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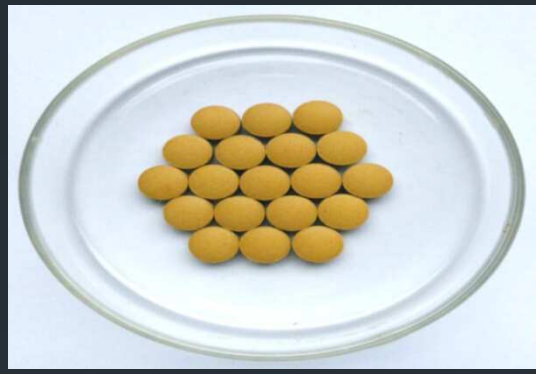
Determination of Antioxidant Activity and Acute Toxicity Study of Pericarp of *Garcinia mangostana* L. (rif*GbDcB Tablet



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INTRODUCTION

- Nowadays, the government has encouraged in Traditional Medicine production with the genuine quality, safety and efficacy.
- Myanmar possesses many indigenous medicinal plants claimed traditionally for health benefits as antioxidant.
- Among them, *Garcinia mangostana* L. (rif;*GwfoD;) are widely used for medicinal purposes.
- It was widely distributed and easily available in Myanmar.

INTRODUCTION (Cont.)

- Mangosteen pericarp is waste product but contained many active principles.
- People in Myanmar use mangosteen pericarp as folklore.[1]
- It claimed to possess mangostin compound and a variety of other xanthones from Mangosteen have been investigated for biological properties including antioxidant, anti-bacterial, anti-inflammatory, and anticancer activities.[2]

1. Ministry of Health. Collection of Commonly used herbal plants. *Health in Myanmar* 2003; 508-510.
2. Ibrahim MY, Hashim NM, Mariod AA, Moham S, Abdulla MA, Abdelwahab SI & Arbab IA. Mangostin from *Garcinia mangostana* L. An updated review of its pharmacological properties. *Arabian journal of chemistry* 2016; 9(3): 317-506.

INTRODUCTION (Cont.)

- In this study, the free radical-scavenging activity in defatted 95% ethanol extract of *Garcinia mangostana* L. pericarp was determined by using *in vitro* DPPH method which is simple and rapid.
- The antioxidant activity of *Garcinia mangostana* L. pericarp was found to be quite all dependent on mangostin and xanthones.

INTRODUCTION (Cont.)

- There is no scientific research conducted in Myanmar with manufacturing of tablets developed for large scale.
- The tablets formulation should be anti-oxidant purpose.
- That was why the tablet as alternative medicine of the *Garcinia mangostana* L. pericarp was researched in this study.

OBJECTIVE

- To determine antioxidant activity and acute toxicity of defatted 95% ethanol extract of *Garcinia mangostana* L. pericarp tablet

MATERIALS AND METHODS

Study period

- From January 2015 to December 2016

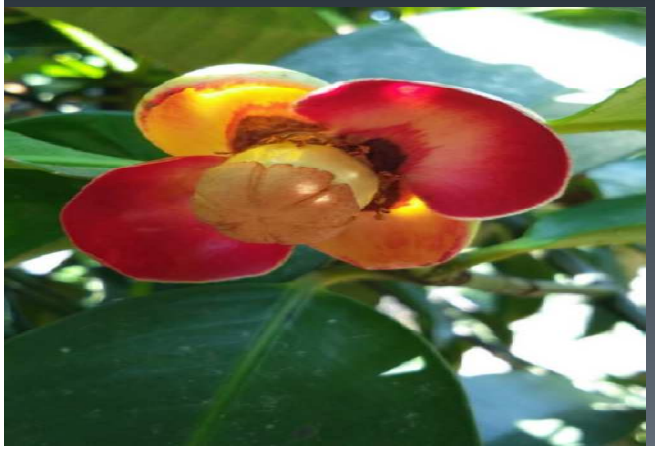
Study design

- Laboratory based experimental study

MATERIALS AND METHODS (Cont.)

Collection of plant specimens

- The plant sample, *Garcinia mangostana* L. pericarp and flowers were collected from Mawlamyaing Township, Mon State during the period of January to June.



MATERIALS AND METHODS (Cont.)

Physicochemical properties (characterization)

were conducted

Central Council for Research in Unani Medicine[3]

WHO Quality Control Methods for Medicinal Plant Materials.[4]

3. Central Council for Research in UNANI Medicine. *Physicochemical standards of Unani formulation*, part 2, New Delhi, India, 1987; 51-55.
4. World Health Organization. Quality control methods for medicinal plant materials. WHO, Geneva 2011; 1-37.

MATERIALS AND METHODS (Cont.)

Phytochemical Investigation (analysis)

was conducted

Central Council for Research in Unani Medicine[3]

Harborne.[5]

3. Central Council for Research in UNANI Medicine. *Physicochemical standards of Unani formulation*, part 2, New Delhi, India, 1987; 51-55.
5. Harborne JB. *Phytochemical Method*, 2nd ed. New York: Chapman and Hall 1998; 38: 196-223.

MATERIALS AND METHODS (Cont.)

Extraction

Garcinia mangostana L. pericarp

↓ washed, air-dried in shadow (room temperature), crushed

Dried pericarp powder

MATERIALS AND METHODS (Cont.)

100 g *Garcinia mangostana* L. pericarp powder

↓ extracted with petroleum spirit (40-60ºC) by Soxhlet apparatus (6 hours)

Filtrate

↓ evaporated by rotary evaporator (60ºC)

Residue

↓ extracted with 50%, 70%and 95% ethanol respectively by Soxhlet apparatus (6 hours)

Filtrates

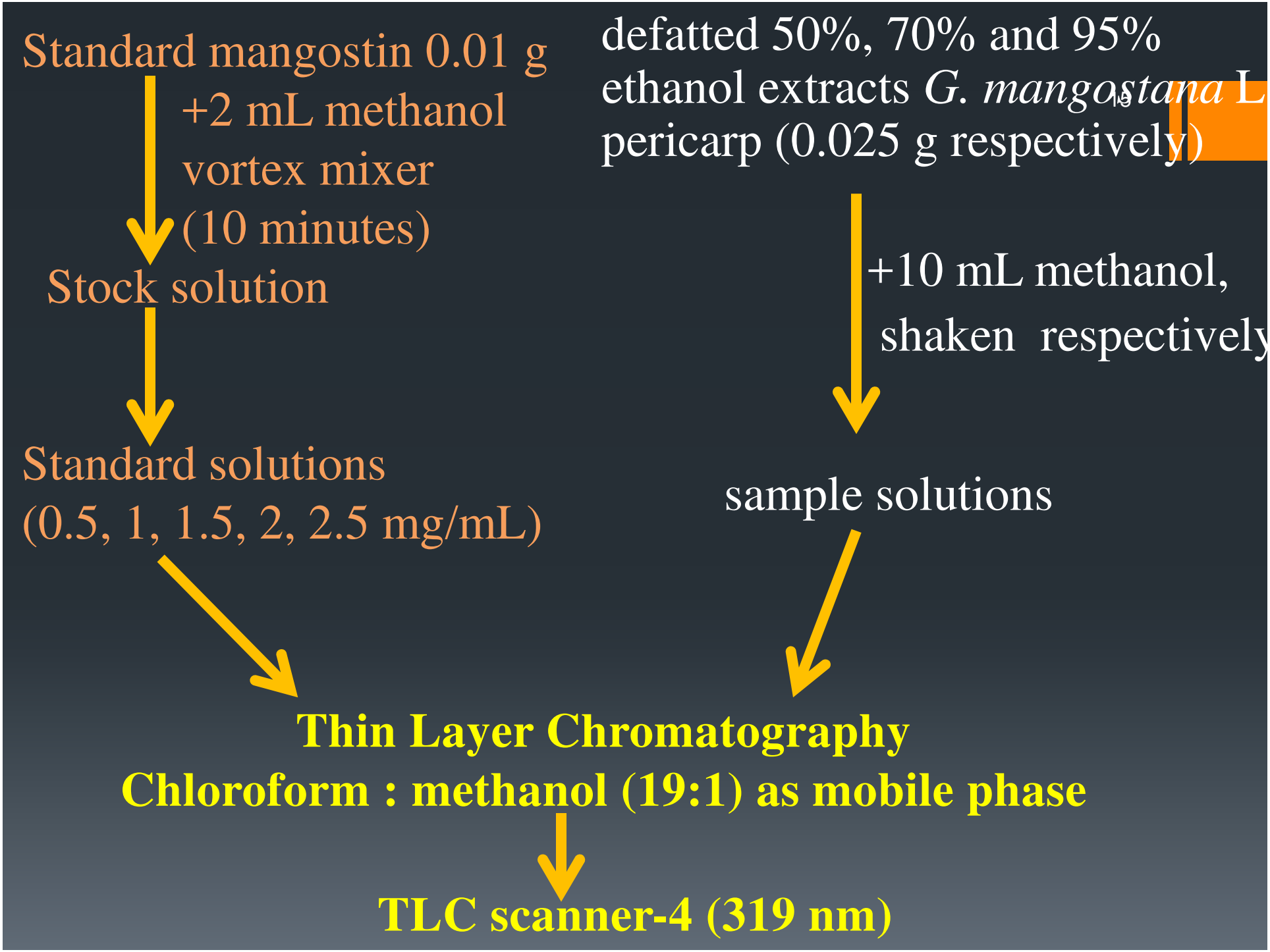
↓ evaporated by rotary evaporator (80ºC)

Extracts

MATERIALS AND METHODS (Cont.)

Evaluation of mangostin content in three different extracts of *Garcinia mangostana* L. pericarp using TLC scanner-4

6. Paramasivam M, Rajlakshmi P & Hezmanta B. Quantitative determination of mangostin in pericarp powder by HPTLC technique. *Current science* 2008; 95(11): 1529-1530.


Standard mangostin 0.01 g
+2 mL methanol
vortex mixer
(10 minutes)
Stock solution
Standard solutions
(0.5, 1, 1.5, 2, 2.5 mg/mL)
defatted 50%, 70% and 95%
ethanol extracts G. mangostana L
pericarp (0.025 g respectively)
+10 mL methanol,
shaken respectively
sample solutions
Thin Layer Chromatography
Chloroform : methanol (19:1) as mobile phase
TLC scanner-4 (319 nm)

MATERIALS AND METHODS (Cont.)

Formulation of *Garcinia mangostana* L. pericarp extract

Defatted 95% ethanol extract (150 mg) +
Microcrystalline cellulose (Avicel) (96.77 mg) [17]

↓ fluidized bed granulator

Starch (66.50 mg) + Paste starch (10.50 mg)

↓ fluidized bed granulator at (50ºC) (15 minutes)

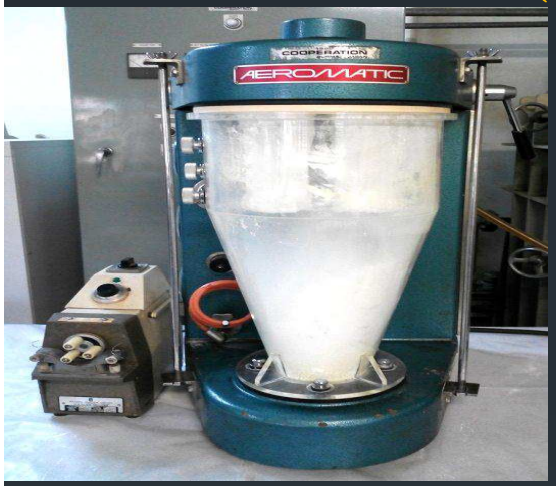
Dried granules

↓ sieved (No.16 mesh)

Glidant (aerosil) (1.75 mg) +
Croscarmellose sodium (10.50 mg) +
Magnesium stearate (3.5 mg)

↓ sieved (No.16 mesh)

Granules



Fluidized bed granulator

Rotary tableting machine

Granules

compressed at room temperature by a rotary tableting machine using 10 mm NC concave punches and dies

Tablets

MATERIALS AND METHODS (Cont.)

Determination of pharmaceutical qualities before compression of pericarp extract tablet

- flowability,
- Carr's index,
- Hausner's ratio,
- moisture content and
- particle size distribution

MATERIALS AND METHODS (Cont.)

Determination of pharmaceutical qualities after compression of pericarp extract tablet

Pharmacopoeial tests

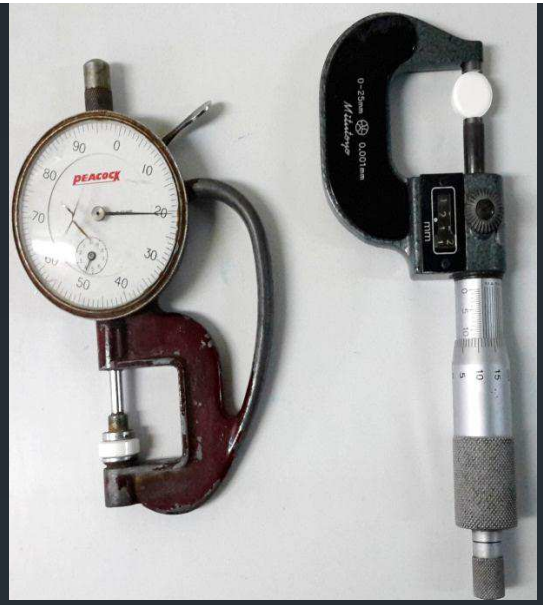
- uniformity of weight
- disintergration time
- dissolution test

Non-pharmacopoeial tests

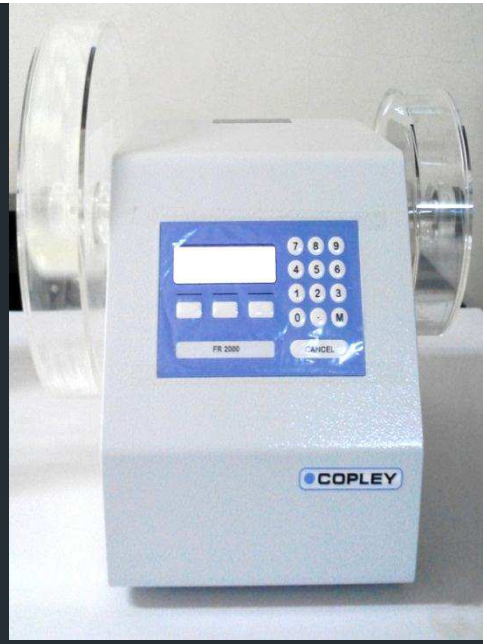
- physical character
- tablet hardness
- friability
- diameter
- thickness

7. ***The Phrmaceutical Codex*. Info Access & Distribution Pte Ltd, Great Britain. 12th ed. 1994; 2, 178-199, 277-321.**

Tablet hardness tester

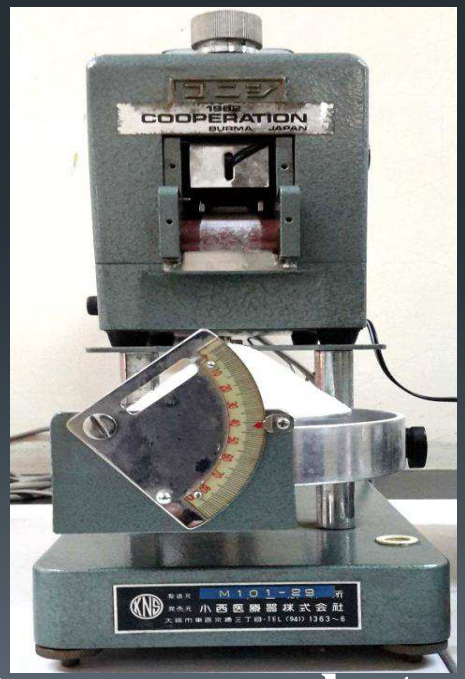


Thickness and diameter tester



Friabilator

Disintegration tester

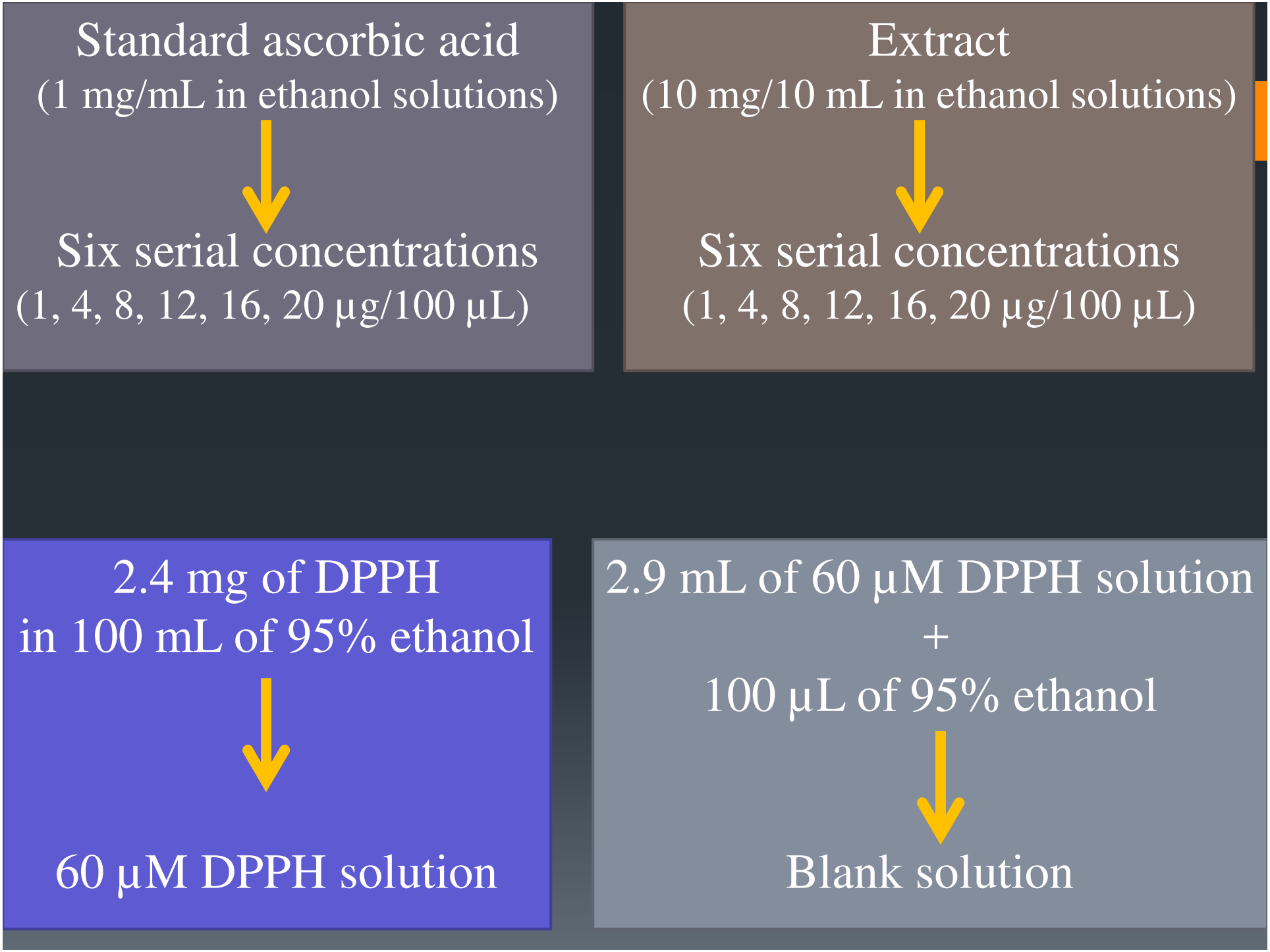


Repose angle tester

MATERIALS AND METHODS (Cont.)

Determination of *in vitro* antioxidant activity of *Garcinia mangostana* L. pericarp extract tablet

8. Khin Tar YarMyint, Mu MuSeihgnMyint, Mar MarMyint, May Aye Than, PhyuPhyu Win, Win Win Maw, Mi Aye Aye Mon & Me Me Thaw. Antioxidant activity, Total Phenolic content and ascorbic content of three different preparations of noni fruit juice. *Myanmar Health Research Journal* 2014; 26(2): 131-132.


Standard ascorbic acid
(1 mg/mL in ethanol solutions)
Six serial concentrations
(1, 4, 8, 12, 16, 20 µg/100 µL)
Extract
(10 mg/10 mL in ethanol solutions)
Six serial concentrations
(1, 4, 8, 12, 16, 20 µg/100 µL)
2.4 mg of DPPH
in 100 mL of 95% ethanol
60 µM DPPH solution
2.9 mL of 60 µM DPPH solution
+
100 µL of 95% ethanol
Blank solution

serial diluted ascorbic acid solution (100 µL respectively)

serial diluted sample solution (100 µL respectively)

1.Added 2.9 mL of 60 µM DPPH solution respectively and mixed thoroughly by a vortex mixer

2.Incubated in the dark (room temperature) (30 minutes)

UV-VIS spectrophotometer (UV – 1240) 517 nm

- Absorbance measurement was done in triplicate.
- Antioxidant activity was determined by calculating the percent inhibition and IC_{50} value by using the following formula.

$$\% \text{ inhibition} = \frac{\text{Abs blank solution} - \text{Abs of sample solution}}{\text{Abs blank solution}} \times 100$$

MATERIALS AND METHODS (Cont.)

Acute Toxicity Study of *Garcinia mangostana* L. pericarp extract tablet

- Acute toxicity test of defatted 95% ethanol extract of *G. mangostana* L. pericarp tablet on albino mice was done according to OECD guideline 423.[11]

11. Organization of Economic, Cooperative and Development. OECD guideline for testing of chemicals2001; 1-14.

Group (1) six mice	Group (2) six mice	Group (3) six mice
pericarp extract tablet 300 mg/kg body weight (orally)	pericarp extract tablet 2000 mg/kg body weight (orally)	pericarp extract tablet 5000 mg/kg body weight (orally)

- Observed first 4 hr continuously for mortality and behavior changes
- Checked the animals for fourteen days
- Noted the mortality during this period

RESULTS AND DISCUSSION

Physicochemical characterization of *Garcinia mangostana* L. pericarp included

- water and volatile matter content (17 %)
- total ash values (3.62 %)
- acid-insoluble ash (0.45 %)
- water-soluble ash (2.86 %)
- foaming index (100)
- swelling index (5 mL)
- extract values (pet-ether extract 1.22 %, ethanolic extract 10.31 %, aqueous extract 9.84 %)
- pH value (1 % solution 6.75, 10 % solution 5.615)

RESULTS AND DISCUSSION (Cont.)

The phytochemical investigation of *Garcinia mangostana* L. pericarp indicated

Present

- alkaloids
- steroids/terpenes
- flavonoids
- polyphenol
- tannins
- saponins
- amino acids
- glycosides
- carbohydrate
- reducing sugars

Absent

- cyanogenic glycosides.

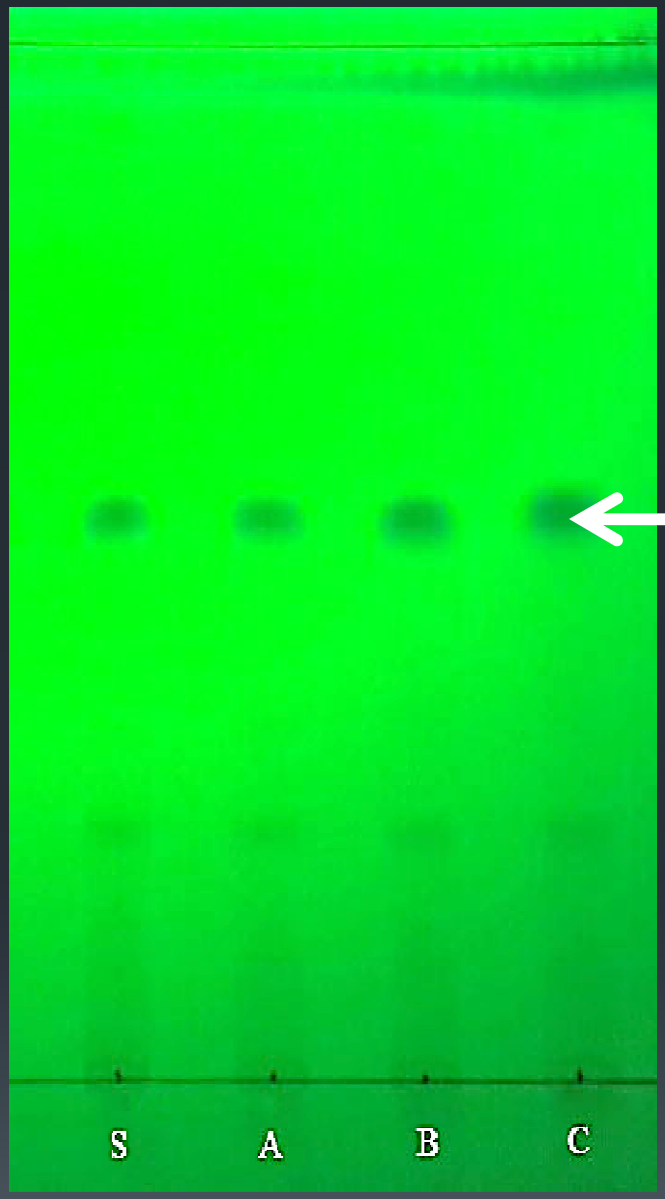
RESULTS AND DISCUSSION (Cont.)

- After defatting with petroleum ether, the defatted 95%, 70% and 50% ethanolic extract yielded 42.6%, 14.54% and 13.46% respectively.

RESULTS AND DISCUSSION (Cont.)

Evaluation of mangostin content in three different extracts of *Garcinia mangostana* L. pericarp using TLC scanner-4

- After thin layer chromatographic plate was developed in the specific solvent system, it was dried in air.
- Standard mangostin compound gave a dark spot under short wave UV light 254 nm and significant spot in each of three different extracts were same R_f value 0.44 as shown in Figure 1.
- This chromatogram was screened by TLC Scanner-4.



R_f = 0.44

S = standard mangostin
A = defatted 95% ethanol extract
B = defatted 70% ethanol extract
C = defatted 50% ethanol extract

Figure 1. Thin layer chromatogram of *Garciniamangostana*L. under UV-254 nm:

RESULTS AND DISCUSSION (Cont.)

- The wavelength of same R_f value (0.44) of the compounds were 241 nm, 257 nm, 319 nm and 353 nm as shown in Figure 2.
- It was agreed with literatures standard mangostin was 243 nm, 259 nm, 318 nm and 351 nm.[11]

11. Windholz, M. (1983) *The Merck Index*: An encyclopedia of chemicals, drugs and biologicals. 10th Edn. Published by Merck & Co., Inc. Rhaway, N.J., U.S.A., p. 818.

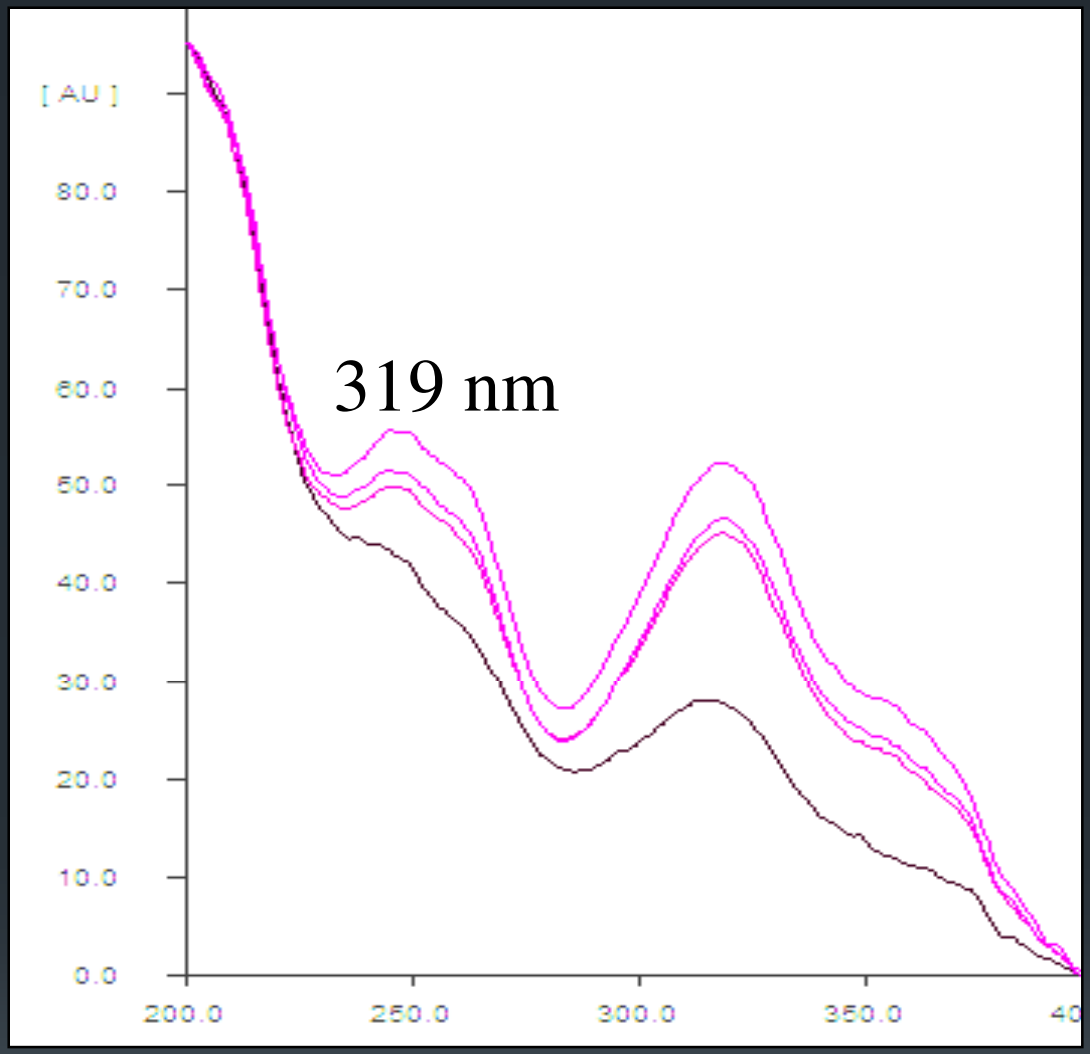


Figure 2. UV spectra of standard mangostin compound and defatted 95%, 70% and 50% ethanol extracts of *Garcinia mangostana* L.

RESULTS AND DISCUSSION (Cont.)

- Mangostin content of defatted 95% ethanol extract of *Garcinia mangostana* L. pericarp was 445.32 mg per gram of raw material, defatted 70% ethanol extract from *Garcinia mangostana* L. pericarp was 325.64 mg per gram of raw material and defatted 50% ethanol extract from *Garcinia mangostana* L. pericarp was 213.28 mg per gram of raw material, respectively.

RESULTS AND DISCUSSION (Cont.)

- Defatted 95% ethanol extract Mangosteen pericarp was compatible with each excipient used in this study.

- The results of in-process control tests for granules were within the acceptable range of specifications in The Pharmaceutical Codex.[7]

7. *The Phrmaceutical Codex*. Info Access & Distribution Pte Ltd, Great Britain. 12th ed. 1994; 2, 178-199, 277-321.

RESULTS AND DISCUSSION (Cont.)

- Formulated Mangosteen pericarp extract tablets were uniformed in shapes and color, with the limited percent for weight deviation for British Pharmacopoeia specification.

RESULTS AND DISCUSSION (Cont.)

- Physicochemical characteristics (thickness, diameter, disintegration time, hardness and friability test) for twenty tablets of formulated tablets were within the acceptable range of specifications described in The Pharmaceutical Codex[7] and British Pharmacopoeia[13] for tablets.

7. *The Phrmaceutical Codex*. Info Access & Distribution Pte Ltd, Great Britain. 12th ed. 1994; 2, 178-199, 277-321.
13. *British Pharmacopoeia.* The general medical council, The Pharmaceutical Press, HMSO. Vol5. 2015.

RESULTS AND DISCUSSION (Cont.)

Evaluation of *in vitro* antioxidant activity of defatted 95% ethanol extract of *Garcinia mangostana* L. pericarp tablet

- Antioxidant activity of defatted 95% ethanol extract of *G. mangostana* L. pericarp tablet was evaluated in comparison with ascorbic acid as a standard antioxidant agent.
- Antioxidant activity was assessed as free radical scavenging activity by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH).

RESULTS AND DISCUSSION (Cont.)

- DPPH assay method was chosen because this method was a widely used model for the evaluation of antioxidant activity since it appeared in many recent research papers. The percent inhibition of pericarp extract tablet and standard ascorbic acid were shown in Table 1 and Figure 3, 4, 5.

Wt. (µg/100 µL)	% Inhibition (Standard ascorbic acid)	% Inhibition (Tablet) [41]
1	0	0.91
4	28.05	5.81
8	58.33	8.08
12	72.47	8.17
16	93.75	11.34
20	99.03	12.70
28	100	18.51
36	100	22.23
44	100	29.58
52	100	33.39
60	100	35.30
68	100	37.30
76	100	41.83
84	100	49.36
92	100	55.96

Table 1. Comparison of Percent inhibition of different dilutions of standard ascorbic acid and tablets

Percent inhibition of standard ascorbic acid and mangosteen tablet

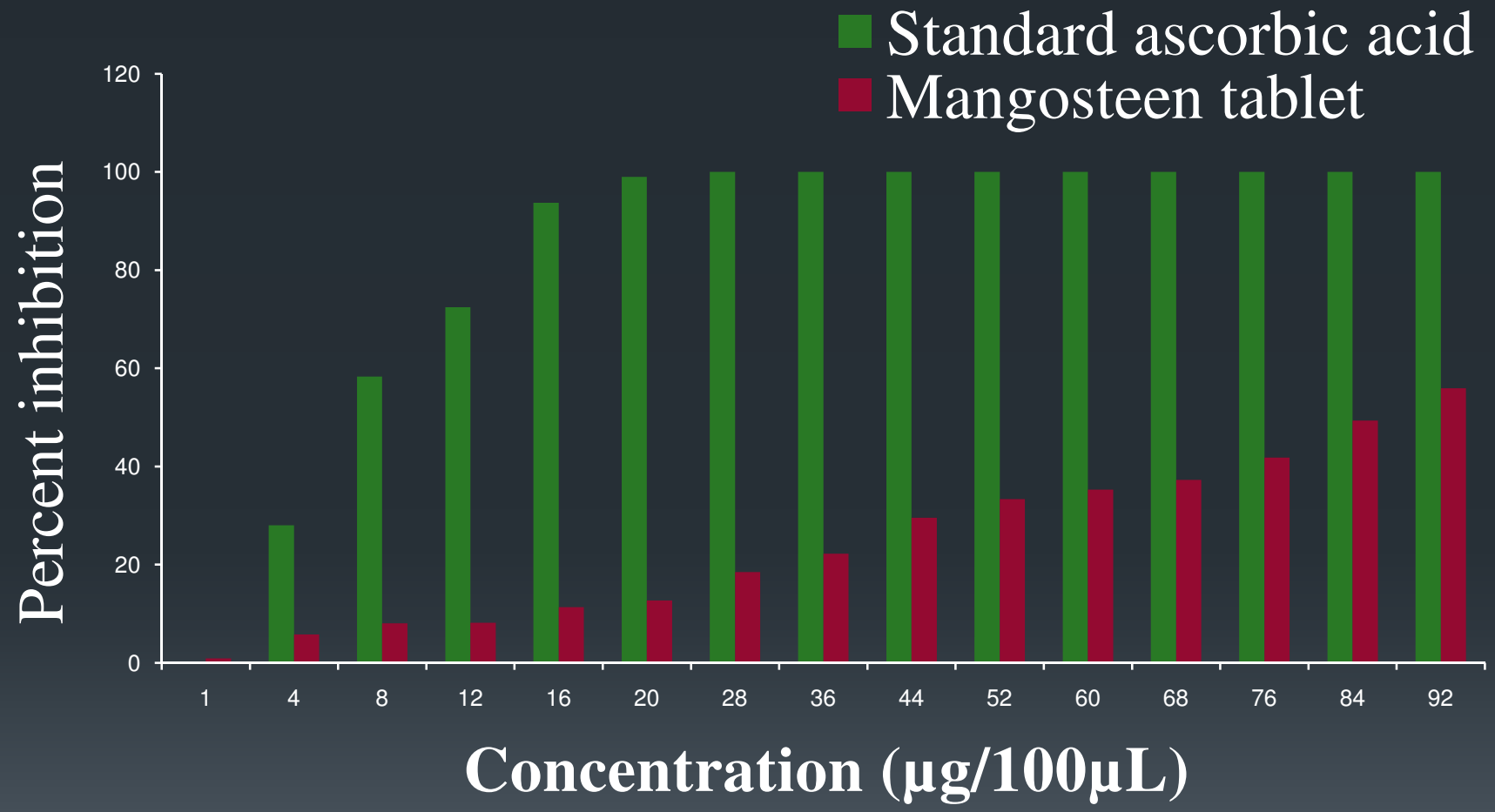


Figure 3. Comparison of Percent inhibition of different dilutions of standard ascorbic acid and tablets

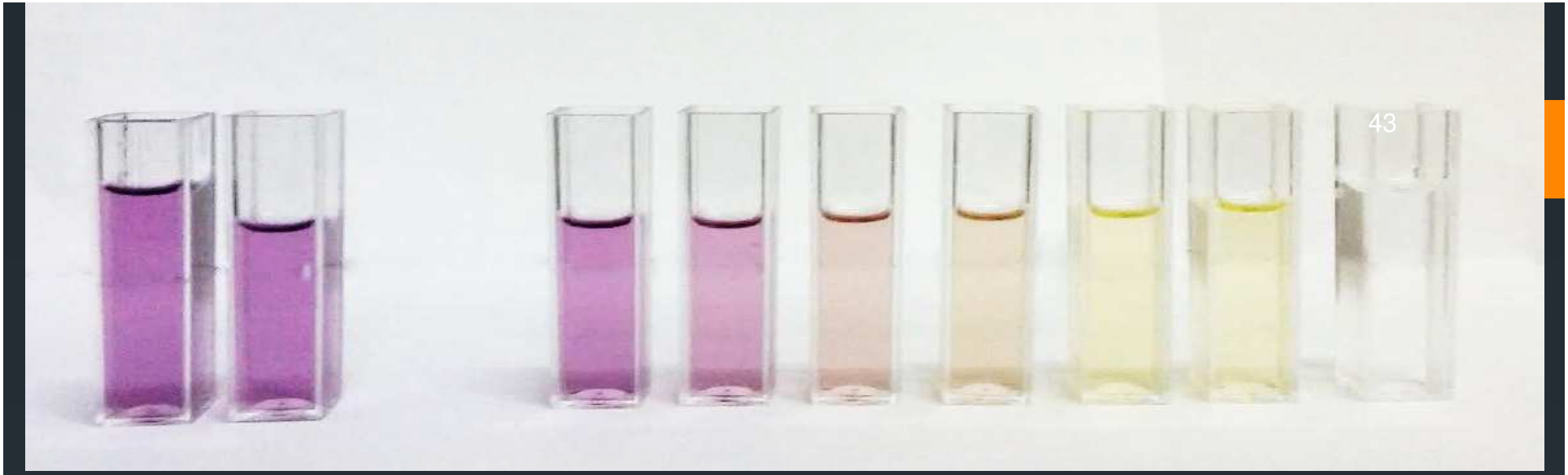


Figure 4. Free radical scavenging activity of standard ascorbic acid

Figure 5. Free radical scavenging activity of tablets

RESULTS AND DISCUSSION (Cont.)

- A lower IC_{50} indicates higher antioxidant activity of the compound.
- In this research, IC_{50} value of pericarp extract tablet was 0.85 mg/mL showed lower antioxidant activity when compared with that of standard ascorbic acid which IC_{50} value was 0.085 mg/mL.

RESULTS AND DISCUSSION (Cont.)

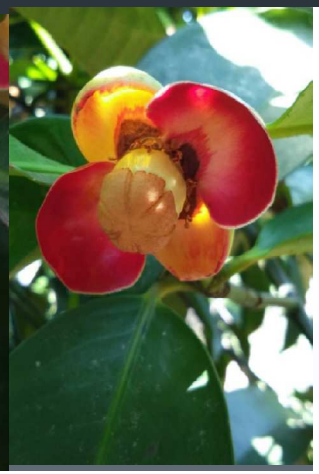
Acute toxicity study of defatted 95% ethanol extract of *Garcinia mangostana* L. pericarp tablets

- The acute toxicity test for estimation of LD_{50} of tablet was done according to the OECD 423 guideline.
- In this study, no toxic signs and lethality were found during the observation period of 14 days with the dose of 300 mg/kg, 2000 mg/kg and also at the maximum dose of 5000 mg/kg.

CONCLUSION (Cont.)

- This study showed the antioxidant activity of defatted 95%ethanol extract of *Garcinia mangostana* L. pericarp tablet which provided for pharmaceutical applications so as to contribute to health.



Thank You!

University of Pharmacy, Yangon

Pharmaceutics Department, University of Pharmacy

Since 1992, Jan 30