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In vitro and *in silico* antioxidant activity of hydrazones and semicarbazones derived from aldehydes found in essential oils. †

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Abstract: The aim of this study was to investigate in vitro and in silico antioxidant properties of four hydrazones and semicarbazones derived from vanillin and cinnamaldehyde, aromatic aldehydes found in essential oils. They were synthesized by condensation of these aldehydes with the corresponding phenylhydrazine and semicarbazide in good yields. The antioxidant properties of the target molecules were determined using the Reducing Power assay (RP) and the hydrogen peroxide scavenging method (HP), and the results were compared with thermodynamic descriptors obtained from theoretical calculations using the DFT method. The target molecules showed to be highly active for the total antioxidant assay and the results of theoretical calculations were consistent with the antioxidant activity observed experimentally, making them a useful tool to understand the mechanism of action. This would also allow theoretical tests of new antioxidant compounds to be carried out in a predictive manner.

Keywords: Antioxidant activity; Phenylhydrazones; Semicarbazones

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1. Introduction

It is widely known that a huge number and varieties of plants and its essential oils have been used worldwide to decrease the risk and to treat diseases and health complications [1]. For example, essential oil of vanillin and cinnamaldehyde obtained as plant extracts has received a lot of attention due to its antioxidant, antimicrobial, and antiinflammatory properties [2,3].

In living systems, the harmful free radicals such as hydroxyl, peroxyl and the superoxide anion are constantly being produced as a result of metabolic reactions. On a daily basis, up to 5% of inhaled oxygen may be converted to reactive oxygen species (ROS). These ROS have the ability to bind to cellular structures, and have been implicated in a number of pathological processes such as aging, inflammation, re-oxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in people [3]. The body has two types of mechanisms to eliminate ROS before they assert any damage. These include: (i) Enzymatic reductions of ROS beyond the regular process, (ii) Scavenging of ROS by anti-oxidant compounds [4].

So, the development of new products with potentially positive effect (scavenging of ROS) is of great interest.

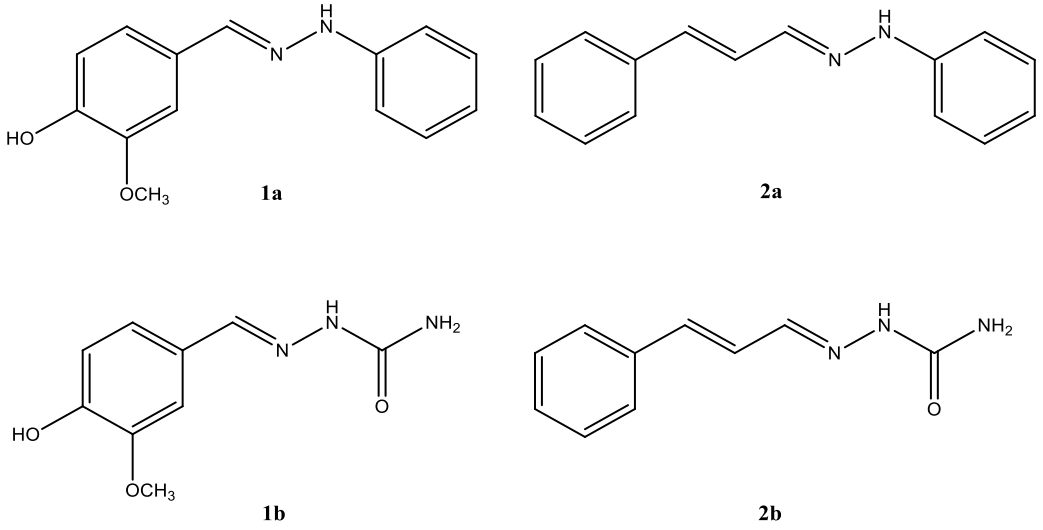
It is known that, the semicarbazone is an electron withdrawing group and exhibited antioxidant activity. Favorable substitution may increase their free radical scavenging ef-

fect [5]. On the other hand, hydrazones are used as building blocks for the development of bioactive molecules showing analgesic, antiinflammatory, antioxidant, antidepressant, anticonvulsant, antiacetylcholinesterase, antitumour, antituberculosis, and broad-spectrum antimicrobial activities [6].

In this sense, the objective of the present work was to synthesize semicarbazones and hydrazones derived from aldehydes found in natural plants, which their antioxi-dant properties were studied *in vitro* and *in silico*.

2. Materials and Methods

Target molecules were synthesized from two aldehydes found in essential oils: vanillin and cinnamaldehyde to obtain their respective phenylhydrazones and semicarbazones (Compounds 1a-b and 2a-b), depicted in Scheme 1.



Scheme 1. Hydrazones and semicarbazones obtained from vanillin (1a-b) and cinnamaldehyde (2a-b).

2.1. Synthesis of phenylhydrazone derivatives

20 mmol of phenylhydrazine were added to a flask containing 50 ml of ethanol. Subsequently, 20 ml of an ethanolic solution containing 20 mmol of the corresponding aromatic aldehyde (vanillin or cinnamaldehyde) was added. Subsequently, 4 drops of glacial acetic acid were added and the reaction was maintained at 50°C for 2 hours. The precipitates were filtered under vacuum and purified by recrystallization in ethanol, and the solid phenylhydrazones obtained (1a and 2a) were placed in a vacuum oven at 40°C for 48 hours.

2.2. Synthesis of semicarbazone derivatives

10 mmol of semicarbazide hydrochloride and 15 mmol of sodium acetate were placed in a flask containing 100 mL of distilled water. A solution of 10 mmol of aromatic aldehyde (vanillin or cinnamaldehyde) dissolved in 25 mL of ethanol was added drop-wise, stirring for 2 hours at room temperature. The products were solids, which were vacuum filtered and purified by recrystallization from ethanol. The solid semicarbazones obtained (1b and 2b) were placed in a vacuum oven at 40°C for 48 hours.

2.3. Evaluation in vitro of antioxidant activity

The reducing power method was used to measure total antioxidant activity. This method is based on the principle of increasing the absorbance of the reaction mixtures because the compound with antioxidant activity reduces Fe^{3+} to Fe^{2+} forming a colored complex [7]. An increase in absorbance indicates an increase in antioxidant activity. Briefly, 1 mL of the targeted compound (20 μg/mL) was placed in a tube and was mixed with 3 mL of buffer phosphate (pH 6.6) and 3 mL of an aqueous solution of $K_3Fe(CN)_6$ (1% w/v). The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of CCl_3COOH (10% w/v). Finally, 2.5 mL of this mixture was collected and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1% w/v). The absorbance was measured at 700 nm against a blank sample overtime.

Also, the hydrogen peroxide scavenging (H_2O_2) assay (HP) was performed according to the method of Ruch et al. [8]. For this, a solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm against blank solution. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = [(A_0 - A_t) / A_0] \text{ X } 100 \quad (1)$$

2.4. Evaluation in silico of antioxidant activity

Antioxidants scavenge free radicals through three main mechanisms: hydrogen atom transfer (HAT), sequential electron and proton transfer (SETPT), and sequential electron transfer and loss of protons (SPLET). These mechanisms are characterized by several thermodynamic descriptors such as BDE (bond dissociation enthalpy), IP (ionization potential), PDE (proton dissociation enthalpy), PA (proton affinity), and ETE (electron transfer enthalpy). The lower the values of the thermodynamic descriptors, the greater the antioxidant activity. Taking this into account and for a better understanding of the antioxidant properties of the synthesized compounds, all the mentioned thermodynamic descriptors were calculated using the DFT method. Computational studies were carried out with the Gaussian 09 program and the B3LYP / 6-31G (d,p) basis set. [9-11].

3. Results

3.1. Preparation of target molecules

The preparation of targeted hydrazones 1a-b and semicarbazones 2a-b was easily achieved. The four synthesized compounds were solid, and their yields, m.p., and NMR data are in accordance with reference methods [12-15]

3.2. Evaluation in vitro of antioxidant activity

The reducing power of the target molecules as a function of time can be observed in Figure 1.a. When analyzing the data obtained, it was found that all compounds have the ability to donate electrons to Fe^{3+} to convert it into Fe^{2+}. This shows that these molecules have antioxidant activity at a concentration of 20 μg/mL.

When comparing the synthesized molecules, it was found that compounds 1b and 2a are the ones that presented the greatest antioxidant activity. This is clearly demonstrated in Figure 1.b, where the increases in % absorbance in relation to the blank at 240 minutes are represented for the compounds evaluated.

The percentage of hydrogen peroxide inhibition for different concentrations of the target molecules are shown in Figure 2. The target molecules studied have the capacity to inhibit the action of H_2O_2 in a proportional relationship to their concentration in the test solutions, these results confirming the antioxidant activity of these compounds. As expected, the compounds that presented the highest percentage of H_2O_2 inhibition were 1b and 2a.

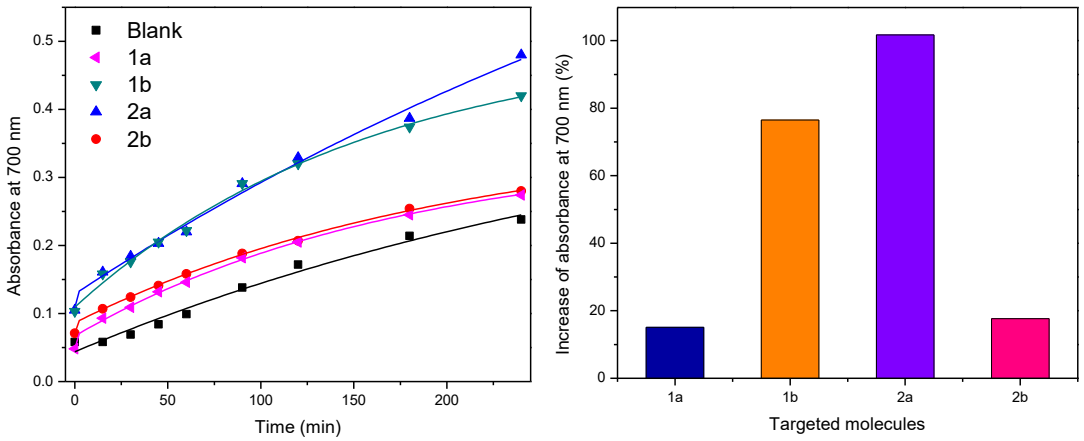


Figure 1. (a) Dependence of the reducing power as a function of time for the target compounds; (b) Increase of absorbance at 700 nm for the targeted molecules after 240 min.

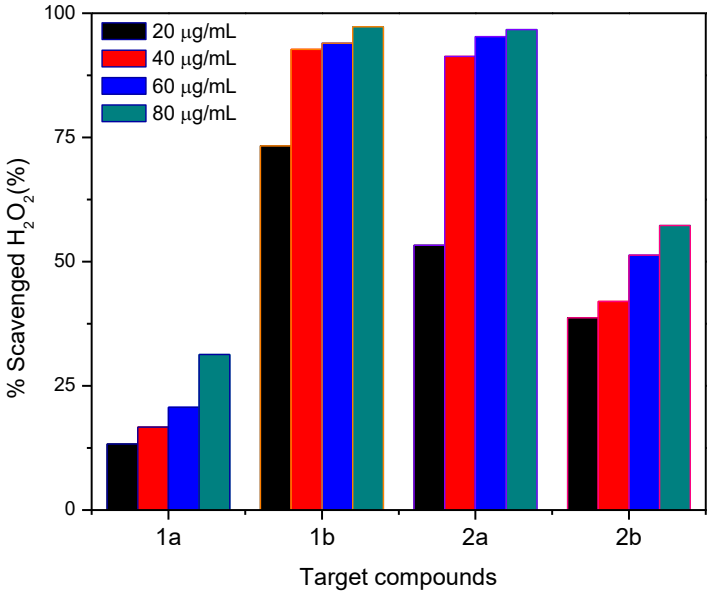


Figure 2. Percentage of H_2O_2 scavenged for the target compounds 1a-b and 2a-b at different concentrations (20-80 µg/mL).

3.3. Evaluation of antioxidant activity with computational studies

DFT calculations were done for antioxidants scavenge free radicals through three main mechanisms, namely hydrogen atom transfer (HAT), sequential electron transfer proton transfer (SETPT), and sequential proton loss electron transfer (SPLET). These mechanisms are characterized by several thermodynamic descriptors such as BDE (bond dissociation enthalpy), IP (ionization potential), PDE (proton dissociation enthalpy), PA (proton affinity) and ETE (electron transfer enthalpy). HAT is characterized by BDE value, SPLET is characterized by IP and PDE values, and finally SETPT is characterized by PA and ETE values. The lower the values of the thermodynamic descriptors, the higher the antioxidant activity.

In order to have a better understanding of the antioxidant properties of the synthesized molecules, and in which mechanism they follow to scavenge free radicals, all the mentioned thermodynamic descriptors (BDE, IP, PDE, PA, and ETE) have been computed for compounds 1a, 1b, 2a and 2b using DFT method at 6-31G(d,p) level of theory.

The obtained results of the thermodynamic descriptors are presented in Table 1.

Table 1. Thermodynamic descriptors.

	BDE	IP	PDE	PA	ETE
1a	78,36	135,46	235,54	358,21	64,33
1b	74,65	159,21	248,91	341,69	58,97
2a	76,82	133,75	232,89	339,65	55,94
2b	75,14	148,37	253,12	361,32	65,59

[1] all values are expressed in kcal/mol.

Thermodynamic parameters calculated with Gaussian software 09 - within the framework of DFT with the B3LYP method and the base set 6-31G(d,p) - turned out to be good indicators of activity antioxidant of hydrazones and semicarbazones derived from aldehydes of natural origin. According to BDE values, the transfer mechanism of a hydrogen atom is greater for semicarbazones as they have the lowest values. Depending on the IP and PDE values, the sequential transfer mechanism of one electron and proton is greater for hydrazones having these the smallest values. 1b and 2a compounds are the ones that presents the minor values in the thermodynamic descriptors in its whole, being the most stable radicals, which indicates its greater antioxidant potential, thus corroborating the data experimental. Even though thermodynamic parameters allow us to distinguish that the 4 target molecules have antioxidant properties, it is challenging to rely solely on these values to differentiate their activities from each other.

5. Conclusions

In this study, the synthesis and antioxidant activity of four hydrazones and semicarbazones derived from aldehydes found in essential oils are described. All investigated compounds exhibited antioxidant capacity in vitro and in silico. Among the target molecules studied, compounds possess the best antioxidant properties among the studied molecules. The results of the theoretical calculations were in general consistent with the antioxidant activity observed experimentally. The hydrogen atom transfer is characterized by the value of the bond dissociation enthalpy, getting the lower values for the semicarbazones and the lowest for the compound **1b**. The sequential electron transfer proton transfer is characterized by the ionization potential value and the proton dissociation enthalpy being the lower the IP and PDE values (and consequently the higher the antioxidant activity through this mechanism) to the compound **2a**. These thermodynamic parameters can be used as a useful tool to understand the mechanism of action.

Supplementary Materials: "Not applicable".

Author Contributions: Conceptualization, C.A.F. and L.G.G.; methodology, C.M.O. and L.G.G.; formal analysis, L.G.G. and C.M.O.; investigation, C.A.F.; resources, C.A.F.; data curation, A.P.R. and V.A.G.; writing—original draft preparation, L.G.G. and C.M.O.; writing—review and editing, C.A.F.; supervision, V.A.G. All authors have read and agreed to the published version of the manuscript.

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