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Evaluation of Direct and Indirect Antioxidant Properties of Selected Four Natural Chemical Compounds: Quercetin, Epigallocatechin-3-Gallate, Indole-3-Carbinol and Sulforaphane 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 a 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.

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 the catalysis process. An accidental generation can cause significant production of accumulated reactive oxygen species [1] which consequently result in oxidative stress [2]. This oxidative may be prevented by antioxidants found in citrus fruits, cruciferous and dark-green vegetables [3]. Therefore, increased consumption of these dietary foods has been inversely associated with a wide range of diseases such as cancers [4,5]. 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 [6]. Several methods have been used to assess the antioxidants activity to scavenge free radicals. Total Phenolics Content (TCP), 2,2-Diphenyl-1-Picryldrazyl (DPPH), and Ferric Reduction Activity Potential (FRAP) are three assays to determine antioxidant activity. TPC assay is usually considered as a marker for antioxidant capacity and commonly used in conjunction with either or both of the DPPH and FRAP assays to increase the

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information database on a specific plant extract. It may consider as a screen to evaluate sections further by either the DPPH or FRAP assays. On the other hand, DPPH and FRAP assays give virtually identical results considerably. They are often being used in parallel and following similar mechanism, by transfer of electrons from the antioxidant to reduce an oxidant [7]. Besides, the antioxidant behaviour in both assays may identify by high phenolics content. Clarks, et al. [8] found that two methodological issues with the FRAP assay which, is the interference caused by the colour in some extracts and slow development of colour which, may reduce the usefulness of this assay during testing plant extracts. Therefore, and according to problems of FRAP colour interference, they reported that the DPPH assay is the preferred assay in a preliminary screening of extracts of plants from the Malaysian rainforest. So, the most trusted, reliable, and common method is DPPH assay, which is based on the scavenging of 2,2-Diphenyl-1-Picryldrazyl (DPPH) radicals [8]. The first conception of the DPPH method was illustrated by Blois in 1958 [9] when DPPH free radicals accepted H atom from cysteine molecule:

$DPPH^{\cdot} + H \rightarrow HDPPHH$

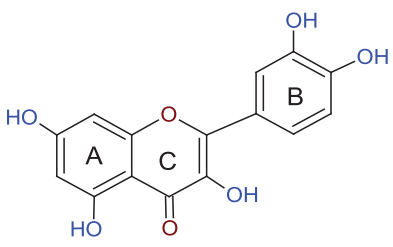
The principle of the assay is based on the fact that DPPH radical accepts hydrogen atoms from the scavenger such as antioxidants to produce DPPHH that appears yellow colour absorbing at 515 nm. This assay has been adopted in different laboratories with some modifications [10]. In this study, we evaluate the antioxidant properties of selected four compounds Quercetin (Q) (Figure 1A), Epigallocatechin-3-Gallate (EGCG) (Figure 1B), Indole-3-Carbinol (I3C) (Figure 1C) and Sulforaphane (SF) (Figure 1D) 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.

MATERIALS AND METHODS

Chemicals and reagents

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.

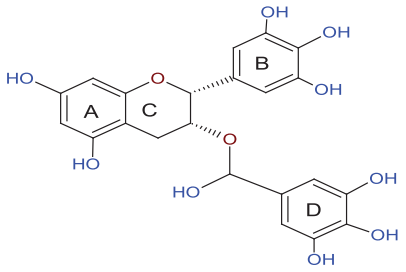
The power of phytochemicals to scavenge free radicals was achieved by using 1,1 Diphenyl-2-Pycrylhydrazl (DPPH) radical. The method was based on that of [11] with some modifications into a 96-well plate in triplicate and for the blank assay, 20 µL of DMSO is added to 180 µL of 0.004% DPPH in methanol 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)



(A) Quercetin (Q)

$C_{15}H_{10}O_7$

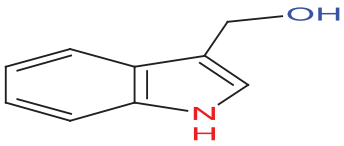
Exact Mass 302.04265



(B) Epigallocatechin-3-gallate (EGCG)

$C_{22}H_{16}O_{12}$

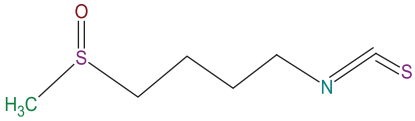
Exact Mass 472.06418



(C) indole-3-carbinol (I3C)

C_9H_9NO

Exact Mass 147.06841



(D) Sulforaphane (SF)

$C_6H_{11}NOS_2$

Exact Mass 177.02821

Figure 1 Chemical structures and molecular weights of Quercetin (A), Epigallocatechin-3 Gallate (B), Indole-3-Carbinol (C), Sulforaphane (D).

was added to 180 µL of DPPH solution. The plate had been left standing for 15 minutes in a 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 antioxidants [12].

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

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

RESULTS

The results for the four selected four compounds demonstrated 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 2).

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 on improving the health of the individual and disease resistance [5]. Therefore, scientists have interested in compounds that possess antioxidant properties [13]. However, their bioavailability is affected by several factors such as plasma protein, where the hydroxyl group in the B-ring of flavonoids has enhanced the binding affinities to proteins [14]. 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 [15]. 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 radicals 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.

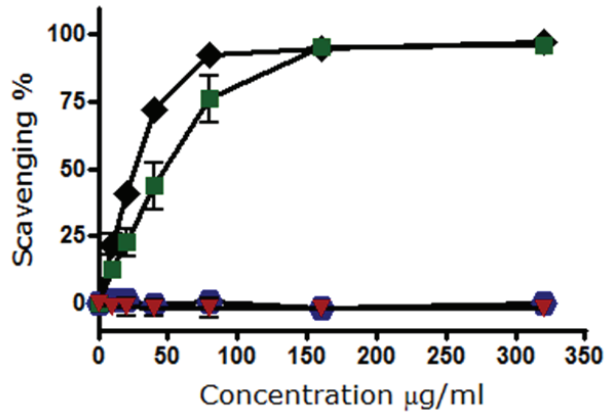


Figure 2 Activities of Q■, EGCG♦, I3C▼ and SF⬣ in scavenging free radicals. Values are mean ± SEM of 3-7 independent experiments.

The free radical scavenging action of Q and EGCG may attribute to the hydroxyl groups present in those compounds. Q has 5 while; EGCG possesses 9 groups on their structure. These groups represent the possible attack sites for the free radicals resulting in the radicalization of all hydroxyl groups [17,18]. This reaction includes the transfer of hydrogen atoms from antioxidant to the active radicals to produce oxidized antioxidant radicals [17], which are less reactive than the active free radical attacker. Scientists have confirmed the power trapping of free radicals by Q during the inactivation of lipid peroxide radicals [15-17]. Trouillas, et al. [19] have reported that the hydroxyl groups on ring B of Q are responsible for the antioxidant properties. RiceEvans, et al. [20] have confirmed that when the 3-OH group on ring B is blocked 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 Salah, et al. [21]. 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 behaviors during DPPH radical scavenging. Thus, any cytoprotection exerted by either I3C or SF would be due to mechanisms other than direct antioxidant mechanisms.

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