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Antioxidant activity of synergistic quercetin resveratrol

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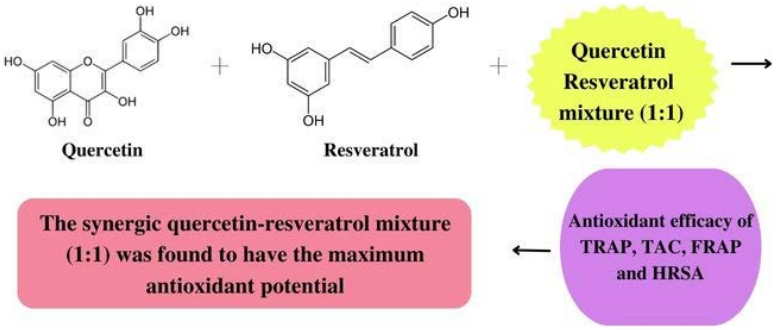
Article

ABSTRACT



Polyphenols, the most prevalent and naturally occurring substances have synergistic qualities that may have positive effects on human's health. When synergistic foods are combined, the evidence for health benefits is greater than when the foods are consumed separately. Nutrient deficiency is a well-known phenomenon in many people, and synergy plays a critical role in combating nutritional deficiency, chronic diseases, and infections and mainly it increases the bioavailability. This research study covers the synergistic interactions of quercetin and resveratrol, and quercetin resveratrol mixtures (1:1) were used to evaluate the TRAP, TAC, FRAP and HRSA antioxidant activity assays. The polyphenolic chemicals that are said to have synergistic effects are very powerful inhibitors of oxidation and a host of other diseases. These results observed the advantages of polyphenol chemicals when combined, which may be helpful for future research.

Keywords: Antioxidants, Synergism, Quercetin, Resveratrol, phytochemicals.

INTRODUCTION

Secondary plant metabolites called polyphenols are frequently used in defense against diseases, infection or UV radiation. Over ten years, the surge in interest in the antioxidant properties of dietary plant polyphenols.[1] Depending on the amount of aromatic (phenolic) rings they contain and the structural components that link these rings, polyphenols are divided into various classes.[2] They can be categorized into lignans, stilbenes, flavonoids, and phenolic acids.[3] Fruits (fruit juice) and drinks (wine, tea, coffee, chocolate, and beer) are the main dietary sources of polyphenols.[4,5]

Quercetin (Q) is a bioactive compound which is mostly present in onions, grapes, berries, cherries, broccoli, and citrus fruits, tomato, apple peel.[6,7] As an antioxidant flavonoid, it is particularly effective, and more specifically as a flavonol (Figure 1). It is a multifunctional antioxidant with the ability to prevent tissue damage brought on by different medication toxicities.[7,8]

The polyphenol resveratrol (R) belongs to the stilbenoids family and has two phenol rings joined by an ethylene bridge (Figure 2). This naturally occurring polyphenol has been discovered in the skin and seeds of grapes, capsicum, berries, cabbages as well as red wines, different human diets, and more. Numerous studies have demonstrated that resveratrol, a naturally occurring dietary component, has a very strong antioxidant activity.[9]

Quercetin (Q) and resveratrol (R) may provide health benefits at the local level when they act directly on the gastrointestinal tract or at the systemic level following absorption. Only 5-10% of the total amount of ingested polyphenols is thought to be able to be absorbed in the small intestine, limiting the digestion and absorption of dietary polyphenols.[10] Two naturally occurring polyphenols, resveratrol and quercetin, are highly concentrated in plants, vegetables and fruits. Both polyphenols are thought to have positive cardiovascular effects, including the ability to relax and act as antioxidants.[11–13] The active ingredients in functional meals are essential for boosting immunity, avoiding infections, and preventing chronic diseases. In order to improve health, synergism is a significant method that lowers the risk of developing cancer and other chronic diseases.[14–16] The

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phytochemicals found in functional meals are crucial in keeping the balance between health and disease. Numerous cellular pathways are disrupted by thousands of phytochemicals that have been identified.[15] Regardless of the person or living situation, it is quite challenging to scientifically address a human being's needs. In order to achieve the best health benefits, this research investigates and reveals the scientific evidence underlying nutrient-nutrient interactions inside food in the human system.[16] Plant-produced primary and secondary metabolites, as well as coexisting constituents, can improve intestinal absorption by improving solubility, inhibiting first-pass elimination mediated by drug-metabolizing enzymes or drug transporters, increasing enterocyte membrane permeability, and reversibly opening the paracellular tight junction between enterocytes.[17] The current study analyses the maximum antioxidant potential of quercetin, resveratrol and quercetin-resveratrol mixtures (1:1).

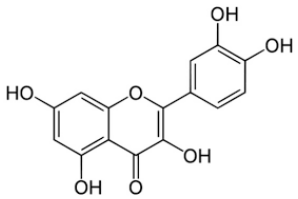

Figure 1. Structure of Quercetin

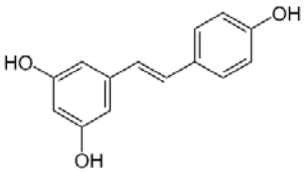

Figure 2. Structure of Resveratrol

EXPERIMENTAL SECTION

Total peroxyl radical-trapping efficiency (TRAP)

TRAP was performed on quercetin, resveratrol and quercetin resveratrol mixtures according to Apak *et al* and Cabrini L with slight modification.[18,19] It was prepared by mixing a different concentrations of quercetin, resveratrol and quercetin-resveratrol mixtures (1:1). Added 20–100 µg/ml of the sample and to this TRAP reagent was added and diluted the same with ethanol to 0.5 ml. Over a period of 45 minutes, the reaction was monitored, and the absorbance was measured at 490 nm against a blank.

Total antioxidant capacity by Phosphomolybdate Assay

The procedure described was slightly modified to determine total antioxidant capacity (TAC) of quercetin, resveratrol and quercetin resveratrol mixtures (1:1) of synergic samples using the phosphomolybdate assay proposed by Prieto P *et al.*[20,21] The concentration (20 - 100µg/ml) of quercetin, resveratrol and quercetin resveratrol mixtures were prepared. 3 ml phosphomolybdate reagent in each test tube (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tube was wrapped in aluminium foil and incubated at 95 ℃for 90 minutes. After allowing the mixture to cool to room temperature, the absorbance at 695 nm was measured. Rutin was used to calibrate standard curve.

Ferric Reducing Antioxidant Power (FRAP)

Fe^{3+} - 2,4,6-Tripyridyl-S-triazine (TPTZ) complexes are reduced to ferrous forms (Fe^{2+}-TPTZ) by reduction to ferric complexes. The FRAP was performed in the concentrations (20 - 100 µg/ml) of quercetin, resveratrol and quercetin resveratrol mixtures. The reagent was prepared by mixing a 25ml acetate buffer, 2.5ml TPTZ solution, and 2.5ml $FeCl_3.6H_2O$ solution in a ratio of 10:1:1. FRAP reagent was mixed with different concentrations of sample solution and the final volume was made upto 1 ml. After 4 minutes, an increase in absorbance of blue-colored ferrous formed was measured at 593 nm. Rutin was used as a standard. FRAP value was expressed as mmol/100 g on a dry weight basis using the calibration curve of Fe^{2+}. [22]

Hydroxyl radical scavenging activity

In a reaction mixture 3.0 ml contained $FeSO_4$ was in amount of 1.5mM, hydrogen peroxide at 6mM, sodium salicylate at 20mM, and quercetin, resveratrol and quercetin resveratrol mixtures (1:1) were taken in various concentrations (20-100µg/ml). After one hour of incubation at 37°C, 562nm was used to detect the absence of the hydroxylated salicylate complex. In order to calculate the percentage scavenging effect, following formula was used.

$$[1-(A1-A2)/A1] \times 100\%$$

Where the control A1 was the absorbance without samples and A2 was the absorbance with samples.[23] Rutin was used as standard to plot the calibration curve.

Statistical Analysis

The results of all experiments were expressed as the Mean ± Standard deviation of three replications. Microsoft Excel was used for statistical analysis.

RESULTS AND DISCUSSION

Antioxidant activity requires a stable and rapid method. Several methods have been developed to determine the bioactive compounds' ability to scavenge free radicals. An effective and reliable method involves the use of a spectrophotometer to determine the disappearance of free radicals. The dietary sources of quercetin, resveratrol and quercetin resveratrol mixtures were assessed *in vitro* antioxidant activity by TRAP, TAC, FRAP and HRSA assays.

Total peroxyl radical-trapping efficiency (TRAP)

TRAP measures the antioxidants' ability to inhibit peroxyl radical reactions with a target molecule.[24] The results of the TRAP anti-oxidant activity are shown in Figure 3, explains the intensity of the antioxidant activity was higher in the quercetin resveratrol mixtures (1:1) than individual compounds. Resveratrol showed less antioxidant intensity than quercetin. This assay causes lipid peroxidation by generating water-soluble peroxyl radicals and is sensitive to all known antioxidants, but it is very complex and time-consuming and requires a high level of expertise and experience.[25] The table 1

shows the gradual increase among all compounds including the synergic, which represents 100 µg/ml.

Table 1. TRAP values

Compounds	100µg/ml
Rutin (Standard)	0.472
Quercetin	0.492
Resveratrol	0.426
Quercetin + Resveratrol (Q+ R 1:1)	0.596

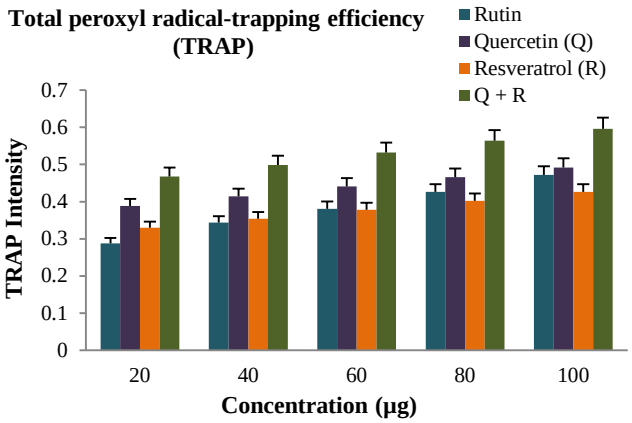

Figure 3. Total peroxyl radical-trapping efficiency (TRAP)

Total antioxidant capacity by Phosphomolybdate Assay

According to Baig *et al.* (2011), the phosphomolybdate method uses the reduction of Mo (VI) to Mo (V), which results in the formation of green-colored phosphomolybdenum (V).[26] Results in figure 4 indicate that the synergistic quercetin resveratrol mixtures (1:1) showed the highest TAC among individual quercetin and resveratrol followed by the standard rutin. Bioactive compounds contain high levels of total phenolics and flavonoids. Phytochemical properties allow phenolic compounds to absorb and neutralize free radicals, quench singlet and triplet oxygen, and decompose peroxides.[27] The table 2 shows the gradual increase among all compounds including the synergic, which represents 100 µg/ml. As a result, phenolic compounds have been classified as potent antioxidants (Rice-Evans *et al.*, 1995).[28] TAC is expected to increase due to antioxidants mechanism. Guanim *et al*. (2011) observed a significant increase in antioxidant capacity in healthy humans after consuming resveratrol with a high-fat meal.[29]

Table 2. TAC by Phosphomolybdate Assay values

Compounds	100µg/ml
Rutin (Standard)	0.629
Quercetin	0.596
Resveratrol	0.616
Quercetin + Resveratrol (Q+ R 1:1)	0.727

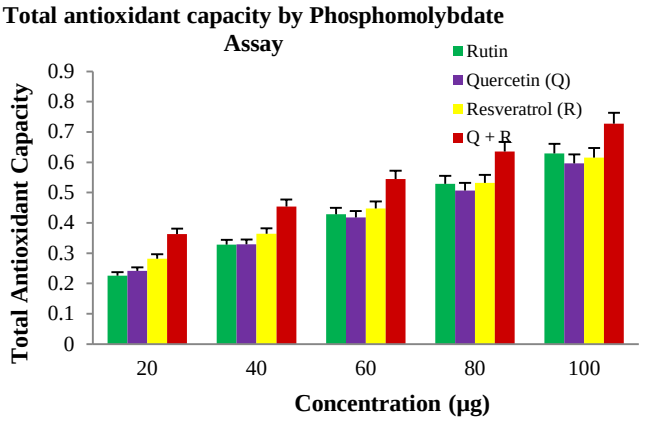

Figure 4. Total antioxidant capacity by Phosphomolybdate Assay

Ferric Reducing Antioxidant Power (FRAP)

A FRAP antioxidant activity has increased antioxidant activity on the synergistic effects of polyphenolic compounds such as quercetin resveratrol mixtures (1:1) and maintained the desired antioxidant activity. In general, values of antioxidant activity of quercetin resveratrol synergies were higher when compared to the individuals of quercetin and resveratrol. Figure 5 shows that the antioxidant activity measured by FRAP was positively correlated with the concentration of polyphenols. The table 3 shows the gradual increase among all compounds including the synergic, which represents 100 µg/ml The antioxidants present in the quercetin and resveratrol act as a reducing agent in the FRAP assay.[30,31]

Table 3: FRAP values

Compounds	100µg/ml
Rutin (Standard)	0.489
Quercetin	0.522
Resveratrol	0.422
Quercetin + Resveratrol (Q+ R 1:1)	0.672

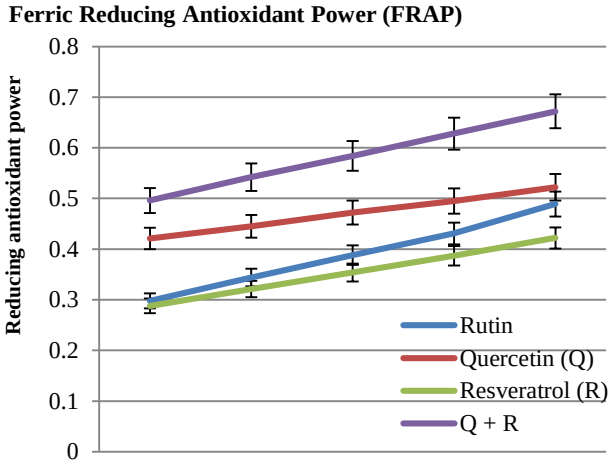

Figure 5. Ferric Reducing Antioxidant Power (FRAP)

Hydroxyl radical scavenging activity

Hydroxyl radicals are the most reactive oxygen-centered species and have the potential to severely damage biomolecules on their surface. As a result of the oxidation reaction, hydroxyl radicals are formed.[32] Compared to individual quercetin and resveratrol, synergistic quercetin-resveratrol mixtures (1:1) displayed the highest hydroxyl radical scavenging activity (Figure 6). The results indicated synergistic values of improved antioxidant efficiency of 5.2 % in HRSA when both compounds were employed in combination. The IC50 values of were 44.47, 35, 41.12 and 24.5 µg/ml for quercetin, resveratrol and quercetin resveratrol mixtures respectively (Table 4).

Table 4: IC50 Values

Compounds	IC50 µg/ml	Antioxidant Activity
Rutin (Standard)	44.47	Very Strong
Quercetin	35	Very Strong
Resveratrol	41.12	Very Strong
Quercetin + Resveratrol (Q+ R 1:1)	24.5	Very Strong

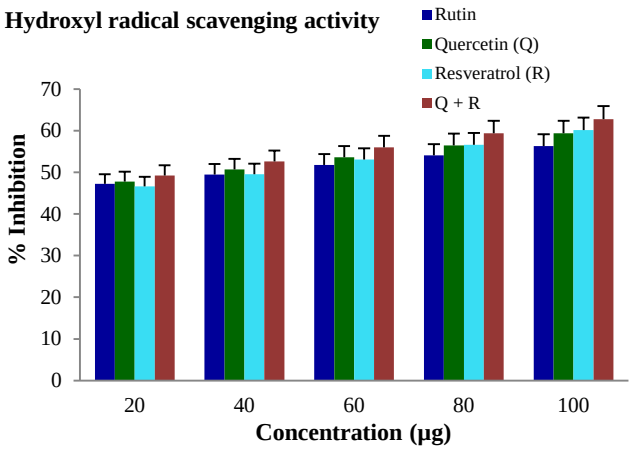

Figure 6: Hydroxyl radical scavenging activity

CONCLUSION

Since quercetin and resveratrol are bioactive flavonoids and phenols, it has antioxidant properties. In previous studies, it has been established that phenolic compounds are directly responsible for reducing oxidative stress. Quercetin, resveratrol, and their synergistic mixtures (1:1) were tested for *in vitro* antioxidant activity by Total peroxyl radical-trapping efficiency (TRAP), Total antioxidant capacity by Phosphomolybdate Assay (TAC), Ferric reducing antioxidant power (FRAP), and Hydroxyl radical scavenging activity (HRSA) tests. It can therefore be concluded that the quercetin-resveratrol mixtures of 1:1 produce the maximum antioxidant potential in synergism.

CONFLICT OF INTEREST

Authors do not have any conflict of interest for this study.

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