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QUALITY CONTROL OF *THUNBERGIA LAURIFORIA* LEAF

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INTRODUCTION

Thunbergia laurifolia Lindl. (Acanthaceae) is commonly known as Rang Jurd in Thai. The aqueous preparations of leaves and root have been used in Thai traditional medicine as anti-inflammatory and antipyretic agents as well as antidote for detoxification of poisons including insecticide, ethyl alcohol, arsenic and strychnine [1,2]. Preparation of the extracts of dried Rang Jurd leaves with boiling water was found to contain apigenin and its glycosides, phenolic acids such as caffeic acid, gallic acid and protocatechuic acid [3]. From its ethnomedical uses as detoxifying herb, Rang Jurd has potential for the treatment and prevention of insecticide poison. Recently, there has been no specification guideline for the quality control of Rang Jurd leaf as a crude drug. In this study, the physical and chemical identifications of Rang Jurd leaves collected from different locations of Thailand were examined. Moreover, DNA fingerprint of the leaves by Random Amplified Polymorphic DNA (RAPD) technique was performed.

MATERIALS AND METHODS

Plant materials: The authentic sample collected from Chiang Mai during April 2012 was identified by Mrs.Vachalee Prachasaisoradej, an Agricultural scientist 8 at Plant Variety Protection Division, Research Unit of Princess Sirinhorn Plant Herbarium Building and was deposited at the same place (VF 064382). The leaves of Rang Jurd were collected from Kanchanaburi, Chachoengsao, Chaiyaphum, Chumphorn, Tak, Narathiwat, Phitsanulok, Ratchaburi, Sa Kaeo and Utaithani from May to August 2012. Fresh leaves were dried in a hot air oven (55°C) for six to eight hours and then moderately powdered.

Macroscopic examination: Macroscopic characteristics of the leaves of Rang Jurd were investigated. Their characteristics were recorded and compared.

Microscopic examination: Powdered drugs of Rang Jurd were microscopically studied using chloral hydrate and aniline sulphate solutions as clearing reagent and staining reagent for lignified tissues, respectively with total magnification of x400.

Thin-layer chromatographic (TLC) fingerprints: five hundred milligram of each powdered sample was separately extracted with 5-ml of methanol in sonication bath for 10 min. Thin-layer chromatography of methanolic extracts of the leaves were performed on a TLC silica gel 60 GF_{254} plate using hexane: ethyl acetate: formic acid: glacial acetic acid (5:25:2:2; solvent I) and toluene: ethyl acetate: formic acid (10:9:2; solvent II), as mobile phases. The application volume of each sample was 20 µL. Polyphenols and Flavonoids were detected under UV 366 nm using a natural product/polyethylene glycol spraying reagent.

RAPD analysis: Genomic DNAs of powdered samples collected from difference locations in Thailand and fresh leaf of authentic sample were extracted using FavorPrep Soil DNA Isolation Kit (Favorgen Biotech Corporation, Taiwan) according to the manufacturer's instructions. Primer PYBC01 (5′-GTTTCTGCGG-3′) was used for RAPD. The 50 µL total volume of RAPD reaction contained 10 ng of DNA, 20 pmol of primer, 80 µM dNTP, 1 × *Taq* reaction buffer, and 2.5 U *Taq* DNA polymerase (RBC Bioscience, Taiwan). The RAPD was started by heating the reactions to 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 1 min at 37°C, and 1.5 min at 72°C, and ending with a final step at 72°C for 3 min. PCR products were separated on a 2% agarose gel and stained with ethidium bromide. RAPD fragments were analyzed using Gel Doc™ XR System PC/Mac (Bio-Rad Laboratories Inc., USA). DNA of *Thermotoga lettingae* was used as a positive control for RAPD amplification.

RESULTS

Physical Identifications: The macroscopic and microscopic characters of the leaves and powdered drugs of Rang Jurd were studied compared to those of authentic sample. Rang Jurd leaves are opposite simple leaf, broadly elliptic to narrowly ovate, 8-15 cm long, 2.5-5.5 cm wide and the leaf margin is entire. The powdered drug occurs in dark greenish powder with odor characteristic. The microscopic characters of the powdered drugs are diacytic stomata, monolayer epidermis cells, monolayer palisade cells, lignified fibers, including reticulate and spiral vessels.

Chemical identification: As described in material and methods section, each extract of leaf was separately applied on precoated silica gel aluminium plate and developed with 2 solvent systems; solvent

system I hexane: ethyl acetate: formic acid: glacial acetic acid (5:25:2:2) and solvent system II : toluene: ethyl acetate: formic acid (10:9:2). All extracts showed blue fluorescence band after detected with NP-PEG reagent under UV 366 nm at Rf value = 0.7 and 0.3 in solvent system I and II, respectively corresponded to standard caffeic acid. However, extract from the leaves of Rang Jurd collected from Chachoengsao province exhibited the weakest band. TLC fingerprints of leaf methanolic extracts are shown in Figure 1.

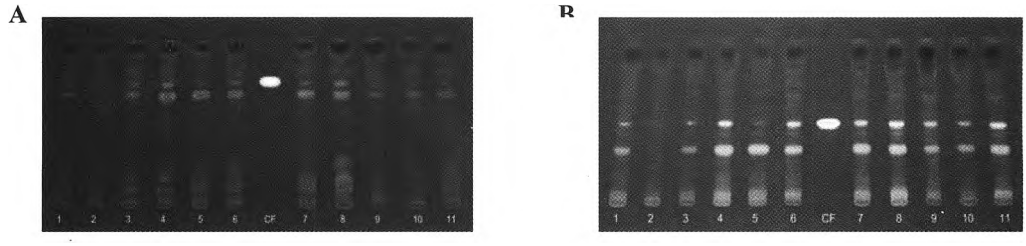

Figure 1 Thin layer chromatograms of methanolic extracts of Rang Jurd leaves collected from difference locations in Thailand detected with NP-PEG reagent using solvent system I (A) and solvent system II (B) 1= Kanchanaburi, 2 = Chachoengsao, 3 = Chaiyaphum, 4 = Chumphorn, 5 = Tak, 6 = Chiang Mai (authentic sample), CF = caffeic acid, 7 = Narathiwat, 8 = Phitsanulok, 9 = Ratchaburi, 10 = Sa Kaeo, 11 = Utaithani

RAPD analysis: The positive control (*Thermotoga lettingae*, P) was given seven bands of RAPD, while the fresh leaf of Rang Jurd (A) was given three bands of RAPD with the size of amplification products of Rang Jurd ranged from 450-650 bp (Figure 2). The results indicated that the PYBC01 primer could be applied for RAPD of bacteria and plant. Interestingly, the powdered samples from Chachoengsao (01) presented only the smallest band (450 bp) of the RAPD.

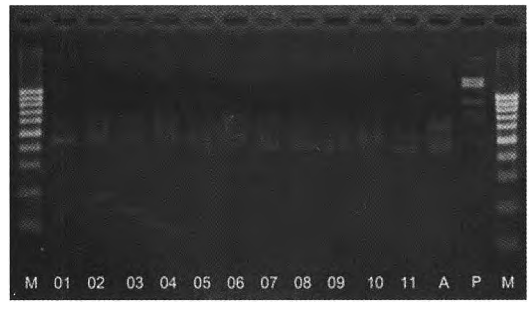

Figure 2 RAPD pattern of Rang Jurd leaves M = 2-Log DNA ladder marker, 01= Chachoengsao, 02 = Chaiyaphum 03 = Chiang Mai (authentic sample), 04 = Chumphorn, 05 = Kanchanaburi, 06 = Narathiwat, 07 = Phitsanulok, 08 = Ratchaburi, 09 =Sa Kaeo, 10 = Tak, 11 = Utaithani, A = Fresh leave of authentic sample, P = Positive control (*T. lettingae*)

DISCUSSION

All Rang Jurd leaves showed similar macroscopic and microscopic botanical characteristics with authentic sample. Thin layer chromatographic analysis using specific spraying reagent indicated that the leaf extracts from every selected samples promoted similar chromatographic fingerprints with the marker bands corresponded to caffeic acid. Moreover, RAPD analysis of all Rang Jurd and authentic sample also exhibited the same RAPD fingerprints with the similar common DNA bands (450 bp). To increase a reproducibility of RAPD, an appropriate protocol for genomic isolation of the powdered drug may be required for further study. From the results, specification of raw material from Rang Jurd leaves can be set up using the specific botanical, chemical and molecular characteristics of plant materials.

CONCLUSION

Macroscopic and microscopic analysis of Rang Jurd leaves confirmed the botanical origin of plant materials while chemical analysis by TLC showed specific chromatographic fingerprints that could be corresponded to biological activities of plant extracts. RAPD analysis of Rang Jurd leaves promoted molecular characteristics that will be useful as DNA marker and for further investigation in biosynthesis of active phytochemicals. Separation, identification and quantitative analysis of active components should

be performed. Moreover, studies for related biological activities and specific biotechnology are also suggested.

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