Pigment Extraction Techniques from the Leaves of *Indigofera tinctoria* Linn. and *Baphicacanthus cusia* Brem. and Chemical Structure Analysis of Their Major Components

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ABSTRACT

Indigofera tinctoria Linn. and Baphicacanthus cusia Brem. are the plants which have been used as natural source of indigo dye and are available in Northern part of Thailand. In this study we carried out methods to find the optimum condition for effective extraction of indigo from the leaves of Indigofera tinctoria Linn. and Baphicacanthus cusia Brem. and investigated chemical constituents including the chemical structure of major components in the water extract from both kinds of plant to develop the extraction method for preparation of ready-to-use natural dyes. It was found that by cutting raw material to small pieces and put into cotton bag before soaking in water for 24 hrs could decrease unpleasant smell and it was easy to get rid of waste. The indigo from fresh Baphicacanthus cusia Brem. fermented for 24 hrs gave the highest amount of indigo (0.005 g/g fresh leaves). Separation of the crude extract by Thin Layer Chromatrography (TLC) using chloroform-hexane-methanol (7:4:1 v/v/v) as solvent system gave two major pigments of blue and red colour which had Rf values of 0.69 and 0.49, respectively. The Rf value, the maximum absorption from UV-Visible spectroscopy and infrared spectrum of blue colour pigment were the same as those of the indigo standard. Moreover, the red pigment extracted from Baphicacanthus cusia Brem. was purified and analysed by UV-Visible spectroscopy, mass spectrum 13C NMR and 1H NMR and showed that it had chemical formula of C16H10N2O2 and chemical structure as indirubin and it was used as indirubin standard. The red pigment from Indigofera tinctoria Linn. was also purified and gave the same Rf value, UV-Visible absorption and IR spectrum as the indirubin standard. Both blue and red pigment could dissolve in chloroform whereas only red pigment could dissolve in methanol. Separation method of the blue and red pigment from the crude extract would be developed to get the powder of the red and blue pigments which could be used to prepare the ready-to-use natural dyes.

Key words : Indigo, Indirubin, Extraction, Structure analysis, *Indigofera tinctoria* Linn., *Baphicacanthus cusia* Brem.