

ABSTRACT

Cleistocalyx nervosum var. *paniala* (Ma kiang) has been used as a health food supplement in northern Thailand. Its ripe berries are rich of purplish red pigments characterized as anthocyanin. The aim of this thesis was to study the suitable method to prepare *C. nervosum* var. *paniala* extracts for nutraceuticals.

The fresh ripe berries of *C. nervosum* var. *paniala* were preliminary extracted by several different methods namely fresh squeezed juice, maceration with 50% ethanol, 95% ethanol water decoction. The extracts were dried by freeze drying, vacuum drying and spray drying methods.

All extracts were subjected to active ingredients determination, namely total monomeric anthocyanin, polymeric anthocyanin, total phenolic compound, ascorbic acid, catechin, cyanidin 3-glucoside, gallic acid, kaempferol and quercetin. Antioxidant activity was determined by DPPH scavenging method and lipid peroxidation on liposomes assay. The extracts were also tested for cytotoxic activities against two different types of human cancer cells; cervical (HeLa) and lung (COR L23) and one type of human normal cell lines (MRC-5 or lung fibroblast) using sulphorhodamine B (SRB) assay.

The 95% ethanol freeze dry extract of fresh berries gave the highest percentage yield (3.22%) and the highest amount of active ingredients (total monomeric anthocyanin = 5.36 ± 0.12 mg/g, total phenolic compound = 111.28 ± 2.54 mg GAE/g, cyanidin 3-glucoside = 1.72 ± 0.09 mg/g, catechin = 0.26 ± 0.02 mg/g, and quercetin = 1.06 ± 0.02 mg/g). It also showed the highest antioxidant activity by DPPH ($EC_{50} = 15.08 \pm 0.74$ μ g/ml), and high antioxidant activity on liposome assay ($IC_{50} = 50.85 \pm 6.72$ μ g/ml). Freeze-dry berries powder from aqueous extraction showed the highest amount of ascorbic acid (0.51 ± 0.04 mg/g) and gallic acid (2.26 ± 0.03 mg/g). It also showed high antioxidant activity by DPPH ($EC_{50} = 18.68 \pm 1.66$ μ g/ml). All extracts exhibited low cytotoxic activities against HeLa, COR L23 and MRC-5 ($IC_{50} > 50$ μ g/ml).

Ninety five percent ethanolic extract and water extract were chosen for large scale extraction to isolate active compounds, nutrition value analysis and stability test of the extract was also investigated.

Cyanidin 3-glucoside was isolated from 95% ethanol extract by reversed phase HPLC. Its chemical structure was elucidated by UV, ¹H NMR, MS and confirmed by spiking using HPLC in 3 different conditions.

The components of the two extracts were carbohydrate (81.6 - 82.1%), phenolics compound (8.5 - 11.13%), moisture (4.1 - 5.5%), crude protein (2.8 - 3.6%), crude fat (0.2 - 0.24%) and total ash (0.1 - 0.12%). They contained sodium (9.1 - 23.7 mg/kg), potassium (10.5 - 15.2 mg/kg), calcium (1.2 - 2.2 mg/kg) and magnesium (0.8 - 2.5 mg/kg). Crude fiber and heavy metals (lead and iron) were not found in the extracts.

The physical properties, stickiness and color changed in accelerated condition (45 °C with 75% relative humidity for 120 days) of the two chosen extracts were investigated. The amount of active ingredients such as total phenolic compound, cyanidin 3-glucoside, and quercetin content of extracts were quickly reduced and cyanidin 3-glucoside content was 0% after 30 days. The antioxidant activity of both extracts were also quickly reduced (EC₅₀ >200 µg/ml, after 45 days). However both extracts were rather stable in ambient temperature condition (25-32 °C with 55-60% relative humidity for 180 days). The stickiness of the extracts changed slowly according to time. The color slowly changed from reddish purple to dark color. The amounts of active ingredients slowly reduced (total phenolics compound were 70.3%, cyanidin 3-glucoside were 31.5% and quercetin were 60.4%) after 180 days. The antioxidant activity of the extracts was also slowly reduced (EC₅₀ = 83.8 µg/ml after 180 days).

Ninety five percent ethanolic extract was the method of choice for *C. nervosum* var. *paniala* berries as it retained the most active ingredients and antioxidant activity. It was not stable in accelerated condition, but can be kept well at 0 °C. Recommendation for future work is extraction method for *C. nervosum* var. *paniala* berries to be kept stable at room temperature.