

# Antioxidant Activities of Cinnamaldehyde Derivatives

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**Abstract.** The modification structures of cinnamaldehyde, which was isolated from cinnamon oil, has been carried out. The synthesized compounds were tested their antioxidant activity by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and the IC<sub>50</sub> was done by spectrophotometric assay method compared with standard vitamin E. The cinnamaldehyde derivatives, e.g. cinnamic acid **2**, methyl cinnamate **3** and cinnamyl alcohol **5** had significantly higher antioxidant activity than that of their starting materials, cinnamaldehyde. However, although cinnamic amine **5** had a hydroxyl group, it gave no antioxidant activity possibly due to its bulky structure.

## 1. Introduction

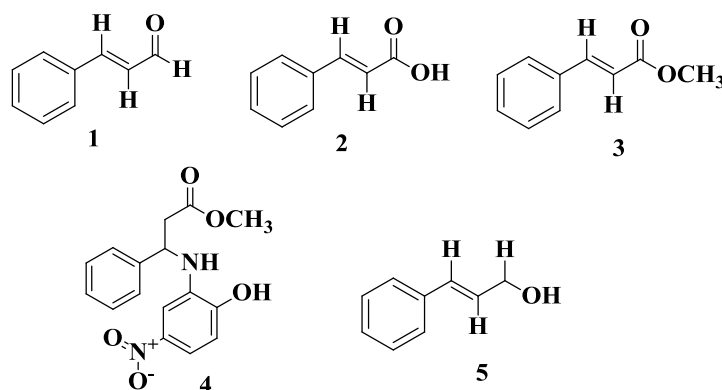
Antioxidants are compounds that are able to trap and deactivate damaging oxygen-centred radicals. Antioxidants have been proven for protection against certain diseases and aging since they have capacity for scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, and therefore there has been a constantly rising interest in the antioxidative constituents of various medicinal plants. Natural occurring antioxidants such as flavonoids from fruits, vegetables and leaves have been consumed for protection against diseases, including cancer, cardiovascular and cerebrovascular, due to their potential in controlling the levels of free radicals and the process of lipid peroxidation [1-5].

It has been established that the cinnamon oil and extract possess antioxidant activity, which is ascribed to the presence of phenolic and polyphenolic substances. Cinnamaldehyde (3-phenyl-2-propanal) **1** is the major component of cinnamon oil or cinnamon bark extract. This compound has various substituents on the aromatic ring, which are useful for starting material in synthesizing of its derivatives. Cinnamaldehyde derivatives have been reported as useful compounds for various application [6-11]. Cinnamic acids derivatives have been studied to have antioxidant, anti-inflammatory, anti-tuberculosis and cytotoxic properties. Cinnamate derivatives have been shown to have anti-inflammatory activity. This study is focused on the antioxidant activities of cinnamaldehyde derivatives. The modification structures of cinnamaldehyde, which have antioxidant activity is need to be done to use them in foods and pharmaceutical preparations as safe synthetic antioxidants.

We have synthesised cinnamaldehyde derivatives **2-4**, e.g. cinnamic acid **2**, methyl cinnamate **3** and methyl-3-(2-hydroxy-5-nitrophenyl amino)-3-phenylpropanoate **4** from cinnamaldehyde **1** [12]. The structures of the synthesized compounds have been established on the basis of UV-Vis, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectral. In this paper, we report the antioxidant properties of those synthesized compounds. We have examined for *in vitro* antioxidant activities of the compounds **1-4** using DPPH



(2,2-diphenyl-1-picrylhydrazyl) radical as a contribution to possible future applications. Additionally, we also synthesised and assessed antioxidant activity of cinnamyl alcohol **5** (Figure 1).



**Figure 1.** The synthesised compounds.

## 2. Experimental

### 2.1 Materials

Cinnamon oil was purchased from CV M & H FARM. Chemicals and solvents were analytical grade, which were obtained from e-Merck or Aldrich.

### 2.2 Methods

**2.2.1 Cinnamaldehyde 1 Isolation.** Cinnamaldehyde **1** was obtained from cinnamon oil by adding sodium bisulphate through a nucleophilic addition reaction. Cinnamaldehyde was separated by solvent extraction using diethyl ether. After evaporation, the cinnamaldehyde was washed with distilled water until neutral and then dried with anhydrous sodium sulphate.

**2.2.2 Cinnamic Acid 2 Synthesis.** Cinnamaldehyde was converted into cinnamic acid by reflux method for 6 h. Cinnamaldehyde (2.46 g) and  $\text{CrO}_3$  (7.9 g) in a mixture of diethyl ether (100 mL) and water (100 mL) in a flask was added four drops of KTF. The mixture was then stirred continuously at a reflux for 6 h. The mixture then was cooled using an ice bath to reach 4-5°C and then allowed to reach room temperature. The mixture was extracted using diethyl ether to separate  $\text{CrO}_3$  from the cinnamic acid.

**2.2.3 Methyl Cinnamate 3 Synthesis.** Cinnamic acid was esterified with methyl alcohol to obtain methyl cinnamate. Cinnamic acid (670 mg) in 10 ml of methanol was added 2 drops of sulphuric acid. The mixture was refluxed for 6 h. The mixture was then evaporated and cooled in room temperature to form precipitation.

**2.2.4 Cinnamic Amine (methyl-3-(2-hydroxy-5-nitrophenylamino)-3-phenylpropanoate) 4 Synthesis.** Reaction between methyl cinnamate and 2-amino-4-nitrophenol to give methyl-3-(2-hydroxy-5-nitrophenylamino)-3-phenylpropanoate. Methyl cinnamate was added with 2-amino-4-nitrophenol in a ratio of 1: 1.2 mmol equivalent. The mixture was refluxed in dichloromethane for 72 h and then evaporated the mixture to remove residual solvent.

**2.2.5 Cinnamyl Alcohol Synthesis 5.** The mixture of 10 mmol cinnamaldehyde and 10 mmol  $\text{NaBH}_4$  were grinded for 10 mins. A saturated  $\text{NaHCO}_3$  solution was added. The mixture was then extracted

with dichloromethane. The organic layer was dried with anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated.

**2.2.6 Antioxidant activity test.** The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to evaluate the free radical scavenging capacity of synthesized compounds [13]. Firstly, the antioxidant activity test was performed by determining the maximum wavelength of DPPH in the range of 800-400 nm. The maximum DPPH wavelength was found to be 516 nm.

The aliquot of the different concentrations of the test sample (12.5, 25, 50, 75 and 100 ppm) was added to 3 mL ethanolic solution of DPPH. Test tubes were kept for 30 mins at room temperature and were recorded at 516 nm by UV-Vis Spectrophotometer. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. The absorbance results were then used to determine the value of  $\text{IC}_{50}$ . The percentage inhibition was calculated using equation 1, where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance of the sample.

$$\% \text{ inhibition} = (A_c - A_s/A_c) \times 100 \quad \dots\dots\dots (1)$$

Then percentage inhibitions were plotted against log concentration of each of the test sample. Then  $\text{IC}_{50}$  value was calculated from the graph. The  $\text{IC}_{50}$  value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

### 3. Result and Discussion

The starting material used was cinnamaldehyde **1** and the reaction was accomplished in three step reactions to give the final product **4**. Cinnamaldehyde was converted into cinnamic acid **2**, which was then esterified with methyl alcohol to obtained methyl cinnamate **3**. The methyl cinnamate was then reacted with 2-amino-4-nitrophenol to give final product of methyl-3-(2-hydroxy-5-nitrophenylamino)-3-phenylpropanoate **4**. The synthetic compounds have been characterized by  $^1\text{H}$  NMR and the spectra showed the right chemical shifts as reported previously [12]. Cinnamaldehyde **1** was obtained as yellowish liquid with the yield of 80%. Cinnamic acid **2** was obtained as white powder with the yield of 64.9%. Methyl cinnamate **3** was obtained as dark brown liquid with the yield of 42%. Cinnamic amine **4** was obtained as brownish-yellow solid with the yield of 68%.

The cinnamyl alcohol showed as yellowish liquid with the yield of 93%. FTIR showed peaks at  $3350 \text{ cm}^{-1}$  for O-H stretching,  $3081$  and  $3059 \text{ cm}^{-1}$  for C-H stretching aromatics,  $1449 \text{ cm}^{-1}$  for C=C aromatic stretching. The  $^1\text{H}$  NMR spectrum of the cinnamyl alcohol compound exhibited 9 protons. A peak at  $\delta = 1.56 \text{ ppm}$  indicated for a proton of hydroxy group. Two peaks at  $\delta = 4.30$  and  $6.57 \text{ ppm}$  showed 2 coupled for proton C = C. Peaks at  $\delta = 7.23$ - $7.37 \text{ ppm}$  showed the existence of 5 protons for aromatic protons.

The DPPH solution was scanned at the wavelength of 800-400 nm and the maximum wavelength of DPPH was obtained at 516 nm. This maximum wavelength can be applied for determination of the antioxidant activity of the synthetic compounds because the maximum wavelength of DPPH exists within the 515-520 nm. DPPH is a stable free radical containing an odd electron in its structure and commonly employed for evaluation of the radical scavenging activity in chemical analysis. The odd electron in the DPPH free radical gives a strong absorption maximum at 516 nm and is purple in colour. DPPH addition and incubation for 30 mins gave different colour from purple to yellow. The colour turned to yellow as the molar absorptivity (optical density) of the DPPH radical at 516 nm reduces when the odd electron of DPPH radical forms the reduced DPPH-H. The resulting decolourization is stoichiometric with respect to the number of electrons captured [14-16]. The absorbance from the several variations concentration at 516 nm was used to determine the percentage inhibition, and plotted with the concentration to obtain the linear equations that was used to determine the  $\text{IC}_{50}$  values. Table 1 showed the antioxidant activities of all compounds.

**Table 1.** Antioxidant potential of the all compounds

Compound	IC <sub>50</sub> Value	Remarks
Vitamin E	7.48	Highly active
Cinnamaldehyde <b>1</b>	95.38	Moderately active
Cinnamic acid <b>2</b>	38.52	Highly active
Methyl cinnamate <b>3</b>	40.76	Highly active
Cinnamic amine <b>4</b>	-37.32	No activity
Cinnamyl alcohol <b>5</b>	21.45	Highly active

Cinnamaldehyde as a starting material had IC<sub>50</sub> value of 95.38, which indicated moderate antioxidant activity. The cinnamaldehyde derivatives **2**, **3** and **5** exhibited IC<sub>50</sub> values of 38.52, 40.76 and 21.45, respectively. These cinnamaldehyde derivatives showed highly antioxidant activities, which were comparable to the antioxidant activity of vitamin E. Furthermore, these IC<sub>50</sub> values showed that these compounds had higher antioxidant capacity than that of starting material **1**. Cinnamyl alcohol **5** was found to have the most efficacious antioxidant among all the synthetic compounds. However, compound **4** gave a negative value of IC<sub>50</sub> which indicated having no antioxidant activity.

As per chemical structural features, there were four different types of compounds synthesized under the study area. It is obvious that structural modification gives about the bioactivity and, of course, structural variations of molecules alters the biological activity in a regular trend [17]. Compound **2** has a hydroxyl group that exerted its antioxidant property. The presence of a hydroxyl group showed upward tendency of antioxidant activity. However, although compound **4** has a hydroxyl group but it does not show antioxidant properties. It might be due to its bulkier structure than that of compound **2**. Although compound **3** does not have hydroxyl group, however, this compound showed highly antioxidant activity. The reason of this finding is still unclear. Compound **5** was found to have the highest IC<sub>50</sub> value among other compounds. It is clearly understood that the alcohol functional group sharply enhanced the antioxidant potency. This is why compounds **5** was found to have highest activity than the others. This study showed that the cinnamaldehyde derivatives as the potent antioxidants and may be efficient as preventive agents in food and drugs.

#### 4. Conclusion

The present investigation emphasized that the modification structure enhanced the antioxidant activity of the cinnamaldehyde. Cinnamaldehyde derivatives **2**, **3** and **5** had significantly higher activities than their starting materials. However, the modification structure for compound cinnamic amine **4** gave no antioxidant activity possibly due to its bulky structure. These findings marked that the cinnamaldehyde derivatives **2**, **3** and **5** are more desirable antioxidant compound than that of their starting material. The results of DPPH assays also demonstrated that compound **5** has the greatest capacity as free radical scavengers, possibly due to the presence of alcohol functional group, which is essential for its antioxidant capacity. However, the potential application of these cinnamaldehyde derivatives in various foods and regulatory acceptance still needs further investigation.

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## References

- [1] Aruoma O I 1998 *J. Am. Oil Chem. Soc.* **75** 199-212.
- [2] Torreggiani A and Tamba, M 2005 *Trends Heterocycl. Chem.* **10** 115–137.
- [3] Wang H, Cao G and Prior R L 1996 *J. Agric. Food Chem.* **44** 701-705.
- [4] Hossain M M, Shaha S H and Aziz F 2009 *Bangladesh Med. Res. Counc. Bull.* **35** 49-52.
- [5] Sadhu S K, Okuyama E, Fujimoto H and Ishibashi M 2003 *Chem. Pharm. Bull.* **51** 595-598.
- [6] Pontiki E, Hadjipavlou-Litina D, Litinas K and Geromichalos G 2014 *Molecules* **3(19)** 9655-9674.
- [7] Godoy M E, Rotelli A, Pelzer L and Tonn C E 2000 *Molecules* **5** 547-548.
- [8] Bairwa R, Kakwani M, Tiwari N R, Lalchandani J, Ray M K, Rajan M G R and Degani M S 2010 *Bioorg. Med. Chem. Lett.* **20(5)** 1623-1625.
- [9] De P, Baltas M and Bedos-Belval F 2011 *Curr. Med. Chem.* **18(11)** 1672-1703.
- [10] De P, Yoya G K, Constant P, Bedos-Belval F, Duran H, Saffon N, Daffé M and Baltas M 2011 *J. Med. Chem.* **54(5)** 1449-1461.
- [11] Yoya G K, Bedos-Belval F, Constant P, Duran H, Daffé M and Baltas M 2009 *Bioorg. Med. Chem. Lett.* **19(2)** 341-343.
- [12] Suryanti V, Wibowo F R, Pranoto, Isnaeni S R, Sari M R K, Handayani S 2016 *IOP Conf. Ser. Mater. Sci. Eng.* **107** 012062.
- [13] Hossain, M M, Shaha S and Aziz F 2009 *Bangladesh Med. Res. Counc. Bull.* **35** 49-52.
- [14] Espin J C, Soler-Rivas C and Wichers H J 2000 *J. Agric. Food Chem.* **48** 648-656.
- [15] Yu L 2001 *J. Agric. Food Chem.* **49** 3452-56.
- [16] Mattha`us B. 2002 *J. Agric. Food Chem.* **50** 3444-3452.
- [17] Jiang H, Sun S-L, Zhang C, Yuan E, Wei Q-Y and Zeng Z 2013 *Trop. J. Pharm. Res.* **12(6)** 1017-1022.