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DPPH RADICAL SCAVENGING ACTIVITY OF SELECTED FLAVONOIDS: EXPERIMENTAL EVALUATION OF ORIENTIN, BREVISCAPINE AND VITEXIN

Abhay Shripad Joshi[1*], Vikas G. Rajurkar[2]

[1]Research Scholar, Dr. Vedprakash Patil Pharmacy College, Chhatrapati Sambhajinagar, Maharashtra, India. Email: abhay.joshirss@gmail.com
[2]Principal , Dr. Vedprakash Patil Pharmacy College, Chhatrapati Sambhajinagar, Maharashtra, India.

ABSTRACT:
Oxidative stress involves reactive oxygen species and other reactive species and flavonoids are widely studied for their ability to neutralize radicals. In this study, we compared the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of orientin, breviscapine and vitexin at concentrations of 1,2,5,10,25, and 50 µg/mL. Absorbance was measured at 517 nm and a sample-specific blank correction was applied before calculating percentage radical-scavenging activity (% RSA). The DPPH control showed an absorbance of 0.8897. All three compounds showed an increase in % RSA in a concentration-dependent manner. Orientin rose from 13.50 ± 0.02% at 1 µg/mL to 74.86 ± 0.30% at 50 µg/mL and passed the 50% response between 10 and 25 µg/mL. Linear interpolation between the experimental concentrations yielded an estimated IC_{50} of 21.3 µg/mL. Breviscapine rose from 13.51 ± 0.35% to 63.03 ± 0.35% across the same concentration range giving an interpolated IC_{50} of 29.3 µg/mL. Vitexin rose from 12.62 ± 0.26%, to 49.33 ± 0.32% and did not reach 50% RSA at the highest tested concentration; therefore Vitexin IC_{50} was reported as >50 µg/mL. Under the present experimental conditions the relative DPPH radical-scavenging activity was orientin > breviscapine > vitexin. The findings provide an experimental comparison of the three compounds while the limited concentration range and triplicate design warrant cautious interpretation of IC_{50} estimates.

KEYWORDS: Oxidative stress; Reactive oxygen species; Flavonoids; Orientin; Breviscapine; Vitexin; DPPH radical-scavenging activity; Antioxidant activity; IC_{50}; Free radical scavenging

INTRODUCTION

Reactive oxygen species and other similar reactive species are always made during cell activity. When too much of them is made it can be more than the bodys natural defenses can handle. This can cause damage to lipids, proteins, and DNA and may contribute to several pathological conditions. (Halliwell and Gutteridge 2007; Sies, 2015). Because of this, research on antioxidants remains important in medicine in food science and, in studies of products. However, the antioxidant activity seen in lab tests does not always match how well the antioxidants work in the body (Prior et al., 2005; Shahidi and Zhong, 2015).

Flavonoids are a group of plant polyphenols. Their ability to fight radicals depends on how many hydroxyl groups they have and how they are arranged. This includes things like conjugation, substitution and glycosylation (Pietta, 2000; Heim et al., 2002; Procházková et al., 2011). Studies that look at structure and activity have found that the way hydroxyl groups are placed especially having two of them next to each other in the B ring can change how well they act as antioxidants (Bors et al. 1990; Rice-Evans et al., 1996; Zheng et al., 2011). Other factors that help stabilize radicals can also have an effect. Glycosylation can change how strong the antioxidant effect is and how fast it works. So even if two flavonoids are similar in structure they might not act the way, in a test (Fukumoto and Mazza 2000; Xiao et al., 2021).

Orientin is a luteolin C-glycoside that comes from medicinal and food plants. It has been studied for its effects and other biological activities. Researchers have looked into it in studies, including those by Miean and Mohamed in 2001 and Ahmed and Rajendran in 2026.

Vitexin is another compound, this one a C-glycoside. It also shows activity. Scientists have tested it in both biological models. Important studies include those by Kawaii and team in 1999 Kim and colleagues in 2005 and He and others, in 2020.

Breviscapine is a flavonoid-based preparation made from the plant Erigeron breviscapus. It has shown activity in several cell-free tests. One of the tests used was the DPPH scavenging assay. This was reported by Wang and team in 2008

The DPPH assay is a commonly used spectrophotometric technique to measure how well substances can scavenge free radicals. DPPH is a nitrogen-centered radical. When DPPH is reduced by compounds that donate hydrogen or electrons the absorbance of the solution drops. This drop is usually measured near 515–517 nm (Blois, 1958; Brand-Williams et al. 1995; Molyneux, 2004). The DPPH assay is easy to perform and cheap. DPPH results can change with reaction conditions the solvent used, how long the reaction goes on the colour of the compound and interference from other colours. Also DPPH itself is not a radical that occurs naturally in the body (Sánchez-Moreno, 2002; Roginsky and Lissi 2005; Gülçin, 2020).

The main goal of this study was to find out how the DPPH radical-scavenging activity of breviscapine, orientin and vitexin changes when the concentration goes from 1 µg/mL to 50 µg/mL all under the same experimental settings. The

study also sought to compare the IC_{50} values that come directly from the data. Only the raw data, in the study dataset were used for analysis. No IC_{50} values were estimated beyond the tested range if the 50 % RSA point had not been reached.

MATERIAL AND METHODS

Chemicals and reagents

DPPH was used as the radical reagent. Orientin, breviscapine and vitexin were evaluated as test compounds. Appropriate solvents were used for the preparation and dilution of the test solutions. Reagents were of suitable experimental grade. The experimental dataset analyzed in the manuscript was obtained from Sheet2 of the study workbook.

Preparation of test solutions

Stock solutions of the three test compounds were prepared at concentrations with the actual stock concentration used in the experiment. These were then serially diluted to obtain concentrations of 1,2,5,10,25 and 50 µg/mL. Each concentration was evaluated in triplicate.

DPPH scavenging assay

The assay was performed using a DPPH radical-scavenging procedure. A DPPH control containing DPPH and solvent without test compound was used to establish the reference absorbance. Sample blanks containing the test compound and solvent without DPPH were used to correct for background absorbance. Absorbance was measured at 517 nm.

The three DPPH control measurements were 0.8624, 0.9051 and 0.9015 giving a mean control absorbance of 0.8897. For each compound and concentration the sample-plus-DPPH absorbance was corrected by subtracting the corresponding sample before calculation of % RSA.

Calculation of radical-scavenging activity

$$\% \text{ RSA} = [(A_{control} - A_{corrected\ sample}) / A_{control}] \times 100 \quad (1)$$

where Acontrol is the mean absorbance of the DPPH control and Acorrected sample is the sample-plus-DPPH absorbance after subtraction of the corresponding sample blank.

Determination of IC_{50}

IC_{50} is the concentration that produces 50% scavenging of the DPPH radical. When the 50% response lay between two concentrations the IC_{50} was found by linear interpolation of those neighbouring points. For vitexin 50% RSA could not be achieved at 50 µg/mL so the IC_{50} for vitexin was conservatively reported as greater than 50 µg/mL instead of an extrapolated figure. Because the concentration range used did not reach response plateaus the nonlinear four-parameter logistic estimates were regarded as exploratory not as the main IC_{50} values.

Statistical analysis

Data are shown as mean ± Standard deviation (SD) with three (n = 3) measurements, for each concentration. The concentration-response relationship was examined in a manner. No inferential p-values were produced because the data include triplicate measurements at each concentration. It is unclear whether these represent independent biological replicates. IC_{50} values were derived from the observed response data using interpolation when it was suitable.

RESULTS

DPPH radical-scavenging activity

We observed that all three investigated flavonoids orientin, breviscapine and vitexin showed increasing DPPH radical-scavenging activity as concentration rose. Orientin displayed the activity throughout the tested range rising from 13.50 ± 0.02 % RSA at 1 µg/mL to 74.86 ± 0.30 % RSA at 50 µg/mL. Breviscapine increased from 13.51 ± 0.35 % to 63.03 ± 0.35 % whereas vitexin increased from 12.62 ± 0.26 % to 49.33 ± 0.32 %.

At 10 µg/mL orientin, breviscapine and vitexin recorded 37.28 ± 0.80 %, 33.77 ± 0.41 % and 26.23 ± 0.43 % RSA, respectively.

At 25 µg/mL orientin, breviscapine and vitexin reached 54.22 ± 0.39 %, 47.30 ± 0.33 % and 35.19 ± 0.34 % RSA.

At 50 µg/mL orientin produced the response at 74.86 ± 0.30 % followed by breviscapine at 63.03 ± 0.35 % and vitexin at 49.33 ± 0.32 %.

These results show a ranking of orientin > breviscapine > vitexin, within the tested range.

Table 1 shows the concentration-response dataset. The low SD values show that the three measurements, at each concentration, are very close together. Low SD values do not automatically mean that the results are biologically reproducible.

Table 1. DPPH radical-scavenging activity of orientin, breviscapine and vitexin. Values represent mean ± SD, n = 3.

Concentration (µg/mL)	Orientin, % RSA (mean ± SD)	Breviscapine, % RSA (mean ± SD)	Vitexin, % RSA (mean ± SD)
1	13.50 ± 0.02	13.51 ± 0.35	12.62 ± 0.26
2	17.78 ± 0.14	17.95 ± 0.33	15.03 ± 0.18
5	25.93 ± 0.48	25.60 ± 0.34	20.69 ± 0.45
10	37.28 ± 0.80	33.77 ± 0.41	26.23 ± 0.43
25	54.22 ± 0.39	47.30 ± 0.33	35.19 ± 0.34
50	74.86 ± 0.30	63.03 ± 0.35	49.33 ± 0.32

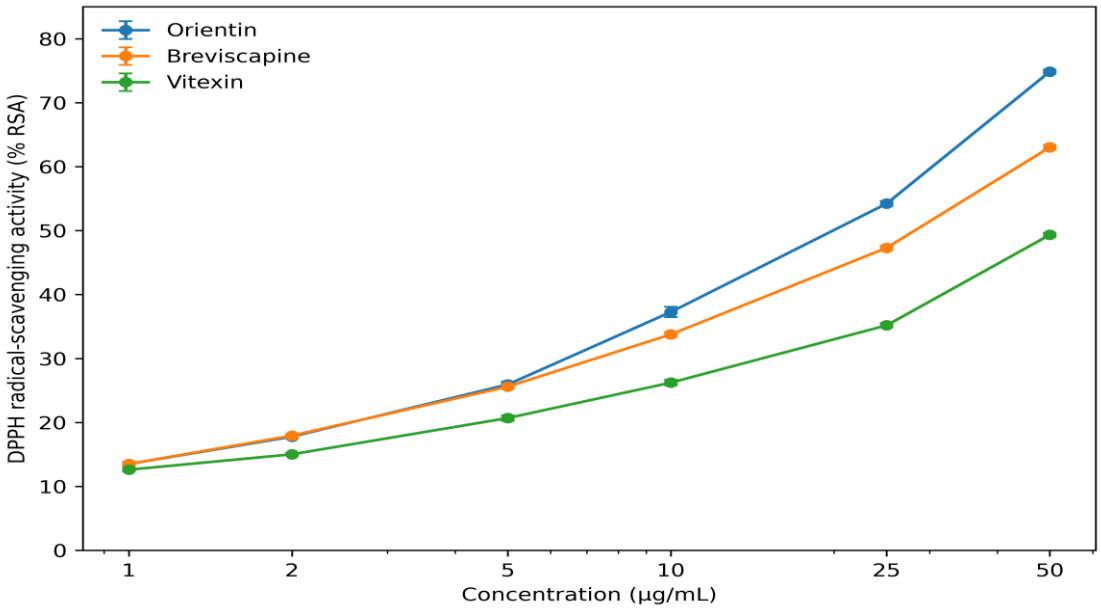

Figure 1. Concentration-dependent DPPH radical-scavenging activity of orientin, breviscapine and vitexin. Points represent mean ± SD (n = 3) at 1, 2, 5, 10, 25 and 50 µg/mL. Concentration is shown on a logarithmic x-axis.

Estimated IC_{50} values

Orientin passed the 50 % RSA threshold between 10 µg/mL (37.28 ± 0.80 %) and 25 µg/mL (54.22 ± 0.39 %). Using interpolation between these two measured concentrations I estimated the IC_{50} for Orientin at 21.3 µg/mL. Breviscapine passed the 50 % RSA threshold between 25 µg/mL (47.30 ± 0.33 %) and 50 µg/mL (63.03 ± 0.35 %) giving an IC_{50} of 29.3 µg/mL. Vitexin reached 49.33 ± 0.32 % RSA at 50 µg/mL so it did not reach the 50 % target; therefore the IC_{50} for Vitexin was reported as greater, than 50 µg/mL (Table 2).

Table 2. Estimated IC_{50} values of the investigated flavonoids. IC_{50} values are based on the observed experimental concentration-response data; no IC_{50} was extrapolated for vitexin beyond the tested range.

Compound	Estimated IC_{50} (µg/mL)	Method	Interpretation
Orientin	21.3	Linear interpolation	50% RSA bracketed by 10 and 25 µg/mL
Breviscapine	29.3	Linear interpolation	50% RSA bracketed by 25 and 50 µg/mL
Vitexin	>50	Not reached	49.33% RSA at 50 µg/mL; endpoint not reached

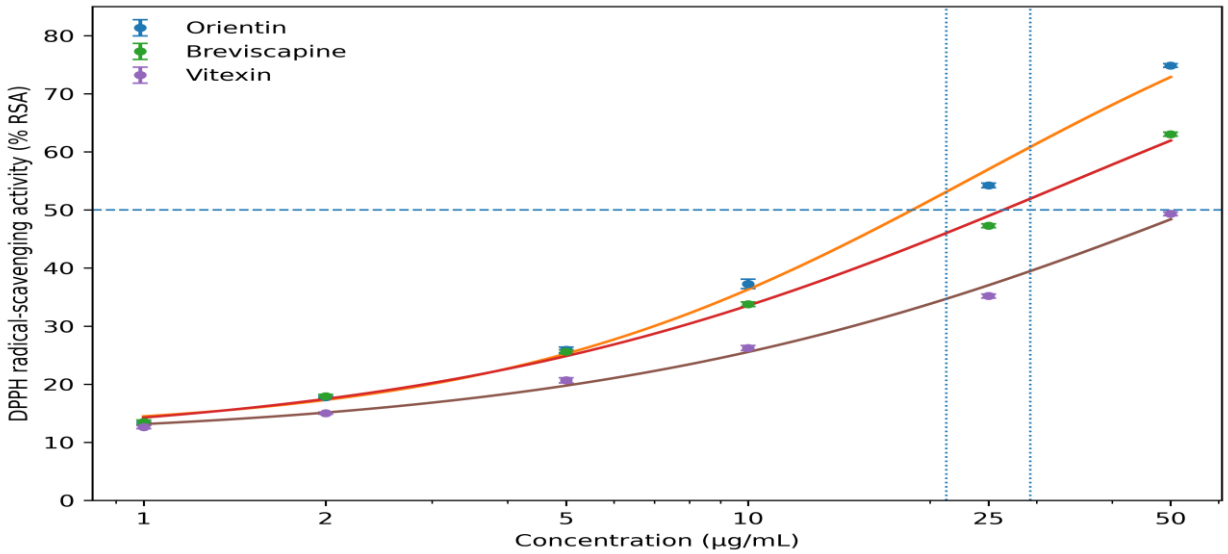

Figure 2. DPPH dose-response curves with exploratory constrained four-parameter logistic fits. Points represent mean ± SD (n = 3). The horizontal dashed line represents 50% RSA. Vertical reference lines indicate the interpolated IC_{50} values for orientin and breviscapine. The vitexin curve did not reach 50% RSA within the tested concentration range and therefore no experimental IC_{50} marker is shown for vitexin.

DISCUSSION

The current study showed that all three investigated compounds exhibited concentration-dependent DPPH radical-scavenging activity. A direct comparison was made under the testing conditions and within the same concentration range. This helped to reduce some of the differences that can make comparisons between antioxidant tests confusing. However the DPPH results are specific to that test. Should not be seen as a direct measure of how well these compounds work in living organisms (Prior et al., 2005; Shahidi and Zhong, 2015; Gülçin, 2020).

Orientin showed the highest activity. It reached 74.86 ± 0.30% RSA at 50 µg/mL. The IC_{50} value was 21.3 µg/mL. Previous studies have shown that orientin has antioxidant properties in both cell-free settings and in living systems. These include studies looking at stress and damage caused by radicals (Uma Devi et al., 2000; Thangaraj et al., 2018). The current results add to these findings by comparing orientin with breviscapine and vitexin under the same DPPH test conditions.

Breviscapine showed moderate radical-scavenging activity. It achieved 63.03 ± 0.35% RSA at 50 µg/mL.The IC_{50} value was 29.3 µg/mL. This matches what has been found in earlier studies where breviscapine showed DPPH and other types of radical removal (Wang et al. 2008). Since this experiment uses the material as it was provided in the data set the result should be seen as a response to DPPH and not as proof of a specific way it works or as evidence of the effect of one part of the mixture.

Vitexin showed concentration-dependent radical-scavenging activity across the tested concentration range. It reached 49.33 ± 0.32% RSA at 50 µg/mL. This was close to the 50% mark but it did not quite reach it. So the safe conclusion is that the IC_{50} is higher than 50 µg/mL. This matches what has been found in earlier reports about vitexins antioxidant activity, including its ability to remove DPPH radicals. However differences in how the tests āre done and the concentrations used can lead to different numbers (Kim et al., 2005; He et al. 2020).

Differences between the three compounds may be due to their structures. The ability to remove radicals depends on how they're built, including where the hydroxyl groups are, how electrons move and where other groups are added or removed (Bors et al., 1990; Rice-Evans et al., 1996; Heim et al., 2002; Zheng et al., 2011). Orientin and vitexin are both C-glycosides. They have different base structures. These differences can affect how they take part in removing radicals. However this test does not prove how the structure affects the activity. To find out more chemical or computer studies would be needed.

The DPPH test has some limits. The speed of the reaction can be very different for each antioxidant. The results can also be affected by the type of solvent, the amounts of the reagents, the reaction time, and other light effects and other light effects (Brand-Williams et al., 1995; Sánchez-Moreno, 2002; Roginsky and Lissi 2005). In this test a blank correction was used to account for these. Using three measurements helps show how variation there is but it does not prove that the results are from real biological samples.

The IC_{50} results were carefully chosen. A full four-parameter model needs data to define both the top and bottom parts of the response and the middle point. This test only used six concentrations. Did not reach a full flat line for any of the three compounds. So the IC_{50} values for orientin and breviscapine 21.3 µg/mL and 29.3 µg/mL are best seen as estimates of how the response changes with concentration not as values for how strong the compounds are. For vitexin saying the IC_{50} is higher than 50 µg/mL is safer because the experiment did not go enough to support a number higher than that.

The main problem with the study is that the concentration range was limited and there were no concentrations above 50 µg/mL. Future experiments should include more concentrations around the 50% response. It would be better to have points to see both ends of the response. Also more experiments should be done to check the results again. Other kinds of tests that use different ways, to work could also help see if the order of the compounds is just because of DPPH or if it shows up in other tests too (Prior et al., 2005; Shahidi and Zhong, 2015).

CONCLUSION

Orientin, breviscapine and vitexin all showed concentration - Dependent DPPH radical-scavenging activity across the tested range of 1 to 50 µg/mL. Orientin had the strongest effect reaching 74.86 ± 0.30% RSA at 50 µg/mL with an estimated IC_{50} of 21.3 µg/mL. Breviscapine showed moderate activity with 63.03 ± 0.35% RSA at 50 µg/mL and an estimated IC_{50} of 29.3 µg/mL. Vitexin had the weakest response achieving only 49.33 ± 0.32% RSA at 50 µg/mL and its IC_{50} was reported as greater than 50 µg/mL. Under the current experimental conditions the order of activity was orientin, then breviscapine, then vitexin. These results reflect DPPH radical-scavenging capacity and should not be taken as a direct measure of antioxidant activity, in biological systems.

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