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Antioxidant activity of a series of amides

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Abstract

Three synthesized amides: benzanilide **1**, dodecanilide **2**, N-cyclohexyloctamide **3**, and two known ones: acetanilide **4** and N-(4-hydroxyphenyl) acetamide (acetaminophen, also known as paracetamol and Tylenol) **5** were investigated for their antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The reduction kinetics, the effective concentration for 50% scavenging (EC_{50}), the time required to reach the steady state of DPPH discoloration at EC_{50} concentration (T_{EC50}) and the antiradical efficiency (ARE) were employed for the antioxidant activity measurement. The results revealed that this activity was more significant for fatty N- aromatic amide. Of these, N-(4-hydroxyphenyl)acetamide, the one with phenol group, showed the highest antioxidant activity. The obtained results were comparable to antioxidant properties of the standard antioxidants: butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT) and ascorbic acid (vitamin C).

Keywords: Amides, Antioxidant, DPPH, Scavenging.

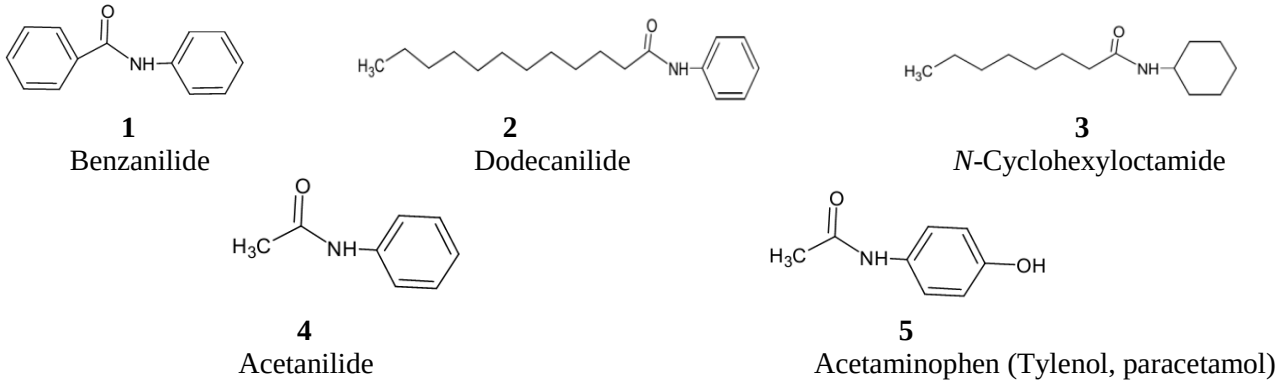
1. Introduction

Antioxidants are organic molecules which can scavenge free radicals and thus avoid or delay the progress of lipid oxidation [1]. Antioxidants are becoming increasingly popular in oxidative stress-related disorders and hold promise as therapeutic agents [2]. Antioxidant capacity is widely used as a parameter for medicinal bioactive components [3]. Several indirect means for quantifying the antioxidant activity have been established [4-6] and, yet, the DPPH method remains the most straightforward and reliable one [7-10]. Needless is to recall the unshakable standing of the amide compounds and their chemistry as, in part; they are linked to polypeptides and proteins. Amides are one of the main building blocks in Nature [11] and play an important role in biological activities [12-17]. Moreover, they are also involved as intermediate products in the synthesis of therapeutic agents [18, 19].

In the last decade, a special interest has been focused on amide derivatives (anilides), because of their peculiar biological activities including anti-bacterial, anti-fungicidal, anti-convulsant, anesthetic, and platelet aggregation [20]. Various anilides have also found a wide applicability as bioactive species (antimicrobial, antioxidant and anti-atherosclerotic agents) [21]. For example, acetanilide, a common precursor for a number of dyes and drugs, and its derivatives such as Tylenol exhibit, besides the analgesic and anesthetic effects, the antibacterial, anti-inflammatory, and antipyretic activities [22-24]. However, a few reports tackled the antioxidant and pharmacological properties of the Tylenol analogs [25-28]. In the herein presented work, investigation of the antioxidant activity of three synthesized amides (**1-3**) and of two known ones (**4, 5**) (Figure 1) was undertaken.

2. Materials and methods

The chemicals were purchased from Sigma-Aldrich, Schuchardt and Merck. They are used without any purification. The solvents used for synthesis were previously dried with standard methods and stored on molecular sieves (4 Å).



1 Benzanilide

2 Dodecanilide

3 *N*-Cyclohexyloctamide

4 Acetanilide

5 Acetaminophen (Tylenol, paracetamol)

Figure 1: The five investigated amides.

The used UV-Visible spectrophotometer was SHIMADZU-1605. Control molecules were ascorbic acid (vitamin C), 2, 6-di-tert-butyl-4-metylphenol (BHT), 2- and 3-tert-butyl-4-methoxyphenol (BHA).

2.1. *General synthetic procedure for **1**, **2**, and **3***

The protocol employed was previously reported [29]. A brief description of the method is as follows: A mixture of aniline and carboxylic acid was heated at a temperature range of 160-200 °C for a time period of 3-4 h. The water from the reaction was continuously distilled off. The produced white solid was recrystallized in ethanol to afford colorless needle-like crystals.

2.2. *DPPH assay*

To a 1 mL of ethanolic solution of the amide **(**10, 15, 25, 35, 50 and 100 µg/mL), was added 1 mL of 0.004% methanolic solution of DPPH. The mixture was then kept under darkness at room temperature.

The kinetics of DPPH reduction by the tested antioxidant at different concentrations was monitored by UV-Visible spectroscopy at λ_{max} = 517 nm, a characteristic absorption band DPPH. The extent of non-reacted DPPH was computed from equation (Eq. 1) [5].

$$\%\ (DPPH)_{NR} = 100 \times (DPPH)_{SS} / (DPPH)_0 \qquad \text{(Eq. 1)}$$

where $\%(DPPH)_{NR}$, $(DPPH)_{SS}$, and $(DPPH)_0$ stand for the extent of non-reacted DPPH, the absorbance of DPPH at steady state (equilibrium), and the absorbance of initial concentration of DPPH, respectively.

The efficient concentrations for scavenging 50% of $DPPH_0$ (EC_{50}) were drawn by extrapolation of the plots of the variations of the $(DPPH)_{SS}$ in function of antioxidant concentration. Also, the antiradical efficiency (ARE), a parameter characterizing the antioxidant power, was calculated from equation (Eq. 2).

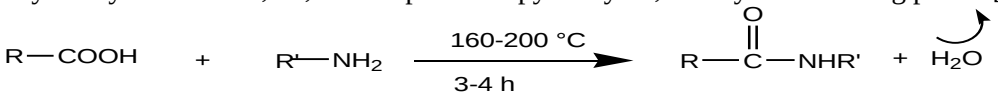
$$ARE = 1/ (EC_{50} \times T_{EC50}) \qquad \text{(Eq. 2)}$$

Where T_{EC50} is the time for reaching EC_{50} [30].

3. Results and discussion

3.1. *Synthesis of derivatives amides **1**, **2**, and **3***

The amides **1**, **2** and **3** were prepared in quantitative yields (Table 1) according to the reaction (Scheme 1). They were analyzed by UV-visible, IR, NMR spectroscopy analyses, and by their melting points [29].



1 R = R' = C_6H_5
2 R = $C_{11}H_{23}$, R' = C_6H_5
3 R = C_7H_{15}, R' = C_6H_{11}

Scheme 1: Synthetic reaction of amides

Table 1: Results of the synthesis of amides **1**, **2**, and **3**.

Amide	R	R'	Yield (%)	m.p. (°C)
1	C_6H_5	C_6H_5	80	163-164 (150-152 [20])
2	$C_{11}H_{23}$	C_6H_5	73	76-78 (78 [31])
3	C_7H_{15}	C_6H_{11}	70	77

3.2. *Antiradical activity*

The kinetic effects of DPPH scavenging at different concentrations and the effective concentration (EC_{50}) of dodecanilide (anilide C_{12}) **2** as an example of amides are illustrated in Figures 2 and 3.

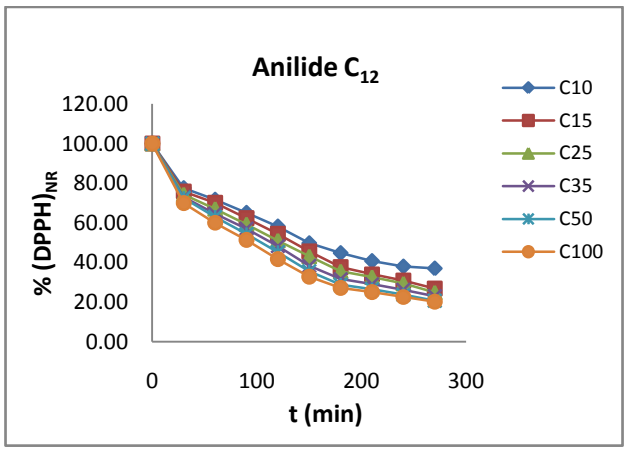


Figure 2: Kinetic of DPPH scavenging effect of dodecanilide **2** (anilide C_{12}).

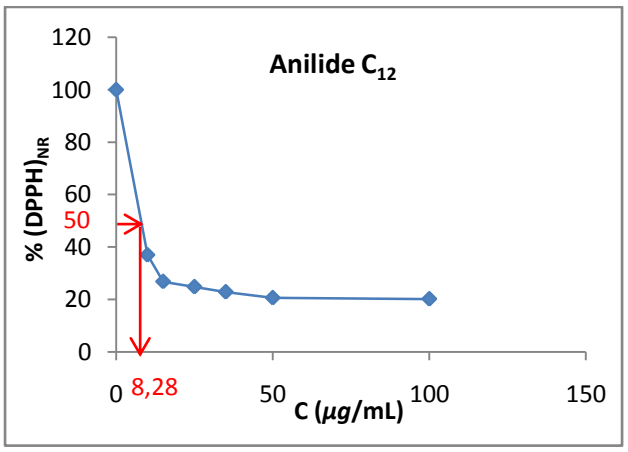


Figure 3: Determination of Effective concentration (EC_{50}) of dodecanilide **2** (anilide C_{12}).

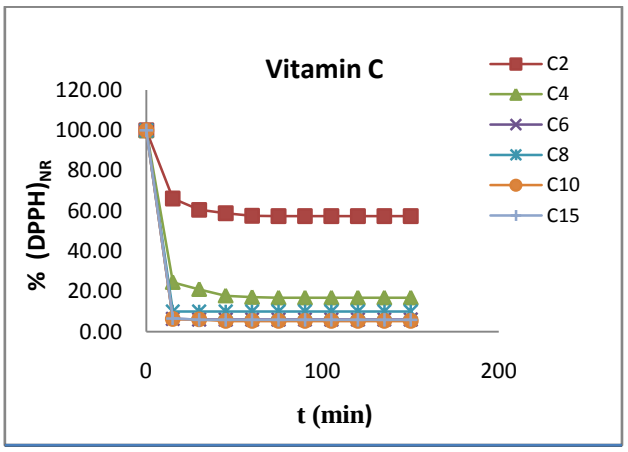


Figure 4: Kinetic of DPPH scavenging effect of ascorbic acid (vitamin C).

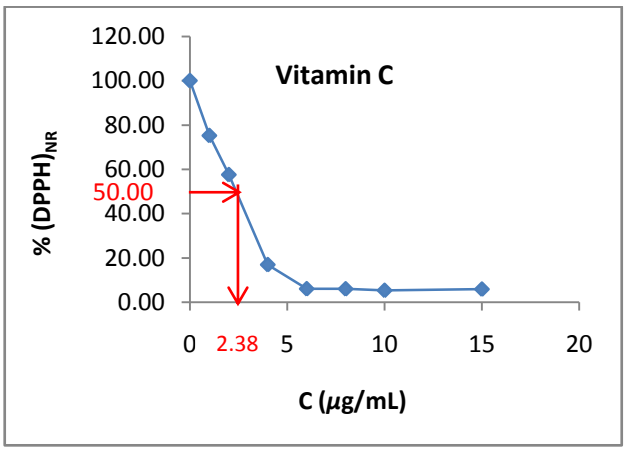


Figure 5: Determination of effective concentration (EC_{50}) of ascorbic acid (vitamin C).

The kinetic effects of DPPH scavenging and the effective concentration (EC_{50}) of ascorbic acid (vitamin C) used as standard are presented in Figures 4 and 5.

The previous figures illustrate the kinetics of the DPPH reduction when mixed with the amides and with the control molecules at different concentrations. The profiles of all the curves would suggest the ability of the experimented amides and the known antioxidants to trap DPPH radicals; a dropping of the DPPH concentration occurred for all tested amides and control molecules, down to steady state values. Indeed, the characteristic pale yellow color of the neutralized form of DPPH, that is H-DPPH, appeared gradually while the deep violet color of free stable radical DPPH faded. This observation clearly indicated the reducing power

of the tested amides by donating protons to DPPH; the reducing power was more or less dependent on the amide concentration.

3.3. *Effective concentration EC_{50} and Antiradical efficiency ARE*

Table 2 gathers the different antioxidant parameters, EC_{50}, T_{EC50}, and ARE. As seen, the EC_{50} and ARE values of amides **1-4** were in the range of 8 - 10 μg.mL^{-1} and 3.40×10^{-4} - 3.70×10^{-4} mL.μg^{-1}.min^{-1}, respectively. Based on the ARE values shown in this table, the antioxidant capacity of the tested amides was lower to that of the control molecules; the antioxidant power of vitamin C is nearly 15-20-fold those of **1-4**, but only 6-fold that of Tylenol **5**. The antioxidant potential is decreasing in this order: Vitamin C > BHT > BHA > Tylenol > dodecanilide > benzanilide > acetanilide > *N*-cyclohexyloctamide.

Table 2: Values of antioxidation parameters

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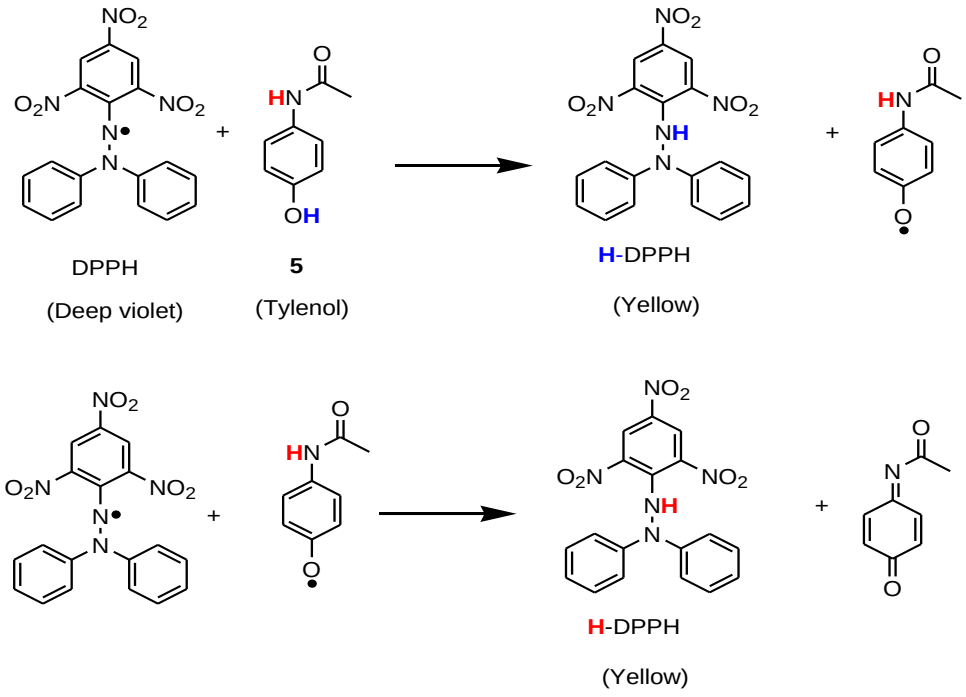
Amide/ Control molecule	EC_{50} (μg.mL^{-1})	T_{EC50} (min)	ARE (mL.μg^{-1}.min^{-1})
1	9.07	300	3.68×10^{-4}
2	8.28	270	4.47×10^{-4}
3	9.80	300	3.40×10^{-4}
4	9.30	300	3.58×10^{-4}
5	5.12	180	1.09×10^{-3}
BHA	1.50	210	4.44×10^{-3}
BHT	1.45	150	4.59×10^{-3}
vitamin C	2.38	60	7.00×10^{-3}

Application to the "structure-chemical reactivity" principle would lessen any surprise as to the above order. Indeed, the enolates hydrogens in vitamin C are by far easier to strip (pK_a = 4.1), and the phenolic hydrogens in BHA, BHT, and Tylenol are ranked in the second position ($pK_a \approx 10$); all these hydrogen abstractions would lead to resonance-stabilized radicals. However, the structures of amides **1-4** reveal only one labile hydrogen, the amide one –CO-NH- ($pK_a \approx 18$); although, the generated radical in nitrogen atom is favorably resonance-stabilized by phenyl and carbonyl groups [32], its abstraction is relatively difficult as its pK_a may indicate. Because it owns both a phenolic hydrogen and an amide hydrogen, the reaction of Tylenol with DPPH could be conceived as the one proposed by Alisi *et al.* [24], shown in Scheme 2.



Scheme 2: Mechanism of scavenging capacity of Tylenol towards DPPH radicals.

Similar mechanism was reported for the enzymatic reaction of Tylenol and its analogs [33, 34] and the theoretical calculations endorsed such mechanism [35, 36]. Thus, the conspicuous antioxidant activity of Tylenol among the examined amides owes to the abstraction propensity of proton of hydroxyl group by DPPH radical as traced in this scheme. Yet, this activity is weaker than that of the control molecules, and this fact can be ascribed to the moderate electron-donating ability of the acetamido group present in the Tylenol molecule, affecting the trapping kinetics. While the phenyl group in **1**-**4** was not greatly discriminatory as to the impact of the resonance-related stabilization of the radical, the effect of lipophilicity manifested in **2** (with undecyl group) vis-à-vis **1** (with phenyl group) and **4** (with methyl group). The amide **3**, however, was the least active because of the limited resonance-stabilized states, that is, stabilization only by the carbonyl group.

Conclusion

The above study revealed that the antiradical activity of a set of amides is mainly due to the amide group. On the other hand, the antioxidant capacity of the amides is linked to the nature of groups **R** and **R'** with regard to the stabilization potential of the free radicals; stabilization by resonance and alkyl length of the groups seem to be the effective parameters.

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