

MALAYSIAN HERBAL MONOGRAPH

Roselle Calyx

Hibiscus sabdariffa L.

Malvaceae



(a)



(b)



(c)

(d)

Figure 1 : *Hibiscus sabdariffa* L. (a) Whole plant, (b) harvested calyces, (c) calyces and (d) flower. (Photos courtesy of Thiyaagu, MARDI, 2012)

DEFINITION

Whole or cut dried calyces and epicalyces of *Hibiscus sabdariffa* L. during fruiting.

SYNONYM

Abelmoschus cruentus (Bertol.) Walp., *Furcaria sabdariffa* Ulbr., *Hibiscus cruentus* Bertol., *Hibiscus fraternus* L., *Hibiscus palmatilobus* Baill., *Sabdariffa rubra* Kostel. [\[1\]](#)

VERNACULAR NAMES

Asam susur (Malay), lou shen hua (Chinese), pulichai kerai (Tamil), rozelle, roselle or red sorrel (English). [\[2,3\]](#)

CHARACTER

The calyx powder is reddish colour with sour smell and acidic taste.

IDENTIFICATION

Plant Morphology

The calyx is joined in the lower half to form an urceolate structure, the upper half dividing to form 5 long acuminate recurved tips. The tips have a prominent, slightly protruding midrib and a large, thick nectar gland about 1 mm in diameter. The epicalyces consist of 8 to 12 small, obovate leaflets which are adnate to the base of the calyx. The calyces and epicalyces are fleshy, dry, easily fragmented and coloured bright-red to deep-purple, somewhat lighter at the base of the inner side. [4]

Microscopy

The powder shows predominantly red coloured fragments of the parenchyma containing numerous crystal clusters of calcium oxalate and, sporadically, mucilage filled cavities, sometimes associated with polygonal epidermal cells and anisocytic stomata; numerous fragments of vascular bundles with spiral and reticulate vessels; sclerenchymatous fibres with a wide lumen; rarely, rectangular, pitted parenchymatous cells; fragments of unicellular, smooth, bent covering trichomes and occasional glandular trichomes ; rounded pollen grains with spiny exine. [4]

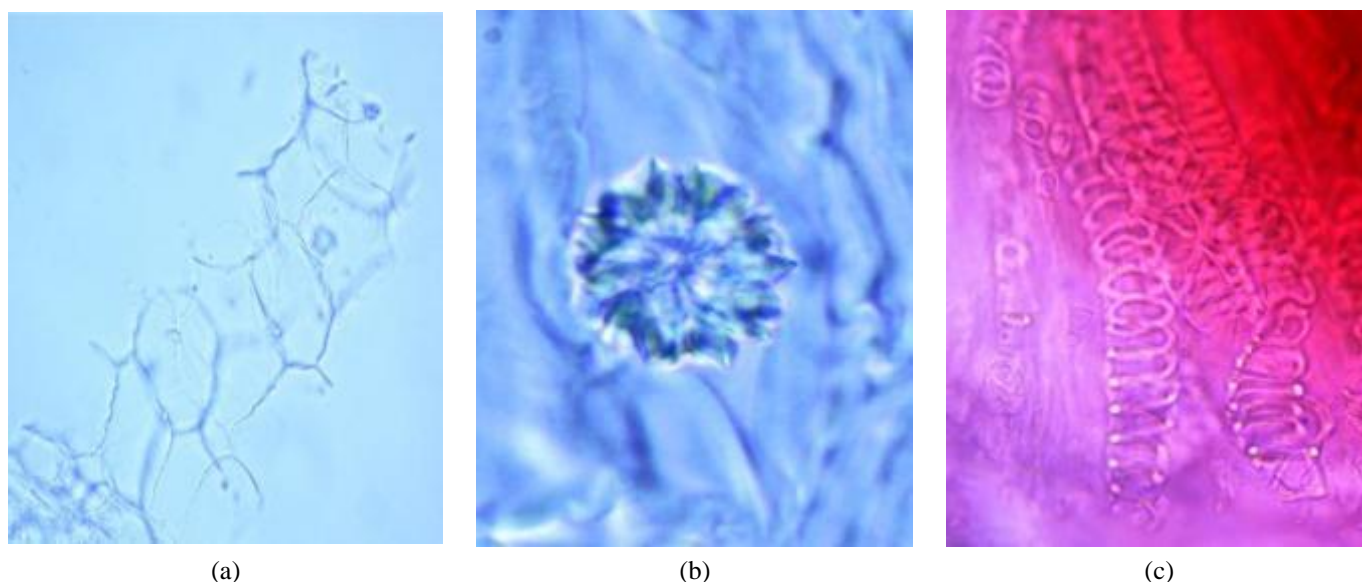


Figure 2 : Microscopic images of *Hibiscus sabdariffa* calyces powder. (a) Parenchymal cells (400X magnification), (b) cluster calcium oxalate crystals (400X magnification) and (c) spiral and reticulate vessels (400X magnification).

Colour Tests

Observed colour changes on treatment with various reagents:

H ₂ SO ₄ (conc.)	:	Brown
HCl (conc.)	:	Red
5% KOH	:	Green to dark green

Thin Layer Chromatography (TLC)

Test Solution	: Weigh about 5.0 g of of <i>H. sabdariffa</i> dried calyces powder in a 250 mL round bottom flask and add 80 mL ethanol into the flask. Reflux the sample for 1 hr and allow to cool. Filter the mixture and evaporate the solvent to dryness. Reconstitute the extract with 10 mL of methanol and use it as the test solution.
Standard Solution	: Dissolve 2.5 mg of delphinidin-3-sambubioside in 10 mL methanol to give 250 µg/mL solution.
Stationary	: HPTLC Silica gel 60 F ₂₅₄ , 10 x 10 cm

Phase

Mobile Phase : Anhydrous formic acid-water-butanol, 10:12:10 (v/v)

Pre-wash the HPTLC plate with chloroform-methanol (5:5, v/v) prior to application of the following solutions:

(a) Delphinidin-3-sambubioside standard solution (S); 5 μ L as a band

Application :

(b) Ethanol extracts of *H. sabdariffa* calyces (L); 5 μ L, as bands

Development

Distance : 8 cm (same direction with pre-wash)

Drying

: Air drying

Detection

: (a) Visible light and (b) UV 254 nm

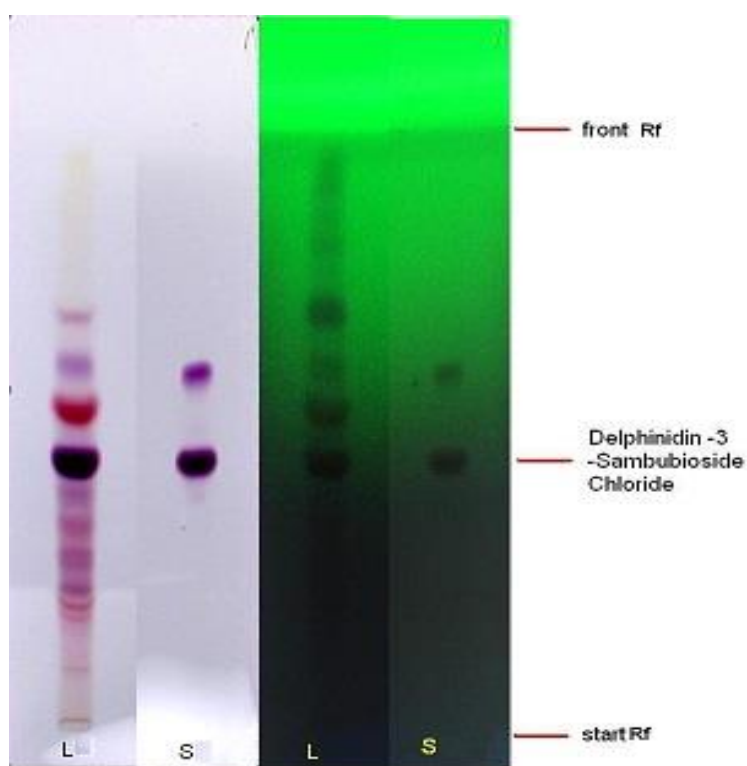


Figure 3 : TLC profiles of delphinidin-3-sambubioside standard (S) and ethanol extract of *Hibiscus sabdariffa* calyces observed under (a) visible light and (b) UV 254 nm.

High Performance Liquid Chromatography (HPLC)

Test Solution : Extract about 1.0 g of *H. sabdariffa* Linn. dried calyces powder with 8 mL of methanol in a 50 mL screw-capped conical flask. Sonicate in a water bath of 40°C for 60 min. Cool the mixture and allow the insoluble matter to settle. Filter 3 mL of the upper solution through a 0.45 μ m syringe filter and inject the filtrate into the HPLC column.

Reference Solution : Dissolve 2.5 mg of delphinidin-3-sambubioside standard with 10 mL of methanol to produce 250 μ g/mL solution.
Detector: UV 520 nm

Chromatographic System : Column: C₁₈ column (5 μ m, 4.6 mm I.D x 150 mm)

Column oven temperature: 30 °C

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Time	0.1% Formic acid in water	Acetonitrile
0.01	100	0
13.00	80	20
20.00	70	30
25.00	20	80
30.00	100	0
35.00	100	0

Perform at least five replicate injections of the delphinidin-3-sambubioside standard solution (250 µg/mL). The requirements of the system suitability parameters are as follow:

- System suitability requirements :
1. Symmetry factor (A_s) is not more than 1.5.
 2. Percentage of relative standard deviation (RSD) of the retention time (t_r) for delphinidin-3-sambubioside is not more than 2.0%.
- Acceptance criteria :
1. Retention time (t_r) of delphinidin-3-sambubioside in the test solution is similar to the t_r of delphinidin-3-sambubioside in the standard solution.
 2. The resolution (R_s) value between peak of delphinidin-3-sambubioside and peak A in the test solution should not be less than 1.5.
 3. The ultraviolet (UV) spectrum of delphinidin-3-sambubioside in the test solution is similar to the UV spectrum of delphinidin-3-sambubioside in the standard solution (optional supportive data).

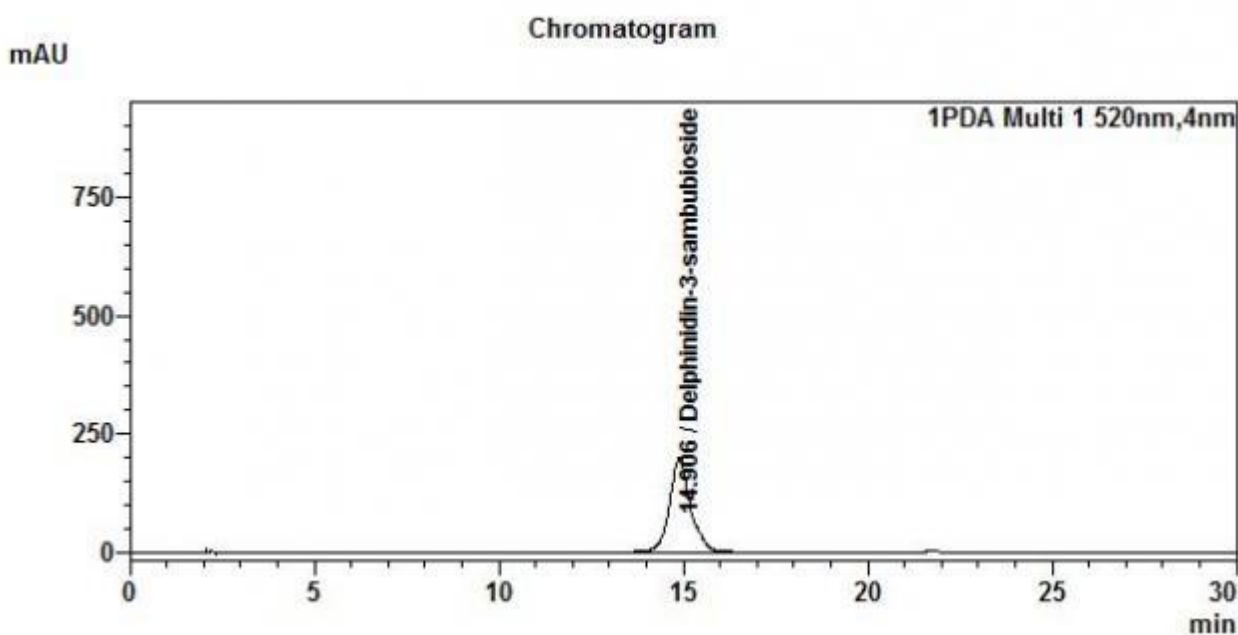


Figure 4 : HPLC chromatogram of delphinidin-3-sambubioside standard solution (250 µg/mL) at $t_r = 14.906$ min

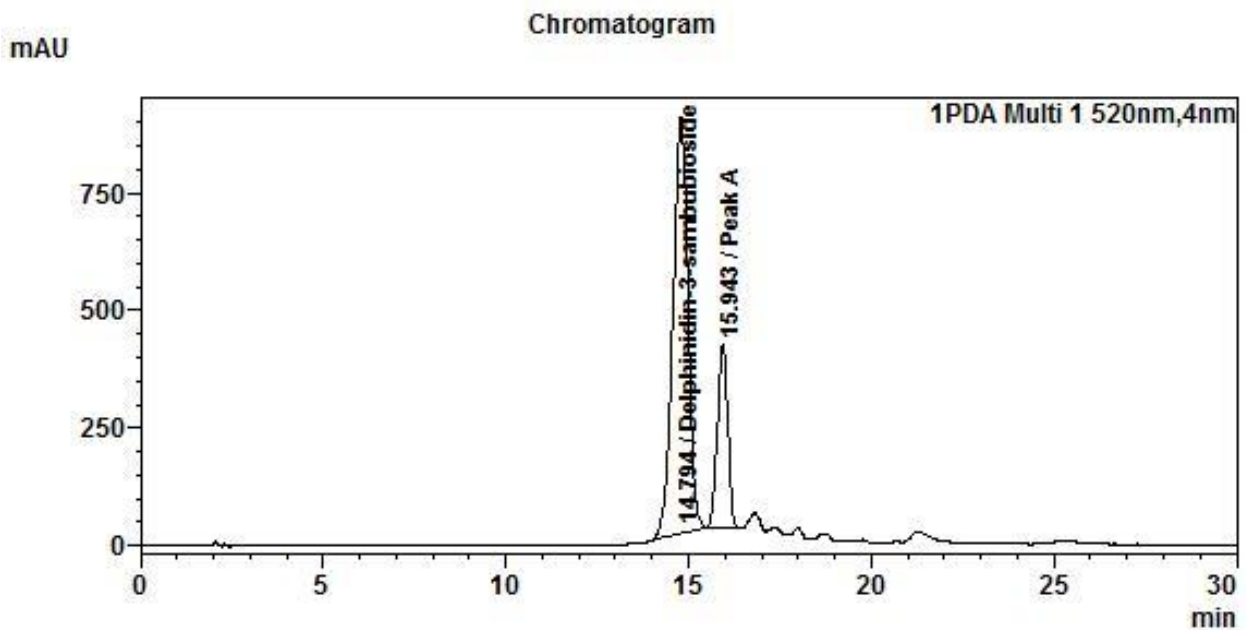


Figure 5 : HPLC chromatogram of methanol extract of *Hibiscus sabdariffa* calyces showing peaks corresponding to delphinidin-3-sambubioside (1) ($t_r = 14.736$ min) and peak A (2) ($t_r = 15.931$ min)

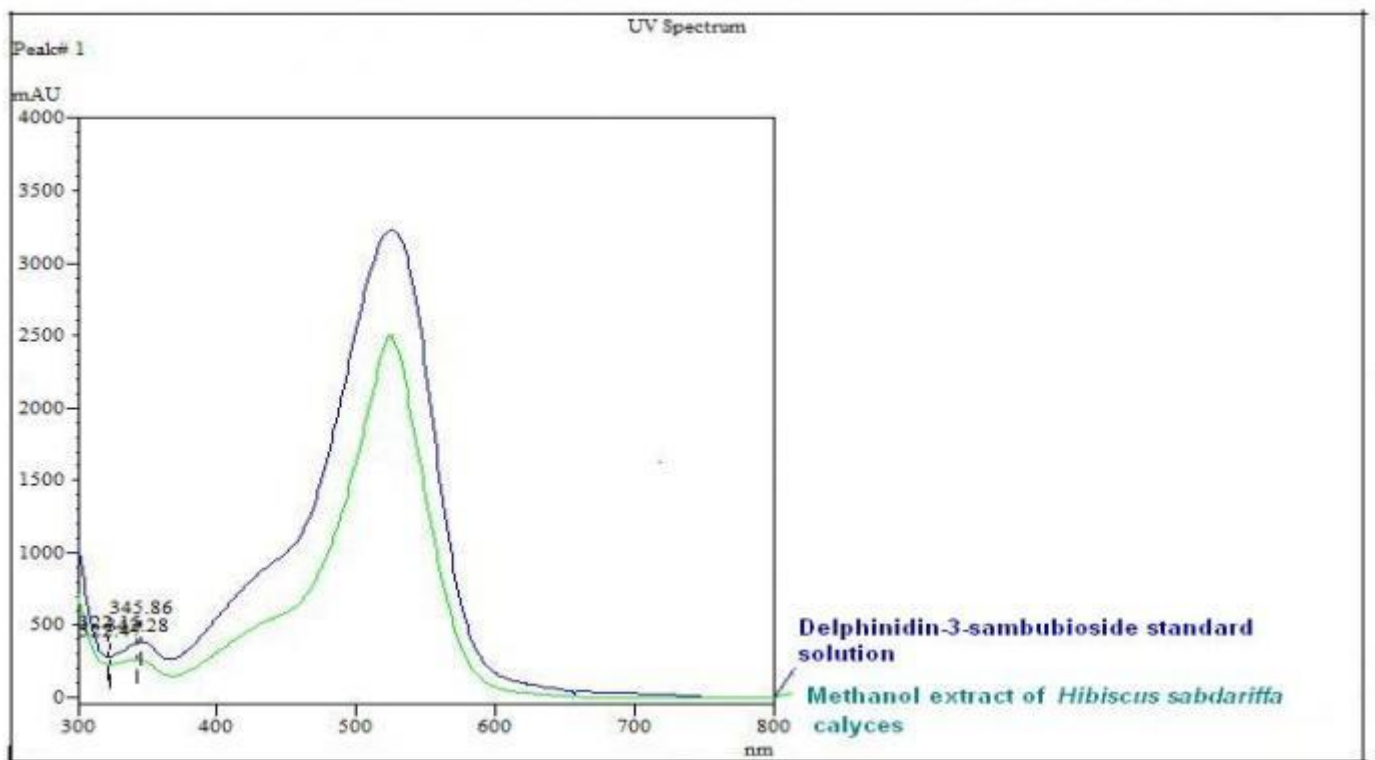


Figure 6 : UV spectrum of delphinidin-3-sambubioside standard solution (250 $\mu\text{g/mL}$) and methanol extract of *Hibiscus sabdariffa* calyces

PURITY TESTS

Foreign Matter
Not more than 2%

Ash Contents
Total ash : Not more than 7%
Acid-insoluble ash : Not more than 1%

Loss on Drying
Not more than 10%

Extractive Values
Water-soluble extracts
Hot Method : Not less than 52%
Cold Method : Not less than 48%
Ethanol-soluble extracts
Hot Method : Not less than 26%
Cold Method : Not less than 21%

SAFETY TEST

Heavy Metals
Arsenic : Not more than 5.0 mg/kg
Mercury : Not more than 0.5 mg/kg
Lead : Not more than 10.0 mg/kg
Cadmium : Not more than 0.3 mg/kg

Microbial Limits
Total bacterial count : Not more than 10^5 cfu/g
Total yeast and mould count : Not more than 10^4 cfu/g
Bile-tolerant gram negative bacteria : Not more than 10^4 cfu/g

Specific Pathogens
Salmonella : Absent in 25 g
Escherichia coli : Absent in 1 g
Staphylococcus aureus : Absent in 1 g
Pseudomonas aeruginosa : Absent in 1 g

CHEMICAL CONSTITUENTS

The calyces have been found to contain acids (e.g. citric acid, malic acid, tartaric acid, hibiscus acid, hydroxycitric acid, chlorogenic acid, chlorogenic acid isomers I and II, 5-O-caffeoylshikimic acid, protocatechuic acid), anthocyanins (e.g. delphinidin-3-O-sambubioside (hibiscin), delphinidin-3-monoglucoside, cyanidin-3-O-sambubioside, cyanidin-3-rutinoside), flavonoids (e.g. hibiscetin-3-monoglucoside (hibiscitrin), gossypetin-7-glucoside (gossypitrin), sabdaritrin, myricetin-3-arabinogalactose, quercetin-3-sambubioside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-O-rutinoside, kaempferol-3-(p -coumarylglucoside), quercetin) and others (e.g. 7-hydroxycoumarin, N-feruloytyramine, alkaloids, β -sitosterol, stearic acid, carotene, wax, mucilage, pectin, calcium citrate, hibiscin chloride). [\[5,6,7,8,9,10,11,12\]](#)

The fresh calyces had proteins, fats, fibres, minerals (e.g. iron, phosphorus, calcium, manganese, aluminium, magnesium, sodium, potassium) and vitamins (e.g. thiamine, riboflavin, niacin, ascorbic acid). [\[13,14\]](#)

MEDICINAL USES

Uses described in folk medicine

Traditionally used as a Thai traditional medicine for kidney stones. It is also used as an antibacterial, antifungal, hypocholesterolemic, diuretic, uricosuric and mild laxative. [\[15\]](#)

Biological and pharmacological activities supported by experimental data

Protective effect of diabetic nephropathy

Polyphenol from methanol extract of dried flower *H. sabdariffa* (up to 200 mg/kg) was given daily for 8 weeks via oral administration to streptozotocin-induced diabetic male Sprague Dawley rats which resulted in improved renal functions (high levels of creatinine and albumin with low level of blood urea nitrogen). Histological examination also showed lower hydropic change of renal proximal convoluted tubules in the rats. The levels of catalase and glutathione were increased whilst the levels of malondialdehyde and lipid peroxidation were lowered in the induced rats. [\[16\]](#)

Antioxidant activity

Anthocyanins of ethanol extracts of dried flower *H. sabdariffa* were able to quench the free radicals of 1, 1-diphenyl-2-picrylhydrazyl (up to 0.50 mg/mL) with an IC_{50} value of 0.02 mg/mL. Whilst the lipid peroxidation which induced by tert-butylhydroperoxide (up to 0.20 mg/mL) on hepatocytes (pre-treated with the same extract) was significantly being inhibited (lowered concentration of malondialdehyde, $p \leq 0.01$). [\[17\]](#)

Hepatoprotective activity

Ethanol extract of dried flower of *H. sabdariffa* (up to 200 mg/kg) was given daily via oral administration to male Sprague Dawley rats and showed that the histological examination revealed no hepatotoxicity and hepatotoxicity reaction was significantly reduced ($p < 0.05$), in rats pre-treated with 100 and 200 mg/kg extracts after 5 days treatments. [\[17\]](#)

Anti-inflammatory activity

Pre-incubated peripheral blood mononuclear cells with water extract of sun-dried calyces *H. sabdariffa* (up to 100 μ g/mL) showed increment on levels of interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 with the presence of H_2O_2 inflammatory stimuli, in a dose-dependent manner. Cell apoptosis was reduced. [\[10\]](#)

Anticholesterol activity

The hot water extract of *H. sabdariffa* flower was given to males New Zealand White rabbits via oral administration daily (up to 1% w/v extract) for 10 weeks exhibited decreased the levels of serum cholesterol, triglycerides and low density lipoprotein-cholesterol. [\[18\]](#)

Polyphenol from methanol extract of dried flower *H. sabdariffa* (up to 200 mg/kg) was given daily for 8 weeks continuously via oral administration to streptozotocin-induced diabetic male Sprague Dawley rats showed reduce serum levels of lipid such as total cholesterol, triglycerides and low density lipoprotein-cholesterol. [16]

Clinical Studies

In a human clinical trial, 10 healthy hospital staffs (5 adult per gender, aged 23-50 years) were ingested a single dose of 10 g of freeze-dried water extract of *H. sabdariffa* before measured levels of oxygen radical absorbance capacity (ORAC) in the plasma specimen and collected their venous bloods and plasma specimens at 0, 1.5, and 3 hour after consumption of the extract. Results showed some anti-oxidant activity in plasma. Whilst the levels of interleukin-6 and interleukin-8 remained low but level of monocyte chemoattractant protein-1 in each subject was decreased. [10]

A study of 36 healthy male Thai medical students (aged 20-30 years) who consumed daily 16-24 g/day of *H. sabdariffa* juice for 7 days showed a decreased excretion of salts in urine such as creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate, except oxalate. [19]

SAFETY INFORMATION

Preclinical toxicology

Acute toxicity

Oral single dose acute toxicity study of female Sprague Dawley rats (aged between 8 and 12 weeks old) using aqueous mixture of *H. sabdariffa* dried calyces showed no toxic effect on the parameters observed which includes behaviors, body weight, food and water intakes. All rats were observed for 14 days prior to necropsy. No death was found throughout the study period. Necropsy revealed no significant abnormality. LD₅₀ > 2000 mg/kg. [20]

Others (Adverse reaction, contraindication, side effect, warning, precaution)

Precaution

Caution use in individuals on diuretics, renally excreted medications, and/or narrow-therapeutic medications (such as digoxin, theophylline and phenytoin), as *H. sabdariffa* extracts have been reported to have a natriuretic effect. [21]

DOSAGE

Information is not available.

STORAGE

Store below 30°C. Protect from light and moisture.

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