

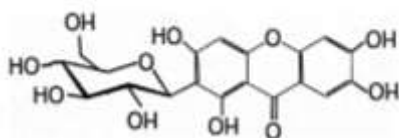
Isolation and Structural Identification of Mangiferin Compound from Natural Medicine of mingut-thee-hsays

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Abstract

Mangosteen fruit contains these powerful antioxidants, but is more in the bark or skin of the fruit. In this paper, the fruit of mangosteen *Garcinia mangostana* L. was collected from Taung-gu, Bago Region. Firstly, the preliminary phytochemical screening of mangosteen was done by test tube method. This fruit contains alkaloid, flavonoid, phenolic compound, polyphenol, glycoside, saponin, tannin, reducing sugar and steroid respectively. And then, mingut-thee-hsay from drug store was selected for chemical analysis. Moreover, the amount of trace elements was determined by using Atomic Absorption Spectrophotometric method (AAS). In addition, a pure compound (Mangiferin) was isolated from mingut-thee-hsay by using Thin Layer Chromatographic (TLC) technique. Finally, the functional groups were determined by FT-IR spectroscopic method.

Key words : phytochemical, AAS, TLC, FT-IR



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Structure of Mangiferin

The mangosteen (*Garcinia mangostana*) is a tropical evergreen. The mangosteen fruit is deep reddish purple when ripe. The taste is sweet and sour. Mangosteen fruit is known as the "Queen of Fruits" in Asia.

The mangosteen contains a large number of biologically active substances such as tannins, quinones, stilbenes, polysaccharides, catechins, proanthocyanindins, sterols, and small amounts of potassium, calcium, fiber, Vitamin-C, phosphorus, sodium, Vitamin B₁, Vitamin B₂, niacin. Mangosteen (*Garcinia mangostana* L.) is one of the most famous fruits in Thailand. Previous studies have shown that the extracts from various parts contain varieties of secondary metabolites such as asprenylated and oxygenated xanthones. Xanthones or Xanthen-9H-ones is a secondary metabolite found in some higher plants that involve mangosteen.

Mangosteen fruit contains these powerful antioxidants, but is more in the bark or skin of the fruit. Nowadays, people know about free radicals and antioxidants. Antioxidants help to repair the cells that are attacked by free radicals almost everyday.

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Botanical Description of Mangosteen



stana L.

Family name : Clusiaceae
Botanical name : *Garcinia mangostana* L.
Local name : mingut-thee
English name : Mangosteen

Medicinal Uses of Mangosteen

Mangosteen have been widely used to treat tuberculosis, malaria, skin diseases, diarrhea and dysentery. The mangosteen is used to treat many diseases and among other things, it is used to maintain a healthy cardiovascular healthcareambut, eyes and skin, helps the immune system, increases the resistance and energy, helps the digestive system, as well as an antidote free radicals. The mangosteen can also be used for the prevention of several life-threatening diseases like heart diseases, diabetes, cancer, arthritis. Mangosteen also contains antioxidants that are good for health and able to fend off free radicals.



Figure 2. Mingut-thee-hsays

Medicinal Uses of Mingut-thee-hsays

The Mingut-thee-hsays have been widely used to treatnee ache, back ache, rheumatic arthritis, gout, spandilitis (cervical & spinal).

Materials and Methods

Sample Collection

The fruis of mangosteen and Mingut-thee-hsays were collected from the local markets, Mandalay Region.

Phytochemical Test of Mangosteen

Phytochemical tests were done on the mangosteen drug to determine the presence or absence of organic constituents in it. The results of phytochemical tests are shown in Table (1).

Determination of Trace Elements by using Atomic Absorption Spectroscopy (AAS)

The amounts of trace elements were quantitatively determined by Atomic Absorption Spectrophotometric method using a Perkin Elmer Analyst 800 atomic absorption spectrophotometer (England) at Universities Research Center, Yangon.

Extraction of the Mingut-thee-hsays

The sample of Mingut-thee-hsays (1.98 g) was percolated with ethylacetate for one month. Then, the extracted solution was filtered through Whatman No. 1 filtered paper. The filtrate was concentrated at room temperature and (0.16 g) was obtained. The ethylacetate extracts were checked by TLC for choosing the solvent system.

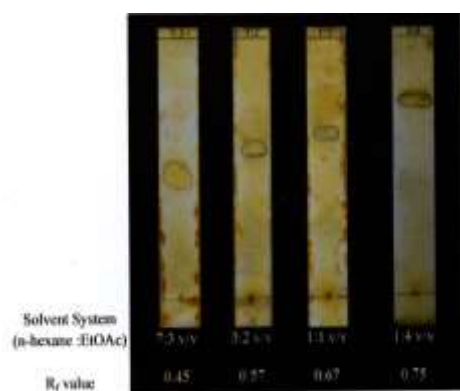


Figure 3. TLC for EtOAc Extract of Mingut-thee-hsays

Isolation of Pure Compound from the Mingut-thee-hsays

The ethylacetate extract solution (2 mL) was streaked onto 1.0 mm thick silica gel G Plates. The plates were developed in n-hexane-ethylacetate (1:4 v/v) for 5 cm length and the bands were located under short-wave ultraviolet light.

Dark brown spot under UV fluorescence was removed from the plates by elution with ethylacetate solution. The ethylacetate eluted extract was rechecked by TLC and trace impurity was removed according to the above procedure.



Pure Compound weight = 0.06 g
Physical properties = Pale yellow
crystal
Melting point = 272-274°C

Figure 4. Isolation of Pure Compound from the Mingut-thee-hsays by TLC

Pure compound pale yellow compound (R_f -0.45, n-hexane : ethylacetate, 7:3 v/v), (R_f -0.57, n-hexane : ethylacetate, 3:2 v/v), (R_f -0.67, n-hexane : ethylacetate, 1:1 v/v) and (R_f -0.75, n-hexane : ethylacetate, 1:4 v/v) were obtained. The yield of pure compound was found to be (0.06 g, 37.5 %).

Phytochemical Test for Isolated Pure Compound

The isolated compound was rechecked by phytochemical test. In pure compound, for flavonoid and phenolic are found to be positive. Hence, pure compound may be a desired phenolic compound (Mangiferin).

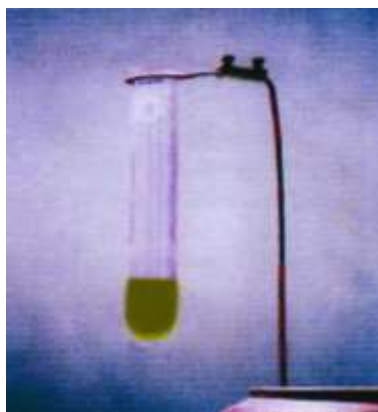


Figure 5. Phenolic Test for Isolated Pure Compound

Melting Point Determination

A small amount of pure compound was inserted into a capillary tube, 3 cm long, and the tube was attached to the thermometer by using a small rubber ring and it was inserted into a round bottomed flask containing liquid paraffin. The thermometer was supported by a cork. After the flask was gently heated, the pure crystal in the capillary tube was melted between (272-274°C), which was identical with the literature value of Mangiferin.



Figure 6. Melting Point Determination Apparatus (SMP-30)

Results and Discussion

Phytochemical Tests of Mangosteen

Preliminary phytochemical analysis was performed in order to find out different types of compounds present in the mangosteen. The results of phytochemical tests are described in Table (1).

Table 1. Phytochemical Screening of the Mangosteen

| No | Constituent | Extract | Reagent used | Observation | Result |
|----|-------------------|--------------|--|------------------------|--------|
| 1 | Alkaloid | 1 % HCl | Dragendroff's reagent | Orange ppt | + |
| 2 | Flavonoid | 95 % ethanol | Conc: HCl, pieces of Mg | Pink colour | + |
| 3 | Phenolic compound | Water | 10 % FeCl ₃ | Purplish colour | + |
| 4 | Polyphenol | 95 % ethanol | 1 % FeCl ₃ , 1 % K ₃ [Fe(CN) ₆] | Greenish blue colour | + |
| 5 | Glycoside | Water | 10 % lead (II) acetate | Yellow ppt | + |
| 6 | Saponin | Water | NaHCO ₃ | Frothing | + |
| 7 | Tannin | 95 % ethanol | 10 % lead acetate | Yellow ppt | + |
| 8 | Reducing sugar | Water | Benedict's solution | Brick red ppt | + |
| 9 | Steroid | 95 % ethanol | Conc: H ₂ SO ₄ | Greenish yellow colour | + |

(+) = presence

According to this table, the fruits of mangosteen extract consist of alkaloid, flavonoid, phenolic compound, polyphenol, glycoside, saponin, tannin and reducing sugar and steroid respectively.

Determination of Trace Elements by using Atomic Absorption Spectroscopy (AAS)

Table 2. Determination of Trace Elements by using AAS from Drug

| No | Trace Elements | Sample | Unit |
|----|----------------|--------|------|
| 1 | Na | 10.900 | mg/L |
| 2 | Mg | 9.007 | mg/L |
| 3 | K | 5.882 | mg/L |
| 4 | Ca | 4.764 | mg/L |
| 5 | Fe | 1.098 | mg/L |
| 6 | Cu | 0.463 | mg/L |
| 7 | Cd | 0.045 | mg/L |

This sample contains the highest amount of sodium which is 10.900 mg/L. Sodium was also required for nerve and muscle functioning. The amount of sodium a person-consumes each day varies with individual and some people get as little as 2 g/day, some as much as 20 grams.

Magnesium content in the sample was 9.007 mg/L. Magnesium maintains healthy bones and helps muscle relax as well as regulating mood, nerve and muscle functions. Humans take between 250 and 350 mg/day of magnesium and need at least 200 mg. Magnesium supplements and medicines can lead to muscle weakness, lethargy and confusion.

Potassium content in the sample was 5.882 mg/L. Potassium can help control blood pressure. Potassium has a balancing relationship with sodium (salt) in the body. It is potassium that helps to control a regular heartbeat. The kidney regulate the level of potassium, sodium and water in the body. Potassium can be found in vegetables, fruits, potatoes, meat, bread, milk and nuts.

Calcium content in the sample was 4.764 mg/L. Calcium is the most abundant metal in the human body and is the main constituent of bones and teeth and it has key metabolic function. It is an essential component for the preservation of the human skeleton and teeth. It also assists the functions of nerves and muscles.

Iron content in the sample was 1.098 mg/L. Iron is an essential part of hemoglobin; the red colouring agent of the blood that transports oxygen through our bodies. A more common problem for humans is iron deficiency, which leads to anaemia. A an needs an average daily

intake 7 mg of iron and a woman 11 mg; a normal diet will generally provided all that is needed.

Copper content in the sample was 0.463 mg/L. Copper helps transport oxygen through the body, maintains hair colour, and is used to make hormones. Long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomachaches, vomiting and diarrhea.

Cadmium content in the sample was 0.045 mg/L. Cadmium is first transported to the liver through the blood. Cadmium accumulates in kidneys, where it damages filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further damage.

FT-IR Spectroscopic Studies of Isolated Pure Compound from Drug

FT-IR Assignment of Isolated Pure Compound

The FT-IR spectrum of pure compound isolated from the Mingut-thee-hsays was measured at the Department of Chemistry, University of Mandalay, and is shown in Figure (7). The functional groups observed in FT-IR spectrum are described in Table (3).

Table 3. FT-IR Assignments of Isolated Pure Compound from Drug

| Absorption band (cm^{-1}) | Assignments |
|--------------------------------------|---|
| 3373.61 | O-H stretching vibration of alcohol or phenolic group |
| 3043.77 | =C-H stretching vibration of sp^2 hydrocarbon |
| 2928.04, 2854.74 | C-H stretching vibration of sp^3 hydrocarbon |
| 1683.05 | C=O stretching vibration of carbonyl group |
| 1616.40, 1550.81 | C=C ring skeletal stretching vibration of aromatic benzene ring |
| 1456.30 | C-H in plane bending vibration of allylic hydrocarbon |
| 1259.56, 1174.69 | C-C-O stretching vibration of alcohol group |
| 1039.67 | C-O-C stretching vibration of ether group |
| 908.04 | C-H out of plane bending vibration of trans or E alkenic group |
| 835.21 | C-H out of plane bending vibration of cis or Z alkenic group |

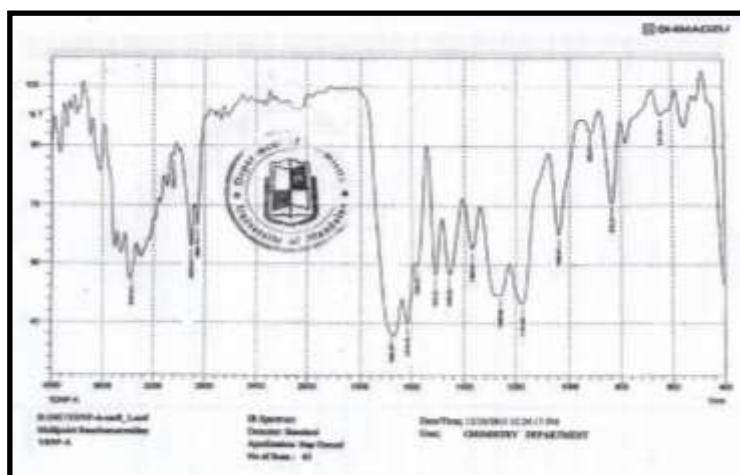


Figure 7. FT-IR Assignments of Isolated Pure Compound from Drug

Conclusion

In this paper, the phytochemical test of mangosteen *Garcinia mangostana* L. was performed. The phytochemical results indicate that fruits contain alkaloid, flavonoid, phenolic compound, polyphenol, glycoside, saponin, tannin, reducing sugar and steroid respectively. The Mingut-thee-hsay was selected for investigation of elemental compositions and chemical constituents. According to AAS results, the ppm amounts of Ca, Mg, Fe, Na, K, Cd and Cu were found to be present in this sample. Among them, Na (10.900 mg/L), Mg (9.007 mg/L) and K (5.882 mg/L) were present as the higher amounts.

In this sample, heavy elements such as Cu and Cd were found to be present in very little amounts. So Mingut-thee-hsay should be taken as a medicine for mee ache, back ache, rheumatic arthritis, gout, spandilitis (cervical & spinal) pure compound (pale yellow crystal) was isolated by solvent extraction and Thin Layer Chromatographic methods. The yield of this compound was found to be 37.5 %. Moreover, the melting point of this pure compound was determined and identified with the literature value of melting point of Mangiferin compound.

Hence, this pure compound may be Mangiferin compound. Finally, the FT-IR spectra of isolated pure compound was measured. According to FT-IR assignments, pure compound of functional groups agrees with the structure of Mangiferin compound.

Acknowledgements

I wish to express my thanks to Rector Dr Aye Kyaw, Pro-rectors Dr Khin Ma Ma Tin and Dr Myinzu Min and Professor Dr Hlaing Hlaing Myat (Head of Professor), Dr Myat Mon (Professor, Department of Chemistry, Yadanabon University) for their kind encouragement carrying out this research.

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Online Materials

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- <http://www.chosemy;late.gov/healthy-eatin-tips/tips-for-vegetarian.html>
- <http://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2357/2>
- <http://lifestyl.iloveindia.com/lounge/health-genefits> of mangosteen