SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY EVALUATION OF THE HYDROXYLATED CHALCONE DERIVATIVE

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Article History: **Received**: 19.06.2022 **Revised**: 18.07.2022 **Accepted**: 11.08.2022

Abstract: The hydroxylated chalcone derivative (**HCD**) has been synthesized efficiently utilizing of Claisen-Schmidt condensation of para-hydroxylated derivatives of both acetophenone and benzaldehyde using a catalyst $SOCl_2$/ETOH. The confirmation and characterization of the chemical structure of the synthesized chalcone derivative was done using melting points, and R_f value as well as FT-IR. The *in vitro* anti-inflammatory activity for the synthesized (**HCD**) was evaluated at different concentrations via Red Blood Cells membrane stabilization test and calculate IC_{50} value by linear regression analysis. Additionally, Ibuprofen was used as a standard drug in this study. The screening data indicated that tested **HCD** showed potent anti-inflammatory activity in comparison with Ibuprofen with the IC_{50} value of 1146.78±0.55µg/ml. The marked anti-inflammatory potential of (**HCD**) has been found as it effectively inhibits heat induced hemolysis in comparison to ibuprofen. The gained results encouraged to use the synthetic (**HCD**) as an effective anti-inflammatory agent instead of NSAIDs to avoid classical side effects of NSAIDs.

Key words: NSAIDs, Ibuprofen, hydroxylated chalcone derivative, anti-inflammatory activity.

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DOI: 10.31838/ecb/2022.11.06.009

INTRODUCTION

Because of their activities as antipyretic, analgesic, anti-inflammatory agents (treatment of inflammatory condition like rheumatoid arthritis, osteoarthritis), the non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed drugs around the world [(1)]. However, their use often causes several complications like gastrointestinal ulceration and perforation which are among the most serious clinical problems concomitant on their long-term use [(2)]. These agents also affect renal and cardiovascular systems [(3)].

Chalcones and its related derivatives have a unique chemical structure that contain a reactive keto ethylenic group (–CO–CH=CH–) linked to two aromatic rings on its both sides. They are precursors for biosynthesis of bioactive flavonoids plant kingdom as well as its chemical ability to play as a lead for drug discovery programs and important therapeutic drugs containing heterocycles [(4)]. Chalcones have demonstrated a variety of protective functions for cells and have therapeutic potential for multiple diseases. For example, these compounds have anti-inflammatory, analgesic [(5)], anticancer [(6)], antiviral and antimicrobial activities [(7)].

For synthesis of chalcones, various methods are employed depending on condensation of an aromatic aldehyde with an aromatic ketone utilizing condensing agents. The simplest method involves Claisen-Schmidt condensation under an acidic condition (produced *in situ*) of an aromatic aldehyde or its derivatives with an aromatic ketone or its derivatives [(8)].

The synthesis and *in vitro* anti-inflammatory estimation of the hydroxylated chalcone derivative (**HCD**) by RBCs membrane stabilization test in comparison with Ibuprofen were the goals of this study.

EXPERIMENTAL

Chemicals And Materials

P-hydroxybenzaldehyde and thionyl chloride were supplied from Fluka AG (Switzerland) and BDH (England) respectively. Ibuprofen was donated thankfully by The State Company for Drug Industries (SDI, Samara, Iraq). R_f values were determined by using ascending TLC, on Kieslgel GF_{254} (60) aluminum plates (E. Merck, Germany) to evaluate the product's purity using petroleum spirit (40-60): ethyl acetate (70:30) as mobile phase. The UV spectrophotometer was used to detect the spots of reactants and product. Bibby-Scientific (England) was used for determination of melting point by capillary tube method. FT-IR spectrophotometer, at college of pharmacy, University of Kufa has been used to determine the product's FT-IR spectrum utilizing KBr disc. The synthesis was done according to the following scheme:

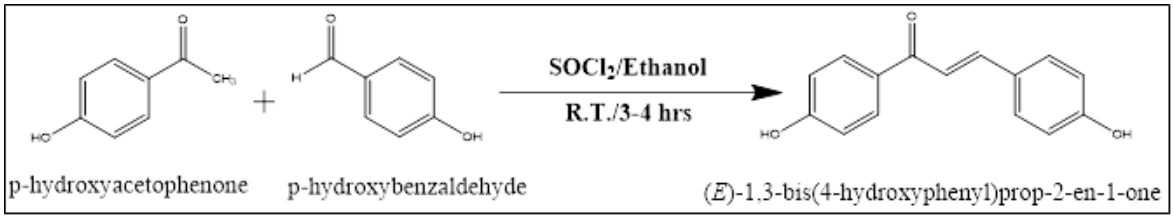


Figure 1. Synthetic scheme for the hydroxylated chalcone derivative (HCD)

Synthesis of the hydroxylated chalcone derivative (HCD): For synthesis of the hydroxylated chalcone derivative (**HCD**), thionyl chloride (8mmol/ 0.5ml) was added to a cooled mixture of the reactants (p-hydroxybenzaldehyde [10mmol/1.22gm] & p-hydroxyacetophenone [10mmol/ 1.36gm]) in 10 ml of dry ethanol, then the mixture was stirred for 5 hours. After completion of the reaction, a 5 ml of cold distilled water was added to the reaction mixture to precipitate and filtrate the solid. Then, washing of the obtained solid was done using cold distilled water (60 ml), cold absolute ethanol (20 ml), and cold diethyl ether (20 ml) respectively and allowed to dry [9].

the product's percent yield and R_f value and other physicochemical properties were given in table (1) whereas table (2), and Figure (1) were showed spectral data of it.

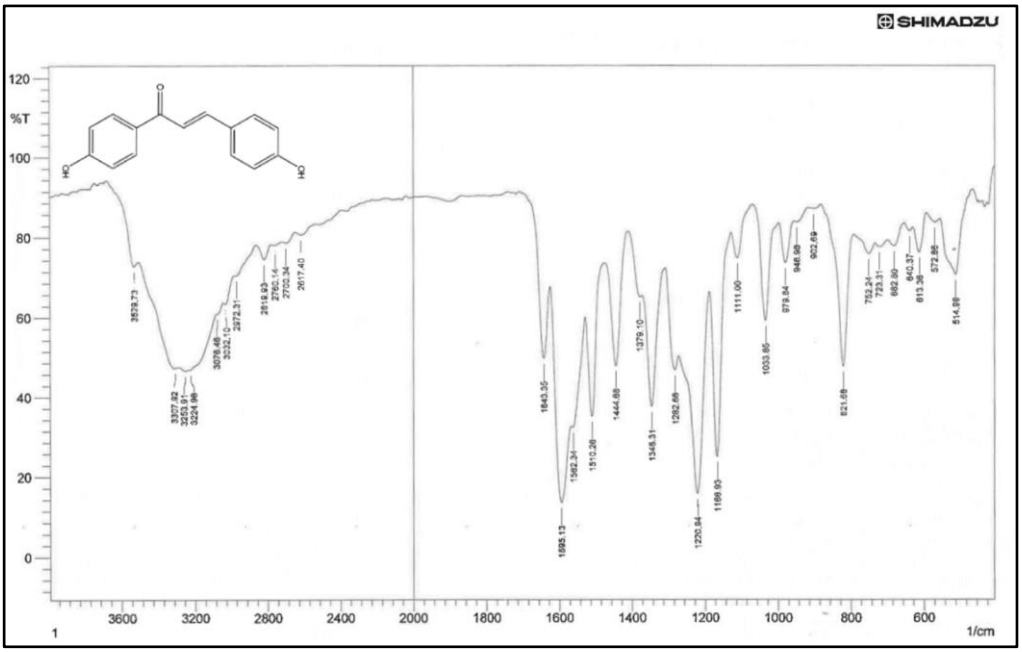


Figure 2. FT-IR spectrum of HCD

Evaluation of the anti-inflammatory activity of HCD:

A. Red blood cells (RBCs) suspension preparation: A 10 ml of fresh human blood was transferred after its collection to the heparinized centrifuged tubes. The tubes were centrifuged for 15 min at 2500 rpm and washed with equal volume of normal saline four times. Then a 10% v/v RBCs suspension in normal saline was prepared [10].

B. Heat induced hemolysis: Several mixtures with volume (2 ml) have been prepared by mixing of 1 ml of test **HCD** solution at different concentrations (250, 500, 1000, 1500, and 2000 µg/ml) and 1 ml of previously prepared 10% v/v RBCs suspension. The control test tubes (-ve control) and (+ve control) were prepared by mixing saline and Ibuprofen (500 µg/ml) solutions respectively with the previously prepared 10% v/v RBCs suspension. All the prepared mixtures have been centrifuged at 3000 rpm for 10 min after its incubation in a water bath at 56oC for 30 min and cooling in tap water. The absorbance of the supernatants was taken at 560 nm [11]. The experiment was performed in triplicates. The following formula have been used to determine percent membrane stabilizing activity:

Percentage membrane stabilization activity = ($Abs_{control}$ −Abs_{sample}) X 100/ $Abs_{control}$

RESULTS AND DISCUSSION

Synthesis of HCD

For synthesis of the hydroxylated chalcone derivative (**HCD**), the condensation by $SOCl_2$/EtOH result in production of HCl (acidic condition) *in situ* by the reaction of the condensing agent ($SOCl_2$) with the solvent (absolute ethanol), then dehydration was occurred to produce the hydroxylated chalcone derivative (**HCD**) with an excellent yield (77%) [12] (Scheme 1). Excellent yields of the chalcone derivatives can be obtained by this simple condensation method without any side product in a short time.

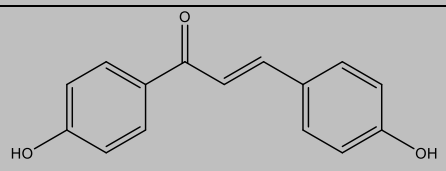
This synthesized chalcone derivative was subjected to physicochemical characterization, the percent yield and R_f value and other physicochemical properties were given in table (1) whereas table (2), and Figure (1) were

showed spectral data of it. In FT-IR spectrum of **HCD**, a conjugated carbonyl group (C=O) and (C=C) of the product were confirmed by presence of the absorption bands at *v* values of 1643, and 1595 cm^{-1} respectively.

Table 1. Physicochemical properties of the HCD

Chemical name	Chemical Formula	Molecular Weight (g/mol)	Physical appearance	Yield (%)	Melting point (ºC)	R_f
(***E***)-1,3-bis(4-hydroxy phenyl)prop-2-en-1-one	$C_{15}H_{12}O_3$	240	Red powder	77	199-200	0.27

Table 2. FT-IR spectral data of the HCD

Chemical structure	IR band [KBr] ν cm^{-1}	Interpretation
	3224	(O-H) stretching vibration of phenolic -OH group
	3032	Aromatic (C-H) stretching vibration
	1643	(C=O) stretching vibration of ketone conjugated to both benzene and alkene
	1595	(C=C) trans stretching vibration of alkene conjugated to both benzene and carbonyl groups
	1562, 1510	Stretching vibration of (C=C) skeleton
	1220	(C-O) asymmetric stretching vibration of ketone
	821	Aromatic (C-H) out-of-plane bending vibration of 1,4-disubstituted benzene

Anti-inflammatory activity of the HCD

The ability of **HCD** to inhibit heat induced hemolysis was studied as an investigation for further anti-inflammatory mechanism. In this evaluation, **HCD** at different concentrations was effective in inhibiting heat induced hemolysis as shown in Figure (2) and Table (3). At the concentration of 2000 µg/ml, maximum inhibition (75.96±0.77%) was obtained. IC_{50} value was found to be 1146.78±0.55µg/ml at correlation coefficient value (r) of 0.989. Ibuprofen showed the inhibition, 65.05±1.30% at the concentration of 500µg/ml.

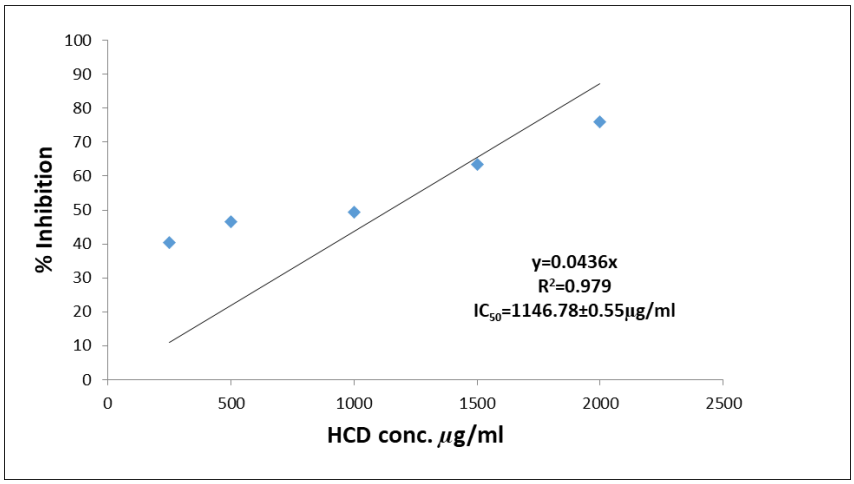


Figure 3. In-vitro anti-inflammatory activity of HCD by membrane stabilization test

Table 3. In-vitro anti-inflammatory activity of HCD by membrane stabilization test

Test	Conc. in (µg/ml)	% Inhibition of heat induced hemolysis
HCD	250	40.34±1.18
	500	46.53±0.90
	1000	49.36±1.22
	1500	63.47±0.68
	2000	75.96±0.77
Correlation coefficient value (r)	----------	0.989
IC_{50} value	----------	1146.78±0.55
Ibuprofen	500	65.05±1.30

Values represent in the results are mean±SD of three replicates; linear regression analysis was used to calculate IC_{50} value

CONCLUSION

In conclusion, the hydroxylated chalcone derivative was synthesized with an excellent yield (77%) employing the Claisen-Schmidt condensation method catalyzing by using $SOCl_2$/ETOH. The FTIR spectroscopy, R_f value, melting point value and other properties were used to characterize this compound. The usage of membrane stabilization test at

different concentrations to estimate *in vitro* anti-inflammatory activity for **HCD** showed a potent anti-inflammatory activity of it by inhibiting the heat induced hemolysis with the IC_{50} value of 1146.78±0.55µg/ml.

ACKNOWLEDGMENT

The authors thank all those who helped carry out this article.

ETHICAL ISSUES

In this study, except for maintaining the secrets of the patient by the Helsinki Treaty, it is assured to patients that their information will be confidential and will be used only for research. In addition, no additional costs were imposed on patients. The proposal is approved by the Ethics Committee of the University of Kufa/College of Pharmacy.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

All participating authors declare no conflict of interest and approve the final article.

AUTHORS' CONTRIBUTIONS

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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