

Comparative study of semi-purification methods of *Caesalpinia sappan* L. extract: Thin layer chromatography and free radical scavenging activity

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The purpose of this study was to compare thin layer chromatography (TLC) fingerprints and free radical scavenging activities of semi-purified *Caesalpinia sappan* L. extracts. Sappanwood crude extract was prepared by maceration of heartwood with 95% ethanol. The crude extract was semi-purified by partition method and ion-exchange chromatography. For partition method, the crude extract was partitioned with deionized water, dichloromethane and ethyl acetate, respectively. For ion-exchange chromatography, Diaion[®] HP-20 was used to semi-purify the extract. Brazilin and protosappanin A reference standards were isolated from ethyl acetate fraction by vacuum liquid chromatography on silica gel and Shephadex LH20. The identification of brazilin and protosappanin A were performed by HR-ESIMS and NMR. All extracts and standards was evaluated for TLC fingerprint. The free radical scavenging activity of all extracts was determined using DPPH assay. Ethyl acetate fraction had the highest percentage of yield (71.05%) followed by Diaion[®] HP-20 fraction (69.5%), water fraction (12.69%) and dichloromethane fraction (5.02%), respectively. The isolation and identification of protosappanin A and brazilin were approved by NMR and MS data with a previous report. All semi-purified extracts showed that brazilin was a major band, whereas protosappanin A was a minor band in TLC fingerprint. The water fraction had significantly the highest DPPH scavenging activity ($IC_{50} = 1.19 \pm 0.02 \mu\text{g/ml}$) followed by Diaion[®] HP-20 ($IC_{50} = 2.03 \pm 0.04 \mu\text{g/ml}$), ethyl acetate fraction ($IC_{50} = 2.08 \pm 0.02 \mu\text{g/ml}$), crude extract ($IC_{50} = 2.72 \pm 0.03 \mu\text{g/ml}$) and dichloromethane fraction ($IC_{50} = 3.95 \pm 0.05 \mu\text{g/ml}$), respectively. The ethyl acetate fraction and Diaion[®] HP-20 fraction had high percentage of yields and similar DPPH scavenging activity. Both fractions showed improved antioxidant activity when compared to crude extract. The TLC finger print showed that brazilin was a major compound of sappanwood. In further study, brazilin content of various sappanwood extracts will be evaluated by using high pressure liquid chromatography.