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MOLECULAR DYNAMICS STUDIES OF FULL HUMAN MATRIX METALLOPROTEINASE 9 LIGANDED WITH N-HYDROXY-2-[(4-PHENYLPHENYL) SULFONYL-PROPAN-2-YLOXYAMINO] ACETAMIDE

Roy Gunawan Wicaksono, Maywan Hariono and Enade Perdana Istyastono*)

Faculty of Pharmacy, Sanata Dharma University, Paingan Maguwoharjo Depok Sleman Yogyakarta, 55282, Indonesia

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ABSTRACT

The research presented in this article aimed to provide a full quarternary structure of human matrix metalloproteinase 9 (MMP9) enzyme with a ligand in the catalytic site for structure-based virtual screening. The enzyme plays an important role in wound healing of diabetic foot ulcer. By employing the primary structure of the enzyme obtained from UniProt database (UniProt:P14780), the theoretical structure of full apoenzyme of the human MMP9 (PDB:1LKG), the crystal structures of the catalytic domain (PDB:4H3X) and the hemopexin domain (PDB:1ITV) of the human MMP9, homology modeling studies have been performed. The ligand N-2-(biphenyl-4-yl-sulfonyl)-N-2-(isopropyloxy)-acetohydroxamic acid (CC27) or N-hydroxy-2-[(4-phenylphenyl)sulfonyl-propan-2-yloxyamino]acetamide (IUPAC version) from PDB:4H3X was embedded in the catalytic site of the enzyme. The modeling made use of the modules of homology modeling in YASARA structure. Subsequently, molecular dynamics (MD) simulations in YASARA structure were performed to examine the stability of the enzyme. The homology model was found stable after 5.05 ns and the lowest energy of the model was found at the 6.40 ns of the MD production run. This lowest energy snapshot was then energetically minimized and analyzed for its applicability for virtual screening. This optimized model was then stored in Mendeley Data (DOI: 10.17632/4gsb4p75gz.1).

Keywords: homology modeling; matrix metalloproteinase 9; molecular dynamics simulations; virtual screening; YASARA structure.

INTRODUCTION

Enzyme matrix metalloproteinase 9 (MMP9) becomes a molecular target of interest in the discovery of therapeutic agents for a diabetic foot ulcers and cancer (Hariono *et al*., 2018). The enzyme comprises a catalytic domain and a hemopexin-like domain, which have different roles (Roeb *et al*., 2002). The catalytic domain degrades the damaged matrix membrane in the wound healing processes (Vandooren *et al*., 2017). Uncontrolled enzyme activity causes the membrane degradation and formation balance disruption in the wound healing processes (Ayuk *et al*., 2016). Inhibitors in the catalytic domain are required to control the balance (Jones *et al*., 2019). On the other hand, although existed in the same MMP9 enzyme, the hemopexin domain has a different role (Dufour *et al*., 2010). Hemopexin domain acts as a messenger receptor by forming dimers (Ezhilarasan *et al*., 2009). The interaction between the hemopexin domain with cell surface receptors triggers cell proliferation (Delozier *et al*., 2011). The growth of cancer cells could, therefore, be inhibited by inhibitors for the hemopexin domain (Alford *et al*., 2017).

The available 3D structures can assist the visualization of the ligand and protein

*Corresponding author: Enade Perdana Istyastono
Email: *enade@usd.ac.id*

interactions in structure-based drug design (SBDD) approaches (Wang *et al*., 2018). The homology comparative modeling method is an approach to predict the 3D structure of protein-based on its amino acid chain. Three-dimensional structures in this approach are made using other similar proteins that the 3D structures are already known as the templates (Cavasotto and Phatak, 2009). The processes of building the structure of the 3D protein model include: (1) Other similar proteins with known the 3D structures are used as templates; (2) The amino acid sequences of protein target are aligned to the templates; (3) The 3D structures of the target are constructed based on the 3D structure of the templates; (4) The MMP9 model resulted are then evaluated, validated, and remodelled until appropriate models are obtained (Mart *et al*., 2000).

There is one publicly available of full MMP9 model (PDB:1LKG). Unfortunately, there is no ligand in the model, which then create difficulties in the binding pocket identification. The aim of the research presented in this article was to provide validated virtual target to discover inhibitors for MMP9. Therefore, homology modeling approaches to construct a 3D structure of full MMP9 with ligands followed by molecular dynamics simulations to examine the stability of the model were performed. The optimized model resulted in this research could be downloaded from Mendeley Data (DOI: 10.17632/4gsb4p75gz.1).

METHODS

Materials and Instrumentations

The human MMP9 primary sequences (UniProt:P14780) were obtained from The Universal Protein Resource (UniProt; https://www.uniprot.org/). Three-dimensional structures from The Protein Data Bank (PDB; https://www.rcsb.org/) with identity PDB:1LKG, PDB:4H3X, and PDB:1ITV were used as the 3D structure templates of human MMP9. The software mainly used was YASARA Structure version 19.1.27 (license number of 394125786). Computational studies for homology modeling were performed in a workstation with Intel® Pentium® Silver N5000 as the processor, 4 GB random access memory (RAM), and Windows 10 Home 64-bit as the operating system. The molecular dynamics simulations and re-docking simulations were performed in a workstation with Intel® CoreTM i5-7500 as the processor, 8 GB RAM, and Windows 10 Professional 64-bit as the operating system.

Procedures

The homology model of the human MMP9 was constructed by employing P14780.fasta obtained from UniProt as the source of the primary sequences and PDB:1LKG (sequences 1 to 93 and 445 to 511), PDB:4H3X (sequences 94 to 444), and PDB:1ITV (sequences 512 to 707) from PDB as the templates. The module homology modeling in YASARA Structure was employed to construct the initial full model of the human MMP9. Subsequently the ligands from PDB:4H3X, and PDB:1ITV were incorporated by aligning the chain A of the structures to the initial model and followed by removing the protein parts of the crystal structures. Focused on the side chain residues of the model, the quarternary structure was then subjected to molecular dynamics simulations with fixed atoms in the main chain of residues number 94 to 444 and 512 to 707. All ligands were also fixed during molecular dynamic simulations. The molecular dynamic simulations were performed in YASARA Structure by employing a modified macro from the default macro md_run.mcr from YASARA Structure (Krieger and Vriend, 2009). The modification was done in line 67, from duration=’forever’ to duration=1000. The molecular dynamics simulations were followed by trajectory analysis using md_analyse.mcr. The final scene file from molecular dynamic simulations resulted from the analysis was unfix and then subjected to energy minimization using energy minimization module. The scene resulted from energy minimization was then saved as mmp9_final.sce. Objects 2 and 3 in the scene were then joined to object 1 by using module Join in YASARA. The object 1 was then

stored in YASARA object and pdb formats as mmp9_final.yob and mmp9_final.pdb, respectively. The model of the human MMP9 was then analyzed by employing module check in YASARA structure and AMBER14 as the force field.

The file mmp9_final.pdb was subsequently employed as the starting point for 20 ns MD simulations using YASARA Structure (Krieger and Vriend, 2009) . The default macro md_run.mcr from YASARA Structure was used with modification in line 67, from duration=’forever’ to duration=20000 and in line 109, from saveinterval=100000 to saveinterval=10000. The MD simulations were followed by trajectory analysis using md_analyse.mcr. The structure of the complex enzyme-inhibitor is considered stable if the deviation of the root-mean-squared-deviation (RMSD) values of at least 5 ns duration of the MD simulations is less than or equal to 1 Å (Liu *et al*., 2017). The first 5 ns of the MD simulations were considered as the production run. The free energy of binding (ΔG) of all snapshots during the production run was calculated by employing a method published by Prasasty and Istyastono (2019). The snapshot with the lowest ΔG value was then minimized and selected to be analyzed for its applicability for being the target in structure-based virtual screening campaigns by performing 1000 times redock simulations using VINA embedded in YASARA Structure. The docking configuration was embedded to the macro file dock_run_1000.mcr developed in this research. The file could be obtained from Mendeley Data ((DOI: 10.17632/4gsb4p75gz.1). The model was considered as applicable for virtual screening if the RMSD values of at least 95% redocking result of the ligand are less or equal to 2 Å.

RESULTS AND DISCUSSION

The research presented in this article aimed to publicly provide a full quarternary structure of human MMP9 with a relevant ligand in the catalytic site to be used further in drug discovery for diabetic wound healing. The result of the homology modeling is available in 3 formats: YASARA scene (mmp9_final.sce), YASARA object (mmp9_final.yob), and PDB file (mmp9_final.pdb). These files were then stored in Mendeley Data (doi:10.17632/xj7yt48jwb.1). The final model applicable for virtual screening is available as a pdb file (mmp9.pdb), which was also stored in Mendeley Data (DOI: 10.17632/4gsb4p75gz.1).

Table 1. Quality of the final model of the full human MMP9.

No	Quality Criteria*)	Value	Reference Value (Krieger *et al*., 2002)
1	Correctness of enantiomers	0.000	> 0 is bad
2	Absence of cis-peptide bonds	2.000 (at the N-terminus of PRO A 553 and of PRO A 598)	> 0 is bad, but cis-prolines are OK
3	Adherence to naming conventions	0.000	> 0 is bad
4	Normality of bond lengths	0.700	< -2 is poor, < -4 is bad
5	Normality of bond angles	0.014	< -2 is poor, < -4 is bad
6	Normality of dihedral angles	0.057	< -2 is poor, < -4 is bad
7	Normality of planar groups	0.573	< -2 is poor, < -4 is bad

*)The criteria of “Normality of water locations” was not checked since the model does not contain any water molecule.

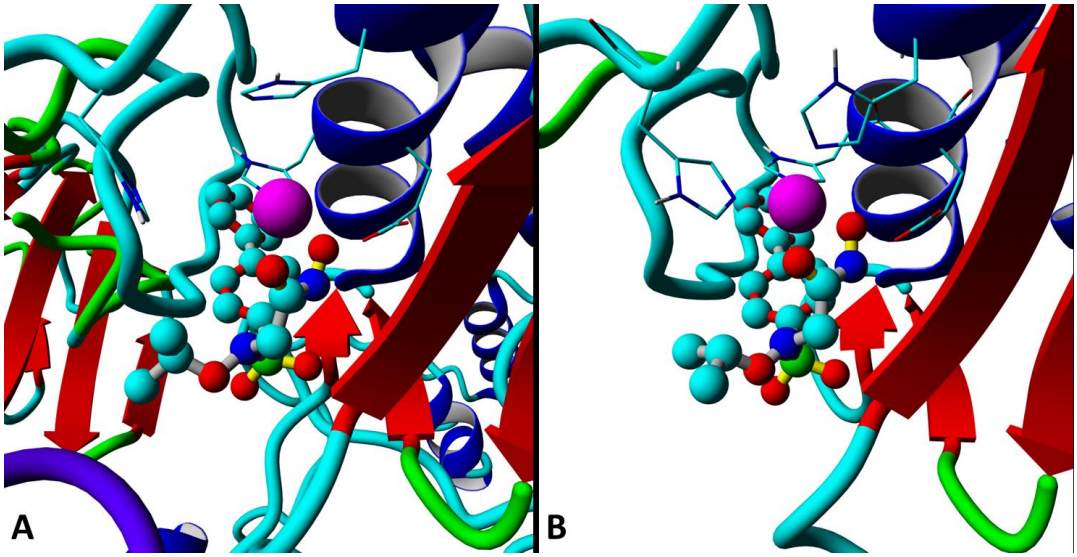

Figure 1. Visualization of the catalytic site of the homology model (A) and PDB:4H3X (B). The figures were built using YASARA Structure in default mode without shadow. The enzyme, the amino acid residues, the ligands, and zinc atom are depicted as ribbon style, stick style, ball-and-stick style and ball, respectively. Only important residues are shown for the shake of clarity.

The analysis of the human MMP9 homology model showed an acceptable deviation from the average structure in all aspects. The results and the details of the analyzed aspects are presented in Table 1. Based on the results, the model of the tertiary structure of the human MMP9 resulted from the homology modeling is acceptable for further application. However, visual inspection of the interactions between the protein, the ligands, and the cofactors should be performed and compared to those from the reference structures, i.e., PDB:4H3X and PDB:1ITV.

The catalytic site of the model (Figure 1A) is slightly different from the catalytic site of the crytal structure PDB:4H3X (Figure 1B). The histidine triad in the model is slightly away from the zinc atom compared to the crystal structure, while the glutamate residue remains similar. Nevertheless, the quantitave approach by aligning the backbone of the model to the backbone of the crystal structure showed the RMSD value of those residues in the model compared to the crystal structure was 0.817 Å. Figure 1 and the alignment show that the model could reproduce the essential interactions for ligands and cofactors with the protein. Unfortunately, the ligands in PDB:1ITV, which was used as one of the templates, are only sulfate ions. Therefore, further investigation in this hemopexin-like domain should be performed.

The reliability of the model to be employed in a further virtual screening campaign will significantly be increased by employing a model with stable protein-ligand interactions (Liu *et al*., 2017). Therefore, further MD simulations for 20 ns with a snapshot in every 10 ps were performed. Figures 2A and 2B show the RMSD values of the backbone atoms during the simulations and the deviation of these RMSD values in the duration of 5 ns started from the initial point, respectively. As depicted in Figure 2, the MMP9-CC27 complex in this research was considered stable starting from 5.05 ns of the MD simulations. The ΔG values of snapshots in 5 ns after the starting stable point were then calculated to identify the snapshot with the lowest ΔG value. Figure 3 shows that the lowest snapshot was identified at the 6.40 ns of the MD production run.

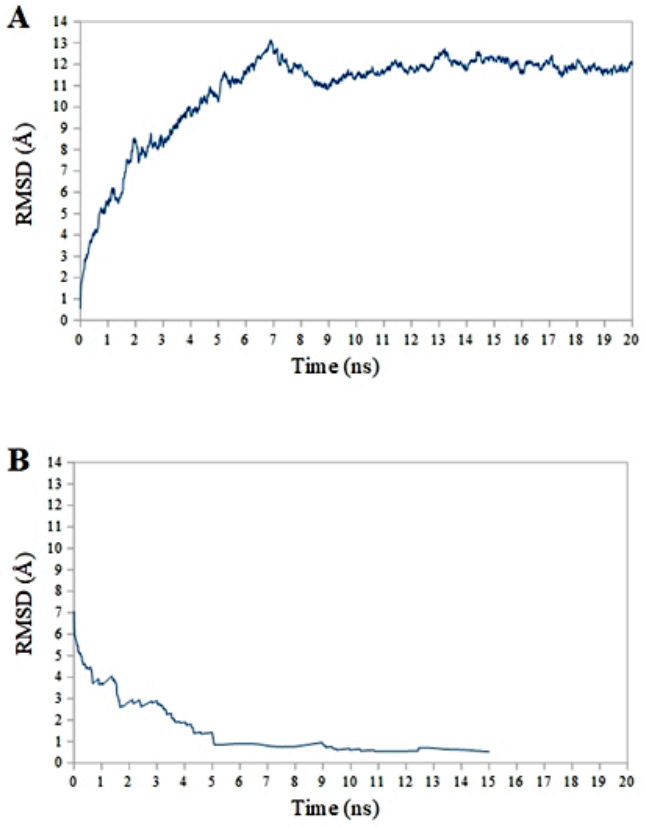

Figure 2. The RMSD values of the MMP9 backbone atoms during MD simulations (A), and the deviation of the RMSD in every 5 ns from the starting point (B).

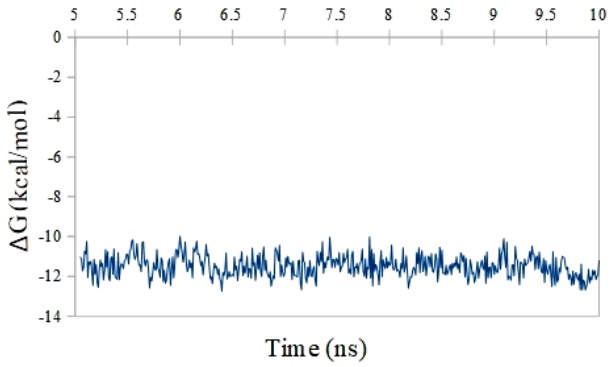

Figure 3. The ΔG values of MMP9-CC27 during the production run of the MD simulations.

The MMP9-CC27 complex from the 6.40 ns of the MD production run was then minimized and exported as a pdb file (mmp9.pdb). The applicability checks of this complex for virtual screening by employing 1000 redocking simulations resulted in RMSD values of less than 2.0 Å for all redocking of CC27 poses. The highest RMSD value was 1.274 Å. Thus, the MMP9-CC27 complex (mmp9.pdb) resulted in this research is highly suggested to be used in the further development of structure-based virtual screening protocols to discover drugs targeting human MMP9.

CONCLUSION

Homology modeling studies followed by 20 ns MD simulations by employing YASARA Structure could result in a proper quaternary structure of full human MMP9 complexed with CC27. The availability of the model offers possibilities to perform structure-based drug design targeting the catalytic site of the human MMP9. Further investigation in the hemopexin domain should be performed to study the inhibitor selectivity of the catalytic site over the hemopexin domain.

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REFERENCES

Alford, V.M., Kamath, A., Ren, X., Kumar, K., Gan, Q., Awwa, M., Tong, M., Seeliger, M.A., Cao, J., Ojima, I., Sampson, N.S., 2017. Targeting the hemopexin-like domain of latent matrix metalloproteinase-9 (proMMP-9) with a small molecule inhibitor prevents the formation of focal adhesion junctions. *ACS Chem. Biol.*, 12 (11), 2788–2803.

Ayuk, S.M., Abrahamse, H., Houreld, N.N., 2016. The role of matrix metalloproteinases in diabetic wound healing in relation to photobiomodulation. *J. Diabetes Res.*, 2016 (2897656), 1-9.

Cavasotto, C.N., Phatak, S.S., 2009. Homology modeling in drug discovery: Current trends and applications. *Drug Discov. Today*, 14 (13-14), 676-683.

Delozier, T.C., Kissling, G.E., Coulter, S.J., Dai, D., Foley, J.F., Alyce, J., Murphy, E., Steenbergen, C., Zeldin, D.C., Goldstein, J.A., 2011. Small molecule anti-cancer compounds selectively target the hemopexin domain of matrix metalloproteinase-9 (MMP-9). *Cancer Res.*, 71 (14), 4977–4988.

Dufour, A., Cao, J., Kuscu, C., Dufour, A., Zucker, S., Sampson, N.S., 2010. Role of matrix metalloproteinase-9 dimers in cell migration. *J. Biol. Chem.*, 285 (46), 35944–35956.

Ezhilarasan, R., Jadhav, U., Mohanam, I., Rao, J.S., Gujrati, M., Mohanam, S., 2009. The hemopexin domain of MMP-9 inhibits angiogenesis and retards the growth of intracranial glioblastoma xenograft in nude mice. *Int. J. Cancer*, 124 (2), 306–315.

Hariono, M., Yuliani, S.H., Istyastono, E.P., Riswanto, F.D.O., Adhipandito, C.F., 2018. Matrix metalloproteinase 9 (MMP9) in wound healing of diabetic foot ulcer: Molecular target and structure-based drug design. *Wound Medicine*, 22 (September 2018), 1–13.

Jones, I., Nguyen, T.T., Peng, Z., Chang, M., 2019. Targeting MMP-9 in diabetic foot ulcers. *Pharmaceuticals*, 12 (2), 79.

Krieger, E., Koraimann, G., Vriend, G., 2002. Increasing the precision of comparative models with YASARANOVA- A self-parameterizing force field. *Proteins*, 47 (3), 393–402.

Krieger, E., Vriend, G., 2015. New ways to boost molecular dynamics simulations. *J. Comput. Chem.*, 36 (13), 996–1007.

Liu, K., Watanabe, E., Kokubo, H., 2017. Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations. *J. Comput. Aided Mol. Des.*, 31, 201–211.

Martí-Renom, M.A., Stuart, A.C., Roberto, S., Melo, F., Andrej, S., 2000. Comparative protein structure modeling

of genes and genomes. *Ann. Rev. Biophys. Biomol. Struct.*, 29, 291–325.

Prasasty, V.D., Istyastono, E.P., 2019. Structure-based design and molecular dynamics simulations of pentapeptide AEYTR as a potential acetylcholinesterase inhibitor. *Indones. J. Chem.*, in press, 1–7.

Roeb, E., Schleinkofer, K., Kernebeck, T., Pötsch, S., Jansen, B., Behrmann, I., Matern, S., Grötzinger, J., 2002. The matrix metalloproteinase 9 (MMP-9) hemopexin domain is a novel gelatin binding domain and acts as an antagonist. *J. Biol. Chem.*, 277 (52), 50326–50332.

Vandooren, J., Knoops, S., Buzzo, J.L.A., Boon, L., Martens, E., Opdenakker, G., Kolaczkowska, E., 2017. Differential inhibition of activity, activation and gene expression of MMP-9 in THP-1 cells by azithromycin and minocycline versus bortezomib: A comparative study. *PLoS ONE*, 12 (4), 1–19.

Wang, X., Song, K., Li, L., Chen, L., 2018. Structure-based drug design strategies and challenges. *Curr. Top. Med. Chem.*, 18 (12), 998–1006.

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THE INFLUENCE OF β-CYCLODEXTRIN CONCENTRATIONS AS LIGANDS ON INCLUSION COMPLEXES TO INCREASE THE SOLUBILITY OF IBUPROFEN

Rahmah Elfiyani*), Anisa Amalia, Alvin Integra

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR HAMKA, Klender-Jakarta Timur, 13460, Indonesia

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ABSTRACT

Ibuprofen is a compound with low solubility but high permeability in water. One method to improve the ability of a substance to dissolve in water is through the formation of inclusion complexes. This study aims to obtain ratio between ibuprofen and β-cyclodextrin which results in inclusion complex with an optimal amount of dissolved ibuprofen. The inclusion complex was made using solvent evaporation method with molar ratio variations of 1: 1, 1: 2, 1: 3, 1: 4 and 1: 5. The results of the inclusion complex were characterized by X-ray diffraction, FTIR, SEM, and DTA. The solubility test was carried out using three different media; they are pH solution 7.4; pH solution 1.5; and distilled water. The solubility test results showed no increase on the ibuprofen solubility of the inclusion complex within medium solutions of pH 7.4 and pH 1.5 whereas in aquades medium there was an increase in the inclusion complex solubility compared to pure ibuprofen. Based on the results, it can be concluded that inclusion complex with molar ratio of 1: 1 shows optimal amount of dissolved ibuprofen compared to other ratio variations in aquadest medium.

Keywords: β-cyclodextrin; ibuprofen; inclusion complexes; solvent evaporation.

INTRODUCTION

In a study conducted by Octavia *et al*. (2015), it was found that pure ibuprofen could not be dissolved for more than 70% in 30 minutes. Such compound requires a method to increase its solubility to improve the bioavailability of drugs (Yasir *et al*. 2010). Other studies investigating the increase of ibuprofen solubility have been carried out in the form of solid dispersion by the methods of solvent evaporation and melting dispersion (Gupta *et al*., 2011). This study uses relatively toxic organic solvents which will leave residue on the results of ibuprofen solid dispersion.

Another way to improve the solubility of drugs that are difficult to dissolve in water is through the inclusion complex, through which can improve the speed of dissolution, absorption, bioavailability, and chemical stability of the drug (Loftsson & Brewster, 1996). Inclusion complex is a form of inserting non-polar compounds (substrates) into a container of another compound (ligand) (Ketan *et al*., 2012). β-cyclodextrin compound is one of cyclodextrin types that can be used as a ligand in the formation of inclusion complex. It is because β-cyclodextrin has relatively large cavity diameter of up to 6Å and good water solubility of 1 part in 20 parts of water (Rowe *et al*., 2012).

Based on the explanation above, this research will produce an inclusion complex with a variation of ibuprofen:β-cyclodextrin ratio using the solvent evaporation method due to an increase in ibuprofen solubility. The purpose of this study is to obtain ratio of ibuprofen:β-cyclodextrin which produces inclusion complex with an optimal amount of dissolved ibuprofen.

*Corresponding author: Rahmah Elfiyani
Email: *rahmahelfiyani@uhamka.ac.id*

METHODS

The materials used in this study are ibuprofen (Hubei granules-biocause pharmaceutical), β-cyclodextrin (BaoJi GuoKang Bio-Technology), alcohol, aquadest, KCl (Merck), HCl (Merck), KH2PO4 (Merck), and NaOH (Merck). In addition, the instruments used in this study include X-ray diffractometer (Pan Analytical empryren), DTA (Mettler Toledo), UV-VIS spectrophotometer, SEM (SEM-eds JEOL JED350), FTIR spectrophotometer (Agilent), magnetic stirrer (Power MS-H-Pro), rotary evaporator (Butchi), and sieving (Retsch AS 200 digits CA).

Production of inclusion complex

The inclusion complex was made into 5 formulas by varying the molar ratio between ibuprofen:β-cyclodextrin which were 1: 1; 1: 2; 1: 3; 1: 4 and 1: 5 (Table 1). Ibuprofen was dissolved in 50 ml of ethanol (M1) while β-cyclodextrin was dissolved in 50 ml of hot water (60°C) (M2). After that, the M2 clear solution was put into M1 clear solution and stirred using a magnetic stirrer at 300 rpm for 30 minutes. The turbid solution is then evaporated (170 bar, 150 rpm, temperature 40°C). The dried powder formed was sieved using a mesh of 100, then dried in a desiccator for 3 hours (Priotta, 2015).

Characterization of inclusion complexes

Infrared spectra of ibuprofen, β-cyclodextrin and inclusion complexes were recorded using FT-IR spectrophotometer by KBr pellet method. Measurements were made at wavenumbers 400 - 4000 cm-1 (Hiremath *et al*, 2008).

DTA would characterize the solid-state interaction of the inclusion complex, β-cyclodextrin and, ibuprofen. The sample used was approximately 5 mg of ibuprofen, β-cyclodextrin, and inclusion complex at warming temperatures from 30 to 400 °C with a heating speed of 10 °C / minute (Ma *et al*., 2012).

XRD patterns of Ibuprofen, β-cyclodextrins and inclusion complexes were recorded using X-ray diffractometer with Cu anode tube at 5-700 / 2θ intervals. The sample was placed on a plate-shaped holder made of aluminum. Diffractogram would be read automatically on the computer (Asih II, 2011).

Particle morphology was observed using SEM. The powder sample (ibuprofen, β-cyclodextrin, inclusion complex) was placed in an aluminum sample holder and coated with gold with thickness of 10 nm. Samples were then observed for the enlargement of SEM devices. The voltage was set at 20 kV and the current was 12 mA (Octavia *et al*, 2015).

Solubility test

A total of 100 mg of standard ibuprofen and inclusion complex results were dissolved into 20 ml of ethanol each, put into a 100 ml volumetric flask and dissolved with the medium (buffer pH 1.5/ distilled water) until the boundary mark. It was stirred using a magnetic stirrer at a speed of 150 rpm for 15 minutes. A sample of 10 ml was taken and filtered with a 0.45 µm filtrate membrane. Absorption was measured at maximum wavelength using spectrophotometer. The solubility test was also carried out on solvent buffer pH 7.4 without the addition of ethanol to help to dissolve ibuprofen.

DATA ANALYSIS

Solubility test data on dissolved ibuprofen levels were analyzed using non-parametric analysis of Friedman because the data were not normally distributed and were not homogeneous.

Tabel 1. Formula of Inclusion Complex

	F 1	F 2	F 3	F 4	F 5
β-cyclodextrine (g)	283,75	567,5	851,25	1135	1418,75
Ibuprofen (g)	51,55	51,55	51,55	51,55	51,55

RESULTS AND DISCUSSION

FTIR

The results of FTIR characterization from ibuprofen, β-cyclodextrin and inclusion complexes can be seen in Figure 1 and Table 2. Inclusion complexes showed the presence of β-cyclodesktrin and ibuprofen groups. In formula 1 there were wavelengths of 1701.5 cm^{-1} and 1459.3 cm^{-1} which were the peaks in ibuprofen, a slight shift in wavelength from 1461 cm-1 to 1459 cm-1 (Barmi *et al*., 2018). In formula 2, a shift in wavelength from 3283.8 to 3280.1 belonged to β-cyclodextrin (Silverstein et.al., 1981), but there was no aromatic peak of ibuprofen. The wavelength of ibuprofen aromatic group was not seen in formulas 3, 4 and 5 as well. Carboxylic group wavelength shift from 1701.5 to 1735.1 belonged to ibuprofen in formula 3, formula 4 and formula 5. This indicated a weak interaction between ibuprofen and β-cyclodextrin (Hiremath *et al*., 2008). From these results, it was known that there was a shift in wavelength and appearance of ibuprofen, and wavelengths of β-cyclodextrin which indicated an interaction between ibuprofen and β-cyclodextrin

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Tabel 2. Wave Number of FTIR

	Distance wave number (cm^{-1})	β-cyclodextryn (cm^{-1})
(O-H)	2500-3300	3263,3
(C-H) aromatis	1000-1275	1099,6
(C-O) alcohol dan fenol	1000-1300	1077,2
	Distance wave number (cm^{-1})	Ibuprofen (cm^{-1})
(C=O) carboxylic acid	1701	1701.5
(C=C) aromatic	1461	1460
	Distance wave number (cm^{-1})	Inclusion complex (cm^{-1})
O-H	2500-3300	3265-3280,1
C-H	1000-1275	1104.1
C-O	1000-1300	1079.1
C=O carboxylic acid	1701	1701.5-1735.1
(C=C) aromatic	1461	1459

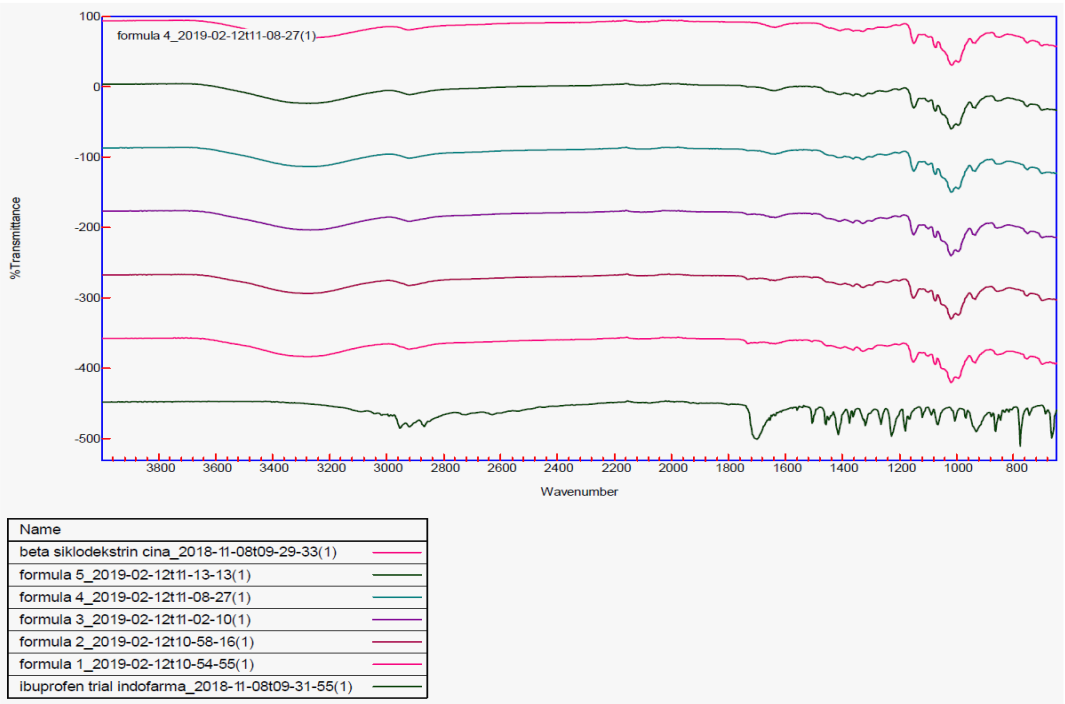
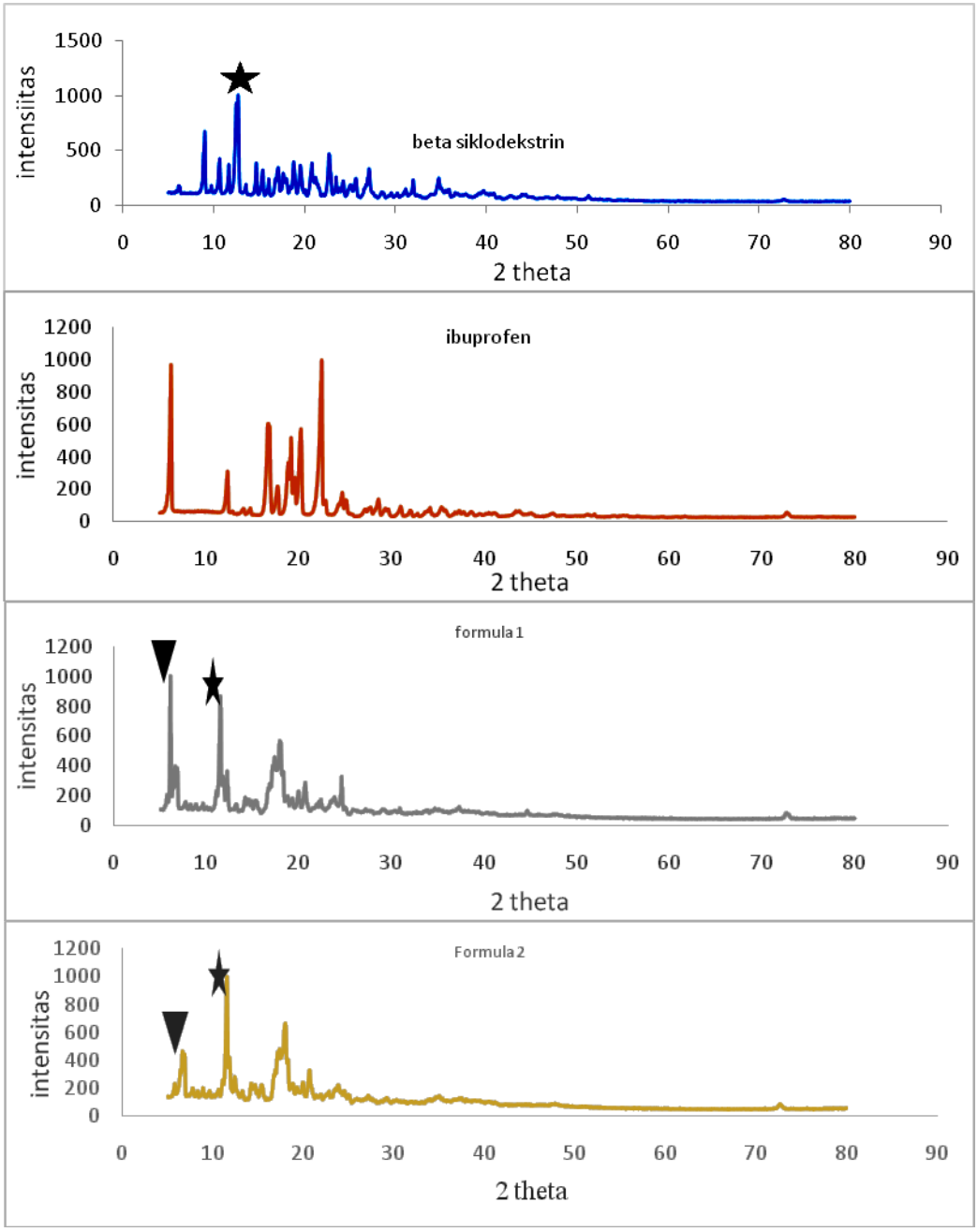


Figure 1. IR Spectrum of ibuprofen, β-cyclodextrin and inclusion complex.

X-ray Diffractometry

It can be seen in Figure 2. In formula 1, the inclusion complex was formed due to the bonding of ibuprofen and β-cyclodextrin so that the peaks in formula 1 and formula 2 were not too many. With the increase on the number of β-cyclodextrin, the peaks were increasingly visible until formula 5, but did not cover the peak of ibuprofen. The diffraction pattern formed from formula 1 to formula 5 was increasingly seen approaching β-cyclodextrin diffraction pattern with increasing peaks similar to β-cyclodextrin. This signifies that ibuprofen molecule has entered the structure of β-cyclodextrin cavity so that it looks dominant β-cyclodextrin diffractogram (Rini *et al*., 2015). The decrease in height of peak intensity indicates a change in the structure so that the results of the inclusion complex are amorphous (Pamudji *et al*., 2014; Barmi *et al*., 2018).



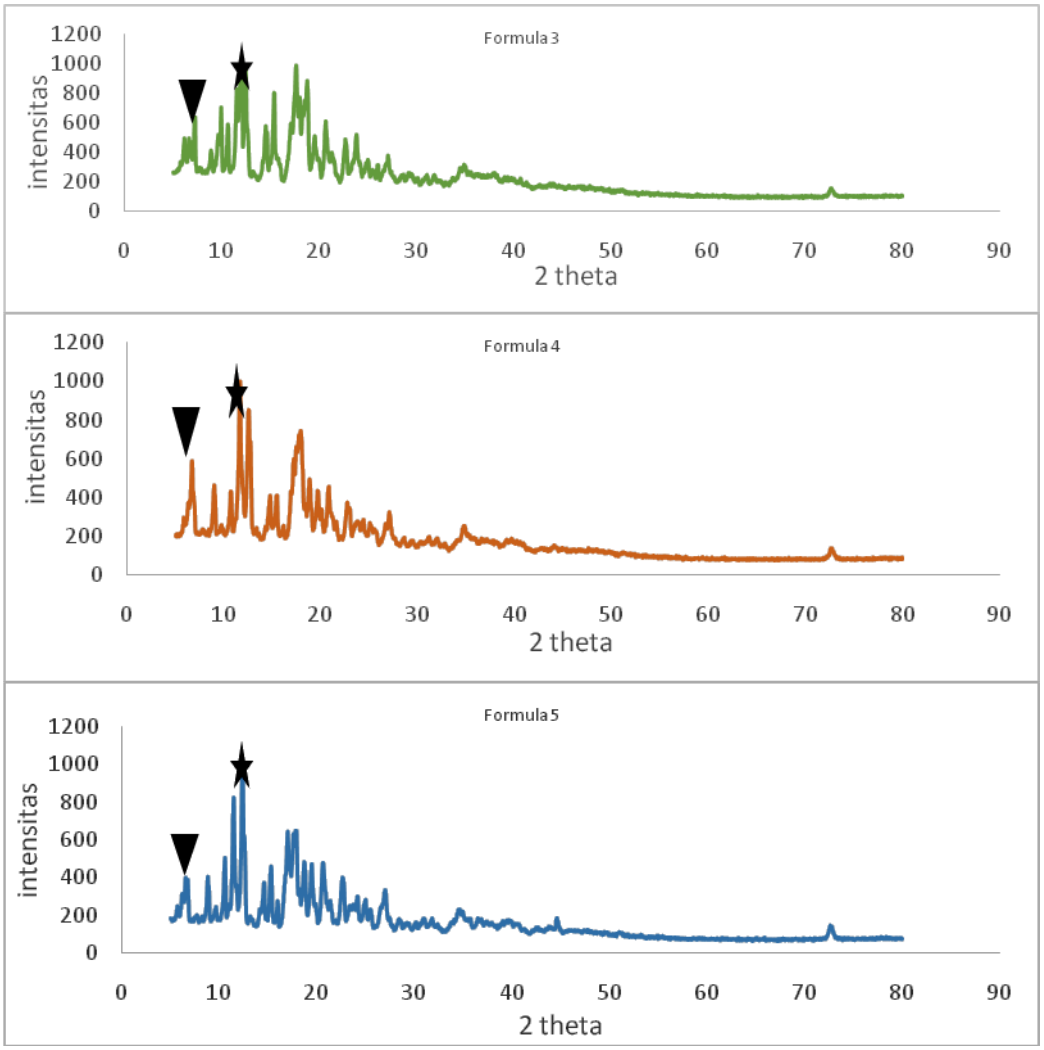


Figure 2. XRD result of ibuprofen, β-cyclodextrin and inclusion complex.

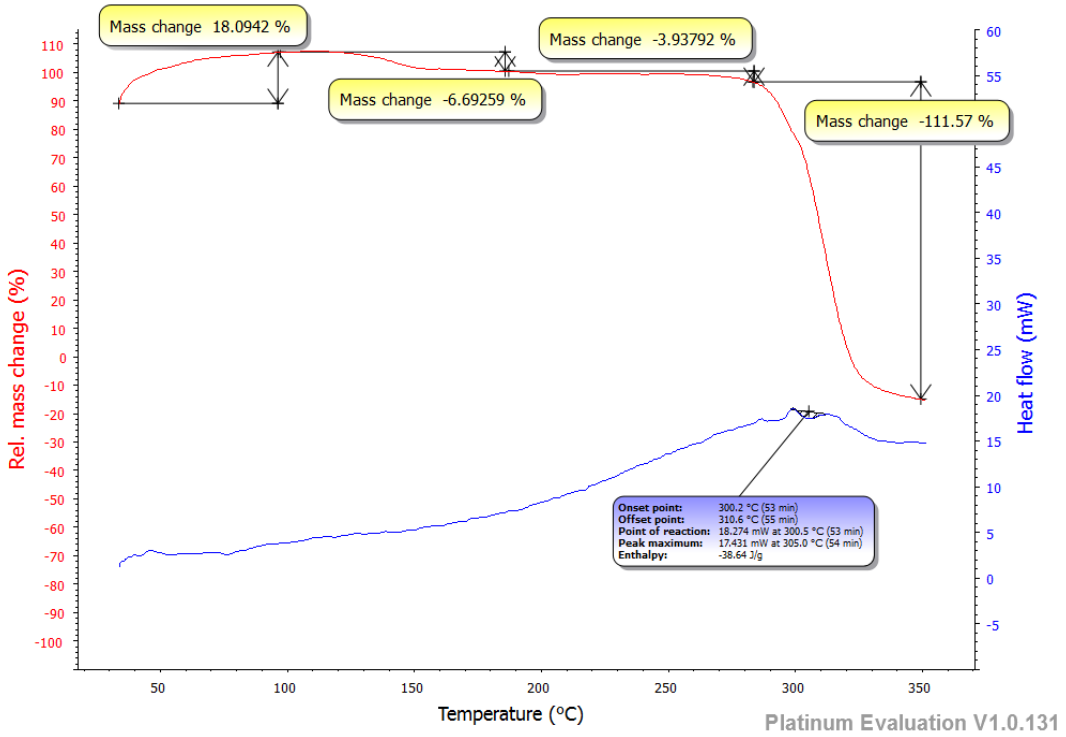
Differential Thermal Analysis

Formula 1 and formula 2 showed an endothermic process at a temperature of 2900°C - 3200°C. Endothermic changes can occur due to oxidation of organic compounds (Setabudi *et al*., 2012). However, formula 3 experienced an exothermic process at a temperature range of 2940°C - 3020°C and at that temperatures there was a change in mass. Meanwhile, in formula 4 and formula 5, exothermic process was directly followed by endothermic process. Exothermic process usually occurs due to the formation of crystals during the heating process which is often referred to solidification.

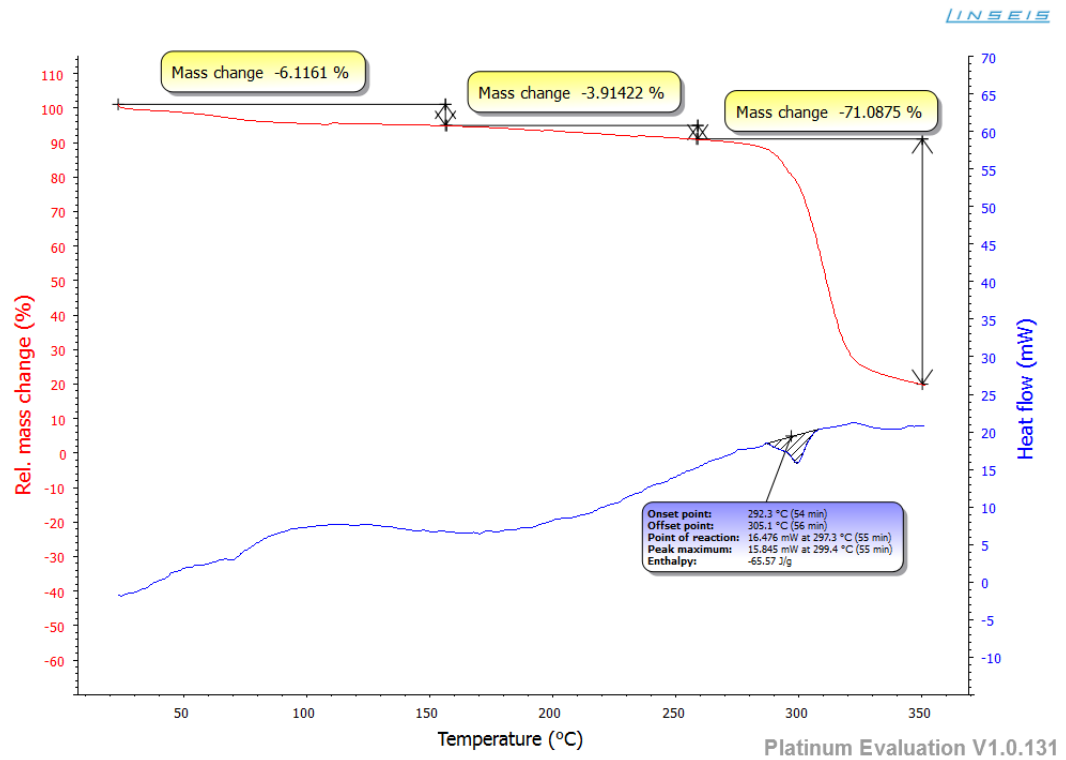
In all results (Figure 3) on inclusion complex, dehydration which usually occurs at temperatures of around 1000°C is absent this time due to the disappearance of ibuprofen melting point. Ibuprofen loses its peak because it has entered the cavity of β-cyclodextrin so that it does not show endothermic points at these adjacent temperatures (Hirameth 2008; Manca *et al*., 2005).

Scanning Electron Microscope

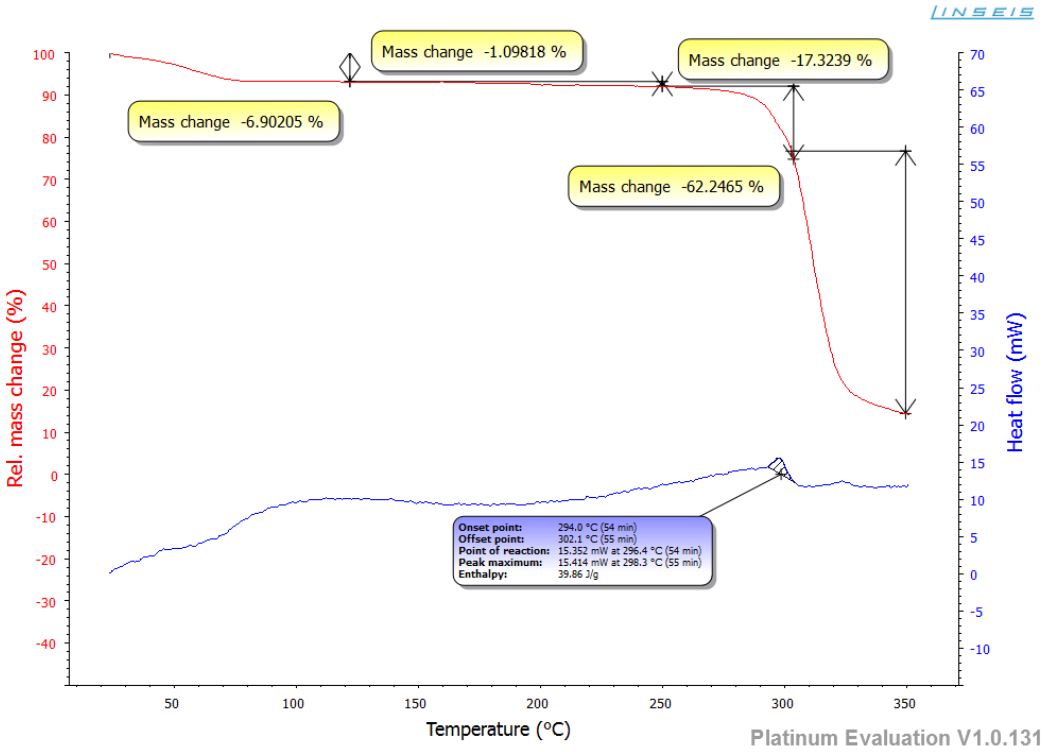
The results of pure ibuprofen examination showed a long cylindrical rod shape while β-cyclodextrin was seen as a lump with a rough and irregular texture. In the results of inclusion complex, all formulas appear to have no form that resembles ibuprofen and β-cyclodextrin. In fact, almost all are irregular in shape (Figure 4).


Mass change 18.0942 %
Mass change -6.69259 %
Mass change -3.93792 %
Mass change -111.57 %
Onset point: 300.2 °C (53 min)
Offset point: 310.6 °C (55 min)
Point of reaction: 18.274 mW at 300.5 °C (53 min)
Peak maximum: 17.431 mW at 305.0 °C (54 min)
Enthalpy: -38.64 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

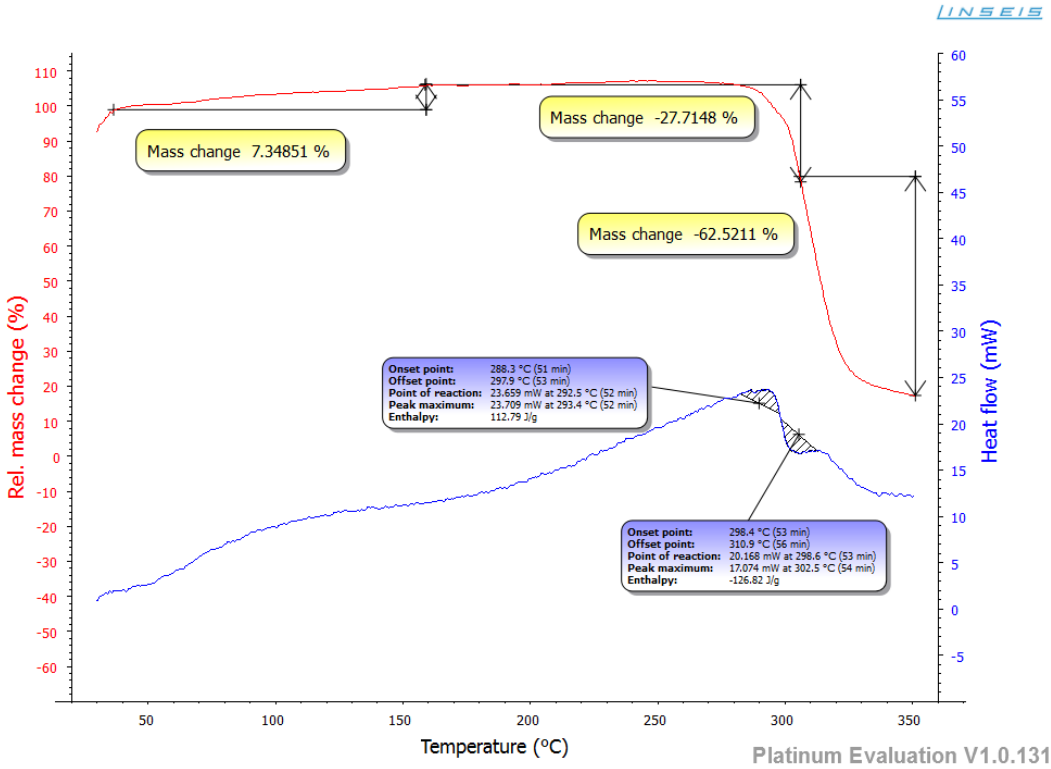
(a)


Mass change -6.1161 %
Mass change -3.91422 %
Mass change -71.0875 %
Onset point: 292.3 °C (54 min)
Offset point: 305.1 °C (56 min)
Point of reaction: 16.476 mW at 297.3 °C (55 min)
Peak maximum: 15.845 mW at 299.4 °C (55 min)
Enthalpy: -65.57 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

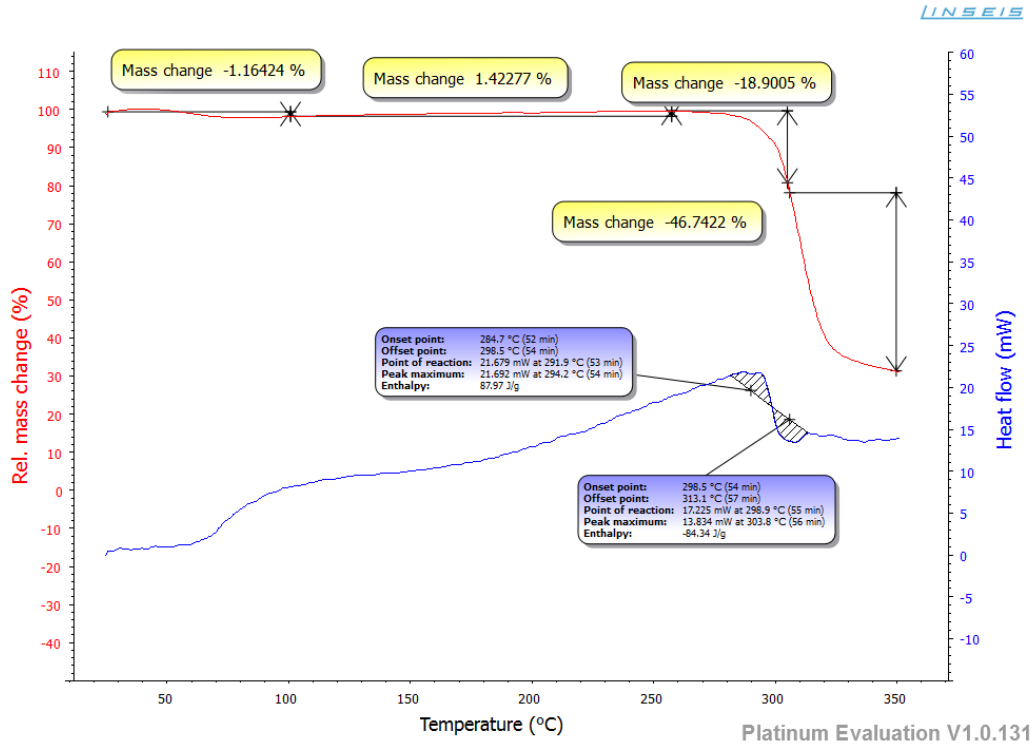
(b)


Mass change -1.09818 %
Mass change -17.3239 %
Mass change -6.90205 %
Mass change -62.2465 %
Onset point: 294.0 °C (54 min)
Offset point: 302.1 °C (55 min)
Point of reaction: 15.352 mW at 296.4 °C (54 min)
Peak maximum: 15.414 mW at 298.3 °C (55 min)
Enthalpy: 39.86 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

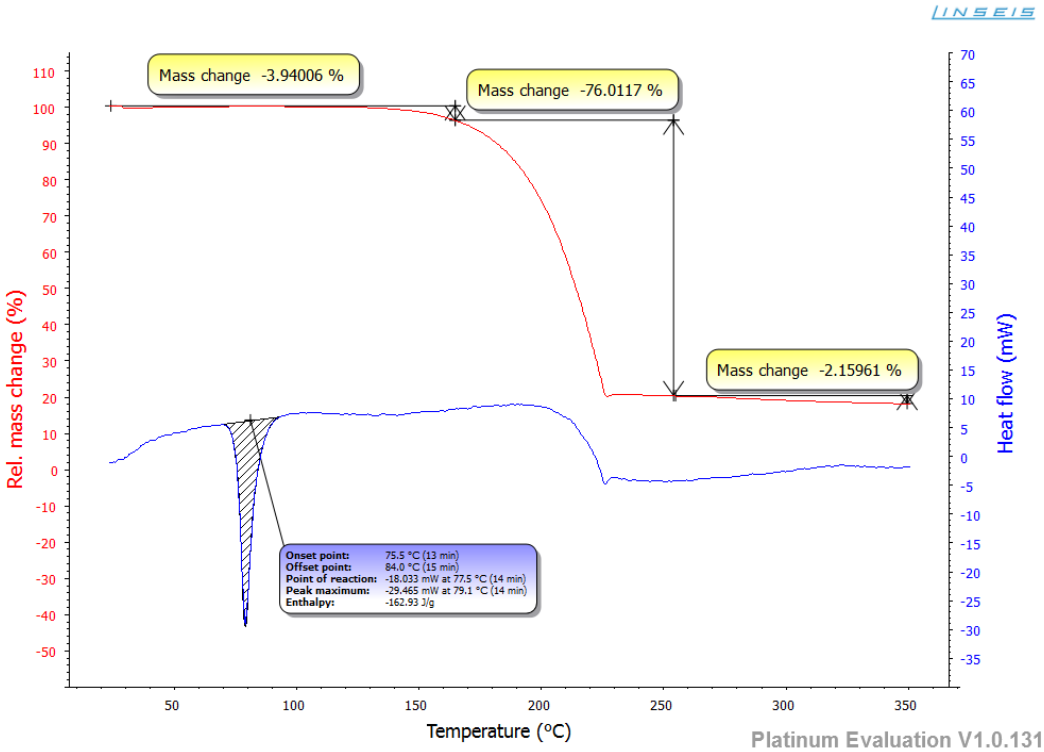
(c)


Mass change 7.34851 %
Mass change -27.7148 %
Mass change -62.5211 %
Onset point: 288.3 °C (51 min)
Offset point: 297.9 °C (53 min)
Point of reaction: 23.659 mW at 292.5 °C (52 min)
Peak maximum: 23.709 mW at 293.4 °C (52 min)
Enthalpy: 112.79 J/g
Onset point: 298.4 °C (53 min)
Offset point: 310.9 °C (56 min)
Point of reaction: 20.168 mW at 298.6 °C (53 min)
Peak maximum: 17.074 mW at 302.5 °C (54 min)
Enthalpy: -126.82 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

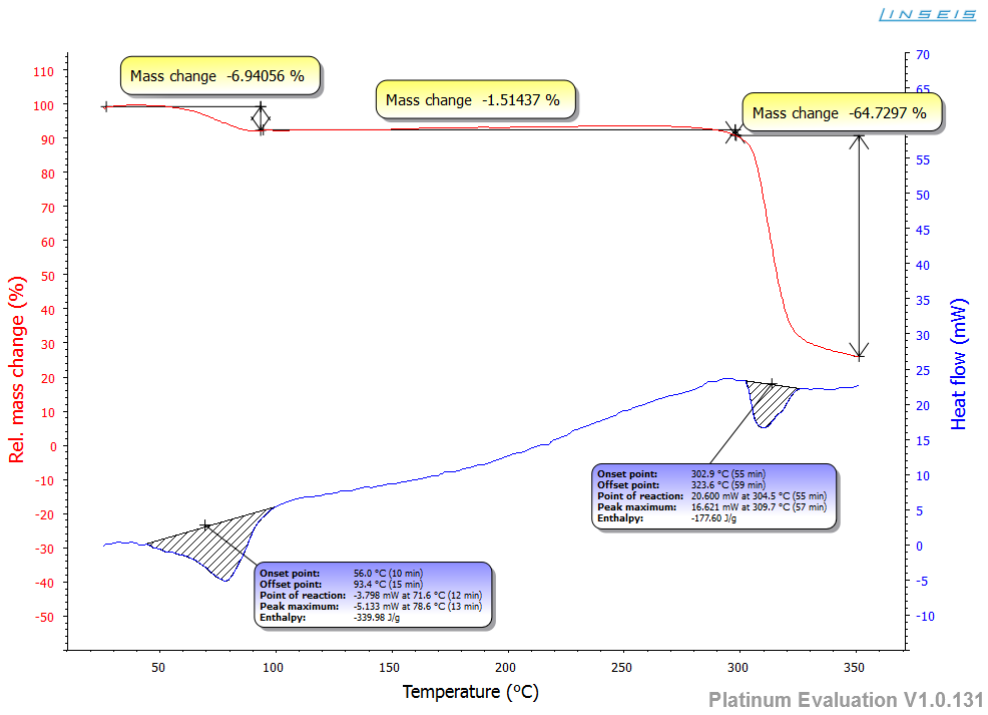
(d)


Mass change -1.16424 %
Mass change 1.42277 %
Mass change -18.9005 %
Mass change -46.7422 %
Onset point: 284.7 °C (52 min)
Offset point: 298.5 °C (54 min)
Point of reaction: 21.679 mW at 291.9 °C (53 min)
Peak maximum: 21.692 mW at 294.2 °C (54 min)
Enthalpy: 87.97 J/g
Onset point: 298.5 °C (54 min)
Offset point: 313.1 °C (57 min)
Point of reaction: 17.225 mW at 298.9 °C (55 min)
Peak maximum: 13.834 mW at 303.8 °C (56 min)
Enthalpy: -84.34 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

(e)

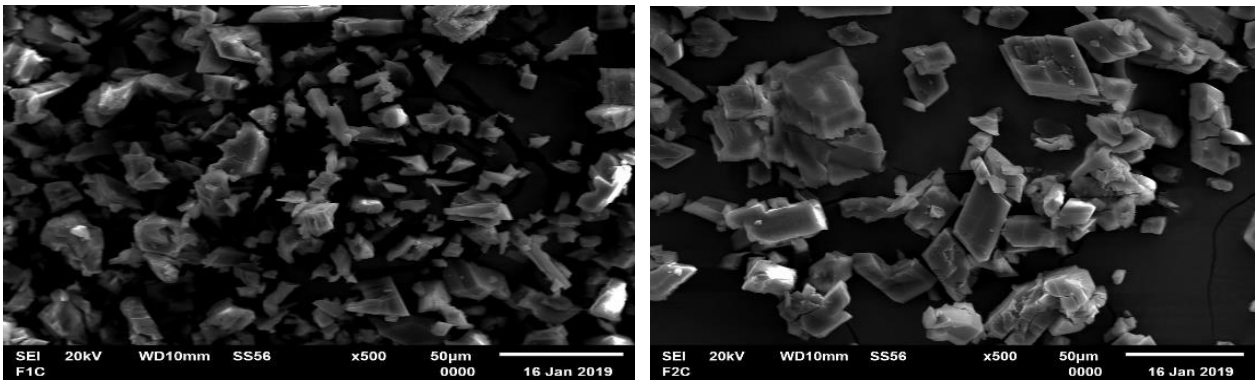

Mass change -3.94006 %
Mass change -76.0117 %
Mass change -2.15961 %
Onset point: 75.5 °C (13 min)
Offset point: 84.0 °C (15 min)
Point of reaction: -18.033 mW at 77.5 °C (14 min)
Peak maximum: -29.465 mW at 79.1 °C (14 min)
Enthalpy: -162.93 J/g
Rel. mass change (%)
Heat flow (mW)
Temperature (°C)
Platinum Evaluation V1.0.131

(f)



(g)

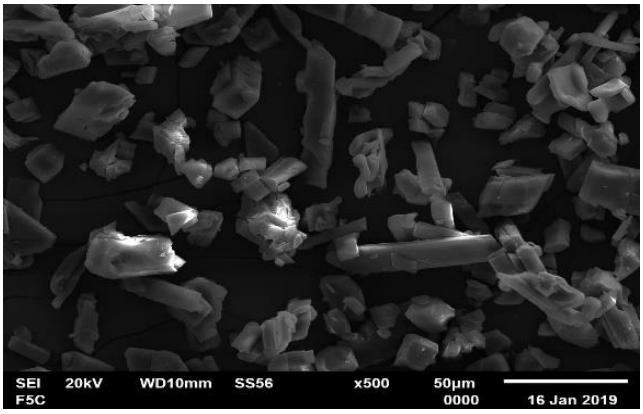
Figure 3. DTA result: (a) Formula 1, (b) Formula 2, (c) Formula 3, (d) Formula 4, (e) Formula 5, (f) ibuprofen, (g) β-cyclodextrin.



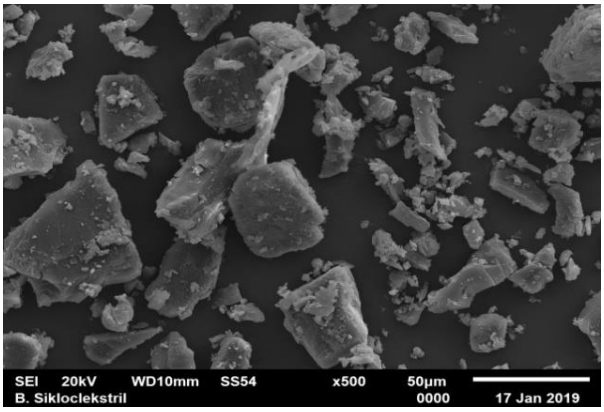
Formula 1 Formula 2

Formula 3 Formula 4

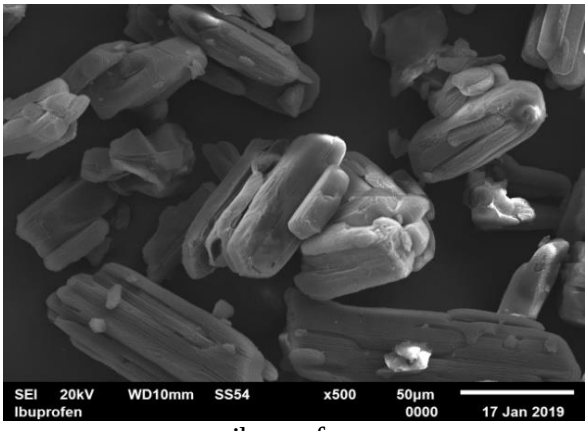
Figure 4. SEM results



Formula 5



β-cyclodextrin



ibuprofen

Figure 4. SEM results (*Continued*)

Solubility Test

One of the most common methods in evaluation inclusion complexation is phase solubility. A phase solubility diagram is constructed by plotting the molar concentration of dissolved solute found on the vertical axis against the concentration of complexing agent added on the horizontal axis. Two general types of phase solubility profiles are generated; Type A where soluble complexes are formed, and Type B where complexes of limited solubility are formed (Higuchi & Connors, 1965).

The results (Figure 5) show that the addition of β-cyclodextrin to the inclusion complex at solution of pH 7.4 and pH 1.5 follows the type BI diagram which means that there is no increase in the solubility of ibuprofen with the addition of β-cyclodextrin at ratios of 1: 1, 1: 2, 1: 3, 1: 4, and 1: 5. As with the aquadest medium, there is an increase in the solubility of ibuprofen in formula 1 (ratio 1: 1) compared to pure ibuprofen, and the solubility type follows type Bs diagram.

Curve Bs shows the formation of a complex that increases the total solubility of the compound (similar to type A diagram). More addition of complexing agents producing solubility of the complex is reached. As additional compound goes into solution, some solid complexes precipitate. Further addition of complexing agents beyond point z results in depletion of the compound from solution by complex formation. Curve BI is interpreted similarly except that the complex formed is so insoluble that no increase in solubility is observed (Mosher & Thompson, 2007).

Statistical analysis of the solubility test results shows that sig < 0.05 based on which it can be concluded that the 2 variables (ratio of ibuprofen:β-cyclodextrin and the type of medium) generate different solubility results

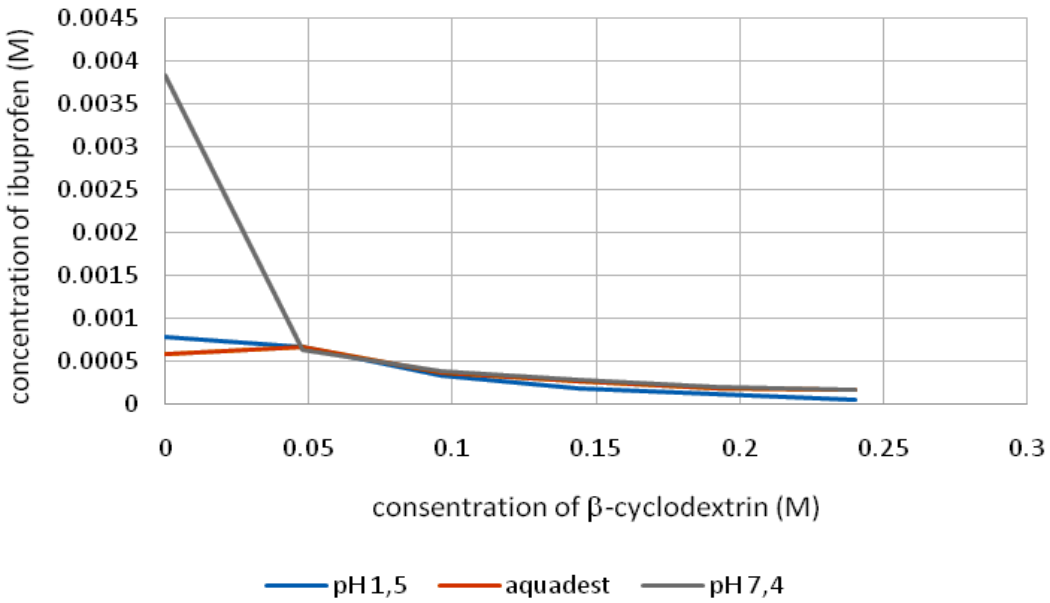


Figure 5. Solubility phase diagram of inclusion complexes

CONCLUSION

The inclusion complex is not able to increase the solubility of ibuprofen within solution medium of pH 7.4 and pH 1.5. Meanwhile, in the aquadest medium, the formation of inclusion complexes can increase the solubility of ibuprofen at ratio of 1: 1.

ACKNOWLEDGMENT

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REFERENCES

Asih, I.I., 2011. Pembuatan Kompleks Inklusi Glikalizid-Beta Siklodekstrin dan Perbandingan Kecepatan Disolusi Glikalizid Dari Sediaan Tablet. *Skripsi*. Fakultas MIPA Universitas Indonesia, Jakarta. pp. 24-25.

Barmi, H., Lia, A., Desi, S., Julia, A.S., 2018. Pembentukan kompleks Inklusi ibuprofen kombinasi polimer β-siklodekstrin dan Hydroxypropyl Metylcelulose (HPMC) menggunakan teknik kneading. Riset Informasi Kesehatan, 7(1).

Gupta, M.M., Patel, M.G., Patel, N.S., Madhulika, K., 2011. Enhancement of Dissolution Rate Of Ibuprofen by Preparing Solid Dispersion Using Different Methods. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(3), 204-206.

Higuchi, T., Connors, K.A., 1965. Phase-solubility Techniques. In Advances in Analytical Chemistry and Instrumentation Vol. 4. John Wiley & Sons, Inc. New York, 117–212.

Hiremath, S.N., Raghavendra, R.K., Suntil, F., Danki, L.S., Rampure, M.V., Swamy, P.V., Bhosale, U.V., 2008. Dissolution Enhancement of Gliclazide by Prepartion of Inclution Complexs with Beta-cyclodextrin. *Asian Journal of Pharmaceuties*, 73-76.

Ketan, T., Salvjni, Anurdha, K., Gajjar, Jignasa, K., 2012. Importance and Enhancment Techniques: A Review. Institute of Pharmacy. Nirma University. Gujarat. India.

Loftsson, T.D., Hrciinsdottir, M., Masson, 1996. Evaluation of Cyclodextrin Solubilization of Drugs, *International Journal of Pharmaceutics, (*302), 18-28.

Ma, S., Yong, W., Xiaojun, S., Fulin, Y., 2012. Formulation of Berberine Hydrochloride and Hydroxypropyl-β-Cyclodextrin Inclusion Complex with Enhanced Dissolution and Reduced Bitterness. *Tropical Journal of*

Pharmaceutical Research, 11 (6), 871-877.

Manca, M.L., Marco, Z., Guido, E., Donatella, V., Chiara, S., Giuseppe, L., Anna, M.F., 2005. Diclofenac-*β*-Cyclodextrin Binary Systems: Physicochemical Characterization and In Vitro Dissolution and Diffusion Studies. *AAPS PharmSciTech*, 2005, 6(3), 464-472.

Mosher, G., Thompson, D.O., 2007. Complexation: Cyclodextrins dalam Encyclopedia of Pharmaceutical Technology, 3rd Edition. New York, 671-676.

Octavia, M.D., Novety, O., Halim A., 2015. Karakterisasi Kompleks Inklusi Ibuprofen Beta Siklodekstrin dengan Menggunakan Teknik Penggilingan Bersama. Sekolah Tinggi Ilmu Farmasi Padang.

Pamudji, J.S., Mauludin, R., Lestari, V.A., 2014. Improvement of Carvedilol Dissolution Rate Trough Formation of Inclusion Complex With β-Cyclodextrin. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(4), 228-233.

Priotti, J., Ferreira, M.J.G., Lamas, M.C., Leonardo, D., Salomon, C.J., Nunes, T.G., 2015. *First Solid-state NMR Spectroscopy Evaluation of Complexes of Benznidazole with Cyclodextrin Deerivatives.* Centro de Qumica Estrutural, Instituto Superior Technico, Lisboa.

Rini, A., Fathya, I.L., Auzal H., 2015. Pembentukan dan Karakterisasi Kompleks Inklusi Fenilbutazon dan β-siklodesktrin dengan Metoda Co-grinding. *Jurnal Ilmiah Farmasi*.

Rowe, R.C., Sheskey, P.J., Cook, W.G., Fenton, M.E., 2012. Handbook of Pharmaceutical Excipient. Seventh Edition. Pharameutical Press. USA.

Setaibudi, A., Rifan, H., Ahmad, M., 2012. Karakterisasi Material: Prinsip dan Aplikasinya pada Peneltian Kimia. First Edition. UPI PRESS. Bandung, 21-22.

Silverstein, R.M., Bassler, G.C., Morrill, T.C., 1981. Spectrometric Identification of Organic Compounds. Fourth Edition. New York, 108-120.

Yasir, M., Asif, M., Kumar, A., Anggarval, A., 2010. *Biopharmaceutical Classification System: An Accunt. Internasional Journal of PharmTech Research.* CODEN. USA, 1681-1690.

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STUDY OF PEAK EXPIRATORY FLOW RATE AMONG VEGETABLE FARMERS USING PESTICIDES IN BOYOLALI DISTRICT, CENTRAL JAVA, INDONESIA

Manggala Sariputri[1*)], Ari Probandari[2], Hartono[3], Eti Poncorini Pamungkasari[2], Isna Qadrijati[4]

[1]Master Program of Family Medicine, Sebelas Maret University, Surakarta, 57126, Indonesia
[2]Department of Public Health, Faculty of Medicine, Sebelas Maret University, Surakarta, 57126, Indonesia
[3]Department of Physiology, Faculty of Medicine, Sebelas Maret University, Surakarta, 57126, Indonesia
[4]Study Program of Occupational Safety and Health, Faculty of Medicine, Sebelas Maret University, Surakarta, 57126, Indonesia

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ABSTRACT

Pesticides exposure affects respiratory system. The study of peak expiratory flow rate (PEFR) as a parameter of lung function changes due to limited pesticide among farmers. This cross-sectional study aims to analyze factors affecting PEFR and formulate a model for predicting PEFR among 76 vegetable farmers using pesticides in Tlogolele Village, Boyolali Regency. Data were collected through questionnaire-based interview and clinical examination. PEFR test was conducted using a peak flow meter while cholinesterase level was investigated using Deutsche Gessellschaftfur Klinische Chemie method. The data were analyzed using unpaired T-test and Pearson test continued by multivariate regression models. The examination showed 75% of subjects had abnormal PEFR. Cholinesterase, body mass index, smoking habits, personal protective equipment usage, pesticide dosage, length of work per day, and last time of spraying did not show a significant correlation with PEFR. On the other hand, age, height, weight, frequency of spraying, and spraying duration showed a significant correlation with PEFR and a prediction equation for PEFR model was obtained as R^2=0.268 ($p<0.001$). The developed model will be useful for early detection of abnormal lung function.

Keywords: occupational health; peak expiratory flow rate; pesticide exposure.

INTRODUCTION

Pesticide exposure is still a health problem throughout the world especially in developing countries. In the past 10 years, there had not been any complete statistical data on morbidity and mortality due to pesticide poisoning globally (Calvert *et al*., 2010; WHO, 2008). The World Health Organization (WHO) estimates that there are around 5 million cases of accidental pesticide poisoning and 20,000 deaths per year due to pesticide poisoning. The incidence of acute pesticide poisoning in the workplace is 1.17 per 100,000 workers and insecticides are the most common cause (49%) of all pesticides. The incidence of pesticide poisoning is higher in agricultural sector compared to non-agricultural sector (Calvert *et al*., 2004; UN, 2017). The level of pesticide poisoning is influenced by various factors including internal and external factors. Internal factors include age, sex, and nutritional status. External factors are pesticide dosage, frequency of spraying, length of work per day, length of service, time of the last spraying, habitual use of personal protective equipment

*Corresponding author: Manggala Sariputri
Email: *man99ala.sariputri@gmail.com*

(PPE), spraying time, and environmental temperature (Djojosumarto P, 2008; Eddleston, 2015; Wudianto, 2007).

Exposure to pesticides in workplace occurs in all agricultural processes such as mixing, spraying, planting, harvesting, weighing, product packaging, and washing agricultural equipment. Spraying is the most important process in pest control (Sapbamrer & Nata, 2014). During the process of pesticide spraying, steam and aerosol droplets from the pesticide solution can enter the body through inhalation. Inhalation of pesticides can cause damage to the nose, throat, and lung tissue because of which lung function changes and the risk of respiratory symptoms and diseases increases (Sapbamrer *et al*., 2019).

Several previous studies have reported an association between exposure to pesticides at work and respiratory disorders such as chronic obstructive pulmonary disease, asthma and chronic bronchitis (Alif *et al*., 2017; Hoppin *et al*., 2009; Lytras *et al*., 2018). Occupational exposure can also induce or exacerbate asthma. Variability of asthma control is worse during periods at work than away from work (Quirce *et al*., 2013; Suhadi *et al*., 2018). Several other studies report that the prevalence of respiratory symptoms is associated with pesticides (Buralli *et al*., 2018; Mathew *et al*., 2015). Evidence of decreased lung function tests associated with pesticide exposure among agricultural workers has also been reported in several studies (Chakraborty *et al*., 2009; Sapbamrer *et al*., 2019). These studies support evidence that exposure to pesticides has an adverse effect on the respiratory system.

Lung function can be assessed through several methods one of which is by peak expiratory flow rate (PEFR). PEFR measures the maximum expiratory speed that can be achieved by someone and is expressed in liters per minute (L/min) or liters per second (L/sec). PEFR mainly reflects the flow in large airways and depends on the conscious effort and muscle strength of the individual. PEFR values are obtained by spirometry test or using a simpler tool namely peak expiratory flow (PEF) meter. PEFR examination with PEF meter is easier, simpler, cheaper, and quantitative (Ismaila *et al*., 2015; Mridha *et al*., 2011; Thorat *et al*., 2017). Differences in PEFR values among populations can be caused by geographical factors, types of environmental and occupational exposure, socioeconomic status, and ethnic differences that affect variations between individuals. Therefore, PEFR prediction model will be more appropriate if each region has its own values (Nayak *et al*., 2016; Prasad *et al*., 2006). Research on the correlation between pesticide exposure and PEFR is still very limited. To the best of our knowledge, there is only one study about the correlation between blood cholinesterase activity and PEFR in Indonesia in which the subject of that study was potato farmers with chronic exposure to organophosphate pesticides. The results indicate a significant positive correlation of a low degree of blood acetylcholinesterase activity with PEFR (Luthfanto *et al*., 2014).

Boyolali Regency is one of regions in Central Java of which the economy relies on agricultural sector. For the last five years, 50 percent of Boyolali's population has worked in agricultural sector. Its best agricultural products include tobacco and vegetables. Selo District is a vegetable producing center in Boyolali (BPS Boyolali, 2018a). Tlogolele is one of villages in Selo District with main commodities of chilies, tomatoes and mustard greens.

Some areas of Tlogolele have agricultural activities throughout the year due to the availability of water in the area (BPS Boyolali, 2018). Based on research on pesticide poisoning in Selo District, data obtained from pesticide poisoning reached 73.9%. Factors related to pesticide poisoning are years of pesticide application, personal protective equipment (PPE), and frequency of spraying (Harriyani, 2016). Based on the explanation above, this study aims to determine PEFR, analyze the factors that affect PEFR and formulate a model of PEFR predictors towards vegetable farmers using pesticides in Tlogolele Village, Boyolali Regency.

METHODS

This study is an observational research applying cross-sectional design with the study permit No. 070/412/VI/39/2019 issued by *Kantor Kesatuan Bangsa dan Politik Kabupaten Boyolali* (The Office of Political and National Unity of Boyolali Regency). The study protocol was approved by the Ethics Commission of Medical Faculty, Sebelas Maret University with the Ethical Clearance No. 261/UN27.06/KEPK/EC/2019. This study was part of the study and development of implemented model for health service of work related illness in national health coverage era for holistic and integrated occupational health efforts.

Selection of Subjects

Samples were taken from farmers who used chemical pesticides in Tlogolele Village, Selo District, Boyolali Regency who met the inclusion and exclusion criteria. Inclusion criteria included staying for minimum one year in Selo District of Boyolali Regency, men aged between 20-65 years, carrying out pesticide spraying activities for at least one year, and willing to be the subject of research by signing informed consent. Exclusion criteria included a history and symptoms of lung and liver dysfunction, signs of malignancy, a history of alcoholic drinking habits, and the use of cholinesterase inhibitors. The sampling technique applied consecutive sampling.

Procedures

The independent variable of the study is blood cholinesterase activity investigated in Laboratory of Health and Medical Devices Testing (*Balai Laboratorium Kesehatan dan Pengujian Alat Kesehatani*) using *Deutsche Gessellschaftfur Klinische Chemie* (DGKC) method whereas the dependent variable is peak expiratory flow rate measured by *Philips Respironics PesonalBest*® peak flow meter. Additional variables were generated by questionnaire-based interview and clinical examination for demographic characteristics, history of pesticides application, and anthropometric variables. Data collection was completed at village hall of Tlogolele. The data collectors consisted of the interviewer and the note documenter who guided the respondents in answering the questionnaires. All data collectors were trained for their reliability in understanding the questions and the respondents' answers before the interview.

Data Analysis

Categorical data including smoking habits, personal protective equipment usage, and pesticide dosage were analyzed using unpaired T-test. Meanwhile, the ratio data of cholinesterase, age, height, weight, body mass index, frequency of spraying, the length of work per day, spraying duration, and last time of spraying were analyzed for their distributions followed by Pearson or Spearman test depending on the normal distribution of the data. Multivariate regression models were performed on the data. All the statistical analyses were performed using IBM SPSS software version 22 (IBM Corp., Chicago, USA). p-values <0.05 were considered significant.

RESULTS AND DISCUSSION

The research subjects consisted of 76 farmers who used chemical pesticides. Demographic and physical characteristics, history of pesticide application, PEFR, and blood cholinesterase are shown in Table 1. The mean age of the study sample was 41.86 ± 10.18. Most of these farmers were graduated from elementary school for as many as 59 (77.6%). The highest income was less than Indonesian rupiah (IDR) 1,000,000 which were occupied by 50 subjects (65.8%). Normal nutritional status was 50 (65.8%) with the mean of body mass index (BMI) of 22.09±2.38 kg/m^2. Smoking habits were performed by 60 subjects (78.9%). The mean height of the study sample was 159.03±7.02 m while the mean body weight was 56.06±8.42 kg.

Data on pesticide application in this study included the use of personal protective equipment (PPE), pesticide dosage, frequency of spraying, length of work, duration of spraying, and last time of spraying (Table 1).

The results showed that there were only 14 subjects (18.4%) used complete PPE. As many as 43 subjects (56.6%) had used the dosage of pesticides according to the rules namely 1.5-2 cc/liters of solvent. The highest frequency of spraying was 2 times/week performed by 37 subjects (48.7%). The longest working duration was 2 hours/day performed by 25 subjects (32.9%). The longest spraying duration was 11-20 years by 34 subjects (44.7%). The closest last time of spraying was 1-7 days for as many as 44 subjects (57.9%).

The results of the examination in this study are 71 subjects (93.4%) with normal cholinesterase levels and 5 subjects (6.6%) with abnormal cholinesterase levels. The results of this study are in line with a study showing normal cholinesterase which is higher than abnormal cholinesterase (Sandra *et al*., 2019). An examination of cholinesterase activity is an assessment of organophosphate poisoning in workers. Decreasing blood cholinesterase activity is reduced due to the inhibition of cholinesterase enzyme activity by organophosphates. However, cholinesterase activity may not always be able to describe the level of chronic organophosphate poisoning especially at low dose exposure because there is a recovery period of cholinesterase activity after exposure. This recovery period ranges from 35-100 days after exposure (Eddleston, 2015; Ye *et al*., 2013).

Based on PEFR normal values guidelines by Indonesian Pneumobile Project Team in 1992 (Alsagaff *et al*., 1992), the PEFR examination in this study showed an abnormal result in 57 subjects (75%). The mean PEFR value of the sample was 407.96 ± 96.04. These results are in line with studies that show higher abnormal lung function than normal lung function in farmers with exposure to pesticides (Luthfanto *et al*., 2014; Sandra *et al*., 2019). This study is also consistent with other studies which figure out a significant decrease in PEFR among farmers compared to the control (Priyadharshini *et al*., 2017). Decreased PEFR values can be a sensitive indicator in predicting the amount of airway obstruction. Pesticide aerosol drops that are sprayed in the air can get in through inhalation which causes irritation and narrowing of the airway (Sapbamrer *et al*., 2019). The results of these studies provide evidence on the adverse effects of exposure to pesticides on the respiratory system.

Bivariate test results (Table 2) in this study showed a significant relationship between age (r=-0,458; p=0,000), height (r=0.434; p=0,000), weight (r=-0,322; p=0.005), spraying frequency (r=-0.279; p=0.015), and spraying duration (r=-0.336; p=0.002) with PEFR. Whereas variables of blood cholinesterase, BMI, smoking habits, PPE usage, pesticide dosage, length of work, and last time of spraying did not show a significant relationship with PEFR. Negative correlation on age variable with PEFR is in line with studies conducted both in the general population and certain occupational exposures (Bhardwaj *et al*., 2014; Price *et al*., 2013). PEFR values decrease with increasing age due to degenerative changes in musculoskeletal system of the thoraco-abdominal compartment which cause a decrease in the strength of respiratory muscles. In addition, the airways also experience increasing resistance due to functional changes with age (Bhardwaj *et al*., 2014; Mukherjee *et al*., 2018).

Height shows a positive correlation with PEFR. This is in line with research conducted on both male and female populations (Garg *et al*., 2015). Taller people have greater chest volume. Also, airway growth and respiratory muscle effort increase with greater body height (Mukherjee *et al*., 2018). Analysis on weight variable with PEFR is in line with studies that show a positive correlation between body weight and PEFR (Bedi & Dang, 2016; Kaur *et al*., 2013). This finding is due to respiratory tract and greater muscular expiratory effort as we gain weight (Mukherjee *et al*., 2018).

The spraying duration in this study shows a negative correlation with PEFR. These results are in line with a research by Fareed *et al*. (2013) which shows a negative correlation between duration of exposure and PEFR (r=-0.41; p<0.01). The duration of exposure in this study is also associated with respiratory symptoms. Research conducted by

Chakraborty *et al*. (2009) also shows results that are consistent with the results of this study. In this study, spraying frequency shows a negative correlation with PEFR. To the best of our knowledge, no previous studies have analyzed the correlation between the frequency of spraying and PEFR.

Table 1. Demographic and Physical Characteristics, History of Pesticide Application, PEFR, and Blood Cholinesterase among Male Sprayers (n = 76)

Variable	n (%)	Mean±SD or Median (Min-Max)
Age		41.86±10.18
Education		
No school	2 (2.6)	
Graduated from elementary school	59 (77.6)	
Graduated from middle school	8 (10.5)	
Graduated from high school	7 (9.2)	
Income		
<IDR 1,000,000	50 (65.8)	
IDR 1,000,000-2,500,000	24 (31.6)	
IDR 2,500,000-5,000,000	2 (2.6)	
Smoking habit		
Yes	60 (78.9)	
No	16 (21.1)	
Nutritional Status / Body Mass Index		
Less	4 (5.3)	22.09±2.38
Normal	50 (65.8)	
More	22 (28.9)	
Height (cm)		159.03±7.02
Weight (kg)		56.06±8.42
Use of PPE		
Complete	14 (18.4)	
Incomplete	62 (81.6)	
Pesticide Dosage		
By the rules	43 (56.6)	
Not according to rules	33 (43.4)	
Spraying Frequency (times/week)		2 (1-4)
Length of Work (hours/day)		2 (1-6)
Spraying Duration (year)		20 (1-40)
Last Time of Spraying (day)		7 (1-196)
Peak Expiratory Flow Rate (L/min)		
Normal	19 (25)	407.96±96.04
Abnormal	57 (75)	
Blood Cholinesterase (U/L)		
Normal	71 (93.4)	5754.00 (2534-9761)
Abnormal	5 (6.6)	

Note: Mean±SD = mean±standard deviation; Median (Min-Max) = median (minimum-maximum); IDR = Indonesian rupiah; PPE = personal protective equipment

Previous studies analyzed the correlation between frequency of spraying and other lung function parameters such as forced expiratory volume in one second/forced vital capacity (FEV_1/FVC), and forced expiratory flow at 25-75% of the pulmonary volume ($FEF_{25-75\%}$). The results of this study indicate a negative correlation between the frequency of spraying and FEV_1/FVC (r=-0.85; p<0.001) and $FEF_{25-75\%}$ (r=-0.62; p<0.001) (Buralli *et al*., 2018). Research using the cumulative exposure index in describing variable of spraying frequency and duration of exposure also shows the results that the cumulative exposure index is a predictor of $FEF_{25-75\%}$ with a value of R^2 50% (r=-0.254; p=0.039) (Hernández *et al*., 2008). The level of pesticide poisoning depends on pesticide toxicity, pesticide formulation, pesticide particle size, PPE usage, duration, frequency, and intensity of exposure. Inhalation of pesticides can cause damage to the nose, throat, and lung tissue which can cause malfunction and increase the risk of respiratory symptoms and diseases (Damalas and Koutroubas, 2016; Mamane *et al*., 2015).

After conducting a bivariate analysis, all variables (use of PPE, pesticide dosage, spraying duration, height, cholinesterase, age, body weight, and spraying frequency) met the criteria for entry into the multivariate analysis (Table 3). With multivariate analysis of linear regression backward method, the PEFR equation is obtained as = 465.537 + (-3.627xage) + (2.389xweight) + (-24.31x frequency of spraying) with an R^2 = 26.8%. Equations consisting of age, body weight, and spraying frequency contributed 26.8% to the dependent variable (PEFR) while the remaining 73.2% was influenced by other variables. In Indonesia, a model to predict PEFR values was carried out by the Indonesian Pneumobile Project Team in 1992. However, the PEFR values in this study were obtained from the general population. The PEFR equation in this study consists of age and height factors (Alsagaff *et al*., 1992).

Table 2. Correlation between blood cholinesterase, demographic and physical characteristics with history of pesticide application and PEFR

Variable	Peak Expiratory Flows (L/min)			
	Mean±SD	Mean difference (95%CI)	p value	r
Cholinesterase (U/L)	-	-	0.145	0.169
Age (years)	-	-	0.000*	-0.458
Height (cm)	-	-	0.000*	0.434
Weight (kg)	-	-	0.005*	0.322
BMI (kg/m^2)	-	-	0.391	0.1
Smoking habit				
Yes	405.67±94.85	-10.90 (-65.04-43.25)	0.69	-
No	416.56±103.13			
Use of PPE				
Complete	441.79±78.58	41.46 (-14.73-97.66)	0.146	-
Incomplete	400.32±98.50			
Pesticide dosage				
By the rules	423.95±94.07	36.83 (-6.93-80.59)	0.098	-
Not according to rules	387.12±95.99			
Spraying frequency (times/week)	-	-	0.015*	-0.279
Length of work (hours/day)	-	-	0.831	0.025
Spraying duration	-	-	0.002*	-0.356
Last time of spraying (days)	-	-	0.400	-0.098

Note: Mean±SD = mean±standard deviation; 95%CI = 95% confidence interval; BMI = body mass index; PPE = personal protective equipment; *p<0.05

Table 3. Model for predicting PEFR of male sprayers (n = 76)

Variable	Unstandardized Coefficients		Standardized Coefficients	p value
	B	Standard Error	Beta	
Constant	465.537	87.323		0.000
Age	-3.627	0.960	-0.385	0.000
Weight	2.389	1.170	0.209	0.045
Spraying frequency	-24.310	13.895	-0.179	0.084

Note: R^2 = 26.8%

Research on cement factory workers in Nigeria shows the results of the PEFR prediction model with the PEFR equation/year of exposure = −69.985 + 3.425xage + 2.922xheight – 2.473xweight + 10.9xyear of exposure (R^2 84.3% ; p<0.001) (Ismaila *et al*., 2015). To the best of our knowledge, there is no PEFR prediction model for farmers with pesticide exposure. The PEFR prediction model is useful in initial screening for respiratory system disorders in farmers using pesticides.

PEFR is an indicator of airway obstruction that is sensitive and accurate. PEFR examination with PEF meters is easier, simpler, cheaper, and quantitative as well as noninvasive (Mridha *et al*., 2011; Thorat *et al*., 2017). PEFR prediction models for farmers using pesticides are useful in initial screening for respiratory system disorders. The results of this study strengthen the evidence of the correlation between pesticide exposure and respiratory system disorders. However, this study has several limitations. First, this research was only conducted in one region/village with a limited sample size. Second, this study was only conducted towards male farmers so that it limits the generalization of research results to the other gender. Third, other laboratory support parameters that might influence the level of cholinesterase were not examined in this study (e.g. hemoglobin, liver function tests, and malignancy markers.

CONCLUSION

The equation model to determine PEFR based on age, weight, and frequency of spraying was obtained with enough coefficients determination. This developed model will be useful in determining the PEFR of vegetable farmers as an effort to access possible medical attention. Multicentre studies with multigender and larger sample sizes are recommended to obtain PEFR predictor models.

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REFERENCES

Alif, S.M., Dharmage, S.C., Benke, G., Dennekamp, M., Burgess, J.A., Perret, J.L., Lodge, C.J., Morrison, S., Johns, D.P., Giles, G.G., Gurrin, L.C., Thomas, P.S., Hopper, J.L., Wood-Baker, R., Thompson, B.R., Feather, I.H., Vermeulen, R., Kromhout, H., Walters, E.H., Abramson, M.J., Matheson, M.C., 2017. Occupational Exposure to Pesticides are Associated with Fixed Airflow Obstruction in Middle-Age. *Thorax*, 72(11), 990–997.

Alsagaff, H., Mangunnegoro, H., Amin, M., Yunus, F., Bernstein, R., Johnson, L., 1992. Nilai Normal Faal Paru Orang Indonesia pada Usia Sekolah dan Pekerja Dewasa Berdasarkan Rekomendasi American Thoracic Society (ATS) 1987. *Paru*, 12(4), 3–18.

Bedi, U., Dang, B., 2016. A Study of Peak Expiratory Flow Rate in Normal Healthy Children of Punjab. *International Journal of Medical and Dental Sciences*, 5(1), 1042–1047.

Bhardwaj, P., Poonam, K., Jha, K., Bano, M.,

2014. Effects of Age and Body Mass Index on Peak Expiratory Flow Rate in Indian Population. *Indian Journal of Physiology and Pharmacology*, 58(2), 166–169.

BPS Boyolali, 2018[a]. Statistik Daerah Kabupaten Boyolali. Badan Pusat Statistika Kabupaten Boyolali, Boyolali.

BPS Boyolali, 2018[b]. Kecamatan Selo dalam Angka 2018. Badan Pusat Statistika Kabupaten Boyolali, Boyolali.

Buralli, R.J., Ribeiro, H., Mauad, T., Amato-lourenço, L.F., Silva, D.S., Remy, J., 2018. Respiratory Condition of Family Farmers Exposed to Pesticides in the State of Rio de Janeiro, Brazil. *International Journal of Environmental Research and Public Health*, 15(1203), 1–14.

Calvert, G., Mehler, L., Alsop, J., De Vries, A., Besbelli, N., 2010. Surveillance of Pesticide-Related Illness and Injury in Humans, in: Krieger, R. (Ed.), Hayes' Handbook of Pesticide Toxicology. Academic Press, USA, pp. 1313–1369.

Calvert, G.M., Plate, D.K., Das, R., Rosales, R., Shafey, O., Thomsen, C., Male, D., Beckman, J., Arvizu, E., Lackovic, M., 2004. Acute Occupational Pesticide-Related Illness in the US, 1998 – 1999 : Surveillance Findings From the Sensor-Pesticides Program. *American Journal of Industrial Medicine*, 23(45), 14–23.

Chakraborty, S., Mukherjee, S., Roychoudhury, S., Siddique, S., Lahiri, T., Ray, M.R., 2009. Chronic Exposure to Cholinesterase-Inhibiting Pesticides Adversely Affect Respiratory Health of Agricultural Workers in India. *Journal of Occupational Health*, 8(3), 1–27.

Damalas, C.A., Koutroubas, S.D., 2016. Farmers' Exposure to Pesticides : Toxicity Types and Ways of Prevention. *Toxics*, 4(1), 1–10.

Djojosumarto, P., 2008. Teknik Aplikasi Pestisida Pertanian. Kanisius, Yogyakarta.

Eddleston, M., 2015. Insecticides: Organic Phosphorus Compounds and Carbamates, in: Hoffman, R.S., Howland, M.A., Lewin, N.A., Nelson, L.S. (Eds.), Goldfrank's Toxicologic Emergencies. McGraw-Hill, USA, pp. 2306–2331.

Fareed, M., Pathak, M.K., Bihari, V., Kamal, R., Srivastava, A.K., Kesavachandran, C.N., 2013. Adverse Respiratory Health and Hematological Alterations among Agricultural Workers Occupationally Exposed to Organophosphate Pesticides: A Cross-Sectional Study in North India. *PLoS ONE*, 8(7), 1–10.

Garg, R., Anand, S., Sehgal, R.K., Singh, H.P., 2015. Normative Data of Peak Expiratory Flow Rate in Healthy School Children of Ghaziabad City—A Pilot Study. *National Journal of Physiology, Pharmacy and Pharmacology*, 5(4), 309–312.

Harriyani, I., 2016. Faktor Risiko Keracunan Pestisida pada Petani Penyemprot Sayuran di Kecamatan Selo Kabupaten Boyolali. Universitas Gadjah Mada.

Hernández, A.F., Casado, I., Pena, G., Gil, F., Villanueva, E., Pla, A., 2008. Low Level of Exposure to Pesticides Leads to Lung Dysfunction in Occupationally Exposed Subjects. *Inhalation Toxicology*, 20(9), 839–849.

Hoppin, J.A., Umbach, D.M., London, S.J., Henneberger, P.K., Kullman, G.J., Coble, J., Alavanja, M.C.R., Beane Freeman, L.E., Sandler, D.P., 2009. Pesticide Use and Adult-Onset Asthma among Male Farmers in the Agricultural Health Study. *European Respiratory Journal*, 34(6), 1296–1303.

Ismaila, S.O., Akanbi, O.G., Olaoniye, W., 2015. Model for Predicting Peak Expiratory Flow Rate of Nigerian Workers in A Cement Factory in Itori, Ogun State, Nigeria. *International Journal of Occupational Safety and Ergonomics*, 21(4), 547–550.

Kaur, H., Singh, J., Makkar, M., Singh, K., Garg, R., 2013. Variations in The Peak Expiratory Flow Rate with Various Factors in A Population of Healthy Women of The Malwa Region of Punjab, India. *Journal of Clinical and Diagnostic Research*, 7(6), 1000–1003.

Luthfanto, H., Indraswari, D., Adyaksa, G., 2014. Hubungan antara Aktivitas Asetilkolinesterase Darah dan Arus

Puncak Ekspirasi Petani Kentang dengan Paparan Kronik Pestisida Organofosfat. Universitas Diponegoro Semarang.

Lytras, T., Kogevinas, M., Kromhout, H., Carsin, A.E., Antó, J.M., Bentouhami, H., Weyler, J., Heinrich, J., Nowak, D., Urrutia, I., Martinez-Moratalla, J., Gullón, J.A., Pereira-Vega, A., Raherison-Semjen, C., Pin, I., Demoly, P., Leynaert, B., Villani, S., Gislason, T., Svanes, C., Holm, M., Forsberg, B., Norbäck, D., Mehta, A.J., Probst-Hensch, N., Benke, G., Jogi, R., Torén, K., Sigsgaard, T., Schlünssen, V., Olivieri, M., Blanc, P.D., Vermeulen, R., Garcia-Aymerich, J., Jarvis, D., Zock, J.P., 2018. Occupational Exposures and 20-Year Incidence of COPD: The European Community Respiratory Health Survey. *Thorax*, 1–8.

Mamane, A., Baldi, I., Tessier, J., Raherison, C., Bouvier, G., 2015. Occupational Exposure to Pesticides and Respiratory Health. *European Respiratory Review*, 24(136), 306–319.

Mathew, P., Jose, A., Alex, R., Mohan, V., 2015. Chronic Pesticide Exposure: Health Effects Among Pesticide Sprayers in Southern India. *Indian Journal of Occupational and Environmental Medicine*, 19(2), 95–101.

Mridha, M.A.-A., Amin, M.R., Kabir, A.L., 2011. Peak Expiratory Flow Rate (PEFR)-A Simple Ventilatory Lung Function Test. *Journal of Shaheed Suhrawardy Medical College*, 3(2), 44–47.

Mukherjee, S., Banerjee, G., Mahapatra, A.B.S., 2018. Peak Expiratory Flow Rate Changes with Relevant Variables in A Population of Eastern India. *Indian Journal of Physiology and Pharmacology*, 62(3), 372–379.

Nayak, P.K., Satpathy, S., Manjareeka, M., Samanta, P., Mishra, J., Pradhan, B.B., 2016. Prediction Models for Peak Expiratory Flow Rate in Indian Population Aged 18-25 Years. *National Journal of Physiology, Pharmacy and Pharmacology*, 6(3), 200–204.

Prasad, R., Verma, S.K., Agrawal, G.G., Mathur, N., 2006. Prediction Model for Peak Expiratory Flow in North Indian population. *The Indian journal of chest diseases & allied sciences*, 48(2), 103–106.

Price, J.P.K., Arthur, N., Macstephen, A.O., 2013. Correlation between Body Mass Index and Peak Expiratory Flow Rate of an Indigenous Nigerian Population in the Niger Delta Region. *Research Journal of Recent Sciences*, 2(2), 28–32.

Priyadharshini, U.K., Latha, R., Kavitha, U., Nirmala, N., 2017. Effects of organophosphorus pesticides on cardiorespiratory parameters among the farmers. *Journal of Clinical and Diagnostic Research*, 11(9), CC01–CC04.

Quirce, S., Muñoz, X., Urrutia, I., Pérez-Camo, I., Sabadell, C., Domínguez-Ortega, J., Barranco, P., Plaza, V., 2013. Changes in the Asthma Control Test Score in Patients with Work-Related Asthma. *Journal of Investigational Allergology and Clinical Immunology*, 23(4), 284–285.

Sandra, P.S.M., Sofiana, K.D., Sutejo, I.R., 2019. Correlation of Cholinesterase Levels to Lung Function in Farmer Exposed by Organophosphate Pesticides in Sukorambi Village, Jember Regency. *Journal of Agromedicine and Medical Sciences*, 5(2), 35.

Sapbamrer, R., Nata, S., 2014. Health Symptoms Related to Pesticide Exposure and Agricultural Tasks among Rice Farmers from Northern Thailand. *Environmental Health Preventive Medicine*, 19, 12–20.

Sapbamrer, R., Thongtip, S., Khacha-ananda, S., Sittitoon, N., Wunnapuk, K., 2019. Changes in Lung Function and Respiratory Symptoms during Pesticide Spraying Season among Male Sprayers. *Archives of Environmental and Occupational Health*, 0(0), 1–10.

Suhadi, R., Fenty, Virginia, D.M., Setiawan, C.H., 2018. The Effect of Health Insurance on Asthma Control in Respondents with Asthma in Yogyakarta, Indonesia. *Jurnal Farmasi Sains dan Komunitas*, 15(2), 62–67.

Thorat, Y.T., Salvi, S.S., Kodgule, R.R., 2017.

Peak Flow Meter with A Questionnaire and Mini-Spirometer to Help Detect Asthma and COPD in Real-Life Clinical Practice: A Cross-Sectional Study. *Primary Care Respiratory Medicine*, 27(1), 1–7.

UN, 2017. Report of the Special Rapporteur on the Right to Food. United Nations.

WHO, 2008. Clinical Management of Acute Pesticide Intoxication: Prevention of Suicidal Behaviours. World Health Organization, Geneva.

Wudianto, R., 2007. Petunjuk Penggunaan Pestisida. Penebar Swadaya, Jakarta.

Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2013. Occupational Pesticide Exposures and Respiratory Health. *International Journal of Environmental Research and Public Health*, 10, 6442–6471.

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HERBS USED AS ANALGESIC BY DAYAK TRIBE IN NORTH KALIMANTAN INDONESIA

Irma Novrianti[1,2*)], Faizal Mustamin[2], Suharjono[3]

[1,3]Department of Clinical Pharmacy, Airlangga University, Surabaya,
[2] Academy of Pharmacy Kaltara Tarakan, Kalimantan Utara

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ABSTRACT

Traditional medicines produced from medicinal plants have played an important role in disease treatment in Dayak Tribe, North Kalimantan. Northern Kalimantan belongs to a remote area where health facilities like healthcare center (*puskesmas – pusat kesehatan masyarakat*) and hospital are not easily accessible. This research aims to investigate herbs used by Dayak tribe in North Kalimantan for treating pain. It is a descriptive research applying two research methods i.e. observation and interview. We directly observed the research object and proposed questions to respondents that were traditional healers from each village. The research was conducted in nine villages within three regencies in North Kalimantan. Meanwhile, 38 herbs collected in this research were from 26 families and 34 species. Four species were still locally named. The highest proportion of the herb family used was *Asteraceae*. The most frequently used part of the herb for medicine was the leaf (60.53%). The main serving methods were by boiling the herb and drinking it (63.15%). Bone pain and stomachache were two main illnesses mostly treated by local herb therapy involving 11 herbs. In conclusion, Dayak tribe in North Kalimantan still used herbs to treat their pain.

Keywords: analgesic; Dayak tribe; herb.

INTRODUCTION

Herbs have been processed into traditional medicine since thousands of years ago. The medicine was in the form of raw medicine such as tincture, tea, poultice, powder, and other herbal formulations (Archana *et al.*, 2011). Indonesia is known as a country with its high diversity of plants. There is 10% of global flowering plant species existing in Indonesia. It is due to natural condition in Indonesia that is different in each island even in each region (Indrawan, 2007). Herbs are plants mainly used for traditional medicine. The use of herb is one of social habits since medicine made from herbs is more natural than modern medicine (Makalalag *et al.*, 2014).

Pain is a body defense mechanism form when the body is having unpleasant emotion and sensor experiences related to potential or real tissue damage. Mediators such as interleukin-1, and TNF-α spread the synthesis, release, and acts of prostaglandin E1 (PGE2) and F2α by endothelium and brain capillary pericytes that stimulate the nerves and may cause pain. Increasing prostaglandin content in peritoneum cavity increases the capillary permeability and causes pain (Nasrin *et al.*, 2015). Conventional therapy used to treat pain such as analgesic, non-steroid anti-inflammatory drugs (NSAID), and corticosteroid have been proven to succeed (Sreekeesoon & Mahomoodally, 2014). The pain may vary from mild to severe. Some therapies such as opioid are alternated for more severe pain (Reid *et al.*, 2015). Aloe vera (L) Burn is used as analgesic in Mauritus to treat leg pain and body pain (Sreekeesoon & Mahomoodally, 2014).

*Corresponding author: Irma Novrianti
Email: *irma.novrianti@gmail.com*

Most residents of Dayak tribe in North Kalimantan live in an area where they cannot easily access health facilities such as hospital and community health center due to the long distance. Living in an area next to the forest, they prefer to use herbs as medicine because of their affordability and accessibility (Yitno, 1991). This research aims to investigate herbs for pain management used by Dayak tribe in North Kalimantan.

METHODS

This research is descriptive in which the data were collected through observation and interview methods. Observation as a data collection technique was completed by the researcher by performing a direct observation towards the research object, while during interview, researchers proposed questions to respondents to investigate herbs used for pain management by Dayak tribe in North Kalimantan. The study was conducted for 3 months from March 2017 to June 2017 with permits directly addressed to customary leaders in their respective regions.

North Kalimantan is geographically located between 114˚35'22"-118˚03'00" east latitude and 4˚24'55"-1˚21'36" north latitude. North Kalimantan has a tropical climate and two seasons i.e. dry season and rainy season, which is similar to any other areas in Indonesia.

Interviews were performed to nine respondents who were traditional healers from several villages i.e. Lembudud, Long Kiwang, and Wayangung in Nunukan; Sekatak, Long Sam, and Pejalin in Bulungan; and Kaliamok, Sembuak Warod, and Tanjung Nanga in Malinau. We identified the respondents based on information given by local people. Data on characteristics of respondents and information related to the use of herbs were recorded. All interviews were conducted in the local language that was Dayak language. Meanwhile, research assistant acted as a translator of Dayak language into Indonesian.

The results of data collection including data on herb type, sample use, and the procedure of use obtained from interviews were further investigated through thorough observation.

Determination of respondents using snowball sampling method started with the Head of Customary Affairs. Afterwards, he gave recommendations and led the researcher to other respondents. Each respondent would be given information about medicinal plants.

Inventory of medicinal plants in the field was carried out by field survey method based on respondent information. Every plant obtained was taken their pictures; and their regional names as well as morphological characters were recoded. Identification process of medicinal herbs was performed by referring to several books entitled *Buku Flora* (Steenis *et al.*, 2005), *Kitab Tanaman Obat Nusantara* (Widyaningrum, 2011) and *Atlas Tumbuhan Obat Indonesia Jilid 4* (Dalimartha, 2006).

RESULTS AND DISCUSSION

The findings were discussed in three subparts i.e. traditional healer, ethnobotanical flora, and type of pain.

Traditional Healer

In this research, researcher collected information about herbs used to treat pain by communicating and interviewing one or two traditional healers of each village. The age range of those traditional healers was 40 up to 70 years old. Our respondents, the traditional healers, obtained their knowledge on herbs from their ancestors. Research on herbal medicine for diabetes conducted in Northeast Iran revealed that 87% of traditional healers learned herbs from their parents or other relatives (Tag *et al.*, 2012). In another study, traditional healers acquired their medicinal plant knowledge by inheriting from their elder lineage (father or grandfather), whereas the rest received his medicinal plant knowledge from dreams (Nasution, Aththorick and Rahayu, 2018). In this research, traditional healers admitted that they received 50-100 patients in one month. Our field observation and interview with traditional healers during survey clarified that patients preferred traditional medicine to

modern drugs due to its effectiveness, affordability, and accessibility.

Patterns of Herbs Used among Dayak Tribe

Information on herbs' scientific name, origin, family, and serving method is fully presented in Table 1. 38 herbs gathered in this research were categorized into 26 families and 34 species. Four herbs were still locally named since they could not be identified by local botanical experts. The highest proportion of the herbs was *Asteraceae* (five species) followed by *Euphorbiaceae, Fabaceae*, *Lamiaceae,* and *Malvaceae*- each with two species and other families with one species for each. *Asteraceae* was the largest family of herb used to reduce pain in North Kalimantan. Based on research on herbs used to reduce pain in Mauritus (2014), it was confirmed that out of 79 herbs found, there were six species from *Lamiaceae* family, followed by *Apiaceae*, *Asteraceae*, *Euphorbiaceae*, and *Poaceae* with five species each (Sreekeesoon & Mahomoodally, 2014). *Asteraceae* was the largest family dominating plant vegetation on earth with the number of members of 24,000-30,000 species and 1,600-1,700 genera globally spread in almost all types of environment (Bisht and Purohit, 2014). Besides, herbs commonly used by eastern people in Amazon came from *Asteraceae*, *Lamiaceae*, *Euphorbiaceae*, *Piperaceae*, and *Verbenaceae* families (Hariyadi, 2011). *Asteraceae* family could be used as traditional medicine since they contained bioactive compound components such as sesquiterpene, lactone, pentacyclic triterpene, alcohol, tannin, polyphenol, saponins, and sterol that could be used as medical ingredients. Due to its bioactive property, *Astereaceae* family was usually used as insecticide, anthelminthic, antimalarial, antiseptic, anti-inflammation, and antioxidant (Wegeira *et al.*, 2012).

Table 1. Types of Herbs and Serving Methods Applied by Dayak Tribe in North Kalimantan

No.	Family	Type	Local Name	National Name	Used Part	Treated Illness	Serving Method
1.	Acanthacea						
		Justicia gendarussa burm	War tonep	Ganda rusa	Leaf	Rheumatic	Boil ten leaves. Drink it thrice a day.
						Bruise and sprain	
2.	Asteraceae						
a.		Blumea BalsamiFera	Ipung	Sembung	Leaf	Stomachache, menstrual pain	Boil five up to seven leaves. Drink it thrice a day.
b.		Pluchea indica	Beluntas	Beluntas	Leaf	Stiffness	Boil a handful of leaves until the water decreases. Drink it thrice a day.
c.		Heliothous anuus L	Bonga mata so'o	Bunga matahari	All parts	Headache, fuzziness, toothache, menstrual pain, stomachache	Take three handfuls of flowers, add one chicken egg (do not break the egg) and three glasses of water. Boil them until the water decreases. Drink it twice a day after meal.

No.	Family	Type	Local Name	National Name	Used Part	Treated Illness	Serving Method
d.		Strobilanthescrispus	Peca beling	Peca beling	Leaf	Stomachache	Boil ten leaves in three glasses of water until the water decreases. Drink it thrice a day.
3.	***Arecaceae***						
		Daemonorops	*Rotan sembulik*	*Rotan jernang*	Stem	Stomachace	Boil and drink the water thrice a day.
4.	***Amaryllidaceae***						
		Crynum asiaticum L	*Kaber lab*	*Daun bakung*	Leaf	Sprain	Grill the leaf and put it on the painful area once a day at night.
5.	***Amaranthi aceae***						
		Gopheruna globasa L	*Bonga derem*	*Kenop*	All parts	Headache	Grind and put it on the painful area.
6.	***Apocynaceae***						
		Plumeria acuminate Ait	*Kamboja*	*Kamboja*	Leaf, stem, root	Toothache	Pick the sap/leaf, grind it and put it on the painful area.
7.	***Balsamin aceae***						
		Impatsens Balsamia	*Kembang pacar*	*Pacar air*	Leaf	Menstrual pain	Grind the leaf and put on the lower abdomen.
8.	***Crassulaceae***						
		Kalanchoe pinnata	*Sosor bebek*	*Cocor bebek*	Leaf	Rheumatic	Boil seven leaves and drink the water twice a day.
						Sprain	Pick several leaves and smear them with oil. Grill then put it on the painful area.
9.	***Cycadaceae***						
		Cycas revolute thumb	*Aka kabuk*	*Akar penawar*	Stem	Pain killer	Cut one cm of the stem, divide it into two, grind it, and soak it into water. Drink the water.
10.	***Euphorbiaceae***						
a.		*Jatropha Curcas L*	*Bua jarak*	*Jarak pagar*	Leaf	Toothache	Heat the sap and smear it on the painful area.
b.		*Phyllantus niruri L*	*Babah anak*	*Meniran*	All parts	Backache	Boil it and drink the water thrice a day.
11.	***Eragrostideae***						
		Eleusin indica	*Unduh gelu'*	*Rumput carulang*	Leaf	Sprain	Grind the leaf and put it on the painful area once a day.

No.	Family	Type	Local Name	National Name	Used Part	Treated Illness	Serving Method
							Grind some leaves. Smear it on the painful area twice a day.
12.	***Fabaceae***						
b.		*Mimosa pudica*	*Uduh suha*	*Putri malu*	Leaf	Rheumatic and back pain	Pick ¾ handful of the leaves and boil them in five glasses of water. Drink it thrice a day.
a.		*Abrus precatorius L*	*Owat dipon*	*Saga rambat*	Stem	Toothache	Grind then put it on the painful area.
13.	***Gesneriaceae***						
		Cyrtandra sarawakensis	*Bura kaguyad ang*	*Cyrtanda sarawak*	The peak of the leaf	Stomachace	Soak it in hot water and drink it thrice a day.
14.	***Labiatae***						
		Orthosiphon stamineus	*Rumput kucing*	*Kumis kucing*	Stem, leaf	Headache	Boil it and drink the water thrice a day.
15.	***Lamiaceae***						
a.		*Coleus parviflorus Benth*	*Dikut pait*	*Kentang ireng*	Stem, leaf	Stomachace experienced by babies	Grind it, add chili leaves then put it on the painful area.
b.		*Orthosphon aristatus*	*Udu pa'sing*	*Daun kumis kucing*	Stem, leaf	Backache	Pick some stems and leaves, boil them and drink the water.
16.	***Loranthaceae***						
		Loranthus	*Ancam*	*Benalu*	All parts	Toothache	Burn it and mix it with oil. Put it on the painful area.
17.	***Malvaceae***						
		Grewia acuminate Juss.	*Bura krotok*	*Akar sekapu*	Stem	Stomachace	Burn it and drink the essence twice a day.
		Hibiscus rosa sinensis L	*Bunga sepatu*	*Kembang sepatu*	Leaf, flower	Headache	Chop the leaf and flower, and boil them in three glasses of water. Drink the water thrice a day.
18.	***Malastomataceae***						
		Melastonia sepfemnerviu	*Jelemuti n*	*Senggani*	Leaf	Stomachache	Boil the leaf. Drink it while still warm.
					Stem	Toothache	Peel and grind the stem. Use it as your mouthwash thrice a day.

No.	Family	Type	Local Name	National Name	Used Part	Treated Illness	Serving Method
19.	***Menispermaceae***						
		Tinospora crispa	*Bundung kemamba ng*	*Brotowali*	Leaf	Stomachache	Pick the stem and cut it. Boil it in three glasses of water then drink it thrice a day.
					Stem	Rheumatic	
20.	**Musaceae**						

		Musa	*Peti*	*Pisang*	Stem	Joint pain and bone pain	Pick a stem and divide it into two. Pick the *umbut* and eat it.
21.	***Myrtaceae***						
		Psiatum guajava L	*Libum*	*Jambu biji*	Leaf	Stomachache	Pick and grind six leaves. Pour some water and squeeze the leaves. Drink the water.
22.	***Pandanaceae***						
		Pandanus amaryllifaius roxb	*Kaber nanung*	*Pandan wangi*	Leaf	Rheumatic and stiffness	Pick and wash three leaves. Cut them into small size and brew them with a half cup of oil. Stir it. Let it cold and smear it on the painful area.
23.	***Poaceae***						
		Cymbopogon nardus L	*Gisumau*	*Sere wangi*	Root, young leaf	Rheumatic	Pick and boil some roots and young leaves in six glasses of water. Boil until the water decreases and drink it thrice a day.
24.	***Rubiaceae***						
		Psychitria sp.	*Concang abang*		All parts	Pain killer	Boil and drink it thrice a day.
25.	***Solanaceae***						
		Physalis angulate L	*Latup*	*Ciplukan*	Leaf	Toothache	Grind then use the leaves as your mouthwash thrice a day.
26.	***Zingiberaeae***						
		Curcuma xanthorrhiza	*Temu lawak*	*Temu lawak*	Root	Backache	Grind the root. Filter and drink it thrice a day.
No.	**Family**	**Type**	**Local Name**	**National Name**	**Used Part**	**Treated Illness**	**Serving Method**
27.	***Unknown***						
		Unknown	*Faling*	Unknown	Root	Kids' stomachache	Dry the root under the sun, grind then brew the powder. Drink it thrice a day.

	Unknown	*Udu pejek*	Unknown	Leaf, root	Sprain	Pick two slices of *udu pejek* and smash it. Twist it on the painful area, wrap the area with cloth.
	Unknown	*Kelepeso*	Unknown	Fruit	Stomachache	Pick and grate two seeds, add some water, and drink it.
	Unknown	*Kayu pela*	Unknown	Stem	Toothache	Pick and break one stem. Wait for two minutes until the sap is out and smear it on the painful area.

The most frequently used parts of the herbs for medicine were the leaf (60.5%) and the stem (26.3%). Meanwhile, the distribution of the herbs' parts used to treat pain is presented in Table 2. To manage pain, Dayak tribe in North Kalimantan preferred to use leaves of the selected herbs. The selection is important because the right part of herbs contains secondary metabolite that helps patients to obtain the desired therapeutic effect. In addition, leaf contains many active phytochemical pharmacologies responsible for curative effects (Sreekeesoon & Mahomoodally, 2014). Ethnobotanical research in Dayak Tribe Seberuang in Ensabang Village conveyed that leaf was mostly used because people found it easier to collect leaves than to collect other parts of herbs that existed underground. Additionally, when they dug the ground, they might destruct the herb root, thus, harmed the herb (Damianus *et al*., 2013). Another research declared that most traditional healers used leaf to substitute part of herb that grew underground to preserve the herb (Traore *et al*., 2013). Besides, leaf is the most accessible part of herb. In addition, leaf could be more easily processed and gave more benefits (Takoy, Linda & Lovadi, 2013). Herbal therapy containing many complex compounds was regarded to provide more action targets to human body (Raja Nasution, Aththorick and Rahayu, 2018).

Herbs could be used as medical treatment through several techniques like by drinking the herbs (63.2%), putting the herbs on the painful area (36.8%), and using the herbs as mouthwash (5.26) for toothache. The fact is shown in Table 3.

Serving methods can be completed by boiling and drinking them, or by rubbing them on the painful area. Boiling was the most common and efficient method (Sreekeesoon & Mahomoodally, 2014). Methods applied to prepare the herbs were boiling (42.1%) and grinding then smearing on the painful area (36.8%), as presented in Table 4.

Types of Pain

Distribution of types of pain and number of herbs for pain management is shown in Table 5. Types of pain experienced were bone pain (rheumatic, sprain, backbone pain, and stiffness), stomachache, toothache, headache, backache, and menstrual pain. Out of those, bone pain and stomachache were two types of pain that were most frequently treated using herbs consisting of 11 herbs for each illness. Especially for headache, it could be cured with a certain therapy using nine types of herb.

One of herbs used to reduce bone pain was *gandarusa*. *Justicia gendarussa Burm F*. (Family: *Acanthaceae*) is widely used in Indian and Chinese traditional medicines and the leaf of the plant is recommended to treat pain such as arthritis, headache, earache, and muscle pain (Jaijesh *et al*., 2009). The herb could be found or cultivated in Indonesia, India, China, Malaysia, Sri Lanka, Philippine, and Bangladesh. The leaf of the herb was reportedly anti-angiogenic, antioxidant, anti-bactericidal, antifungal, anti-arthritis, anti-inflammatory, anti-nociceptive, and anti-sickling, and it

showed anthelmintic, cytotoxicity, larvicidal, and adulticidal activity (Ningsih *et al.*, 2015). Additionally, *gandarusa* contained flavonoid compound (Gustina, 2017) known to cure inflammatory disorders (Wegeira *et al.*, 2012). Hence impeding Cox-2 formation and preventing prostaglandin formation.

Another herb used as a therapy for stomachache and menstrual pain was *sambong* (*Blumea BalsamiFera*). *Blumea balsamifera (L.) DC. (Asteraceae)*, or *sambong* has been widely used in many countries such as Chinese, Malaysia, Thailand, Vietnam, and Philippine for years. The herb is widely used in Indonesian traditional medicines; the leaf of the plant is recommended to treat pain such as arthritis, headache, earache, and muscle pain (Rahardjo, 2016). It is the most important member in *Blumea* genus and the origin herb of tropical and subtropical Asia, particularly China. It grows in the edge of the forest, underneath the forest, river bed, valley, and meadow. All parts of the herb, including the leaves, were used as a traditional therapy to cure eczema, dermatitis, beriberi, backache, menorrhagia, rheumatic, and damaged skin, and as an insecticide. The main active compound contained in *Sembong* (*Blumea BalsamiFera*) is L-borneol characterized by high volatility. Besides, *Sembong* contained essential oil, flavonoid, and terpenoid with some different biological activities (Pang *et al.*, 2014). It has many flavonoid compounds. There were 27 of 29 flavonoid compounds identified in *Sembong* (*Blumea balsamifera (L.) DC*) including 21 analog flavonoids, five derivative CQAs, and one coumarin (Pang *et al.*, 2014). Anti-inflammatory activity mechanism of flavonoid is restricting eicosanoid to produce some enzymes such as phospholipase A2, cyclooxygenases, and lipoxygenase, hence, reducing both leukotriene and prostanoid concentrations. Other mechanisms are histamine and phosphodiesterase restriction, protein kinase releases, and transcriptase activation. Restricted eicosanoid enzyme would restrict phospholipase A2 enzyme formation so that cyclooxygenase and lipoxygenase would be unable to be formed which cancelled the production of prostaglandin compound (Rathee *et al.*, 2009).

Table 2. Distribution of the Used Parts of the Herbs

No.	Part of the Herbs Used	Number	Percentage (%)
1	Leaf	23	60.5
2	Stem	10	26.3
3	Flower	1	2.6
4	Fruit	1	2.6
5	Root	2	5.3
6	Flower	1	2.6
7	All parts	2	5.3
8	Herb	1	2.6

Table 3. Distribution of the Technique in Herbs Use

No.	Technique to Use	Number	Percentage (%)
1	Oral	24	63.2
2	Topical	14	36.8
3	Mouthwash	2	5.3

Table 4. Distribution of Herbs Serving Methods

No.	Serving Method	Number	Percentage (%)
1	Boiling	16	42.1
2	Grinding and smearing on the painful area	14	36.8
3	Soaking into water	3	7.9
4	Burning	1	2.6

5	Grinding and using the herb as mouthwash	2	5.3
6	Mashing up by using a blending machine	1	2.6
7	Shredding and adding water	1	2.6
8	Brewing	1	2.6
9	Eating	1	2.6

Table 5. Types of Pain and Herbs

No.	Name of Illness	Number of Herbs	Herbs (Local Name)
1.	Bone pain (rheumatic, sprain, stiffness, backbone pain)	11	*Ganda Rusa, Sere Wangi, Cocor Bebek, Putri Malu, Beluntas,* leaf of *Bakung, Pandan Wangi, Pisang, Brotowali, Rumput Carulan, Udu Pejek*
2	Stomachache	11	*Sambong, Rotan Jernang, Kentang Ireng,* root *of Sekapu, Cyrtanda Sarawak, Senggani, Jambu Biji, Peca Beling, Brotowali, Faling, kelepeso.*
3	Toothache	9	*Kamboja, Ciplukan, Senggani, Jarak Pagar, Bunga Matahari, Tahi kotok, Saga, Benalu, Kayu Pela*
4	Headache	4	*Kumis Kucing, Kenop, Bunga Matahari, Kembang Sepatu*
5	Backache	3	*Temu Lawak,* Leaf of *Kumis Kucing, Meniran*
6	Menstrual pain	2	*Pacar Air, Sembung*
7	Pain killer	2	Root of *Penawar, Concanga-bang*

One of the herbs used by Dayak people in North Kalimantan to cure toothache was *benalu* (*Loranthus europaeus,* family: *Loranthaceae*). World Health Organization (WHO) estimates that more than 80% of world population relies on traditional medicines and the market is rapidly growing. Lots of medicinal plants like *Loranthus europaeus* are recommended to treat pain (Nasrin *et al.*, 2015). The herb is rich of flavonoid, alkaloid, terpenoid, and polysaccharide, and shows expressed gen toxicity, cytotoxic and antioxidant properties. Flavonoid is known for its ability to impede prostaglandin synthesis involved in the final phase of acute inflammation and pain perception. It can also increase the number of endogen serotonin or interact with 5-HT2A and 5-HT3 receptors involved in central analgesic activity mechanism. Terpenoid is also reported to show a significant analgesic activity whereas alkaloid can also prevent inflammation by blocking the metabolic path of arakidonat acid. *Loranthus europaeus* contains flavonoid, alkaloid, terpenoid, polysaccharide, and other phytochemicals that cause analgesic activities. All parts of herbs can be used as medicine. However, in all of our experiments, extract of the leaves gave better effect than the stems which might be due to its higher phytochemical content (Nasrin *et al.*, 2015).

CONCLUSION

This research figured out that Dayak people in North Kalimantan still used traditional medicine to cure pain and other illnesses. Traditional healers collected herbs in the forest and processed them into medicine and gave it to their patients. The most frequently used herb by Dayak tribe in North Kalimantan was from *Asteraceae* family while the most frequently used part was leaf. Furthermore, types of pain experienced by Dayak tribe in North Kalimantan were bone pain, stomachache, toothache, headache,

backache, and menstrual pain. Effectiveness and safety of herbs reported to be potentially analgesic shall be evaluated within phytochemical and pharmacological studies to determine the dosage, minimum restriction concentration, bioactive compound, and toxicity.

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REFERENCES

Aleebrahim-Dehkordy, E., Mohammad, R.T., Nasri, H., Azar, B., Parto, N., Sara, B., 2017. Review of Possible Mechanisms of Analgesic Effect of Herbs and Herbal Active Ingredient. *J Young Pharm*, 9(3), pp.303-06.

Archana, Jatav, S., Paul, R., Tiwari, A., 2011. Indian Medicinal Plants: A rich source of natural immune-modulator. *Int J Pharmacol 7(2)*, pp.198-205.

Gustina, A.Y., 2017. *Analisis Kandungan Flavonoid pada Berbagai Usia Panen Gandarusa (Justicia gandarussa Burm.F.) Secara Spektrofometri*. Thesis. Yogyakarta: Universitas Sanata Dharma Program Study Pendidikan Biologi Universitas Sanata Dharma.

Hariyadi, B., 2011. Medicinal Plants and Traditional Medicine of Serampas – Jambi. *Biospecies*, 4(2), pp.29-34.

Indrawan, M., 2007. *Biologi Konsenvasi*. Jakarta: Yayasan Obor.

Jaijesh, P. *et al*. 2009. Anti-Arthritic potential of the plant Justicia gendarussa Burm F, *Clinics*, 64(4), pp. 357–360.

Kumar Bisht V, Purohit, Vineet, 2014. Medicinal and Aromatic Plants Diversity of Asteraceae in Uttarakhand. *Nature and Science*, pp.121-28.

Nasrin, F., Hakim, L., Pinon, R.M., Chakraborty, M., 2015. Analgesic Study of Methanolic Extracts of *Loranthus Europaeus* Leaf and Stem. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(07):11–9.

Makalalag, I., Wirnangsi, D.U., Baderan, D.W., 2014. Inventarisasi Jenis Tumbuhan Obat Tradisional di Kecamatan Pinolosian Kabupaten Bolaang Mongondow Selatan. *Thesis*. Gorontalo: Universitas Negeri Gorontalo Fakultas Matematika dan Ipa.

Ningsih, I.Y., Purwanti, D.I., Wongso, S., Prajogo, B.E.W., Indrayanto, G., 2015. Metabolite profiling of Justicia gendarussa Burm. F. leaves using UPLC-UHR-QTOF-MS. *Sci Pharm*, 83(3):489–500.

Pang, Y., Zhang, Y., Huang, L., Xu, L., Wang, K., Wang, D. *et al.*, 2017 Effects and mechanisms of total flavonoids from Blumea balsamifera (L.) DC. on skin wound in rats. *Int J Mol Sci*, 18(12):1–12.

Rahardjo, S.S., 2016. Review Tanaman Sembung [Blumea balsamifera (L.)], *Proceeding of Mulawarman Pharmaceuticals Conferences*, 3(April), pp. 18–28.

Raja Nasution, B., Aththorick, T.A., Rahayu, S., 2018. Medicinal plants used in the treatment of diabetes in karo ethnic, north sumatra, indonesia. *IOP Conf Ser Earth Environ Sci*, 130(1).

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., Kohli, K., 2009. Mechanism of action of flavonoids as anti-inflammatory agents: A review. *Inflamm Allergy - Drug Targets*, 8(3):229–35.

Reid, M.C., Eccleston, C., Pillemer, K., 2015. Management of chronic pain in older adults. *BMJ*, 350:1–10

Sivasankari, B., Anandharaj, M., Gunasekaran, P., 2014. An ethnobotanical study of indigenous knowledge on medicinal plants used by the village peoples of Thoppampatti, Dindigul district, Tamilnadu, *India. J Ethnopharmacol*, 153(2):408–23.

Sreekeesoon, D.P., Mahomoodally, M.F., 2014. Ethnopharmacological analysis

of medicinal plants and animals used in the treatment and management of pain in Mauritius. *J Ethnopharmacol*, 157:181–200.

Tag, H., Kalita, P., Dwivedi, P., Das, A.K., Namsa, N.D., 2012. Herbal medicines used in the treatment of diabetes mellitus in Arunachal Himalaya, northeast, *India*. *J Ethnopharmacol*, 141(3):786–95.

Takoy, D.M., Linda, R., Lovadi, I., 2013. Tumbuhan Berkhasiat Obat Suku Dayak Seberuang di Kawasan Hutan Desa Ensabang Kecamatan Sepauk Kabupaten Sintang. *J Protobiont*, 2(3);122–8.

Traore, M.S., Baldé, M.A., Diallo, M.S.T., Baldé, E.S., Diané, S., Camara, A. *et al*., 2013. Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. *J Ethnopharmacol*, 150(3):1145–53.

Wegiera, M., Smolarz, H.D., Jedruch, M., Korczak, M., Koproń, K., 2012. Cytotoxic effect of some medicinal plants from Asteraceae family on J-45.01 leukemic cell line - Pilot study. *Acta Pol Pharm - Drug Res*, 69(2):2.

Yitno, A., 1991. *Gambaran Kehidupan Masyarakat Kalimantan*, Universitas Gadjah Mada, Yogyakarta.

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COMBINATION UV-VIS SPECTROSCOPY AND PARTIAL LEAST SQUARE FOR DETECTING ADULTERATION PARACETAMOL AND PIROXICAM IN TRADITIONAL MEDICINES

Pretty Falena Atmanda Kambira, Dion Notario*), Untung Gunawan, Sherina Dhamayanti, Raymunanda W.K. Ningrum, Sondang G.O. Ambarita, Grafty Polin

Department of Pharmacy, Faculty of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jalan Pluit Raya no 2, Jakarta Utara, 14440, Indonesia

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ABSTRACT

An analytical method based on combination UV-Vis spectroscopy and chemometric was developed for detecting commonly listed adulterants such as paracetamol and piroxicam simultaneously in traditional medicines. No complex sample preparation and separation are required except grinding, dissolving, and filtering. The spectral interferences were resolved by multivariate techniques. Wavelengths selection and number of components optimization were performed by a combination of Genetic Algorithm and Partial Least Square (GA-PLS) followed by backward elimination through Jack-Knife Partial Least Square Regression (JK-PLSR). The capability PLSR model for quantitative analysis was assessed from the coefficient of determination (R^2) and root mean square of error prediction/cross-validation (RMSEP/RMSECV) dan predicted residual sum of square (PRESS). Classification performance of PLS Discriminant Analysis (PLS-DA) was evaluated from the area under the receiver operating characteristic curve (AUROCC). For ensuring the sensitivity of the method, the detection limits from the two pseudo-univariate lines were estimated. The R^2, RMSEP, RMSECV, AUROCC, and detection limit obtained from the selected models of paracetamol and piroxicam were >0.999, <0.25 mg/L, <0.15 mg/L, 100%, and <0.4 mg/L respectively. Therefore, the proposed method is suitable for the rapid screening of adulterated herbal medicine.

Keywords: adulteration; paracetamol; partial least square; piroxicam; UV-vis spectroscopy.

INTRODUCTION

Traditional herbal medicines have been used by the Indonesian population for a long time on any occasions whether to boost immune systems or improve health or to treat diseases. The abundant source of Indonesian medicinal plants makes traditional herbal medicine still commercially available and consumed today. However, to enhance its efficacy and boost sales, chemical adulterants are often added by some manufacturers to their herbal products. Indonesian National Agency of Drug and Food Control (NADFC) found an illegal traditional medicine production facility in Tangerang, West Java, in June 2019. The manufacturer added paracetamol as a chemical adulterant in their products (BPOM, 2019). Paracetamol and piroxicam are listed as common chemical adulterants in traditional herbs for treating pain, gout, and rheumatic (Ching *et al*., 2018).

Consumption of adulterated herb leads to therapeutic error and may cause the patient to worsen dependent on what kinds of adulterant(s). Paracetamol may lead to hepatotoxicity caused by its reactive metabolite N-acetyl-para-benzo-quinone imine. Piroxicam, a non-steroid anti-inflammatory drug (NSAID), induces peptic ulcer and gastrointestinal hemorrhages (Drini, 2017; Tittarelli *et al*., 2017). Some adverse events associated with NSAIDs were reported in

*Corresponding author: Dion Notario
Email: *dion.notario@atmajaya.ac.id*

patients who took adulterated herbal medicines, including Cushing Syndrome (Xu *et al*., 2018).

Most of the chemical adulterants are mixed homogenously in traditional herb preparation, so it is difficult to distinguish an adulterated powdered herb sample without modern analytical techniques. Some analytical methods for detecting paracetamol and piroxicam in traditional herb preparation were developed, such as thin-layer chromatography (Mustarichie *et al*., 2017) and liquid chromatography coupled with tandem mass spectrometry (Ching *et al*., 2018; Kim *et al*., 2014). However, the previous methods were time-consuming due to complicated sample preparation steps, not eco-friendly because they used toxic organic solvent, and not practical for initial screening since they required complex instrumentations. For this reason, a new simple analytical method is necessary to provide rapid detection of these adulterants.

Ultraviolet-visible (UV-Vis) spectroscopy provides a rapid and sensitive detection based on light-absorbing properties of chemical adulterants in the UV-Vis region. Lack of specificity due to spectral interferences can be resolved by multivariate techniques (Contreras *et al*., 2010). The strength of combination spectroscopy and chemometric techniques are no separation processes required, more rapid processing provided, and non-toxic solvent used so that moving forward to the green chemistry. However, the appropriate wavelength selections are essential to generate an accurate model.

The visual observations by comparing multispectral data are practical and commonly used to perform variables selection (Rahayu *et al*., 2018; Rohman *et al*., 2017), but the holistic changes in spectra cannot be elucidated clearly by visual observation only (Wang *et al*., 2017). The stepwise variable elimination/addition according to Jack-Knife Partial Least Square Regression (JK-PLSR) was recommended as the best variable selection technique based on the significance test (Westad and Martens, 2000). Nevertheless, the selection of optimal variables with stepwise elimination/addition is time-consuming due to the variables elimination/addition that must be done each in order. The limitation of stepwise variable elimination/addition can be overcome by performing Genetic-Algorithm-PLS (GA-PLS). Based on natural selection, the variables with a higher likelihood to "persist" (fit to the data) would be selected (Mehmood *et al*., 2012). GA-PLS would be able to screen a large number of variables, then stepwise variable elimination/addition by JK-PLSR would be performed more efficiently. In this study, we apply GA-PLS and JK-PLSR to the UV-Vis spectroscopy data for screening and eliminating non-significant variables at once to build the optimum quantitative models. The absorbances of the selected wavelengths were re-processed with PLS Discriminant Analysis (PLS-DA) for identifying adulterated and non-adulterated samples (qualitative analysis). As far as we know, the wavelength selection strategy through combination GA-PLS and JK-PLSR has not yet been implemented in UV-Vis spectroscopy to analyze a mixture of paracetamol and piroxicam in herbal medicine. Therefore, we applied these chemometric techniques in combination with UV-Vis spectroscopy to develop a fast method for the detection of paracetamol and piroxicam in traditional medicine based on the pattern recognition of the UV-Vis multispectra data.

METHODS

Chemicals NaOH (Merck, Germany), piroxicam (98.5%, Putra Bakti Niaga, Indonesia), paracetamol (99.9%, Putra Bakti Niaga, Indonesia), double distilled water, drug-free herbs, and commercial samples obtained from local markets in Jakarta and Tangerang in August-September 2019.

Instrumentation

The absorbances of calibration and sample solutions were measured by Shimadzu UV-1800 UV/VIS spectrophotometer with 1 cm path length quartz cell under scan-mode from 200 – 500 nm with an interval of 1 nm. Data acquisition was conducted by UVProbe

2.52 (Shimadzu) software and saved in .spc file format until the next analysis step.

Calibration solutions

Five hundred mg piroxicam and paracetamol reference standards were weighed at the operating range of an analytical balance, dissolved in 0.1 N NaOH, and diluted with double distilled water in an appropriate volumetric flask to obtain concentration about 0.25 mg/mL accurately. Meanwhile, a blank sample solution, extracted from drug-free herbs powder, was prepared in the same way as the standard solutions. Standard solutions and blank solutions were mixed in certain proportions and diluted to the desired concentration previously designed by central composite design (CCD) with additional sampling points (Table 1).

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Table 1. Calibration curve design by central composite design and additional sampling points

run.order	x1.coded	x2.coded	x3.coded	x1	x2	x3
1	0.000	0.000	1.414	15	15	22
2	-1.000	-1.000	-1.000	10	10	10
3	0.000	0.000	-1.414	15	15	8
4	1.000	1.000	-1.000	20	20	10
5	-1.414	0.000	0.000	8	15	15
6	1.000	-1.000	-1.000	20	10	10
7	-1.000	1.000	1.000	10	20	20
8	-1.000	1.000	-1.000	10	20	10
9	0.000	1.414	0.000	15	22	15
10	1.000	-1.000	1.000	20	10	20
11	1.000	1.000	1.000	20	20	20
12	0.000	-1.414	0.000	15	8	15
13	0.000	0.000	0.000	15	15	15
14	-1.000	-1.000	1.000	10	10	20
15	1.414	0.000	0.000	22	15	15
Additional sampling points						
16	NA	NA	NA	20	0	22
17	NA	NA	NA	20	0	22
18	NA	NA	NA	20	0	22
19	NA	NA	NA	20	23	0
20	NA	NA	NA	20	23	0
21	NA	NA	NA	20	23	0
22	NA	NA	NA	20	0	0

x1 = approximate concentration of drug-free herbs in working solution (mg/L); x2 = concentration of piroxicam in working solution (mg/L); x3 = concentration of paracetamol in working solution (mg/L)

Sample preparation

Samples were homogenized and approximately 500 mg homogenates were weighed in the operating range of an analytical balance, dissolved in 0.1 N NaOH, and diluted with double distilled water to obtain concentration about 10-20 mg/L. Each sample was analyzed in triplicates.

Data preparation and statistical analysis

Multispectral data in .spc format were converted to .csv and exported to R software for further analysis. Calibration data were divided into two groups labeled as data train and data test. Data train (n=17) were used for generating a regression model by PLSR technique and data test (n=5) for performing external validation. Wavelength selection was conducted by GA-PLS and optimized with backward elimination after performed a t-test for each wavelength by JK-PLSR. Root mean square error of cross-validation (RMSECV) and prediction (RMSEP) was calculated by applying eq. 1 to leave-one-out crossvalidation (LOO-CV) of data train and back-calculation from data test respectively. The lowest number of the components and wavelength resulting coefficient of determination (R^2) above 0.999, smaller RMSECV/RMSEP, and lowest predicted residual sum of square (PRESS) was selected (Rohman *et al*., 2016). For discriminating between adulterated and non-adulterated samples, qualitative analysis based on PLS-DA was performed and visualized in biplot of two first principal components. The sensitivity of the proposed method was assessed by calculating the limit of detection from pseudo-univariate linear regression (LOD_{pu}) according to eq. 2 (Allegrini and Olivieri, 2014; Oleneva *et al*., 2019). All of the statistical operations including variable selection, PLSR, and PLS-DA calculation performed by plsVarSel, pls and mixOmics package installed in R 3.6.0 (Mehmood *et al*., 2012; Mevik and Wehrens, 2007; R Core Team, 2019; Rohart *et al*., 2017).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(y_{predicted} - y_{actual}\right)^2}{N}} \qquad \text{.....1}$$

RMSE is the root mean square error (mg/L); $y_{predicted}$ and y_{actual} are predicted and calculated drug concentration in spiked standard solution (mg/L); *N* is the number of calibration samples.

$$LOD_{pu} = 3.3 s_{pu}^{-1}\left[(1 + h_{0min} + 1/I) var_{pu}\right]^{1/2} \qquad \text{.....2}$$

The terms LOD_{pu}, s_{pu}, and var_{pu} are limit of detection, slope, and residual variance calculated from the pseudo-univariate line between $y_{predicted}$ and y_{actual} respectively; h_{0min} is the lowest projected leverage; *I* is the number of the calibration points.

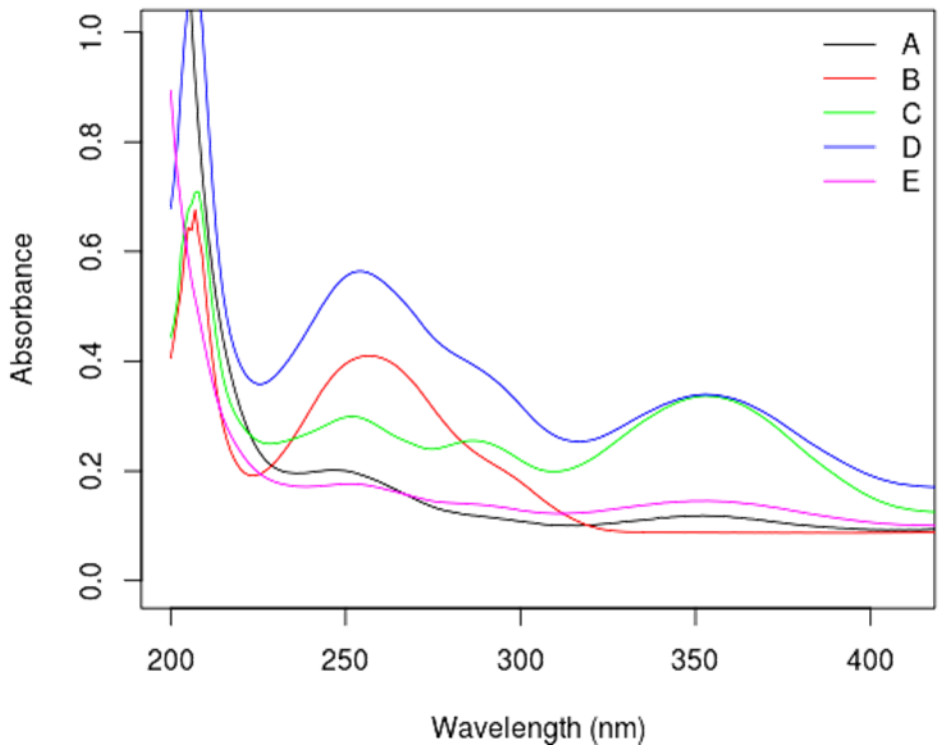

Figure 1. UV-Vis spectrum of blank (A), paracetamol (B), piroxicam (C), a mixture of paracetamol and piroxicam (D), and representative commercially available sample (E)

RESULTS AND DISCUSSION

A traditional medicine preparation adulterated with pharmaceutical substances such as paracetamol and piroxicam is challenging to detect just by relying on visual observation. Nevertheless, the light-absorbing properties of paracetamol and piroxicam in aqueous solution can be used to identify them in herbal medicine. The molecular structure of paracetamol and piroxicam contain conjugated double bonds that π electrons are in. Conjugated double bonds are essential for absorbing light in the UV-Vis region (chromophore) due to π→π* electronic transition (Rafi *et al*., 2018). Besides, the presence of the auxochrome group (-OH) in the chromophore system may strengthen the light-absorbing properties of paracetamol and piroxicam. Differences in a chromophore-auxochrome system of paracetamol, piroxicam, and endogenous compound in herbs cause variation of spectral patterns (Figure 1), from which adulterated and non-adulterated herbal medicine are discriminated.

In the case of adulteration of traditional edicines with chemicals, the composition of natural ingredients with counterfeiting materials correlates with each other. When chemicals are added in a higher proportion, then the ratio of natural component is lower and *vice versa*. This correlation can lead to prediction errors due to two dependent variables correlated with each other. The use of an appropriate experimental design aims to prevent the effects of undesirable correlations within dependent variables due to the components are arranged in a structured and statistically unrelated way (Tomuta *et al*., 2017). At present, some types of experimental designs that were used initially to optimize responses such as CCD are widely utilized to design calibration curves in multivariate analysis (Hassaninejad-Darzi *et al*., 2016; Pinto *et al*., 2019).

The relationship between the concentration of paracetamol and piroxicam with absorbance in multiple wavelengths were modeled by PLSR. The first model of paracetamol and piroxicam which all wavelengths included showed a good fit to data; however, the accuracy of prediction could be improved by optimizing wavelength selection (Table 2). Wavelength selection through GA-

PLS was able to extract more than one hundred variables to be less than 20 variables (Mehmood *et al*., 2012) so that it was more efficient to re-optimize variables selection through backward elimination after jack-knifing (Mevik and Wehrens, 2007). The number of components also optimized by assessing the plot between RMSECV/RMSEP and PRESS against the number of components. The number of components resulting in minimum RMSECV/RMSEP and PRESS was selected (Table 2).

Table 2. Validation parameters of PLSR

Model	Compound	Wavelengths (nm)	Ncomp	RMSECV	RMSEP	R^2 (CV)	R^2 (EV)	PRESS
1	Piroxicam	all wavelengths	4	0.541	0.326	0.9952	0.9984	4.98
2	Piroxicam	213*, 298*, 351, 353*, 365*, 369*, 393*, 398*, 404*, 414*, 417*, 476	4	0.141	0.208	0.9997	0.9993	0.34
3	Piroxicam	213*, 298*, 351, 353*, 365*, 369*, 393*, 398*, 404*, 414*, 417*	4	0.124	0.217	0.9997	0.9993	0.26
4	**Piroxicam**	**213*, 298*, 353*, 365*, 369*, 393*, 398*, 404*, 414*, 417***	**4**	**0.125**	**0.212**	**0.9997**	**0.9993**	**0.26**
5	Paracetamol	all wavelengths	3	0.225	0.362	0.9991	0.9979	0.86
6	Paracetamol	239*, 253*, 263*, 335, 351*, 378, 381, 455, 500	4	0.090	0.128	0.9999	0.9997	0.14
7	Paracetamol	239*, 253*, 263*, 335*, 351*, 381, 455, 500	4	0.090	0.128	0.9999	0.9997	0.14
8	Paracetamol	239*, 253*, 263*, 335*, 351*, 381, 455	4	0.096	0.128	0.9998	0.9997	0.16
9	**Paracetamol**	**239*, 253*, 263*, 335*, 351*, 381***	**3**	**0.087**	**0.157**	**0.9999**	**0.9996**	**0.13**

*Variable is significant at 95% confidence interval ($p<0.05$); CV = cross validation; EV = external validation; Ncomp = number of components; RMSECV = root mean square error of cross validation; RMSEP = root mean square error of prediction; PRESS = predictive residual sum of square; Selected models were typed in bold letter.

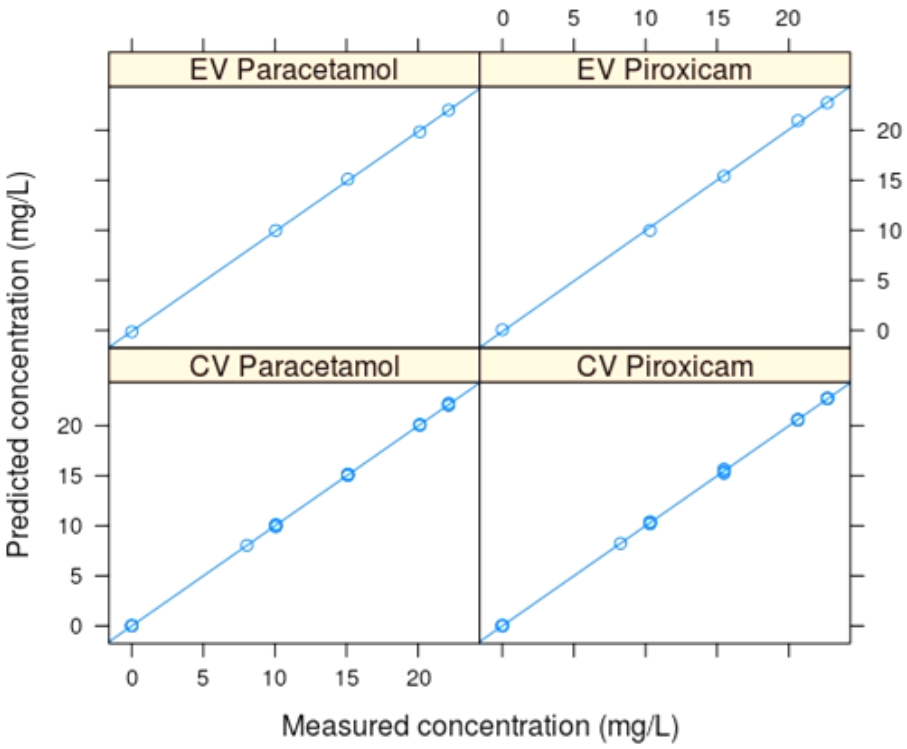

Figure 2. Validation plot of two selected regression models. CV = internal validation by leave-one-out cross-validation; EV = external validation by using back-calculation obtained from data test.

The goodness of fit of the multivariate regression is assessed by considering R^2, RMSEP, RMSECV, and PRESS values. Generally, the acceptance criteria of R^2 and RMSEP/RMSECV are above 0.999 and less than 0.4, respectively (Rohman and Man, 2010). Internal and external validation parameters showed a high linear correlation from the selected models, and no overfitting was found due to the significance of all variables in the selected model (Table 2 & Figure 2). Variable wavelength selection through GA-PLS followed by jack-knifing and backward elimination proved capable of generating a more accurate calibration model than using all of the wavelengths without selection and overcoming visual wavelength selection limitation, which very subjective wavelength selection is performed. The optimum wavelength selection resulted in a calibration model with high accuracy so that no data pre-processing such derivative curve, smoothing, or mean centering required.

The sensitivity of the models was assessed by calculating the detection limit from the pseudo-univariate line. The detection limit values of paracetamol and piroxicam were found in a low concentration of 0.23 and 0.36 mg/L, respectively. A low detection limit showed that the proposed analytical method is considered sensitive for detecting paracetamol and piroxicam commonly added to herbal preparation in medium to high proportion.

Classification of multispectral data conducted by PLS-DA at the selected wavelengths showed an excellent performance. The high discriminant capacity of the PLS-DA model was confirmed by the receiver operating characteristic (ROC) curve located closer to the top left corner and quantitatively represented by the area under the ROC curve (AUROCC) (Hajian-Tilaki, 2013). Based on AUROCC values (Figure 3), the chances of the PLS-DA model to discriminate spectral patterns between one counterfeit to the others were about 77 – 83%, while non-adulterated with adulterated samples was distinguished correctly (AUROCC = 1). Therefore, this method can be considered selective for determining adulterated and non-adulterated samples. The two first principal components carried out about 98% variances, which means almost all variance of the data can be explained by PLS-DA. There was shown that adulterated and non-

adulterated herbs could be distinguished with good separation on the biplot of two first principal components. In addition, based on our findings, there was no adulteration found in the commercial samples (Figure 4).

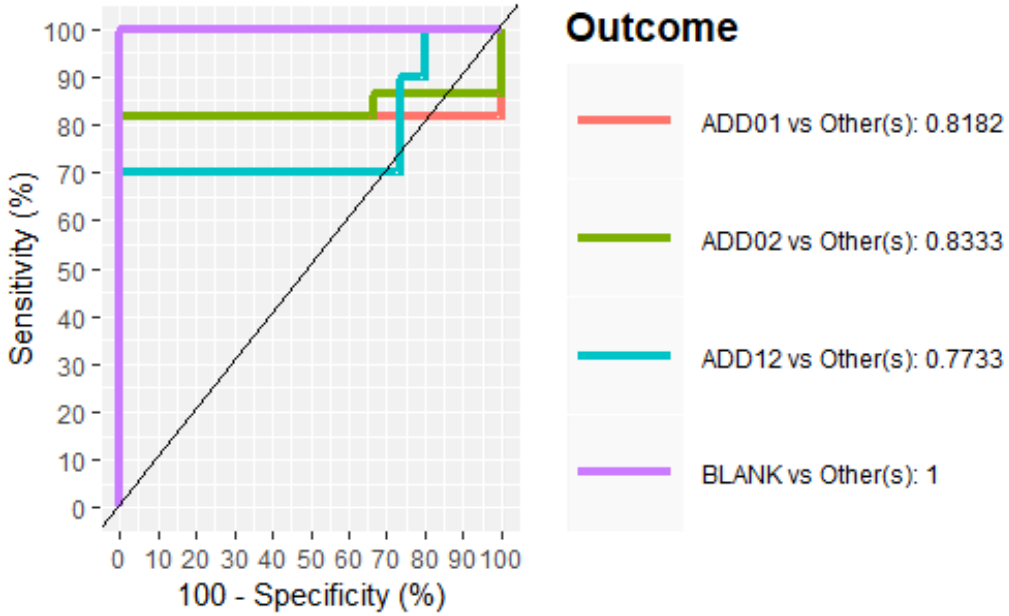

Figure 3. Receiver operating characteristic (ROC) curve of the PLS-DA model. Blank samples (BLANK), spiked samples with paracetamol (ADD01), piroxicam (ADD02) and both paracetamol and piroxicam (ADD12)

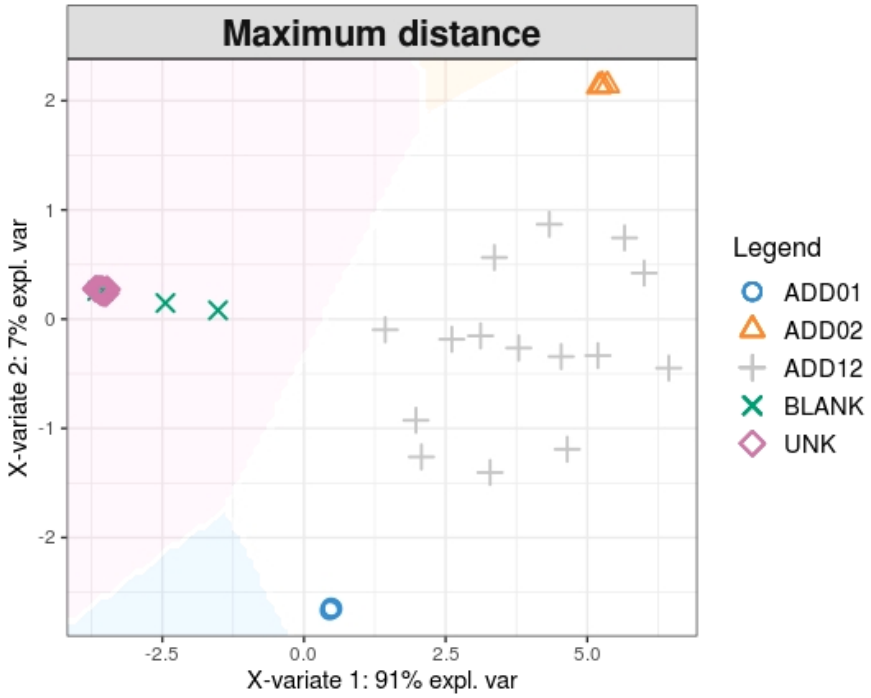

Figure 4. Classification of multispectral data obtained from blank samples (BLANK); unknown commercial samples (UNK); spiked sample with paracetamol (ADD01), piroxicam (ADD02) and both of paracetamol and piroxicam (ADD12)

CONCLUSION

A selective, sensitive, rapid, and accurate analytical method based on UV-Vis spectroscopy and chemometric for detecting paracetamol and piroxicam in herbal medicine is successfully developed. Variable selection enhances the performance of the calibration model so that data pre-processing was not necessary. The limitation of this method is restricted only for paracetamol and piroxicam quantities. Therefore, enclosing other commonly chemical adulterants would be better for creating a vast database for future research.

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REFERENCES

Allegrini, F., Olivieri, A.C., 2014. IUPAC-Consistent Approach to the Limit of Detection in Partial Least-Squares Calibration. *Anal. Chem.*, 86, 7858–7866.

BPOM, 2019. Balai POM di Serang Temukan Sarana Produksi Obat Tradisional Ilegal di Wilayah Tangerang [WWW Document]. URL https://www.pom.go.id/new/view/more/pers/315/-NADFC-PROVINCIAL-OFFICE-IN-SERANG-FOUND-ILLEGAL-TRADITIONAL-MEDICINE-PRODUCTION-FACILITY-IN-TANGERANG.html (accessed 10.31.19).

Ching, C.K., Chen, S.P.L., Lee, H.H.C., Lam, Y.H., Ng, S.W., Chen, M.L., Tang, M.H.Y., Chan, S.S.S., Ng, C.W.Y., Cheung, J.W.L., Chan, T.Y.C., Lau, N.K.C., Chong, Y.K., Mak, T.W.L., 2018. Adulteration of Proprietary Chinese Medicines and Health Products with Undeclared Drugs: Experience of a Tertiary Toxicology Laboratory in Hong Kong. *Br J Clin Pharmacol*, 84, 172–178.

Contreras, U., Barbosa-García, O., Pichardo-Molina, J.L., Ramos-Ortíz, G., Maldonado, J.L., Meneses-Nava, M.A., Ornelas-Soto, N.E., López-de-Alba, P.L., 2010. Screening Method for Identification of Adulterate and Fake Tequilas by Using UV–Vis Spectroscopy and Chemometrics. *Food Research International*, 43, 2356–2362.

Drini, M., 2017. Peptic Ulcer Disease and Non-Steroidal Anti-Inflammatory Drugs. *Aust Prescr*, 40, 91–93.

Hajian-Tilaki, K., 2013. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. *Caspian J Intern Med*, 4, 627–635.

Hassaninejad-Darzi, S.K., Samadi-Maybodi, A., Nikou, S.M., 2016. UV-Vis Spectrophotometry and Multivariate Calibration Method for Simultaneous Determination of Theophylline, Montelukast and Loratadine in Tablet Preparations and Spiked Human Plasma. *Iran J Pharm Res*, 15, 379–391.

Kim, H.J., Lee, J.H., Park, H.J., Kim, J.-Y., Cho, S., Kim, W.S., 2014. Determination of Non-Opioid Analgesics in Adulterated Food and Dietary Supplements by LC-MS/MS. *Food Additives & Contaminants: Part A*, 31, 973–978.

Mehmood, T., Liland, K.H., Snipen, L., Sæbø, S., 2012. A Review of Variable Selection Methods in Partial Least Squares Regression. *Chemometrics and Intelligent Laboratory Systems*, 118, 62–69.

Mevik, B.-H., Wehrens, R., 2007. The pls Package: Principal Component and Partial Least Squares Regression in R. *Journal of Statistical Software*, 18, 1–23.

Mustarichie, R., Ramdhani, D., Indriyati, W., 2017. Analysis of Forbiden Pharmaceutical Compounds in Antirheumatic Jamu. *AJPCR*, 98–101.

Oleneva, E., Khaydukova, M., Ashina, J., Yaroshenko, I., Jahatspanian, I., Legin, A., Kirsanov, D., 2019. A Simple Procedure to Assess Limit of Detection

for Multisensor Systems. *Sensors*, 19, 1359.

Pinto, L., Stechi, F., Breitkreitz, M.C., 2019. A simplified and versatile multivariate calibration procedure for multiproduct quantification of pharmaceutical drugs in the presence of interferences using first order data and chemometrics. *Microchemical Journal*, 146, 202–209.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Viena, Austria.

Rafi, M., Jannah, R., Heryanyo, R., Kausta, A., Septaningsih, D., 2018. UV-Vis Spectroscopy and Chemometrics as a Tool for Identification and Discrimination of Four Curcuma Species. *IFRJ*, 25, 643–648.

Rahayu, W.S., Rohman, A., Martono, S., Sudjadi, S., 2018. Application of FTIR Spectroscopy and Chemometrics for Halal Authentication of Beef Meatball Adulterated with Dog Meat. *Indonesian Journal of Chemistry*, 18, 376–381.

Rohart, F., Gautier, B., Singh, A., Cao, K.-A.L., 2017. mixOmics: An R Package for 'Omics Feature Selection and Multiple Data Integration. *PLOS Computational Biology*, 13, e1005752.

Rohman, A., Man, Y. bin C., Ismail, A., Hashim, P., 2017. FTIR Spectroscopy Coupled with Chemometrics of Multivariate Calibration and Discriminant Analysis for Authentication of Extra Virgin Olive Oil. *International Journal of Food Properties*, 20, S1173–S1181.

Rohman, A., Man, Y.B.C., 2010. Fourier Transform Infrared (FTIR) Spectroscopy for Analysis of Extra Virgin Olive Oil Adulterated with Palm Oil. *Food Research International*, 43, 886–892.

Rohman, A., Windarsih, A., Riyanto, S., Sudjadi, Shuhel Ahmad, S.A., Rosman, A.S., Yusoff, F.Mohd., 2016. Fourier Transform Infrared Spectroscopy Combined with Multivariate Calibrations for the Authentication of Avocado Oil. *International Journal of Food Properties*, 19, 680–687.

Tittarelli, R., Pellegrini, M., Scarpellini, M.G., Marinelli, E., Bruti, V., di Luca, N.M., Busardò, F.P., Zaami, S., 2017. Hepatotoxicity of Paracetamol and Related Fatalities. *Eur Rev Med Pharmacol Sci*, 21, 95–101.

Tomuta, I., Porfire, A., Casian, T., Gavan, A., 2017. Multivariate Calibration for the Development of Vibrational Spectroscopic Methods. Calibration and Validation of Analytical Methods - A Sampling of Current Approaches.

Wang, Q., He, H., Li, B., Lin, H., Zhang, Y., Zhang, J., Wang, Z., 2017. UV–Vis and ATR–FTIR Spectroscopic Investigations of Postmortem Interval Based on the Changes in Rabbit Plasma. *PLoS One*, 12(7): e0182161.

Westad, F., Martens, H., 2000. Variable Selection in Near Infrared Spectroscopy Based on Significance Testing in Partial Least Squares Regression. *Journal of Near Infrared Spectroscopy*, 8, 117–124.

Xu, Y., Patel, D.N., Ng, S.-L.P., Tan, S.-H., Toh, D., Poh, J., Lim, A.T., Chan, C.-L., Low, M.-Y., Koh, H.-L., 2018. Retrospective Study of Reported Adverse Events Due to Complementary Health Products in Singapore from 2010 to 2016. *Front Med*, 5:167.

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THE EFFECT OF TEMPEH EXTRACT GEL ON WOUND HEALING IN DIABETES RAT: OVERVIEW OF TISSUE COLLAGEN, WOUND CLOSURE, EPITHELIALIZATION AND CAPILLARIZATION

Ignatius Adi Kurniawan, Rini Dwiastuti, Sri Hartati Yuliani*)

Magister Program of Pharmacy, Universitas Sanata Dharma, Kampus III Paingan Maguwoharjo Depok Sleman Yogyakarta, 55282, Indonesia

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ABSTRACT

The development of wound healing preparations in diabetes conditions needs to be done because the wound healing process in the respective conditions requires longer time. The aim of this study is to measure the effect of *tempeh* extract gel containing genistein on wound healing in diabetes rats by observing the response parameters of collagen expression, wound closure speed, epithelialization, and capillarization of wound tissue. The results of testing wound closure using a base gel treatment, *tempeh* extract gel 2.5%, 5%, 7.5% and without treatment indicated that the treated groups of *tempeh* extract gel 5% and 7.5% have significant effect on diabetes wound healing in the tested animals. This indication is strengthened by the results of the non-treatment groups statistical test with the 5% and 7.5% *tempeh* extract gel treatment which showed significant difference in influencing the wound closure response, epithelialization and collagen formation in tissue. The results of the statistical in group without treatment with the base treatment group without extract gel and *tempeh* extract gel 2.5% showed no significant difference in influencing the wound closure response, epithelialization and collagen formation. Statistical test results on capillarization responses indicated that the results were not significantly different from all treatment groups.

Keywords: diabetes; gel; *tempeh* extract; wound closure; wound healing.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder caused by a decrease in the amount of insulin production from pancreatic cells. It is a chronic metabolic disorder in which the pancreas does not produce enough insulin or the body cannot use the available insulin effectively. This condition could increase glucose concentration in the blood (hyperglycemia). Indonesia ranks number 7 for the number of people with DM in the world. The cases of diabetes ulcer were found in 15% of people with DM in the world (Utami and Karim, 2014). DM conditions can cause disruption on wound healing process. Longer wound healing time is influenced by the increase of matrix metalloproteinase (MMP) and fibroblast activities in forming collagen (Sharp and Jane, 2011).

Tempeh is food made of soy that is very easy to get in Indonesia. The process of boiling and processing soybeans into *tempeh* can increase the level of isoflavones from soybeans (Utari, 2010). Genistein is one of the isoflavone compounds contained in soybeans that can be extracted and isolated (Agrawal *et al*., 2015). Genistein is known to have an influence on wound healing through various mechanisms. Administration of gel containing genistein can accelerate the wound healing process in experimental animals (Emmerson *et al*., 2010). The treatment of genistein is known to influence the wound healing process in test animals with DM conditions (Tie *et al*., 2013).

*Corresponding author: Sri Hartati Yuliani
Email: *srihartatiyuliani@usd.ac.id*

Genistein contained in *tempeh* extract needs to be developed into a topical dosage form. So, it is necessary to conduct a test to measure *tempeh* extract effect in topical preparations on wound healing process in experimental animals with DM. The effect of administration is seen from the amount of collagen formed in wound healing.

The development of wound healing preparations in diabetes conditions needs to be done because wound healing process in diabetes conditions often encounters obstacles. It causes healing process to take a longer period. These obstacles include an increasing potential for infection due to decreasing neutrophil migration and prolonged inflammation. In this study, the observation of wound healing activities in diabetic rats was done by giving *tempeh* extract gel. This study aims to look at the effect of *tempeh* extract gel preparations to wound healing in diabetic rats with qualitative and quantitative observation parameters. The measured parameters to be observed were the collagen expression response, wound closure, epithelialization, and capillarization of wound tissue in the presence of *tempeh* extract gel containing genistein. The preparations used in this study were hydrogels, a form of preparation that can be used for wound care. This preparation has several benefits in closing wounds, maintaining moisture, and delivering medicinal ingredients (Gupta *et al*., 2011).

METHODS

Materials and Instrument

The materials included enistein-standardized *tempeh* extract with levels of 429,055 µg/ mL, ethanol, water, buffer citrate, streptozotocin (Cayman Chemical_USA), injection ketamine (Generic®), neutral buffer formalin 10%, and xylene. Test animals of male Wistar strain rats aged 2 months with a weight range of 150-180g was obtained from the Center for Food and Nutrition of Gadjah Mada University Yogyakarta, masson thricrome (Bio-Optica_Italia).

Test Animal

The tested animals were male Wistar strain rats placed in individual cages maintained at room conditions of 25 + ± 2 ° C with a relative air humidity of 75%. The weight control of the tested animals was carried out by weighing them from time to time. Tested animals' bodies weighed between 170-200g.

Induction of animal diabetic try

Experimental animals were induced with Streptozotocin (STZ) with a single dose of 45mg/kg. Induction was carried out by injection of i.p STZ solution in citrate buffer (0.1M) pH 4.5. The STZ solution was made an hour before the induction by dissolving STZ in a cold buffer solution (Etuk, 2010).

Measurement of blood glucose levels in experimental animals

Glucose level measurements were performed after three days of induction with STZ. Blood sampling was done 4 days after STZ induction treatment. The rat blood was taken from the retro-orbital plexus, held in a 1.5ml microtube, and let standing for 30 minutes. Blood samples were centrifuged for 15 minutes at 4,000 rpm. The obtained serum was measured on its glucose levels based on the workings of the GOD FS reagent (Diasys®). Blood sugar levels used in this study were >200 mg/dl (Martsiningsih 2016).

Wound making and measurement of test responses

Experimental animals were anesthetized with ketamine injection at a dose of 50 mg/kg by administering intramuscular (i.m) (Kintoko, 2017). After the animal lost consciousness, the rat's back hair was shaved and wounded using a 5 mm diameter punch biopsy (Júnior *et al*., 2011).

Grouping of Tested Animals

Tested animals were grouped into five. Each group made replication of three tested animals. The division of the observation group is as follows: DM1 group: Basis Treatment; DM2 group: Formula 1 (F1) with extract content of 2.5%; DM3 group: Formula 2 (F2) with extract content of 5%; DM4 group: Formula 3 (F3) with extract content of 7.5%; and DM5 group: No Treatment. Administration

of preparations containing genistein gel was done every 12 hour in the morning and evening. The amount applied was as much as ± 25 mg in the wound area.

Observation of collagen expression and epithelialization

Injured skin samples were fixed using 10% of formalin neutral buffer. Then, the sample was put in distilled water for 15 minutes and conditioned dehydrated with the addition of ethanol in stages; 70%, 80%, and 96%. The samples were clarified using xylol and printed on paraffin blocks. The slide cutting was done using a microtome with a thickness of 5-6 µm. Paraffin pieces were stretched in warm water to prevent creases. The preparation was removed using a beaker and dried in 60°C oven. HE staining was carried out on preparations to evaluate closure of dermis tissue and perform the remodeling stage (Febram, 2010). Collagen observations were carried out by Masson Thricrome staining based on the workings of the Masson Thricrome Goldner (Bio-Optica®) kit (Suvik, 2012). Furthermore, observations were made using a microscope with a magnification of 10 x 10 times to see the collagen and epithelialization scores. The result is shown in Table 1.

Observation of wound closure

The observation of wound closure was done by measuring the diameter of the initial wound area and wound area on the day of observation. The diameter was obtained from the average of two wound areas on the right and left-back of the tested animal (Kintoko, 2017). The percentage measurements of wound closure (WC) were calculated using the following formula:

$$WC = \frac{\text{initial wound area} - \text{wound area on the day}}{\text{initial wound area}} \times 100\% \quad \text{.....1}$$

Table 1. Histological assessment

Parameter and description	Score
The degree of collagen formation	
a. Collagen appears very dense/ small visual field (density <75%)	4
b. Collagen appears solid/ small visual field (density> 50% - 75%)	3
c. Collagen appears to be less dense/ small visual field (density> 25% -50%)	2
d. Collagen appears to be very dense/ small visual field (density <25%	1
The degree of epithelialization	
a. High epithelialization/ microscope field of view	4
b. Normal epithelialization/ microscope field of view	3
c. Low epithelialization/ microscope field of view	2
d. There is no epithelialization/ microscope field of view	1
Total new blood vessel formation	
a. More than 4 new blood vessels/ microscope field of view	4
b. 2-4 new blood vessels/ microscope field of view	3
c. 1-2 new blood vessels/ microscope field of view	2
d. There are no new blood vessels/ microscopic field of view	1

Data analysis

The results of the research data were analyzed using the Shapiro-Wilk test to determine the normality of data distribution. If abnormal data distribution was found, a Kruskal-Wallis analysis was used and a significant difference in results was followed by a Post-Hoc Test

RESULTS AND DISCUSSION

Blood glucose levels in experimental animals

Diabetes in rat tested animals is a condition in which the rats have blood glucose levels above 200 mg/dl. In this study measurements of serum blood levels of rats were carried out to measure blood glucose levels on day 0, 3, 7 and 14. The blood sugar level tests on the animals within measurements of 0, 3, 7, 14 were >200 mg/dl. This condition was created to ensure that the tested animals experienced diabetes during wound healing test period.

High glucose level in diabetes has an effect on the wound healing process. High blood glucose causes decreasing neutrophils and macrophages migration leading to increasing potency for infection and prolongation of inflammatory conditions. The expression of TNF-α (Tumor Necrosis Factor-Alpha) will increase during ulcers. The high TNF-α increases apoptosis and decreases fibroblast proliferation (Rosyid, 2016). Decreasing number of fibroblasts causes the synthesis of collagen in the wound area will also decrease. The high expression of MMP (Matrix Metalloproteinase) in a diabetes condition has worsen the wound healing process. MMP has cause some ECM (extracellular matrix) degradation including collagen.

Response toward wound closure, collagen synthesis, epithelialization, and tissue capillarization

The stages of the wound healing process are grouped into four stages namely, hemostasis, inflammation, proliferation, and remodeling (Velnar *et al*., 2009). Hemostasis stage occurs in the initial stage after the injury. Inflammation in the wound area is characterized by the increase of neutrophil and macrophage activity in the wound area. Formation of new blood vessels and fibroblasts occurs at the stage of proliferation. In addition, epithelial tissue begins to form from the edge of the wound. In the remodeling stage, collagen synthesized by fibroblasts will bond together and crowd increasingly. At this stage, the epithelial tissue forms thickens and closes the wound surface area (Baltzis *et al*., 2014).

The speed of wound closure is indicated by the closure of the wound surface caused by the process of epithelial cell formation on the wound surface. From the results of experiments on the gel base group, there are test subjects who did not experience the wound healing process. This condition is shown by the the size of the wound diameter which did not decrease. The highest rate of wound closure was achieved in the 7.5% extract gel group.

From the results of the measurement of wound closure speed, the calculation results are obtained as in Table 2. The results of statistical analysis using the Kruskal-Wallis test ($p < 0.05$) shows a significant difference in the treatment group of 5% extract gel, and 7.5% extract gel against the group without treatment. In 2.5% extraction gel treatment group and base treatment group, there was no significant difference in the group without treatment.

Genistein is the main isoflavone compound contained in *tempeh* extract. The compound belongs to the phytoestrogen group found in soybean seeds. Estrogen affects the speed of wound closure by increasing mitogenesis and migration of keratinocytes. Estrogen interacts with Estrogen Receptor Beta (ERβ) thereby affecting the pattern of differentiation and proliferation activity of keratinocytes (Horng *et al*., 2017). Keratinocytes has migrated from the edge of the wound area to the center. Increased proliferation and migration activity of keratinocytes will accelerate the narrowing of the wound area resulting in wound closure.

Table 2. Result of wound closure, collagen synthesis, epithelialization, and capillarization.

Group	Wound Closure (%) ±SD	Collagen Score ± SD	Epithelialization Score ± SD	Capillarization Score ± SD
DM1	16,23±4,07	1,11±0,33	6,00±2,64	8,33±4,61
DM2	48,99 ± 7,72	1,22±0,44	6,66±0,57	9,66±3,21
DM3	91,89±14,05 *	3,00±0,50*	7,33±2,31*	8,66±3,05
DM4	94,68 ±9,21*	3,67±0,50*	7,33±1,53*	7,66±3,21
DM5	33,82 ±10.30	1,67±1,00	5,00±1,73	5,66±2,51

Information: *significant difference ($p < 0.05$) to the DM5 group.

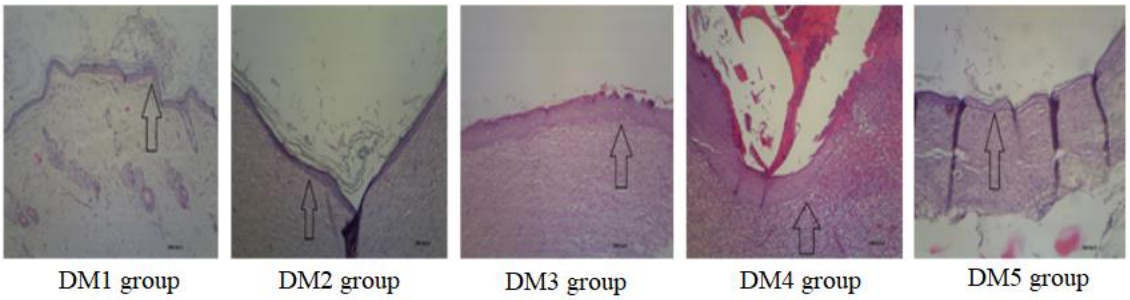

Figure 1. Microscopic images of epithelialization in each group

Histological observation of tissue samples was made to observe the wound healing process from the process of epithelial tissue formation, blood vessel formation, and collagen. This observation is followed by a scoring assessment of the formation of epithelial cells, blood vessels and collagen.

Wound closure can occur when epithelial cells, formed due to keratinocyte differentiation, migrate over the provisional matrix headed to the middle of the wound. Epithelial cells will meet in the middle area of the wound, so that cell migration will stop. Then, the formation of bacialis membrane will start accordingly. The thickness of the epithelial tissue is done by measuring the image using the help of image raster. The appearance of epithelial tissue can be shown by the arrows in Figure 1.

The results of statistical analysis using ($p < 0.05$) showed a significant difference in the degree of epithelialization from the formula 5% extract content and 7.5% extract content of the treatment group. Whereas in the test group with basis application, giving formula 2.5% extract content did not have significant differences in the group without treatment.

Giving *tempeh* extract with levels of 5% and 7.5% extract contents has an influence on the degree of epithelialization in the wound healing process. *Tempeh* extract contains genistein which has a mechanism for increasing the degree of epithelialization that depends on keratinocyte cells, fibroblasts, and macrophages. Migration of epithelial cells into the wound surface is one of the keys in the process of re-epithelialization. The speed of cell migration is influenced by the presence of estrogen (Emmerson *et al*., 2010).

Formation of new blood vessels has a major role in the process of wound healing. The presence of blood vessels in the wound area will increase the wound healing process. Blood vessels contribute to the supply of blood, oxygen, and nutrients to the injured area. Blood vessels will be seen by the presence of red blood cells in the observation of histological preparations as shown in Figure 2. From the results of the study, there were no significant differences in the formation of blood vessels from each test group. Giving *tempeh* extract gel preparations in the base treatment group, levels of 2.5%, 5%, and 7.5% extract content did not provide significantly different results compared to the group without treatment.

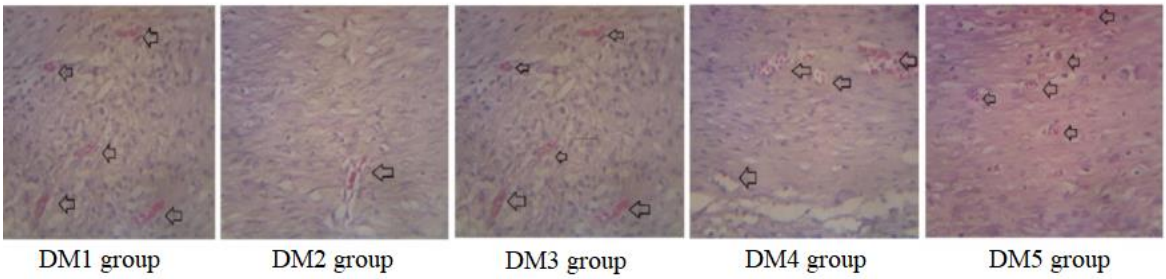

Figure 2. Capillary microscopic depictions of each group

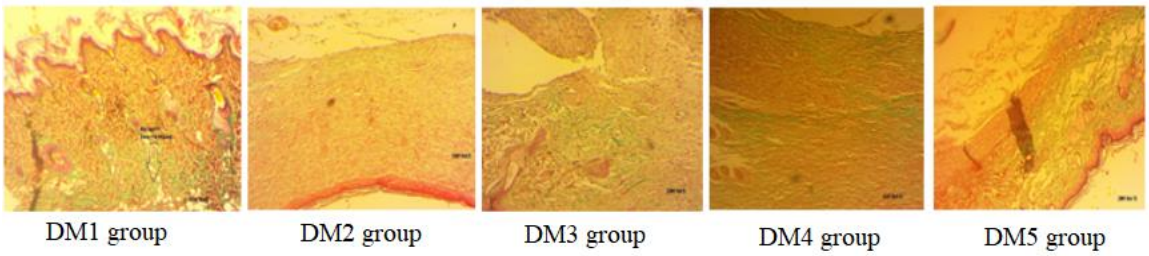

Figure 3. Microscopic picture of collagen in each group

Collagen is one of the proteins that make up the skin. Collagen fibers have a strong resistance to pressure. The more collagen found in the tissue, the stronger the tissue is against pressure. Collagen observations were carried out by staining using Masson Trichrome Goldner. The results of collagen staining provide a visualization of the green color in the histological preparations as shown in Figure 3. The more green color that appears shows the greater amount of collagen contained in the test tissue.

From the results of collagen analysis, it showed a significant difference in collagen expression in the test group. The 5% formula group and 7.5% extract content formula group had significant differences in the expression of collagen groups without treatment. The test group giving the basis and formula 2.5% extract content did not show any significant difference to the group without treatment.

The content of genistein contained in *tempeh* extract has an influence on collagen synthesis. From previous studies, genistein has an effect on collagen synthesis by binding to estrogen receptors and inhibiting tyrosine kinase (Irrera *et al.*, 2017). The effect of genistein in *tempeh* extract on collagen synthesis is thought to be related to the mechanism of shortening the inflammatory phase so that the synthesis of fibroblasts in skin tissue increases. Fibroblasts are cells that produce collagen, so an increase in the number of fibroblasts in the tissue causes an increase in the amount of collagen.

In the condition of diabetes, the formed collagen is degraded due to high MMP in the tissue. The reduced amount of collagen will make the tissue more fragile. Hence, the wound that has been covered becomes more easily open again. With the high amount of collagen in the wound area, it is expected to increase the tissue's resistance to pressure on the skin.

From the results of testing, the wound closure using a gel base treatment, gel 2.5% *tempeh* extract content, gel 5% *tempeh* extract content, gel 7.5% *tempeh* extract content and without gel treatment, indicating the treatment group gel 5% and 7.5% *tempeh* extract content treatment can affect diabetes wound healing in test animals. This is based on the results of the statistical test of the untreated group with the treatment group of gel 5% *tempeh* extract content and 7.5% *tempeh* extract content treatment showing significantly different results in influencing the wound closure response, epithelialization and collagen formation. While the statistical results of the untreated group with the base treatment group without extracts and gel 2.5% *tempeh* extract content treatment showed no significant difference in influencing the wound closure response, epithelialization and collagen formation. The results of statistical tests in

influencing capillarization responses indicate the results were not significantly different from all treatment groups.

CONCLUSION

The results of testing the wound closure using a base gel treatment, gel 2.5% *tempeh* extract content, gel 5% *tempeh* extract content, gel 7.5% *tempeh* extract content and without treatment, indicate that the treatment group gel 5% *tempeh* extract content and 7.5% *tempeh* extract content treatment have a significant effect on diabetes wound healing in test animals based on the results of statistical tests. The untreated group with the treatment base gel without extract and gel 2.5% *tempeh* extract content treatment showed no statistically significant test results in influencing the wound closure response, epithelialization and collagen formation. In addition, all treatment groups did not show significant capillarization responses.

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REFERENCES

Agrawal, S.S., Saxena, S., Sharma, A., 2015. Phytoestrogen " Genistein ": Its Extraction and Isolation from Soybean Seeds. *International Journal of Pharmacognosy and Phytochemical Research*, 7 (6), 1121–1126.

Anwar, K., 2018. Aktivitas Gel Ekstrak Etanol Umbi Akar Tawas Ut (Ampelocissus rubiginosa L .) Terhadap Penyembuhan Luka Insisi Pada Tikus Wistar. *Traditional Medicine Journal*, 23 (April), 30–39.

Baltzis, D., Eleftheriadou, I., Veves, A., 2014. Pathogenesis and Treatment of Impaired Wound Healing in Diabetes Mellitus: New Insights. *Advances in Therapy*, 31 (8), 817–836.

Emmerson, E., Campbell, L., Ashcroft, G.S., Hardman, M.J., 2010. The phytoestrogen genistein promotes wound healing by multiple independent mechanisms. *Molecular and Cellular Endocrinology*, 321 (2), 184–193.

Etuk, E.U., 2010. Animals models for studying diabetes mellitus Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University,. *Agriculture and Biology Journal of North America*, 1 (2), 130–134.

Febram, B., 2010. Aktivitas sediaan salep ekstrak batang pohon pisang ambon (musa paradisiaca var sapientum) dalam proses persembuhan luka pada mencit (*Mus musculus albinus*), *Majalah Obat Tradisional*, 15(3), pp. 121–137.

Gupta, B., Agarwal, R., Alam, M.S., 2011. Hydrogels for wound healing applications. Biomedical Hydrogels: *Biochemistry*, Manufacture and Medical Applications. Woodhead Publishing Limited.

Horng, H. *et al*., 2017. 'Estrogen Effects on Wound Healing', International Journal of Molecular science, pp. 1–14.

Irrera, N., Pizzino, G., Anna, R.D., Vaccaro, M., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., 2017. Dietary Management of Skin Health : The Role of Genistein. *MDPI*, 1–10.

Júnior, M.C.S., Colchon, P.H., Portes, A.G., 2011. Histological evaluation on Brazilian green propolis effect in tissue repair of wistar rats cutaneous wounds Histological Evaluation on Brazilian Green Propolis Effect in Tissue Repair of Wistar Rats Cutaneous Wounds, *Lat. Am. J. Pharm*. 30 (2): 383-7.

Kintoko, 2017. Effect of Diabetes Condition on Topical Treatment of Binahong Leaf Fraction in Wound Healing Process Pengaruh Kondisi Diabetes pada Pemberian Topikal Fraksi Daun Binahong dalam. *Majalah Obat Tradisional*, 22 (August), 103–110.

Martsiningsih, M.A., 2016. Gambaran Kadar Glukosa Darah Metode GOD-PAP (Glucose Oxsidase – Peroxidase Aminoantypirin) Sampel Serum dan

Plasma EDTA (Ethylen Diamin Tetra Asetat), *Jurnal Teknologi Laboratorium*, Vol.5, No.1, Maret 2016, pp. 45-48.

Rosyid, F.N., 2016. Role of TNF-α in Diabetic Ulcer Healing Process. *In*: *The 2nd International Conference on Science, Technology, and Humanity*. 142–149.

Sharp, A.N.U., Jane, C., 2011. Diabetes and its effects on wound healing. *Nursing Standard*, 25 (45), 41–47.

Suvik, A., 2012. The Use Of Modified Masson's Trichrome Staining In Collagen Evaluation In Wound Healing Study, *Malaysian Journal of Veterinary Research*, Volume 3 No.1 January 2012, 39-47.

Tie, L., An, Y., Han, J., Xiao, Y., Xiaokaiti, Y., Fan, S., Liu, S., Chen, A.F., Li, X., 2013. Genistein accelerates refractory wound healing by suppressing superoxide and FoxO1/iNOS pathway in type 1 diabetes. *Journal of Nutritional Biochemistry*, 24 (1), 88–96.

Utami, D.T., Karim, D., 2014. Diabetes mellitus dengan ulkus diabetikum, *Jom Psik* Vol. 1 No. 2 Oktober 2014, 1–7.

Utari, 2010. Tempe Terhadap Kadar Isoflavon, *PGM* 2010, 33(2): 148-153.

Velnar, T., Bailey, T., and Smrkolj, V., 2009. The wound healing process: An overview of the cellular and molecular mechanisms. *Journal of International Medical Research*, 37 (5), 1528–1542.

Yuliani, S.H., Gani, M.R., Istyastono, E.P., and Riswanto, F.D.O., 2018. Optimization of genistein and daidzein extraction from a *tempeh*-fermented product of soybean, *Journal of Pharmacy & Pharmacognosy Research*, 6 (4), 231–241.

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THE USE OF INTERNET AND SOCIAL MEDIA FOR DRUG INFORMATION SERVICES IN PHARMACIES IN YOGYAKARTA PROVINCE: A STUDY OF ASTHMA CARE

Fajar Ira Juwita, Aris Widayati*), Enade Perdana Istyastono

Faculty of Pharmacy, Universitas Sanata Dharma, Kampus III Paingan Maguwoharjo Depok Sleman Yogyakarta, 55282, Indonesia

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ABSTRACT

Industrial revolution 4.0 is the process of digitizing the industry that leds health services in the era of application of Information and Communication Technology (ICT) called e-health. The application of ICT in Pharmacy is known as e-pharmacy. The role of pharmacists in Drug Information Services by utilizing ICTs has an effect on e-health literacy which ultimately supports the success of patient therapy. This is very important for patients with chronic diseases who are receiving treatment for a long time, one of which is asthma patients. Therefore, this study aims to explore pharmacist perceptions regarding the use of the internet and social media for drug information services, with a case study of services in asthma patients. This research is descriptive with a qualitative approach. Data is collected by interviews using an interview guide that has been validated in a professional judgment. Interviews were conducted with pharmacist participants who provided pharmacy services to asthma patients, who were selected purposively in March to July 2019. Data from interviews were transcribed verbatim, then a thematic analysis was performed. Ethical clearance has been obtained under number 945/ C.16/ FK/ 2019. The results of the study mention the use of the internet and social media indicate the potential capabilities of pharmacists in health services and also the challenges of transformation to the role of pharmacists in the e-pharmacy era. This study also mentions the urgency of establishing regulations regarding e-pharmacy that is driven by the violation from the internet and social media use. Improvement of ICT infrastructure in the pharmacy field as well as the pharmacist's contribution on the provision of drug information for chronic diseases, including asthma, are urgently required.

Keywords: asthma; drug information services; e-health, e-pharmacy; internet; social media.

INTRODUCTION

The use of Information and Communication Technology (ICT) has been growing up incredibly. This phenomenon is part of the era of industrial revolution 4.0 (Hermann n.d., Bigirimana and Chinembiri 2015, Rüßmann *et al.* 2015). The use of ICT in health services is known as e-health, which also has been developing extremely (Ruxwana *et al.* 2010(Board 2004)). E-health covers supportive, promotive, preventive, and curative, also rehabilitative activities (World Health Organization 2015, Lee and Lim 2017).

Pharmacist is one of health professionals who has been familiar with the use of ICT for the services, especially for purchase and storage of medicines (Webster and Spiro 2010, Westerling *et al.* 2011). The use of ICT in pharmaceutical areas is known as e-pharmacy (European commission 2012). Examples of e-pharmacy includes e-purchasing, e- prescribing, and e-dispensing (Nanji *et al.* 2009, Webster and Spiro 2010, Malathi *et al.* 2018). The use of ICT in pharmaceutical services can help to improve patient's medication adherence through drug information services (Goundrey-Smith 2014).

*Corresponding author: Aris Widayati
Email: *ariswidayati31@gmail.com*

For example, a reminder tool based on internet use has been developed in Pennsylvania USA to improve medication adherence for asthma patients (Pool *et al.* 2017). In Canada, there has been a community of asthma patients who interact each other through a social media platform with a health professional as a guide (Letourneau *et al.* 2012).

Indonesia is one of the top five most populated countries in the world. As a consequence, a huge amount of internet users resides in Indonesia (Internet World Stats 2016, Kominfo 2017). In the health care context, especially pharmaceutical care services, the use of internet could help in improving health services to people. Patients with chronic disease are a group of population who requires a special service to improve their quality of life. Since they commonly receive more than three types of medicines for long duration of medication, the use of internet for drug information service could facilitate patient's medication adherence. Asthma is one of chronic diseases in Indonesia with a prevalence escalation, and therefore it requires more attention. The use of ICT on asthma care seems promising, as has been done in the USA and Canada mentioned earlier. Drug information service for asthma patients applying an internet platform is required to be developed. However, study on the use of ICT on asthma care in the Indonesia context is very rare. Therefore, this study aimed in exploring pharmacists' perceptions on the use of ICT platform to provide drug information service, with asthma pharmaceutical care as a model in this study.

METHODS

Study design and variables

This is an observational study with a qualitative approach (FHI 2005). The qualitative approach applied in this study aimed to explore in more details regarding the use of internet and social media for providing drug information to asthma patients from the perspectives of pharmacists who practice in pharmacies in Yogyakarta.

Exploration on the use of internet and social media to provide drug information for asthma patients from pharmacists' perspectives were focused on these variables: 1) capability of use; 2) advantages and disadvantages of use; 3) barriers and expectations of use.

Data collection technique

Data were gathered using interview technique. This technique provides opportunity for participants to give their thought regarding the topics questioned freely and responsively (Kallio *et al.* 2016). An interview guideline was formulated based on a theoretical framework named COM-B (Capacity, Opportunity, Motivation to perform a Behavior) (Eliasson *et al.* 2011). This theoretical framework was applied to assist in guiding questions to explore perceptions on the use of internet and social media to deliver drug information to asthma patients based on pharmacists' views. The interview guideline was assessed using a professional judgement approach. A pharmacist who is expert in using this theoretical framework and is familiar with pharmacist's standard of practice, especially in delivering drug information was asked to assess the guideline.

Sampling technique and recruitment of the participants

Participants of this study were pharmacists who met the inclusion criteria, which is those who practice in pharmacies in Yogyakarta and had served their asthma patients at least one month before. The participants were selected non-random purposively. The purposes in selecting the participants were: 1) selecting those who would provide detailed explanation on the use of internet and social media for drug information service, especially for their asthma patients; 2) selecting those to fulfil variations as much as possible, in term of gender, age, experience, and location of the pharmacies.

Recruitment of the participants was conducted followed these steps: 1) identified pharmacists who met the inclusion criteria and listed as potential participants; 2) contacted the potential participants and approached them to confirm their voluntary participation in this study; 3) made appointment for interview with those who agreed to participate in this study.

Data collection process

Interviews were conducted during March to July 2019. A trained interviewer met a potential participant in a scheduled time. A brief information of this study were delivered to the potential participant. After that, the potential participant was asked to sign an inform consent to confirm her/his voluntary participation. Interviews were done face-to-face for about 45 to 60 minutes per-participant. Interviews were audio-taped subject to participants' approval. The process of data collection was discontinued after data saturation was achieved. This mean that there is no new type of information given by at least the last three interviewees (Saunders *et al*. 2018).

Data analysis

Results of the interviews were transcribed verbatim and were analyzed thematically. The steps of thematic data analysis are as follows: 1) repetitive reading of the verbatim; 2) coding ideas found in the reading; 3) grouping the almost similar ideas with a new code; 3) extracting the grouped ideas into a theme and drawing the emerged themes into a theme's map. Since constructs of the COM-B theoretical framework informed the interview guideline, the theme's map were referred to those constructs.

Ethical clearance and research permit

Ethical clearance was obtained from the Ethic Committee of the Faculty of Medicine Duta Wacana Christian University (UKDW) with No. 945/C.16/FK/2019. Research permit was sought from *Badan Kesatuan Bangsa dan Politik DIY* with No. 074/2052/Kesbangpol/2019.

RESULTS AND DISCUSSION

Participants involved in this study were 15 pharmacists. Participants' characteristic were described in Table 1. While the themes emerged through the interviews were defined as follows:

The first theme: Pharmacist's capability in using internet and social media to support the services

Results of the study indicate several potencies in using internet and social media to support the provision of services.

To support communication to patients, pharmacists, and other health care professionals

All participants in this study stated that they preferred to use particular social media, i.e.: *WhatsApp*, to communicate with their patients. The *WhatsApp* platform allows them to provide information to their patients in the various ways, such as photos, pictures, or other types of interesting visual media.

Table 1. Participants' characteristics of the study of pharmacists' perception in using internet on social media to deliver drug information to asthma patients in Yogyakarta

Characteristics	Number (N=15)
Age	
< 35 years	7
≥ 35 years	8
Gender	
Male	14
Female	1
Length of practice as a pharmacist	
< 10 year	5
≥ 10 year	10
Type of the pharmacy	
Individual pharmacy	7
Chain pharmacy	4
Pharmacy at Primary Health Centre	4

"…. soalnya kalau telepon gitu kan sudah tidak jamannya, mahal juga, kalau SMS [Short Message Service] juga tidak bisa memuat gambar, tapi kalau WA [WhatsApp] itu kan bisa lebih fleksibel apapun bisa dimasukkan kesitu…." (R13J)

Eight participants even stated that they have been communicating regularly with their asthma patients.

"Kalau kemarin dengan Bu P. A [nama pasien asma rutin] ini justru kita malah via Whatsapp.... Itu saya hubungi dan beliau juga sudah kasih nomornya ke saya, lalu saya hubungi via WA [Whatsapp]." (R6P)

All participants also mentioned that they can benefit from the use of social media to improve their knowledge by reading some specific information shared by other pharmacists from around the country, with no barriers of time and place.

"Terus mungkin juga bagusnya itu, ... soalnya media sosial membahas tentang kayak pertemuan-pertemuan ilmiah rutin. Tapi lewat medsos kan tidak terbatas ruang dan waktu ... lebih fleksibe lah istilahnya, daripada harus ada pertemuan itu, harus ke tempat itu, butuh waktu." (R2NJ)

To support advertisements of pharmacy and pharmacist's activities

Some of the participants put a particular information in their social media status or profile picture, such as in *Instagram, Facebook, Whatsapp*. They used the specific feature in the social media as a tool to spread their activities related to their service provisions.

".....Whatsapp itu kan apabila kita minta nomer kontaknya (pasien) terus sengaja kita upload story tentang PIO [Pelayanan Informasi Obat] itu akan sangat mudah menyebar dan dibaca oleh yang punya kontak kita, kalau ada keluarga atau ada teman atau ada sanak saudara yang membutuhkan mereka bisa kembali men-share kepada yang bersangkutan…." (R15P)

Intrenet and social media is also seen by all the participants as an opportunity to introduce pharmacists' role to society.

"Yang maksud satu arah itu contohnya itu kayak informasi yang diberikan itu arah dari kita (apoteker) itu bisa sih di media sosial lewat media sosial untuk memperkenalkan apoteker juga bisa" (R12NJ)

To support searching of sources of drug informartion

All the participants agreed that internet helped them in searching information easily and quickly.

"Kalau keuntungannya [penggunaan internet] sih kita bisa tau apa-apa ya …. Ditanya sama pasien gitu kan kita bisa langsung cari [informasi], langsung nemu... (R5J)

To support other pharmaceutical service provisions

Most of the participants mentioned that their pharmacy has been supported by wireless internet connection. They convinced that such facility can help to provide services in their pharmacy.

"Terus pelayanan informasi obat untuk mencari obat baru misalnya ada permintaan resep tapi di sini gak ada obatnya ya kita searching dengan bantuan wifi atau internet, bisa dicari lewat komputer atau pakai handphone. Ya banyak dimudahkan lah dengan adanya wifi kita untuk searching informasi." (R11NJ)

Some participants said that they did partnership with a particular provider of internet-based marketing platform to improve their sales; for example, the HaloDoc.

"Kalau jualan online, kami kerjasama sama HaloDoc. Untuk meningkatkan omset sangat bermanfaat." (R1NJ)

The second theme: Challenges of pharmacist's roles transformation in the era of e-pharmacy

As a reliable drug informer

Some participants said that mostly patients prefer to search drug information through internet, especially for self medication instead of having consultation with pharmacists. Participants see this fact as a potential disadvantage of the use of internet as information accessed through the internet is not always valid and reliable.

"Kadang orang tidak mau bertanya kepada tenaga kesehatan, mereka lebih memilih untuk cari dulu di internet, nah ketika sumber yang dicari itu istilahnya tidak punya basic secara ilmu kesehatan kadang kan berbeda cara pandangnya." (R1NJ)

"Jadi kalau ruginya itu terkait apa yang mereka buka sendiri di internetnya. Kadang ada informasi yang belum valid nah mereka kadang cuma baca aja dan mereka bilang mau ini [obat tertentu]." (R8J)

The fake and misleading health information potentially leads to jeopardize to the society.

"Kerugiannya adalah kalau saya melihat secara umum atau untuk kasus ini, ya kadang terdapat berita yang simpang siur atau hoax gitu ya. Yang malah bingung. kita [apoteker] itu sudah betul-betul on the track apa belum, jadi kita [apoteker] bingung sendiri, ini bener nggak sih?" (R2NJ)

As an authority person of online pharmacy

Participants who work at a chain pharmacy mentioned that there is a special group of pharmacists in their corporation, who has a specific duty regarding digital marketing. This team is specifically responsible to the management of online pharmacy.

".....memang itu apoteker semua mbak, jadi tugas kami ini ada pengenalan produk (iklan) kemudian ada produk diskon, lalu job karir ya macam-macam itu apoteker semua yang mengerjakan. Digital marketing ini istilahnya, kami ada transformasi mbak, transformasi untuk dibuat job-job apa yang harus dilakukan salah satunya digital marketing, tugasnya ya itu tadi [salah satunya] untuk membuat PIO [Pelayanan Informasi Obat]." (R13J)

As an educator to improve society's health literacy

Participants mentioned that pharmacist should have capability and capacity to select a qualified information retrieved from the websites or social media. They have to assure that information obtained through the internet must be qualified, valid, and reliable to be shared to the society.

"Kalau kekurangan nya sih itu tadi kalau kita [apoteker] tidak bisa menyaring informasi, kita [apoteker] jadi memberikan informasi yang salah. Makanya itu menjadi tepat dan cepat kalau kita [apoteker] bisa memilih sumber informasi yang benar dari internet gitu." (R12NJ)

The third theme: urgency of regulation regarding e-pharmacy (online pharmacy)

Most participants stated that there are a lot of online shops that sell medicines, not only the over-the-counter medicines but also the prescription only medicines which must be obtained using prescription. Even, the online shops are run without supervision from an authorized person, i.e.: pharmacist. Further, participants stated that regulation regarding online pharmacy is urgent.

"Bahwa itu enggak tepat kalau seperti itu itu loh, kalau mau ada online sih oke, tetapi betul-betul harus di handle oleh orang yang memang berwenang di situ dan yang berkompeten di situ itu. Jangan hanya penjual lepas gitu kan, tidak ada kejelasan apapun dan sebagainya. Regulasinya harus betul-betul ketat dan tegas. Tapi kan lebih bagus kalau ini bisa berlaku secara nasional ada peraturan perundang-undangan lah atau dari Kementerian Kesehatan seperti itu kan?" (R2NJ)

Further, participants expressed their concern regarding the missuse of the internet for selling medicines in a website.

"Terus juga kalau menurut saya negatifnya adalah karena di situ [toko online] ini tidak menutup kemungkinan juga untuk pemanfaatan media sosial dan internet ini juga untuk untuk kasus-kasus kejahatan tertentu juga, atau mungkin bisa juga pemanfaatan penipuan dan sebagainya, ini yang sangat tidak diharapkan." (R2NJ)

The fourth theme: improving the ICT facilities

Participants who work in Primary Health Centre (PHC) said that there are barriers in using internet and social media to support their pharmaceutical care service. The main barrier they mentioned are the unstable internet

connection that causes delay or suboptimal of the service.

"Tapi kadang kendalanya ya mungkin ada kendala jaringan juga. Sehingga itu yang dirasa kita sebagai kerugiannya, misalkan pas ada kendala-kendala... Jadi bisa tidak cepat juga, kayak gitu, kalau ketika ada kendala seperti itu." (R6P)

They also convinced that there are still many PHCs in remote rural areas that have not been reached by the internet, in other words internet network infrastructure is still limited.

"Terkait dengan akses informasi artinya di Puskesmas sendiri di setiap desa belum tentu menggunakan internet yang memiliki akses yang cepat, hanya orang-orang tertentu yang bisa mengakses internet itu masih sangat terbatas sehingga untuk infrastruktur internet masih sangat terbatas." (R14P)

The fifth theme: pharmacist's contribution on people's e-health literacy regarding chronic diseases.

Participants in this study stated that pharmacists can contribute on the improvement of people's literacy regarding health and the use of internet and social media to search qualified health information. For example, information about medication adherence.

"Bisa untuk lebih meningkatkan tingkat pemahaman dari dari masyarakat ya tentang kesehatan, tentang penyakit, tentang pengobatannya. Kemudian ya terus terutama juga tentang bisa memberikan masukan juga tentang kepatuhan. Kepatuhan dalam hal melakukan terapi itu harapannya sih seperti itu dan bisa diakses secara luas dan secara mudah, secara murah gitu ya." (R2NJ)

Some participants who work at retail pharmacies expected to use internet and social media to support their professional role. They expected that the use of internet and social media would help them to improve patients' knowledge regarding medicines and medication, to improve patient's medication adherence, including asthma medication.

"Ayo bikin ini, untuk diposting, misalkan seperti itu. Pada prinsipnya tetap ingin menggunakan internet sebagai media untuk kita bisa terus memberikan informasi obat kepada masyarakat, kepada pasien sehingga bisa ikut meningkatkan kepatuhan penggunaan obat dan juga meningkatkan pengetahuan tentang obat gitu ya, terhadap asma juga." (R5P)

Participants stated that pharmacists must increase their contribution on delivering drug information using an internet and social media. They expected that through the use of internet and social media to communicate with patients and people and improve patient's quality of life, the role of pharmacist will be appreciated by society.

"Saya berharap dengan adanya pasien tahu informasi obat di internet, ya walaupun masih dalam kendali kita ya, artinya tidak seluruhnya. Apoteker dalam membuat ini itu semuanya disampaikan. Berharap pasien ini quality of life nya tetap bagus ya." (R13J)

"Semoga pasien asma bisa mengontrol kondisi kesehatannya karena kalau asma kan tidak bisa disembuhkan, hanya bisa dikontrol. Meningkatkan kualitas hidup aja sih… dan ketika dia mendapatkan informasi yang sesuai, dia akan bisa menjaga, istilahnya menjaga menjauhkan diri dari alergen atau dapat tahu cara penggunaan inhaler maupun alat yang digunakan untuk kesehatannya dengan baik." (R1NJ)

Participants were aware that information regarding chronic diseases, including asthma, is easily searched via internet. However, they underlined that further detailed consultation must be handled by pharmacists as a health professional who are expert in medicines.

"Misalnya mencari kata Asma ya pakai yang mudah dipahami masyarakat terus kemudian bisa disitu penanggungjawabnya boleh sih kalau misalnya itu jadi ada kontak, mungkin beberapa udah ada ya misalnya kayak contact person atau registrasi email jadi kalo kita mau berhubungan dengan orang yang memposting informasi itu." (R9P)

This qualitative study underlines five themes from pharmacists' perspectives regarding the use of internet and social media to deliver drug information to asthma patients as a model. The five themes are: 1) Pharmacist's capability in using internet and social media to

support the services; 2) Challenges of pharmacist's roles transformation in the era of e-pharmacy; 3) Urgency of regulation regarding e-pharmacy (online pharmacy); 4) improving the ICT facilities; 5) Pharmacist's contribution on people's e-health literacy regarding chronic diseases.

The first theme emerged through this study is pharmacist's capability in using internet and social media to support pharmaceutical care practice. Pharmacist's capability in using internet and social media is a strength, especially in the era of "internet of things". On the other side, there is a big need from society to receive reliable information regarding medicines, especially through online communication (Leonita and Jalinus 2018). For examples: the use of video uploaded in a website to educate asthma patients regarding the use of inhaler and other asthma medical devices (Benetoli *et al.* 2017) and the use of internet-based integrated information system in hospital and community pharmacy to improve health services, including pharmaceutical care services (Lalitaphanit 2016). Internet and social media also provide a huge opportunity for pharmacists to improve and share their knowledge with their colleagues, even with other health professionals without any significant boundaries (Webster and Spiro 2010, Leonita and Jalinus 2018). Online communication using internet can minimize barriers of time and location (Ruxwana *et al.* 2010). Therefore, pharmacists should equip themselves with adequate knowledge and skill regarding the use of internet and social media to deliver drug information to their patients as well as to have professional communication with other health professionals (Eliasson *et al.* 2011).

The second theme is the need of transformation of pharmacist's role from "off-line pharmacist" to "online pharmacist". In the era of "internet of things" there is a need and challenge for pharmacists to transform their roles, especially regarding the use of ICT to improve pharmaceutical care services (Bigirimana and Chinembiri 2015). The need of transformation is triggered by the increase of internet used by society to search information regarding medication and medicines. Social media and website become the most popular sources of health information accessed by people. The online and user friendly sources of information become a popular choice when people got difficulty in meeting pharmacists face-to-face (Crilly *et al.* 2019). There is also a tendency of people to share their experiences regarding their own health problems. Information shared by lay people based on their own experience tend to be trusted by society (Bhaskaran *et al.* 2017). However, there is a crucial problem when people are not able to differentiate between qualified information and false information (Prasanti 2018). Therefore, pharmacist must take a role in helping people to get a valid and reliable information they search via internet. In this case, pharmacists must play their role as an educator to improve people's health literacy using internet-based platforms (MacLure and Stewart 2018). Further, challenge of the pharmacist transformation role is how to develop pharmaceutical care service using internet-based platforms (Webster and Spiro 2010, MacLure and Stewart 2018). For example, in Scotland pharmacists has been supported by government to transform in managing e-prescribing and e-Health Record in collaboration with other health professional in hospital (MacLure and Stewart 2018).

The third theme is urgency of regulation regarding e-pharmacy. Authorized online pharmacy has been growing up very fast. On the other hand, selling medicines on the websites illegally is also common (Ebner 2012). Obtaining medicines sold in illegal websites will jeopardize consumers, especially regarding the risk of selecting and using the purchased medicines inappropriately (Chaturvedi *et al.* 2015). Therefore, there is an urgent need to regulate online medicines trading via websites.

The fourth theme is the prerequisite to improve ICT facilities. The ICT will revolutionize the way to deliver pharmaceutical care services the patients (Ruxwana *et al.* 2010). However, ICT infrastructures in some extends is still inadequate (Benetoli *et al.* 2017). In the Indonesia context, fast connection remains a problem of the ICT facilities.

The fifth theme is pharmacist's contribution regarding people's e-health literacy especially in managing chronic diseases. Pharmacists must take their role as an educator to improve people's health literacy. Pharmacist must also design and develop new ways to engage with society members to improve people knowledge and awareness regarding the use of medicines safely (Benetoli *et al*. 2017). The use of internet creates a huge opportunity for pharmacists to get involve in improving the society's literacy regarding health (MacLure and Stewart 2018).

This study is not without its limitation. The nature of interview method in gathering data would likely to provide a big chance for participants to answer the question openly and freely. This could lead to a response bias. In this study, although questions were queried using a guideline with emphasizing on asthma care, yet participants tended to answer using a perspective of chronic diseases as general. The inclusion criteria of recruitment of participants, which was pharmacists who had experience in delivering pharmaceutical care service to asthma patients within a month before the interview, was an anticipating approach to minimize such a response bias, however. Furthermore, as asthma care in this study only becomes a model, the participants' responses could possibly describe chronic diseases as general.

CONCLUSION

Using the asthma care as a model this study concludes that the use of internet and social media to support pharmaceutical care services is promising. There are challenges and opportunities for pharmacists to take an advanced role in the era of "internet of things" to improve patient's quality of life. Further research is needed to develop new and appropriate internet-based platforms that can be used to support the pharmaceutical care services.

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REFERENCES

Benetoli, A., Chen, T.F., Schaefer, M., Chaar, B., and Aslani, P., 2017. Do pharmacists use social media for patient care? *International Journal of Clinical Pharmacy*, 39, 364–372.

Bhaskaran, N., Kumar, M., Janodia, M.D., 2017. Use of Social Media for Seeking Health Related Information – An Exploratory Study, 9 (2), 267–271.

Bigirimana, S., Chinembiri, M., 2015. Towards E-Pharmacy: The Future Information and Communication Technologies Needs for Community Pharmacies in Harare, Zimbabwe. *International Journal of Economics, Commerce and Management*, III (4), 1–26.

Board, E., 2004. eHealth: Report by the Secretariat (Executive Board EB115/39 115th Session, Provisional agenda item 4.13), (1), 1–6.

Chaturvedi, A., Kumar, A., Noida, G., 2015. Online pharmacy: an e-strategy for medication, (April 2011).

Crilly, P., Hassanali, W., Khanna, G., Matharu, K., Patel, D., Patel, D., Rahman, F., Kayyali, R., 2019. Research in Social and Administrative Pharmacy Community pharmacist perceptions of their role and the use of social media and mobile health applications as tools in public health. *Research in Social and Administrative Pharmacy*, 15 (1), 23–30.

Ebner, N., 2012. Electronic copy available at: http://ssrn.com/abstract=2170559. *Signal*, 27 (2), 10–14.

Eliasson, L., Barber, N., Weinman, J., 2011. Applying COM-B to medication adherence work tended to focus on the role and its effects on patient, 7–17.

European commission, T.H.E., 2012. EU

eHealth Action Plan 2012 to 2020.

FHI, I.F.H., 2005. Qualitative Research Methods: A Data Collector's Field Guide.

Goundrey-Smith, S., 2014. Examining the role of new technology in pharmacy: now and in the future [online]. Available from: https://www.pharmaceutical-journal.com/examining-the-role-of-new-technology-in-pharmacy-now-and-in-the-future/11134174.article.

Hermann, M., n.d. Design Principles for Industrie 4.0 Scenarios: A Literature Review.

Internet World Stats, 2016. Internet World Stats [online]. *Internet World Stats*. Available from: http://www.internetworldstats.com/ [Accessed 8 Nov 2018].

Kallio, H., Pietilä, A.M., Johnson, M., Kangasniemi, M., 2016. Systematic methodological review: developing a framework for a qualitative semi-structured interview guide. *Journal of Advanced Nursing*, 72 (12), 2954–2965.

Kominfo, 2017. Jumlah Pengguna Internet 2017 Meningkat, Kominfo Terus Lakukan Percepatan Pembangunan Broadband [online]. Available from: https://kominfo.go.id/content/detail/12640/siaran-pers-no-53hmkominfo022018-tentang-jumlah-pengguna-internet-2017meningkat--kominfo-terus-lakukan-percepatan-pembangunan-broadband/0/siaran_pers/ [Accessed 23 Nov 2018].

Lalitaphanit, T., 2016. Factors affecting community pharmacy customers' decision to use personal health records via smartphone. *Thai Journal of Pharmaceutical Sciences*, 42.

Lee, J.-Y., Lim, J.-Y., 2017. The Prospect of the Fourth Industrial Revolution and Home Healthcare in Super-Aged Society. *Annals of Geriatric Medicine and Research*, 21 (3), 95–100.

Leonita, E., Jalinus, N., 2018. Peran Media Sosial dalam Upaya Promosi Kesehatan : Tinjauan Literatur, 18 (2), 25–34.

Letourneau, N., Stewart, M., Masuda, J.R., Anderson, S., Cicutto, L., McGhan, S., Watt, S., 2012. Impact of Online Support for Youth With Asthma and Allergies: Pilot Study. *Journal of Pediatric Nursing*, 27 (1), 65–73.

MacLure, K., Stewart, D., 2018. A qualitative case study of ehealth and digital literacy experiences of pharmacy staff. *Research in Social and Administrative Pharmacy*, 14 (6), 555–563.

Malathi, S., Priadarsini, M., Dharshana, M., Agathiya, T., 2018. Big Data and CPS (Cyber Physical System) used in Pharmacy to Alert on Expiration of Medicine, 8 (4), 16946–16948.

Nanji, K.C., Cina, J., Patel, N., Churchill, W., Gandhi, T.K., Poon, E.G., 2009. Overcoming Barriers to the Implementation of a Pharmacy Bar Code Scanning System for Medication Dispensing: A Case Study. *Journal of the American Medical Informatics Association*, 16 (5), 645–650.

Pool, A.C., Kraschnewski, J.L., Poger, J.M., Smyth, J., Stuckey, H.L., Craig, T.J., Lehman, E.B., Yang, C., Sciamanna, C.N., 2017. Impact of online patient reminders to improve asthma care: A randomized controlled trial. *PLoS ONE*, 12 (2), 1–17.

Prasanti, D., 2018. Literasi Informasi Kesehatan sebagai Upaya Pencegahan Informasi Hoax dalam Penggunaan Obat Tradisional di Era Digital Health Information of Literation as Prevention Processes of Hoax Information in the Use of Traditional Medicine in Digital Era, 3 (1), 45–52.

Rüßmann, M., Lorenz, M., Gerbert, P., Waldner, M., Justus, J., Engel, P., Harnisch, M., 2015. Industry 4.0: The Future of Productivity and Growth in Manufacturing Industries. *Business and Information Systems Engineering*, 6 (4), 239–242.

Ruxwana, N.L., Herselman, M.E., Conradie, D.P., 2010. ICT applications as e-health solutions in rural healthcare in the Eastern Cape Province of South Africa. *The HIM journal*, 39 (1), 17–26.

Saunders, B., Sim, J., Kingstone, T., Baker, S., Waterfield, J., Bartlam, B., Burroughs, H., Jinks, C., 2018. Saturation in qualitative

research: exploring its conceptualization and operationalization. *Quality and Quantity*, 52 (4), 1893–1907.

Webster, L., Spiro, R.F., 2010. Health information technology: A new world for pharmacy. *Journal of the American Pharmacists Association*, 50 (2), e20–e34.

Westerling, A.M., Haikala, V., Airaksinen, M., 2011. The role of information technology in the development of community pharmacy services: Visions and strategic views of international experts. *Research in Social and Administrative Pharmacy*, 7 (4), 430–437.

World Health Organization, 2015. Regional Strategy for Strengthening eHealth in the South-Asia Region 2014-2020.