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# Development and Validation of High Performance Thin-Layer Chromatographic Method for Determination of α-Mangostin in Fruit Pericarp of Mangosteen Plant (*Garcinia mangostana* L.) using Ultraviolet – Visible Detection

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**Abstract:** A simple, fast and precise quantitative high performance thin-layer chromatographic method has been developed for quantitative estimation of α-mangostin in fruit pericarp of *Garcinia mangostana* L. (Hypericaceae). Best solvent for extraction of α-mangostin optimized after screening with five solvents under same conditions using hot solid-liquid extraction through soxhlet apparatus. Methanol and chloroform gave highest and second highest recovery of α-mangostin, respectively. Plates were developed in chloroform-methanol in the ratio of 27-3 (v/v). Post-chromatographic derivatization performed using anisaldehyde-sulphuric acid reagent and scanned at 382 nm in ultraviolet-visible mode. The developed method was found to be linear in the range 1.0 to 5.0 µg spot$^{-1}$, limits of detection and quantitation were 150 and 450 ng spot$^{-1}$. The developed method was validated in terms of system suitability, specificity and robustness.

**Keywords:** *Garcinia mangostana* L.; Hypericaceae; α–Mangostin; HPTLC determination.

## 1. Introduction

*Garcinia mangostana* L. (Hypericaceae alternatively known as Clusiaceae and Guttiferae) plant is a tropical evergreen tree, believed to have originated in the Sunda Islands and the Moluccas. It was first discovered in Burma and Siam (now known as Thailand). The tree grows from 7 to 25 meters tall. This exotic edible mangosteen fruit is deep reddish purple when ripe. In Asia, the mangosteen

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fruit is known as the "Queen of Fruits". The mangosteen tree requires a warm, very humid, equatorial climate all year round. Many people have tried to grow *Garcinia mangostana* in warm places such as California and Florida or in special greenhouses outside South East Asia, but they experienced little success because of the different environmental factors. The mangosteen tree requires abundant moisture and only grows well in a tropical environment [1].

The mangosteen fruit grown in Thailand is harvested in the season of optimum ripeness for maximum efficacy. The xanthone rich pericarp (the outer rind) is then separated from the pulp and freeze-dried. The outer shell or rind of the mangosteen fruit (called the pericarp) is rather hard, typically 4 to 6 centimeters in diameter, resembling a spherical, reddish-black, cartoon-style bomb. By cutting through the shell, one finds a very pale and fleshy fruit 3 to 5 centimeters in diameter. Depending on its size and ripeness, there may or may not be pits in the segments of the fruit. The number of fruit pods is directly related to the number of petals on the bottom of the shell. Commonly, the average mangosteen will have 5 fruit pods [1]. Mangosteen tree, flower and fruit have been shown in Figure 1.

[A] [B] [C]

**Figure 1.** *Garcinia mangostana* L. [A]: Whole plant with different parts; [B]: Flower and [C]: Fruit.

The shell of the mangosteen fruit looks tough and hard, but is easy to open. Care must be taken when opening the fruit, as the reddish-black husk outside produces a purplish, inky juice that stains fabric and can be almost impossible to remove (the reason why they are banned from some hotels in countries where they are available). To open a mangosteen, the shell is usually broken apart, not cut. Holding the fruit in both hands, press it gently (thumbs on one side, the other fingers on the other) until the shell cracks. It is then very easy to pull the halves apart along the crack and remove the fruit without staining.

One of the most-praised of tropical fruits, and certainly the most-esteemed fruit in the family Hypericaceae, the mangosteen is almost universally known. But there are numerous variations in nomenclature: among Spanish-speaking people the fruit is called mangostan; to the French it is mangostanier, mangoustanier, mangouste or mangostier; in Portuguese it is mangostao, mangosta or mangusta; in Dutch it is manggis or manggistan; in Vietnamese it is mang cut; in Malaya it may be referred to in any of these languages or by the local terms mesetor, semetah, or sementah; and in the Philippines it is mangis or mangostan. Throughout the Malay Archipelago of Malaysia, there are many different spellings of names for mangosteen similar to most of those mentioned above.

Mangostin (Molecular formula: $C_{24}H_{26}O_6$; Mol. Wt. = 410.46) is a natural organic compound isolated from mangosteen plant. It is a yellow colored, crystalline solid with a xanthone core structure [2]. Mangostin and a variety of other xanthones from mangosteen have been investigated for biological properties including antioxidant [3, 9], antibacterial [4], antifungal [5], anti-inflammatory [6], antileukemic [7] and anticancer [8-10] activities. Its pericarp (fruit hull) contain xanthones, α-, β- and γ-mangostin together with other xanthones. *In vitro* tests have shown that the constituent xanthone "γ-mangostin" is more potent antioxidant than vitamin-E. *Garcinia mangostana* extract produced strong inhibition zones against *Propionibacterium acnes* and *Staphylococcus epidermidis* [11]. Medicinal properties of mangosteen recently reviewed by Pedraza-Chaverri et *al.* 2008 [12].

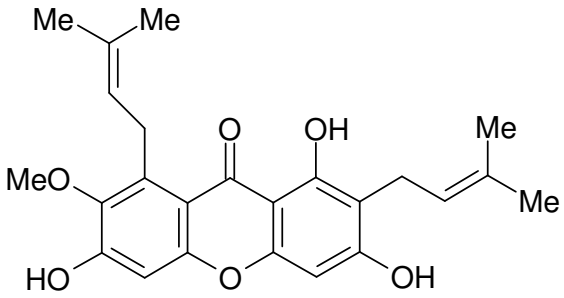



**Figure 2.** Chemical structure of α - mangostin

Computational planar chromatography has been the essential tool for analyzing as well as authenticating active principles of natural origin. Developed analytical methods ensures efficacy of plant drug under tests [13-17]. Our group is actively involved in the development of analytical methodologies for the same purpose [18-20]. To the best of our knowledge, there is no report on thin-layer chromatographic method for quantitative analysis of mangostin in crude extracts of mangosteen, although few workers have developed HPLC [21, 22], HPLC–ESI–MS [23]. Here, we have developed an HPTLC method for quantitative estimation of α - mangostin (Figure 2). The developed method was validated in terms of system suitability, specificity, linearity, LOD, LOQ and robustness.

## 2. Materials and Methods

### *2.1. Plant material*

The plant of *Garcinia mangostana* L. (Hypericaceae) was collected from Nilgiri Hills nearby Bangalore (India) and obtained with thanks from Dr. Binit K. Dwivedi of Sanat Products Ltd. The plant was identified by Dr. H. B. Singh, *Scientist*, Raw Materials Herbarium & Museum, NISCAIR (CSIR), New Delhi, India and a voucher specimen (reference number NISCAIR/RHMD/Consult/-2009-10/1230/34) has been deposited in the herbarium.

### *2.2. Chemicals and reagents*

All solvents used in this study were of analytical grade. Reference standard of α - mangostin (HPLC purity 66.0 %) was contributed by one of the author Dr. Binit K. Dwivedi. Post-chromatographic derivatization of developed TLC plates done using anisaldehyde – sulphuric acid reagent. Anisaldehyde-sulphuric acid reagent was prepared by mixing 5 mL anisaldehyde in 500 mL

glacial acetic acid kept in a beaker and stirred well for 5 minutes. Now, 10 mL of concentrated $H_2SO_4$ (98 %) added slowly to the above solution through sidewall of beaker and stirred well.

*2.3. Apparatus*

A computerized TLC scanner 3 with *winCATS* online *Planar Chromatography Manager version 1.4.2* (CAMAG, Switzerland) was used for quantitative chromatographic evaluation of test spots. Camag's *Linomat 5* was utilized for nitrogen gas-assisted and controlled application of sample spots on to TLC plate.

*2.4. Optimization of mobile phase for chromatographic separation and determination of appropriate wavelength*

Few mobile phases like, *n*-hexane-ether, benzene-acetone and chloroform-methanol were tried for separation and we opted for $CHCl_3$ – $CH_3OH$ (9:1). A qualitative TLC run performed using standard and extract in the selected mobile phase and scanned under 540 nm after post-chromatographic derivatization and its spectrum was scanned, which clearly indicated its $\lambda_{max.}$ at 382 nm with satisfactory peak purity data.

*2.5. Preparation of standard α-mangostin and calibration curve*

10 mg of standard α-mangostin was dissolved in 10 mL of methanol in a volumetric flask and sonicated for 5 minutes for homogenizing it completely. A calibration curve was plotted between 1µg to 5 µg $spot^{-1}$. A linear relationship obtained with correlation coefficient (r) 0.98189 (1µg to 5 µg), 0.99037 (1 µg to 4 µg) and 0.99570 (1 µg to 3 µg) with standard deviation 8.51, 6.13 and 4.04 %, respectively.

*2.6. Extraction and preparation of test samples*

500 mg of powdered pericarp was taken in five different extraction thimbles and extracted via Soxhlet extraction for 6 hours using chloroform, ethyl acetate, acetone, methanol and ethanol. Extracted samples were dried under vacuum to dryness and re-dissolved in methanol and volume of each test sample made up to 50 mL and sonicated for 5 minutes. These test solutions were spotted against standard α-mangostin for assay.

*2.7. Chromatographic analysis*

Thin-layer chromatography was performed on aluminum backed HPTLC plates (60 $F_{254}$ , E. Merck, Germany, 200 x 100 mm). 2 µL of test and standard sample spots were applied through camag's Linomat 5 as 6.0 mm wide bands at the height of 10 mm from base; spots were simultaneously dried with $N_2$ gas supply on to HPTLC plates. Plates were developed in a camag twin trough chamber of size more than 200 x 200 mm at 95 mm from base using chloroform : methanol (90 : 10, v/v) as mobile phase. Plates were air-dried for complete evaporation of mobile phase and post-chromatographic derivatization performed with anisaldehyde – sulphuric acid reagent and followed by heating at 110° C for 10-15 minutes. HPTLC plate was stabilized by putting it to room temperature for 30 minutes and then scanned using camag's TLC scanner 3 equipped with *winCATS* software in absorption – reflection detection mode at 382 nm (using deuterium and tungsten lamps). Chromatographic separation of α-mangostin ($R_f$ = 0.46) is shown in Figure 3.

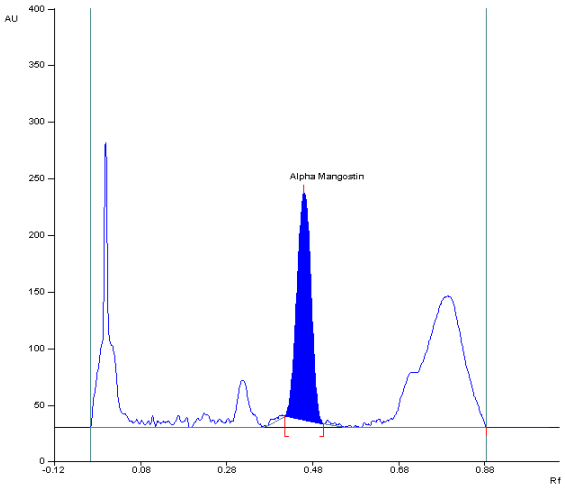



**Figure 3.** High-performance thin-layer chromatographic separation of α-mangostin

## 3. Method Validation

*3.1. System suitability*

System suitability of test was used to ensure reproducibility of the equipment. The test was carried out by injecting 2 µL of the standard solution of α-mangostin (1.0 mg $mL^{-1}$) five or six times. The RSD was found to be less than 2%.

*3.2. Specificity*

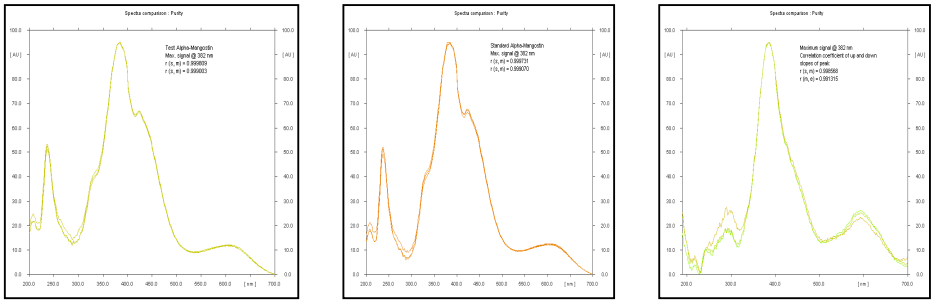
The developed method was found to be specific as no interfering or contaminating peak (s) was detected as was also evidenced from peak purity data (Figure 4 and Table 2).

*3.3. Linearity*

For the evaluation of linearity five different standard solutions of α-mangostin were spotted in increasing amount from 1.0 to 5.0 µg $spot^{-1}$. A linear relationship between the peak area and the concentration of α-mangostin was observed for determining range of 1.0 to 5.0 µg $spot^{-1}$, but a good linearity obtained between 1.0 to 3.0 µg $spot^{-1}$. The equation of linear regression curve obtained was Y = mX + C = 451.5 X + 137.6, with a correlation coefficient (r) = 0.99570 (sdv = 4.04%).

*3.4. Limits of detection and quantitation*

The signal-to-noise ratio of 3:1 and 10:1 were used to establish LOD and LOQ, respectively. The LOD and LOQ of α-mangostin were 150 and 450 ng $spot^{-1}$.



**[A]** **[B]** **[C]**

**Figure 4.** Overlay absorption spectra of α-mangostin after post-chromatographic derivatization with anisaldehyde – sulphuric acid reagent showing purity of up and down slopes. [A] Peak purity of test α-mangostin eluted from extract; [B] Peak purity of standard α-mangostin and [C] peak purity of α-mangostin eluted from another test extract, showing slight deviation in absorption pattern but at the same absorption maxima.

*3.5. Chromatographic assay*

2 µL each of the standard solution of α-mangostin (1.0 mg $mL^{-1}$) and plant extract (10 mg $mL^{-1}$) were injected, separately. The peak areas of α-mangostin were measured. This procedure was repeated seven times and in each case the amount of α-mangostin in the sample solution was determined using linearity equation. The retardation factor ($R_f$) of α-mangostin sample solution was 0.46 and that of α-mangostin in the standard solution was found to be 0.45. The mean assay value of α-mangostin were determined in different solvents was shown in Table 3.

*3.6. Precision and accuracy*

The intra-day and inter-day precision was used to study the variability of the method. The %RSD for intra-day and inter-day precision were 0.94 and 1.19 %, respectively. Accuracy of the method was studied using standard addition method. Standard α-mangostin solutions were added to the extract of the fruit pericarp powder of *Garcinia mangostana* and the percentage recovery was determined at three different levels. The results of the recovery analysis are shown in Table 1.

**Table 1.** Recovery data as calculated from methanolic extract.

| Amount of α-mangostin present in preanalyzed extract (µg/mL) | Amount of α-mangostin added (µg/mL) | Amount of α-mangostin in Mixture (µg/mL) | Amount of α-mangostin detected in Mixture (µg/mL) | % Recovery |
|---|---|---|---|---|
| 429.8 | 250 | 339.9 | 332.3 | 97.76 |
| 429.8 | 500 | 929.8 | 902.3 | 97.04 |
| 429.8 | 1000 | 1429.8 | 1381.2 | 96.60 |

*3.7. Robustness*

Robustness of the method was determined by performing small variations in mobile phase ratio, height of the plate development and TLC tank saturation time. The results indicated insignificant differences in assay and thus indicative of a robust method.

**Table 2.** Summary of validation parameters for α-mangostin

| **Parameters** | **Results** |
|---|---|
| Linearity | |
| Range (µg/spot) | 1-3 |
| Linear equation | Y = m X + C |
| Slope (m) | 451.5 |
| Intercept (C) | 137.6 |
| Correlation coefficient (r) | 0.99570 |
| Product of correlation coefficient ($r^2$) | 0.99141 |
| Standard deviation (sdv) | 4.04 % |
| Peak purity of eluted test α-mangostin spot | |
| Correlation coefficient, r (s, m) | 0.999809 |
| Correlation coefficient, r (m, e) | 0.999003 |
| Peak purity of eluted standard α-mangostin spot | |
| Correlation coefficient, r (s, m)† | 0.999731 |
| Correlation coefficient, r (m, e)‡ | 0.999070 |
| Precision (%RSD) | |
| Intra day (n = 3) | |
| Repeatability of Samples | 0.94 |
| Repeatability of peak area | 1.93 |
| Repeatability of $R_f$ | 1.55 |
| Inter day (n = 3) | |
| Repeatability of Samples | 1.19 |
| Repeatability of peak area | ND* |
| Repeatability of $R_f$ | 1.62 |
| Limit of detection (LOD) | 150 ng/spot |
| Limit of quantification (LOQ) | 450 ng/spot |
| Recovery | 96.60 – 97.76 % |
| Specificity | specific |

† r (s, m) is correlation coefficient of up slope (i.e., peak start to peak middle).
‡ r (m, e) is correlation coefficient of down slope (i.e., peak middle to peak end).

## 4. Results and Discussion

Extraction of α-mangostin was performed from powdered pericarp of fruits of *Garcinia mangostana* using different solvents such as chloroform, ethyl acetate, acetone, ethanol and methanol. Results of quantitative high-performance thin-layer chromatographic analysis (Table 3) reveals that chloroform and methanol recovered highest amounts of α-mangostin.

The decreasing order of α-mangostin recovery is:

Methanol > Chloroform > Ethanol > Acetone > Ethyl acetate.

Either of these two solvents (i.e., methanol or chloroform) can be used for the quantitative analysis as well as isolation purposes. Although methanol is the solvent of choice for authors since chloroform is toxic in nature as unstabilized chloroform produce toxic gas phosgene (a culprit gas for Union Carbide pesticide plant, Bhopal, tragedy in India), small amounts of free radical and hydrochloric acid, when heated in presence of oxygen [24].

**Table 3.** Screening of solvents for best extraction of α-mangostin.

| Extraction solvent | α-Mangostin, % (Dry wt. basis) n = 3 | Mean content of α-mangostin, % | ±SD[a] | RSD[b] |
|---|---|---|---|---|
| Chloroform | 4.318 | | | |
| | 4.128 | 4.222 | 0.095 | 2.250 |
| | 4.220 | | | |
| Ethyl acetate | 2.026 | | | |
| | 2.061 | 2.038 | 0.020 | 0.981 |
| | 2.028 | | | |
| Acetone | 2.778 | | | |
| | 2.832 | 2.803 | 0.027 | 0.963 |
| | 2.800 | | | |
| Ethanol | 4.000 | | | |
| | 3.967 | 3.959 | 0.046 | 1.162 |
| | 3.910 | | | |
| Methanol | 4.309 | | | |
| | 4.260 | 4.298 | 0.033 | 0.768 |
| | 4.324 | | | |

[a] Standard Deviation; [b] Relative standard deviation

## 5. Conclusion

The developed HPTLC method was precise and accurate based on validation parameters determined. Screening of extraction power of different solvents towards α-mangostin shows methanol as the solvent of choice for future development of analytical as well as industrial plant based developments.

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