

## CHAPTER 6: CONCLUSION

Results from this study found that three different variety of *H. sabdariffa* used were possessed different characteristics. UKMR-2 calyx sample contain highest percentage of water followed with UKMR-1 and Arab. The average yield of crude extract from 100 g of Arab, UKMR-1 and UKMR-2 dried calyx shows that the Arab variety gives the highest percentage of crude extract yield with 5.82g of crude extract was obtained from 100g of dried calyx. Meanwhile 5.41g and 4.30g of crude extract were obtained from each 100g of UKMR-2 and UKMR-1 dried sample. *H. sabdariffa* from Arab variety also exhibit better quality as antioxidant compared to UKMR-1 and UKMR-2 variety. The water extract of Arab variety shows good free radicals scavenger and metal chelator in DPPH radical scavenging assay and metal chelating assay. However it shows poor reducing power ability reducing power assay. In contrast, the extract of UKMR-1 and UKMR-2 variety exhibit poor antioxidant in all antioxidant assays.

The present study was also designed to investigate and evaluated the toxicity effect of UKMR-1 and UKMR-2 variety compared to its parent Arab variety in rat through repeated oral administration for 14 days at dose of 500 mg/kg bw/d and 1000 mg/kg bw/d. This study found no death and no abnormalities in clinical sign, body weights and necropsy finding in all groups of rats used. Test on total renal function and total liver function also shows no sign of toxicity effect except in several mild elevation of renal and liver serum biochemical analysis of UKMR-1 variety. Thus, it is conclude that the lethal dose with 50% mortality rate (LD<sub>50</sub>) of this new UKMR-1 and UKMR-2 variety of *H. sabdariffa* and its parent Arab variety are greater than 1000 mg/kg. This study also showed that consumption of diets containing up to 1000 mg/kg bw/d of Arab, UKMR-1 and UKMR-2 aqueous

extracts for 14 days were not associated with any obvious signs of toxicity in Sprague-Dawley rats, thus the no observed adverse effect level (NOAEL) for these extracts are considered to be greater than 1000 mg/kg/day.

The anticholesterol effect study of these three varieties of *H. sabdariffa* extracts on induced hypercholesterolemic New Zealand White rabbits found that at the end of the induction period, body weight of the animals, triglyceride, total cholesterol, HDL and LDL level in blood serum were significantly increase compared to the normal control. The elevation of cholesterol level in blood serum also induces atherosclerosis development on intimal surface of aorta. Though that, repeated oral administration for 60 days at dose of 250 mg/kg bw/d of Arab and UKMR-2 extracts were resulted in slightly reduction in percentage of atheromatous plaque. Animals treated with Arab and UKMR-2 extracts were recorded  $62.68 \% \pm 3.44$  and  $64.46 \% \pm 2.19$  of aorta covered with atheromatous plaque, more 4 % and 2.5 % less than recorded by cholesterol control group. Meanwhile, hypercholesterolemic induced animals treated with UKMR-2 extract did not show any reduction in atheromatous plaque formation.

The chemical constituent in the aqueous extract of Arab, UKMR-1 and UKMR-2 also were analyzed. The chemical constituents in these extract were prescreen using TLC method before further indentified using LCMS/MS technique. Results from TLC analysis revealed those phenol and flavonoids groups are the major chemical constituent in aqueous extracts of Arab, UKMR-1 and UKMR-2. LCMS/MS analysis on aqueous extract of Arab variety found the present of gossypetin-3-O-glu-7-O-xylo, herbacetin-8-O-xylo-3-O-glu, delphinidin, delphinidin-3-sambubioside, cyanidin 3-sambubioside, and kaempferol-3-O-rutinoside were present in Arab extracts. Phytochemical analysis on UKMR-1 extract found

the present of delphinidin-3-sambubioside, hibiscetine, delphinidin, gossypetin, herbacetin-8-O-xylo-3-O-glu, and quercetin rutinoside. While UKMR-2 sample extract contains hibiscetine, delphinidin, herbacetin-8-O-xylo-3-O-glu and quercetin rutinoside.