International Journal of Chemistry and Applications.
ISSN 0974-3111 Volume 4, Number 3 (2012), pp. 271-277

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Quantitative Analysis of a-Mangostin in Fruit Hulls of *Garcinia Mangostana* Extract and it's Content in Formulation by a Validated RP-HPLC Method

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Abstract

The present study involves the optimization of different solvent extract and Standardization of α-mangostin from fruit hulls of *Garcinia mangostana* was studied by gradient reverse-phase high-performance liquid chromatographic (HPLC) method for quality control and quantity determination. Chromatographic separation was carried out on Develosil RP C-18 (5 μm, 4.6 x 150mm) at room temperature using a (Gradient phase) mobile phase consisting of flow rate of 1.0mL/min, with UV detection at 245nm. The method has been studied for linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The linearity of the proposed method was found in the range of 10-200 ug mL^{-1} with regression coefficient 0.9998. The method was also applied for the determination of mangostin in three samples collected from three different places and two herbal formulation. The proposed HPLC method was simple, accurate, precise and it is suitable for performing the quality control of crude extract and herbal formulation.

Keywords: Garcinia mangostana, α-Mangostin, RP-HPLC, Quality control, Validation method.

Introduction

Mangosteen or *Garcinia mangostana* Linn. belongs to the family Guttiferae and it is widely cultivated throughout Southeast Asian countries, especially in eastern and southern parts of Thailand. It has been used as traditional medicine to treat skin infections, wounds, and diarrhea (Mahabusarakam, et al., 1987). There are over 50 natural xanthones (Pedraza, et al.,2008) reported in mangosteen. Xanthones are secondary metabolites commonly occurring in a few higher plant families, and in fungi and lichens. Due to its pharmacological activities, it is popularly applied to herbal cosmetics and pharmaceutical products. Xanthones and tannins assure astringency to discourage infestation by insects, fungi, plant viruses, bacteria and animal predation while the fruit is immature (Akao, et al., 2008).). The xanthones, a- and c-mangostins, are major bioactive compounds found in the fruit hulls of the mangosteen (Jinsart et al., 1992; Chairungsrilerd et al., 1996a,b,c). Moreover, a- and c-mangostins can inhibit both human immunodeficiency virus (HIV) infection (Chen et al., 1996; Vlietinck et al., 1998), and topoisomerases I and II (Tosa et al., 1997). The mangosteen has long been widely used as an antiinflammatory, anti-diarrhea, and anti-ulcer agent in Southeast Asia (Lu et al., 1998; Harbborne and Baxter, 1993). However, there is limited information for quality and quantity determination of α-mangostin (Figure 1) in mangosteen extract. So, analytical methods play an important role in the quality control of its raw materials and products.

The present work is to determine the content of α-mangostin (fig 1) in formulated product which is available in market and extraction of Mangostin from fruit hulls of G. mangostana by different solvent from non-polar to high polar extracts and the presence of mangostin in all extracts has initially revealed by TLC with Ethylacetate and n Hexane as mobile phase followed by determining the percentage content in all extracts by using High performance Liquid Chromatography (HPLC). In order to evaluate the content in different region the Fruit was procured from three different origins and its content was determined.

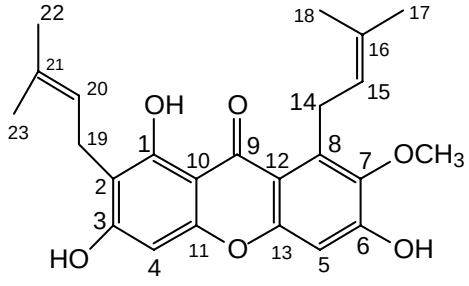

Figure I: Structure of α-Mangostin

Materials and Methods

Chemicals and Reagents

α-Mangostin was purchased from Sigma Aldrich. All solvents used were HPLC grade, and the reagents were of Analytical grade. Purified Milliq water has been used.

Solvents used for the mobile phase were filtered through membrane filter (0.45-µm pore size) and degassed before use.

Plant material

The Fruits of G. mangostana were purchased from local market , different places (Bankog, Kodaikanal and Tenkasi) was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai 600 045. A specimen has been stored at the herbarium unit of Asthagiri Herbal Research Foundation (AHRF), perungudi, Chennai – 600 096. The fruits were cleaned and dried in a room temperature. The dried sample were grounded into powder using electronic blender. The sample were separately kept in air tight container until use.

HPLC and Chromatography conditions

HPLC method was performed on waters Alliance equipped with photo diode array detector, UV 2695 and with autosampler, cooler. The column selected is Develosil UG-5 150*4.6*5µ. The elution was carried out with gradient Solvent systems with a flow rate of 1 mL min^{-1} at ambient temperature (25-28^{0}C). The mobile phase was consisted of 0.02 mole of Potassium dihydrogen ortho phosphate adjusted pH to 7.0 with Potassium hydroxide (1N solution). The sample injection volume was 20uL while the wavelength of the UV-vis detector was set at 245 nm. The chromatogram has been acquired by using waters EMPOWER software.

Time	Channel A	Channel B	Mode
0	50	50	Isocratic
5	50	50	Isocratic
20	20	80	Linear
30	50	50	Linear

Preparation of Standard Solution

A stock solution of alpha-Mangostin reference standard (purity 99%) was prepared by dissolving an accurately weighed 25 mg into a 50 mL Volumetric flask add 5.0 mL of Methanol and dilute to volume with diluent to get the stock solution with 1000µg/ml concentration, then further diluted to 5.0 mL to 10 mL with diluent .

Preparation of Sample Solution from Extract

Each sample (100g) was extracted with 500 mL of different solvents varying polarity viz. Hexane, Benzene, Ethyl acetate, Acetone and Ethanol in a soxhlet apparatus for 6h. Each extraction was filtered through a whattman no.1 filter paper by suction. The filtrate was concentrated under reduced pressured at 60^{0}C using a rotary vacuum evaporator. The final weight of the crude extract was weighed and calculated for the yield.

Each dried extract (10mg) was accurately weighed and transferred in a 10mL volumetric flask and 5.0 mL of methanol was added then make upto volume with diluent further dilute 5.0 mL to 10 mL with diluent. Prior to analysis, the solutions were filtered through 0.45µm membrane filters.

Method validation
Validation of the analytical method was done according to the International Conference on Harmonization guideline (ICH, 1996). The method was validated for Linearity, Precision, Specificity, Limit of detection (LOD) and Limit of quantitation (LOQ).

Linearity
Linearity was established by preparing serial dilution of α-Mangostin standard solution as from 10 to 200 µg mL-1. The calibration graphs were obtained by plotting the peak area versus the concentration of the standard solutions.

Precision
Repeatability of the sample application and measurement of peak area were carried out using five replicates of the same sample and was expressed in terms of percent relative standard deviation (%RSD)

Specificity
The specificity of the method was ascertained by analyzing the standard drug (i.e., α-Mangostin) and the crude herb powder. The peak for α-Mangostin in the sample was confirmed by comparing the retention times of the sample peak with that of the standard. The peak purity of the α-Mangostin was assessed by comparing the spectra at two levels, viz; peak start (S) and peak end (E) positions. The purity of peak is passing, and there is no interference from Blank.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use recommendations, the approach based on signal to noise ratio method.

Results and Discussion

Quantitative Analysis of α-Mangostin Content – Quantitative Determination
The contents of α-mangostin in the extracts and the fruit rind were expressed as gram per 100 grams of the extract and of the dried powder, respectively. A simple, precise, accurate and rapid high pressure liquid chromatographic method has been developed and validated for the estimation of α-mangostin in different extract of *G. mangostana*. Comparative HPLC chromatograms show that α-mangostin very well separated from other constituents of *G. mangostana*. Optimization of various solvent extracts of *G. mangostana* were analyzed by the proposed method and the data are recorded in which Ethylacetate extract was showing more content of α-mangostin compare with the other extracts (Table I & Fig II). Region variation of α-Mangostin content was found by purchasing *G. mangostana* from three different places (Bangkok, Kodaikanal and Tenkasi) and extracted with Ethyl acetate, in which α-Mangostin found more content in Kodaikanal region (Table II & Fig III). This proposed method was also applied for the formulation brought from market in united states – Solaray (475mg per capsule) , Mangosteen (500 mg per capsule - Doctor Formulated) and

proceeded in similar conditions, which is showed 3-4 % of α-Mangostin in Formulation I and II (Table III and Fig IV).

The Limit of detection (LOD) was obtained by successively decreasing the concentration of α-Mangostin as long as a signal to noise ratio of 3:1 appeared. The LOD was found to be 0.02 ppm and the limit of quantification (LOQ) was found to be 0.04 ppm of α-Mangostin. The calibration graph for α-Mangostin was within the concentration range of 10.5 – 200 µg mL^{-1}, with a correlation coefficient (r2) of 0.9998 and the calibration graph was obtained by plotting peak area versus the concentrations of the standard solution.

Table I: Content of α-Mangostin in different extracts

Name of the Extract	Yield of crude extract (%w/w of dried powder)	Alpha-mangostin content (%w/w)	
		In dried powder	In extract
Hexane	22.2	6.74	30.4
Benzene	16.2	5.33	32.9
Ethyl acetate	26.3	12.83	48.8
Acetone	10.0	3.08	30.8
Ethanol	15.2	3.91	25.7

Table II: Content of α-Mangostin in three different regions

% Alpha Mangostin Content Obtained from Different Region			
	Tenkasi	Bankog	Kodaikanol
Assay (In extract) (%w/w)	29	39	48
Crude Extract Weight	110 g	130g	150g

Table III: Content of α-Mangostin in two different formulations

	% Alpha Mangostin Content in Formulation	
	Formulation 1	Formulation 2
Assay (%w/w)	3.1	3.9

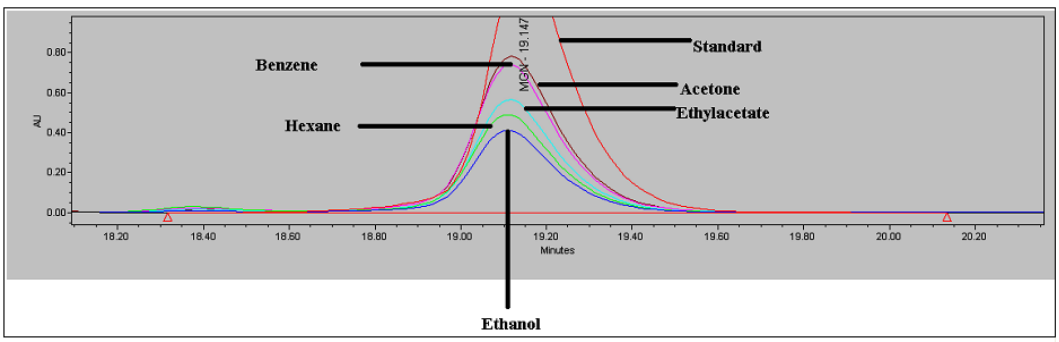

Figure II: Comparison of Standard α-Mangostin with different extracts

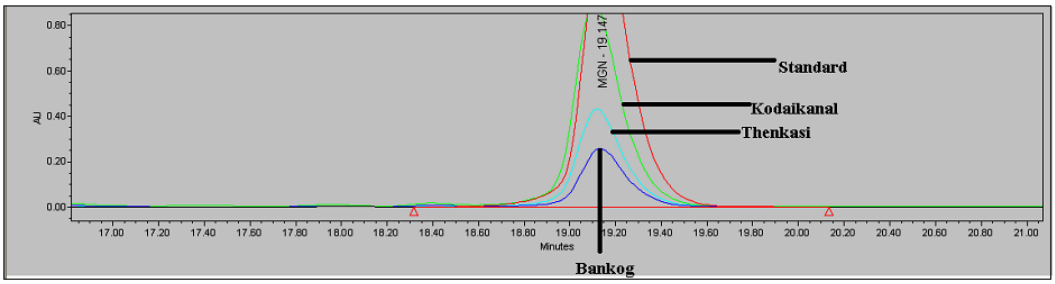

Figure III: Comparison of Standard α-Mangostin with different regions

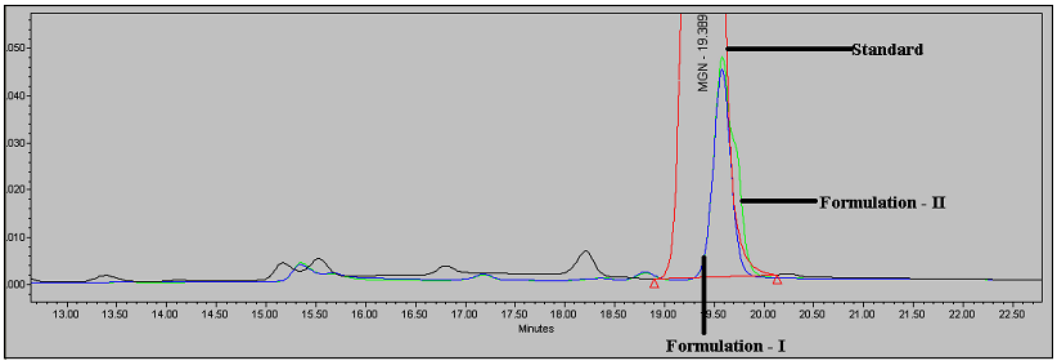

Figure IV: Comparison of Standard α-Mangostin with different formulation

Conclusions

The need for quality assurance, including conformation of the label strength and content uniformity has long been recognized even for herbal medicinal products. A high- performance liquid chromatography method has been developed for the detection and quantification of α-Mangostin in different extracts from the fruit hulls of Garcinia mangostana. The method was found to be specific and suitable for routine analysis because of its simplicity, and reproducibility. This proposed method will be useful for quantitative analysis in standardization and quality assessment of *G. mangostana* for pharmaceutical and cosmetic uses.

Acknowledgements

We are thankful to the management of Orchid Chemicals and Pharmaceutical Ltd Chennai, for the excellent support to perform the research project.

References

[1] Mahabusarakam, W., Iriyachitra, P., Taylor, W.C., 1987, "Chemical constituents of *Garcinia mangostana"* J. Nat. Prod., 50, pp. 474-478.

[2] Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., Pérez-Rojas, J. M., 2008, "Medicinal properties of mangosteen (*Garcinia mangostana*)," *Food Chem. Toxicol*., 46, pp. 3227-3239.
[3] Akao,Y., Nakagawa,Y., Iinuma,M., Nozawa.Y., 2008, "Anti-cancer effects of xanthones from pericarps of mangosteen," Int. J. Mol. Sci., 9, pp. 355-370.
[4] Jinsart, W., Ternai, B., Buddhasukh, D., Polya, G.M., 1992, "Inhibition of wheat embryo calcium-dependent protein kinase and other kinases by mangostin and c-mangostin," Phytochemistry 31, pp.3711–3713.
[5] Chairungsrilerd, N., Furukawa, K.I., Ohta, T., Nozoe, S., Ohizumi, Y., 1996a, "Histaminergic and serotonergic receptor blocking substances from the medicinal plant Garcinia mangostana," Planta Med, 62, pp.471–472.
[6] Chairungsrilerd, N., Furukawa, K.I., Ohta, T., Nozoe, S., Ohizumi, Y., 1996b, "Pharmacological properties of a-mangostin, a novel histamine H1 receptor antagonist," Eur. J. Pharmacol, 314, pp.351–356.
[7] Chairungsrilerd, N., Takeuchi, K., Ohizumi, Y., Nozoe, S., Ohta, T., 1996c, "Mangostanol, a prenyl xanthone from Garcinia mangostana," Phytochemistry 43, pp.1099–1102.
[8] Chen, S.X., Wan, M., Loh, B.N., 1996, "Active constituents against HIV-1 protease from Garcinia mangostana," Planta Med. 62, pp.381–382.
[9] Vlietinck, A.J., Bruyne, T.D., Apers, S., Pieters, L.A., 1998, "Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection," Planta Med. 64, pp.97–109.
[10] Tosa, H., Iinuma, M., Tanaka, T., Nozaki, H., Ikeda, S., Tsutsui, K., Tsutsui, K., Yamada, M., Fujimori, S., 1997, "Inhibitory activity of xanthone derivatives isolated from some guttiferaeous plants against DNA topoisomerases I and II," Chem. Pharm. Bull. 45, pp.418–420.
[11] Lu, Z.X., Hasmeda, M., Mahabusarakam, W., Ternai, B., Ternai, P.C., Polya, G.M., 1998, "Inhibition of eukaryote protein kinases and of a cyclic nucleotide-binding phosphatase by prenylated xanthones," Chem.-Biol. Interact. 114, pp.121–140.
[12] Harbborne, J.B., Baxter, H., 1993, "Phytochemical dictionary. A Handbook of Bioactive Compounds from Plants," Taylor and Francis, London.