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Antioxidant Activity of Resveratrol Analogs

Natalia Oliveira Calil[1], Gustavo Senra Gonçalves de Carvalho[2], Danielle Cristina Zimmermann Franco[1], Adilson David da Silva*[,2] and Nádia Rezende Barbosa Raposo*[,1,3]

[1]*NUPICS, Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Juiz de Fora, M.G. 36036-330, Brazil*

[2]*Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Juiz de Fora, M.G. 36036-900, Brazil*

[3]*Laboratório de Neurociências, LIM 27, Universidade de São Paulo, São Paulo, SP.*

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Abstract: This study evaluated the antioxidant activity of five resveratrol analogs by relating the activity of the molecule with its chemical structure. The five resveratrol analogs were synthesized and the antioxidant activity was evaluated using the DPPH method. The resveratrol was used as the reference standard. A descriptive statistical analysis and ANOVA followed by the Tukey test, with the aid of software. The antioxidant activity of resveratrol analogs was considered statistically different, with the analog **A** which showed activity superior to the others. The five analogs presented lower antioxidant activity than the reference standard ($p < 0.001$). According to the findings, hydroxylation was the molecular modification that gave the best evaluated antioxidant activity result. Resveratrol analogs may have an important anti-oxidative activity, but with the one with the higher IC_{50} was presented by the natural compound.

Keywords: Antioxidants, Chemical Synthesis, 2,2-diphenyl-1-picrylhydrazyl, Hydroxylation, Resveratrol, Structure-Activity Relationship.

INTRODUCTION

The search for a healthy life has stimulated habit changes in the world population, as the growing interest in the use of natural products [1]. Flavonoids are worth mentioning because they cover a wide range of substances capable of meeting this need [2], one of them being resveratrol (3,5,4'-trihydroxyestilbene) [3].

Regarded as a chemo preventive agent, polyphenolic phytoalexin this belongs to the class of stilbenes, actively protecting cells against oxidative damage observed in several diseases caused by reactive oxygen species (ROS) [4] and reactive nitrogen species (RNS) [5].

Resveratrol is particularly present in the grape and its derivatives which has become a composite of interest for its broad spectrum of action as an antioxidant, cancer [6] and aging preventive [7], cardioprotective [3], anti-inflammatory [4] and its ability to reduce obesity [8]. Studies suggest an important participation of this substance as a protector of acute kidney disease [4] and Alzheimer's disease [9]. As an anti-proliferative agent in certain types of tumors, resveratrol works by inducing apoptosis [10]. The combination of this drug with certain drugs [11], ionizing radiation, or cytokines may be a way to optimize anticancer therapy [12].

Another related benefit of resveratrol is the reduction of hepatotoxic effects caused by isoniazid and rifampicin, drugs used to treat tuberculosis [13]. Its antioxidant activity also gives it the ability to selectively inhibit the monoamine oxidase enzyme type A (MAO-A) [14], the target of drugs used as antidepressants [15].

However, according to some studies, stilbenes, despite being well absorbed, have low bioavailability [12], which is one of the limiting factors for its wide use as medicine. This emphasizes the importance of searching for molecules similar to resveratrol that retain antioxidant activity, but with improved bioavailability.

This study evaluated the antioxidant activity of five resveratrol analogs by relating the activity of the molecule with its chemical structure.

MATERIAL AND METHODS

Chemistry

A series of azaresveratrol derivatives were synthesized and their antioxidant activities were examined. The structure of this compounds, 1-N-(4'-hydroxy-benzylidene) aniline (A), 1-N-(4'-carboxy-benzylidene) aniline (B), 1-N-(4'-nitro-benzylidene) aniline (C), 1-N-(4'-methoxy-benzylidene) aniline (D), 1-N-(4'-dimethylamino-benzylidene) aniline (E) are show in the Table **1**.

All compounds were characterized by ^{1}H and ^{13}C NMR, and melting point (Table **2**) and were in accord with data in the literature [16-19].

Antioxidant Activity

The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, USA) assay, described by Sreejayan and Rao [20], with minor modifications. This method is based on the DPPH reduction

*Address correspondence to these authors at the NUPICS, Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Juiz de Fora, M.G. 36036-330, Brazil. Tel/Fax: +55 32 21023809. E-mail: nadiafox@gmail.com

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Juiz de Fora, M.G. 36036-900, Brazil. Tel: +55 32 21023310. E-mail: david.silva@ufjf.edu.br.

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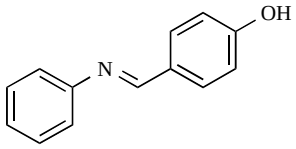
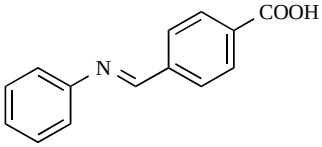
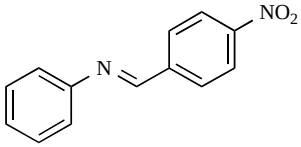
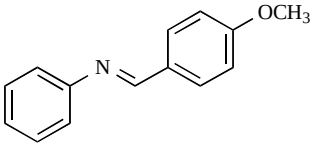
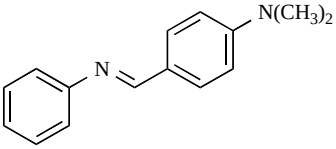
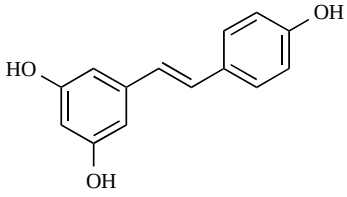
Table 1. Concentration of Synthetic Resveratrol Analogs and of the Standards that Inhibits 50% of DPPH Radicals

Sample	Chemical Structure	IC_{50} (µg/mL)*
A		103.8
B		279.7
C		356.6
D		528.0
E		609.7
Resveratrol		8.5

* $p < 0.05$ for comparison between average values of IC_{50} of resveratrol and resveratrol analogs.

Table 2. Spectral Data of Aza-Stilbene Derivatives

Compounds	δ H_{imine}	δ $C{=}N_{imine}$	Melting point
A	8.44	160.0	89.2-90.7
B	8.72	159.8	220.4-221.6
C	8.80	158.8	89.6-90.7
D	8.51	159.8	61.4-62.1
E	8.39	159.9	96.8-97.3

The NMR experiments were performed at 300 MHz for ^{1}H and 75 MHz for ^{13}C in DMSO-d6 (ppm) and melting point (ºC).

in the presence of an antioxidant (AH) a proton donor (H^{+}) for a non-radical (DPPH-H) [21].

A 150 µL of a DPPH ethanolic solution of 0.05 mM were added to 50 µL of ethanol solution of resveratrol analogs at concentrations from 0.97 to 250 µg /mL in 96-well plates. Resveratrol (trans-resveratrol 99.0%, Attivos Magistrais, Brazil) was used as a standard at the same concentrations.

Reactions elapsed at room temperature for 30 minutes in the dark and then the absorbance was read in a spectrophotometer (λ= 510 nm).

Inhibition of DPPH radical was calculated using the equation:

$$\% \text{ of inhibition} = 100 \times (A_0 - A_s) / A_o$$

Being: A_o absorbance as the negative control and A_s a absorbance of test samples.

The IC_{50} value was calculated from the line in the equation of the linear dispersion graph. All tests were performed in triplicate.

Statistical Analysis

We performed a descriptive statistical analysis and ANOVA followed by the Tukey test, with the aid of Statistical Package for Social Sciences (SPSS) v.14.0 for Windows software, to compare the average values of IC_{50} calculated between the resveratrol analogs *versus* the standard. The level of significance was 5%.

CONCLUSION

The IC_{50} results revealed that substance **A** was the analog which needed the lowest concentration to inhibit the DPPH radical, but higher than the concentration shown by the reference standards and the other analogs showed no significant values (Table **1**).

The antioxidant activity of resveratrol analogs was considered statistically different, with the analog **A** showed activity superior to the others.

The antioxidant activity of resveratrol analogs *versus* reference standard showed that these are statistically different bearing, the analogs, presented lower antioxidant activity than the resveratrol ($p < 0.001$).

Polyphenolic compounds such as resveratrol and flavonoids, have reducing properties and chemical structures that play an important role in neutralizing free radicals and chelation of transition metals, both in the initiation step as in the propagation of oxidative stress [22]. The intermediaries formed by the action of phenolic antioxidants are relatively stable due to the resonance of the aromatic ring in the structure of these substances [23].

Barreiros and colleagues describe five factors besides the chemical structure, which may influence flavonoid antioxidant activities. They are a reactive agent as a proton and electron donor agent, the stability of the flavonoil radical formed, reactivity with other antioxidants, the ability to chelate transition metals and solubility and interaction with membranes [24].

For Baur and Sinclair, in the case of resveratrol in mammals, it should be remembered that the compound has extremely low bioavailability and undergoes rapid clearance from the bloodstream [6]. Studies have shown that resveratrol is rapidly absorbed but quickly metabolized, usually in the first hour following oral administration in humans [25]. Some alternatives to improve resveratrol bioavailability were blocking its metabolism, the discovery of more potent compounds that mimic its effects or developing analogs with improved bioavailability [6].

Resveratrol has been shown to be extensively metabolized through glucuronidation and sulfation [25-27]. Where as glucuronidation was considered the main conjugation pathway [25]. Glucuronides and sulfates may bind one or two hydroxyl residues [28]. The sites of glucuronidation in the resveratrol molecule there are the 3 and 4' positions and major site of sulfate conjugation is the 4' position [29]. Resveratrol metabolites have a very short half-life and are subjected to rapid urinary elimination [25].

Based on this information the hydroxyl groups in 3 and 5 positions were replaced by hydrogen atoms in order to minimize the formation of metabolic conjugates. In addition, evaluated functional groups were inserted in position 4'. According to Walle and colleagues, methylation of the polyphenols effectively blocks the metabolic conjugation reactions, thereby dramatically increasing both metabolic and stability [30]. This substitution was present in the analog **D**.

Based on the concept of bioisosterism [31] the basic skeleton of trans-stilbene was modified by replacement of the central C=C linkage with a C=N double bond.

In an attempt to preserve the resveratrol antioxidant activity, the five analog molecules in this compound kept the two aromatic rings and double bonds; they can form relatively stable intermediates due to resonance of the ring [32].

However, the compounds tested, only analog **A**, which has a hydroxyl as differential grouping in their structure, it presented satisfactory results as an antioxidant. This can be attributed to the reducing power of the aromatic hydroxyl that reduces reactive free radicals and produces the phenoxyl radical, stabilized by resonance [5]. The behavior of analog **A** also supports studies showing that the degree of antioxidant activities of is directly related to the number of hydroxyl groups present in the molecule [33, 34].

Regarding the structure of the analog **B**, uptake of free radical DPPH was due to the presence of the group carboxyl in C-4, but this occurred in a less effective manner than of the analog **A**, allowing to infer that the phenolic hydroxyl has greater potential to reduce. But for Rosa and colleagues the carboxyl groups in some locations can interact with metal ions, responsible for initiating the Fenton reaction, responsible for production of hydroxyl radical. Thus, molecules containing carbonyl group would be useful as metal chelators [35].

The analog **C** is a nitro compound and presented intermediary antioxidant activity. Most of the nitro compounds showed a bio enzymatic reduction mechanism and these results in the formation of free radicals, but with preferential toxicity to bacteria and parasites [36].

The analogs **D** and **E** had no significant results on the antioxidant activity test, which can be explained by the fact of not presenting the above mentioned analog groups and possibly by the lower polarity presented by methoxyl groups and amine, respectively. According to Chun and colleagues, the hydrophilic and hydrophobic behavior of phenolic compounds influence the antioxidant activity given by the DPPH assay, which primarily measures the antioxidant activity of soluble phenols in water [22].

It was found that the inclusion of carboxyl, nitro, methoxy and amine groups were not modifications of interest for the antioxidant activity improvement.

Although there were none, in this study of sufficiently large structural diversity, it is possible to observe that molecules with polar groups such as hydroxyl and carboxyl, confer a higher antioxidant activity than analogs with fewer polar groups such as methoxy and amine, highlighting the importance of a polar cluster in this position.

According to the findings, hydroxylation was the molecular modification that gave the best evaluated antioxidant activity result by the DPPH method. However, this is lower than the reference standards tested.

Resveratrol analogs may have an important antioxidant activity, but with the one with higher IC_{50} presented by the natural compound.

Studies arising from the relationship between the properties of the analogs chemical structure and coupled with new molecular changes may contribute to the development of more effective bioactive molecules.

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CONFLICT OF INTEREST

The authors have reported no conflict of Interest.

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