SCREENING OF SOME BIOACTIVITIES AND ISOLATION OF MANGOSTIN FROM *GARCINIA MANGOSTANA* L. (MANGOSTEEN) PERICARP

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Abstract

The aim of present study is to screen some bioactivities and to isolate mangostin from the pericarp of *Garcinia mangostana* L.(Mangosteen). Firstly, the phytochemical constituents were investigated by the reported chemical methods. The qualitative elemental analysis was done by EDXRF technique. The extractable matter contents were determined by extracting sample with polar solvents such as ethanol and water. In the study of the antioxidant activity, ethanol and watery extracts were screened by DPPH radical scavenging assay. The ethanol extract (IC_{50} = 8.15 µg/mL) was found to be more potent than watery extract (IC_{50} = 12.3 µg/mL) in antioxidant activity. In addition, the antitumor activity of ethanol and watery extracts was investigated by Potato Crown Gall (PCG) test and both extracts exhibited antitumor activity with the doses of 0.15, 0.1 and 0.05 g/disc. Furthermore, the mangostin (0.09 %, 180-181°C) was isolated from the ethanol extract by column chromatographic separation technique. The isolated compound was identified by spectroscopic techniques compared with authentic mangostin.

Keywords: *Garcinia mangostana* L., antioxidant activity, antitumor activity, mangostin

Introduction

Nowadays, the government has encouraged in traditional medicine products with the genuine quality, safety and efficacy. In Myanmar, many indigenous medicinal plants have been used in traditional medicine. Among them *Garcinia mangostana* L. (mangosteen) is widely used for medicinal purposes. Mangosteen is a tropical fruit originated from South East region. It is dark purple to red purple fruit. The edible fruit aril is white, soft and juicy with a sweet, slightly acid taste and a pleasant aroma. It is also known as " Queen of fruits " (Martin, 1980). It has been established that mangosteen contains a variety of active ingredients, including xanthones, phenolic acids, polysaccharides and pigments. Xanthones are the main active substances in mangosteen, where in α–mangostin is one of the most important natural xanthone derivatives (Ibrahim *et al*., 2016). Scientists who studied the properties of xanthones in mangosteen found that they exhibited potent free radical scavenging activity. Xanthones absorb free radicals and stop cellular damage that leads to many types of diseases. Several studies have shown that obtained xanthones from mangosteen have remarkable bioactivities such as antioxidant, antitumor, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral activities (Suksamrarn *et al*., 2006). The presence of these bioactivities in mangosteen makes it very potent immune system booster that promotes good health and wellness. Recently, products such as mangosteen juices or dietary supplements have begun to be wide spread around the world. In Myanmar, mangosteen is widely cultivated in Mon State and Tanin-thayi Region. The photograph of fruits *Garcinia mangostana* L. (mangosteen) is described in Figure 1. The research has focused on screening of some bioactivities and isolation of mangostin from *Garcinia mangostana* L. (mangosteen) pericarp.

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Botanical Aspects of *Garcinia mangostana* L. (mangosteen)

Genus	:	*Garcinia*
Species	:	*mangostana*
Family	:	Clusiaceae
Botanical name	:	*Garcinia mangostana* L.
English name	:	Mangosteen
Myanmar name	:	Mang khut thee
Part used	:	pericarp

Figure 1 Photograph of fruits of *Garcinia mangostana* L. (Mangosteen)

Materials and Methods

In this research work, *Garcinia mangostana* L. (Mangosteen) fruit was collected from Mawlamying Township, Mon State, during the period of September to November, 2017. After collection, the sample was identified at Botany Department, Taungoo University. The pericarp of the fruit was separated from edible part and dried at room temperature. The dried sample was ground into powder form and stored in airtight container to prevent moisture changes and other contaminations. The reagents used in this research were analar grade chloroform, methanol, ethanol, 2,2-diphenyl-1-picryl hydrazyl, DPPH (BDH), ascorbic acid (BDH), dimethyl sulfoxide, DMSO (BDH), sodium hypochlorite, iodine and agar powder. The instruments used were UV - visible Spectrophotometer (Shimadzu UV-240) , Shimadzu FT IR- 8400 Spectrophotometer and EDXRF (Shimadzu EDX-7000 spectrometer).

Phytochemical Investigation of Pericarp of *Garcinia mangostana* L.

In order to find out the types of phytochemical constituents present in pericarp of *G.mangostana*, phytochemical investigation was carried out by chemical methods (Harborne, 1984; Robinson, 1983; Vogel, 1966).

Qualitative Elemental Analysis of Pericarp of *Garcinia mangostana* L.

The elemental contents in pericarp of *G.mangostana* were determined by EDXRF spectrometer. For this measurement, pellet of the sample (2.5 cm diameter) was first made. X-ray fluorescence spectrometer (Shimadzu EDX-7000) can analyze the element from Na to U under vacuum condition. It uses x-ray to excite an unknown sample. The sample was placed in the chamber and pumped up to vacuum. The vacuum pressure was about 38 Pa and the detector temperature is about 170 ºC. Therefore, liquid nitrogen needs to be added at the time of analysis. The measurement condition of x-ray spectrometer was used Rh target. The sample was run for a counting time of about 100 s and the spectrum obtained was stored and analyzed in PC based multi-channel analyzer using EDX-7000 software.

Determination of Extractable Matter Contents of Pericarp of *Garcinia mangostana* L.

Dried powdered sample (100 g) was percolated with ethanol (400 mL) for three days and then filtered. The same procedure was repeated three times. The combined filtrate was

concentrated under vacuum rotatory evaporator. The dried filtrate was transferred to a weighed porcelain basin and evaporated to dryness on water-bath to obtain ethanol extract.

For water soluble extractable matter, dried powdered sample (100 g) was boiled with distilled water (300 mL) for 2 h. The extract was then filtered and transferred to a weighed porcelain basin and evaporated to dryness on water-bath. The dried filtrate was placed in oven, maintained till constant weight, at 100 °C. The two extracts were stored in a desiccator containing dry silica gel prior using in each experiment.

Investigation of Bioactivities of Pericarp of *Garcinia mangostana* L.

Investigation of antioxidant activity by DPPH assay

The antioxidant activity of ethanol and watery extracts of pericarp of *G. mangostana* was determined by radical scavenging (DPPH) assay. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol. The sample solution was also prepared by mixing thoroughly 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature and shaken on shaker for 30 min. The absorbance was measured at 517 nm using UV-7504 spectrophotometer. Absorbance measurements were done in triplicate for each sample and the mean values so obtained were used to calculate % radical scavenging activity (% RSA) by the following equation:

$$\% \text{ RSA} = [\{(A_{DPPH} - A_{Sample}) - A_{Blank}\} / A_{DPPH}] \times 100$$

A_{DPPH} = the absorbance of the control solution

A_{Sample} = the absorbance of the sample solution

A_{Blank} = the absorbance of the blank solution

Then, IC_{50} value was calculated by linear regressive excel program and the mean % RSA and standard deviation was also calculated by excel program.

Investigation of antitumor activity by Potato Crown Gall test

Tumor producing bacteria, *Agrobacterium tumefaciens* (Smith and Townsend) Conn. was obtained from the extraction of the leaf of *Sandoricum koetjape* Merr. (Thitto), family Meliaceae. All of these strains have been maintained as solid slants under refrigeration. For inoculation into the potato discs, 48 h broth culture containing 9×10^9 cell/mL was used by the method of Galsky *et al*., 1980. Tubers of moderated size obtained from fresh potato were surface sterilized by immersion in 50 % sodium hypochlorite (clorox) for 20 min. The ends were removed and soaked for 10 min more in clorox. A core of the tissue was extracted from each tuber by using surface sterilized (ethanol and flame) 1.0 cm wide cork borer. Pieces of 2 cm were removed from each end and discarded. The remainder of the cylinder is cut into 0.5 cm thick discs with a surface sterilized cutter. The discs were then transferred to agar plants (1.5 g of agar was dissolved in 100 mL distilled water, autoclaved for 20 min at 121°C, 20 mL was poured into each petri dish). Each plate contained three potato discs and three plates were used for each sample dilution.

Samples (0.15 g, 0.1 g, 0.05 g) of ethanol and watery extracts were respectively dissolved in dimethyl sulphoxide (DMSO) (2 mL) and filtered through Millipore filter (0.22 μm) into sterile tube. Each solution (0.5 mL) was added to sterile distilled water (1.5 mL) and broth culture (2 mL) of *A.tumefaciens* strains. Controls were made in this way, DMSO (0.5 mL) and

sterile distilled water (1.5 mL) were added to the tube containing broth culture (2 mL) of *A.tumefaciens* strains. By using a sterile disposable pipette, one drop (0.5 mL) from these tubes was used to inoculate each potato disc spreading it over the discs surface. After inoculation, petri dishes were sealed by paraffin and incubated at 27-30 °C for 3 days. Tumors were observed on potato discs after 3 days under stereomicroscope followed by staining with Lugol's solution (5 % I_2 and 10 % KI) after 30 min and compared with control. The antitumor activity was examined by observation of crown gall produced or not.

Isolation and Identification of Phytoconstituent from the Ethanol Crude Extract

The ethanol crude extract was subjected to chromatography over a silica gel column. The column was initially eluted with $CHCl_3$:CH_3OH (19:1 v/v) solvent system and the fractions were collected at the rate of one drop per second. Gradient elution was performed successively with $CHCl_3$:CH_3OH (19:1, 9:1, 4:1 and 1:1). A quantity of 10 mL was collected for each fraction and chromatographic separation was monitored by TLC. Spots on TLC were examined under UV lamp (254 and 365 nm). Fractions that showed similar TLC pattern were combined to provide four main fractions. Fraction F_2 was crystallized in benzene to provide yellow crystalline solid compound.

The isolated compound was characterized by melting point determination, TLC examination and spectroscopic techniques such as Shimadzu UV-240 UV–visible Spectrophotometer and Shimadzu FT IR-8400 Fourier Transform Infrared Spectrophotometer.

Results and Discussion

Phytochemicals Present in Pericarp *Garcinia mangostana* L.

Phytochemicals or phytoconstituents are non-nutrient plant chemical compounds and are responsible for protecting the plant against microbial infections (Doughari *et al*., 2009). The phytochemical investigation of pericarp of *G.mangostana* showed that α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, saponins, tannins, steroids and terpenoids were present. However, alkaloids, cyanogenic glycosides, reducing sugars and starch were not detected.

Some Elements Present in Pericarp of *Garcinia mangostana* L.

The relative abundance of elements in dried powder sample was determined by using EDXRF spectrometer and the EDXRF spectrum is described in Figure 2. It can be found that the sample contains K (0.354 %), S (0.074 %), Ca (0.046 %), Mn (0.003 %), Fe (0.003 %), Cu (0.001 %) and Zn (0.001 %) respectively. According to the results, potassium was found to be the principal element. Potassium is a vasodilator, meaning that it relaxes blood vessels and by rigidity, it increases blood flow and reduces the strain on the cardiovascular system. It is an essential component of neural activity and the passage of fluid and blood in the brain.

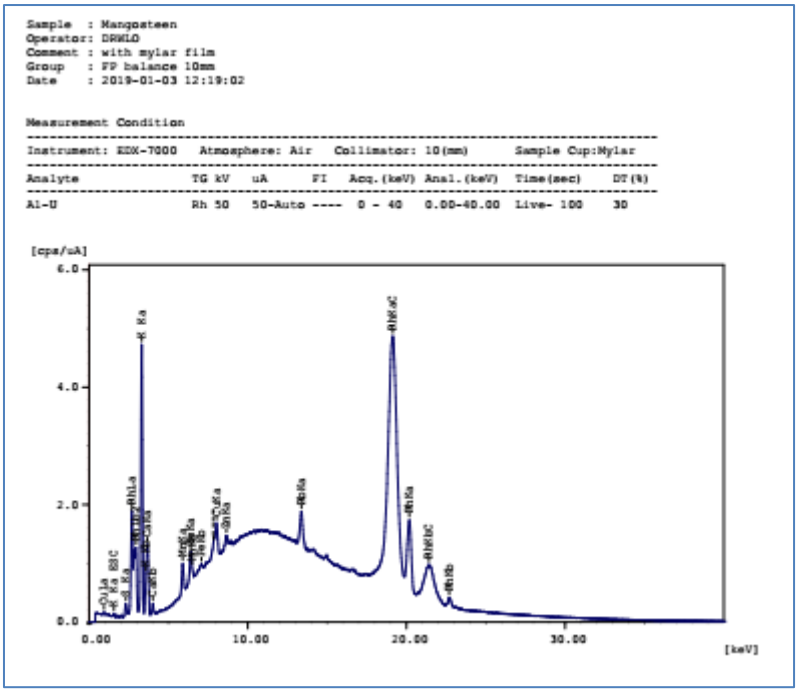


Figure 2 EDXRF spectrum of pericarp of *Garcinia mangostana* L.

Table 1 Some Elements Present in Pericarp of *Garcinia mangostana* L.

No.	Elements	Relative Abundance (%)
1	K	0.354
2	S	0.074
3	Ca	0.046
4	Mn	0.003
5	Fe	0.003
6	Cu	0.001
7	Zn	0.001

Extractable Matter Contents of Pericarp of *Garcinia mangostana* L.

After performing the preliminary phytochemical tests, it is required to investigate some organic constituents present in pericarp of *G.mangostana*. Ethanol and watery crude extracts were prepared and the percentage of crude extract yield was derived from the weight of dried and ground plant material. According to the results, it was observed that the yield of ethanol extract (15.2 %) was higher than that of watery extract (12.3 %). It can be concluded that the amount of active constituents contained in ethanol extract was higher than watery extract.

Antioxidant Activity of Pericarp of *Garcinia mangostana* L.

Antioxidant activity screening of ethanol and watery extracts of pericarp of *G. mangostana* was carried out by determining the DPPH free radical scavenging property using the UV spectroscopic method. DPPH free radical scavenging assay has been widely used especially evaluation of antioxidant potential in food system. DPPH radical is reduced to the corresponding to hydrazine when it reacts with hydrogen donors that can be phenolic compounds. Plant phenolic contributes one of the major groups of antioxidants acting as free

radical terminators. In this study, six different concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/mL) of each crude extract were prepared by serial dilution method. Ascorbic acid was used as standard to be compared with the samples and ethanol without sample was employed as control. After mixing with the DPPH solution, the absorbance of each solution was measured at 517 nm using UV-visible spectrophotometer.

For both extracts, the percent inhibition of DPPH radical was plotted as a function of concentration in order to determine the IC_{50} value, which is defined as the necessary sample concentration to reduce 50. The results of % RSA and IC_{50} were shown in Figure 3 and Table 2. According to the results, it was observed that ethanol extract (IC_{50} = 8.15 µg/mL) of pericarp of mangosteen contained higher scavenging activity of DPPH radical in comparison with the watery extract (IC_{50} = 12.3 µg/mL), due to the lower value of IC_{50}. The ethanol extract contains a high quantity of bioactive compounds able to capture free radicals like DPPH. However, it was observed that ethanol extract has the lower radical scavenging activity than standard ascorbic acid (IC_{50} = 4.52 g/mL).

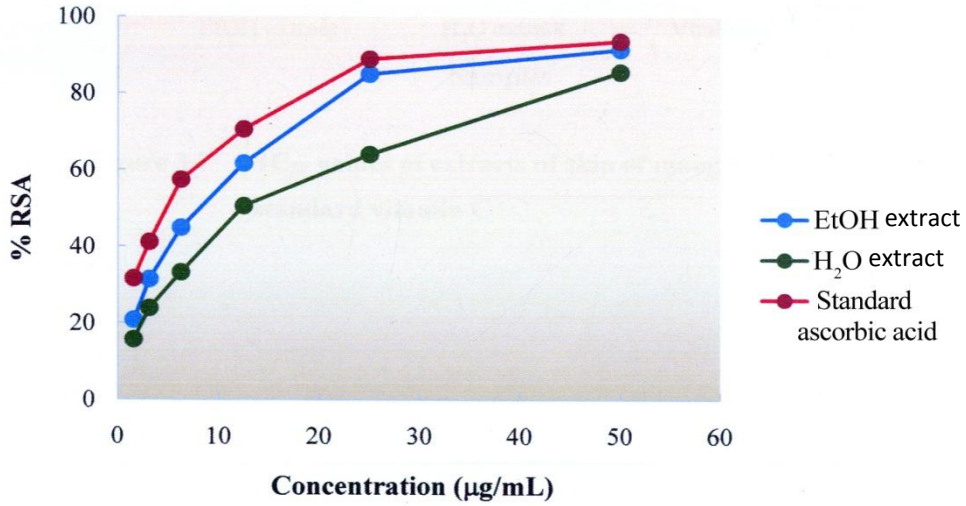


Figure 3 Percent radical scavenging activity vs concentration of crude extracts of *Garcinia mangostana* L. pericarp and standard ascorbic acid

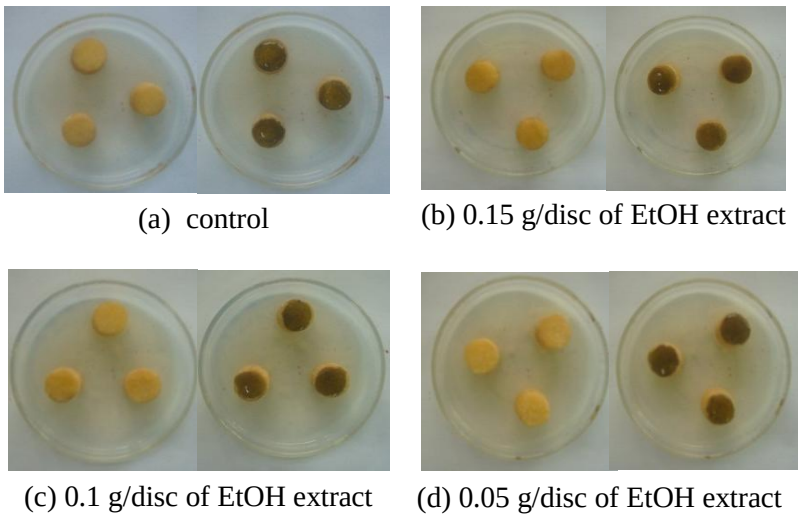
Table 2 Percent Radical Scavenging Activity and IC_{50} Values of Crude Extracts of *Garcinia mangostana* L. Pericarp and Standard Ascorbic acid

Sample	% RSA ± SD at different concentrations (µg/mL)						IC_{50} (µg/mL)
	50	25	12.5	6.25	3.125	1.5625	
EtOH extract	91.46 ± 0.39	84.99 ± 0.97	61.71 ± 0.78	44.90 ± 1.56	31.40 ± 3.51	20.80 ± 2.92	8.15
H_2O extract	85.54 ± 0.58	64.05 ± 2.14	50.55 ± 1.75	33.20 ± 2.92	23.83 ± 3.31	15.70 ± 3.51	12.3
Ascorbic acid	93.66 ± 0.78	88.98 ± 0.39	70.66 ± 0.97	57.44 ± 2.53	41.18 ± 3.31	31.68 ± 2.34	4.52

Antitumor Activity of Pericarp of *Garcinia mangostana* L.

The antitumor activity of ethanol and watery extracts of mangosteen pericarp was investigated by Potato Crown Gall test with *Agrobacterium tumefaciens* (Smith and Townsend) Conn. isolated from *Sandoricum koetjape* Merr. (Thitto) leaf. For inoculation of the potato discs, 48 h broth cultures containing $5x10^9$ cells/ mL were used. The tested samples were dissolved in DMSO and the diluted samples were mixed with the bacterial culture for inoculation. After preparing the inoculations, the bacterial suspensions was inoculated on the cleaned and sterilized potato discs, and incubated for 3 days at room temperature. After that, the tumor on potato discs were checked by staining the Knob with Lugol's solution (5 % I_2 and 10 % KI). In control disc, formation of white Knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The tested samples have no tumor formation on the potato discs and their surfaces remained blue as shown in Figures 4 and 5. In this study, it was found that ethanol and watery extracts of mangosteen pericarp were effective for preventing the tumor formation with the doses of 0.15, 0.1 and 0.05 g/disc and the results are shown in Tables 3 and 4. This experiment revealed that ethanol and watery extracts of mangosteen pericarp can inhibit the tumor growth.

Antitumor potato disc assay is valuable tool that indicates antitumor activity of test by their inhibition of formation of characteristic crown galls induced in wounded potato tissues by *A.tumefaciens* (Smith and Townsend) Conn. Tumor formations were inhibited by the plant extracts only for the presence of bioactive compounds (Inayatullah *et al*., 2007). Potato disc bioassay was based on *A.tumefaciens* (Smith and Townsend) Conn. infection on potato disc; it becomes useful for checking antitumor properties of biological and synthetic bioactive compounds. This assay has advantages of being short period, inexpensive, simple and reliable pre-screen for antitumor activity.



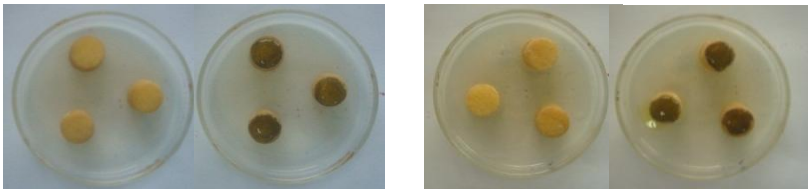
(a) control (b) 0.15 g/disc of EtOH extract

(c) 0.1 g/disc of EtOH extract (d) 0.05 g/disc of EtOH extract

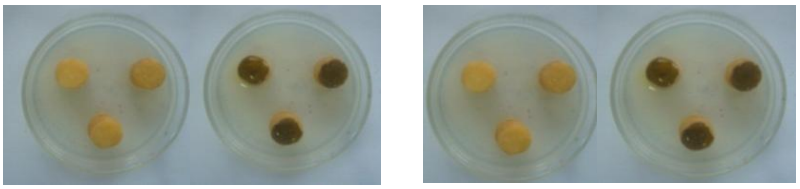
Figure 4 Tumor inhibition by EtOH extract of pericarp of *Garcinia mangostana* L.

Table 3 Antitumor Activity of EtOH Extract of *Garcinia mangostana* L. Pericarp

No.	Tested sample	Concentration of sample (g/disc)	Tumor	Remarks
1.	Control	0	++	Tumor occur significantly
2.	EtOH	0.15	-	No tumor occur
3.	EtOH	0.1	-	No tumor occur
4.	EtOH	0.05	-	No tumor occur



(a) control (b) 0.15 g/disc of H_2O extract



(c) 0.1 g/disc of H_2O extract (d) 0.05 g/disc of H_2O extract

Figure 5 Tumor inhibition by H_2O extract of pericarp of *Garcinia mangostana* L.

Table 4 Antitumor Activity of H_2O Extract of *Garcinia mangostana* L. Pericarp

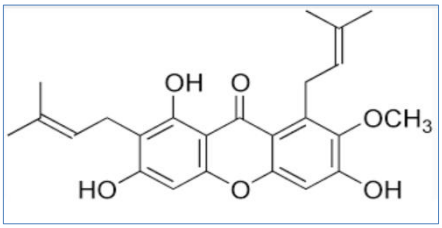
No.	Tested sample	Concentration of sample (g/disc)	Tumor	Remarks
1.	Control	0	++	Tumor occur significantly
2.	H_2O	0.15	-	No tumor occur
3.	H_2O	0.1	-	No tumor occur
4.	H_2O	0.05	-	No tumor occur

Identification of the Isolated Compound

The yellow crystalline solid compound (0.09 % yield) was isolated from fraction F_2 of the ethanol extract on silica gel by column chromatography using $CHCl_3$:MeOH (9:1) solvent system. The melting point of isolated compound was found to be 180-181 °C which consistent with reported value of mangostin (Windholz, 1983). Isolated compound provided deep blue colour with 5 % ferric chloride solution and its R_f value was 0.44 in $CHCl_3$:CH_3OH (9:1) solvent system. The ultraviolet spectrum of isolated compound in methanol solvent is shown in Figure 6. The wavelengths of maximum absorption were found to be 241, 257, 319 and 353 nm which agreed with reported value of mangostin (Windholz, 1983).

The FT IR spectrum of isolated compound is shown in Figure 7. Absorption bands appeared at 3419 cm^{-1} and 3255 cm^{-1} were attributed to O-H stretching vibration of phenolic OH

group. Aliphatic C-H stretching vibrations of CH_3 and CH_2 groups were observed at 2962 cm^{-1}, 2918 cm^{-1} and 2852 cm^{-1}, respectively. The C=O stretching vibration of xanthone ring observed at 1643 cm^{-1}. Absorption bands at 1610 cm^{-1} and 1460 cm^{-1} were assigned to the aromatic ring stretching vibration. The O-H bending vibration was observed at 1377 cm^{-1} and stretching vibration of C-O group was observed at 1284 cm^{-1}. The absorption band at 852 cm^{-1} was assigned as aromatic C-H out of plane bending (Silverstein and Webster, 1998). According to the melting point determination, UV and FT IR spectral data, isolated compound may be identified as mangostin with the following molecular structure. It was further confirmed by Co TLC with authentic mangostin.



(Mangostin)

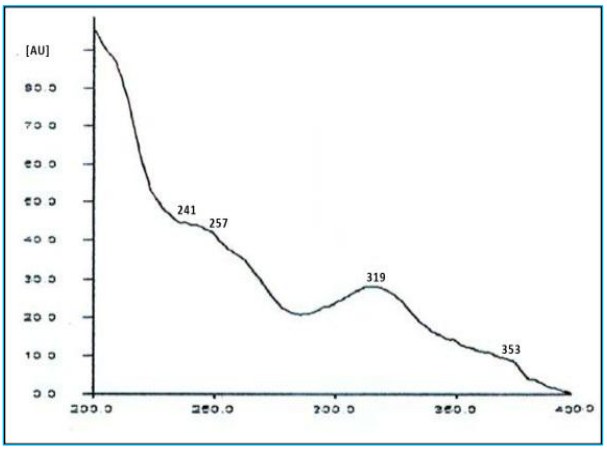


Figure 6 UV spectrum of the isolated compound (MeOH)

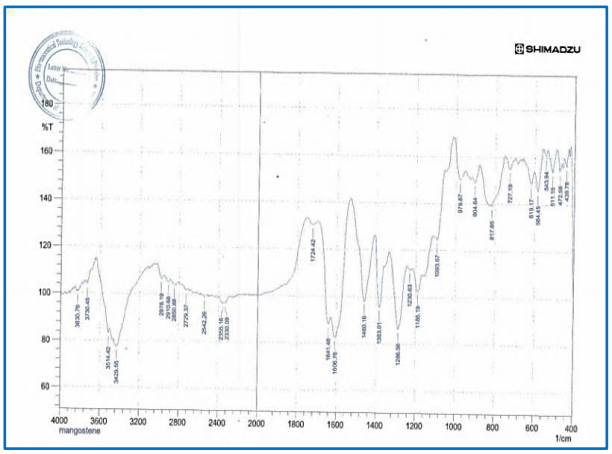


Figure 7 FT IR spectrum of the isolated compound (KBr)

Conclusion

From the preliminary phytochemical investigation, it was found that α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, saponins, tannis, steroids and terpenoids were present in pericarp of mangosteen. However alkaloids, cyanogenic glycosides, reducing sugars and starch were not detected. In the qualitative elemental analysis, content of K is more significant than other elements such as S, Ca, Mn, Fe, Cu and Zn. In extractable matter contents, the percentage of ethanol extract (9.34 %) was found to be higher than watery extract (5.98 %). In screening of antioxidant activity using DPPH assay, it was observed that free radical scavenging efficacy of ethanol extract (IC_{50} = 8.15 µg/mL) was better than watery extract (IC_{50} = 12.3 µg/mL). The high antioxidant activity of the ethanol extract at low concentration makes the pericarp of mangosteen as a potential source of antioxidant. In addition, the antitumor activity of ethanol and watery extracts was also screened by Potato Crown Gall test. In this study, both extracts were effective for preventing the tumor growth with

the doses of 0.15 , 0.1 and 0.05 g/disc. On silica gel column chromatographic separation, mangostin (0.09 %, 180-181 °C) was isolated from ethanol extract and then identified by UV and FT IR spectroscopic methods compared with authentic mangostin. According to the results obtained from this research, it can be hoped that the waste product of mangosteen pericarp is beneficial to use for the people. In Myanmar, mangosteen is locally abundant and seasonally available in markets. So, mangosteen should be consumed for health.

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