

## Phytochemical Compounds of *Garcinia mangostana*-Linn Pericarp Fractions from Ethanolic Extract

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### Abstract

The use of drugs derived from plant-based materials or treatment derived from natural products, is currently being developed, including in Indonesia. Indonesia is a country with various biodiversity. About 30,000 species of plants identified, and of all the species, 7,000 species are medicinal plants. One of the plants that are medicinal plants is mangosteen or in Latin, known as *Garcinia mangostana*-Linn (GML). Some literatures suggest mangosteen has the properties as a medicinal plant. The purpose of this study was to determine the phytochemical compounds of GML fraction of the pericarp ethanol extract. Phytochemical compounds of chloroform fraction, ethyl acetate fraction and ethanol fraction of ethanolic extract of GML pericarp were identified by gas chromatography – mass spectrometry (GC-MS).

The result of this study indicates that the GML fractions, from ethanolic extract have various phytochemical compounds that potentially for development new medicine.

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### Introduction

Current research uses of plants for the treatment of various diseases are being developed. According to World Health Organization (WHO), 80% people around the world have used traditional medicine for the primary health care.<sup>1</sup> Plants have used as traditional medicine with less adverse effect. One plant origin from Indonesia rain forest is mangosteen, or *Garcinia mangostana*-Linn (GML) belonging to the family Guttiferae. It cultivated in Southeast Asia rainforest, like Indonesia, Malaysia, Srilangka, Phillipines, and Thailand.<sup>2,3</sup> Besides as a fresh fruits, the GML has long history of folk medicine in the world.

Many previous studies have shown secondary metabolites or phytochemical compounds of the GML plants have been used as medicine, such as anti diarrhoea, dysentery, skin infection, and as anti-inflammatory agent, cytotoxic, antioxidant, antitumoral, immunomodulatory, neuroprotective, anti-allergic, antiviral properties.<sup>3-6</sup> The objective of this present study is to identify the phytochemical compound from three different fractions (chloroform, ethyl acetate and ethanol fraction) from an ethanol extract by using gas chromatography – mass spectrometry (GC-MS) technique. This present study is interesting, because the significance of research on natural medicine plants is important. There is growing the awareness in correlating the phytochemical compounds of a GML with its pharmacological activity.

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### Materials and methods

#### Preparation of *G. mangostana*-Linn extraction and fractionation

*Garcinia mangostana*-Linn (GML) was collected from Puspahiang Village in West Java,

Indonesia. We separated the pericarps and the arils. The pericarp were dried at room temperature and blended to become powder. We extracted pericarp powder with ethanol 75% and macerated for 48 hours at room temperature. The solvent was evaporated with rotary vacuum evaporator at 50°C. After that, the crude extract was chromatographed on silica gel column pack column (Merck), eluted with three different solvent, ethyl acetate, chloroform, and ethanol, become 3 fractions.

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analyses were conducted to the 3 fractions of GML pericarp. GC-MS analysis was carried out on a Shimadzu GCMS-QP2010 Ultra, with ion source temperature 200 °C, interface temperature 240 °C and solvent cut time 2.5 minutes. Separation was performed using a 30 m x 0.25 mm x 0.25 mm RXi-1MS silica-fused capillary column coated with 0.25 µl films of poly (dimethylsiloxane) as the stationary phase (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at an average flow rate of 28 cm<sup>3</sup> per minute. The injector and the transfer line temperatures were kept at 240°C. The column oven temperature program used was 120°C - 260°C at a rate of 20°C per 10 minutes. Initial and final temperatures were held for 2 minutes and 10 minutes. The injections were carried out in a split mode with a split ratio of 20:1, split flow: 1ml/min, scan mass range 50m/z-500m/z.

The mass spectrometry was operated with an ionization voltage of 235 eV and electron multiplier voltage of 1700 V and was scanned from 50 m/z to 500 m/z at 0.8 s per scan. The volume injected samples ranged from 1 µl to 2 µl. GC-MS performed based on the database having more than many patterns.

The spectrum of the unknown compound was compared with the spectrum of the known compound in the library based on retention time and mass spectra. The phytochemical compounds of GML pericarp fractioned were identified using the mass spectral library Willey 7 and NIST 11.

### Results

In this study, we analysis, GC-MS of the phytochemicals compounds in three different fractions (chloroform, ethyl acetate and ethanol fractions) using column chromatography of

ethanol extract of GML pericarp. We have identified five phytochemical components from chloroform fraction, presented in tables 1.

Peak no	Name of the compound	Molecular formula	Molecular weight	Area %
1	Hexadecanoic acid methyl ester	C17H34O2	270	4.16
2	Hexadecanoic acid, ethyl ester	C18H36O2	284	2.75
3	Hexanedioic acid, bis(2-ethylhexyl)ester	C22H42O4	370	53.34
4	Bis(2-ethylhexyl)phthalate	C24H38O4	390	31.30
5	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	C24H38O4	390	8.46

**Table1.** Phytochemical compounds of Chloroform Fraction from GML Pericarp Ethanol Extract.

The major chemical compound of chloroform fraction is hexanedioic acid, bis(2-ethylhexyl)ester (53.34%).

Besides chloroform fraction, we have identified four phytochemical components from ethyl acetate fraction, presented in table 2 below.

Peak no	Name of the compound	Molecular formula	Molecular weight	Area %
1	Ethyl.alpha.-d-glucopyranoside	C8H16O6	208	2.63
2	3-Methyl-1H-indene	C10H10	130	0.93
3	Hexanedioic acid, bis(2-ethylhexyl)ester	C22H42O4	370	10.50
4	1,2Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	85.94

**Table 2.** Phytochemical Compounds of Ethyl-Acetate Fraction from GML Pericarp Ethanol Extract.

We have identified 4 phytochemical compounds in ethyl acetate fraction, and one of the component, hexanedioic acid, bis(2-ethylhexyl)ester was also identified from chloroform fraction. The major compound from ethyl acetate fraction are 1,2Benzenedicarboxylic acid, diisooctyl ester(CAS) Isooctyl phthalate (85.94%) and Hexanedioic acid, bis(2-ethylhexyl)ester (10.50%).

From ethanol fraction, 12 phytochemical compounds were identified. The major components are 9-Octadecenamide, N,N-dimethyl-(CAS) N,N-Dimethyl Octadec-9-Enamide (29.13%) and 9-Octadecenamide,N,N-dimethyl- (22.31%) (Table 3).

Peak no	Name of the compound	Molecular formula	Molecular weight	Area %
1	Phenol,2,4-bis(1,1-dimethylethyl)-	C14H22O	206	1.73
2	Hexadecanoic acid, methyl ester	C17H34O2	270	3.43
3	1-Nonadecene	C19H38	266	3.61
4	Hexaethylene glycol,	C12H26O7	282	2.13
5	9-Octadecenoic acid (Z)-,methyl ester	C19H36O2	296	2.37
6	Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	298	2.12
7	1-Heptacosanol	C27H56O	396	2.29
8	N,N-Dimethylpalmitamide	C18H37NO	283	11.65
9	9-Octadecenamide, N,N-dimethyl	C20H39NO	309	29.13
10	9-Octadecenamide, N,N-dimethyl-	C20H39NO	309	22.31
11	N,N-Dimethylpalmitamide	C18H37NO	283	4.30
12	Bis(2-ethylhexyl) phthalate	C24H38O4	390	14.91

**Table 3.** Phytochemical compounds of Ethanol Fraction from GML Pericarp Ethanol Extract.

GC-MS chromatogram of the ethanol fraction of GML pericarp extract, showed twelve distinct peaks, and the major compound is 9-Octadecenamide, N,N-dimethyl-(CAS) N,N-Dimethyl Octadec-9-Enamide (29.13%) and 9-Octadecenamide,N,N-dimethyl (22.31%).

## Discussion

Secondary metabolites have biological activities in physiological systems. Previous research has identified that pericarp of GML revealed the presence of prenylated xanthenes, benzophenones, bioflavonoids and triterpenes.<sup>3</sup> Therefore, studies, have reported many biological activities of phytochemical compounds from mangosteen, such as anti-oxidant, anti-tumor, anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, anti-malaria, anti-HIV, anti-histamine, anti-ulcerative activities, CNS-depressant, cardiovascular, and cytotoxic.<sup>2</sup> GC-MS is a valuable tool for phytochemical component identification. In this present study, we identified twenty-one phytochemical compounds from three different fractions of GML pericarp were detected in the GC-MS

chromatograms in this study. Based on the GC-MS analysed, we had found phthalate compounds from chloroform fraction (Bis(2-ethylhexyl)phthalate; 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester); from ethyl acetate fraction (Hexanedioic acid, bis(2-ethylhexyl)ester; 1,2Benzenedicarboxylic acid, diisooctyl ester(CAS)Isooctyl phthalate); and from ethanol fraction (Bis(2-ethylhexyl) phthalate).

Phthalate is a well-known synthetic plasticizer.<sup>7</sup> More than 18 billion pounds of phthalate are used as plasticizer of polyvinyl chloride products every year, and about 6 million ton of plasticizers are consumed every year. Phthalates used in large number of product, including pharmaceutical pills, supplemens, and also applied in several commercial compounds.<sup>8</sup> It is interesting, although phthalate toxicity levels have varied, depending on its structures, many studies have identified the presence of phthalate on medicinal plants, which most often exhibited antimicrobial activities.

Hence the controversy on the safety of phthalates in medicine plants, the safety usage of the plant products should be first tested in animals to evaluate their toxicity. Our previous study, in acute oral toxicity of ethyl acetate fraction of GML pericarp ethanolic extract in Spraque-Dawley rats, reveal that the LD<sub>50</sub> is greater than 15,480 mg/kg body weight,<sup>9</sup> and the cytotoxicity study stated, ethyl acetate fraction from ethanolic extract of GML pericarp was not toxic to HaCaT cell line for 24 h at concentration 500ppm, 1000ppm, and 2000ppm.<sup>4</sup> Thus, the ethyl acetate fraction of ethanolic fraction of GML pericarp is still within safe limits on experiments with animals and to the HaCat cell.

Here are various medicinal plants, with secondary metabolites similar to that found in this present study and its medicinal activities. Phylanthus, have been reported produce phthalates (bis)2-ethyloctyl phthalate and bis (2-ethylicosyl)phthalate) which is an antimicrobial. The leaves of *Pongamia pinnata*, had been reported consist of phthalates, and also have antimicrobial activities.<sup>7</sup> Other medicinal plants is flowers of *Calotropis gigantea*, has antimicrobial activities against pathogenic bacteria and fungi.<sup>10</sup>

*Eucommia ulmoides*, native medicinal plant from China, has been used for Traditional Chinese Medicine for thousand years and consist of Hexanedioic acid, bis (2-ethylhexyl) ester, one

of phthalate, which has anti-tumour, anti-inflammation, anti-cancer activities. Hexadecanoic acid plays an important role as anti-inflammation process, by helping to make the design of specific inhibitor of phospholipase A.<sup>11</sup> Furthermore, in this present study, GC-MS analysis showed 1,2 -Benzendicarboxylic acid, mono(2-ethylhexyl)ester from chloroform and ethyl acetate fraction. Govindappa M, et al have reported the effect of 1,2 -Benzendicarboxylic acid, mono(2-ethylhexyl)ester, 9-Octadecenoic acid (Z)-methyl ester, hexadecanoic acid as antiviral, antimicrobial, antifungal, antioxidant and anti-inflammatory, but were isolated from different natural sources and extracted with different material.<sup>12</sup> Cytotoxic activity of bioactive compound 1,3-Benzene dicarboxylic acid, mono 2 ethylhexyl ester, , extracted from a marine derived *Streptomyces* sp, have also been reported.<sup>13</sup>

Phytochemical compounds from chloroform and ethanol fraction, such as Hexadecenoic acid methyl ester has antiinflammation and antifibrotic activities, 9-hexadecenoic acid methyl ester (Z)- and octadecanoic acid methyl ester have antioxidant activities, and antimicrobial activity.<sup>12</sup>

## Conclusion

The finding of this study indicates the presence of twenty-one phytochemical compounds of three fractions of GML pericarp ethanol extract, which may be responsible for the pharmacological activity. All the phytochemical compounds were needed further research for development of new medicine.

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## Declaration of Interest

The authors report no conflict of interest.

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