Abstract

The study on pharmacognostic characteristics and specifications of raw materials and extracts of tea (*Camellia sinensis* var. *assamica*), horsetail (*Equisetum debile* Roxb.) and arils of gac fruit (*Momordica cochinchinensis* (Lour.) Spreng.) used in cosmeceuticals of The Royal Project was aimed to establish the pharmacognostic characteristics together with specifications and to isolate chemical markers of those medicinal plants for use in quality control of raw materials and extracts used in the preparation of herbal products of The Royal Project. In this study, each raw material was collected from 3 different locations in nothern Thailand including Chiang Mai, Chiang Rai and Lampun provinces.

Macroscopical characters of tea, arils of gac fruit and horsetail were examined. The results displayed that they had different botanical characteristics. Powdered drugs of fresh tea and fermented tea are both brownish green in color, while those of horsetail and gac fruit arils were brownish green and brownish red, respectively. Under microscopical character investigation, it was found that tea and horsetail had the same basic plant cells in common, but they were differed in that tea had sclereid, rosette crystal and trichome whereas horsetail had obvious pith cavity. Microscopical character of tea exhibited the presence of stomas, epidermal cells, fragments of fibers, filiform sclerieds, unicellular trichomes and spiral vessels. Thai of horsetail consisted of stomas, multicelluar trichome, fragments of epidermal cells, fragments of parenchymas, fragments of fibers and spiral vessels. Gac fruit arils' microscopical character displayed the presence of oil droplets, prism crystals of calcium oxalate, starch granules, reserved parenchymas, sclereids and spiral vessels.

The phytochemical screening of tea (both fresh and fermented) and horsetail revealed that they had the same main chemical constituents which included alkaloids, flavonoids, tannins, cardiac glycosides and saponins, while those of arils of gac fruit contained only flavonoids and saponins.

The investigation on pharmacognostic characteristics of the plants including moisture content, total ash, acid-insoluble ash, extractive value and volatile oil content was conducted based on Thai Herbal Pharmacopoeia and WHO guidelines. The results exhibited that the moisture and total ash contents of fresh tea were higher than those of fermented tea, but their acid-insoluble contents were the same with 0.03 \pm 0.02 % w/w. Horsetail and gac fruit arils had moisture contents with 7.16 \pm 0.37 and 5.50 \pm 0.77 % w/w, respectively. It was noteworthy that horsetail had total ash and acid-insoluble ash contents much higher than those of the others with

21.83 \pm 0.16 and 15.78 \pm 0.48, respectively. This would be due to the presence of inorganic silicon compounds in epidermal cells of horsetail; the compounds can be not decomposed under 500 °C conducted in the experiment. After hydrodistillation of the four plants, it was found that no volatile oil was obtained from fresh tea, whereas fermented tea, horsetail and gac fruit arils afforded the oils in minute amount with less than 0.10 mL; fermented tea and horsetail oils' physical appearances were greenish having thin films covering a surface, and that of gac fruit arils was deep orange with a greasy appearance at room temperature. From the extractive value study in 95% EtOH, MeOH, EtOAc/hexane (30:70) and water, fermented tea yielded a greater amount than that of fresh tea in the four different solvents. The highest yield was obtained from water (22.23 \pm 0.21 % w/w) followed by MeOH, 95% EtOH and EtOAc/hexane (30:70), respectively. The same result was observed in horsetail and gac fruit arils having the highest yield in water with 15.76 \pm 0.42 and 50.97 \pm 0.21 % w/w, respectively.

The isolation of major chemical compounds from the extracts using chromatographic techniques and structural elucidation of isolated compounds by spectroscopic evidences yielded caffeine and (–)-epicatechin gallate (ECG) from the fresh tea extract, β -carotene from the gac fruit aril extract and an unidentified glycoside from the horsetail extract. The isolated compounds were used as chemical markers in TLC chromatographic fingerprints of those four plants, each of which was collected from three different locations, and the fingerprints together with suitable mobile phases for TLC development were obtained.

From this investigation, the pharmacognostic characteristics of fresh tea, fermented tea and gac fruit arils can be used to establish the specifications of raw materials and extracts from those three plants.