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Evaluation of direct and indirect antioxidant properties of selected four natural chemical compounds: quercetin (Q), epigallocatechin-3-gallate, (EGCG), indole-3-carbinol (I3C), and sulforaphane (SF) by DPPH radical scavenging assay

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Abstract

The main characteristic of antioxidants is the capacity to scavenge free radicals produced during cell metabolism, and thus they prevent oxidative stress, which may reduce the risk of many diseases. In this study, we evaluate the antioxidant properties of selected four compounds quercetin (Q), epigallocatechin-3-gallate, (EGCG), indole-3-carbinol (I3C), and sulforaphane (SF) by DPPH assay. The view is to establish the distinction between direct and indirect antioxidants, which would be the form of the basis for subsequent cellular antioxidant assays in our further studies. For sample assay: 20 µl of antioxidant solutions of Q, EGCG, I3C, and SF was added to 180 of 2,2- diphenyl-1-picrylhydrazyl (DPPH) solution. For blank solution, DMSO was used. Leaving the plate for 15 min in dark place and measure the absorbance at 540 nm. The results demonstrated that Q and EGCG possess direct antioxidant properties, which can be used in further cellular studies. I3C and SFN did not appear to possess any direct antioxidant behaviours during DPPH radical scavenging.

Key words: DPPH assay; quercetin (Q), epigallocatechin-3-gallate, (EGCG), indole-3-carbinol (I3C), and sulforaphane (SF), direct and indirect antioxidants

Introduction

Free radicals are produced in animal cells either deliberately or accidentally. The deliberate production yields profitable entities if they are targeted correctly, such as utilizing free radicals by enzymes at their active sites during catalysis process. An accidental generation can cause significant production of accumulated reactive oxygen species [10], which consequently result in oxidative stress [7]. This oxidative may be prevented by antioxidants found in citrus fruits, cruciferous and dark-green vegetables [1]. Therefore, increased consumption of these dietary foods has been inversely associated with a wide range of diseases such as cancers [2 ,11]. The main characteristic of antioxidants is the capacity to scavenge free radicals, and thus they contribute to the lower risks of many diseases such as neurodegenerative and cardiovascular diseases [19]. Several methods have been used to assess the antioxidants activity to scavenge free radicals. The most trusted, reliable and common method is (DPPH) assay, which is based on the scavenging of 2,2-diphenyl-1-picryldrazyl (DPPH) radicals and ferric reduction activity potential (FRAP) assays which are often being used in parallel whilst measure the total phenolics content (TPC) is usually considered as marker for antioxidant capacity (5). The first conception of DPPH method was illustrated by Blois in [3] when DPPH. free radicals accepted H atom from cysteine molecule: DPPH+ H DPPHH

The principle of assay is based on the fact that DPPH. radical accepts hydrogen atom from the scavenger such as antioxidant to produce DPPHH that appears yellow colour absorbing at 515 nm. This assay has been adopted in different laboratories with some modification [8]. In this study, we evaluate the antioxidant properties of selected four compounds quercetin (Q) (Fig.1), epigallocatechin-3-gallate, (EGCG) (Fig.2), indole-3-carbinol (I3C) (Fig.3), and sulforaphane (SF) (Fig.4) by DPPH assay. The view is to establish the distinction between direct and indirect antioxidants, which would be the form of the basis for subsequent cellular antioxidant assays in our further studies.

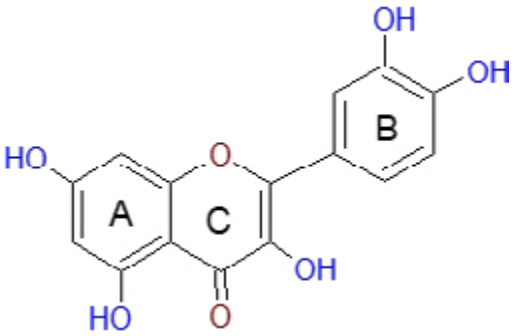

Figure:1 Quercetin (Q)

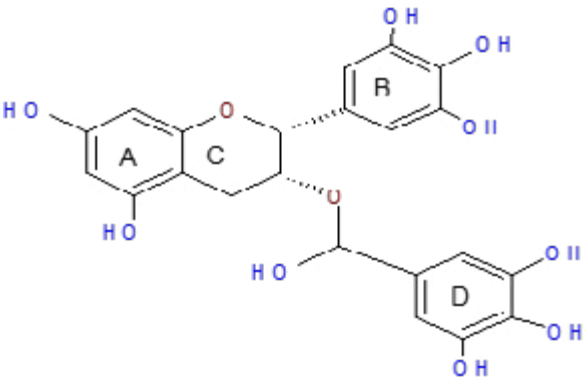

Figure:2 Epigallocatechin-3-gallate (EGCG)

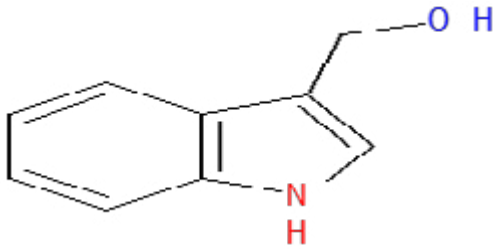

Figure:3 indole-3-carbinol (I3C)

Figure:4 Sulforaphane (SF)

Materials and methods
Chemicals

All chemicals used in this study were obtained from Sigma Chemical Co. Ltd., Poole, Dorset. UK, unless otherwise noted. Stock solutions of Q, EGCG, I3C, and SF were prepared in DMSO at µg/mL concentration units and stored at 4°C until use. The four selected chemicals were of >95% purity, as specified by the supplier.

Materials and methods

The power of phytochemicals to scavenge free radicals was achieved by using 1,1 diphenyl-2-pycrylhydrazl (DPPH) radical. The method was based on that of [9], with some modifications into 96-well plate in triplicate and for the blank assay, 20 µl of DMSO is added to 180 µl of 0.004% DPPH in methanol working solution. For sample assay 20 µl of antioxidant solution Q, EGCG, I3C, and SFN (320 µg/ml, 160 µg/ml, 80 µg/ml, 40 µg/ml, 20 µg/ml and 10 µg/ml) was added to 180 µl of DPPH solution. The plate had been left standing for 15 minutes in dark place to avoid dissociation; the absorbance was measured spectrophotometrically at 540 nm after shaking for one minute. The scavenging of DPPH radical percentage was calculated from the difference between the control run with no antioxidant addition and the absorbance in the presence of antioxidant [9].

% Scavenging=100 x [A0-(A+DPPH - A-DPPH)]/A0

Where A0 is an absorbance of sample solvent (DMSO) plus DPPH, A-DPPH is an absorbance of DMSO in methanol, and A+DPPH is an absorbance of sample (i.e. phytochemicals) with DPPH.

Results

The results for the four selected four compounds demonstrated that that Q and EGCG possessed radical-scavenging activity and act as direct antioxidants, with 100% scavenging being achieved at a concentration of 160 µg/ml. While SF and I3C did not display any antioxidant activity in that both failed to scavenge DPPH radicals and remained inactive in the concentrations range 0-320 µg/ml.

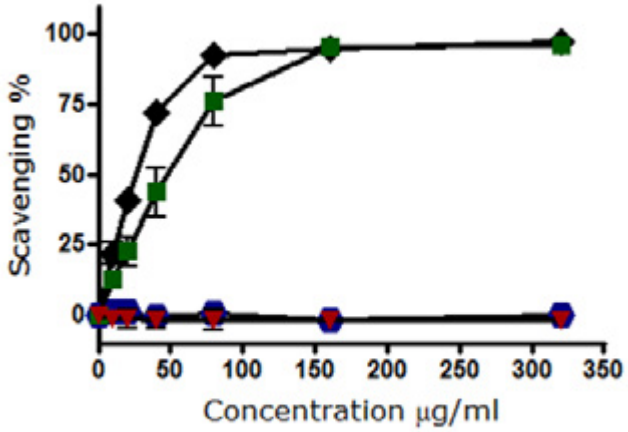

Figure:5 Activities of Q ■, EGCG ◆, I3C ▼, SF ⬬ in scavenging free radials. Values are mean ± SEM of 3-7 independent experiments.

Discussion

The daily consumption of vegetables and fruits rich with antioxidants such as onion, garlic, green tea, citrus fruits, and cruciferous vegetables has a clear impact in improving the health of the individual and disease resistance [11]. Therefore, scientists have interested in compounds that possess antioxidant properties [15]. However, their bioavailability is affecting by several factors such as plasma protein, where hydroxyl group in the B-ring of flavonoids has enhanced the binding affinities to proteins [18]. Moreover, plasma proteins may influence the cytoprotective effect of these compounds such as Q and EGCG during human hepatoma HepG2 cells exposed to oxidative stress elicited by t-BHP [6]. In this study, we have selected four natural chemical compounds Q, EGCG, I3C, and SF to assess their ability in scavenging free radicals when they possess antioxidant properties. The mechanism of direct trapping action of free radical is based on the structure of the antioxidant and hydroxyl groups in particular. Therefore and relying on our results, Q and EGCG have exhibited a notable action in trapping free radicals confirming that they possess direct antioxidants activity, while I3C and SF are not.

The free radical scavenging action of Q and EGCG may attribute to the hydroxyl groups present in those compounds. Q has 5 while; EGCG possess

es 9 groups on their structure. These groups represent the possible attack sites for the free radicals resulting in the radicalization of all hydroxyl groups [4, 13]. This reaction includes the transfer of hydrogen atoms from antioxidant to the active radicals to produce oxidized antioxidant radicals, which are less reactive than the active free radical attacker. Scientists have confirmed the power trapping of free radicals by Q during inactivation of lipid peroxide radicals [4, 16] have reported that the hydroxyl groups on ring B of Q are responsible for the antioxidant properties. [12] have confirmed that when the 3-OH group on ring B is blocking by adding sugar as in rutin, which causing the antioxidant activity decreased significantly.

Our results for the capacity of EGCG to scavenge free radicals were compatible with [14]. They have attributed this superior action to the contribution of multiple numbers of hydroxyl groups when the ortho-dihydroxyl groups on ring B confer high stability for oxidized EGCG in particular. On the other hand, I3C and SF didn't display any direct action in scavenging DPPH radicals. I3C has only one hydroxyl group on its structure lead to insufficient attacking sites by free radical atoms. According to this, the radicalization of the hydroxyl group is absent. The story of SF looks different, as its structure has no hydroxyl group, then any donation for the hydrogen atom is missing resulting in that SF is inactive completely and DPPH radicals are accumulated without any trapping.

Conclusion

In conclusion, this investigation indicates that Q and EGCG possess direct antioxidant properties, which can be used in further cellular studies. I3C and SF did not appear to possess any direct antioxidant behaviours during DPPH radical scavenging. Thus, any cytoprotection exerted by either I3C or SFN would be due to mechanisms other than direct antioxidant mechanisms.

Future study

In the next paper, the effect of direct antioxidants (Q) and (EGCG) and the indirect antioxidants,(SF) and (I3C) will assess in a cellular protection assay. This assay includes cytoprotection provided by these compounds against oxidative stress induced by t-BHP under particular conditions and will provide further insights into the mechanism of toxicity.

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References

1. Agudo A, Cabrera L, Amiano P, et al. (2007) Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). American Journal of Clinical Nutrition 85(6):1634-1642.
2. Barrera LN, Cassidy A, Johnson IT, Bao YP, Belshaw NJ (2012) Epigenetic and antioxidant effects of dietary isothiocyanates and selenium: potential implications for cancer chemoprevention. Proceedings of the Nutrition Society 71(2):237-245 doi:Doi 10.1017/S002966511200016x.
3. BLOIS, M. Antioxidant Determinations by the Use of a Stable Free Radical.Nature 181, 1199–1200 (1958). https://doi.org/10.1038/1811199a0.
4. Chiodo SG, Leopoldini M, Russo N, Toscano M (2010) The inactivation of lipid peroxide radical by quercetin. A theoretical insight. Physical Chemistry Chemical Physics 12(27):7662-7670 doi:Doi 10.1039/B924521a.
5. Clarke G, Ting K, Wiart C, and Fry J (2013) High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. Antioxidants 2013, 2, 1-10; doi:10.3390/antiox2010001.
6. Hashim MJ and Fry JR (2019) Influence of Extracellular Protein on the Cytoprotective Effects of Two Model Phytochemicals Mol Biol, vol 8(1) DOI: 10.4172/2168-9547.1000227.
7. Lima CF, Valentao PC, Andrade PB, Seabra RM, Fernandes-Ferreira M, Pereira- Wilson C (2007) Water and methanolic extracts of Salvia officinalis protect HepG2 cells from t-BHP induced oxidative damage. Chem Biol Interact 167(2):107-15 doi:10.1016/j.cbi.2007.01.020.
8. Mishra K, Ojha H, Chaudhury NK (2012) Estimation of antiradical properties of antioxidants using DPPH center dot assay: A critical review and results. Food Chemistry 130(4):1036-1043 doi:DOI 10.1016/j.foodchem.2011.07.127.
9. Nara K, Miyoshi T, Honma T, Koga H (2006) Antioxidative activity of bound-form phenolics in potato peel. Bioscience Biotechnology and Biochemistry 70(6):1489-1491 doi:Doi 10.1271/Bbb.50552.
10. Reichard P, Ehrenberg A (1983) Ribonucleotide Reductase - a Radical Enzyme. Science 221(4610):514-519 doi:DOI 10.1126/science.6306767
11. Riboli E, Norat T (2003) Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. American Journal of Clinical Nutrition 78(3):559s- 569s
12. RiceEvans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20(7):933-956
13. RiceEvans C, Miller N (1997) Measurement of the antioxidant status of dietary constituents, low density lipoproteins and plasma. Prostaglandins Leukotrienes and Essential Fatty Acids 57(4-5):499-505 doi:Doi 10.1016/S0952-3278(97)90435-X
14. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Riceevans C (1995) Polyphenolic Flavanols as Scavengers of Aqueous-Phase Radicals and as 189 Chain-Breaking Antioxidants. Archives of Biochemistry and Biophysics 322(2):339-346 doi:DOI 10.1006/abbi.1995.1473
15. Stanner SA, Hughes J, Kelly CNM, Buttriss J (2004) A review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutrition 7(3):407- 422 doi:Doi 10.1079/Phn2003543
16. Trouillas P, Marsal P, Siri D, Lazzaroni R, Duroux JL (2006) A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: The specificity of the 3-OH site. Food Chemistry 97(4):679-688 doi:DOI 10.1016/j.foodchem.2005.05.042
17. Wagner C, Fachinetto R, Dalla Corte CL, et al. (2006) Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. Brain Research 1107: 192-8.
18. Xiao JB, Kai GY (2012) A Review of Dietary Polyphenol-Plasma Protein Interactions: Characterization, Influence on the Bioactivity, and Structure-Affinity Relationship. Critical Reviews in Food Science and Nutrition 52(1-3):85-101 doi:Doi 10.1080/10408398.2010.499017
19. Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D (2007) Berry anthocyanins as novel antioxidants in human health and disease prevention. Molecular Nutrition & Food Research 51(6):675-683 doi:DOI 10.1002/mnfr.200700002