CHARACTERIZATION OF A BIOACTIVE SUBSTANCE.
α-MANGOSTEN ISOLATED FROM THE HULL OF
GARCINIA MANGOSTANA L.

KARAKTERISASI SINYAWA BIOAKTIF α-MANGOSTIN
DARI KULIT BUAH GARCINIA MANGOSTANA L.

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ABSTRAK
Kebutuhan obat baru telah meningkat secara kualitatif maupun kuantitatif. Sumber-sumber alami seperti obat tradisional secara luas telah dimanfaatkan oleh masyarakat untuk mengobati berbagai penyakit masalah kesehatan yang sulit diatasi oleh obat-obat barunya. Pencarian obat baru dapat dimulai dari isolasi dan identifikasi kandungan tanaman dari bahan alami. Kail buah Garcinia mangostana L. (Manggis) telah digunakan dalam obat tradisional untuk mengatasi gangguan pendarahan. α-Mangostin, senyawa deriva t kautum dari kandungan utama dalam kulit buah G. mangostana dapat digunakan sebagai senyawa awal untuk pengembangan obat-obat baru. Dalam artikel ini, dilaporkan isolasi dan identifikasi α-mangostin (LC-50 = 1.02 μg/ml, dengan BST) dari kulit buah G. mangostana secara spektroskopis (UV, IR, MS dan 'H-NMR) yang dibandingkan dengan spektra dari α-mangostin baku.

Kata kunci: Garcinia mangostana, α-mangostin, data spektroskopis

ABSTRACT
The need of new medicines is increasing qualitatively as well as quantitatively from time to time. Natural resource such as traditional medicines that have been extensively utilized to treat various diseases, is the ultimate alternative to fulfill the need. Searching for a new medicine can be initiated by isolating and identifying the major compounds present in the natural resources. The hull of Garcinia mangostana L. (Manggis) has been used traditionally to treat respiratory disorders in Indonesia. α-Mangosten, a xanthone type of compound, is a major substance present in the hull of Garcinia mangostana L. can be used as a starting material for development of new medicines. In this paper, isolation and purification procedures of α-mangosten (LC-50 = 8.02 μg/ml on BST) from the hull of G. mangostana are reported. Characterization is carried out by spectroscopic methods (IR, UV, MS and 'H-NMR) compared with standard spectra of α-
mangosten.

Key words: Garcinia mangostana, α-mangosten, spectroscopic data
INTRODUCTION

The need for new drugs is increasing qualitatively as well quantitatively these days. Increasing amount of resistance to antibiotics requires a new type of antibiotics that have different mechanisms of action. Recent recognition of new diseases requires intensive drug search to fight: while some of the deadly diseases remain uncured such as cancer. The plant kingdom is one of some alternatives for a new drug resource, and in fact higher plants have a large amount of secondary metabolites that may be used as alternative new drugs.

Searching for new drugs are indeed time consuming and very costly. The search is initiated by pharmacological guided extraction, partition, isolation, and followed by identification of the active compounds. When the amount of the active substance is not sufficient, total synthesis of the active substance is required. A longer time is needed by the latter step especially when dealing with a complicated chemical structure. This problem can be overcome by selecting the plant material having high concentration of secondary metabolites such as the hull of "Manggis" (Caricae mangostanae L. fam. Ceciaceae). This species demonstrates several interesting pharmacological activities such as antileucemic, monoamine oxidase inhibitors (Robinson, 1963), antibacterials (Sundaram et al., 1983), and antioxidant (Yoshikawa et al., 1994.) Antispasmodic (Brise, 1989), immunopathologic inhibitors (Gopalakrishnan et al., 1980) and spasmodogenic activities (20%) measured on the isolated states of guinea pig have been reported (Wahyono et al., 1997).

Literature studies indicate that G. mangostana contains a variety of compounds such as terpenoids, tannins (o-catechol derivatives), yellowsins and also a high concentration of xanthone type of compound, mangosteen which is characteristic substance for this species. Several xanthone type of compounds have been isolated from the hull of G. mangostana, such as mangosteen (a prenyl xanthone) (Chairangkird et al., 1996) and garcinone (Sen et al., 1982).

Considering these interesting pharmacological activities demonstrated by the hull of G. mangostana and the high concentration of the xanthone compounds in the hull, therefore this species is expected to be a potential candidate for a new drug resource. This paper reports a simple isolation, identification of α-mangosteen, its toxicity on BST (Briones Shrimp Leukemia Virus) and its ability to inhibit the growth of mouse lymphocyt cell lines.

METHODOLOGY

Isolation of α-mangosteen. The fruit of G. mangostana were collected from BPTO Taranganamu, Surakarta on January 20, 1997. The meat was separated and the hull was collected, dried in the oven (40°C), ground on a grinder (Retsch Mibue, Germany). The resulted powder material (300 g) was extracted by n-hexane followed by ethanolic to give n-hexane (5.05 g, 1%) and ethanolic (105.9 g, 21%) extract respectively. The ethanolic extract (5 g) that contained major compound (I) was partitioned with diethyl ether (Et2O) to give Et2O insoluble and Et2O soluble fractions. The later that contained I was partitioned further by Vacuum Liquid Column Chromatography (SiO2 GF254 (E Merck) 15 g) eluted with petroleum ether and increasing amount of EtOAc (Ceil and Bowden, 1986). Eight fractions were collected 30 ml each and fractions (1-4) consisted of I were combined and I was separated by preparative TLC (SiO2 GF254 (E Merck); Petroleum ether - EtOAc: 1:3.75 v/v). Compound I was crystallized in a mixture of CHCl3 and MeOH at equal volume, and it appeared as bright yellow powder.

General Analytical Procedures. The UV spectra was recorded on UV-Via spectrophotometry (Melvyn Roy 3000); the Infrared spectra was run on FT-IR 1000 PC spectrophotometer, the low
Electron Impact (EI) Mass spectra was recorded on a Varian MAT 311A double focusing spectrometer (direct inlet probe), and the 1H-NMR spectra were recorded on Bruker WM 250 with TMS as the internal standard.

Identity of the isolated substance was confirmed by comparing spectra of α-mangostone standard.

Bratts-Kinney Lethality Test. The test followed the procedure described by Meyer et al., (1982) with the LD₅₀ value was determined from 24 hours counts using the probit analysis method described by Finney (1971).

Cytotoxicity testing: The cytotoxicity test was performed on mouse lymphocytic cell lines on 24 wells and each well contained 6x10⁵ cells. Detail of the test won’t be discussed in this paper.

RESULTS AND DISCUSSION

The major compound (1) appeared as an amorphous yellow powder, with a single homogeneous spot on TLC with various solvent systems. UV spectra (MeOH) showed absorption at λ_max 350 (s), 317, 259 (s) and 244 nm which indicated the presence of conjugated double bonds that is specifically as substituted aromatic group. The IR spectra of 1 (KBr, cm⁻¹) (Figure 1) showed a broad absorption bands at 3423 (OH), 1650 (C=O, 1600 (C=O), 1600 (Ar C=C), 1475 and 1375 (geminal di-C-CH), 1157 and 1281 (C-O-C) (Silverstein et al., 1991; Dyer, 1965).

![Image of IR spectra](image-url)
Figure 2. Low Resolution EI Mass spectra of compounds 1

The low resolution EIMS of 1 (Figure 2) displayed a molecular ion peak at m/z 410 (71%) and was identified as C_{28}H_{43}O by the high resolution EIMS. The spectra displayed major peaks at m/z 395 (10%), m/z 410 (13%) due to the loss -C_6H_5 group; m/z 367 (30%), m/z 410 (33), 351 (36%), m/z 367 (35%), 339 (true peak, 100%), m/z 367 (35%), 311 (29%), m/z 339 (28). The low resolution EIMS spectra of 1 was compared to that of reference standard (α-mangostin), they turned out to be superimposable. This result indicated that 1 was positively α-mangostin.

The identity of 1 was determined further by 1H-NMR and the proton chemical shift was presented in Table 2. The 1H-NMR (CDCl_3) spectra of 1 (Figure 3) demonstrated the presence of four allylic methine, two of them were chemically equivalent [δ, 1.84 ppm (d, 6 Hz, H-19 & H-20)] and the other two were chemically inequivalent [δ, 1.85 (d, 3 H, d, H-14 & H-15 interchangeable). Two aromatic methylene groups appeared at δ 7.40 ppm (d, 7.1 Hz) of H-11 and δ 7.47 ppm (d, 7.0 Hz) of H-16. Each of these two methylene groups were attached to two functional groups, and the H-16 was more downfield compared to H-11 due to inductive effect of two -OH groups (C-1 & C-3) causing H-16 to more deshielded than H-11. Two allylic methine protons (H-12 & H-17) appeared at δ 5.13 ppm, t (δ, 6.9 Hz), and two aromatic proton (H-4 & H-5) appeared at δ, 6.43 & 6.88 ppm (q). In addition, there two characteristic signals were present in this spectra. A sharp signal at δ, 3.84 ppm (s) integrated as 7 protons was identified as an hydroxy group, and a very down field signal at δ, 13.60 ppm was identified as an acyl proton signal of the phenolic -OH. This 1H-NMR spectrum was identical to the reported 1H-NMR of α-mangostin by Jefferson et al. (1970); therefore, compound 1 was confirmed as α-mangostin.
Compound I was toxic to Brine Shrimps as shown in Table 2. The Brine Shrimps Lethality test (BST) has been used to screen the presence of anticancer compounds from natural origin by some workers (Meyer et al., 1982; McLaughlin & Ferrugia, 1983; Gu et al., 1995; Eder et al., 1998a). Compound I was tested initially at the dose of 100, 75, 25 and 10 μg/ml at the lowest dose indicated, 62% nauplii were killed and based on Probit analysis, compound I had LC₅₀ at 8.92 μg/ml in the Brine Shrimps Lethality Test.

Cytotoxicity test was performed on mouse lymphocyt cells line at the Inter University Center, Gadjah Mada University using 24 wells (6 x 10⁵ cells/well). The test was observed after 24 hours incubation. Up to 0.3 μg/ml 1.65 x 10⁻⁶ μg/ml compound I was still able to inhibit (70%) the growth of the mouse lymphocyt cells line.
Table I. $^1$H-NMR (250 MHz), chemical shifts (δ, CDCl$_3$ + TMS) and coupling constants (in parentheses, Hz) for compound I

<table>
<thead>
<tr>
<th>H</th>
<th>Compound I</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-4</td>
<td>6.43 ppm (s)</td>
</tr>
<tr>
<td>H-5</td>
<td>6.83 ppm (s)</td>
</tr>
<tr>
<td>H-11</td>
<td>3.40 ppm, d (7.1)</td>
</tr>
<tr>
<td>H-12 &amp; H-17</td>
<td>3.37 ppm, t (6.9)</td>
</tr>
<tr>
<td>H-14 &amp; H-15</td>
<td>1.98 (s) &amp; 1.77 (s) ppm (interchangeable)</td>
</tr>
<tr>
<td>H-16</td>
<td>4.17 ppm, d (7.0)</td>
</tr>
<tr>
<td>H-19 &amp; H-20</td>
<td>1.84 ppm (s)</td>
</tr>
<tr>
<td>- OCH$_3$</td>
<td>3.84 ppm (s)</td>
</tr>
<tr>
<td>- OH (phenolic)</td>
<td>13.62 ppm (s)</td>
</tr>
</tbody>
</table>

Table II. Blue Shrimps Lethality test (GST) data of compound I (α-mangosteen, n=6)

<table>
<thead>
<tr>
<th>Dose (μg/ml)</th>
<th>Death (%)</th>
<th>Probit Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>6.960</td>
</tr>
<tr>
<td>75</td>
<td>96</td>
<td>6.736</td>
</tr>
<tr>
<td>25</td>
<td>66</td>
<td>5.544</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>5.308</td>
</tr>
<tr>
<td>LC$_{50}$</td>
<td>8.02 (calculated)</td>
<td>5.000</td>
</tr>
</tbody>
</table>

α-Mangosteen

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CONCLUSIONS

1. The major compound present in the leaves of *G. mangle* was identified as α-mangosteen based on its spectroscopic data in comparison with spectra of α-mangosteen standard.
2. α-Mangosteen demonstrated LC50 = 8.02 μg/ml on the BST bioassay
3. It’s up to the dose 1.65 x 10^7 μg/ml α-mangosteen was still able to inhibit (70%) the growth of the mouse lymphocytic cells line, observed after 24 hours incubation.

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LITERATURE

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