

Optimization of flavonoids loaded ophthalmic formulation

Geetanjali sahu^{1*}

Shri Shankaracharya Professional University, Junwani Line, Bhilai, Chhattisgarh.

Dr. Swarnali das paul²

Shri Shankaracharya Professional University, Junwani Line, Bhilai, Chhattisgarh.

Abstract

Diabetic Retinopathy (DR) remains one of the most severe complications of diabetes, often resulting in vision impairment and blindness. The progression of DR is heavily influenced by oxidative stress, inflammation, and neovascularization. Quercetin, a flavonoid present in many fruits and vegetables, has gained attention for its antioxidant, anti-inflammatory, and anti-angiogenic properties. This updated review focuses on quercetin's therapeutic potential in preventing or slowing the progression of DR, discussing the evidence from recent experimental and clinical studies.

Keywords: *Flavonoids, Retinopathy, Ophthalmic preparations, Polymers, optimization, zeta, particle size, drug release.*

Introduction

Flavonoids are organic compounds characterized by a range of phenolic structures, which are abundantly present in various natural sources such as fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine¹. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves². Flavonoids have been used in natural dyes^{6,7}, in cosmetics and skin care products^{8,9}, and anti-wrinkle skin agents¹⁰. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc.³⁻⁵. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. These biological activities depend upon the type of flavonoid, its (possible) mode of action, and its bioavailability. These cost-effective medicinal components have significant biological activities, and their effectiveness has been proved for a variety of diseases.

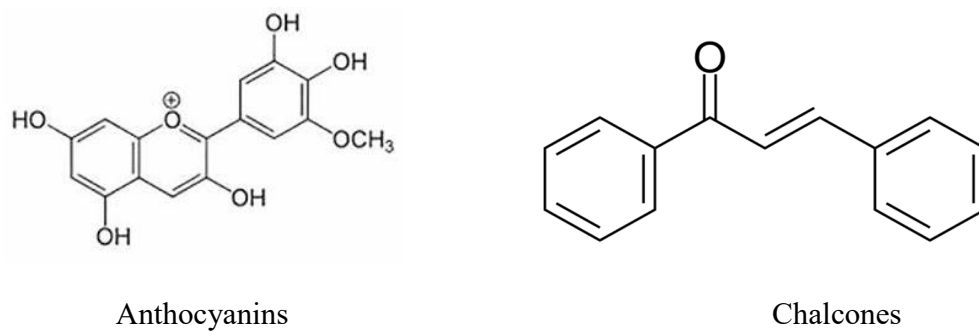
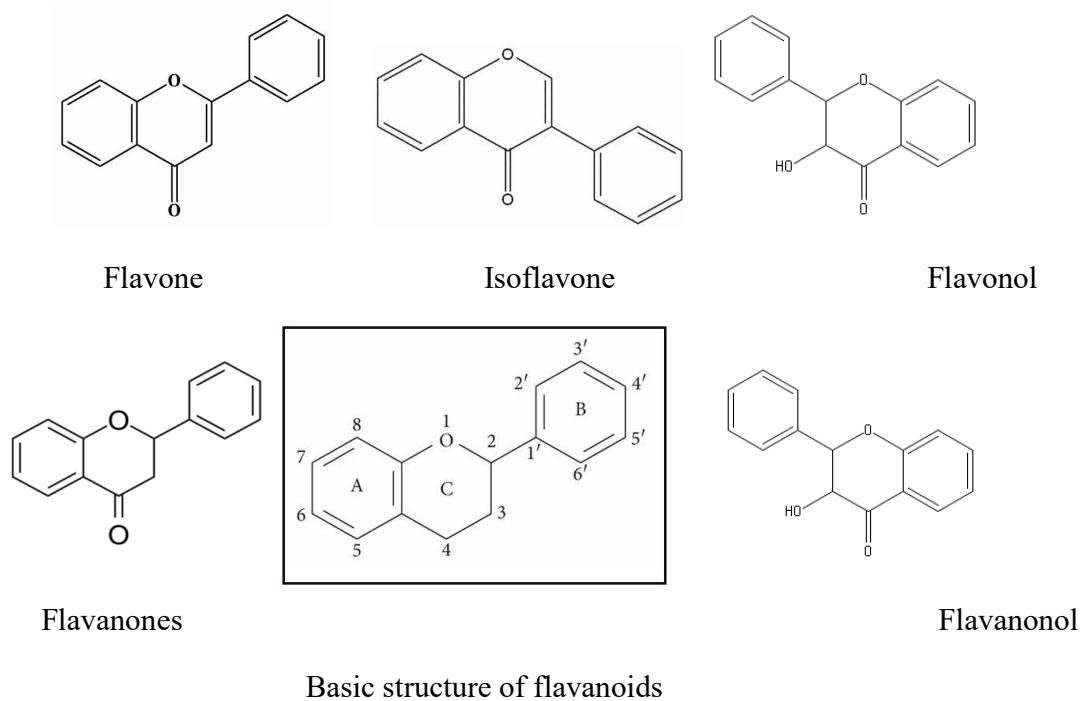
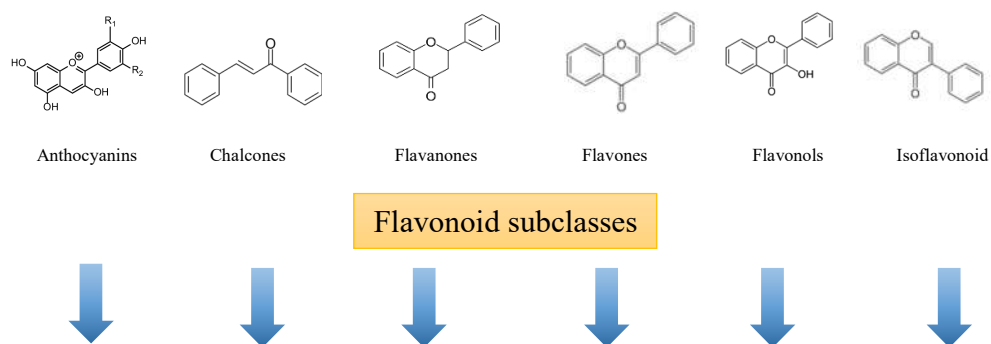


Figure 1. Chemical structures of important bioactive flavonoids.



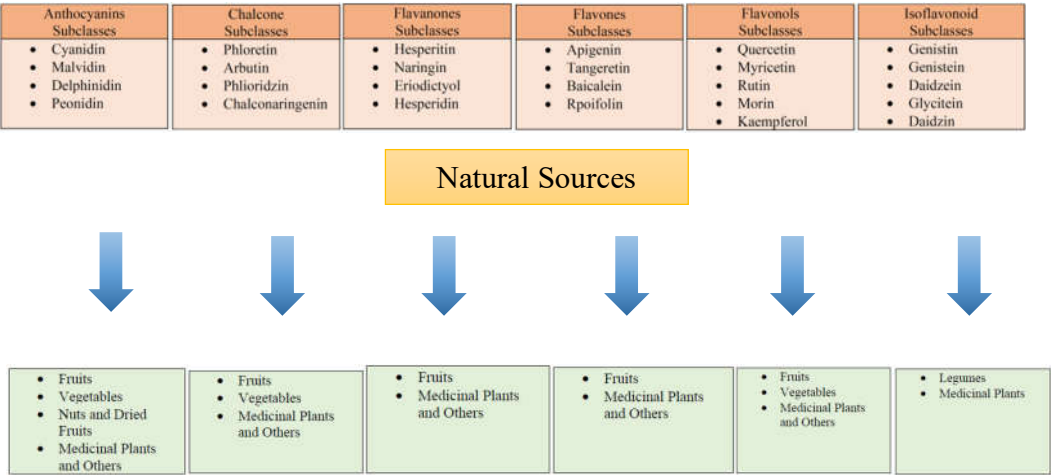


Figure 2. Flavanoids classification

Materials and methods

Table 1 Materials

S.N.	Materials	Manufacturer
1	Quercetin	Central Drug House, New Delhi
2	Dimethyl Sulfoxide	Lobachemie, Mumbai
3	Polysorbate 80	Lobachemie, Mumbai
4	Polyvinyl Pyrrolidone K-25	Pallav Chemicals, Boisar
5	Benzalkonium Chloride	Lobachemie, Mumbai
6	Ethylene Dimethyl Tetra acetate	Lobachemie, Mumbai
7	Hydroxypropylmethylcellulose	Suvidhinath laboratories, Baroda
8	Sodium Hydroxide	Pallav Chemicals, Boisar
9	Sodium Chloride	Molychem, Mumbai
10	Boric acid	Pallav Chemicals, Boisar

Method

- Quercetin was dissolved in Dimethyl Sulfoxide (DMSO) and passed the solution through 0.2-micron filters to sterile the solution with continue mixing. 1% (w/v) PVP K-30 solution was passed through 0.2-micron filter to sterile the solution and transferred it to above solution drop wise. When all solution consumed, resultant mixture was passed through 0.2-micron filter. Filtrate (active material) was washed with purified water previously passed through 0.2-micron filter.
- In an appropriate amount of purified water, HPMC was slowly dispersed and hydrated.
- In next step, following ingredients were added in an appropriate amount of purified water in a separate beaker, (in an order allowing each to dissolve completely before adding the next) Edetate Disodium, Boric Acid, Benzalkonium

- Chloride, Sodium Chloride, Sodium Borate, Polysorbate 80 and finally hydrated HPMC solution was added and pH was adjusted.
- Solution was passed through 0.2-micron filter and collected in a suitable calibrated vessel for made up the final volume.

Results

Optimization

Table 2: Data feeded for optimization (Using Design expert version 13 Software)

Run	Factor 1 A: Polymer	Factor 2 B: Time	Response 1 Particle size	Response 2 Zeta	Response 3 Drug release
1	C	30	607	12.61	51.28
2	E	30	736	13.52	45.48
3	C	40	509	12.61	51.32
4	H	30	341	17.31	71.68
5	E	40	592	13.52	47.88
6	H	40	336	17.31	81.16
7	E	20	801	13.52	39.56
8	H	20	392	17.31	63.92
9	C	20	750	12.61	49.76

Particle Size

ANOVA Table

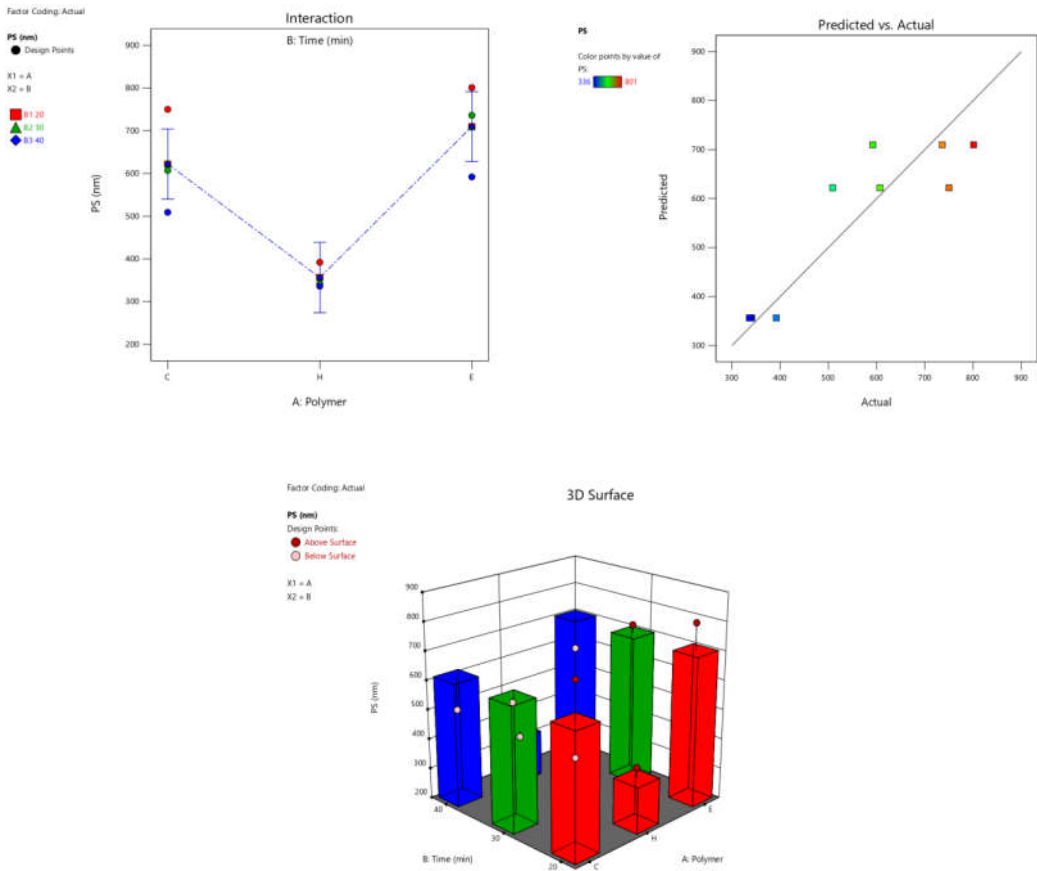
Source	Sum of squares	df	Mean square	F- value	P-value	
Model	2.031F+05	2	1.016E+05	11.5	0.0093	Significant
A Polymer	2.031F+05	2	1.016E+05	11.5	0.0093	
Residual	54179.33	6	9029.89			
Cor Total	2.573E+05	8				

The Predicted R^2 of 0.5262 is in reasonable agreement with the Adjusted R^2 of 0.7192; i.e. the difference is less than 0.2.

Polynomial equation

$PS = 562.67 + 59.33 A[1] - 206.33 A[2]$ (Coded Equation)

Interaction (Polymer vs Time)



% Drug Release

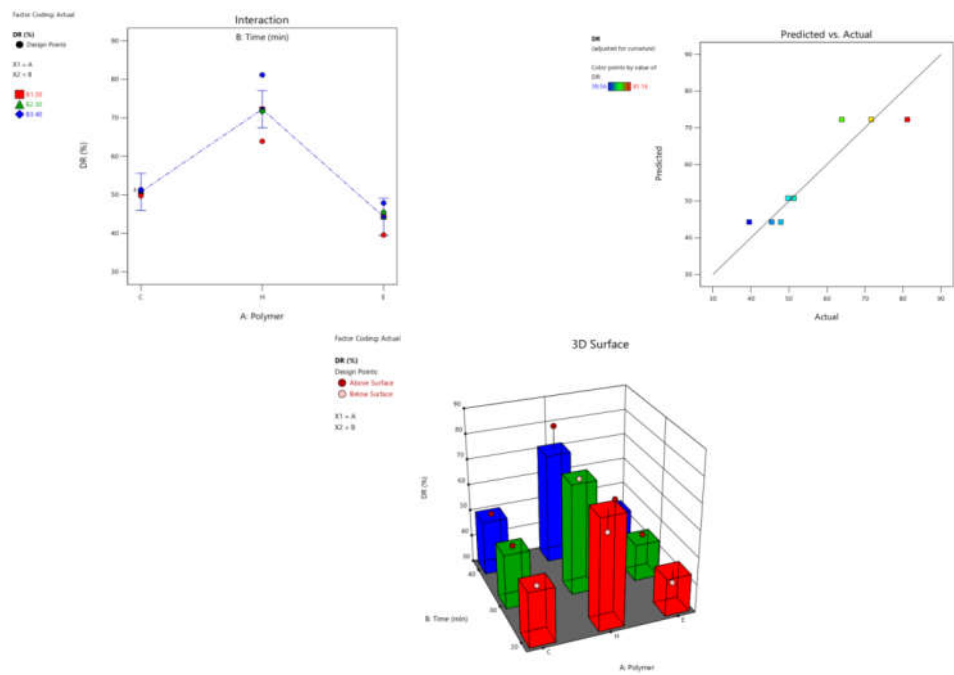
ANOVA Table

Source	Sum of squares	df	Mean square	F- value	P-value	
Model	1283.82	2	641.91	20.56	0.0021	significant
A Polymer	1283.82	2	641.91	20.56	0.0021	significant
Residual	187.36	6	31.23			
Cor Total	1471.18	8				

The Predicted R² of 0.7135 is in reasonable agreement with the Adjusted R² of 0.8302; i.e. the difference is less than 0.2.

Polynomial equation

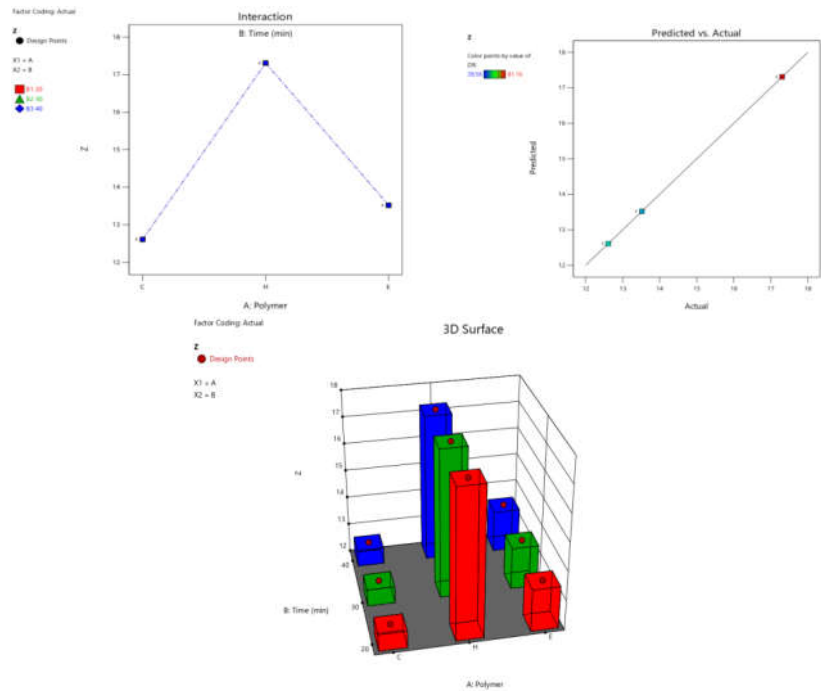
$DR = 55.78 - 5.00 A[1] + 16.47 A[2]$ (Coded Equation)



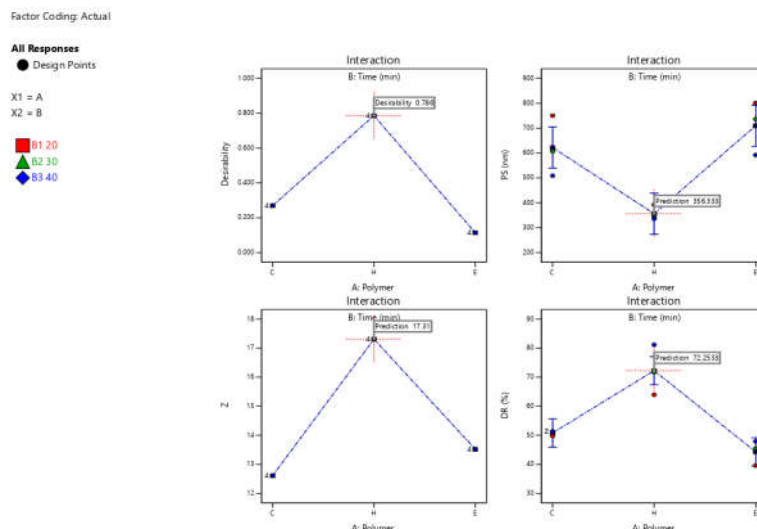
Zeta Potential

Polynomial equation

$Z = 14.48 - 1.87 A[1] + 2.83 A[2]$



All Factors interaction



Conclusion

Flavonoid-based ophthalmic preparations are increasingly recognized as beneficial in the management of diabetic retinopathy (DR) due to their multifaceted therapeutic properties.. Research indicates that flavonoids such as quercetin can mitigate oxidative stress and inflammation in retinal tissues. Quercetin has been reported to attenuate high glucose-induced injury in retinal pigment epithelial cells by modulating microRNA-29b expression, leading to reduced inflammation and oxidative damage. This research based on preparation and optimization of prepared formulation using design expert.

Hydroxypropyl methylcellulose (HPMC) is a water-soluble polymer that exhibits thermal gelation in aqueous solutions often preferred over carboxymethylcellulose (CMC) and ethyl cellulose (EC) for use in pharmaceutical suspensions due to its superior stabilizing properties, biocompatibility and mucoadhesive nature. HPMC exhibits good solubility in water and forms a gel-like network that controls the rheological properties of suspensions. Unlike CMC (which is sensitive to acidic solution). HPMC exhibits stability across a broad pH range (3-11). Unlike Ethyl cellulose which is poorly soluble in water, HPMC excels in water retention and thickening making it ideal for optimum and control drug release over extended period of time in pharmaceutical preparation. Due to this characteristic property of HPMC, on increasing sonication time it shows better particle size reduction and higher drug release rate as compare to formulation prepared by using CMC and Ethyl Cellulose.

Acknowledgement

I Ms. Geetanjali Sahu confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation. I would also like to thank my guide Dr. Swarnali Das Paul for her continuous guidance and my colleague Mrs. Suruchi Prasad for her consistent support throughout completion of this article.

Declaration of conflict of interests

The author declared no potential conflict of interest with respect to the research, authorship and/or publication of this article.

Funding

The author received no financial support for the research, authorship and/or publication of this article.

References

- [1] I.Hasin Hasnat, Suriya Akter Shompa, Md. Mirazul Islam, Safaet Alam, Fahmida Tasnim Richi, Nazim Uddin Emon, Sania Ashrafi, Nazim Uddin Ahmed, Md. Nafees Rahman Chowdhury, Nour Fatema, Md. Sakhawat Hossain, Avoy Ghosh, Firoj Ahmed. "Flavonoids: A treasure house of prospective pharmacological potentials", Cellpress, Heliyon 10 (2024) e27533.
- [2] de Groot H, Rauven U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundam Clin Pharmacol* 1998;12:249–55.
- [3] Lee Y, Yuk D, Lee J, et al. (2009) Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of β -amyloid generation and memory deficiency. *Brain Res* 1250, 164–174.
- [4] Metodiewa D, Kochman A & Karolczak S (1997) Evidence for antiradical and antioxidant properties of four biologically active N, N, diethylaminoethyl ethers of flavanone oximes: a comparison with natural polyphenolic flavonoid (rutin) action. *Biochem Mol Biol Int* 41, 1067–1075.
- [5] Hayashi T, Sawa K, Kawasaki M, et al. (1988) Inhibition of cow's milk xanthine oxidase by flavonoids. *J Nat Prod* 51, 345–348.
- [6] Villela, A.; van Vuuren, M.S.; Willemsen, H.M.; Derksen, G.C.; van Beek, T.A. Photo-stability of a flavonoid dye in presence of aluminium ions. *Dyes Pigment*. **2019**, 162, 222–231.
- [7] Paramita, V.; Kusumayanti, H.; Amalia, R.; Leviana, W.; Nisa, Q.A. Application of Flavonoid and Anthocyanin Contents from Rambutan (*Nephelium lappaceum*) Peel as Natural Dyes on Cotton Fabric. *Adv. Sci. Lett.* **2018**, 24, 9853–9855.
- [8] Lanzendörfer, G.; Stäb, F.; Untiedt, S. Cosmetic and Dermatological Preparations with Flavonoids. WO/1996/018379, 20 June 1996.
- [9] Danihelová, M.; Viskupičová, J.; Šturdík, E. Lipophilization of flavonoids for their food, therapeutic and cosmetic applications. *Acta Chim. Slovaca* **2012**, 5, 59–69.
- [10] Chuarienthong, P.; Lourith, N.; Leelapornpisid, P. Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. *Int. J. Cosmet. Sci.* **2010**, 32, 99–106.
- [11] Andreeva OA, Ivashev MN, Ozimina II, et al. (1998) Diosmetin glycosides from Caucasian vetch: isolation and study of biological activity. *Pharm Chem J* 32, 595–597.
- [12] Cai H, Al-Fayez M, Tunstall RG, et al. (2005) The rice bran constituent triclinicin potentially inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in ApcMin mice. *Mol Cancer Ther* 4, 1287–1292.
- [13] Kayoko S, Hisae O, Michiyo F, et al. (1998) Intestinal absorption of luteolin and luteolin 7-O- β -glucoside in rats and humans. *FEBS Lett* 438, 220–224.
- [14] López-Lázaro M (2009) Distribution and biological activities of the flavonoid luteolin. *Mini Rev Med Chem* 9, 31–59.
- [15] Hertog MG, Hollman PC & Van De PB (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 41, 1242–1246.
- [16] Mani R, Natesan V (2018) Chrysin: sources, beneficial pharmacological activities, and molecular mechanism of action. *Phytochemistry* 145:187–196
- [17] Felgines C, Texier O, Morand C, et al. (2000) Bioavailability of the flavanone naringenin and its glycosides in rats. *Am J Physiol Gastrointest Liver Physiol* 279, G1148–G1154.

- [18] Rathmell WG & Bendall DS (1971) Phenolic compounds in relation to phytoalexin biosynthesis in hypocotyls of *Phaseolus vulgaris*. *Physiol Plant Pathol* 1, 351–362.
- [19] Hvattum E (2002) Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. *Rapid Commun Mass Spectrom* 16, 655–662.
- [20] Khan MT, Orhan I & Enol SS (2009) Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. *Chem Biol Interact* 181, 383–389
- [21] Cruickshank IA, Biggs DR, Dawn PR, et al. (1974) Phaseollin and phaseollidin relationships in infection-droplets on endocarp of *Phaseolus vulgaris*. *Physiol Plant Pathol* 4, 261–276.
- [22] Chang S, Tan C, Frankel E, et al. (2000) Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected clingstone peach cultivars. *J Agric Food Chem* 48, 147–151.
- [23] Ross JA & Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* 22, 19–34.
- [24] Basli A, Soulet S, Chaher N, et al. (2012) Wine polyphenols: potential agents in neuroprotection. *Oxid Med Cell Longev* 2012, 805762.
- [25] Calderon-Montaña JM, Burgos-Moron E, Perez-Guerrero C, et al. (2011) A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem* 11, 298–344.
- [26] Liu RH (2013) Health-promoting components of fruits and vegetables in the diet. *Adv Nutr* 4, 384S–392S.
- [27] Kim SH & Choi KC (2013) Anti-cancer effect and underlying mechanism(s) of kaempferol, a phytoestrogen, on the regulation of apoptosis in diverse cancer cell models. *Toxicol Res* 29, 229–234.
- [28] Sahu BD, Kalvala AK, Koneru M, et al. (2014) Ameliorative effect of fisetin on cisplatin-induced nephrotoxicity in rats via modulation of NF- κ B activation and antioxidant defence. *PLOS ONE* 9, e105070.
- [29] Leung LK, Su Y, Chen R, Zhang Z, et al. (2001) Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J Nutr* 131, 2248–2251.
- [30] Eng Khoo H, Azlan A, Teng Tang S, Meng Lim S (2017) Food & nutrition research anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res* 61:1361779
- [31] Truong V-D, Deighton N, Thompson RT, et al. (2010) Characterization of anthocyanins and anthocyanidins in purplefleshed sweetpotatoes by HPLC-DAD/ESI-MS/MS. *J Agric Food Chem* 58, 404–410.
- [32] Thompson LU, Boucher BA, Liu Z, et al. (2006) Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutr Cancer* 54, 184–201.
- [33] Umpriss ST, Murphy SP, Franke AA, et al. (2005) Isoflavone content of foods with soy additives. *J Food Comp Anal* 18, 533–550.
- [34] Krenn L, Unterrieder I & Rupprechter R (2002) Quantification of isoflavones in red clover by high-performance liquid chromatography. *J Chromatogr B* 777, 123–128.