, juneberry, sumacberry, sloeberry, turkeyberry, huckleberry, salmonberry, saskatoonberry, maquiberry, marionberry, cloudberry, pineberry, seaberry, tayberry, coralberry, yewberry, tart cherries, Concord and Norton grapes, black plums, black corn, black beans, purple onions, red radish, red currant, red cabbage, red onions, red lettuce, red-skinned potato, broccoli, rhubarb, fennel, lettuce, brown beans, seabuckthorn, purple sweet potatoes, peach, tomato, pistachio nut, pomegranate, nectarine, apples, turnip, European and Mediterranean olives, blood orange, purple carrot, black carrot,

tea, coffee beans, and black rice, etc., have been found to be rich in anthocyanins contents [4][5][6][7][8][9][10][11][12][13][14][15][16][17][18].

The anthocyanins distribution has been followed from the beginning of their discovery as pigments from plants, and information on finger-printings obtained through the chemical profiling of different extracts of several fruits and other plant parts have led the way to identify, compare, and establish the anthocyanins presence in several plant species. The use of HPLC, LC-MS [19], HPLC-DAD-ESI/MS/MS [4][20], and other different mass (MS) techniques, including soft ion bombardment, electrospray, and TOF (time of flight) techniques have contributed immensely toward the anthocyanins discovery and structure elucidations [12][21][22][23][24][25]. Among the other techniques, through established protocols of the methods, the chromatographic and spectro-analytical methods were also employed. The UV-visible spectrophotometry in conjunction with pH variability [26], mass spectrometry (MS) [27], and NMR (nuclear magnetic resonance) techniques have been utilized and anthocyanins presence have been defined at large scale [28]. The concentrations and structurally varied anthocyanins have been reported from various sources through advancements in techniques and methodology development [29]. For instance, the anthocyanins level in fresh berries (gooseberry and chokeberry) has been found varying from 0.7 to 1480 mg/100 g, while the most abundant anthocyanin-based compounds were found as the cyanidin, delphinidin, petunidin, pelargonidin, peonidin, and malvidin-based glycosylated molecules [4][30][31]. Nonetheless, the concentration of anthocyanins and their specific structural types differ in various different sources of the plant kingdom. The anthocyanins are highly affected by the temperature, light, and agronomic factors, which have been reported as major reasons for their considerable variations in the anthocyanins' contents, as well as their structure-based variations among several fruits and vegetable types [32]. In this regards, the intersecting anthocyanins cluster distribution in the plant kingdom based on the Phenol Explorer Online Database, has been performed by Mannino et al., [33], which demonstrated the anthocyanins concentrations in different plants in ascending order from 15 mg (e.g., grape fruits, date, and red onion) to 500 mg/100 g (e.g., black chokeberry, black raspberry, and evergreen huckleberry) of the plants [33]. The exercise has provided a useful and predictive range of product types and their probabilistic as well as expected concentrations.

3. Anthocyanins Roles in the Plants

The anthocyanins play beneficial role as a protective barrier for plants against high influx of light intensity, and the UV-B light [33]. The anthocyanins as antioxidant plant contents have primary defensive role in plants against various abiotic stress, including drought and high salinity conditions, as well as work against heat and light stresses. The anthocyanins are also involved in functioning and regulations of senescence, leaf temperature, osmotic balance, monosaccharides transport, and camouflage [33]. Therefore, the anthocyanins play definite part in protecting the photosynthetic apparatus of the plants from high light radiation flux [34], and avoid the damage to the plant DNA. The anthocyanins also protect against water, cold, heat, and drought stress, as well as help regulate the hemostasis of the plants [35]. Besides, the anthocyanins role in the protection of plants against different biotic stresses, e.g., microbial and insect attacks, have also been reported [36][37]. The anthocyanins contents in plants vary by the plant species and its variety, the prevalent environmental factors, plants' growth stages, and the plant-products' storage [38]. Anthocyanins are also accumulated over time in the plant vacuoles, thereby reaching maximum at ripening age of the fruits, and are also distributed in different plants parts, including in the autumn [39][40].

4. Chemistry of the Anthocyanins, Structures, and the Structural Variants

Structurally, the anthocyanins are part of the flavonoid series of plants products, and have been categorized as part of C_6-C_3-C_6 molecular framework. The anthocyanin products are found as the sugar-bonded counterparts (glycosides) of the anthocyanidins (aglycones) analogs [41], in which fifteen carbon atoms-based framework skeleton, flavylium cation, is arranged in three rings denoted as A, B, and C, which form the part of the basic skeleton of the anthocyanin compounds (**Figure 1**). The anthocyanin compounds usually have multi-hydroxylation patterns distributed along the three-ring structure of the compound, especially at the C-3 position of the ring C; C-5, C-6, and C-7 positions of the ring A; and C-3′, C-4′, and C-5′ positions of the ring B [42]. The sugar moieties are attached to the anthocyanidins' structure through the formation of acetal linkage with one, or more of the mentioned hydroxyl groups. The most common and abundant anthocyanin glycosides are produced by the glycosylation of the 3-OH group to form the 3-O-β-glucosides derivatives, e.g., cyanidin-3-O-β-glucoside and peonidin-3-O-glucoside (**Figure 1**) [43].

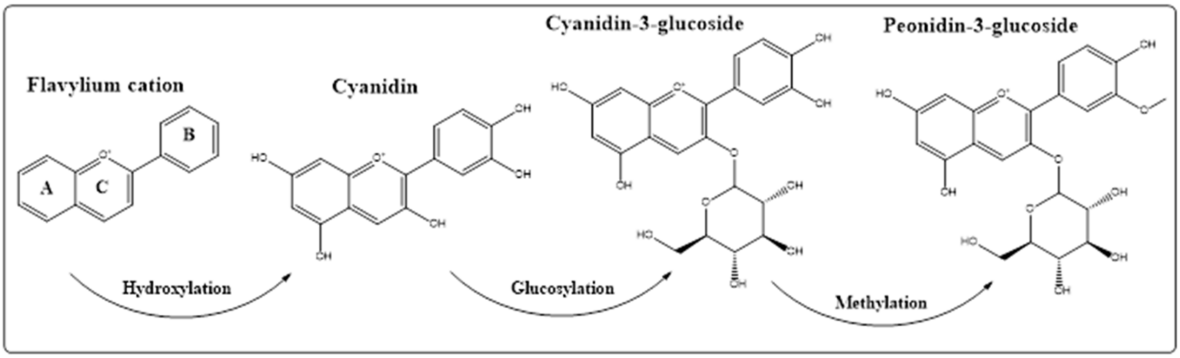

Figure 1. Basic skeleton and common biogenetic pathway of the anthocyanins.

The anthocyanin compounds provide different colors to the plants which are attributed to the highly resonating electrons around the flavylium ion structure [44][45]. Besides, the variety of the hydroxylation patterns of the anthocyanins in different positions along the three rings (rings A, B, and C) of the compounds, and also, the types and positions of the glycosylation, and the carboxylates attached to the sugar moieties, contribute to the diversification of huge varieties of the identified anthocyanin compounds, and their colors [41].

Based on structural variations, over 700 anthocyanin compounds have been identified from different plant sources, and categorized under different compound types. Anthocyanidin, the aglycone molecular framework constituting the anthocyanins, have been identified based on their 27 distinct types, which produce the different anthocyanins by virtue of their structural variations. Interestingly, the anthocyanins are rarely encountered as aglycone in nature, and nearly all of them exist as glycosides, of which nearly half are acylated in their structures. The chemistry of anthocyanin colors' involve the pH-based modulations [46]. The quinonoid base (pH 8–10) changes to pH < 2 of the flavylium cation with red to orange color which upon hydration produces the carbinol pseudo-base between pH range 3–6 with a colorless hue, and then the chalcone pseudo-base is produced which also remains colorless (**Figure 2**). However, the color and stability of these products are also controlled by the presence of other anti-oxidants, oxygen, moisture, enzymes, metals, light exposure, and temperature, including the factors of pH and the structure of anthocyanins, of which the presence of hydroxyls and methoxy groups lowers the stability of the anthocyanins [47]. Moreover, the tannin-anthocyanin co-conjugates increases the color stability at the lower pH [48]. Moreover, the co-pigmentation of the anthocyanin aglycone with flavonoids, facilitated by metallic ions presence, also stabilizes the color. The acetylation and glycosylation of the anthocyanidins immensely contribute to the color up-keep of the glycosylated products, the anthocyanins [49]. The anthocyanins are highly sensitive compounds, and their color can also be flocculated by the presence of other compounds, such as proteins, phenolic acids, and enzymes [41][50]. However, at very high pH, the anthocyanin compounds lose their color due to their degradation [51].

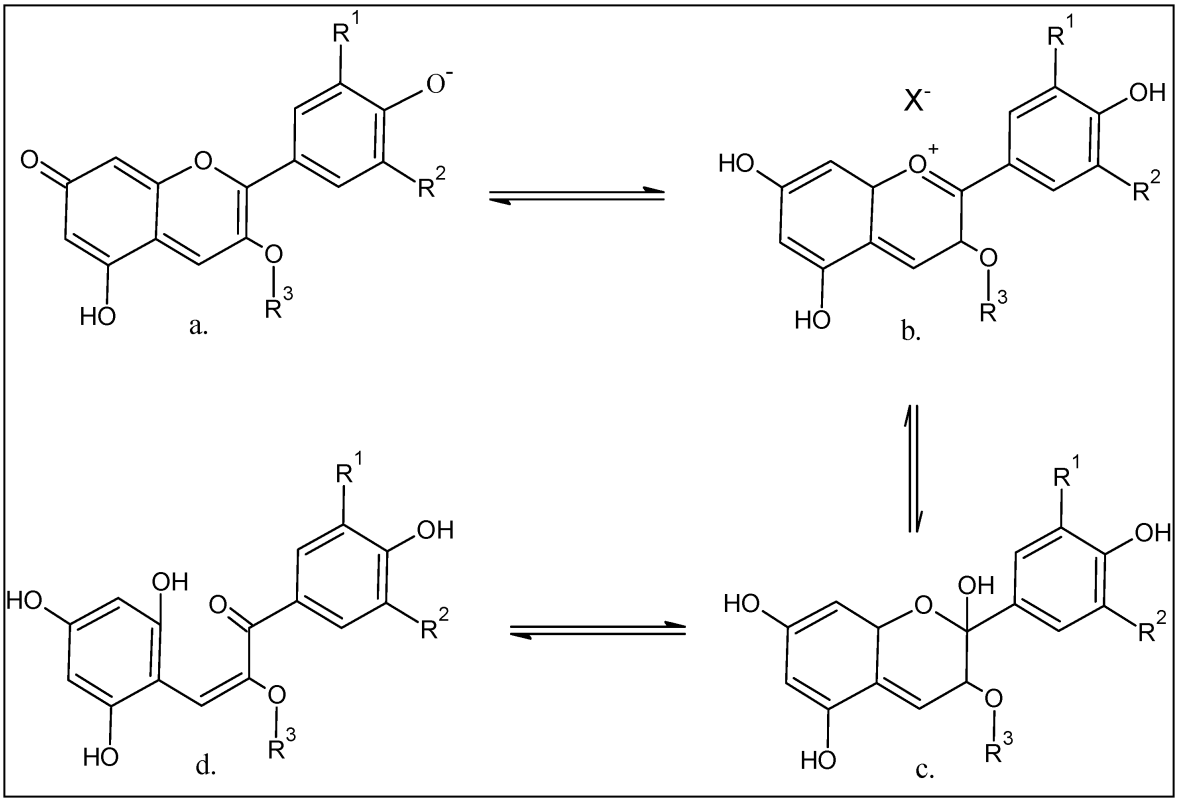

Figure 2. Effects of pH conditions on colors of anthocyanins: R^1 = R^2 = OH/O-CH_3/OC(O)CH_3; R^3 = glycoside; (**a**) quinonoid base (pH 8–10) changes to, (**b**) flavylium cation (pH < 2, red to orange color), (**c**) carbinol pseudo-base (pH range 3–6, colorless), (**d**) chalcone pseudo-base (high pH, pale yellow to colorless). The X^- refer to the anionic entity, usually a halide ion.

5. Anthocyanins Extraction, Purification, and Structure Determinations

Total anthocyanidins are conventionally extracted in polar organic solvents. Acetone and their mixture with water, also, usually together, with some acid contents are used to keep the ionization state of the flavylium forms intact. A mixture of ethanol, or methanol, and water (70 to 95%, and 30 to 5%), together with hydrochloric, formic, citric, and other organic acids have been employed [52][53][54]. Lipophilic organic solvents under ultra-sonication, and slightly elevated temperature than the RT have also been utilized for seeds anthocyanins extraction [53][55]. Natural deep eutectic solvents (NADES) were used since they are biocompatible in nature, green, environment-friendly, recyclable, and sustainable. They have also been proven to be *at par* with the organic-solvents-aqueous-acid extraction media [56][57][58][59]. The methodology needed to remove the chlorophyll, and process the extraction soup for the anthocyanins rich portion, which was carried out by chromatographic means utilizing several stationary phase materials, e.g., reverse phase silica C_{18}, and Sephadex® to fractionate the material based on molecular weight/molecular size [60][61]. Normal silica, the SiO_2 gel, cation exchange material, Amberlite® IRC 80, Amberlite® XAD-7HP, and DOWEX® 50WX8 resins have also been used for the purpose [62]. A high-speed counter-current chromatography (CCC) run following the resin XAD-7® treatment of the crude/semi-processed extract was also employed. Solvent system comprising *n*-butanol, ethyl acetate, and 0.5% acetic acid in 3:1:4 ratio, *n*-butanol, *tert*-butyl methyl ether, acetonitrile in ratios of 6:5:2:1 with 0.2% trifluoro acetic acid were also demonstrated to be the best solvent mixture, as also confirmed by the HPLC analysis of the obtained anthocyanin products [63][64][65].

The extraction of anthocyanin products of the grape marc were obtained from acetone-water in different ratios of the acetone (5,7, and 10) at varying temperatures (20 and 60 °C) through the high-pressure CPF (concentrated powder form) technique to yield the anthocyanins extracts in powder form. The starch and silica were used as carrier materials, and colorimetric analysis was performed to check the extraction products quality. The storage of thus extracted materials provided stable color as compared to the non-CPF extracted material containing the anthocyanins for a longer period of time [66].

In a more simplified extraction method, avoiding the methanol as the toxic entrant, water and ethanol based extractions were considered as green solvent, and were used in conjunction with UV-VIS-based spectrophotometric contents determination at different pH values [67]. The difference in the λ_{max} absorption values at pH 1 and pH 4.5 in the visible range of the UV-VIS absorption range, provided an accurate estimation of the total monomeric anthocyanins in presence of other colored materials, conjugates, and polymeric entities in the diluted extract. The cyanidin-3-O-β-D-glucoside was used as an equivalent for unknown samples [68]. An estimation of the degraded and polymerized anthocyanin-based products contributed to the color intensity, and the bisulfite based reaction was employed for estimating their contribution toward color, and ensured the monomeric anthocyanin contents, which reacted with the bisulfite reagent, thereby producing the sulfonic acid adducts that did not contribute to the anthocyanin-based color, and helped in isolating the anthocyanin based fraction [69][70][71].

Critical extraction with acidified water (0.01% HCl, pH~2.3) at 110–160 °C under 40 bars of pressure had been used, and has proved to be a highly efficient procedure [72]. Anthocyanins' structure stabilization by sulfur dioxide with high diffusion coefficient had increased the anthocyanins solubility with water [73], and the crude mixture was subjected to various chromatographic separations including, preparative TLC, normal silica gel, cellulose, and RP column chromatography, vacuum liquid chromatography, Sephadex, CCC, ion-pair and resins-based chromatography, HPLC and UPLC analyses at laboratory and extended scales, of which the HPLC and UPLC were also used for the known contents' quantification. The use of GC has also been recommended [74][75][76].

The purified anthocyanins structures elucidations have been achieved using various spectro-analytical techniques. Ultra-violet (UV) spectrophotometry, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, high-resolution (HR), and tandem mass spectrometry as well as X-ray diffractions (XRD) have been utilized in structure determinations. The HR-MS, and tandem mass methods have been pressed to identify the specific fragmentation pathways [77][78]. The aglycones, anthocyanidins, have been identified after the sugar loss in mass fragmentations, and also differentiated the structural types by the MS/MS fragmentations observations. The cross-ring cleavages, specifically for the ring-C of the anthocyanidin structures, produced different oxonium fragments ions. The mass fragments produced by the other structure parts, the structures of the ring A and B also helped to distinguish the substitution patterns and the

substitution groups [79], while the NMR protons pattern accounted for the substitution pattern/designs of the anthocyanins/anthocyanidins rings. Functional groups differentiation for 1072 cm^{-1} bending vibration for C-O-C groups for the ethereal C-O-C bond of the aglycone-sugar attachments were specific for the anthocyanin structures [80]. The NMR-based identification of the known and unknown anthocyanins with the diagnostic peaks for H-4 at downfield shift at 8.6–9.1 ppm as a singlet signal identifying the flavylium salt presence was conclusive evidence [41][81]. The sugar signals, anomeric protons, β, or of α configurations of the linked glycoside(s) provided the structure of the common molecular framework-based anthocyanin framework. A 2D-NMR spectral analyses involving homo and hetero-COR (correlation spectroscopy) NMR experiments, e.g., HMBC, HSQC, TOCSY, NOESY/ROESY had been used to identify the structurally complex anthocyanin structures with certainty [82].

6. Herbal Medicines Traditional Uses, Toxicity, Liver Disorders, and Anthocyanins

The use of plant-based products is from antiquity, and plants have entered the traditional system of medicine as well as in the folklores of various civilizations and cultures around the world. Moreover, the old-age knowledge about the toxicity of the herbal products is also obscure, and limited. Certain plants products, their derived nutraceuticals, and food supplements, as well as dietary supplements extracts and powders have been found detrimental to health, damaging the liver and disrupting its functioning. The use of ma-huang, germander, valerian, mistletoe, skullcap, chaparral, comfrey, kava, pennyroyal oil, and excessive intake of vitamin-A are among these products [83][84]. There are also reports of excessive iron, potassium, calcium, vitamins C, niacin, folate, green tea, ginseng, black cohosh, and anabolic steroids causing the liver damage [85][86]. Certain antidepressants, antibiotics, anti-epileptics, synthetic hormones, antifungal and anti-microbial drugs [87][88][89] are also reported to cause liver damage upon their excessive uses. Indirect implications of the methotrexate, valproic acid, tamoxifen, estrogen, diltiazem, and antiretroviral drugs have been implicated in liver disorders, especially the non-alcoholic fatty liver disease (NFLD) and the liver tissue damage [90][91].

There are several liver diseases, and multiple types of liver-based malfunctioning, that are hard to diagnose, hence an early diagnosis is recommended by physicians. Nonetheless, the liver diseases can also be inherited, i.e., hemochromatosis, Wilson's disease (copper storage in liver), and α-1 antitrypsin deficiency. The hyperoxaluria, a condition when urine contains high levels of urea as a consequence of liver making excess oxalate, owing to certain genetic mutation, and which leads to kidney failure, together with excessive oxalate accumulations in several organs. The other condition, hemochromatosis, manifests itself when excess iron is stored-up from the food, and the excessive iron is accumulated in liver, including heart, and other organs, and which leads to liver disorders, as well as cardiovascular conditions and diabetes [92]. There are several contributing factors to liver diseases, and malfunctioning, including fat accumulation (5–10%) in non-alcoholic liver, called nonalcoholic fatty liver disease (NAFLD), parasitic and viral infections, i.e., hepatitis A, B, and C, excessive weight gain, and permanent obesity, excessive alcohol abuse by individuals, different drugs' abuse and their adverse reactions, exposure to toxins, certain harmful herbal products, and the immune attacks led liver disorders, i.e., auto-immune hepatitis, primary biliary cholangitis, primary sclerosant cholangitis, and type-2 diabetes, as well as malignancies causing liver tissue and bile duct cancers, and the liver adenoma [93][94]. Some persisting adverse conditions, malfunctioning, and infections, including chronic inflammation can also lead to liver cirrhosis, a life-threatening situation, which can be controlled, also due to self-regeneration capacity of the liver tissue. However, the warning signs of looming liver disorders include jaundice (yellow coloration of eyes and skin), abdominal pain and swelling, darker urine, pale stool, nausea, chronic fatigue, loss of appetite, and itchy skin, etc. Most of the liver diseases can be confirmed by blood tests, scanning CT (computed tomography), and MRI (magnetic resonance imaging), ultrasound, and the biopsy. However, the treatment for liver disorders depends on the diagnosis and the disease condition. Life-style modifications of removal of alcohol consumption, weight loss, control of diabetic conditions, removal of processed carbohydrates, red-meat, *trans*-fat, and high-fructose corn syrup from the diets, light exercise (30 min/day) have also been recommended to help [95]. Among short-term liver disorders, the acute liver failure, where liver functioning is severely affected, or stopped within days, or weeks, and which is caused by overdose of prescription and OTC drugs, acetaminophen overdose, as well as severe infection, or chemicals led damage, i.e., cyclophosphamide, acrylamide, endotoxin, d-galactosamine, palmitic acid, and carbon tetra chloride, are known. Among the herbal products, used traditionally for a long time for liver disorders include *Phyllanthus niruii*, *Silybum marianum* (milk thistle), *Glycyrrhiza glabra* (licorice root extract, and glycyrrhizin), and berry-based products [96]. The use of carom seeds, papaya, cumin seeds, garlic, and carrot is also recommended as part of the traditional plants-based products for liver therapy [97]. In this context, the colored plants have been used by humans in different aspect, i.e., foods, medicines, to enhance the mood, as well as remove the environmental stress. The anthocyanins, therefore, have been used as counterpart of the human diets long back, and have been utilized in the ancient traditional applications in treatment of various diseases (**Table 1**). For instance, anthocyanins-rich plant parts, e.g., berries, fruits, seeds, and leaves, have been used by the North American Red Indians, Europeans, and the Chinese

as part of their traditional herbal medicines to cure and prevent several other diseases, though at times, included their use in liver disorders [98].

Table 1. Plants and their parts used in treatment of various diseases and the identified anthocyanin contents.

Plant's Name	Folklore Medicinal Uses, Other than Liver Disorders	Plant Parts Used	Major Identified Anthocyanins	Refer
Hibiscus sabdariffa	Hypertension, pyrexia	Calyx, Epicalyx	Cyanidin-3-O-β-glucoside, and delphinidin-3-glucoside	[99] [100]
Cichorium intybus	Inflammation	Leaves	Cyanidin-3-*O*-(6″-malonyl-β-glucopyranoside)	[101]
Garcinia indica	Male digestion, flatulence, and constipation.	Fruits	Cyanidin-3-O-β-glucoside, and cyanidin-3-O-sambubioside	[102]
Raphanus sativus		Roots	Pelargonidin derivatives	[103]
Morus alba (Mulberry), & other species	Cardiovascular diseases, nephritis, thirsty, constipation	Fruits	Cyanidin-3-O-rutinoside, cyanidin-3-O-glucoside	[104] [105]
Cornus mas (cornelian cherry)	Diabetes, diarrhea, fevers, rheumatic complains, skin diseases and urinary tract infections	Fruits	Cyanidin-3-O-galactoside, pelargonidin-3-O-galactoside, delphinidin-3-O-galactoside, cyanidin-3-O-rutinoside, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, pegonidin-3-O-glucoside	[106]
Lannea microcarpa	Scurvy, rickets and cough.	Fruits	Cyanidin-3-O-(2-O-β-D-xylopyranosyl)-β-D-galactopyranoside, and cyanidin-3-O-β-D-galactopyranoside.	[107]

As the current concern deals with the traditional uses of various anthocyanins-rich plants, the **Table 1** provides examples of the uses of the anthocyanins rich plants for prevention and treatment of various disorders. The major anthocyanins' structures listed in **Table 1** are presented in **Figure 3**.

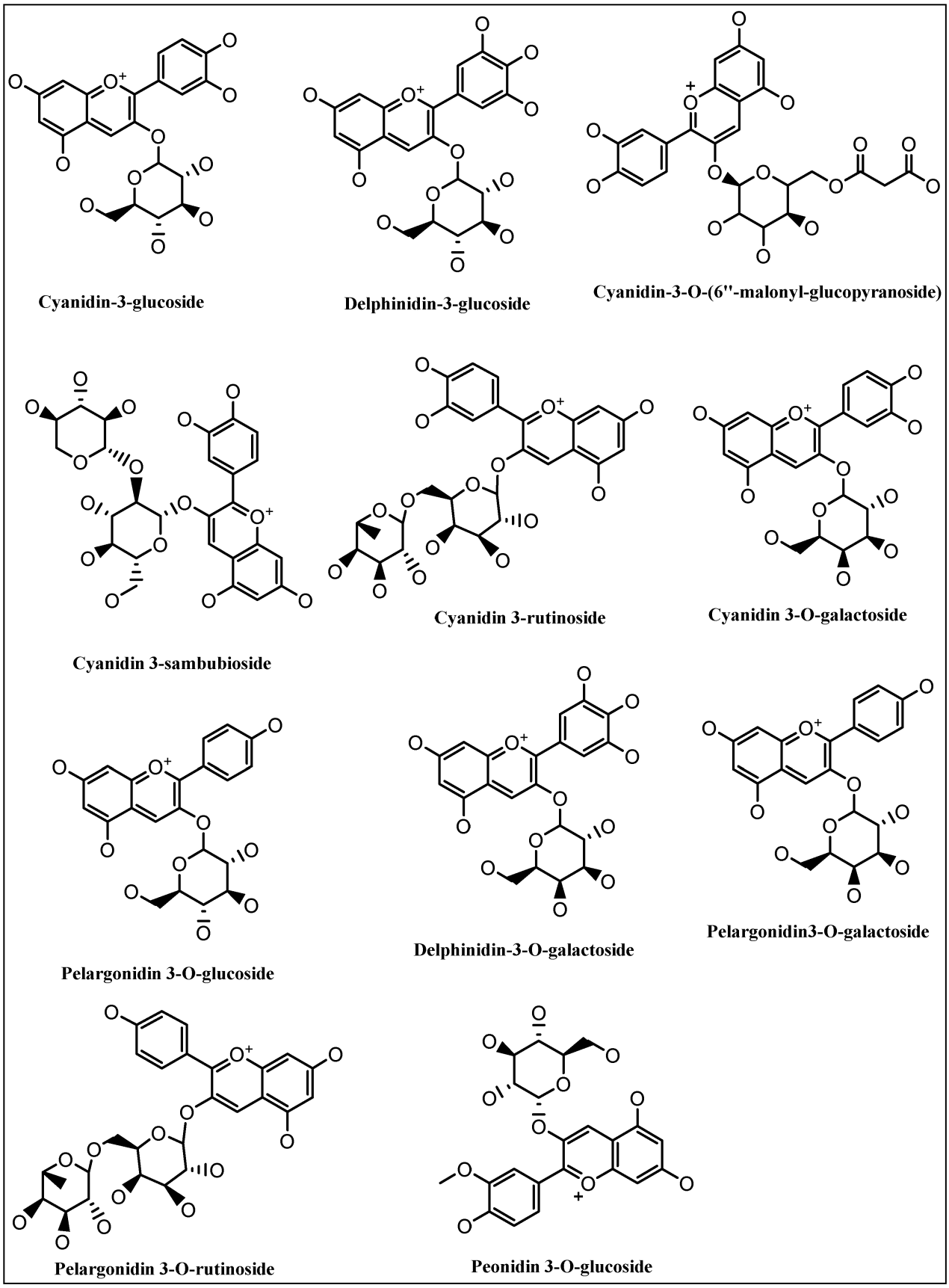

Figure 3. Common anthocyanins present in plants used in the treatment of liver disorders.

7. Anthocyanins' Metabolism in Liver

Following the anthocyanins consumption through oral route, the absorption is followed by the stomach, and the gastrointestinal tract (GIT), where the distal lower region absorbs the majority of the products and the metabolism of the product takes place. The anthocyanins undergo extensive microbial transformation and lead to phase II metabolism in humans. The microbial–human hybrid metabolites also passed through the GIT lumen, and increase the already lowered bioavailability, and its subsequent metabolic products presence in the systemic circulation [42]. These metabolites include phenolic acid, phenolic-conjugate products, hippuric acid, phenyl acetic acid, and phenyl propionic acid, as obtained from the major anthocyanin product, cyanidin-3-O-β-glucoside, from the anthocyanins mixtures. However, the delphinidin-3-O-

rutinoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside from blackcurrant are directly absorbed in their molecular form, and are excreted through urine as the intact glycosylated with other metabolites [69]. The anthocyanins outreach to the liver is followed through systemic supply, and according to the observations by several research groups [108][109][110][111], the anthocyanins could be considered as liver-protecting agents, with specific mechanism, and their high antioxidant potential. However, there is an important question that needs to be answered about the anthocyanins' safety and efficacy as well, in detail. This question includes the structure(s) and nature of the anthocyanins metabolites in liver, and what is their safety/toxicity status? As a part of the answer to this question, Curtis et al., conducted a randomized, placebo-controlled trial to evaluate the safety of chronic consumption of anthocyanins on the heart, liver, and kidney biomarkers in 52 healthy postmenopausal women volunteers [112]. The study established the safety of chronic consumption of anthocyanins-rich plants, as the liver, kidney, and heart's functions biomarkers were measured, and were found within the acceptable range after 12 weeks of chronic consumption of elderberry extract [112]. The study highlighted the safety of the dietary anthocyanins for post-menopausal women without any added cardio-protective benefits of the berry. Additionally, the anthocyanins accumulation, and degradations have been investigated in different animal-models; for instance, the accumulation of anthocyanins in pigs supplemented with blueberries for four weeks were investigated by Wilhelmina, et al. [113], and it was found that the anthocyanins were accumulated as an intact product in the liver, eyes, and brain tissues. The absorption and metabolism of the cyanidin-3-O-β-glucoside was investigated by Tsuda et al. in rats. The rats were subjected to hepatic ischemia-reperfusion as an oxidative stress model. The cyanidin-3-O-glucoside, and protocatechuic acid were detected in the plasma of the rats, however, the methylated form of the cyanidin-3-O-β-glucoside was also detected as metabolite of the cyanidin-3-O-β-glucoside in the liver, and kidneys [114]. Furthermore, the methylated and glucuronidated metabolites of the anthocyanins were also detected in the liver of rats fed with the blackberry extracts. The **Figure 1** depicts the major sites of anthocyanins absorption and metabolism which were mainly absorbed from the stomach and colon [115]. The absorbed anthocyanins reach the vital organs, i.e., liver, and kidneys, through systemic circulation, where their common metabolites, methylates and glucoronates are also found [115]. Part of the anthocyanins metabolism by the gut microbiota includes de-glycosylation (conversion of cyanidin-3-O-rutinoside into cyanidin-3-O-β-glucoside, and cyanidin aglycone), and the anthocyanin products degrade to small molecules, e.g., protocatechuic acid, gallic acid, syringic acid, and 3-O-methylgallic acid (**Figure 4**), which supposedly contribute to reported health benefits, and biological activities of the anthocyanin molecules [116][117][118][119]. Protocatechuic acid, the main metabolite of anthocyanins [120][121], exhibits antioxidant and anti-inflammatory activities, and has been demonstrated to provide liver-protecting effects in different models of liver injury [122][123][124][125].

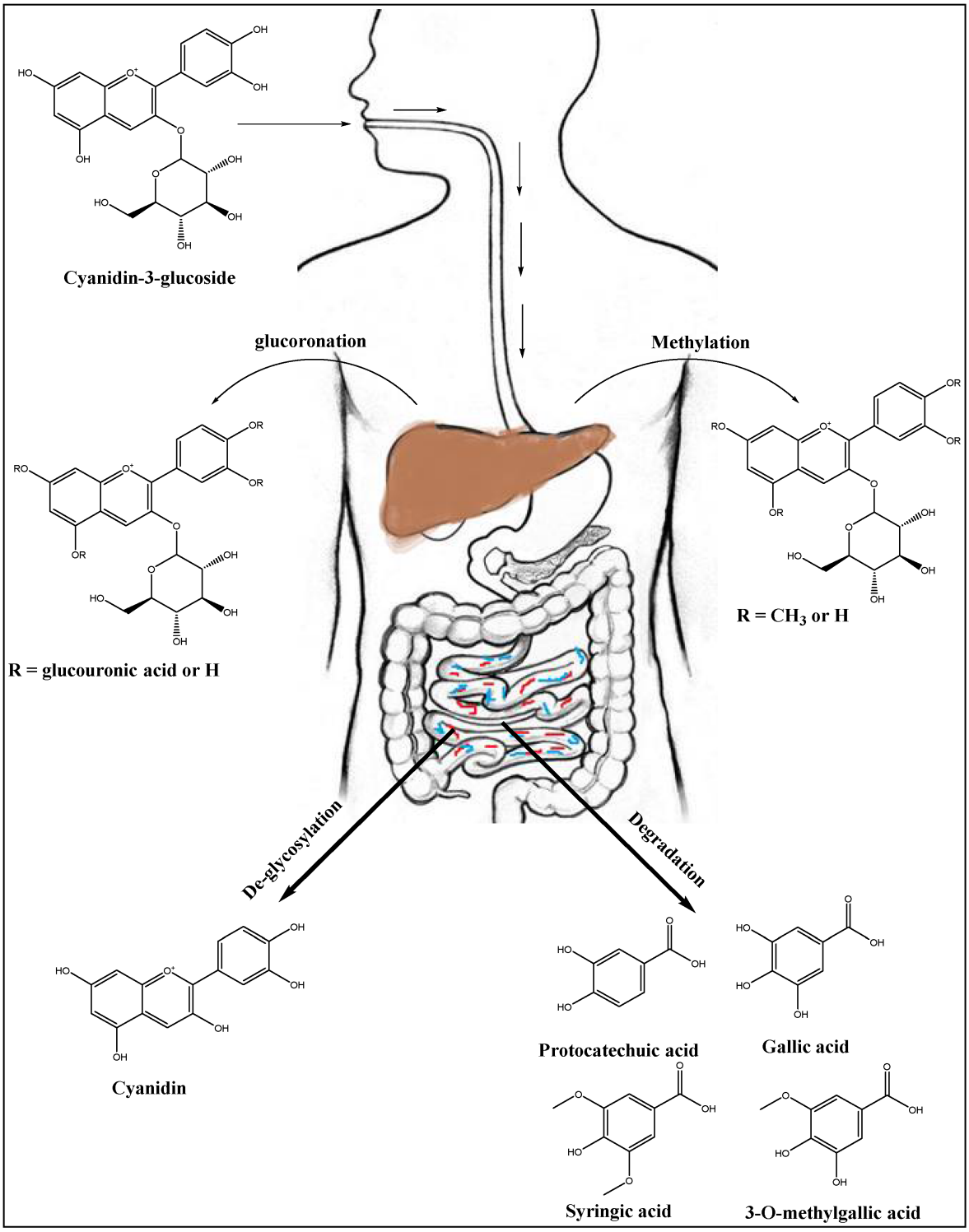

Figure 4. Proposed metabolic products of anthocyanins in humans.

8. Anthocyanins and Liver Disorders

The liver is one of the important organs of human body with capacity to regenerate. The liver is highly sensitive to xenobiotic entities, oxidative stress, and presence of toxins. It is also well-known that the liver regenerates itself, and resizes its portions after partial hepatectomy. The activation of hepatocyte proliferation, modification of the enlarged liver mass, and correction to the apoptosis process are also known [126][127]. The role of oxidative stress in restricting the liver cells regeneration is acknowledged [128][129], together with its contributions to induction, propagation, and catapult to liver diseases related complications plausible removal have been discussed [129][130][131][132]. The reactive oxygen and nitrogen species (ROS and RNS) produced as a part of normal metabolic functions are under limits to take part in physiological functions in the body, and are considered significant as primary elements in the inflammation responses in the innate immunity mechanism [129][133]. They also have physiological roles in processing of signal transduction, and normal process of ageing and cell death. The excessive production of these critical species, ROS and RNS, particularly associated with the mitochondrial dysfunctions, are also responsible for the endogenous production of ~90% ROS through the oxidative phosphorylation type of metabolic process [134]. The excessive production of ROS is associated with

initiation of lipid peroxidation, DNA damage, glycoxidation, and protein oxidations, of which all are linked to promoting of several degenerating diseases, and soft tissues injury [135].

Among the body's tissues, the liver is highly susceptible to aggressive injuries caused by the processes of oxidative stress [128], and the excessive production of ROS is linked to liver inflammation and fibrosis [128]. In addition, the oxidative stress is hallmark of chronic liver disease, regardless of the cause of the injury and the inducer [136]. In liver, the parenchymal cells, mitochondrion, and endoplasmic reticulum produce ROS which are primarily associated with the liver's fatty acid oxidation activity. The largest population of resident tissue macrophages in the liver, and Kupffer cells, are highly sensitive to oxidative inducers, which derives the initiation and development of hepatic inflammation, and consequently, the fibrosis [129]. Since the body's metabolic processes mainly occurr in the liver, and the liver cells are susceptibility to oxidative stress, there is a greater need for the presence of self-defensive mechanism in liver to scavenge ROS. The nuclear related factor 2 (Nrf2) works in the liver as a cellular redox status sensor, in which the higher levels of ROS-induced Nrf2 are released by sequestration, and translocate to the nucleus, wherein it promotes the transcription of cytoprotective antioxidant genes, as well as this activity promotes the liver cells regeneration which almost takes place through the activation of the antioxidant response element (ARE) [136]. The impairment of the Nrf2 defensive system of the liver is considered as the direct cause to increase the hepatocytes damages in response to the oxidative stress inducers, such as, toxins and high-fats diets, which are the reasons to elevate the mitochondrial production of ROS [136]. Therefore, certain agents that alleviate the reduction in Nrf2 protein levels are the promising therapeutic candidates for liver diseases treatment, and also for liver protection against oxidative stress, as well as oxidative stresses-led liver's lipid peroxidation [137] (**Figure 5**).

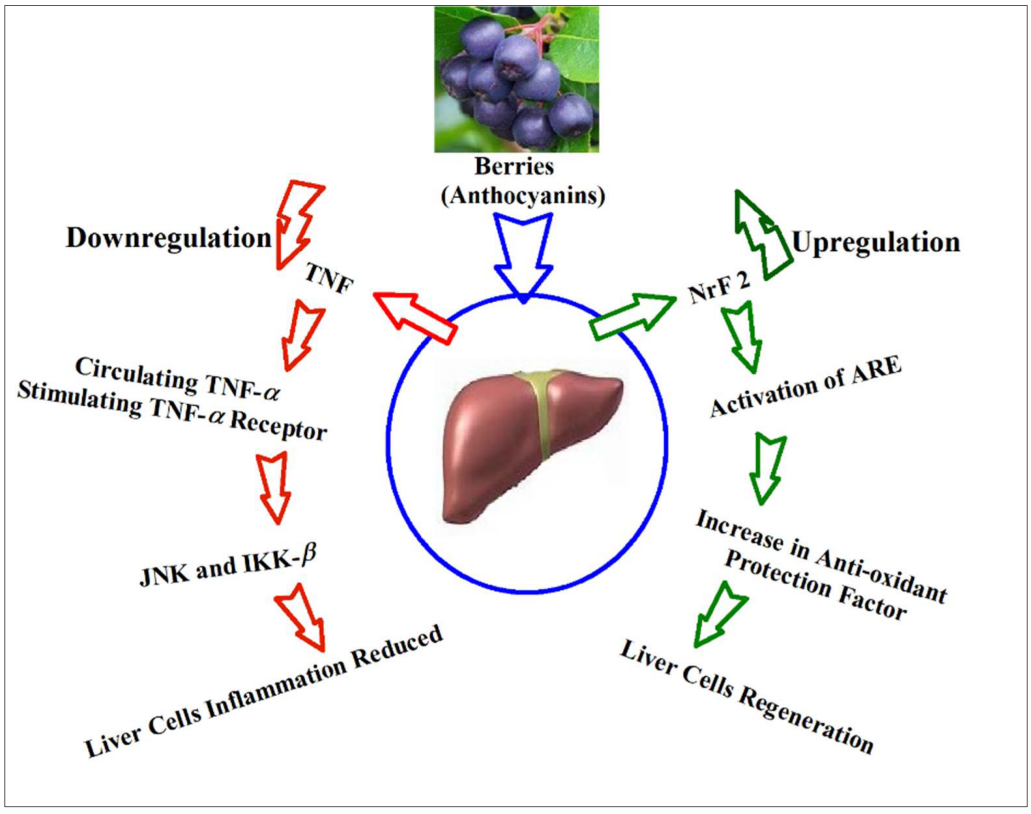

Figure 5. Diagrammatic representation of major hepatoprotective pathways of anthocyanins.

The pro-inflammatory cytokines group, TNF, have also been established for their role in the activation of liver diseases. Therefore, the TNF inhibitors are expected to be protective agents against liver injuries, as the increased levels of circulating TNF-α stimulates the TNF-α receptors located on cells surface, and leads to activation of the stress-related protein kinases, JNK and IKKβ. The activation of JNK and IKKβ upregulates the production of inflammatory cytokines leading to subsequent liver injury as the resultant action [138][139].

The plants-based liver prophylactic, and treatment therapies are well-known in the medicinal market, and are prescribed nowadays for the treatment of liver diseases, alone, or in combination, with other drugs [96]. Certain medicinal plants have also been consumed by different societies and traditional groups as remedies for liver complaints [140][141][142]. It is also reported that some vegetables, fruits, cereals, and flowers have ability to scavenge free radicals, and protect the liver cells from oxidative stress [143][144].

9. Anthocyanins' Suggestive Roles through Hepatic Biomarkers Regulation, and Biomechanistics Outlook

The anthocyanins support of the liver is still debated, and have been opined confirmed in some recent observations. The anthocyanins in general, and cyanidin-3-O-β-glucoside in particular, reduced the ALT and AST levels in serum, as well as malondialdehyde and protein contents levels in the liver homogenate of the experimental animals [145]. The reduced levels/activities of MCP-1, IL-1β, MIP-2, collagen III, and *α*-SMA were also obtained in the rodent liver fibrosis model. The cyanidin-3-O-β-glucoside also showed strong anti-atherogenic activity [146]. The cyanidin-3-O-glucoside and other anthocyanins enhanced the cell-based AMPK activity, and ACC phosphorylation together with the carnitine palmitoyltransferase-1 (CPT-1) expression, thereby leading to increased oxidation of the fatty acids in HepG2 cells [147] [148]. The attenuation of liver steatosis, and reduction of white adipose tissue messenger RNA levels of MCP-1, TNF-*α*, IL-6, and serum concentrations of TNF-*α*, IL-6, MCP-1, as well as reduction of macrophage infiltration in adipose tissue were also observed. The cyanidin-3-O-glucoside also exhibited fasting glucose levels reductions. The cyanidin-3-O-glucoside also lowered the oxidative stress through GSH (glutathione)-based antioxidant defense mechanism, and thereupon lowered the ROS production, and subsequently the hyperglycemia-induced hepatic oxidative damage. In addition to the regulation of the thermogenic and secretory functions of BAT (brown adipose tissue), it also lowered ROS production, and oxidative stress [149][150].

In context with the liver infections, steatosis is histological outcome of the chronic hepatitis C viral, and at times severe bacterial infections together with as an outcome of host's metabolic risk factors involving resistance to insulin, obesity, type 2 diabetes, and hyperlipidemia. The phenomenon tends to accumulate lipids in the intracellular spaces, and it is associated with liver fibrosis, and diminished response to antiviral therapy [151]. However, simple steatosis is benign, but a synergistic combination of cellular adaptation, and oxidative damage together with the steatosis, aggravates the injury in the liver, and may lead to chronic fibrosis and hepatic carcinoma. The heightened oxidative stress, augmented vulnerability to apoptosis, and uncontrolled cells activity have been implicated in steatosis severity [152].

Anthocyanins-rich plants have been used in folk-medicine as remedies for several diseases including protection and treatment of liver disorders. The plants organs' rich in anthocyanins as well as pure anthocyanin entities have also been extensively evaluated for their in vivo hepatoprotection effects against several hepatocyte oxidative stress inducers, e.g., carbon tetrachloride (CCl_4), ethanol, acetaminophen, thioacetamide (TAA), *tert*-butyl hydro-peroxide (t-BHP), and dimethyl-nitrosamine. Certain reports have also evaluated the liver protection effects of anthocyanins-rich plants extracts using in vitro cell line models, e.g., inducing oxidative stress in HepG2 cell lines (**Table 2** and **Table 3**).

Table 2. Anthocyanins modes of action as liver-protecting agents against induced liver injuries.

Anthocyanins	Experimental Protocol	Mode of Action	Refer
Cyanidin-3-O-β-glucoside	In vivo CCl_4-induced liver damage in mice and in vitro H_2O_2-induced oxidative stress in HepG2 cells apoptosis	Enhance the antioxidant enzymes activities and upregulating Nrf2-antioxidant pathway.	[153]
Delphinidin	In vitro H_2O_2-induced oxidative stress in HepG2 Cells	Enhance the expression of Nrf2 and promoted Nrf2 nuclear translocation. Increase expression of antioxidant protein HO-1 (Nrf2-related phase II enzyme heme oxygenase-1). Alleviate the reduction of Nrf2 protein levels and the accumulation of intracellular ROS levels in Nrf2 knockdown HepG2 cells.	[154]

Anthocyanins	Experimental Protocol	Mode of Action	Refer
Mixture of cyanidin-3-O-β-glucoside, delphinidin-3-O-rutinoside, and malvidin-3-O-galactoside	In vivo CCl_4-induced human embryonic-liver (L-02) cells toxicity	Reduce the percentage of hypo-diploid cells and decrease in caspase-3 protein expression	[155]
Cyanidin-3-O-β-glucoside, and peonidin-3-O-glucoside	In vitro human embryo non-malignant liver tissue cell line (L-02). Hepatoprotection	Exhibited higher cell viability, decreased aminotransferase activity and enhanced cellular antioxidant status. Furthermore, Cy-3-G showed much stronger hepatoprotective activity than Pn-3-G at the same concentration.	[110]

Nonetheless, the upregulation of Nrf2 protein and the down regulation of the pro-inflammatory cytokines, TNF-*α*, are two possible mechanisms adopted by the liver-protecting agents. Anthocyanins have shown potential activity in both the mechanisms.

The effects of anthocyanins as a liver-protecting agent against several types of experimentally induced liver injuries are summarized in **Table 2** and **Table 3**.

Table 3. Anthocyanins-rich fractions/extracts of plants as liver-protecting agents against experimentally induced liver injuries.

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Morus alba, and species	Mulberry anthocyanins	CCl_4 (carbon tetrachloride), in vivo model. Hepatoprotection	Cyanidin-3-O-β-glucoside	Decreased the ALT (alanine transaminase), AST (aspartate transaminase), hyaluronidase, hydroxyproline, and collagen type-III in the injured rats	[156]
Ipomoea batatas L.	Anthocyanins rich purple sweet potato extract	CCl_4, in vivo model. Hepatoprotection	Peonidin-3-caffeoyl-feruloyl sophoroside-5-glucosid, peonidin 3-caffeoyl-p-hydroxy benzoyl sophoroside-5-glucoside, peonidin 3-dicaffeoyl sophoroside-5-glucoside	Reduced the AST and ALT enzymes and MDA (malondialdehyde) level; Increased the SOD (superoxide dismutase), and GSH (glutathione) levels compared to the injured CCl_4 administered group of animals	[157]

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Oryza sativa	Anthocyanins rich black rice bran extract	CCl_4, in vivo model. Hepatoprotection	Cyanidin-3-O-β-glucoside, and peonidin-3-O-glucoside	Reduced aminotransferase activity in serum, enhanced SOD and glutathione peroxidase (GSH-Px) activities, thiobarbituric acid reactive substances (TBARS), and 8-hydroxy-20-deoxyguanosine levels significantly decreased as compared to the CCl_4 intoxicated group. Liver histopathology confirmed pathological gains by ARBE administration	[110]
Ipomoea batatas	Anthocyanins rich fraction of purple sweet potato extract	In vivo, ethanol, acetaminophen, and, CCl_4. Hepatoprotection, and treatment	3-O-(6-O-trans-caffeyt-2-O-~-glucopyranosyl/3-glucopyranoside)-5-O-glucosides of cyanidin, and peonidin	Treatments of mice with anthocyanins fraction in dose dependent manner, and reduced the CYP2E1-dependent aniline hydroxylation, and CYP2E1 protein levels. Antioxidant effects on hepatic GSH level, and GSH S-transferase activity were up-regulated in $FeCl_2$/ascorbate-induced lipid peroxidation in mouse liver homogenates, also showed superoxide radical scavenging activity.	[158] [159]
Hibiscus sabdariffa L.	Anthocyanin-rich extract	In vivo, thioacetamide (TAA)-induced hepatotoxicity. Hepatoprotection	Cyanidine, delphinidin derivatives, cyanidin-3,5-O-di-glucoside, cyanidin-3-O-sophoroside-5-glucoside	Reduced the serum levels of ALA, AST, and hepatic malondialdehyde, decreased hepatic inflammatory markers, including TNF-α, interleukin-6, and INF-γ, decreased the immuno-positivity of NF kappa-B, and CYP2E1 in liver tissues	[109]
		In vivo *tert*-BHP-induced cytotoxicity in rat			[100]
		CCl_4 in vivo model. Hepatoprotection			[160]

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Aronia melanocarpa	Fruit juice	CCl_4, N-nitroso diethyl amine, Paracetamol in vivo model. Hepatoprotection	Cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-xyloside and cyanidin-3-O-β-glucoside	Reduced necrotic changes in rat liver and inhibited increase of plasma AST and ALT activities, MDA formation induced by CCl_4. Increased liver GSH contents. Decreased the activities of enzymatic markers of cytochrome P450, CYP1A1 and 1A2.	[161] [162] [163]
Justicia spicigera	Ethyl acetate fraction	CCl_4 in vivo model. Hepatoprotection	Peonidin 3,5-O-di-glucoside, malvidin 3,5-O-di-glucoside, and petunidin 3,5-O-di-glucoside	Improvement in liver function indices and oxidative stress markers. Increased SOD and GSH, and decreased MDA.	[108]
Vaccinium sp.	Berry pomace extract	In vitro hepatic cell line HepG2 proliferation. Hepatic cells protection	Procyanidin dimers	Protects hepatic cells from oxidative damage.	[164]
Solanum tuberosum L.	Purple potato's anthocyanins rich extract	In vivo, alcoholic liver disease mouse model. Hepatoprotection	Petunidin-3-coumaroyl-rutinoside-5-glucoside, peonidin-3-coumaroyl-rutinoside-5-glucoside, petunidin-3-O-glucoside, petunidin-3-rutinoside-5-glucoside, pelphinidin-3-coumaroyl-rutinoside-5-glucoside	Higher levels of SOD and reduced GSH enzymes, reduction in formation of malondialdehyde, protected against alcohol-induced detrimental levels, maneuvered the activity of cytochrome P450 2E1 (CYP2E1)	[165]
Ipomoea batatas	Anthocyanin fraction	Dimethyl nitrosamine-induced liver injury in rats. Hepatoprotection	Cyanidin-3-O-β-glucoside chloride, malvidin-3-O-glucoside, pelargonidin-3-O-glucoside chloride, and peonidine-3-O-glucoside chloride	Induced Nrf2 mediated antioxidant enzymes, and reduced the COX-2, and iNOS expressions, reduced inflammation through NF-KB inhibition	[166]

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Hibiscus sabdariffa	Water extract, and anthocyanins	Paracetamol-induced hepatotoxicity in rats. Hepatoprotection	Anthocyanins	Increased GSH and SOD levels, decreased ALT and AST	[167]
Colocasia antiquorum	Ethanolic extract	Paracetamol, and CCl_4 toxicated rats. Hepatoprotection	Cyanidin-3-O-β-glucoside, pelargonidin-3-O-glucoside and cyanidin-3-O-rhamnoside	Decreased ALT, and AST levels	[168]
Vaccinium myrtillus and *Ribes nigrum*	Anthocyanins-rich extracts	Acetaminophen-induced hepatotoxicity in rats. Hepatoprotection	Glycosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin	Normalized activities of glutamate oxaloacetate and glutamate pyruvate transaminase, prevented APAP-induced plasmatic and tissue alterations in biomarkers of oxidative stress	[169]
Raphanus sativus L. (Red radish)	Anthocyanins fraction	CCl_4 in vivo model. Hepatoprotection	Pelargonidin derivatives	Reversed the alteration of biochemical parameters to normal	[103]

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Raphanus sativus L. var. *niger*	Fermented roots	In vivo model for the methionine, and choline-deficient, diet-induced non-alcoholic fatty liver in mice. Hepatoprotection	Pelargonidin derivatives	Decreased lipids in 3T3-L1 adipocytes by downregulating adipogenic transcription factors, sterol regulatory element-binding protein 1c, CCAAT/enhancer-binding protein α, peroxisome proliferator-activated receptor γ, and lipid accumulation-related genes adipocyte protein-2, as well as fatty acid synthase. Decreased ALT, AST, TG levels. Deceased expression of iNO synthase, suppression of the inactivation of macrophages, and Kupffer cells in liver. Inhibition of α-smooth muscle actin, transforming growth factor β-1, and collagen type-I α-1 chain leading to reduced liver fibrosis.	[170]
Raphanus sativus L. var. *niger*	Aqueous extract of roots	In vitro model in HepG2 cells. Hepatoprotection	Pelargonidin derivatives	Induced quinone reductase activity, and expression of multiple phase I, II detoxification enzymes in the HepG2 human hepatoma cell line	[171]
Malvaviscus arboreus Cav	Aerial parts extracts	CCl_4 in vivo model. Hepatoprotection	Cyanidin-3-sambubioside	EtOAc (ethyl acetate), and CH_2Cl_2 (Dichloromethane) extracts significantly reduced the liver injury in rats as indicated by the reduced levels of ALT, AST, ALP, TB, and MDA, comparatively the EtOAc fraction enhanced total antioxidant capacity of liver at the maximum.	[172]

Plant's Name	Used Extracts, and/or Pure Compounds	In Vivo/In Vitro Models and Bioactivity	Major Anthocyanins	Biomarkers, and Mode/Mechanism of Action	Refer
Cornus mas L.	Anthocyanins rich fraction	Lipid peroxidation, oxidative stress in the livers of cholesterol-fed rabbits	Delphinidin 3-O-galactoside, cyanidin-3-O-galactoside, Cyanidin-3-O-robinobioside, pelargonidin-3-O-galactoside, pelargonidin-3-O-robinobioside, cyanidin, and pelargonidin.	Decreased lipid peroxidation, decreased MDA levels, and reduced oxidative stress, an increase in liver GSH found.	[173]

10. Anthocyanins Roles in Hepatocellular Longevity, Hepatic Carcinoma and Liver Cancer

Anthocyanins have also been reputed with liver longevity. Kunming mice administered with D-galactose to accelerate ageing were intervened with anthocyanins administrations, and liver histology and functions were evaluated after eight weeks. Western blot analysis was used to assess the genes involved in DNA damage signaling pathways. Hepatic tissue injury, fibrosis were found reduced, while the liver functional biomarkers were found to be delayed in their levels' reductions. The anthocyanins administrations maintained the stability of the GSH redox system (GSH-PX, T-SOD and MDA), as found in the plasma and liver, together with the reduced levels of the inflammatory factors, i.e., IL-1, IL-6, and TNF-α were observed. Expression levels of the sensors (ATM, ATR), mediators (H2AX, γ-H2AX), and effectors (Chk1, Chk2, p53, p-p53) of the DNA-damage signaling pathways were found reduced [174].

Anthocyanins role in cancer prevention and cure has been much deliberated by the researchers. Anti-cancer effects of the anthocyanins have been suggested to be connected to a number of different biological activities mediation including the anti-inflammatory, antioxidant, anti-mutagenesis, inhibition of cells proliferation through modulating the signal transduction pathways, cell cycles arrest, inhibition to induction of cells differentiation, as well as apoptosis, and autophagy of the cancer cells. The reversal of drug resistance, increased sensitivity to chemotherapeutic agents, anti-invasion and anti-metastasis have also been suggested to be involved in ameliorating the cancerous situations. A data analysis of the basic findings, in vivo and in vitro, inferences from clinical trials, as well the herbalists and traditional healers practices based information was analyzed [175]. The anti-cancer effects of anthocyanins have also been reported by Longo et al. The anthocyanins-rich extracts obtained from the Mediterranean ever-green shrubs' berries from *Phillyrea latifolia* L., *Pistacia lentiscu* L., and *Rubia peregrina* L. were examined for their anticancer activity, and autophagy inhibition enhanced anthocyanin-induced apoptosis in hepatocellular carcinoma was observed to be working as the mechanistic aspect of the anti-cancer action. The autophagy was established through observation of up-regulation of the autophagy inducer, elF2α, and down-regulation of the autophagy inhibitors, i.e., mTOR and Bcl-2 which led to enhanced expressions of LC3-II. The autophagy was replaced with the apoptosis, also confirmed by the activation of Bax, cytochrome *C,* and caspase 3. The terminal deoxy nucleotide transferase mediated dUTP nick-end labeling–positive fragmented nuclei, and cancer cells with sub-G_1 DNA contents that were prevented by z-VAD, confirmed the notion. The autophagy inhibition either by 3-methyladenine, or Atg5 small interfering RNA, prompted the anthocyanin-led apoptosis. Hence, intervention of autophagy inhibitors in combination with anthocyanins and anthocyanins rich extract/products can be beneficial in controlling hepatic cancer [176].

On the mechanistic front, the cytochrome P450 family enzymes CYP1, CYP2, and CYP3 played major roles in metabolism of ~75% of all administered drugs of herbal and synthetic origins, together with other chemical entities reaching the liver. The NAFLD disorder represented a noticeable reduction in these vital enzymes. In an experiment dealing with microsomes isolated from human liver samples, the microsomal CYP1A2, CYP2D6, and CYP2E1 mRNA levels were found to be decreased with the NAFLD progression, while the CYP2A6, CYP2B6, and CYP2C9 mRNA expressions were found increased. The microsomal protein expression of CYP1A2, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 reduced with the progressing NAFLD. The enzymatic activity of CYP1A2 and CYP2C19 were increased with the progressing NAFLD, while the activity of CYP2A6, and CYP2C9 were found increased with NAFLD severity with different

drugs metabolism. Pro-inflammatory cytokines, TNF-alpha, and IL-1beta were observed along with the decreased P450 enzymatic activity. The increased enzymatic activity of the CYP2C9 during higher degrees of NAFLD progression related with the increased hypoxia-induced factor-1alpha expression in NAFLD's late stage [177]. The roles of CYP 450 enzymes is also more pronounced in the detoxification of the xenobiotic materials [178][179][180]. The oxidation of the heme-thiolate cysteine to a sulfenic acid (-SOH) and the heme-thiolate insensitive routes are the key step in the oxidative step involving the CYC 450 family of enzymes. This is a redox-regulated process [181]. Obesity, considered to be related to a decrease in CYP2C and CYP2E1 activities, is also regulated through the liver conditions [182]. Moreover, the CYP enzymes isoforms, with respect to genic polymorphisms, and drug metabolism have major roles in metabolism and cancer initiation. They can activate pro-carcinogens to ultimate carcinogens through exogenous substrates which constituted the majority of drugs, and other known chemical carcinogens through, primarily, in liver, but also in other organs. The clinically most relevant CYP2D6, CYP2A6, CYP2C19, CYP2C9, CYP1B1, and CYP1A2 enzymes have been in focus [183].

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