Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(3): 198-205

ISSN- 0975 1556

Research Article

Synthesis of Ferulic Acid and Its Non Covalent Inclusion with Hydroxypropyl-β-Cyclodextrin

Juni Ekowati[1*], Rahman S.D.[1], Isadiartuti D[2], Widyowati R[3], Budiati T[1]

[1]*Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam Street, Surabaya 60286, Indonesia*

[2]*Departement of Pharmaceutics, Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam Street, Surabaya 60286, Indonesia*

[3]*Departement of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam Street, Surabaya 60286, Indonesia*

Available Online:29th February, 2016

ABSTRACT

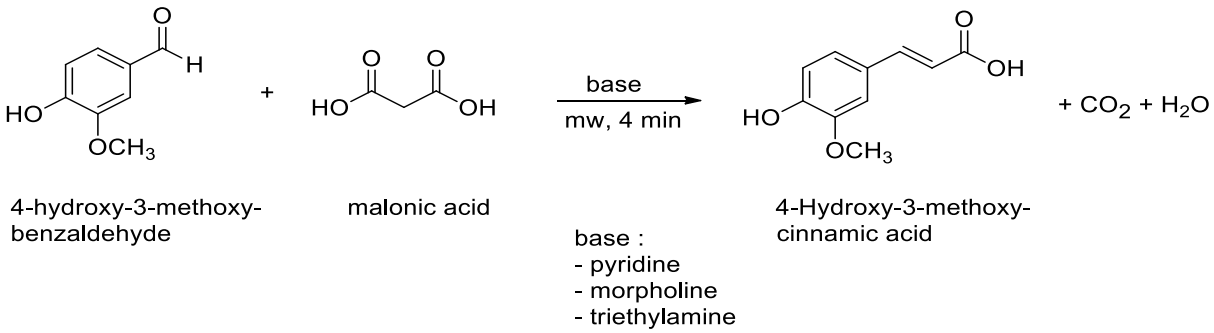
Ferulic acid (FA) is a highly effective compound with many activities, but it is poorly suited for pharmaceutics application because of its low physicochemical properties. In the present study we synthesized FA through microwave assisted Knoevenagel condensation using malonic acid and 4-hydroxy-3-methoxybenzaldehyde, in the present of organic base as catalyst. The optimization of the synthesis method was done by comparing the base catalyst; that are pyridine, triethyl amine and morpholine. The structure of FA was determined through UV, FTIR, ^{1}H-NMR, ^{13}C-NMR and MS spectroscopic methods. Improvement the physicochemical properties of FA was carried out through the establishment of the non covalent inclusion of FA with hydroxypropyl-β-cyclodextrin (HP-β-CD) by the kneading method. Its physicochemical characterizations were performed by FTIR and ^{1}H-NMR spectroscopic methods, ROESY experiment, differential thermal analysis (DTA), X-Ray diffractometry (XRD) and dissolution rate. Microwave irradiation that assisted synthesis of FA using morpholine as catalyst produced the highest yield compared to other catalysts. All of the characteristic data showed that non-covalent inclusion of FA/HP-β-CD has been formed. The dissolution rate of inclusion complex of FA/HP-β-CD was increasing significantly compared with FA.

Keywords: Knoevenagel condensation, hydroxypropyl-β-cyclodextrin, inclusion complex, microwave irradiated

INTRODUCTION

Ferulic acid (FA) is an interesting and an important phenolic acid compound in natural source, such as *Lactobacillus acidophilus*[1], *Impatient bicolor*[2], and wheat bran[3]. It has been reported that FA was utilized for its many biological effects, such as antimicrobacterial[4], anti-inflammatory activities (inhibition of COX-2), chemopreventive action and anti-proliferative agent for cancer[5-7]. The anticancer effect of FA is partly attributed to its antioxidant activity since it forms a resonance-stabilized radical phenoxyl and the high degree of conjugated unsaturation and permeability on the skin[2]. FA also has been used as a sunscreen in many countries[8]. Beside obtaining from natural source, it is very important to obtain FA by simple and effective methods. It was recently reported that synthesis of some cinnamic acid through Knoevenagel condensation could be quickly done to achieve a satisfactory yield[9,10]. Since green chemistry by the solvent free reaction is the leading issue in our lab, it has motivated us to present our results of the synthesis of FA by Knoevenagel condensation under microwave irradiation. To optimize the reaction condition, we comparing three different base catalysts, that are pyridine (pKb = 8.75), morpholine (pKb = 5.6) and triethylamine (pKb = 4.5)[11]. Despite many benefits on the biological activities, the therapeutic application of FA are imperfect because of its unpleasant physicochemical properties, especially its very poor water solubility and low stability[12,13]. Some pharmaceutical excipients, such as aerosil, avicel, and CMC are not compatible with FA, it causes many complications in its formulation[14]. Many compounds

**Author for Correspondence*



Sch. 1. The scheme of microwave assisted synthesis ferulic acid by Knoevenagel condensation

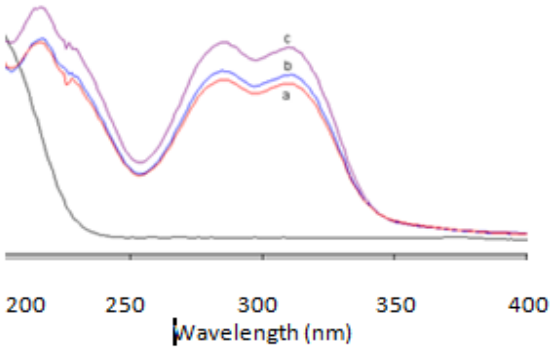


Figure 1: UV spectrum of FA (a), HP-β-CD (b), CF (c) and KI (d) in water solution

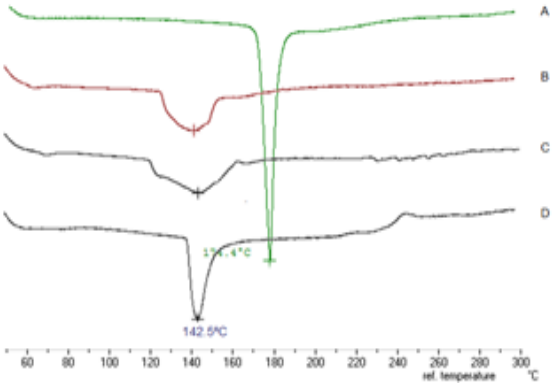


Figure 2: DTA peaks of FA (A), HP-β-CD (B), CF (C) and KI (D)

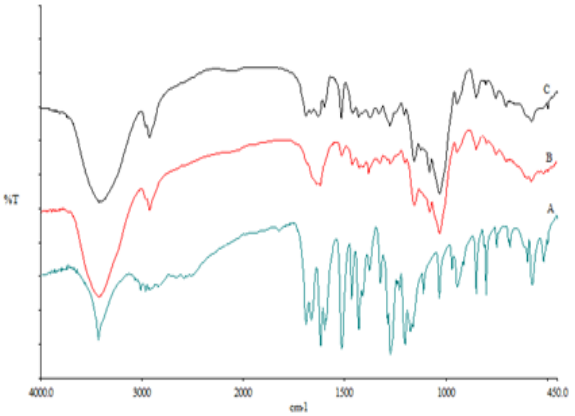


Figure 3: IR spectra study of FA (A), CF (B) and KI (C)

can tackle this problem by complexation or non covalent inclusion with HP-β-CD[15,16]. The HP-β-CD is hydroxyalkyl derivatives of cyclodextrin (CD), nonmutagenic macrocyclic oligosa-charides created by seven glucose units and consisted of α-1,4-linked α-D-glucopyranose moiety. It has no less than 10.0 percent and no more than 45.0 percent hydroxypropoxy moieties attached at primary hydroxyl, with a hydrophilic in exterior and a hydrophobic in interior site. The complex inclusion of these host-guest systems contained various non covalent interaction, such as hydrophobic and van der waals interaction, electrostatic attraction and hydrogen bonding[16,17]. The object of this study is divided into two parts. In the first part, we obtained FA and its inclusion complex of FA/HP-β-CD (KI) through kneading method. In the second part, we investigated the physicochemical characters of KI, compared with FA and FA/HP-β-CD physical mixture (CF), including the dissolution rate and their characterization profile using different analytical techniques including Ultra Violet (UV) and Fourier transform infrared (FT-IR) spectrophotometry, differential thermal analysis (DTA), X-ray diffractometry (XRD), ^{1}H-NMR contained ROESY experiment.

Experimental

Synthesis reaction was carried out in Microwave oven SANYO EM-S2612S with power 400W. Melting points were determined on an Electrothermal melting point apparatus and without correction. TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60 GF254 (Merck) in an appropriate solvent. Both ^{1}H and ^{13}C-NMR spectra were taken on a Bruker Ultrashield 600 spectrometer at 600 MHz and 150 MHz, respectively with TMS as an internal standard. The ^{1}H and ^{13}C-NMR data of CD_3OD peaks determined at δ_H 3.31 and δ_C 49.5 ppm. The IR and UV spectra were recorded on Perkin-Elmer Spectrum One and on Perkin-Elmer Lambda EZ-201, respectively. The synthesis of non covalent inclusion of FA results also performed by Differential Thermal Analyzer (DTA) Mettler Toledo, difractometer PXRD (Phillips X'Pert) and Erweka DT-700 dissolution tester. All chemicals and solvent were analytical grade. The chemicals included malonic acid, 4-hydroxy-3-methoxybenzaldehyde, morpholine, pyridine, triethylamine and HCl were purchased from E.

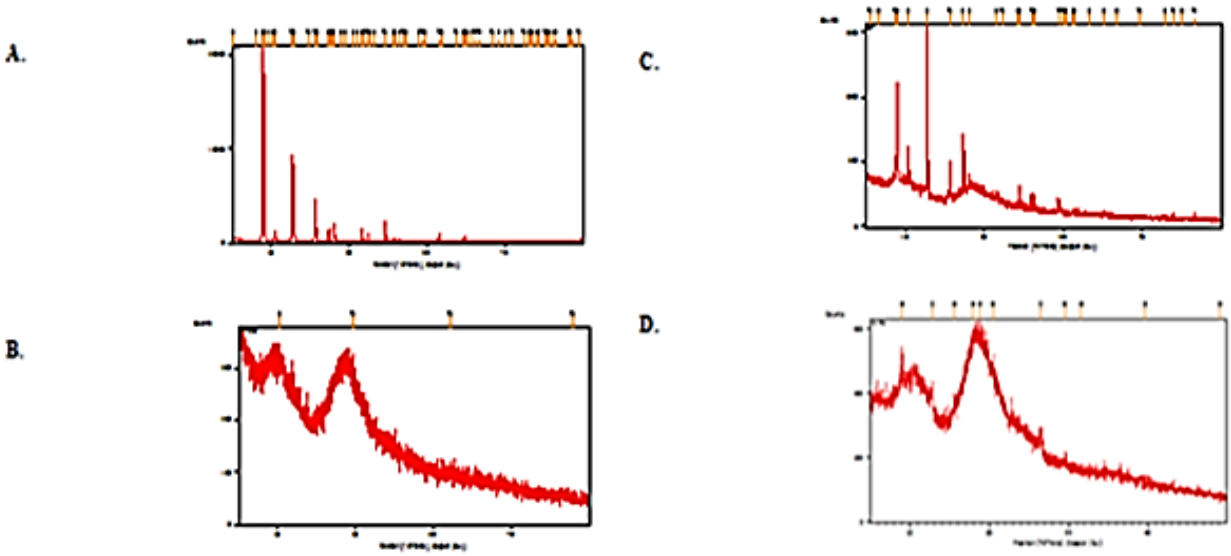


Figure 4: XRD pattern of FA (A), HP-*β*–CD (B), CF (C) and KI (D)

Merck. Hydroxypropyl-*β*–cyclodextrin (HP-*β*-CD) was obtained from WAKO Chemical.

Procedure of Synthesis of FA

A mixture of a 4-hydroxy-3-methoxybenzaldehyde or vanillin (8 mmol), malonic acid (16 mmol) and pyridine (0.04 mmol) was irradiated and heated in household microwave oven with 70%P of 400 W for 4 minutes. The reaction was monitored by TLC and stopped until the vanillin didn't show on the plate. At the end of exposure to microwave, the reaction mixture cooled at room temperature and then acidified by HCl 2N solution until the precipitated of FA formed. The precipitated of FA was recrystallized from ethanol. The structure of the product was determined through spectroscopic method, that is UV, IR, ^{1}H-NMR, ^{13}C-NMR and HR-ESI-MS.

Ferulic acid, 4-hydroxy-3-methoxycinnamic acid (FA)

Light yellow needle; m. p. = 170–171ºC; positive HR-ESI-MS *m/z* 217.0469 $[M+Na]^{+}$(calcd. for $C_{10}H_{10}O_4Na$: 217.0471); UV (ethanol) λmax: 322 nm; FTIR (KBr) ν_{max} cm^{-1}: 3436 (OH), 2941 (C-H), 2595 (OH) 1666 (C=O), 1586 (C=C), 1276 (C-O); ^{1}H-NMR (600 MHz, CD_3OD, δ ppm): 3.89 (3H, s, OCH_3), 4.86 (1H, s, OH), 6.31 (1H, d, *J* = 18 Hz, CH), 6.81 (1H, d, *J* = 6 Hz, CH), 7.05 (1H, d, *J* = 6 Hz, CH), 7.16 (1H, s, CH), 7.60 (1H, d, *J* = 18 Hz, CH), ^{13}C-NMR (150 MHz, CD_3OD, δ ppm): 56.6 (Ar-OCH_3), 111.9 (C-6), 116.1 (C-3), 116.6 (C-9), 124.1 (C-4), 127.9 (C-5), 147.1 (C-8), 149.5 (C-1), 150.6 (C-2), 171.1 (C-10).

Preparation of inclusion complex of FA and HP-β-CD (KI)

The complex of FA and HP-*β*-CD was synthesized by mixing FA and HP-*β*-CD (at 1:1 M ratio) corresponding to the kneading method[22]. A mixture of HP-*β*-CD (0.005 mol) and FA (0.005 mol) was dissolved in 50 ml of ethanol : water (1:1). The mixture was grinded continuously at room temperature until dry precipitated formed, and then stored for 48 h at the exicator. Its physicochemical properties were characterized by DTA, XRD and spectroscopic methods, including FTIR, ^{1}H-NMR and ROESY.

Preparation of physical mixture of FA and HP-β-CD (CF).

FA and HP-*β*-CD were separately mixed in mortars, and then the determined amounts of both compounds were weighted out (at 1:1 M ratio) and blended together until a homogeneous form was taken. Its organoleptic of the CF was identified and its physicochemical properties were characterized by DTA, XRD and spectroscopic methods, including spectrophotometer UV and FTIR[12].

Physicochemical characterization

UV–visible spectroscopy

The UV–visible absorption spectra were verified for FA, their physical mixture (CF) and the inclusion complex (KI) through a UV–visible recording spectrophotometer Perkin-Elmer Lambda EZ-20[12]. The water solution (5 mmol) of each sample was prepared at the room temperature then was examined, respectively, in the range from 200 to 400 nm to obtain the UV–visible absorption spectra.

Differential Thermal Analysis (DTA)

DTA analysis was carried out for FA, HP-*β*-CD, CF and KI with Differential Thermal Analyzer (DTA) Mettler Toledo. All samples were previously dried for 24 h at 110ºC. Approximately powder 5 mg of sample is introduced into an aluminium pan and heated into DTA instrument. Tool set at a scanning rate of 10ºC/min in 50 and 300ºC, then thermogram profiles were analyzed.

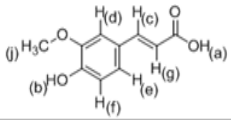
Fourier transformer infrared (FT-IR) spectrum analysis

The FT-IR spectra of FA, HP-*β*-CD, CF and KI were treasured between 4000 and 450 cm^{-1}. Each sample was crushed with spectroscopic grade potassium bromide (KBr) powder and then compelled into 1 mm pellets (1% sample in dry KBr). A blank KBr disk was handled as background[12].

X-ray diffraction (XRD) analysis

XRD pattern of FA, HP-*β*-CD, CF and KI were measured

Table 1: Chemical structure of FA and ^{1}H-NMR chemical shifts of the protons of FA free and after complexation with HP-β-CD (1:1 Molar ratio) in D_2O.



Proton	δ_{free} (ppm)	$\delta = \delta_{complex}$ (ppm)	$\delta = \delta_{complex} - \delta_{free}$ (ppm)
H (c)	7.38	7.50	0.12
H (d)	7.21	7.17	- 0.04
H (e)	7.08	6.98	- 0.10
H (f)	6.88	6.84	- 0.04
H (g)	6.33	5.95	- 0.39
H (b)	4.84	4.82	- 0.02
H (j)	3.85	3.90	0.05

by Differential Thermal Analyzer (DTA) Mettler Toledo. Sample is blended in a mortar until smooth, and be recorded in X-ray diffractometer in the 2θ angel range between 5^0 and 50^0 with the speed of change in angle of 0.01-0.02/sec.

^{1}H-NMR analysis

The alterations in chemical shifts and interaction the guest and the host on each other, the formation of FA/HP-β-CD inclusion complex (KI) has also been investigated via ^{1}H-NMR. The spectra were recorded at 26.85ºC on Bruker Ultrashield 600 MHz (^{1}H) spectrometer. Chemical shifts were measured from TMS used as external standart and the resonance set as 0 ppm. Penetration of FA at the FA/HP-β-CD inclusion complex (KI) was explained through ROESY experiment using D_2O as solvent.

Dissolution test

Dissolution test was directed for FA, CF and KI. The samples was equivalent to 50.0 mg FA was weighed, and put it into 900 ml of CO_2-free water dissolution medium, then adjusted at pH of 6.8 ± 0.05. The temperature is set at 37 ± 0.5°C and the paddle-type stirrer rotated at 50 rpm. The sampling of aliquot (5 ml) were taken at 5, 10, 15, 30, and 60 minutes, filtered by 0.45 µm pore filter, and suitably diluted with dissolution medium. The concentration of FA was determined using a UV spectrophotometer at λ maximum. The dissolution test was performed triplicate.

RESULTS AND DISCUSSION

Chemistry

In this present study we report the synthesis of FA by Knoevenagel condensation between malonic acid that has properties of C-Hα with benzaldehyde derivatives, i.e. 4-hydroxy-3-methoxybenzaldehyde or vanillin in the presence organic base as catalyst. Knoevenagel condensation on malonic acid and carbonyl compounds is an important route for the synthesis α,β-unsaturated acids especially for cinnamic acid derivatives[9,18]. This method involves heating the aromatic aldehyde that is vanillin and malonic acid in the presence of a basic solvent to facilitate the decarboxylation reaction. The addition of a catalyst in a Knoevenagel condensation would accelerate the rate of reaction and to optimize the time and cost efficiency. In optimizing the condition reaction, we based on our previous works in time consuming reaction and power of microwave irradiation. The general scheme of transformations is shown below: UV spectrum of FA exposed λmax at 322 nm that is longer than λmax of vanillin (230 nm) due to a conjugated vinylic double bond with aromatic ring of FA. The IR spectrum of FA displayed peaks at 1666 cm^{-1} (unsymetrical conjugated C=C of alkene), 1586 and 1473 cm^{-1} (C=C aromatic ring) (Fig.3a). The unsymetrical conjugated vinylic double bond and aromatic ring were approved by ^{1}H-NMR and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of FA presented three aromatic proton at δ_H 6.81 (1H, d, J = 6 Hz), 7.14 (1H, d, J = 6 Hz) and 7.09 (1H, s), and two protons doublets with J = 18 Hz at δ_H 6.31 and 7.60 that indicated the protons of vinylic double bound in *trans* form isomer. The IR spectrum of FA reveals of a sharp peak at 3436 cm^{-1}, assigned to O-H phenolic vibration, which is proved at δ_H 4.86 ppm of ^{1}H-NMR spectrum. The peak at 1276 cm^{-1} of IR spectra indicated C-O vibration of methoxy moiety and validated by ^{1}H-NMR and ^{13}C-NMR spectra at δ_H 3.89 and δ_C 56.6 ppm, respectively. The IR spectrum of FA confirmed the substituted moieties at 690-880 cm^{-1}. The carbonyl moiety of carboxylate group approved by peak at 1692 cm^{-1} of IR spectrum and the chemical shift of ^{1}H-NMR and ^{13}C-NMR spectra at δ_H 3.89 and δ_C 56.6 ppm, respectively. The GC-MS spectrum through EI mode of FA showed one peak at 10.87 min and its base peak at *m/z* 150 verified FA lost the CO_2 molecule. While HR-ESI-MS data showed base peak at *m/z* 217.0469 [M^++Na]. All data recommended the agreement with the suggested structure of FA (4-hydroxy-3-methoxy cinnamic acid). This results showed physicochemical properties that were similar to the product reported by Shahwar et al. (2010)[2] and Sajjadi et al.[19]. Different catalysts will give diverse yields, it is due to their distinction physical and chemical properties. The yields of FA using catalysts i.e. pyridine, morpholine and triethyl amine are 76%, 84% and 71%, respectively. Differences of alkalinity properties of each catalyst is highly influenced the speed of the reaction. This catalyst caused the displacement of protons in the formation of carbanions of malonic acid which acts as a nucleophilic carbon. This carbanion attacked the carbonyl moiety of derivates of benzaldehyde such as vanillin so nucleophilic attack on the carbonyl group can be taken[20]. The factor that may affect the yield is the chemical structure of catalyst. In the review of its structure, morpholine having secondary amine and in the form of a ring that causes obstruction the free rotation around that nitrogen atom and cause small steric hindrance as well. In addition to morpholine, the nitrogen atom of amine moiety has the sp^3 hybridization properties. Alkalinity the sp^3 hybridization of morpholine is stronger than sp^2 owned by pyridine structure. While the sp^3 hybridization of triethylamine has the same shape as morpholine, but the structure of triethylamine has tertiary amines which have higher steric obstacle causes difficulty to receive a proton from Hα of malonic acid. So, its yield is less than the other catalyts. Bath et al. (2014)[20] reported that synthesis annulated uracil derivatives through Knoevenagel reaction used triethylamine, which is a weak catalyst base, give a

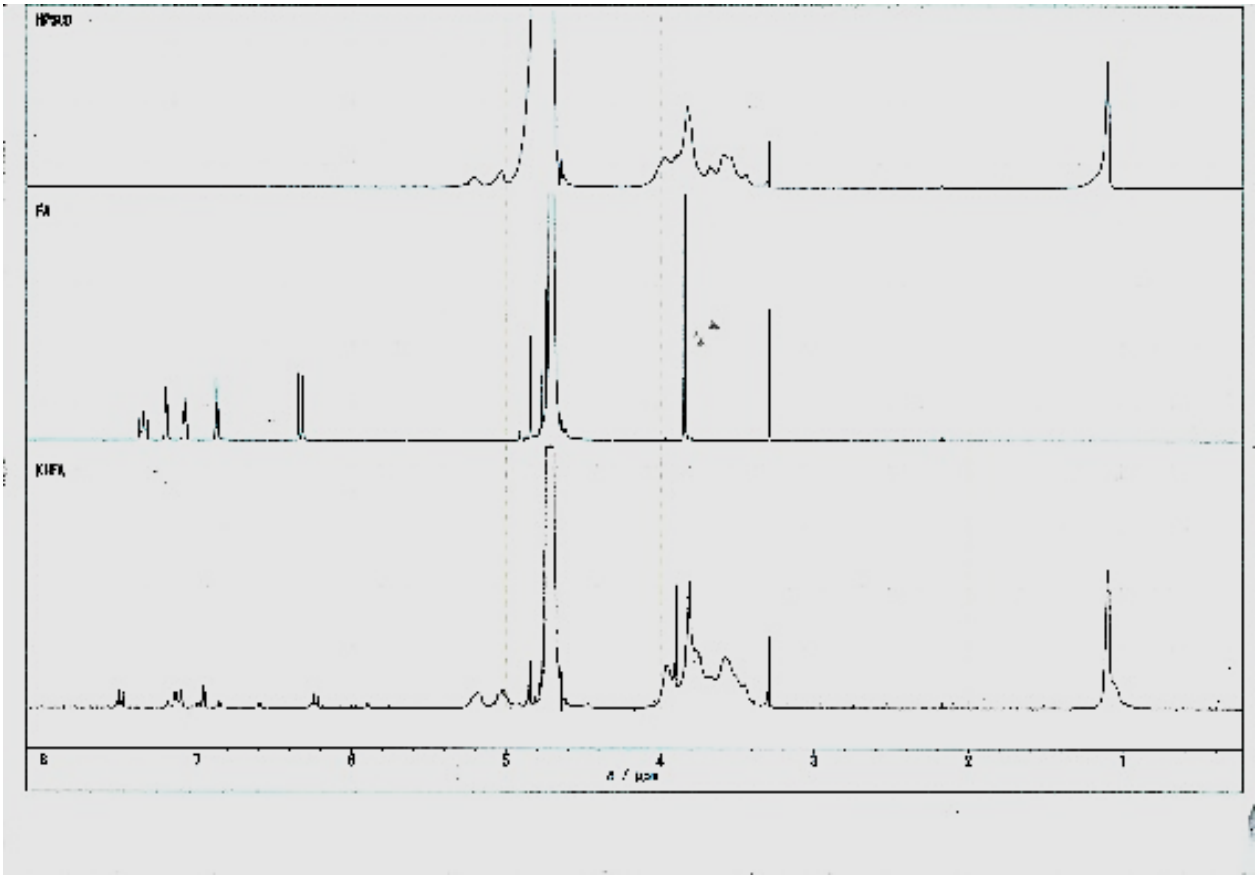


Figure 5: ^{1}H-NMR spectra of of HP-β-CD (upper), FA (middle), KI (lower) in D_2O

good yield.

Characterization of CF and KI of FA

UV spectroscopy analysis

UV spectroscopy is one of the crucial instruments to analysize the complexation of FA with HP-β-CD. HP-β-CD had no UV absorption because it does not have a chromophore group. Fig. 1 displayed the spectra of FA, CF and KI in water solution. Fig. 1b showed three peaks at 310, 283, and 216 nm of CF, it was similar to FA (Fig. 1a). Whereas Fig. 1c showed the highest absorbance among the other sample explained the largest concentration solute in water solution. All spectra present a high peak at 217 nm, which verify that it is impervious to HP-β-CD[21]. But this result has not yet proof the formation of inclusion complex. Furthermore the inclusion complex of FA/HP-β-CD will be characterized by using X-Ray Diffractometry, FTIR, ESI-MS and ^{1}H-NMR spectroscopy including ROESY.

DTA analysis

FA, HP-β-CD, physical mixture of FA/HP-β-CD (CF) and the complex of FA/HP-β-CD (KI) prepared by kneading method were subject to DTA analysis. The thermal curves were given in Figure 2. The DTA curve (Fig. 2A) displayed one characteristic of sharp endothermic peak at 174.4-ºC which indicates the melting point of FA (Fig. 2A). The DTA curve at Fig. 2B, 2C and 2D showed similar wide endothermic peak at 142.5ºC of HP-β-CD, CF and KI, respectively. Their peak shifted to 31.5ºC and no peak shown at 174.4ºC indicate that the inclusion complex does not contain much residue of FA, thus it suggests that the compound is well dispersed in HP-β-CD cavity.

IR spectra analysis

The IR spectrum of FA reveals a sharp peak at 3436 cm^{-1}(O-H phenolic vibration), 3016-2595 cm^{-1} (O-H of carboxylate group overlaps with the C-H absoption of aromatic moiety), 1692 cm^{-1} (carbonyl moiety of carboxylate group), 1666 cm^{-1} (unsymetrical conjugated C=C of alkene), 1586 and 1473 cm^{-1} (C=C aromatic ring), 1276 cm^{-1} (C-O vibration of methoxy moiety) and the substituted moieties at FA confirmed at 690-880 cm^{-1} (Fig. 3A). According to reference, the IR spectra of HP-β-CD showed intense band at 3300-3500 cm^{-1} (OH streching vibration) and 2800-3000 cm^{-1} (-CH and -CH_2 groups)[22]. Afterward the IR spectra of physical mixture (Fig. 3B) and complexation (Fig. 3C) had not show the aromatic and alkene moieties of FA. The carbonyl and O-H phenolic band were slightly shifted the wave number and the show of different pattern from FA indicated there is an appearance of host-guest interaction. These suggest the possibility of formation of hydrogen bonds between the hydroxyl groups of host cavities and carbonyl moiety of carboxylate group and between the hydroxyl groups of host cavities and hydroxyl phenolic of FA. The bands of aromatic and alkene which are not shown at Fig 3B and 3C,

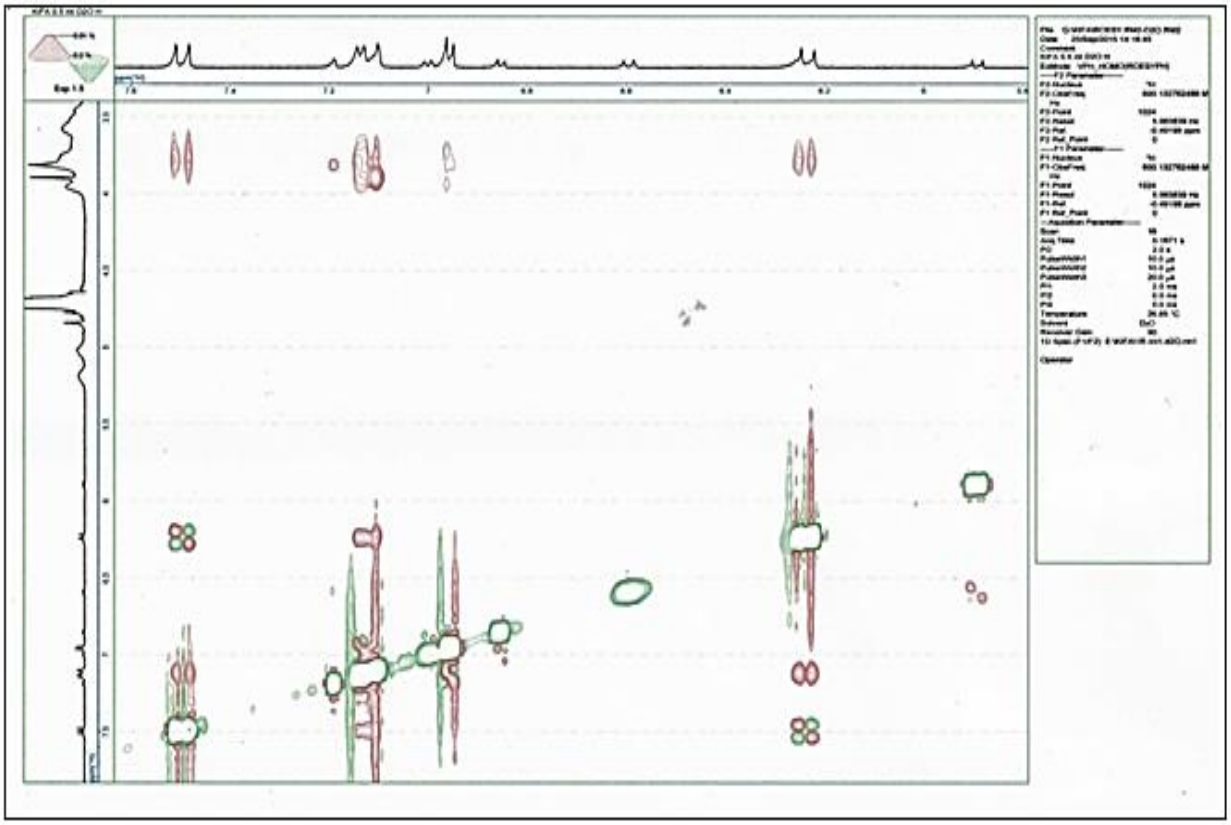


Figure 6: ROESY spectrum of FA/HP-*β*-CD inclusion complex (KI) in D_2O

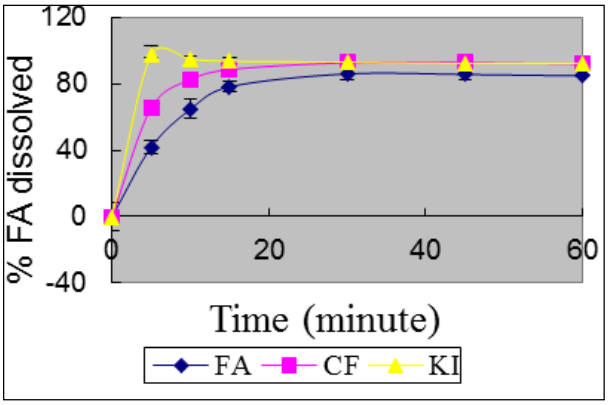


Figure 7: Profile Dissolution of FA, CF and KI

indicate van der walls interaction between –CH and $–CH_2$ group of host cavity occured.

X-Ray Diffractometry (XRD) analysis

XRD study was performed in Fig. 4, while Fig. 4A showed intense and sharp peaks of FA that confirm the crystalline nature of compound, whereas Fig. 4B displayed a wide peak indicating amorphous material of HP-*β*-CD. The XRD pattern of CF (Fig. 4C) and KI (Fig. 4D) displayed distinctive peak compared to FA. The peak corresponding to 9.0' (2θ) showed less intense in CF than FA and the shortest in KI, whereas the peak of 12.8' (2θ) in FA had less intense in CF and had not shown in KI. The peaks of 15.6' and 24.6' in FA were not displayed in both diffractogram of CF and KI, but there were the new short and broad peaks shown at 17.3' in CF and 17.9' and 18.8' in KI. The KI pattern (Fig. 4D) showed quite similarity with HP-*β*-CD (Fig. 4B). Fig. 4C informed two patterns of some peaks from crystalline FA and the amorphous of HP-*β*-CD. The XRD results prove the confidential change at lattice level during inter-penetration of two substance by kneading method and huge similarity with amorphous character in XRD pattern of inclusion complex of FA with HP-*β*-CD through freeze-drying method reported by Wang et al. (2011)[12].

^{1}H-NMR studies

^{1}H-NMR spectra of the inclusion complex between FA and HP-*β*-CD cavity in D_2O were performed at Fig. 5, whereas the chemical shift of FA, FA in KI and differences of the chemical shift of both of them were given in Table 1. Data in Table 1 presented the chemical shift and their changes upon complexation for FA protons are presented, where $\Delta\ \delta = \delta_{complex} - \delta_{free}$ FA. There are some differences of chemical shift protons of FA in inclusion complex form compared with FA, especially its significance for the protons of vinylic double bond and aromatic ring. The peaks H(c) and H(j) of the signals displayed downfield frequency field, while the rest showed upfield. The upfield movement of FA protons is caused the disparity of local polarity and the weak interaction with HP–*β*-CD cavity hydrogen atom. This result described some difference with FA/*β*-CD inclusion complex form informed by Anselmi et al. (2008)[13]. Nicolaescu et al. (2010)[22] reported that the steric hindrance is an important factor in the formation of

inclusion complex. The highest variation of chemical shifts has been recorded for the unsubstituted molecule of *β*-CD. The structure of HP-*β*-CD had a large substituent on the glucose units, so maybe it makes difficulty for the guest i.e. FA to come into HP-*β*-CD cavity. The 2D NMR (ROESY) was also presented at Fig. 6 to explain the inclusion complex form. There were strong cross-peaks at protons of vinylic double bond and aromatic ring of FA with protons of HP-*β*-CD. It indicated the protons of vinylic double bond that was conjugated with ring aromatic moiety of FA are located inside the HP-*β*-CD cavity. No cross-peaks were detected for the methoxy protons of FA and the HP-*β*-CD protons and this is maybe due to the location of the methoxy and phenol group in proximity to either the primary or secondary rim of HP-*β*-CD. These result are similar with FA/*β*-CD inclusion complex form[13]. The interpretation of ^{1}H-NMR and ROESY experiment clarified and proved that FA/HP-*β*-CD inclusion complex formed by kneading method.

Dissolution Rate analysis

The dissolution rate profiles of FA, CF and KI were displayed at Fig. 7. Physical mixture of FA/HP-*β*-CD (Fig. 7B) has a dissolution rate faster than the FA (Fig. 7A) even if the physical mixture is not formed inclusion complex. Physical mixture between the compound soluble or insoluble in water such as FA with cyclodextrin compounds rather than a single compound because the compound is dispersed in a hydrophyllic matrix. The compound will be carried passively into a dissolution medium as the matrix dissolves so that it will increase the rate of dissolution[23]. Cyclodextrin compounds have the ability to form inclusion complex in situ in the dissolution medium so the dissolution rate of drugs increased even though the state is not formed on the inclusion complex[24]. The profile of dissolution rate displayed KI (Fig. 7C) have the highest dissolution rate as related to CF and pure FA at DP5, DP10 and DP15 ($p<0.05$). Whereas at DP30, DP45 and DP60 dissolution rate of CF and KI showed significant effect with respect to FA, however no significantly different between CF and KI ($p<0.05$). The XRD study approved the improvement in dissolution rate related the formation of inclusion complex in the solid state with reduction in the crystallinity of FA. The characterization of dissolution profiles was carried out by comparing dissolution efficiency (DE), that is the area under a dissolution curve between described time points, and the fit factors (*f1* and *f2*) [25]. Dissolution efficiency for 60 minutes (DE60) of KI is the highest and significantly difference ($p<0.05$) from CF and FA. That is due to interaction between FA and HP-*β*-CD in CF form only in dipersion form, so the contact is not optimum. Fernandes et al. (2002)[26] reported greater hydrophilicity, higher wetting effect and mechanical treatment caused the amplified of dissolution rate for inclusion complex. It is due to improving the contact between the compound and the carrier. By kneading method, FA and HP-*β*-CD dissolved in ethanol, so molecular interaction occurred and the contact between them is optimum. These results indicated, there was a change in the crystalline structure of FA in the form of FA/HP-*β*-CD inclusion complex to be amorphous form to improving of dissolution rate and solubility rather than before [27].

CONCLUSION

Ferulic acid (FA) can be synthesized by Knoevenagel reaction using microwave irradiation. Preparation the inclusion complex of FA/HP-*β*-CD through kneading method increased dissolution rate of FA significantly.

ACKNOWLEDGMENTS

The authors would like to express their sincere thanks to Indonesian Directorate General of Higher Education (DIKTI) and Rector of Airlangga University Indonesia for financial support by PUPT Grant in year 2015.

REFERENCES

1. D. Szwajgier. The Use of An Extracellular Ferulic Acid Esterase from *Lactobacillus acidophilus* K1 for The Release of Phenolic Acids during Mashing. esides, *J. of The Institute of Brewing*. 2011, *117(3)*, 427-434.
2. D. Shahwar, S.U. Rehman, M.A. Raza. Acetyl cholinesterase inhibition antioxidant activities of ferulic acid isolated from *Impatients bicolor* Linn. *J. of Medicinal Plants Research.* 2010, *4(3)*, 260-266.
3. P.K. Sarangi, H.P. Sahoo. Ferulic Acid Production from Wheat Bran using *Staphylococcus aereus*. *New York Science Journal*. 2010, 79-81.
4. A. Borges, C. Ferreira, M.J. Saavedra, M. Simoes. Antibacterial activity and mode of action of ferulic acid and gallic acids against pathogenic bacteria. *Microb. Drug. Resist.* 2013, *19(4)*, 256-265. Doi:10.1089/mdr.2012.0244.
5. M. Bilecova-Rabajdova, A. Birkova, Urban, K. Gregova, E. Durovcova, M. Marekova. Naturally occurring substances and their role in chemo-protective effects. *Cent. Eur. J. Public Health*. 2013, *21(4)*, 213-219.
6. S. Manoharan, T. Rejitharaji, M.M. Prabhakar, A. Manimaran, R.B. Singh. Modulating Effect of Ferulic Acid on NF-kB, COX-2 and VEGF expression pattern during 7,12-dimethylbenz(a) anthracene induced oral carcinogenesis. The Open Nutraceutical Journal. 2014, 7, 33-38.
7. N. Kumar, V. Pruthi. Structural Elucidation and Molecular Docking of Ferulic Acid from *Parthenium hysterohorus* possessing COX-2 inhibition activity. *3 Biotech*. 2015, 5, 541-551. Doi:10.1007/s13205.014-0253-6.
8. C. Oresajo, T. Stephen, P.D. Hino, R.M. Law, M. Yatskayer, . Foltis, S. Pillai, S.R. Pinnell. Protective effects of a topical antioxidant mixture containing vitamin C, ferulic acid and hloretin against ultraviolet-induced hotodamage in human skin. *J. of Cosmetic Dermatology*. 2008, 7, 290-297.
9. E. Pontiki, D. Hadjipaviou-Litina, K. Litinas, G. Geromichalos. Novel Cinnamic Acid Derivatives as Antioxidant and Anticancer Agents : Design, Synthesis and Modelling Studies. *Molecule*, 2014, *19*, 9655-9674.Doi: 10.3390/molecules 19709655.

10. M. Gupta, B.P. Wakhlo. Tetrabutyl ammonium bromide mediat*ed* Knoevenagel condensation in water : Synthesis of cinnamic acids. *Arkivoc,* 2007,i, 94-98.
11. S. Budavari, M. J. O'neil, A. Smith, P.E. Heckleman, J.F. Kinneary. The Merck Index : Encyclopedia of Chemical, Drug and Biologicals. 2001.13th Ed, New York: Merc & Co Inc.
12. J.Wang, Y. Cao, B. Sun, C. Wang. Characterisation of Inclusion Complex of *trans*-ferulic acid and hydroxypropyl-*β*-cyclodextrin. *Food Chemistry*. 2011,
15. M.F. Canbolat, Celebioglu, T. Uyar. Drug delivery system based on cyclodextrin-naproxen inclusion complex incorporated in electrospun polycaprolactone nanofibers. Colloids and Surfaces B. *Biointerfaces*. 2014, *115*, 15-21.
16. L. Lawtrakul, K. Inthajak, P. Toochinda. Molecular calculation on *β*-cyclodextrin inclusion complex with five essential oil compounds from *Ocimum basilicium* (sweet basil). *Science Asia*. 2014, *40*, 145-151.
17. S. Talegaonkar, A.Y. Khan, R.K. Khar, F.J. Ahmad, Z.I. Khan. Development and characterization of paracetamol complexes with hydroxypropyl-β-cyclodextrin. *Iranian Journal of Pharmaceutical Research*. 2007, *6(2),* 95-99.
18. M. Rudyanto, L. Hartanti. Synthesis of some cinnamic acid derivatives : Effect of groups attached on aromatic ring to reactivity of benzaldehyde. *Indo. J. Chem*. 2008, *8(2),* 226-230.
19. S.E. Sajjadi, Y. Shokoohinia, N.S. Moayedi. Isolation and identification of ferulic acid from aerial parts of *K.odoratissima* Mozaff. *Jundishapur J. Pharmaceutical Products*. 2012, *7(4),* 159-162.
20. R. Menegatti. Green chemistry-aspects for Knoevenagel reaction. http://cdn.intechopen.com/pdfs-wm/33320.2012:13-32. Accessed on 15 October 2015.
21. A.R. Bhat, R.S. Selokar, J.S. Meshram, R.S. Dongre. Triethylamine : an efficient N-base catalyst for 124, 1069-1075. Doi: 10.1016/ j.foodchem.2010.07.080.
13. C. Anselmi, M. Centini, M. Ricci, A. Buonocore, P. Granata, T. Tsuno, R.M. Facino. Analytical characterization of ferulic acid *γ*-cyclodextrin inclusion complex. *J. of Pharmaceutical and Biomedical Analysis*. 2006, *40,* 875-881.
14. Y.T. Sohn, J.H. Oh. Characterization of physicochemical properties of ferulic acid. *Arch. Pharm. Res*. 2003, *26(12),* 1002-1008.
synthesis of annulated uracil derivatives in aqueous ethanol. *J. Mater. Environ. Sci*. 2014, *5(5),* 1653-1657.
22. C. Nicolescu, C. Arama, C.M. Monciu. Preparation and characterization of inclusion complexes between repaglinide and *β*-cyclodextrin, 2-hydroxypropyl*β*-cyclodextrin and randomly methylated *β*-cyclodextrin. *Famacia*. 2010, *58(1),* 78-88.
23. O. Bekers, E.V. Uijtendaal, D.A. Beijnen, A. Bult, W.J.M. Underberg. Cyclodextrin in pharmaceutical field. *Drug Dev. Ind. Pharm.* 1991, *17(11),* 1503-1548.
24. C.M. Fernandes & F.J.B. Veiga. Effect of the hydrophobic nature of triacetyl-*β*-cyclodextrin on the complexation with nicardipine hydrochloride : Physicochemical and dissolution properties of the kneaded and spray-dried complexes. *Chem. Pharm. Bull.* 2002, *50(12),* 1597-1602.
25. L. Kassaye & G. Genete. Evaluation an comparison of in vitro dissolution profiles for different brands of amoxicillin capsules. *African Health Science*. 2013, *13(2),* 363-375.
26. C. Challa, A. Ahuja, J. Ali, R.K. Khar. Cyclodextrin in drug delivery : An updated review. *AAPS Pharm. Sci. Tech*. 2005, 13.
27. J.S. Pamudji, R. Mauludin, Nurhabibah. Influence of *β*-cylodextrin on cefixime stability in liquid suspension dosage form. *Procedia Chemistry*. 2014, 13, 119-127.