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REVIEW ON PHARMACOLOGICAL ACTIVITY AND PHARMACOKINETIC OF ANTHOCYANIN

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ABSTRACT

Anthocyanins are natural products that give color to plants. As natural plant pigments, anthocyanins also have a series of health-promoting benefits. Many researchers have proved that anthocyanins have therapeutic effects on diseases, such as urinary and immune systems. Additionally, a large number of studies have reported that anthocyanins have an anticancer effect through a wide range of antiinflammatory and antioxidant effects. The anti-disease impact and mechanism of anthocyanins are diverse, so they have high research value. This review summarizes the research progress of anthocyanins on the pharmacological agents of different diseases to provide references for subsequent research.

KEYWORDS: Anti Fungal, Anti Oxidant, anti-microbial, anti-cancer, anti-viral.

INTRODUCTION: Disease prevented by anthocyanins

ANTHOCYANIN CHEMISTRY[18,3]

Formula: C15H11O6N

IUPAC name: 2-phenyl-1λ^4-chromen-1-ylium

Molar mass: 471.28 g/mol

Density: 0.55 g/cm

Melting point: ≤ 25°C

Boiling point: Decompose at high temperature

Odour: Rose-like

Appearance: White

State: Semi-solid

pH: 4.5 – 6

Solubility: Slightly soluble in water

Highly soluble in organic solvent (Ethanol, Chloroform)

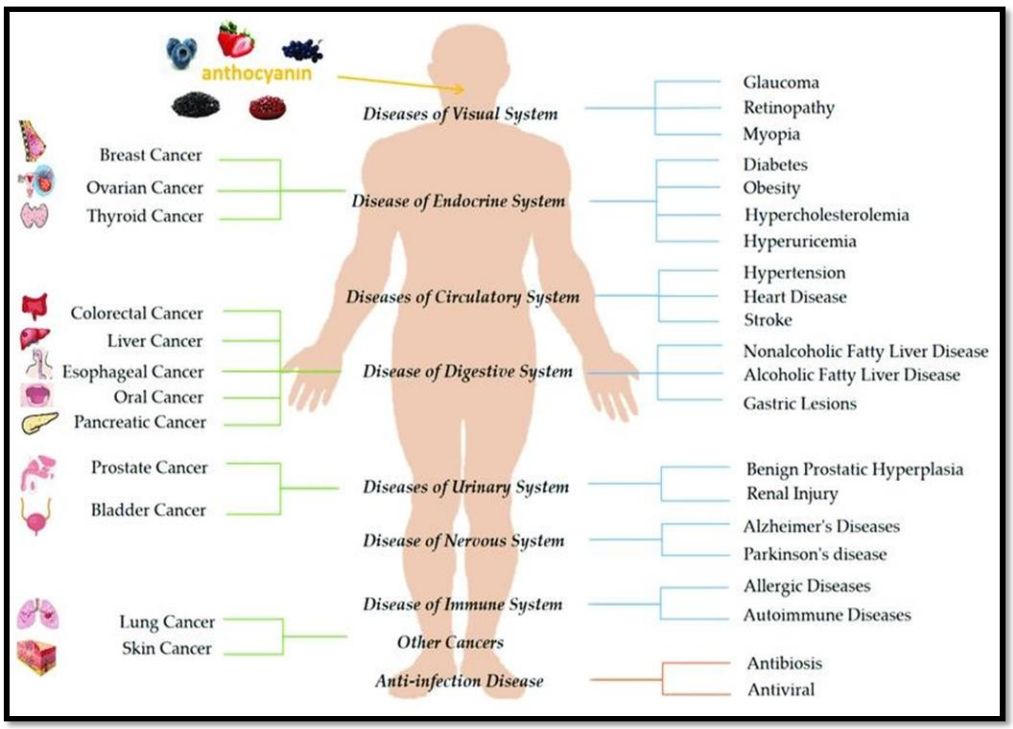
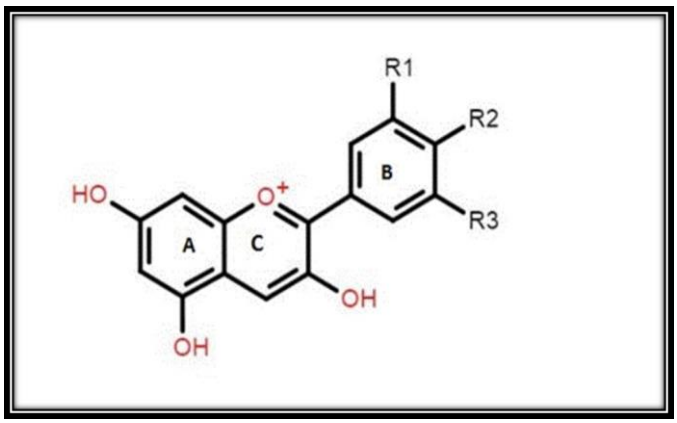


Figure 1: Disease prevented by Anthocyanin.[9]

STRUCTURE OF ANTHOCYANINS



Structure 1: 2- phenyl-1λ⁴-chromen-1-ylium []

BIOSYNTHESIS OF ANTHOCYANIN

The phenylpropanoid route or the combined action of TAL and 4CL, respectively, converts the general precursor phenylalanine or tyrosine obtained from the shikimate pathway to 4-coumaroyl-CoA. In order to create one molecule of naringenin chalcone, one molecule of 4-coumaroyl-CoA is condensed with three molecules of malonyl-CoA. This molecule is then transformed to naringenin by CHI. The main intermediate, naringenin, goes through multiple hydroxylation processes to produce different anthocyanidins. The anthocyanidin molecules are further glycosylated and decorated to produce anthocyanins.[6,3.5]

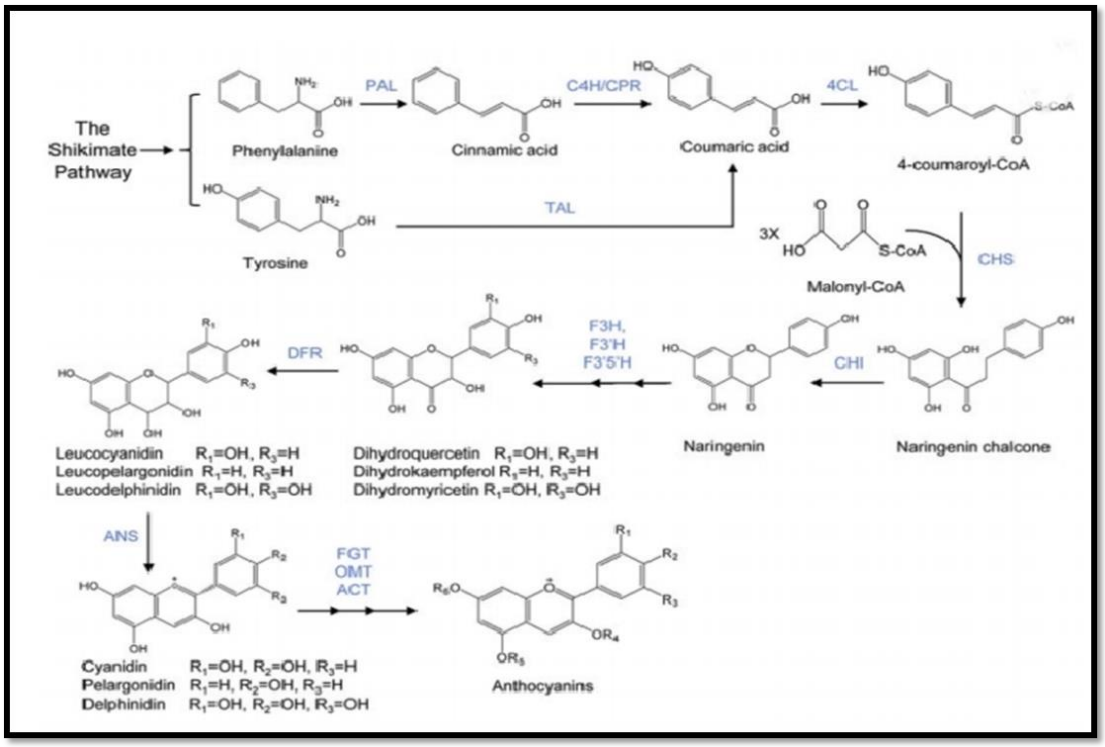


Figure 2: Schematic synthesis of Anthocyanin.[3]

ABSORPTION OF ANTHOCYANIN

Flavonoids are mostly present in foods as glycosides (Hollman & Katan, 1998a). Glycosylation influences their biological properties, having the greatest effect on partitioning coefficients. This property is important in determining mechanisms of absorption, i.e. whether a compound will passively diffuse across a biological membrane and how it might partition internally within various cell phases. Aglycones are primarily hydrophobic and can passively diffuse through biological membranes. The linkage with sugars increases their water solubility and limits passive diffusion. Diffusion of a hydrophilic flavonoid glycoside across biological membranes is not likely to occur. Therefore, the absorption of an

anthocyanin glycoside probably requires either a specific active transport mechanism or hydrolysis of the b-glycoside before absorption.

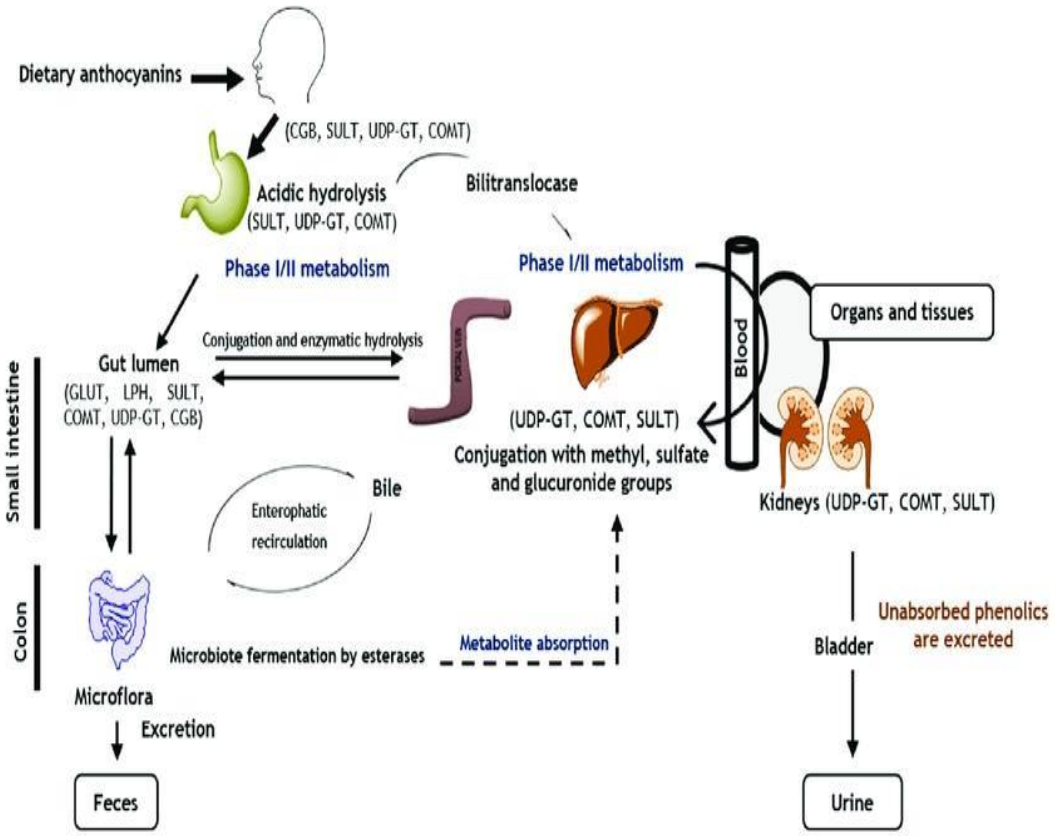


Figure 3: Schematic Absorption of Anthocyanin.[3]

Human studies within the last decade have established that the absorption of most flavonoids occurs in the small intestine however, the exact mechanisms involved in flavonoid absorption are a matter of much debate. It is speculated that the absorption of nonglycosylated flavonoids occurs in the small intestine via passive diffusion while it has yet to be definitively established how flavonoid glycosides enter the enterocyte. The question is whether flavonoid glycosides enter the enterocyte as intact structures and are cleaved before passage across the basolateral membrane or if they are cleaved at the cell interface and move passively across the luminal membrane The two proposed mechanisms involved in the transport of flavonoid glycosides are: either the transport of the intact glucoside by a sodium – glucose co-transporter, or the extracellular hydrolysis of the glycoside via lactate phlorizin hydrolase at the brush border, followed by passive diffusion of the aglycone.[17,19]

In addition, a fraction of flavonoids will escape absorption in the upper small intestine and undergo bacterial metabolism in the lower intestine where the compounds will be

deglycosylated and the aglycones will be subjected to transport or further metabolism. The mechanisms involved in the absorption and metabolism of anthocyanins are probably similar to those of other hydrophilic flavonoid glycosides, and may occur by one or both of the above suggested routes (Fig. 3). First, anthocyanin glycosides may be hydrolysed at the mucosal brush-border membrane by lactate phlorizin hydrolas. Once at the brush border, the aglycone may diffuse into the enterocyte where it could either enter the portal circulation as an aglycone, or be conjugated before transference across the basolateral membrane into the serosal fluid ,Currently there is little direct evidence of anthocyanin aglycones existing in the circulation or urine of humans Also, if anthocyanin glycosides are hydrolysed at the mucosal brush-border membrane by lactate phlorizin hydrolase before absorption and conjugation with glucuronic acid, one would not expect to find parent anthocyanin glycosides in biological fluids. Therefore a second pathway must exist, as many investigators have identified anthocyanin glycosides in human blood and urine The second possible route of absorption is likely to occur via transport of the intact anthocyanin glycoside into the enterocyte, possibly by sodium – glucose co-transporter, as suggested for other flavonoids Supplementation of anthocyanins with various sugars has been observed to reduce the excretion of anthocyanins, suggesting the potential role of sodium – glucose cotransporter in the transport of anthocyanin glycosides. Once inside the cell, the intact glycoside could either directly cross the basolateral membrane into the portal circulation, or be hydrolysed by cytosolic b-glucosidasen before intestinal metabolism and transport. Saturation of the above detailed pathways could account for the large variation in findings presented in the literature. The theory of a saturatable transport mechanism involving an Nadependent transporter has been previously proposed for the absorption of quercetin glycosides. Even though evidence presented by Ne`meth et al. suggests that cyanidin and delphinidin glucosides are not substrates for lactate phlorizin hydrolase and cytosolic b-glucosidase, the fact remains that cyanidin glycosides are absorbed and appear as parent glycosides as well as glucuronide derivatives in the circulation, suggesting both transport of intact compound and hydrolysis before transport are possible. As both anthocyanin glycosides and glucuronide derivatives have been identified in human plasma and serum the current evidence suggests that both pathways are involved in the absorption of anthocyanins. It is clear that much further research is needed to resolve the mechanisms of anthocyanin absorption. The stomach has also been proposed as a site of absorption of anthocyanins however, direct evidence of this occurring in humans has not been presented and requires further investigation.[19]

METABOLISM OF ANTHOCYANIN

Many flavonoids are extensively metabolised in humans with less than 5– 10 % of the ingested parent (intact) compounds excreted in the urine. It has been reported that as much as 52 % of an oral dose of radiolabelled quercetin was exhaled as 14CO2 in humans, indicating that significant absorption and extensive metabolism had occurred. Even though many flavonoids are reported to have low bioavailabilities as a result of their extensive metabolism, their metabolites may persist in the circulation for long periods of time and consequently have significant bioactivity This was observed for quercetin, as its glucuronidated metabolites retained much of their antioxidant capacity. This may also be the case for anthocyanins, as metabolites identified by our group were reported to retain their basic anthocyanidin structures, therefore probably preserving much of their bioactivities. In order to form a working hypothesis of the metabolic fate(s) that anthocyanins may have in the body, it is necessary to first review the basic processes associated with flavonoid and polyphenol metabolism. Research conducted within the last decade suggests that the majority of flavonoids are found in the circulation and urine as methylated, sulfated, glucuronidated and glycated conjugates with only 0·1 –1·5 % of ingested dietary quercetin reported to be excreted unmetabolised Glucuronide conjugation is regarded as the major conjugation reaction involved in flavonoid metabolism.[17]

There are two main reasons for the widespread utilisation of the glucuronidation pathway: first, glucuronic acid is derived directly from glucose, and its store, glycogen, and is therefore readily available; second, glucuronic acid has the capacity to be conjugated with a wide range of compounds. O-glucuronides (linkage through an oxygen atom) are the most common form of glucuronide conjugation, making the highly hydroxylated flavonoids prime targets for glucuronidation. The glucuronidation reaction is catalysed by UDPglucuronosyltranferases which is found in high concentrations in the liver, intestine and kidneys. Of all the tissues, the liver has the greatest capacity for glucuronidation, although more and more evidence points toward the intestine as being the initial and principal site for flavonoid glucuronidation in humans following typical dietary consumption). Methylation appears to be the second most significant conjugation reaction involving flavonoids. Methylation is driven by a group of enzymes referred to as methyltransferases. These non-specific enzymes are found in many tissues including the liver and intestine. The most common methylation reaction associated with flavonoid metabolism is O-methylation. Omethylation is catalysed by catechol-O-methyltransferase utilising S-adenosy methionine as a cofactor. The liver has the highest

catecholO-methyltransferase activity and is the main organ responsible for methylation. Thehydroxylation pattern of a flavonoid's ring structure will determine the primary site of methylation. Studies reveal that quercetin is extensively methylated following low oral doses in humans and in animals. Sulfation or glycination are also common conjugation reactions which predominate when low doses of phenolic drugs are administered. Sulfation reactions are catalysed by sulfotransferases, which are a small group of cytosolic enzymes widely distributed throughout the body. They utilise phosphoadenosine-50- phosphosulfate as a cofactor and their known substrates include phenols and polyphenols (i.e. flavonoids), iodothyronines, 4-nitrophenol, and hydroxyarylamines. Additionally, sulfation, as a conjugation reaction, is relatively costly in ATP and sulfate and is more likely to be rapidly limited by aglycone loading than is glucuronidation. Therefore, sulfate conjugation is regarded as a highly saturatable pathway, making it difficult to identify flavonoid sulfides as a result of their relatively low concentrations in the blood and urine. The body of knowledge regarding the subject of anthocyanin metabolism is much less extensive than that of many other flavonoids, with the metabolic consequence(s) of anthocyanins still a matter of much debate potential pathways in the body). The presence of unmetabolised (parent) anthocyanin glycosides in human blood and urine has been extensively documented However, even though many investigators suggest that anthocyanins are not metabolised before release into the systemic circulation, recent evidence indicates otherwise. The detection of glucuronide, methyl and sulfoconjugates has recently been documented, with investigations reporting.[6,8]

Between 68 % and 80 % of anthocyanins in the urine as metabolised derivatives. Studies identifying anthocyanins exclusively as unmetabolised parent compounds probably result from either saturation of metabolic pathways following megadose interventions, insufficient extraction procedures, and misidentification as a result of insufficient detection methods (i.e. using UV-visible HPLC exclusively for identification).[1,3]

ELIMINATION OF ANTHOCYANINS: The route of elimination of anthocyanins and flavonoids. will ultimately depend on the type of conjugates produced and their site of production. Glucuronides formed in the intestine tend to enter the systemic circulation directly and are not readily available for biliary excretion unlike those newly formed in the liver, which are predominantly excreted into the bile. This implies that glucuronidation in the intestinal cells facilitates the loss of flavonoids from enterohepatic circulation (EHC) towards the systemic circulation. Compounds undergoing EHC may also undergo further sulfation in

the intestine or hepatocyte, which would divert them from biliary excretion toward eventual urinary elimination, as enteral reabsorption of strongly polar compounds is negligible. EHC prolongs the residence of a chemical or xenobiotic in the body by reducing its faecal excretion. If a compound undergoing EHC is also available to the systemic circulation, this will result in a longer plasma elimination half-life (t1/2;. EHC is likely to be a major factor in the metabolism of many flavonoids and is responsible for their low but consistent levels in the circulation long after their time to reach maximum concentration (tmax) is achieved (for example, quercetin tmax 1– 2 h; t1/2 11– 28 h). Although it appears that various forms of quercetin may undergo EHC, current evidence suggests that this is probably not a major route for the elimination for highly polar anthocyanin glycosides as their t1/2 is reported to be relatively short (tmax 1– 4 h; t1/2 1 – 4 h) (Table 1). This, however, is only speculatory as the biliary excretion of anthocyanins in humans is currently unexplored. The urinary excretion of most flavonoids is relatively low (for example, quercetin about 2·5 %) with the exception of the isoflavonoids (for example, genistein and daidzein about 5– 50 %) Similarly the urinary excretion of anthocyanins is generally reported between 0·01 and 3 % of the ingested dose. In addition, the urinary elimination of colonic metabolites of anthocyanins is largely unknown and may be significant. Colonic bacteria are likely to play a major role in the metabolism of anthocyanins. Many flavonoids are extensively metabolised by colonic bacteria, leaving a range of phenolic acids which may be reabsorbed Colonic bacteria cleave glycosides from their parent compounds for catabolism, leaving the resulting aglycones for reabsorption through the intestinal wall or further degradation. In a study conducted by utilising radiolabelled catechin, phenolic acid metabolites were found to be reabsorbed and excreted in the urine of rats post-bacterial degradation. Conversely, in a study by utilising colonic bacteria isolated from human faeces, quercetin-3-glucoside was observed to be degraded to quercetin; however, the majority of quercetin aglycone was left intact. The extent to which flavonoids are eliminated in the. Faeces of humans is undetermined for many flavonoids including anthocyanins. reporte a significant increase in plasma concentrations of protocatechuic acid following the administration of cyanidin glycosides to rats. Theresearchers suggested that the protocatechuic acid may be derived from the breakdown of anthocyanins. The concentration of protocatechuic acid in the plasma was reported to be eight-fold higher than the parent anthocyanins. Additionally a recent in vitro fermentation study has confirmed that protocatechuic acid is a major metabolic byproduct of anthocyanins by human faecal bacteria Radiolabelling studies are required to establish the extent to which this occurs in humans in vivo. If anthocyanins are readily absorbed and less than 2 % are

generally identified in the urine, it is possible that a significant proportion of anthocyanins exist as colonic metabolites in the circulation. Additionally, if a large conversion of anthocyanins to phenolic acids and phenolic acid residues does occur, these compounds would not be easily identified in biological fluids using traditional HPLC techniques and would probably go undetected. Varying proportions of flavonoids present in food will also pass through the gastrointestinal tract unabsorbed and be eliminated in the faeces. Studies feeding oral doses of radiolabelled quercetin and catechin to rats reported 30– 32 % of the radiolabelled compounds to be excreted in the faeces unmetabolised. Empirical evidence for the excretion of anthocyanins in human faeces is currently not available. The lung is responsible for the elimination of volatile substances from the body and is a major site of excretion for many xenobiotics., 44 % of the radioactivity of 14C-labelled quercetin was detected in the gastrointestinal tract of rats and 11 % was detected in the lungs. More recently, it has been reported that as much as 52 % of an oral dose of radiolabelled quercetin in humans was exhaled as 14CO2. Unfortunately, the relative respiratory excretion of many flavonoids, including anthocyanins, is still unknown.[8,19]

PHARMACOLOGICAL ACTIVITY OF ANTHOCYANIN

ANTI-FUNGAL ACTIVITY

The different samples were screened for antifungal activity by agar well diffusion method. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of nine different compounds were introduced serially after successful completion of one compound analysis. Incubation period of 24-48 hours at 28□C was maintained for observation of antifungal activity of compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

ANTI-HYPERTENSIVE ACTIVITY

Anthocyanins and flavonoids may prevent hypertension. Anthocyanin-rich berries and red grapes/wine significantly reduce blood pressure, particularly in elderly individuals over 50 years old Observations on tens of thousands of women and men for 14 years indicated that

people who consume more anthocyanins had a lower risk of hypertension An increased risk of cardiovascular disease is linked to endothelial dysfunction. Endothelium-derived nitric oxide (NO) deficiency is closely associated with hypertension Black jamun extract (BjE) contains high anthocyanins concentrations and increases NO synthesis via endothelial nitric oxide synthase, which are critical regulators of cardiovascular disease Anthocyanin-enriched extracts of Odenton strictum flowers can block the contraction of aortic rings because anthocyanin (400 µg/mL) inhibits the effects of CaCl2 and a thromboxane A2 analog agonist (U46619) in physiological salt solution. Blood pressure was significantly reduced after consumption of 300 mL of anthocyanin-rich cherry in older adults. the content, and bioavailability of anthocyanins and individual differences in anthocyanin absorption and metabolism are significant factors conducive to the beneficial effect of anthocyanins on blood pressure.[7,22,23]

ANTI-DIBETIC ACTIVITY

DM is a serious chronic hereditary endocrine system disease characterized by high blood glucose concentrations. Mulberry anthocyanin extract can alleviate pathological changes in diabetic mice by activating the PI3K/AKT pathway and reducing insulin resistance in HepG2 cells Black jamun seed extract whichcontains cyanidin-3-glycoside and proanthocyanidins, improved insulin sensitivity and reduced blood sugar levels in type 2 DM mice. BjSE may regulate GLUT4 and gluconeogenesis in skeletal muscle by activating AMPK. BCE is rich in anthocyanins, including delphinidin 3-rutinin (D3R), and may help reduce DM medications and prevent diabetes. This is consistent with the fact that BCE stimulates glucagon-like peptide-1 (GLP-1) expression and induces insulin secretion to significantly improve glucose tolerance Differentiation of fat cells into smaller insulin-sensitive fat cells is also an important strategy for the treatment of diabetes. BJSE and its active ingredient C3G reduced 3T3-L1 preadipocyte differentiation, activated skeletal muscle metabolism, and exerted antidiabetic effects in db/db mice. Therefore, anthocyanin-rich extracts from plants have noticeable benefits for the treatment of diabetes.[2,3]

ANTI-MICROBIAL ACTIVITY

Anthocyanins can be used as alternative antimicrobial agents. Blueberry anthocyanins interfere with Staphylococcus aureus and Escherichia coli growth, inhibit the formation of biofilms, and hinder bacterial adhesion without reducing bacterial growth, which is the mechanism by which anthocyanins prevent thedevelopment of drug resistance and infection.

Anthocyanins extracted from jamun seeds and an antibiotic for urinary tract infections synergistically and significantly inhibited the formation of monoculture biofilms in 11 tested strains. Anthocyanidins from black mulberries (M. nigra) exert strong analgesic and antimicrobial effects against S. aureus, Pseudomonas aeruginosa and E. coli by inhibiting the expression of proinflammatory cytokine-, iNOS and NF-κB pathway-related proteins. Anthocyanins from Syzygium cumini can be used as novel agents for sensing regulatory phenotypes based on a reduction in violacein production, biofilm formation and EPS production of Klebsiella pneumoniae in a concentrationdependent manner. Anthocyanin extracts from bilberry (V. myrtillus) and blueberry (Vaccinium corymbosum)have antimicrobial properties involving.[11,13,14]

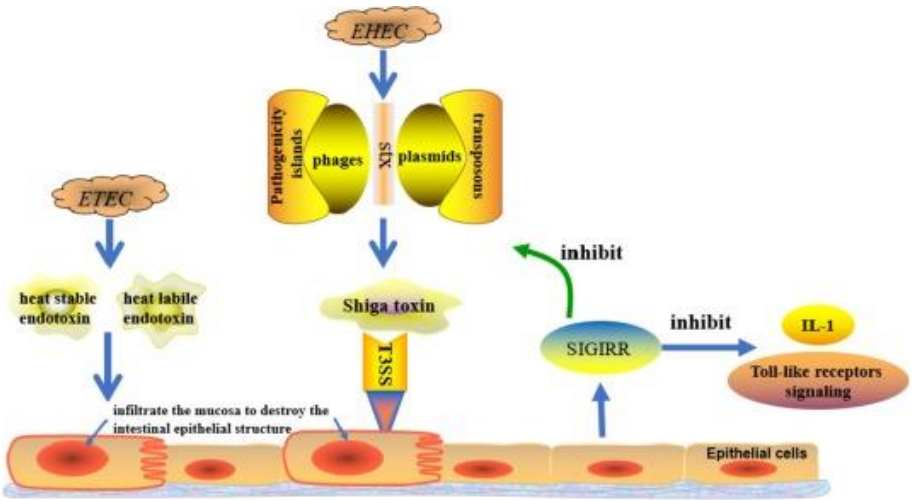


Figure 4: Antimicrobial activity of Anthocyanin.[9]

ANTI- OXIDANT ACTIVITY Anthocyanin extract from black flour can inhibit the growthof Candida albicans, P. aeruginosa, E. coli, and S. aureus. Aichinger et al., studied the effect of altertoxin II on the cytotoxic effects of Dp on HT-29 colon cancer cells and showed that the concentration of mycotoxin altertoxin II is reduced in the presence of anthocyanins and that anthocyanins can protect the gut tract fromgenotoxicity induced by altertoxin II Therefore, the natural antimicrobial properties of anthocyanins expand their application prospects in the pharmaceutical and food industries.[9,10]

ANTI-CANCER ACTIVITY

1. Pancreatic Cancer: Pancreatic cancer is an aggressive type of cancer characterized by metastasis, which involves cell adhesion, invasion, migration and the expression and secretion of several extracellular matrix-degrading proteolytic proteases. Kuntz et al.,

reported that anthocyanins and their metabolites isolated from the plasma of healthy subjects who ate anthocyanin-rich fruits reduce pancreatic cancer cell migration in vitro, as determined by cell phenotypes.[20]

2. Oral Cancer: Anthocyanins, from a species of black rice can suppress the in vitro migration and invasion of human oral cancer CAL 27 by reducing MMP-2, MMP-9, and NF-kB p65 expression through the suppression of the PI3K/Akt pathway and inhibition of NF-kB expression. The blueberry anthocyanins can inhibit the proliferation of oral cancer KB cells in a dose-dependent manner by inducing G2/M cell cycle arrest and apoptosis, and downregulating the methylation of p53. Yue et al., found that anthocyanins promote the death of oral squamous cell carcinoma cells by activating.[20]

3. Breast Cancer

Anthocyanin extract is a potential adjuvant therapy for breast cancer. Anthocyanins from grape skin can markedly increase intracellular ROS levels and apoptosis of MCF-7 breast cancer cells and arrest cells in the G2/M phase. In addition, Alba strawberry anthocyanin extract can induce apoptosis and death of breast cancer cells by exerting antioxidant activity and downregulating AMPK expression, which plays a role in resisting breast cancer. Eugenia jambolana fruit extract, which contains 3.5% anthocyanins, exhibits proapoptotic effects against breast cancer cells but not against normal breast cells .The anthocyanin cya-3-O-sam, extracted from the fruit of Acanthopanax sessiliflorus, inhibits metastasis of breast cancer cells by suppressing neovascularization and the gelatinolytic activity of MMP- 9. Additionally, black rice anthocyanins inhibit the metastasis of breast cancer cells by targeting the Ras/Raf/MAPK pathway.[21,27,28]

ANTI-VIRAL ACTIVITY

The chemical structure of anthocyanins plays a crucial role in their ability to inhibit viral activity. Hayashi et al., found that Pg-type anthocyanins isolated from redfleshed potato can inactivate influenza viruses A and B. Kannan et al., demonstrated that viruses are susceptible to natural cyanidin-3-sabubiocide and that cyanidin-3-sabubiocide can treat H1N1 subtype influenza virus. Anthocyanins from elderberry fruit have potential as antiviral drugs for SARS CoV-2 by preventing reproduction via budding from the host cell of the virus. Some anthocyaninrelated substances in small red beans (Vigna angularis) can affect the early stage of rabies virus infection and the infectivity of the rabies virus. In addition, oligomeric

proanthocyanidins from Crataegus sinaica have apparent inhibitory effects on herpes simplex virus type.[24,25,26]

CONCLUSION

Anthocyanins are natural flavonoids that can alleviate a variety of systemic diseases and cancers and have antiviral and bacterial properties. Anthocyanins have pharmacological potential for diseases of the circulatory, endocrine, digestive, urinary, sensory, nervous and immune systems. Studies have shown that anthocyanins can alleviate circulatory system diseases, mainly by increasing NO synthesis and inhibiting the jnk-p53 signaling pathway and antioxidative stress. To treat endocrine system diseases, anthocyanins can ameliorate insulin sensitivity, activate the PI3K/Akt pathway and reduce insulin resistance in HepG2 cells. Adipocyte differentiation is inhibited, the AMPK signaling pathway is activated, urate reabsorption is decreased and urate excretion is increased. Anthocyanins can also be used to treat diseases of the digestive and urinary system by acting as anti-inflammatory and antioxidant agents to activate the AMPK pathway and promote apoptosis by exerting anti-lipid peroxidation effects. Anthocyanins can alleviate visual diseases, mainly by affecting blood circulation and exerting antioxidative effects and can combat immune system diseases by reducing eosinophil infiltration and Th2 and Th17 cell development.

REFERENCES

1. Millar, C.L.; Duclos, Q.; Blesso, C.N. Effects of dietary flavonoids on reverse cholesterol transport, hdl metabolism, and hdl function. Adv. Nutr, 2017; 8: 226–239.[CrossRef]
2. Kurimoto, Y.; Shibayama, Y.; Inoue, S.; Soga, M.; Takikawa, M.; Ito, C.; Nanba, F.; Yoshida, T.; Yamashita, Y.; Ashida, H.; et al. Black soybean seed coat extract ameliorates hyperglycemia and insulin sensitivity via the activation of Amp activatedprotein kinase in diabetic mice. J. Agric. Food Chem, 2013; 61: 5558–5564. [CrossRef] https://en.m.wikipedia.org/wiki(pharmacy
3. Bueno JM, Saez-Plaza P, Ramos-Escudero F, Jimenez AM, Fett R, Asuero AG Analysis and antioxidant capacity of anthocyanin pigments. Part II: chemical structure, colour, and intake of anthocyanins, 2012.
4. Winkel-Shirley, B. Flavonoid Biosynthesis. A Colourful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. Plant Physiology, 2001.
5. Holton, T. A., & Cornish, E. C. Genetics and Biochemistry of Anthocyanin Biosynthesis. The Plant Cell, Jousilahti, P.; Vartiainen, E.; Tuomilehto, J.; Puska, P. Sex, age,

cardiovascular risk factors, and coronary heart disease: A prospective follow-up study of 14,786 middle-aged men and women in Finland. Circulation, 1999; 99: 1165–1172.

6. Manolescu, B.N.; Oprea, E.; Mititelu, M.; Ruta, L.L.; Farcasanu, I.C. Dietary anthocyanins and stroke: A review of pharmaco kinetic and pharmacodynamic studies. Nutrients, 2019; 11: 1479. [CrossRef] [PubMed] Google scoller. Com
7. Bueno JM, Saez-Plaza P, Ramos-Escudero F, Jimenez AM, Fett R, Asuero AG Analysis and antioxidant capacity of anthocyanin pigments. Part II: chemical structure, colour, and intake of anthocyanins Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of Syzygium cumini (L.) (Myrtaceae) leaves extracts. Int J Pharm Tech Res, 2010; 2(2): 1569–1573.
8. Nemes, A.; Homoki, J.R.; Kiss, R.; Hegedus, C.; Kovacs, D.; Peitl, B.; Gal, F.; Stündl, study on antibacterial activity, 2011.
9. L.; Szilvássy, Z.; Remenyik, J. Effect of anthocyanin-rich tart cherry extract on inflammatory mediators and adipokines involved in type 2 diabetes in a high fat diet induced obesity mouse model. Nutrients, 2019; 11: 1966.
10. Bag, A., Bhattacharyya, S.K., Pal, N.K., Chattopadhyay, R.R. In vitro antibacterial potential of Eugenia jambolana seed extracts against multidrugresistant human bacterial pathogens. Microbiological Research, 2012.
11. Andersen, Ø.M.; Jordheim, M. The anthocyanins. In: Flavonoids – Chemistry, Biochemistry and Application, Andersen ØM.; Markham, K.R., Eds.; Taylor & Francis, Boca Raton, London and New York, 2006; 471-551.
12. Giusti, M.M.; Wrolstad, R.E. Acylated anthocyanins from edible sources and their applications in food systems. Biochem Eng J., 2003.
13. Ichiyanagi, T; Shida, Y.; Rahman, M.M.; Hatano, Y.; Konishi T. Bioavailability and tissue distribution of anthocyanins in bilberry (Vaccinium myrtillus L.) extract in rats. J Agric Food Chem., 2006; 54: 6578-6587.
14. Stoner, G.D.; Sardo, C.; Apseloff, G.; Mullet, D.;Wargo,W.; Pound, V.; Singh, A.; Sanders, J.; Aziz, R; Casto, B.; Sun, X. Pharmacokinetics of anthocyanins and ellagic acid in healthy volunteers fed freeze-dried black raspberries for 7 days. J. Clin. Pharmacol, 2005; 45: 1153-1164.
15. Morazzoni, P. Vaccinium myrtillus anthocyanosides pharmacokinetic in rats. Arzneim. Forsch, 1991.

16. Huang, S.; Bucana, C.D.; Van Arsdall, M.; Fidler, I.J. Stat1 negatively regulates angiogenesis; tumorigenicity and metastasis of tumor cells. Oncogene, 2002; 21: 2504-2512
17. Kovacic, B.; Stoiber, D.; Moriggl, R.; Weisz, E.; Ott, R.G.; Kreibich, R.; Levy, D.E.; Beug, H.; Freissmuth, M.; Sexl, V. STAT1 acts as a tumor promoter for leukemia development. Cancer Cell, 2006; 10: 77-87.
18. Igwe, E.O.; Charlton, K.E.; Probst, Y.C. Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults. J. Hum. Nutr. Diet., 2019.
19. Aedín, C.; O'Reilly, E.J.; Kay, C.; Sampson, L.; Franz, M.; Forman, J.; Curhan, G.; Rimm, E.B. Habitual intake of flavonoid sub classes and incident hypertension in adults. Am. J. Clin. Nutr, 2011; 93: 338 347.
20. Salamon, I. Elderberry, Its Anthocyanins, as Antiviral Drugs (Comprising the Novel Virus SARS CoV-2). Biological Activities of Medicinal Plants; University of Presov: Presov, Slovakia, 2020.
21. Kawai, A.; Fujita, K. Small red bean (azuki) sheds biologically active substances as a prerequisite step for germination, one of which displays the antiviral activity against the rabies virus infectivity and infections in culture. Microbiol. Immunol, 2007; 51: 1071–1079.
22. Shahat, A.A.; Cos, P.; Bruyne, D.T.; Apers, S.; Hammouda, F.M.; Ismail, S.; Azzam, S.; Claeys, M.; Goovaerts, M.; Pieters, L.Antiviral and antioxidant activity of flavonoids and proantho cyanidins from Crataegus sinaica. Planta Med, 2002; 68: 539–541.[CrossRef] [PubMed]
23. Tan, J.Q.; Li, Q.; Xue, H.K.; Tang, J.T. Ultrasound-assisted enzymatic extraction of anthocyanins from grape skins: Optimization, identification, and antitumor activity. J. Food Sci., 2020; 85: 3731–3744.
24. Mazzoni, L.; Giampieri, F.; Suarez, J.M.A.; Gasparrini, M.; Mezzetti, B.; Hernandez, J.M.A.; Battino, M.O. Isolation of straw berry anthocyanin-rich fractions and their mechanisms of action against murine breast cancer cell lines. Food Funct, 2019; 10: 7103–7120.