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## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF $\alpha$ -MANGOSTIN IN THE RIND EXTRACT AND FRACTIONS OF *GARCINIAMANGOSTANA* L. AND THEIR CYTOTOXIC ACTIVITY ON T47D BREAST CANCER CELL LINE

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### ABSTRACT

**Objective:** To develop a cheap, accurate, precise, linear and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of  $\alpha$ -mangostin in the rind extract and fraction of mangosteen (*Garcinia mangostana* L.) as well as to determine their cytotoxic activity against T47D breast cancer cell line.

**Methods:** The optimized method uses a reverse phase column, Shimadzu <sup>®</sup>Shimp-pack VP - ODS (4.6 x 250 mm; 5 $\mu$ ), a mobile phase of 0.1 % v/v H<sub>3</sub>PO<sub>4</sub> in water: acetonitrile (15:85), flow rate of 1 ml/min and a detection wavelength of 243,2 nm using a UV detector. The cytotoxic activity against breast cancer cell line T47D was determined as percentage of cell viability by using MTT (Microculture Tetrazolium Assay) colorimetric assay and IC<sub>50</sub> (concentration that inhibits cell growth by 50%) were calculated.

**Results:** The developed method resulted in  $\alpha$ -mangostin eluting at 8.87 min.  $\alpha$ -Mangostin exhibited linearity in the range 0.5 - 30  $\mu$ g mL<sup>-1</sup>, and precise (intra-day variation  $\leq$  0.10 %, inter-day variation  $\leq$  2.28 %). The average percentage mean recovery was 94.41-102.01 %, during accuracy studies. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.2807 and 0.9357  $\mu$ g mL<sup>-1</sup> respectively. The concentration of  $\alpha$ -mangostin in the 70% ethanol extract, n-hexane fraction, ethyl acetate fraction and n-butanol fraction were 50.73; 11.12; 98.66; 2.29 % w/w, respectively. This extract and fractions had IC<sub>50</sub> of 1.375; 5.879; 0.463; and 51.839  $\mu$ g mL<sup>-1</sup> against T47D cell line respectively.

**Conclusion:** A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of  $\alpha$ -mangostin in the rind extract and fraction as per ICH guidelines and hence it can be used for the quality control of crude extract and herbal formulation. The strongest cytotoxic activity was showed by an ethyl acetate fraction of fruit rind of *Garcinia mangostana* L, against T47D cell line. These results were in agreement with the concentration of  $\alpha$ -mangostin.

**Keywords:** *Garcinia mangostana* L, Mangosteen, RP-HPLC, Cytotoxic activity, T47D cell line, MTT assay.

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### INTRODUCTION

The rind of mangosteen (*Garcinia mangostana* L) has many pharmacological activities [1]. It contains abundant xanthenes, such as:  $\alpha$ -mangostin,  $\beta$ -mangostin,  $\gamma$ -mangostin, gartanin, 8-deoxygartanin, and mangostanol [1-4].  $\alpha$ -Mangostin is the major component. It has been used worldwide as traditional medicine for anti-inflammatory [5], antibacterial [6,7], and anticancer effects [8,9]. Since  $\alpha$ -mangostin represents the majority of the clinical benefits of this traditional medicine, several studies were carried out to determine the concentration of  $\alpha$ -mangostin in mangosteen rind extract [10].

This paper reports a new, cheap, precise, accurate and linear isocratic RP-HPLC method for the quantitative estimation of  $\alpha$ -mangostin in the rind extract and fractions of *Garcinia mangostana* L. as well as to determine their cytotoxic activity on T47D breast cancer cell line.

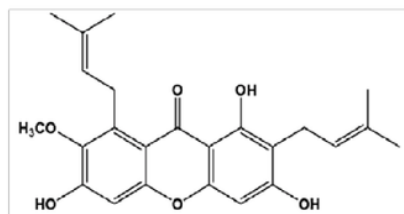


Fig. 1: Structure of  $\alpha$ -mangostin

### MATERIALS AND METHODS

#### Chemical and reagents

Standard  $\alpha$ -mangostin was purchased from Wuxi Gorunjie Natural-Pharma Co., Ltd (Jiangsu, China; purity of 90.3 %). The chemicals and solvents used in this experiment were acetonitrile (HPLC grade), ortho phosphoric acid, methanol (AR grade) were obtained from Merck. High purity aqua bidestilled water was obtained from Otsuka. Solvents used for the mobile phase were filtered through membrane filter (0.45- $\mu$ m pore size) and passed before use.

Methyl Thiazol Tetrazolium (MTT) was obtained from Sigma (Germany). Methanol, dimethyl sulfoxide (DMSO) and all other organic solvents (AR grade) were purchased from E. Merck (Germany). Fetal bovine serum (FBS), penicillin/streptomycin solution (PS) and the Roswell Park Memorial Institute (RPMI)-1640 cell culture medium were purchased from GIBCO BRL.

#### Cell culture

T47D cell line was obtained from Tissue Culture Laboratory of Faculty of Medicine, Gajah Mada University, Jogjakarta, Indonesia and was cultured in RPMI-1640 with 10% fetal Bovine Serum (Gibco) dan 1% Penicillin-Streptomycin.

#### Instrumentation and chromatographic condition

HPLC method was performed on a Shimadzu (Kyoto, Japan) liquid Chromatograph system, equipped with a model LC-20 AD pump, UV-Vis SPD M-20A Diode detector. Separation was performed in a reversed-phase column Shimadzu <sup>®</sup>Shimp-pack VP - ODS (4.6 x 250

mm). The elution was carried out with isocratic solvent using 0.1% v/v H<sub>3</sub>PO<sub>4</sub> in water-acetonitrile (15:85) with a flow rate 1 ml/minute. The solvents used for the mobile phase were filtered through membrane filter (0.45-µm pore size) and degassed before use. Total running time was 15 minutes and the sample injection volume was 20 µL while the wavelength of the UV-VIS detector was set at 243.2 nm. The compound was quantified using CLASS VP software.

#### Plant materials

Ripened *G. mangostana* fruits were collected from Batu Busuk, Limau Manis, West Sumatera, Indonesia and identified at Herbarium Universitas Andalas (ANDA), Indonesia. The fruit rinds were separated from the edible part, chopped using an electric grinder, and dried in a hot oven at 50°C for 72 hours. The dried samples were ground into powder, passed through a sieve (20 meshes). The samples were separately kept in air tight container and protected from light until use.

The powder of fruit rind of *Garcinia mangostana* L. (1253 g) was macerated with 70% ethanol at room temperature (3x5 days). The extract was separated using the rotary evaporator and consequently partitioned between *n*-hexane, ethyl acetate, and butanol. Each fraction evaporated with rotary evaporator and has been stored at refrigerator.

#### Preparation of standard solutions and sample solutions

A stock solution of α-mangostin reference standard was prepared by dissolving an accurately weighed 10 mg of α-mangostin in 10 mL of methanol in a volumetric flask. Various concentrations of the standard solutions were diluted to obtain final concentrations at 0.5; 1; 5; 10; 20; and 30 µg mL<sup>-1</sup> with methanol. The sample extracts were prepared at 1 mg mL<sup>-1</sup> in the same solvent, and were further diluted to obtain 200 µg mL<sup>-1</sup>. The stock solutions were filtered through 0.45 µm syringe filters.

### RESULT AND DISCUSSION

#### Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of α-mangostin at 8.87 min. Figure 2 chromatogram of standard solution (20 µg/ml). The total run time is 25 minutes.

Table 1: System suitability data of the proposed method

Parameters*	α-mangostin
Retention time (min)	8.87
Number of theoretical plates (N)	12202.8
Tailing factor (T)	1.041
Height Equivalent to a Theoretical Plate (HETP)	0.0942

\*Mean of three injections

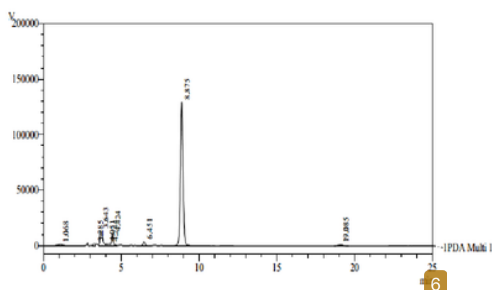


Fig. 2: Chromatogram of the standard α-mangostin. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (R<sub>t</sub>), number of theoretical plates (N), peak Tailing factor (T) and Height Equivalent to a Theoretical Plate (HETP) were evaluated for three replicate injections of the standard at the working concentration.

#### Validation of the method

The described method was validated according to the ICH guidelines [12]. The following validation characteristics were evaluated: linearity, precision, accuracy and the limits of detection and quantification (LOD and LOQ).

#### Linearity

Linearity was determined by using α-mangostin standard solution of 1000 µg/mL in methanol. 0.5 to 30 µg/ml of the standard solution was prepared (n = 3). The calibration Graphs were obtained by plotting the peak area versus the concentration of the standard solutions. The correlation coefficient of α-mangostin is 0.99955 (Table 2), which meet the method validation acceptance criteria and hence the method is said to be linear.

#### Limit of detection (LOD) and limit of quantitation (LOQ)

According to ICH [12], the LOD and LOQ were calculated through the slope and standard deviation method using the following formula: LOD = (3.3 × δ) / S, and LOQ = (10 × δ) / S, Where: δ is the standard deviation of the Y intercept of the linear regression equations. S is the slope of the linear regression equations. The LOD and LOQ for α-mangostin were found to be 0.2807 and 0.9357 µg mL<sup>-1</sup>, respectively, which indicate a high sensitivity of the method (table 2).

#### Accuracy and precision

Intra- and inter-day precision and accuracy were evaluated at three different levels of standard α-mangostin concentrations (5.0, 10.0, and 20.0 µg mL<sup>-1</sup>). Intra- and inter-day assay precision were determined as relative standard deviation (RSD), and intra and inter-day assay accuracies were expressed as percentages of theoretical concentration, as accuracy (%) = (found concentration/theoretical concentration) × 100%. Intra-day assay involved three replicates per day and inter-day assay were performed on three separate days. The inter-day and intra-day precisions of α-mangostin are presented in Table 3 and Table 4. All these data indicated good precision and accuracy. The results showed acceptable precision of the method.

#### Recovery

The recovery of the method was tested by performing recovery studies at 3 levels of α-mangostin reference standard added to the samples. Three different levels concentration (2; 5; and 10 µg mL<sup>-1</sup>) of the standard solution in methanol were added to the sample solution (11.63 µg mL<sup>-1</sup>) and analyzed by the proposed HPLC method. The recovery and average recovery were calculated. Three determinations were performed for each concentration level.

The recoveries of α-mangostin were calculated as the following equation:

$$\text{Recovery (\%)} = \frac{C_{\text{obs}} - C_s}{C_{\text{st}}} \times 100\%$$

where: C<sub>obs</sub> is the observed concentration of α-mangostin detected in the sample solution after added standard α-mangostin solution (µg mL<sup>-1</sup>). C<sub>s</sub> is the concentration of α-mangostin detected in mangosteen peel extract sample solution without added standard α-mangostin solution (µg mL<sup>-1</sup>). C<sub>st</sub> is the actual concentrations of standard α-mangostin solution (µg mL<sup>-1</sup>). The recoveries at 3 different levels of α-mangostin was 94.41, 99.21, and 102.01%, with an average of 98.54% (Table 5). These values indicate good recovery of the method.

#### Determination of α-mangostin content in the extracts of *G. mangostana* rind

HPLC method with isocratic elution was developed for the determination of α-mangostin in *G. mangostana* rind extracts. The mixture of 0.1% ortho phosphoric acid and acetonitrile (15:85) gave

optimum chromatographic separation of  $\alpha$ -mangostin with the other peaks in the extract (Figure 2). The wavelength at 243,2 nm was used for all measurements due to its maximum absorption. The percentage of  $\alpha$ -mangostin in the extract was calculated based on the peak area using its calibration curve. The content of  $\alpha$ -mangostin in the extract was expressed as gram per 100 grams of the extract. Each determination was carried out in triplicate.

**Table 2: Validation parameters for quantification of  $\alpha$ -mangostin**

Parameters	Results
Linear range ( $\mu\text{g mL}^{-1}$ )	0.5 – 30
Regression equation*	$y = 77486. X - 7998.3$
Correlation coefficient	0.99955
LOD ( $\mu\text{g mL}^{-1}$ )	0.2807
LOQ ( $\mu\text{g mL}^{-1}$ )	0.9357

\* x is the concentration of  $\alpha$ -mangostin in  $\mu\text{g mL}^{-1}$ , Y is the peak area at 243,2 nm

**Table 3: Intra-day precision and accuracy of the method**

Intra- day (n=3)			
CA ( $\mu\text{g/ml}$ )	CF (mean $\pm$ SD)	RSD (%)	Accuracy (%)
5	5.0426 $\pm$ 0.00	0.02	100.85
10	9.9865 $\pm$ 0.01	0.10	99.86
20	19.3381 $\pm$ 0.02	0.10	96.69

\* Mean of triplicate analyses in a day.

**Table 4: Inter-day precision and accuracy of the method**

Inter- day (n=9)			
CA ( $\mu\text{g mL}^{-1}$ )	CF (mean $\pm$ SD)	RSD (%)	Accuracy (%)
5	5.1788 $\pm$ 0.12	2.28	103.57
10	10.1502 $\pm$ 0.21	2.04	101.50
20	19.5089 $\pm$ 0.27	1.36	97.54

† Mean of triplicate analyses per day over three days,  $\pm$  CA = concentration added and CF = concentration found.

**Table 5: Recovery study of  $\alpha$ -mangostin in mangosteen rind extract**

Spike Level ( $\mu\text{g mL}^{-1}$ )	*Mean Recovery (%)
2	94.41 $\pm$ 0.00
5	99.21 $\pm$ 0.02
10	102,01 $\pm$ 0.13

\*The results are mean  $\pm$  SD of 3 experiments

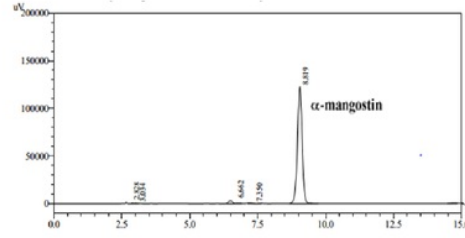
**Table 6:  $\alpha$ -Mangostin concentration in *G. mangostana* fruit rind extract and fractions**

Extracts	* $\alpha$ -mangostin (% w/w)
70 % Ethanol	50.73 $\pm$ 0.12
n-Hexane fraction <sup>a</sup>	11.12 $\pm$ 0.09
Ethyl acetate fraction <sup>b</sup>	98.66 $\pm$ 0.23
Butanol fraction <sup>c</sup>	2.29 $\pm$ 0.01

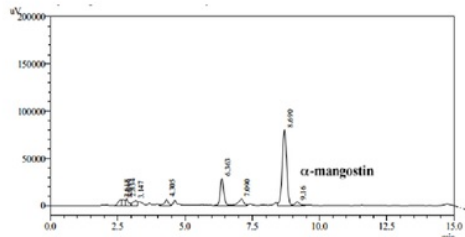
<sup>a-c</sup> refer to sub-extracts of the *G. mangostana* 70 % ethanol extract, \*The results are mean  $\pm$  SD of 3 experiments mangostin

Mangostin content in the samples of *G. mangostana* fruit rind were determined by the developed HPLC method is given in Table 2, based on the peak area. The contents of  $\alpha$ -mangostin in the ethanol

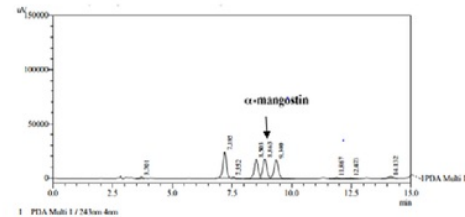
extract, n-hexane fraction, ethyl acetate fraction and butanol fraction were 50.73; 11.12; 98.66; 2.29% w/w, respectively (Table 6).



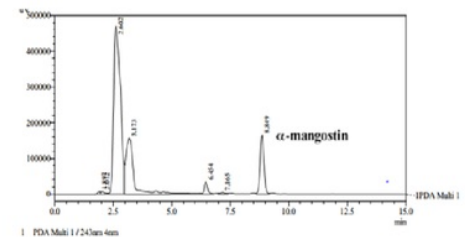
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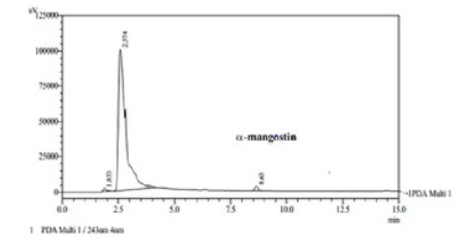
B



C



D



E

**Fig. 3: HPLC chromatograms of *G. mangostana* L. extracts at 243,2 nm. (A) standard mixture of  $\alpha$ -mangostin, (B) ethanol extracts, (C) n-hexane fraction, (D) ethyl acetate fraction, (E)butanol fraction**



The highest concentration of  $\alpha$ -mangostin was obtained in the ethyl acetate fraction. Statistical test results obtained value of  $p < 0.01$ , means at  $\alpha = 0.05$  there is a significant difference between the concentration of  $\alpha$ -mangostin in ethanol fraction, hexane fraction, ethyl acetate fraction and butanol fraction. HPLC chromatograms of all extracts showed the similar pattern with a major peak of  $\alpha$ -mangostin at retention time of 8.87 min. (Figure 2). The identity of the peak of  $\alpha$ -mangostin in the sample chromatograms was confirmed by spiking with its standard and determination of retention time.

#### Cytotoxicity assay

Cytotoxic activity was measured using modified MTT assay [13].  $2 \times 10^3$  cells/well were plated in 96-well plates (Nunc, Denmark) and incubated for 24 h before the addition of drugs in incubator (temperature 37°C). In second day, cell is added with extract and fraction. 20  $\mu$ L extract and fraction 0.1; 1; 10; and 100  $\mu$ g mL<sup>-1</sup> in concentration that has dissolved in DMSO completely were added into 180  $\mu$ L cell suspension in RPMI media. Incubation is continued in 37°C until third day. After 24 h of incubation in T47D cells, 20  $\mu$ L of MTT (Merck, Germany) reagent (5 mg mL<sup>-1</sup>) in phosphate buffered saline (PBS) was added to each well. The plates were incubated at 37°C for 4 h. The medium was discharged and the purple precipitate, which had been formed in the cells, were dissolved with 100  $\mu$ L dimethyl sulphoxide (DMSO). After the incubation at 37°C for 10 min, the absorbance was measured by ELISA microtiter plate reader at wave length 550 nm. In this experiment, ethanol extract and its fractions were tested for their effects inhibition of cell growth against breast cancer cell line T47D, over a concentration range (0.1-100  $\mu$ g mL<sup>-1</sup>) to determine their potency (IC<sub>50</sub>-50% inhibition of cell growth). Assay was performed in vitro on exponentially growing cells. Percentage of cell viability was evaluated by measuring the levels of surviving cell after incubation for 24h with the test samples, using the MTT colorimetric assay. In this assay, Yellow MTT is reduced to a purple formazan dye by mitochondrial enzymes in actively respiring but not necessarily proliferating cells. The intensity of the color formed can be correlated to untreated controls to obtain the IC<sub>50</sub> value by reading the absorbance at wave length of 550 nm. Profiles obtained from cell viability MTT test showed that the ethanolic extract and fractions (n-hexane, ethyl acetate, and butanol) of fruit rind of *Garcinia mangostana* L. in general may decrease the viability of cells T47D cells compared with controls. The resulting cytotoxic effects depending on the level of concentration of the sample where the greater concentration of the sample so the greater the levels of cytotoxic effect.

The IC<sub>50</sub> values clearly indicated that ethyl acetate fraction of mangosteen rind (IC<sub>50</sub> = 0.463  $\mu$ g/mL) had a much more potent effect than the T47D breast cancer cell line tested than ethanol extract, hexane fraction, and butanol fraction (Figure 5). However, butanol fraction has no cytotoxic activity (IC<sub>50</sub> = 51.839  $\mu$ g/mL). According to The National Cancer Institute's America, an extract is said to have cytotoxic activity when IC<sub>50</sub> values <20  $\mu$ g mL<sup>-1</sup> [14]. Statistical analysis showed that the sample type and concentration have significant effect on the percentage of viability ( $p < 0.005$ ) also found an interaction effect between the type of sample and the concentration on the percentage of viability ( $p < 0.005$ ).

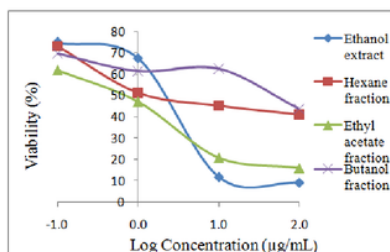


Fig. 4: Viability (%) of T47D cells in different concentrations of *G. mangostana* L.

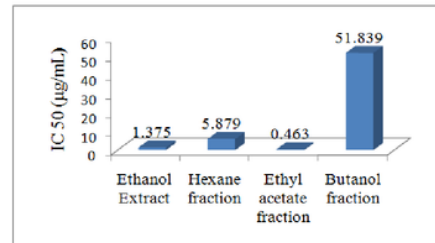


Fig. 5: IC<sub>50</sub> value of ethanol extract and fractions of *G. mangostana* L. against T47D cell line

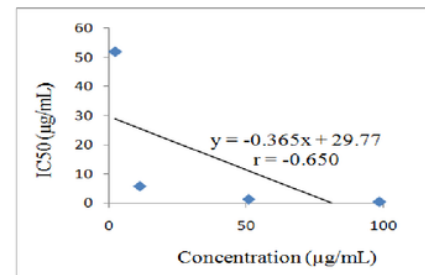


Fig. 6: Correlation between  $\alpha$ -mangostin concentration and IC<sub>50</sub>

It can be seen from Figure 6, the concentrations of  $\alpha$ -mangostin have a close relationship with the IC<sub>50</sub>, where  $r = -0.650$ . The higher the concentration of  $\alpha$ -mangostin then the lower the IC<sub>50</sub> value, or increased cytotoxic activity. Statistical analysis show there is not a significant association ( $p > 0.005$ ).

#### Statistical analysis

Statistical analysis was carried out with the SPSS 15.0 for Windows software package. The results are expressed as the mean  $\pm$  SD. One way ANOVA and two way ANOVA with Tukey HSD test were used to compare the means, and differences were considered significant at  $p < 0.05$ .

#### CONCLUSION

The proposed HPLC method promoted high precision, sensitivity and accuracy for quality control of extract of *G. mangostana* L. rind. This proposed method will be useful for quantitative analysis in standardization and quality assessment of extract of *G. mangostana* L. fruit rind for pharmaceutical uses.

Ethyl acetate fraction from fruit rind of *Garcinia mangostana* L. showed highest activity and potential to find bioactive compound against T47D cell line. Strongest cytotoxic activity of ethyl acetate fraction of *Garcinia mangostana* L. may be due to the high content of  $\alpha$ -mangostin in this fraction.

#### CONFLICT OF INTERESTS

The authors have no conflict of interest

#### ACKNOWLEDGMENT

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#### REFERENCES

- Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. *Garcinia mangostana* L: a phytochemical and pharmacological review. *Phytother Res* 2009;23(8):1047-65.
- Bennett GJ, Lee HH. Xanthenes from guttiferæ. *Phytochem* 1989;28:967-98.

- 20
3. Suksamrarn S, Suwannapoch N, Ratananukul P, Aroonlerk N, Suksamrarn A. Xanthenes from the green fruit hulls of *Garcinia mangostana*. J Nat Prod 2002;6:761-3.
  4. Mahabusarakam W, Iriyachitra P, Taylor WC. Chemical constituents of *Garcinia mangostana*. J Nat Prod 1987;50:474-8.
  5. Gopalakrishnan G, Shankaranarayanan D, Kameswara L, Nazimudern SK. Effect of mangostin, a xanthone from *Garcinia mangostana* Linn in immunopathological and inflammatory reactions. Indian J Exp Biol 1980;18:843-6.
  6. Iinuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, Miyauchi K. Antibacterial activity of xanthenes from guttiferaceous plants against methicillin-resistant *Staphylococcus aureus*. J Pharm Pharmacol 1995;48:861-5.
  7. Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuhiranlert J, Ratananukul P, Chimnoi N, *et al.* Antimycobacterial activity of prenylated xanthenes from the fruits of *Garcinia mangostana*. J Pharm Bull 2003;51:857-9.
  8. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J Ethnopharmacol 2004;90:161-6.
  9. Sumoto K, Akao Y, Kobayashi E, Ohguchi K, Ito T, Tanaka T, *et al.* Induction of apoptosis by xanthenes from mangosteen in human leukemia cell lines. J Nat Prod 2003;66:1124-27.
  10. Yodhnu S, Sirikatitham A, Wattanapiromsakul C. Validation of LC for the determination of  $\alpha$ -mangostin in mangosteen peel extract: a tool for quality assessment of *Garcinia mangostana* L. J Chromatogr Sci 2009;47:185-7.
  11. Pothitirat W, Gritsanapan W. Thin layer chromatography densitometric analysis of  $\alpha$ -mangostin content in *Garcinia mangostana* fruit rind extracts. J AOAC Int 2008;91:1145-8.
  12. International conference on harmonization of note for guidance on validation of analytical procedures: text and methodology, Step 5 of the ICH Process; 1995.
  13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assay. J Immunol Methods 1983;65:55-63.
  14. Boik J. Natural compounds in cancer therapy. 1<sup>st</sup> ed. Oregon Medical Press, LLC, Princeton, Minnesota, USA; 2001. p. 6-8.

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Peerapattana, Jomjai, Kuniko Otsuka, Yusuke Hattori, and Makoto Otsuka. "Quantitative analysis of  $\alpha$ -mangostin in hydrophilic ointment using near-infrared spectroscopy", Drug Development and Industrial Pharmacy, 2015.

Publication

---

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John A. Manthey, Najla Guthrie. "Antiproliferative Activities of Citrus Flavonoids against Six Human Cancer Cell Lines", Journal of Agricultural and Food Chemistry, 2002

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Taher, Muhammad, Tg Muhamad Faris Syafiq Tg Zakaria, Deny Susanti, and Zainul Amiruddin Zakaria. "Hypoglycaemic activity of ethanolic extract of *Garcinia mangostana* Linn. in normoglycaemic and streptozotocin-induced diabetic rats", BMC Complementary and Alternative Medicine, 2016.

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P. A. Boakye. "5-HT3 and 5-HT4 receptors contribute to the anti-motility effects of *Garcinia buchananii* bark extract in the guinea-pig distal colon : 5-HT3 and 5-HT4 receptors contribute to the anti-motility effects of *Garcinia buchananii*", *Neurogastroenterology & Motility*, 01/2012

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Dellai, Afef, Dorra Dridi, Valerie Lemorvan, Jacques Robert, Ameer Cherif, Ridha Mosrati, and Hedi Ben Mansour. "Decolorization does not always mean detoxification: case study of a newly isolated *Pseudomonas peli* for decolorization of textile wastewater", *Environmental Science and Pollution Research*, 2013.

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Kamatou, G.P.P.. "In vitro evidence of antimicrobial synergy between *Salvia chamelaeagnea* and *Leonotis leonurus*", *South African Journal of Botany*, 200611

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Publication

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42

DEGHRIGUE, MONIA; DELLAI, AFEF and BOURAQUI, ABDERRAHMAN. "IN VITRO ANTIPROLIFERATIVE AND ANTIOXIDANT ACTIVITIES OF THE ORGANIC EXTRACT AND ITS SEMI-PURIFIED FRACTIONS FROM THE MEDITERRANEAN GORGONIAN

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EUNICELLA SINGULARIS", International Journal of Pharmacy & Pharmaceutical Sciences, 2013.

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Pothitirat, Werayut, Mullika Traidej Chomnawang, Roongtawan Supabphol, and Wandee Gritsanapan. "Free radical scavenging and anti-acne activities of mangosteen fruit rind extracts prepared by different extraction methods", Pharmaceutical Biology, 2010.

Publication

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Gerald N Teke. "Antimicrobial and antioxidant properties of methanol extract, fractions and compounds from the stem bark of Entada abyssinica Stend ex A. Satabie", BMC Complementary and Alternative Medicine, 2011

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Wiratchanee Mahavorasirikul. "Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro", BMC Complementary and Alternative Medicine,

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---

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Bao, Y.. "Quantitative determination of anti-tumor agent bis(4-fluorobenzyl)trisulfide, fluorapacin and its pharmaceutical preparation by high-performance liquid chromatography", *Journal of Pharmaceutical and Biomedical Analysis*, 20080107

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