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Research Article

INVESTIGATION OF ANTIOXIDANT POTENTIAL OF QUERCETIN AND HESPERIDIN: AN *IN VITRO* APPROACH

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ABSTRACT

Objective: The study was carried out to evaluate the antioxidant potential of quercetin and hesperidin.

Methods: The antioxidant potential of quercetin and hesperidin was estimated using free radicals in *in vitro* condition. Antioxidant activity of quercetin and hesperidin was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, nitric oxide, hydroxyl radical, hydrogen peroxide radical, and reducing power assay.

Results: Quercetin and hesperidin have high efficiency in scavenging free radicals. Both the compounds are more efficient in scavenging DPPH (IC50 value 36.15±0.30 and 41.55±0.49 mg/ml) and superoxide radicals (IC 50 value 19.3±0.26 and 28.08 ±0.18 mg/ml) have high reducing capacity when compared to the standards ascorbic acid (IC50 value 31.45±1.31 and 23.2±0.20 mg/ml).

Conclusion: The present study suggests that quercetin and hesperidin have significant antioxidant activities and could be easily accessible to the immune system.

Keywords: Quercetin, Hesperidin, 1,1-diphenyl-2-picrylhydrazyl, Radical scavenging activity.

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INTRODUCTION

In the living system, free radicals of different forms are frequently generated for specific metabolic requirements. When the generation of these species exceeds the levels of antioxidant mechanism, they cause wide-ranging damage to the cells leading to oxidative damage that in saw in the disease conditions, especially degenerative diseases. The endogenous antioxidants act as intracellular protection systems protecting the cells from free radical damage and extensive lysis. Scavenging and retreating the formation of oxygen-derived species if not 100% efficient. Different disease conditions are associated with free radical oxidative stress. Herbal drugs containing free radicals scavengers such as phenolics, tannins, flavonoids and flavanones are renonowned for their therapeutic acitivity [1]. There are thousands of medicines and natural products that have been closely linked through the use of traditional medicines. Organic products represent a wealthy source of biologically active compounds with conventional potential for drug discovery and development [2]. In flowers also have a free radical activity such as *Jasminum grandiflorum* (L.) flower [3]. The best described property of quercetin is its ability to act as an antioxidant. It appears to be the most powerful flavonoid for protecting the body against reactive oxygen species (ROS), produced during the normal O_2 metabolism or induced by exogenous damage [4]. These include enzymatic and non-enzymatic antioxidants that remain in check the ROS level and repair the oxidative cellular damage. The major enzymes, constituting the first line of defense, directly involved in the neutralization of ROS are superoxide dismutase, catalase and glutathione peroxidase. Repair and de novo enzymes act as the third line of defense by repairing damage and reconstituting membranes which include lipases, proteases, DNA repair enzymes and transferases [5]. Quercetin acting as a free radical scavenger was shown to exert a protective effect in reperfusion in ischemic tissue damage [6] by various ways. It prevents free radical induced tissue injury. One way is the direct scavenging of free radicals, by scavenging free radicals, flavonoids particularly can inhibit low-density lipoprotein oxidation *in vitro* [7]. Quercetin leads to a reduction in ischemia-reperfusion injury by interfering with inducible nitric oxide (NO) synthesis activity [8]. It causes scavenging of free radicals, and therefore can no longer reacts with NO, ensuing less damage [9]. Free radicals are very stable and react speedily with other compounds, trying to capture the needed electron to gain stability. Generally free radicals attack the nearest stable molecules, "stealing" its electron. When the attack molecules lose its electron it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade finally resulting in the disruption of living cells. Some free radicals arise normally during metabolism. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals. ROS include hydroxyl radicals (OH), superoxide anion (O_2), hydrogen peroxide (H_2O_2), NO, peroxynitrite and nitrosyl with the OH being the most harmful. Quercetin is one of the most frequently studied bioflavonoid in its class of flavonol. It has been shown to have cytoprotective effects in preventing endothelial apoptosis caused by oxidants [10]. Quercetin is a more potent antioxidant than other antioxidant nutrients, such as vitamin C, vitamin E, and β-carotene, and it can chelate transition metal ions, including iron, thus preventing the iron-catalysed Fenton reaction [11,12]. Hesperidin is a naturally occurring flavanone that exists in citrus and other plants [13]. Hesperidin is reported to exert a wide range of pharmacological effects [14]. It has been demonstrated that hesperidin can protect neurons against various types of insults associated with many neurodegenerative diseases [15]. Hence, it was planned to investigate on the antioxidant potential of quercetin and hesperidin.

METHODS

Ascorbic acid, nicotinamide adenine dinucletide (NADH), nitro blue tetrazolium (NBT), phenazine methosuphate (PMS), EDTA, sulphanilamide, N-(1-naphthyl) ethylene diamine dihydrochloride were purchased from Hi-Media-Ltd., Mumbai, India. 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin and hesperidin were purchased from Sigma Chemical co., St. Loius, MO, USA. All other chemicals used were of analytical grade (Sigma Aldrich).

DPPH
The antioxidant activities of quercetin and hesperidin was determined in term of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Mensor *et al.* [16]. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the sample dissolved in ethanol at different concentration (10-50 µg/ml). The mixture was added and incubated in dark for 30 minutes and 1 ml of ethanol served as control. The change in color was observed in terms of absorbance using a spectrophotometer at 517 nm. Ascorbic acid was invoked as standard. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$(\%) = [(Ac - A_T/A_C] \times 100$

Superoxide radical (SO) scavenging activity
The SO scavenging activity was studied by using the method of Liu *et al*. [17] SOs are generated in PMS–NADH systems by oxidation of NADH and assayed by the reduction of NBT. Different concentration (10-50 µg/ml) of the sample solution was taken in a series of test tubes. SO were generated by 1 ml of Tris–HCL buffer (16 mM, pH - 8), 1 ml of NBT (50 µM), 1 ml of NADH (78 µM) solution and 1 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 minutes and the absorbance was measured at 560 nm in spectrophotometer. The IC50 values of quercetin and hesperidin were calculated and compared with ascorbic acid (standard) solution. The percentage inhibition of O_2 generation was calculated as follows:

% Inhibition = [(Ac–At)/Ac] × 100

NO radical scavenging activity
NO radical - scavenging activity was measured spectrophotometrically depending to the method described by Govindarajan *et al.* [18]. When sodium nitroprusside was mixed with an aqueous solution at physiological pH, it generated NO, which reacts with oxygen to produce nitrite ions that can be estimated using the Greiss reagent. To 2 ml of sodium nitroprusside (5 mM), 0.5 ml of phosphate buffer (pH - 7.4) was mixed with different concentrations of the quercetin and hesperidin (10-50 µg/ml). The tubes were then incubated at 25°C for 2.5 hrs. After incubation 0.5 ml of the reaction mixture, 1.0 ml of sulphanilic acid was added and incubated at room temperature for 5 minutes for complete diazotization. Finally, 1.0 ml of 5% N-napthyl ethylene diamine dihydrochloride was added, mixed and incubated at room temperature for 5 minutes to form pink coloured chromphore. The absorbance was then measured at 546 nm against the equivalent blank solution, decreased absorbance of the reaction mixture indicated increased scavenging activity. The IC50 value of the test solution was calculated and compared with that of ascorbic acid.

Hydroxyl radical scavenging assay
Hydroxyl radicals (OH) are generated from Fe2+-EDTA – H_2O_2 system (Fenton's reaction) The hydroxyl radical-scavenging activity of quercetin and hesperidin was measured according to the method of Halliwell *et al.* [19] stock solutions of EDTA (0.1 mM) were prepared in dimethyl sulfoxide and $FeCl_3$ (0.1 mM), ascorbic acid (0.1 mM), H_2O_2 (4 mM) and deoxyribose (2.8 mM) were prepared in distilled water. The method was carried out by adding 100 µl of EDTA, 10 µl of $FeCl_3$, 100 µl of H_2O_2, 360 µl of deoxyribose, and different concentrations (10-50 µg/ml) of compounds was dissolved in distilled water, 330 µl of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid. This mixture was then incubated at 37°C for 10 minutes. 1 ml of the incubated mixture was mixed with 1 ml of 10% trichloroacetic acid and 1 ml of 0.5% thio barbituric acid was added and the tubes were boiled for 30 minutes. After incubation, colour was developed and measured at 532 nm in spectrophotometer. The IC50 values of the test sample were calculated and compared with ascorbic acid (standard). The values are expressed as percentage of inhibition.

H_2O_2 scavenging activity
Scavenging of H_2O_2 was determined by the method of Ruch *et al.* [20]. The procedure was started with different concentrations (10-50 µg/ml) of test solution, 500 µl of buffer and 400 µl of 2 mM H_2O_2 were added. The mixture was kept at the room temperature for 5 minutes. After incubation 2 ml of dichromate acetic acid reagent was added and colour intensity was measured at 570 nm. The blank solution contains 2 ml of dichromate acetic acid alone. Whereas the reaction mixture without compound served as control. The percentage of inhibition was calculated and compared with Ascorbic acid as the standard.

Reducing power assay
The reducing power was determined according to the method of Oyaizu [21]. The different concentrations of test solution (10-50 µg/ml) were added to 0.1 ml of DTC reagent contain (0.4 g of thiourea, 0.05 g of copper sulphate and 3 g of DNPH) and incubated at 37°C for 3 hrs. To the mixture 0.75 ml of 85% HCl was added and incubated at room temperature for 30 minutes. Ascorbic acid served as control. Intensity of colour developed was read at 520 nm using a spectrophotometer. The reducing power was calculated and compared with ascorbic acid.

RESULTS

The compound such as quercetin and hesperidin was investigated for the effects on the *in vitro* generation of free radicals. The results of the study showed that these two compounds could inhibit the free radicals such DPPH, SOs, NO, hydroxyl radicals and H_2O_2.

DPPH
DPPH free radical compound has been widely used to test the free radicals scavenging ability of various food samples. The antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow by reading at 517 nm. The DPPH radical-scavenging activity of quercetin and hesperidin was detected and compared with standard antioxidant–ascorbic acid. Both compounds tested revealed that they possessed excellent antioxidant capacity and it was dose dependent. The scavenging activity of ascorbic acid was high. The IC50 values of quercetin (36.15 µg/ml) and hesperidin was 41.55 µg/ml (Fig. 1).

SO scavenging assay
The result of SO scavenging activity showed that both compounds could efficiently scavenge and it was dose dependent. In the present study, quercetin and hesperidin were found to be a notable scavenger of SOs generated in the reduced NBT. The IC50 values of quercetin was 19.3 µg/ml and hesperidin 28.08 µg/ml when compared with the ascorbic acid (23.2 µg/ml) (Fig. 2).

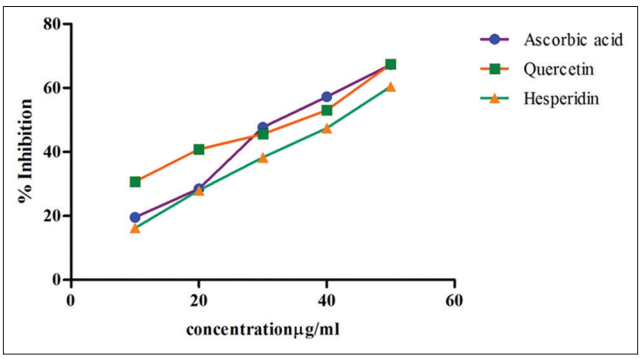

Fig. 1: The scavenging activity of quercetin and hesperidin as determined by on 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. Values are expressed in mean ± standard deviation (n=6)

Nitric oxide radicals scavenging assay

Compound such as quercetin and hesperidin effectively reduced the generation of NO from sodium nitroprusside. IC50 value of quercetin and hesperidin at the concentration of 49.35 µg/ml and 45.11 µg/ml while the standard ascorbic acid was 35.56 µg/ml. Scavenging of NO radical is based on the generation of NO from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore was measured at 546 nm in the presence of the test solution proved the decreased amount of nitrite generated by the decomposition of sodium nitroprusside *in vitro* (Fig. 3).

Hydroxyl radical scavenging assay

In the present study, the hydroxyl radical-scavenging effect of the test compounds at different concentrations was studied with ascorbic acid was standard. It is found to be significantly effective in inhibition of hydroxyl radicals (Fig. 4). The IC50 value of quercetin, hesperidin and ascorbic acid was 31.4 µg/ml and 37.36 µg/ml and 27.01 µg/ml respectively.

Scavenging of H_2O_2

Scavenging of H_2O_2 by two compounds namely of quercetin and hesperidin is presented (Fig. 5). The percentage of H_2O_2 scavenging activity of quercetin and hesperidin was found to be 38.35 µg/ml and 46.1 µg/ml which is compared to antioxidant activity of standard ascorbic acid (11.15 µg/ml).

Reducing power assay

In the present study the reducing power of the compounds was found to be excellent and steadily increased in direct proportion to the increasing concentration of the compounds (Fig. 6). Which was relatively more pronounced than that of standard ascorbic acid. The reducing power of quercetin and hesperidin was found to be 50.06 µg/ml and 47.46 µg/ml while the standard antioxidant ascorbic acid was of 35.35 µg/ml.

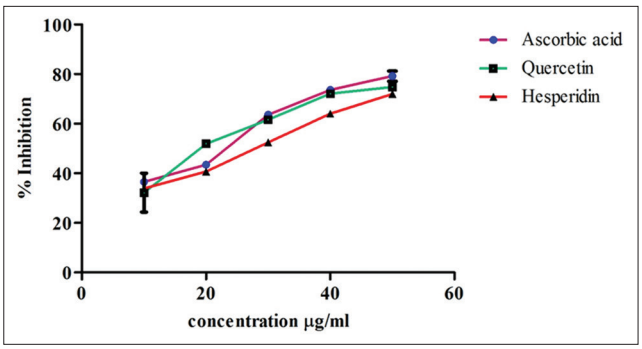

Fig. 2: The superoxide radical-scavenging activity of Quercetin and Hesperidin as determined. Values are expressed in mean ± standard deviation (n=6). Nitric oxide radicals scavenging assay

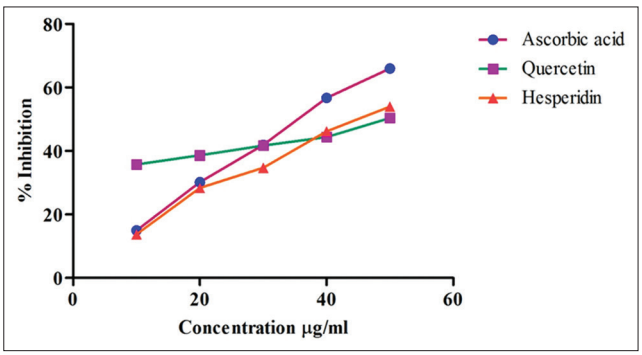

Fig. 3: Nitric oxide scavenging activity of quercetin and hesperidin as determined. Values are expressed in mean ± standard deviation (n=6)

DISCUSSION

Stratil *et al.* [22] reported that DPPH is relatively stable and hence it is a less reactive free radicals, so it can be reduced primarily by more reactive reducing components such as phenolic substance. Kalaivani *et al.* [23] say that the DPPH is a free radical which has been widely accepted as a tool for estimating free radicals scavenging activities of antioxidants. SOs is known to be a very harmful species to cellular compounds as a precursor of more reactive species is reported by Halliwell and Gutteridge [24]. Esmaeili and Sonboli [25] suggested the H_2O_2 itself is not very reactive, but some times it is toxic to cell. So removing of H_2O_2 is very important for antioxidant defence in cell system. Polyphenols have also protect mammalian cells from damage induced by H_2O_2, especially compounds with the orthohydroxyphenolic compounds like quercetin, gallic acid, ceiffecic acid and catechin. Hofseth *et al.* [26] reports NO formed during their reduction with oxygen or with superoxide. These radicals are responsible for altering the structure and functional

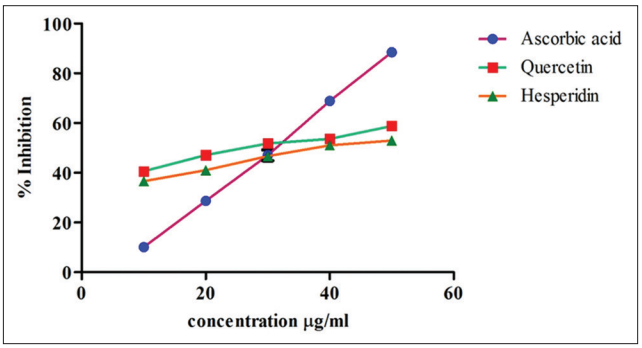

Fig. 4: Sacavenging activity of quercetin and hesperidin as determined by hydroxyl radical scavenging assay. Values are expressed by mean ± standard deviation (n=6)

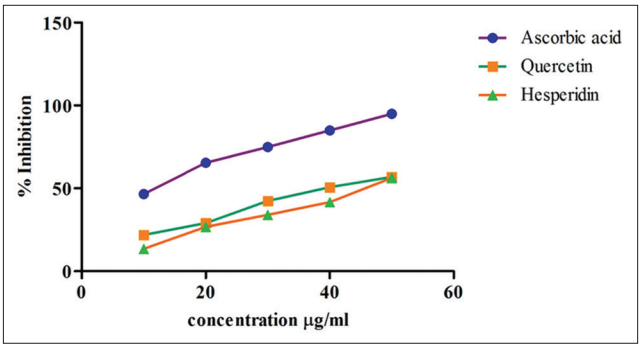

Fig. 5: The scavenging activity of quercetin and hesperidin as determined by H2O2. Values are expressed in mean ± standard deviation (n=6)

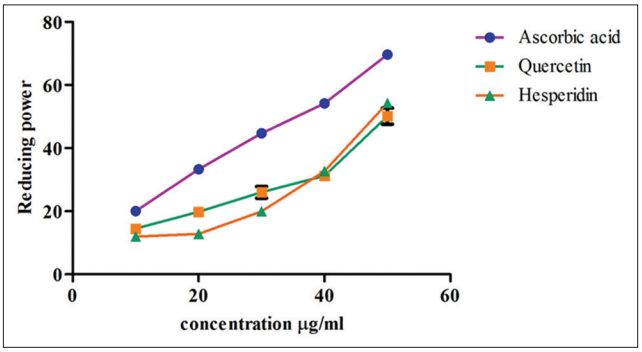

Fig. 6: The reducing power assay of quercetin and hesperidin. Values are expressed by mean ± standard deviation (n=6)

behavior of many cellular compounds. NO is also implicated for inflammation, cancer and other pathological conditions. Sulaiman *et al.* [27] say that the antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atom to free radicals. In addition they posssess ideal structural activities. Mahadevan *et al.*, reported that *Mimosa pudica* exerts have a neuroprotective effects [28]. This study reported that selenium enriched mushrooms also have prevention effect against neurodegenerative diseases [29] in treating oxidative stress related diseases.

CONCLUSION

The results obtained from the study suggest that both the compounds quercetin and hesperidin have significant antioxidant activities. Hence they could be helpful for the immune system against oxidative stress and its related disorder such as neurodegenerative diseases.

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