The Antioxidant Activity from Hydroquinone Derivatives by the Synthesis of *Cinnamomum Verum J.Presl* Bark's Extracted

Uthumporn Kankeaw and Ekkalak Masong

Abstract—Hydroquinone is polyphenol compound which was used as antioxidant. In this study, the hydroquinone was synthesized via aldol condensation between 1,4-cyclohexanedione and cinnamaldehyde which was extracted from the bark of *Cinnamomum verum J.Presl* by stream distillation and using LiCl as a catalyst in pyridine. The hydroquinone derivatives showed the dark brown crystal with Rf 0.50 (hexane : ethyl acetate : methanol 5 : 4 : 1). These products were characterized by FT-IR spectroscopy which showed spectrum at 3300 - 3500 cm^{-1} (O-H stretching), 3025 cm^{-1} (C-H stretching of aromatic), 1660 cm^{-1} (C=C stretching of alkene) and 1450, 1502 cm^{-1} (C=C stretching of aromatic), respectively. The characterization by $^1\text{H-NMR}$ spectroscopy expressed $^1\text{H-NMR}$ (ppm): 9.70 (d, 2H, OH), 6.35-7.56 (m, 8H, aromatic protons) and 3.50 (d, 2H, $-\text{CH}_2-$), respectively. The synthesized product was tested the antioxidant activity by using DPPH radical scavenging assay and the IC_{50} was done by spectrophotometric assay method compared with standard gallic acid. The antioxidant activity revealed that IC_{50} were 14.15 ppm.

Index Terms—Cinnamaldehyde, antioxidant, hydroquinone derivatives, *Cinnamomum verum J.Presl*.

I. INTRODUCTION

Cinnamomum Verum J.Presl is an evergreen tropical tree, belonging to the *Lauraceae* family [1]. Cinnamon barks is widely used as flavoring agent in foods and for various applications in medicine, anticancer, antioxidant and antibacterial properties [2]. The essential oil from bark is rich in *trans*-cinnamaldehyde with antimicrobial effects against animal and plant pathogens, food poisoning and spoilage bacteria and fungi [3]-[5]. Until now more than 300 volatiles were found as constituents of essential oils of cinnamon [6]. The essential oil derived from cinnamon leaves is rich in eugenol, moreover from the roots in camphor and that from the barks express a high amount of sesquiterpenes [7]. It has been established that the oils and extracts from cinnamon possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances [8]. Free radicals are generated in consequence of the metabolism of normal or pathological cells.

In the last years there has been a constantly rising interest in the antioxidative constituents of various medicinal plants, due

to their potential in controlling the levels of free radicals and the process of lipid peroxidation. This study is focused on the modification of cinnamaldehyde which was extracted from cinnamon bark to use them in foods and pharmaceutical preparations in order to replace synthetic antioxidants.

The modification of the structure to improve antioxidant activity was done by aldol condensation between 1, 4-cyclohexanedione and cinnamaldehyde express in Fig. 1.

Fig. 1. Aldol condensation between 1, 4-cyclohexanedione and cinnamaldehyde.

II. MATERIAL AND METHOD

A. Materials

The dry bark of *Cinnamomum Verum J.Presl* from Sri Lanka.

B. The Essential Oil Extraction

The volatile oils of the cinnamon bark were obtained by hydrodistillation [9]. 1 kg of each of the cinnamon bark powders with 3L of distilled water followed by heating at 100 °C for 8h. The volatile products were extracted from the water phase three times using methylene dichloride. The essential oil was characterized the structure by FT-IR spectroscopy.

C. The Synthesis of Hydroquinone Derivative

The synthesis strategy leading to the target compounds are illustrated in Fig. 1. The hydroquinone derivative was synthesized by 0.0065 mol of 1, 4-cyclohexanedione and 0.0065 mol of cinnamaldehyde with the presence of LiCl in 5 mL of pyridine at reflux temperature for 8 h. The product was extract by Ethyl acetate and 10% HCl and purified by column chromatography. The characterization of product was done by spectroscopic method FT-IR spectroscopy and $^1\text{H-NMR}$ spectroscopy.

D. Antioxidant Studies: DPPH Assay

The method of Brand-Williams *et al.*, [10] based on the reduction of DPPH radical solution in the presence of hydrogen donating antioxidants, was used with some modifications. One ml of 0.3 mM alcoholic solution of DPPH

was added to 2.5 ml of the samples with different concentration of hydroquinone derivatives. The samples were kept at room temperature in the dark and after 30 min. the optic density was measured at 518 nm. The optic density of the samples, the control and the blank was measured in comparison with ethanol. One synthetic antioxidant, Gallic acid was used as positive control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 518 nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

III. RESULTED AND DISCUSSTION

A. The Essential Oil Extraction

The volatile oils of the cinnamon bark were obtained by hydrodistillation give the liquid yellow with unique aromatic scent (see Table I).

TABLE I: THE QUALITY OF THE VOLATILE OIL OF THE CINNAMON BARK BY HYDRODISTILLATION

	Weight of bark (kg)	Weight of volatile oil (g)	% yield
1	1.06	4.52	0.43
2	1.15	4.95	0.43
3	1.03	5.40	0.52
average	1.08	4.96	0.46

The volatile oil was characteristic by IR spectroscopy compare with reference spectrum is presence in Fig. 2.

Fig. 2. The FT- IR spectrum of volatile oil.

It was found that the volatile oil is cinnamaldehyde because there are the present peak of 1676 cm^{-1} (C=O stretching), $2742, 2846 \text{ cm}^{-1}$ (C-H stretching of aldehyde), 1625 cm^{-1} (C=C stretching of alkene) and $1450, 1502 \text{ cm}^{-1}$ (C=C

stretching of aromatic), respectively. When compare the spectrum with the reference found that there's dissimilar.

B. The Synthesis of Hydroquinone Derivative

The hydroquinone derivative was done by aldol condensation between 1, 4-cyclohexanedione and cinnamaldehyde, the result show in Table II.

TABLE II: THE SYNTHESIS OF HYDROQUINONE DERIVATIVE

	R_f^*	melting point (°C)
2-(cinnamyl) hydroquinone	0.64	162-166

* hexane: ethyl acetate: methanol (5 : 4 : 1)

The hydroquinone derivative was purified by column chromatography method and the yield of product is 21.96%. The mechanism of the reaction can be express in Figure3. In the experiment, pyridine was used as catalyst which perform as base to increase the nucleophile activity of 1, 4-cyclohexanedione.

The synthesis product was characterized by FT-IR spectroscopy which showed spectrum at $3300 - 3500 \text{ cm}^{-1}$ (O-H stretching), 3025 cm^{-1} (C-H stretching of aromatic), 1660 cm^{-1} (C=C stretching of alkene) and $1450, 1502 \text{ cm}^{-1}$ (C=C stretching of aromatic), respectively. The characterization by $^1\text{H-NMR}$ spectroscopy expressed $^1\text{H-NMR}$ (ppm): 9.70 (d, 2H, OH), 6.35-7.56 (m, 8H, aromatic protons) and 3.50 (d, 2H, $-\text{CH}_2-$), respectively. From the FT-IR spectrum, it was found that C-H stretching of aldehyde was disappear in the other hand, OH stretching which is the character of hydroquinone was appeared. Moreover, the $^1\text{H-NMR}$ spectrum show 2H of CH_2 at 3.5 ppm which was connected between 1, 4 cyclohexanedione and cinnamaldehyde together. It can be concluded that the modified structure was followed by expect reaction.

C. Antioxidant Studies: DPPH Assay

The antioxidant activity of hydroquinone derivative is mainly due to the active of OH and the functional group in their structure [11]. In this study, the derivative was compare with gallic acid standard which was the hydroquinone derivative and also acts as an antioxidant and helps to protect human cells against oxidative damage [12] by DPPH free radical scavenging assay, the results are shown in Fig. 4, Fig. 5 and Table III.

A significant correlation was observed between the concentration and the scavenging of DPPH radical in the hydroquinone derivative (Fig. 5) ($r = 0.993$) indicate that the radical scavenging capacity of each extract might be related to their concentration of the hydroquinone derivative. The results obtained from the DPPH as well as the liquid per oxidation assay employed to test the antioxidant activity of hydroquinone derivative demonstrated that the derivative give less IC_{50} than gallic acid but not much difference because the OH in the derivative is less than that reference.

Fig. 3. The mechanism of the synthesis of hydroquinone derivative.

Fig. 4. Correlation between antioxidant activity and concentration of gallic acid.

Fig. 5. Correlation between antioxidant activity and concentration of 2-(cinnamyl)hydroquinone.

TABLE III: ANTIOXIDANT ACTIVITY OF HYDROQUINONE DERIVATIVE AS DETERMINED BY DPPH FREE RADICAL SCAVENGING ASSAY

	IC ₅₀ (ppm)
2-(cinnamyl) hydroquinone	14.15
Gallic acid	9.80

IV. CONCLUSION

This present study has revealed that the antioxidant activity of hydroquinone derivative which was synthesis by the aldol condensation between 1,4-cyclohexanedione and cinnamaldehyde. This study showed the potent antioxidants and may be efficient as preventive agents in and can be seen as a potential source of useful food and drugs. Further studies have to be carried out to modification of the structure for higher antioxidant activity.

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REFERENCES

- [1] J. Seidemann, *Cinnamon-Lauraceae*, Heidelberg: Springer-Verlag, 2005, pp. 100-104.
- [2] V. Katalinic, M. Milos, T. Kulicic, and M. Jukic, "Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols," *Food Chem*, vol. 94, pp. 550-557, 2004.
- [3] T. Hatano, M. Takagi, and T. Yoshida, "Phenolic constituents of liquorice. VII. A new calcone with a potent radical scavenging activity and accompanying Phenols," *Chem. Pharm. Bull.*, vol. 45, pp. 1485-1492, 1997.
- [4] M. Lis-Balchin, S. Deans, and E. Eaglesham, "Relationship between bioactivity and chemical composition of commercial essential oils," *Flavour Fragr. J.*, vol. 8, pp. 98-104, 1998.
- [5] M. Mastura, M. A. N. Azah, S. Khozirah, R. Mawardi, and A. A. Manaf, "Anticandidal and antidermatophytic activity of Cinnamomum species essential oils," *Cytobios*, vol. 98, pp. 17-23, 1999.
- [6] L. Yan-Qua, K. De-Xin, and W. Hong, "Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy," *Industrial Crops and Product*, vol. 41, pp. 269-278, 2013.
- [7] W. Brand-William, M. Cuelier, and M. E. Berset, "Use of free radical method to evaluate antioxidant activity," *Lebensm. Wiss. U. Technol.*, vol. 28, pp. 25-30, 1995.
- [8] G. Singh, S. Maurya, M. P. Lampasona, and C. A. N. Cantalan, "A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents," *Food and Chemical Toxicology*, vol. 45, pp. 1650-1661, 2007.
- [9] L. F. Yamaguchi, H. G. Lago, T. M. Tanizaki, P. D. Mascio, and M. J. Kato, "Antioxidant activity of prenylated hydroquinone and benzoic acid derivatives from Piper crassinerium Kunth," *Phytochemistry*, vol. 67, pp. 1838-1843, 2006.
- [10] S. Sasaki, T. Tozawa, Y. Matsushita, and T. Satoh, "Anovel diterpene para-hydroquinone compound derived from cryptoquinone protects neuronal cells against oxidative stress," *Neuroscience Letters*, vol. 548, pp. 132-136, 2013.
- [11] H. Bagheri, M. Yazid, B. A. Manap, and Z. Solati, "Antioxidant activity of Piper nigrum L. essential oil extracted by supercritical CO₂ extraction and hydro- distillation," *Talanta*, vol. 121, pp. 220-228, 2014.
- [12] P. M. Dewick and E. Haslam, "Phenol biosynthesis in higher plants. Gallic acid," *Biochemical Journal*, vol. 113, pp. 537-542, 1969.

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For this research, she is working with Ekkalak Masong, the student who studied in Maejo University moreover the research is the part of senior project.