論　文　内　容　要　旨

PHYTOCHEMICAL INVESTIGATION OF INDONESIAN PLANT, *CURCUMA HEYNEANA* AND ITS CHEMICAL CONVERSION STUDY

（インドネシア産ウコン *Curcuma heyneana* の含有成分と化学的変換研究）

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Indonesia, one of Southeast Asia country, has an abundant amount of medicinal plant resources. Most are used as folk medicine, which is passed from generation to generation. *Curcuma heyneana,* locally known as Temugiring, is Indonesian native plant. It is traditionally used as skin scrub to treat skin disease and also as anthelmintic. Leishmaniasis is one of neglected-tropical diseases (NTD) categorized by WHO. It is estimated up to one million new cases and 65,000 mortality cases arise per year. Recently, a concern about the difficulty of discovery of novel bioactive compounds is increasing. In the last decade, it has been introduced an approach to produce bioactive compounds *via* chemical modification of crude natural extracts. In this study, we conducted phytochemical analysis of raw and chemically converted extracts of *Alpinia galanga* and *Curcuma heyneana* as a promising method to obtain novel bioactive "unnatural" natural products with anti-Leishmania activity.

Curcuma heyneana rhizomes were collected from a cultivated area in Java, Indonesia. The powder of dried rhizomes was extracted with ethanol, and then partitioned with ethyl acetate and 1-butanol, successively. By using bioassay-guided isolation procedure (Fig.2), ethyl acetate extract of *Curcuma heyneana* was subjected to investigation. Then the extract was separated on several chromatographic techniques, including silica gel and octadecylsilane column chromatography. The chemical structures of the isolated compounds were investigated by spectroscopic data analyses; consist of 1D and 2D NMR, FT-IR, UV and HR-ESI-MS measurements. The inhibitory activity of these compounds against *Leishmania major* parasites was examined by the colorimetric method, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide(MTT) assay. In this study, the cytotoxicity evaluation against A549 human lung cancer cell was also carried out.

On our phytochemical investigation of ethyl acetate fraction of *C.heyneana* rhizomes, we have isolated 15 known compounds. The investigation revealed the presence of seven guaiane-type sesquiterpenes (procurcumenol, aerugidiol, zedoarondiol, isozadoarondiol, curcumenol, 4-epicurcumenol and curcuzedoalide), two curcuminoids (curcumin and gingerol), three labdane-type diterpene, two known fatty acids (eicosadienoic acid and octadecatrienoic acid) and also adenosine.

The isolated compounds from *C. heyneana* were evaluated their inhibitory activity against *L. major* parasites and human lung cancercell, A549. Eicosadienoic acid (**7**),

octadecatrienoic acid (**8**), curcumin (**9**), gingerol (**10**) and zeramin A (**12**) showed high inhibition against *L. major* (100 μg/ml). Meanwhile the compounds **7**, **8**, **9**, **10** and curcuzedoalide (**11**) were found to be toxic to A549 cell (100 μg/ml). These activities of the extract and the isolated compounds were confirmed for the first time in this study.

As preliminary study, we've conducted chemical conversion of the extract of *Alpinia galanga*, zingiberaceae family plant, by the treatment with Oxone® (potassium peroxymonosulfate). In this study, the significant increase of inhibitory activity was observed on the chemically converted extract (CCE) of *Alpinia galanga*, in contrast to the original extract (ethyl acetate extract) that didn't show any activity against *L.major*. The bioactivity-guided fractionation and purification resulted in thesuccessful isolation of six new compounds (**14-19**) along with four known compounds, including hydroquinone (**20**), 4-hydroxy-(4-hydroxyphenyl)- methoxy)benzaldehyde (**21**), Isocoumarin *cis* 4-hydroxymelein (**22**) and humulene triepoxide (**23**) (Fig. 4).From these compounds, hydroquinone (**20**) showed the highest inhibitory activity against *L. major* parasites with IC_{50} 0.37 ± 1.37μg/ml, higher than the positive control, miltefosine (IC_{50} 7.47 ± 0.30μg/ml).

The 1-BuOH extract of *Curcuma heyneana* had no activity against *L. major*. Therefore, we tried to increase the activity of 1-BuOH extract of *C. heyneana* by chemical conversion process to generate "unnatural" natural products from the extract of *C.heyneana*. In this case, the extract was simply heated (160 ℃) in DMSO for 8 hr. The result showed that the CCE of *C.heyneana* had higher activity against *L.major* parasites than intact 1-BuOH extract. This finding indicated that the usefulness of heat treatment to modify the activity of crude extract, and the chemical conversion can be potential and powerful method on the discovery of new drug candidates, such as Leishmaniasis.