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LETTERS

In-vitro evaluation of synergism in antioxidant efficiency of Quercetin and Resveratrol

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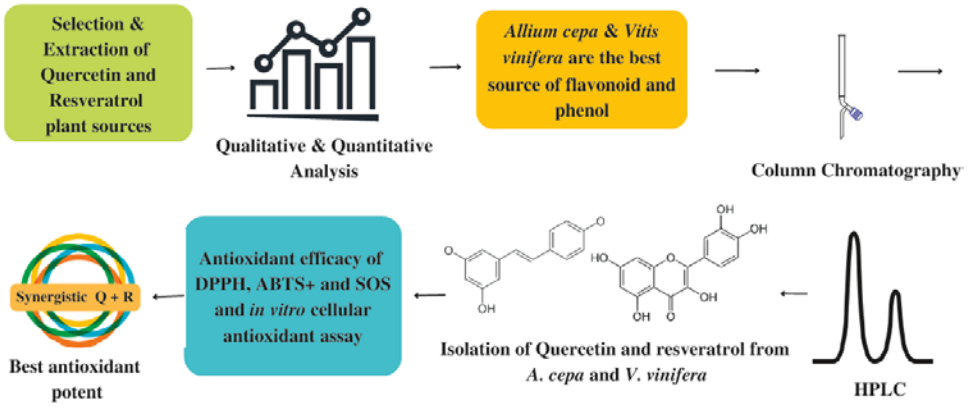
Article

Abstract



Plants serve as an excellent source of therapeutic molecules that help in medicinal treatments. The production of large amounts of pure phytocompounds from plant sources for human consumption and the nature of phytocompounds exhibiting toxicity issues at higher dosages lead to the challenge of increasing the therapeutic effect by using low dosages. This current study focuses on extracting two active antioxidant compounds, quercetin (Q) and resveratrol (R), from plant sources and evaluating their ability to exhibit antioxidant synergism through *in vitro* models. Quercetin and resveratrol were extracted using an ethanol-solvent extraction procedure from *Allium cepa,* and *Vitis vinifera* peels, respectively. The extracts were subjected to qualitative and quantitative analysis, column chromatography and then High-Performance Liquid chromatography for purification. DPPH, ABTS^{+}, SOS, and cellular antioxidant assays evaluated the synergistic antioxidant activity of the quercetin and resveratrol complex. The results showed synergistic antioxidant efficacy values approximately as follows: 5.37 % in DPPH, 15.26 % in ABTS+, 11.99 % in SOS, and 19.13 % in cellular antioxidant assays when both molecules were used combinedly. The results promisingly pave the way for a new dimension in nutraceuticals formulation parameters which could trigger combined molecular usage to achieve better results at low dosages.

Keywords: Antioxidant activity, Synergism, Quercetin, Resveratrol, Nutraceuticals

INTRODUCTION

People have been using medicinal plants worldwide as a source of healing since ancient times. The fact that medicinal plants serve as the primary healthcare delivery system for 85% of the world's population and as a source for drug discovery for 80% of synthetic pharmaceuticals shows that these plants are still significant even today.[1] Medicinal plants have many natural bioactive compounds found in the plant kingdom. Due to compounds such as phenols, flavonoids, anthocyanin, and other phytochemicals, medicinal plants play a very important role in preventing and treating several disorders.[2]

Plants contain bioactive compounds that are secondary metabolites for favourable outcomes for human and animal health.[3] Research has consistently demonstrated that daily intake of foods high in bioactive compounds, such as vitamins and phytochemicals, has a positive effect on human health and may reduce the risk of a variety of diseases, including cancer, heart disease, stroke, Alzheimer's disease, diabetes, cataracts, and age-related functional impairment.[4] The flavonoids and polyphenols exhibit different medicinal properties, which can cure different human diseases. One major therapeutic efficiency is their ability to act as antioxidants.

Antioxidants from plants have a variety of biological and pharmacological actions.[5] These antioxidants reach the body as dietary compounds. They are involved in scavenging and neutralizing free radicals, which are unstable agents, produced in the body through metabolic processes and can have the potential to damage cell membranes and other structures. To combat free radicals, the body contains natural antioxidants such as vitamins A, C, and E and minerals such as copper, zinc, and selenium,

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among others. Dietary antioxidants from plants act as non-nutrient antioxidants (for example, lycopene in tomatoes and anthocyanins in cranberries) and aid the body in disease prevention and control alongside natural antioxidants. Though dietary supplements have many positive effects, they still contain negatives, including overdose side effects. A drug overdose occurs when the dosage of a drug or other substance exceeds the recommended amounts. An overdose may cause a toxic state, pose health risks, and even lead to death.[6] Reducing the overdose can be practiced using different dimensions, known as synergism.

Synergy is a natural process in which two or more substances work together to produce a combined effect more significant than the sum of their individual effects.[7] Plant compounds are reported to have synergistic activity when combined. This current research work combines two critical dietary antioxidants, quercetin and resveratrol, and evaluates their synergistic antioxidant activities when combined.

This current research focuses on two important dietary supplements, quercetin and resveratrol, and their antioxidant activities.

Quercetin and resveratrol are phenolic, bioactive compounds found in fruits and vegetables.[8] Quercetin eliminates free radicals and strengthens the body's antioxidant defense systems. It scavenges free radicals and binds transition metal ions and inhibits lipid peroxidation. Resveratrol has anti-inflammatory, anti-cancer, and antioxidant properties and is used in a wide variety of foods, including red wine, grapes, berries, and peanuts.[9] Resveratrol contributes substantially to the health benefits because of its antioxidant properties.[10] Resveratrol's antioxidant properties are also linked to the activation of enzymes responsible for removing reactive oxygen species produced in the body.

In this study, Quercetin and resveratrol were isolated from fresh vegetable and fruit sources and analyzed the possibility of their combination in producing synergic ability in increasing the antioxidant potential at low doses through *in vitro* experiments.

EXPERIMENTAL SECTION

Collection of plant materials

Quercetin-rich sources such as *Allium cepa* (Small onion), *Solanum lycopersicum* (Tomato) and *Malus domestica* (Apple peel) and Resveratrol rich sources such as – *Vitis vinifera* (Grape peel), *Vaccinium cyanococcus* (Blueberry), and *Capsicum annuum* (Yellow pepper) were obtained from a local market. The plant materials were washed with running tap water and used as moisture-free samples in subsequent experiments.

Plant authentication

The samples were authenticated (BSI/SRC/5/23/2021/Tech/16-21) by Dr M.U. Sheriff PhD, Joint Director & Botanist, Botanical Survey of India, Coimbatore, Tamil Nadu.

Extraction of Quercetin from different plant samples

A. cepa (small onions) were taken, and the skin was peeled, washed with running tap water, and cleaned.[11] From that, 20g samples were weighed and ground using mortar and pestle. The paste was subjected to extraction with 100 mL of 80% hexane. The onion paste with the hexane mixtures was stirred using a magnetic stirrer for 24 hours at room temperature and then filtered using Whatman No. 1 filter paper. The filtrate was then concentrated at 65 °C under reduced pressure using a rotary evaporator as per the method of Muhammad Abdul Qadir *et al.* (2017) with slight modifications.[12] The same extraction procedure was carried out on a fresh tomato sample. The apple peel was manually removed and immediately boiled for 30 seconds to inactivate polyphenol oxidase activity, as described by Sekhon-Loodu *et al.* (2013), with slight modifications.[13] The boiled peels were then dried for 8 hours at 60 °C in a hot air oven, ground, and sieved to obtain powder using a 1 mm sieve. 10 g of powdered apple peel was extracted using 100 ml of hexane at room temperature in an ultrasonic bath and sonicated at the maximum power (50–60 Hz) for 20 minutes. The sonication was repeated twice and centrifuged for 10 minutes at rpm. The obtained supernatants were collected and stored at 4 °C. The above extraction procedure was repeated with different solvent substitutions, namely petroleum ether (40-60 °C), ethanol (80%), DMSO, toluene, and deionized water, in all plant samples.

Extraction of Resveratrol from different plant samples

20 g of frozen grape peel was homogenized for 30 seconds in a blender with 100 ml of hexane (80:20 v/v) and maintained at 60 °C for 30 minutes with gentle stirring. The extract was filtered through a stainless-steel colander and concentrated by rotary evaporation (in vacuo) at room temperature. The concentrated extracts were filtered through Whatman No. 1 filter paper.[14] The same extraction procedures were performed on fresh, whole blueberries and yellow peppers. The above extraction procedure was repeated with different solvents, namely, petroleum ether (40-60 °C), ethanol (80%), DMSO, toluene, and deionized water, in the remaining two plant samples.

Qualitative phytochemical analysis

(a) Test for flavonoids

To 1 ml of the extract, a few drops of 1% sodium hydroxide (NaOH) was added and observed the color changed to yellow. After a minute, a few drops of dilute hydrochloric acid were added and observed for the disappearance of the yellow color. The procedure was performed on all six extracts.

(b) Test for phenols

To 1 ml of the extract, 20µl of 1% Ferric chloride was added and observed the change of blue color was to a bluish-black precipitate. This confirms the presence of phenolic compounds. The procedure was performed on all six extracts.[15]

Quantitative phytochemical analysis

(a) Estimation of total flavonoid content

The Total flavonoid composition was analyzed using the aluminium chloride colorimetric method based on Chang *et al.* (2002) with a slight modification.[16] The extract was taken at 100, 200, 300, 400 and 500 µg/ml and made up to 1 mL of 96% ethanol. Added 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. The mixture was incubated at room temperature for 10 minutes with intermittent shaking. The absorbance was measured at 376 nm against a blank

using a UV-Vis spectrophotometer. Results were tabulated. Quercetin was used as the standard. The total flavonoid content expressed as quercetin equivalent was calculated by:

$$C=(c\times V)/m$$

Where,
C= total content of flavonoid compound, mg/g plant extract, in quercetin equivalent (QE);
c = the concentration of quercetin sample, mg/ml;
V = the volume of extract, ml;
m = the weight of extract, gm

(b) Estimation of Total Phenolic Content

The Folin Ciocalteu assay was used to calculate the total phenolic content. To 100, 200, 300, 400, and 500 µg/mL of extract, 1 ml of Folin's Ciocalteu reagent was added and left for 10 minutes, then 0.8 ml of Na_2CO_3 solution was added and placed in the dark for 30 minutes. The reaction turns the samples blue color depending on the phenolic content. The mixture was read in a UV spectrometer at 743 nm.[17] Resveratrol was used as the standard. The total phenol content in the extract expressed as resveratrol equivalent was calculated by:

$$C=(c\times V)/m$$

Where,
C= total content of flavonoid compound, mg/g plant extract, in resveratrol equivalent (RE);
c = the concentration of resveratrol sample, mg/ml;
V = the volume of extract, ml;
m = the weight of extract, gm

Thin Layer Chromatography

TLC was used to examine the presence of phenolic compounds of extracts using Vanillin: Ethanol as a mobile phase as per Sharma Archana *et al.* (2011) with slight modification.[18] The plate was kept under UV light and observed.

Column Chromatography

A glass column packed with 5 g silica gel in hexane and ethanol in a ratio of 1:1 was used. The sample was loaded. Fractions were collected and qualitative and quantitative tests were performed to confirm flavonoid and phenol content in the extracts, respectively. The fraction was then partially purified using the re-crystallization method.[19]

HPLC

The column fractions were further analyzed and purified using HPLC analysis. Agilent HPLC instrument was used for the purpose with column Luna 18 (2) (250 mm × 2.0 mm, 5 µm) in conjunction with a Luna C18 (2) (30 mm × 2.0 mm, 5 µm) guard cartridge. The column temperature was maintained at 30 °C. Elution was performed using a mobile phase made up of 1% (v/v) formic acid aqueous solution acetonitrile-2-propanol (70:22:8)- at a flow rate of 0.2 mL/min. Chromatograms were recorded at 306 and 370 nm for quercetin and resveratrol, respectively. Analytes in each sample were identified by comparing their retention times and UV visible spectra in the 220-450 nm range.[20]

Evaluation of synergism in antioxidant efficiency of quercetin and resveratrol complex

Radical scavenging activity using DPPH (Diphenyl-2-picrylhydrazyl) method: The DPPH radical method was used to determine the free radical scavenging activity of quercetin, resveratrol, and the quercetin-resveratrol complex (1:1). DPPH solution (0.1 mM) in ethanol was prepared, and 1.0 ml of the solution was mixed with 3.0 ml of the sample solution in water at various concentrations (20–100 μg). The absorbance was measured at 517 nm after 30 minutes. Higher free radical scavenging activity is indicated by the relatively low absorbance of the reaction mixture. Rutin was used as a standard. The following equation was used to calculate the ability to scavenge the DPPH radical.

% of DPPH of radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

The mean values were calculated from three replicate experiments.[21]

$ABTS^+$ radical scavenging assay

$ABTS^+$ radical scavenging assay was performed on quercetin, resveratrol, and the quercetin-resveratrol complex (1:1). ABTS radical was freshly prepared by adding 4.9 mM ammonium persulfate solution to 14 mM ABTS solution and kept in the dark for 16 hours. An absorbance of 0.396 at 734 nm was obtained by diluting the solution with 99.5% ethanol. A reaction mixture containing 950 µl ABTS radical solution and 50 µl sample solutions (20–100 µg/ml) were vortexed for 10 seconds. The absorbance was measured at 734 nm after 6 minutes and compared with that of the control ABTS solution. The percentage inhibition was calculated based on the formula.[22] The assay was carried out in triplicate. Rutin was used as a standard.

% inhibition= [1− absorbance of test/absorbance of control ×100]

Superoxide anion scavenging activity assay (SOS)

The scavenging activity of the samples against superoxide anion radicals was determined using Nishimiki *et al. with a* slight modification.[23] 1 ml of nitro blue tetrazolium solution, 1 ml of nicotine amide adenine dinucleotide solution, and 0.1 ml of quercetin, resveratrol and quercetin resveratrol mixtures and standard concentrations in water were mixed. 100 µl of phenazine methosulphate (PMS) solution (60 M) in 100 mM phosphate buffer (pH 7.4) was added to the mixture. The reaction mixture was incubated at room temperature for 5 minutes, and the absorbance was measured at 560 nm using a spectrophotometer against a reagent blank. The superoxide anion scavenging activity was calculated using the equation.

% Inhibition = control – test/control × 100

where the absorbance without samples was the control and the absorbance with samples was the test. The experiment was carried out in triplicates. Rutin was used as a standard.

Cellular Antioxidant Assay

Cellular antioxidant analysis was carried out through an *in vitro* antioxidant assay coupled with an MTT assay with slight modifications by H. Boutennoune *et al.*[24] A flat bottom culture plate with wells was taken and added to the HaCAT cell along with the culture medium (4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate) in the 6 wells (3 sets) and incubated for 24 hours at 37 °C with 5% CO2 in a humidified atmosphere. After incubation, the medium was removed and washed with

Phosphate Buffer Saline (PBS) to remove non-adherent cells and dead cells. Fresh media was added to each well and incubated for 1 hour at 37 °C. 100 µl quercetin was added to the set 1 wells, 100 µl resveratrol was added to the set 2 wells, 100 µl of the quercetin-resveratrol (1:1) complex was added to the set 3 wells, and 100 µl of deionized water was added to the control well in all the sets. The plates were incubated for 1 hour at 37 °C. After incubation, the cells were washed with Phosphate Buffer Saline (PBS). 100 µl of free radical generator sodium persulfate was added and kept for 15 min without disturbance. After the waiting period, the cells were washed immediately with PBS (pH 7.4) and subjected to the MTT (3-(4, 5-dimethylthiazol-2-yl) - 2, 5-diphenyltetrazolium bromide assay) to assess the viability. 20µL of MTT solution (5 mg/mL in PBS) was added to each well. The plates were then allowed to stand at 37 °C in the dark for 2 hours. The formazan crystals were dissolved in 100µL DMSO, and the absorbance was read spectrophotometrically at 570 nm using a microplate reader.[24] The cell viability was calculated using the following formula:

Cell viability (%) = ((Absorbance of sample)/ (Absorbance of control)) X100

Statistical Analysis

The results of all experiments were expressed as the Mean ± Standard deviation. GraphPad Prism version 9.4.1 and Microsoft Excel software were used for statistical analysis and representation.

Results and Discussion

Flavonoids and polyphenols are highly effective free radical scavengers and contribute significantly to antioxidant activity. Their redox properties play a major role in neutralizing and quenching free radicals, quenching singlet oxygen and triplet oxygen, and decomposing peroxides.[25] High dose-dependent side effects are a common phenomenon noted with phototherapeutic. The synergistic effects of phytochemicals could produce efficient results at a lower dosage. This current research was focused on evaluating the isolated bioactive compounds quercetin and resveratrol for their synergistic antioxidant properties.

Qualitative and quantitative analysis

The solvent extraction method was used to obtain crude extracts from the six plants, namely *M. domestica*, *S. lycopersicum*, *A. cepa* of quercetin, *V. vinifera*, *V. cyanococcus*, *C. annuum* of resveratrol sources. The qualitative profiling of flavonoids and phenols was conducted in different solvents such as hexane, petroleum ether 40-60 °C, ethanol, DMSO, toluene, and deionized water. Among these, ethanol was found to be best for extracting quercetin and resveratrol from the selected plant sources.

The results are summarized in Tables 1 and 2. Ethanol was reported as a better solvent for the extraction of phytocompounds due to its effectiveness in extracting substances and safety for human consumption.[26] Kiassos *et al.* (2009)[26] reported that increasing the ethanol concentration resulted in better extraction of phenolic compounds from different parts of onions.

Table 1: Qualitative screening for Flavonoids

S.No	Solvents	*M. domestica*	*A. cepa*	*S. lycopersicum*
1	Hexane	+	++	-
2	Petroleum ether 40-60° C	+	+	+
3	Ethanol	+++	+++	+++
4	DMSO	++	++	+
5	Toluene	+	+	+
6	Deionized water	++	++	+

(+)Low, (++) Moderate, (+++) High, (-) Nil

Table 2: Qualitative screening for Phenols

S.No	Solvents	*V. vinifera*	*C. annuum*	*V. cyanococcus*
1	Hexane	+	+	+
2	Petroleum ether 40-60° C	+	-	+
3	Ethanol	+++	+++	+++
4	DMSO	++	+	+
5	Toluene	+	+	+
6	Deionized water	+	+	+

(+)Low, (++) Moderate, (+++) High, (-) Nil

The retention factors (Rf) of ethanolic extracts of small onions (*A. cepa*) and grape peel (*V. vinifera*) *showed* a band identical to standard quercetin and resveratrol in table 3. Onion and grape peel extract bands and retention factor (Rf) was compared with standard quercetin and resveratrol bands to confirm their identities. TLC has qualitatively confirmed the phytochemical flavonoids and phenols present in the plant extracts by comparing the Rf value of unknown compounds with the Rf of a known reference compound.[27]

Table 3: Thin Layer Chromatography

Extracts	Rf value
Small onion	0.6
Grape peel	0.5
Standard Quercetin	0.61
Standard Resveratrol	0.56

The ethanolic extracts were subjected to quantitative analysis to estimate flavonoids and polyphenols. The total flavonoid content was high in small onions with 26.66 mg QE/g, compared to the levels in ethanolic apple peel and tomato extracts (Figure 1). The total phenolic content was highest in grape peels with 79.61 mg RE/g ion compared to the yellow pepper and blueberry (Figure 2). The Previous study by Slimestad R *et al.* identified the total flavonoid content of small onions ranging from 50 to 1300 mg/kg-1 FW. The total phenol content of grape peels was reported to range from 44 to 189 mg GAE/100g.[28] Many research reports have concluded that a plant sample has a linear relationship between its phenolic compound concentration and its antioxidant potential. The antioxidant responses of plant extracts are largely correlated with their polyphenol content.[29]

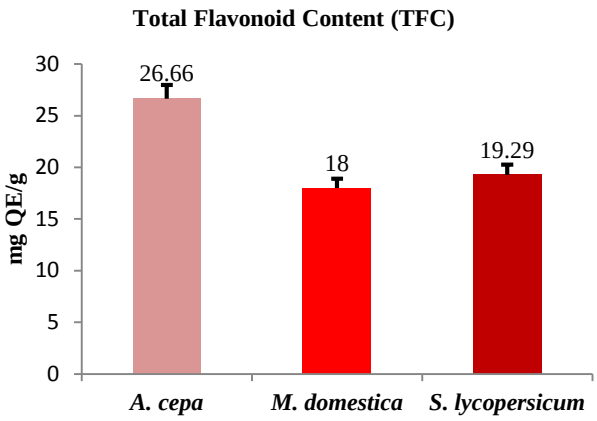

Figure 1: Total flavonoid content in plant extracts

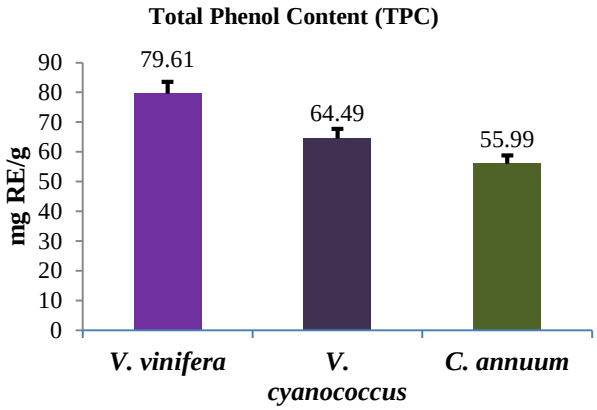

Figure 2: Total Phenol content in plant extracts

Partial purification of quercetin and resveratrol using chromatography

Small onion and grape peel extracts (5 ml each) were subjected to column chromatography on silica gel (100–200 mesh) separately. The fractions were subjected to a quantification assay. Among the fractions of onion extract, F3 showed a high level of flavonoid content of 11.96 mg QE/g **(**Figure 3). Among the fractions of grape peel extract, F2 contained a high level of phenols (26.37 mg RE/g (Figure 4)**.** The adsorbent-based stationary phase of this chromatography was composed of silica gel particles with 40–63 µm.[30] A low- or medium-polar constituent such as flavonoids can be isolated using this silica gel stationary phase.[31] Separation of bioactive compounds using column chromatography relies mainly on adsorption processes governed by hydrogen bonds forming between proton donors and acceptors in resins, as well as hydrophobic interactions with aromatic rings of phenolic compounds.[31] The fractions F3 (small onion) and F2 (grape peel) were further characterized by HPLC analysis to compare with reference standards and confirm their identity. The flavonoids were eluted from the column fraction of onion extract with a retention time of 6.5 minutes, with reference quercetin compound eluted at a retention time of 6.4 minutes (Figure 5 & 6). Studies have shown that quercetin has a retention time between 2.78 and 7.4 minutes and can be detected at 280 nm.[32] The phenols were eluted from the column fraction of grape peel extract with a retention time of 5.5 minutes, and the reference resveratrol compound was eluted at a retention time of 5.5 minutes (Figure 7 & 8). Singh G *et al*. research studies have shown that resveratrol has a retention time between 3.94 minutes to 7.86 minutes and can be detected at 360 nm.

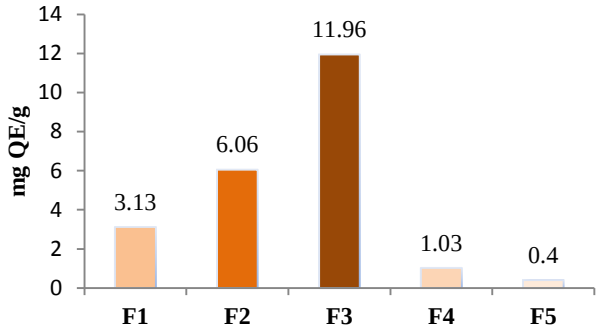

Figure 3: Quantification of flavonoids in small onion extract fractions

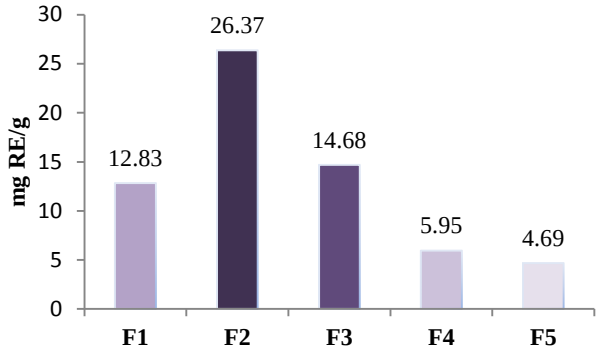

Figure 4: Quantification of phenols in grape peel extract fractions

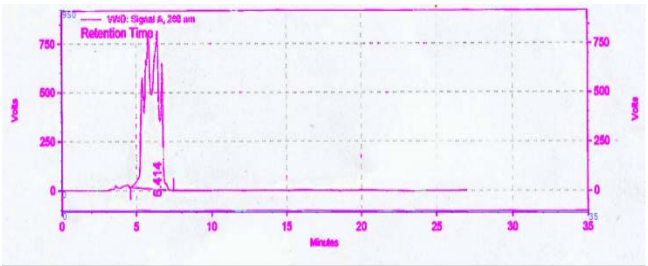

Figure 5: Quercetin Standard

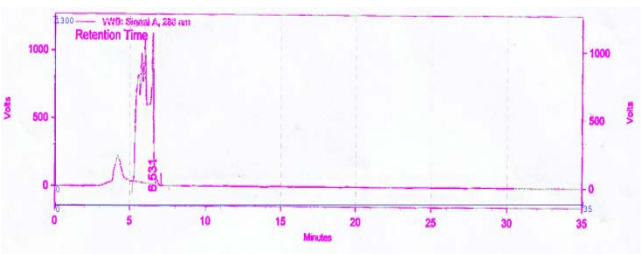

Figure 6: HPLC of fraction F3 (small onion extract)

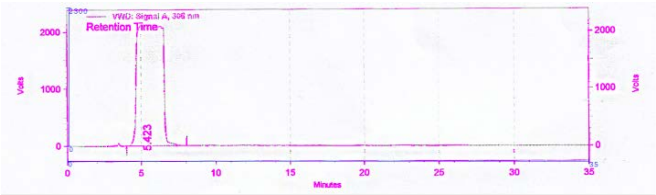

Figure 7: Resveratrol Standard

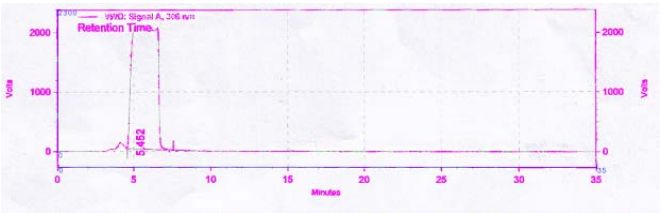

Figure 8: HPLC of fraction F2 (grape peel extract)

Evaluating synergism in antioxidant efficiency through *in-vitro* chemical and cellular methods

An evaluation of the antioxidant efficiency of the individual and combined formulations of quercetin and resveratrol was analyzed using the DPPH (Diphenyl-2-picrylhydrazyl) method, the $ABTS^+$ radical scavenging assay, and the superoxide anion scavenging activity assay (SOS). The results have shown that quercetin, resveratrol, and a quercetin-resveratrol composition (1:1) can act as effective antioxidants. The percentage of inhibition observed by all antioxidant models indicated that free radicals were scavenged in a concentration-dependent manner. The results have shown positively that the two molecules quercetin and resveratrol exhibit synergistic effects in increasing the antioxidant capacity of their composition. The results showed synergistic values of increased antioxidant efficacy of 5.37 % in DPPH (Figure 9), 15.26 % in $ABTS^+$ (Figure 10), and 11.99 % in SOS (Figure 11) when both molecules were combinedly used. In all antioxidant assays, the concentration of the antioxidant material required to scavenge 50% of the initial radicals (IC50) has been calculated. The results have shown that when combined (1:1), the antioxidant quantity required to attain the IC50 was considerably reduced. It required 21%, 40%, and 62% less potent compounds to achieve the IC50 to scavenge DPPH, $ABTS^+$, and SOS, respectively (Table 4 and Figure 13).

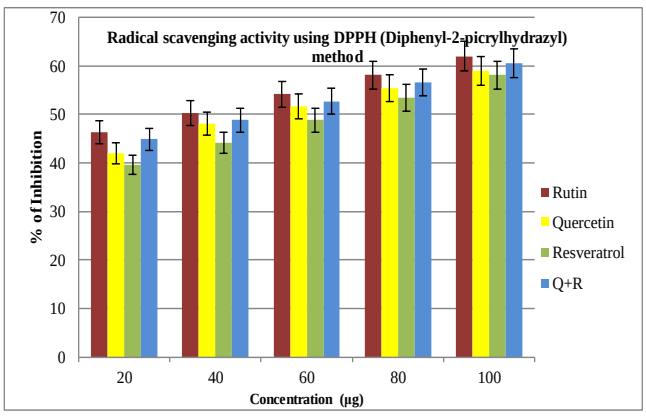

Figure 9: DPPH Activity

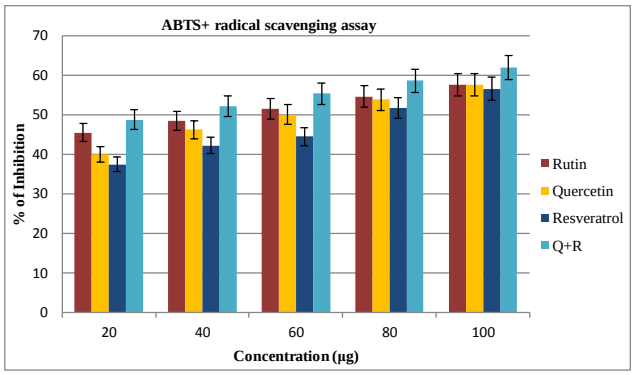

Figure 10: $ABTS^+$ radical scavenging activity

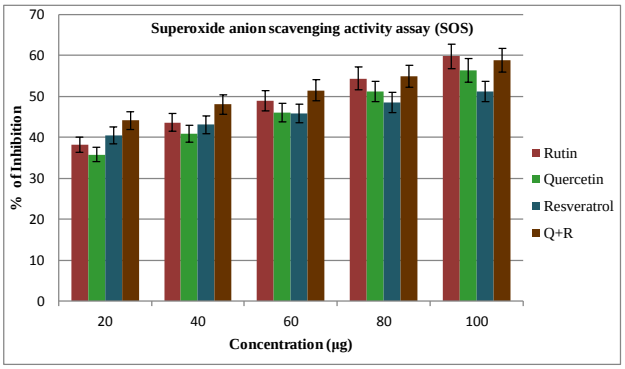

Figure 11: Superoxide anion scavenging activity assay (SOS)

Further evaluation of synergism in antioxidant efficiency has been analyzed through the *in vitro* cell line method. The antioxidant experiments were carried out on healthy HaCAT cell lines. The oxidant stress was created on the actively growing pretreated cells with different compositions of quercetin and resveratrol antioxidants through sodium persulfate. The efficiency of the antioxidant-pretreated and non-treated cells in fighting the antioxidant stress was analyzed and measured using the MTT assay. The results from the cellular antioxidant assay correlated with the *in vitro* chemical-based antioxidant analysis.[33] Both experiments exhibited dose-dependent increases in the antioxidant power of the tested individual compounds and the complex. Both quercetin and resveratrol exhibited strong synergism in protecting the cells from free radical stress. There is an increase of 19.13% in the antioxidant power exhibited by the quercetin and resveratrol complex in cellular protection (Figure 12).

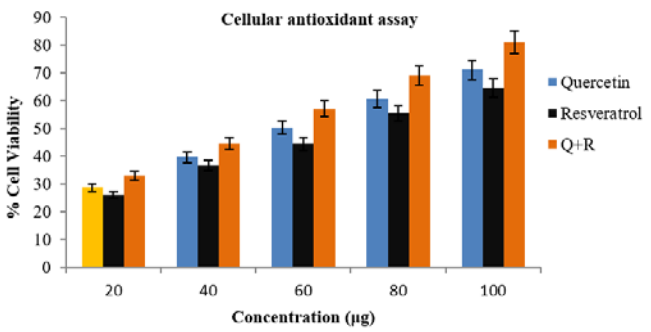

Figure 12: Cellular antioxidant assay

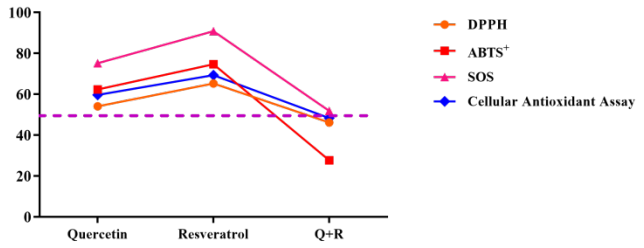

Figure 13: Comparison of IC50 requirements of individual compounds and complex

Table 4: Antioxidant required reaching IC_{50}

Compound required for	DPPH assay	ABTS assay	SOS assay	Cellular antioxidant assay
IC50 of Quercetin	54.11 µg	62.35 µg	75.24 µg	59.69 µg
IC50 of Resveratrol	65.27 µg	74.73 µg	90.86 µg	69.40 µg
IC50 of Q + R (1:1)	46.23 µg	27.68 µg	51.89 µg	48.40 µg

Several research reports have previously declared the antioxidant efficacy of the quercetin and resveratrol compounds. The bioactive compounds function through various chemical mechanisms to exhibit their antioxidant efficiency, including hydrogen atom transfer, single electron transfer, and their ability to chelate transition metals, which allows them to function as antioxidants.[34] The mechanisms of synergism can also vary from situation to situation. Lui *et al.* reported synergistic effects of phytochemicals showed the increasing potent antioxidant activities.[35] Nishtha S Srivastava *et al.* research proved that the antiproliferative properties of two phyto compounds are partly attributed to their synergistic effects on cancer cell proliferation.[36]

Noemí Arias *et al.* studies indicated that quercetin and resveratrol phytocompounds had synergistic effects in inhibiting adipose tissue triacylglycerol synthesis. Researchers justify that the phytochemicals found in polyphenol-containing foods can protect against cancer not by their individual effects but by their synergistic effect.[37] The current study results also coincide with the existing research outcomes that the synergic combination of quercetin and resveratrol can offer a higher antioxidant potential in relatively lesser doses through the mechanism of synergism. The results promisingly pave a new way in the nutraceuticals formulation parameters that could trigger combined molecular usage to achieve better results at low dosages, which can overcome many dose-dependent side effects and help reduce the cost of the medicine.

Conclusions

Plants possess immense amounts of compounds that exhibit the best therapeutic action inside the body when consumed. The activity of these compounds, however, is highly dose-dependent. At higher doses, the action of these therapeutic compounds has a higher chance of triggering side effects in the body. The major focus of this study is to find the ability of two compounds, quercetin and resveratrol, to exhibit synergism in producing antioxidant activity when combined. The *in vitro* research results have shown that the two plant-based compounds could produce synergistic results when exhibiting antioxidant activity. This synergism can be considered to further design the nutraceuticals formulations, which could supply dietary supplements to the body at a lower level yet maintain the same therapeutic efficacy. It could be a potential alternative to combat the dose-dependent toxicity challenges of phytocompounds.

Conflict Of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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