STUDY ON BIOACCESIBILITY AND ANTIOXIDANT CAPACITY OF FLAVONOIDS IN *CITRUS HYSTRIX* USING *IN VITRO* METHODS AND THEIR ANTICLASTOGENIC POTENTIAL USING THE ERYTHROCYTE MICRONUCLEUS ASSAY IN THE MOUSE

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ABSTRACT

The objective of this study was to evaluate the effect of 3 different processing methods, fresh (F), boiling (B) and deep fat frying (DF), used on the leaf of *Citrus hystrix* on *in vitro* total antioxidant capacities (TAC), total polyphenols content, types and amounts of flavonoids and bioaccessibility. F was assessed *in vivo* for its anticlastogenic potential using the erythrocyte micronucleus assay in mice.

Water- and lipid-soluble extracts of freeze-dried samples were evaluated for antioxidant activities by 3 different assays, i.e. ORAC, FRAP, and scavenging effect on the DPPH● free radical. The results demonstrated that DF had the highest TAC values on the assays, followed by F and B samples, in order. The amount of total flavonoids as their aglycones calculated from the sum of 9 flavonoids (theobromine, cyanidin, myricetin, peonidin, quercetin, luteolin, hesperetin, apigenin and isorhamnetin) determined by HPLC was shown to be 1110 ± 74.1, 556 ± 29.7 and 1235 ± 102.5 mg/100 g solid freeze-dried weight (d.w.) for F, B, and DF samples, respectively. Hesperetin (Hesp) is the predominant flavonoid. The total phenolics content was 2.0, 1.8 and 1.9 g GAEs/100 g solid fresh weight in F, B and DF samples, respectively. Bioaccessibility was investigated by measuring the transfer of flavonoids from digesta into the aqueous fraction using an *in vitro* digestion system. Then, flavonoids uptake was evaluated by adding the aqueous fraction (from the *in vitro* digestion) to human intestinal cells (Caco-2). The results showed that Hesp was hydrolysed during the *in vitro* digestion and the bioaccessibilities of F (73 ± 28.1%), B (89 ± 15.6%) and DF (113 ± 26.2%) were not significantly different ($p \geq 0.05$). The uptake of quercetin, peonidine and Hesp by Caco-2 cells were 89 ± 10.4%, 7.9 ± 1.5% and 0.8 ± 0.4%, respectively. Regarding the antimicronucleus evaluation, F was administered by gavage to mice in two doses per day (0.2 or 0.4 g/ kg BW per day) over 14 days and their anticlastogenicity was evaluated on 7,12 dimethylbenz[a]anthracene (DMBA, 40 mg/kg BW) or mitomycin C (MMC, 1 mg/kg BW) -induced genotoxicity. Peripheral blood samples were collected at 0, 24, 48 h. following treatment. The result demonstrated that F at the dose of 0.2 g/kg BW tended to reduce the clastogenic effects in MMC-treated and DMBA-treated groups at 24 h and 48 h, respectively, after administration of clastogens, but they were not significantly different ($p \geq 0.05$).

These results suggest that processing methods, particularly boiling, can have significant effects by decreasing the content of flavonoids and TAC values in *C. hystrix* leaf. Although Hesp is the predominant flavonoid in *C. hystrix* leaf, it does not show a preferential uptake by Caco-2 cells. *C. hystrix* leaf slightly decreases the clastogenicity induced by both direct acting carcinogen, MMC and indirect acting carcinogen, DMBA at the low dose tested (0.2 g/kg BW/ day.