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Gas Chromatography and Gas Chromatography-mass Spectrometry Analysis of Indonesian *Croton tiglium* Seeds

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Abstract: The result of phytochemical analysis showed that the hexane-soluble extract of seeds of *C. tiglium* contained fatty acids, terpenoids and alkaloids, while the ethanol-soluble extract of the seeds contained alkaloids, flavonoids, steroids, terpenoids and saponins. Moisture and proximate analysis showed that the seeds contained fat up to 40,1%, protein 26%, carbohydrate 15.51 and other elements such as fiber, moisture and ash. Gas Chromatography (GC) analysis on hexane-soluble extract of the seeds, using available instrument and reference-standards in our laboratory, showed 17 peaks indicating that at least the fat contained 17 compounds. Part of the compounds, 8 of them were identified as fatty acids and the 9 were unknown. The highest fatty acid level was linoleic acid (43.67%), oleic acid (19.98%) and myristic acid (7.64%). The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the hexane-soluble extract showed at least 32 compounds and the ethanol-soluble extract showed at least 25 compounds.

Key words: *Croton tiglium*, bioactive, extract, GC, GC-MS

INTRODUCTION

Back to nature has recently become the trend of life in our society. It believes that consuming natural medicines is relatively safer than consuming synthetic drugs. This has caused high demand of natural medicine in the world. Therefore, the prospect of medical plant market in Indonesia is open wide (Kardono *et al*., 1990).

Indonesia is one of the countries, which is rich on medicinal plants. Its biodiversity is the second largest after Brazil. Thirty thousands out of forty thousand types of plants can be found in Indonesia and 940 of them are known to have restorative power. They have been used as traditional medication by many generations of many ethnics in Indonesia. One of them is *Croton tiglium* that is widely believed to function as laxative (Dictionary of Natural Products, 1982).

Indonesian medical plants have been fascinating to many researchers from industrial countries due to the discovery of bioactive compounds, which have potentials to be developed as drug elements for industry. They come from Japan, France, Netherland, Australia, Germany, Switzerland and United States of America, England with Japan dominating 60% of the researches (Kardono, 1991).

C. tiglium is found in many regions in Indonesia and named differently in each region. In Sumatra it is called Simalakian, Ceraken in Java, Roengkok in North Sumatra, Semoeki in Ternate, Kowe in Tidore and Kamadrah in Central Kalimantan (Duke, 1983). In the last mentioned region, the seed is widely used because it is believed to have healing power and function as laxative (Sangat *et al*., 2000). However their knowledge of the plant is merely not in depth that they inherited from their previous generation. Its dosage and active materials contained have not yet been found (Duke, 2001; Frederiksen *et al*., 2004). It would be interesting if the explored plant becoming a product useful for a pharmaceutical industry, an added value to agroindustrial products (Heyne, 1988).

Most of traditional medicines are developed from nature. They have not yet fulfilled the scientific

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requirements needed to be developed and classified as modern medicines (Gupta, 1994; Quisumbing, 1951). To scientific back up, a research is needed to search their bioactive components, their efficacy and safety (Dipalma, 1971; Farnsworth *et al*., 1985). Usually, most compounds useful for medicinal purpose are secondary metabolites (De Padua *et al*., 1999; Jamaran, 1995).

In the development of medicinal plant industry, Indonesian traditional plant medicines are classified into three groups, herbs (*jamu*), standardized extracts and phytopharmaceuticals (Anonymous, 1982).

There are some strict requirements for standardizing the extracts (Anonymous, 1982). Some of them are: correctness and restorative power proven, uniformity of active constituents, its efficacy, safety and its assurance, both in quality and quantity (Corral *et al*., 1988; Guerrero *et al*., 1990).

The purpose of this research is to analyze the constituents of *C. tiglium* seed extracts to give contribution for qualitative standardized extract development, as laxative.

MATERIALS AND METHODS

Plant materials: *C. tiglium* seeds, were collected from its natural habitat in Tamiang layang and Muara Teweh forest, in the boundary of South Kalimantan and Central Kalimantan.

Instrument and general procedures: Gas Chromatography (GC) (Shimadzu-GC-HP 6890 Series), Gas Chromatography-Mass Spectrometry (Shimadzu GCMS-QP2010), magnetic stirer, buchner funnel, filter paper, analitical balance, candle jar, candle, swap, blender, centrifuse, ice box, stirer, autoclave, incubator, laminar flow, microscope, object glass, micro pippet, magnifying glass, petridish, colony counter, reaction tube, sterilized bottle and measurement flask.

GC and GCMS experiment conditions

Determining moisture content and proximate analysis: Determining the moisture content was done on its weight based on its drying porcelain plate at temperature above 100°C for 30 min. After it cooled down in exicator, then was weighed. Materials that have been weighed were put into the porcelain and dried in an oven at 105°C for 2 h.

Proximate analysis was conducted for ash, fat, protein, fiber and carbohydrate contents.

Preparation of *C. tiglium* seeds organic-soluble extracts: The seed is refined, sun-dried and grinded into powder. About 200 g powdered seeds were extracted continuously by masseration using hexane solvent (3×1 L) and evaporated at 35°C to yield the hexane-soluble extract. The residues (5 g) were masserated with 50 mL ethanol for 24 h, repeated for 3 times and then filtered. The filtrate was evaporated and sticky ethanol-soluble extract was obtained. The sticky extract was dried using vacuum-oven at 35°C.

Phytochemical analysis: Phytochemical analysis was conducted to explore the secondary metabolites, such as, alkaloid, quinone, flavonoid, saponin, tanin and triterpenoid, based on an established procedure (Harborne, 1973).

GC-MS identification of secondary metabolites: Gas Chromatography-Mass Spectrometry (GC-MS) was applied to identify seed components of hexane- and ethanol-soluble extracts. About 1 μL of each extract was is injected into GC-MS column inlet port and was analysed using instrument standard procedure resulted the Total Ion Chromatogram and GC-MS ion spectra (Ubhaysekera *et al*., 2004). Interpretation on mass spectrum GC-MS was conducted using computer database software class 5000 (Shimadzu) and database of National Institute Standard and Technology (NIST) 12, 62 having more than 62.000 patterns. The retention time indicated the score of every spectrum done by calculation using retention time data from standard n-alkane (C_8-C_{22}) injected with concentration 0.1% at the same condition with sample injection condition.

The spectra then compared to those of pattern spectra available from data base literature. The compound identity was confirmed by comparing Linier Retention Indeces (LRI) of calculation result components with the score of LRI of certain component from the literature. Identified compound can be confirmed if the score of the LRI of the calculation result equal to that of the literature (Ubhaysekera *et al*., 2004).

RESULTS AND DISCUSSION

The results of moisture and proximate analysis showed that the seed of *C. tiglium* contained highest fat level up to 40.01%, while ash content only 14%. The seeds major constituents were fat, protein and carbohydrate. The results is showed in Fig. 1.

The phytochemical analysis data indicated that the seeds containing various secondary metabolites, such as, alkaloid, flavonoid and saponin as showed in Table 1.

The hexane-soluble extract contained less various components. Most of the compounds were fatty acids.

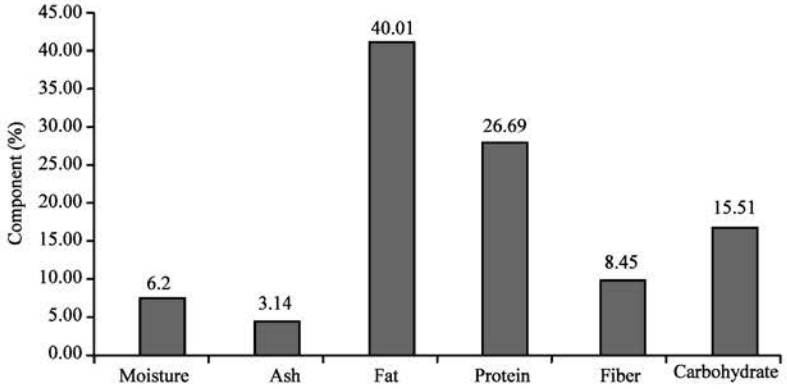

Fig. 1: Moisture and proximate analysis results

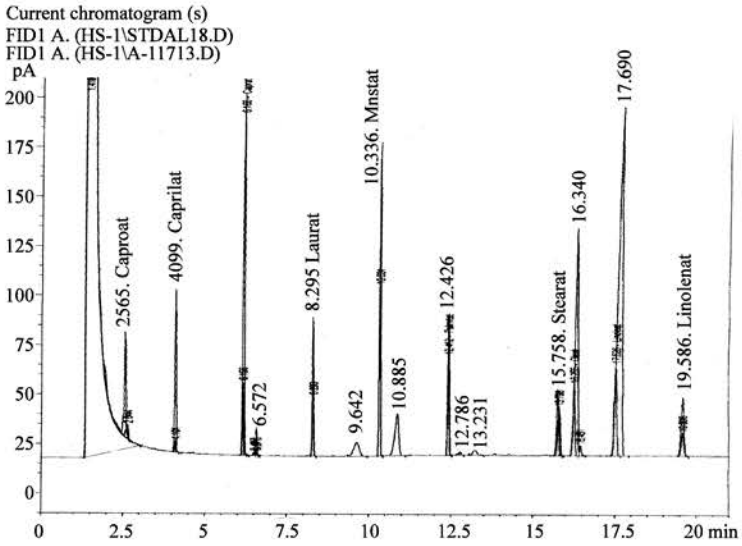

Fig. 2: Chromatogram GC on fatty acid from *C. tiglium* seeds

Table 1: Result phytochemical analysis of *C. tiglium* seeds

Phytochemistry test	Result test	
	Hexane-soluble extract	Ethanol-soluble extract
Alkaloid		
Dragendorf	-	+
Mayer	+	++
Wagner	+	+++
Quinon	-	-
Flavonoid	-	+
Saponin	-	+
Tanin	-	-
Triterpenoid	-	-

-: unknown, +: little, ++: sufficient, +++: much

It is likely, that the laxative potentials as traditional application of the seeds are probably the ethanol-soluble extracts containing alkaloids, flavonoids or saponins (Hutapea, 1994).

Fatty acid gas chromatogram (GC) of the hexane-soluble extract of *C. tiglium* seed showed 17 peaks with 8 identified peaks in various percentage of fatty acid degree, show in Fig. 2.

Table 2 showed that linoleic acid is the highest component up to 43.67% followed by oleic acid and myristic acid with 19.98 and 7.64%, respectively.

Based on GC-MS library search data, the hexane-soluble extract showed 32 constituents as presented in Fig. 3. The main components were 9,12-octadecadienoic acid appeared at retention time (rt.) 73.163 min. Octadec-9-enoic acid appeared at rts 73.498, 9,12-actadecadienoic acid 70.721, hexadecanoic acid and unknown compounds rts. 65.005, 65.132 and 65.241, octadecanoic acid and unknown compounds rts. 56.967, 57.442 and 56.561, 9-octadecenoic acid, rt. 71.130 min, respectively.

The mass spectrums that gave the major compound with Mol Weight (MW) 294. Figure 4 show chromatogram

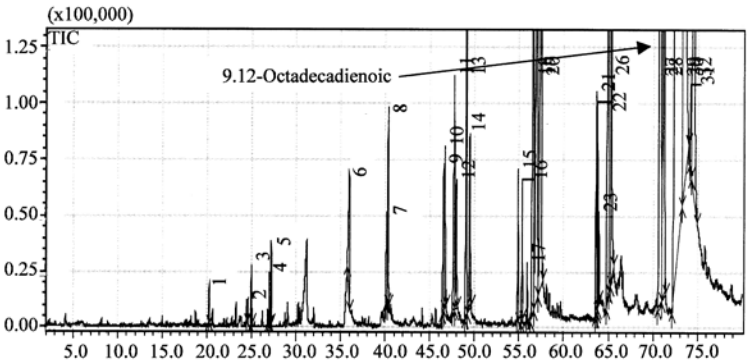

Fig. 3: GC-MS chromatogram of hexane-soluble extract

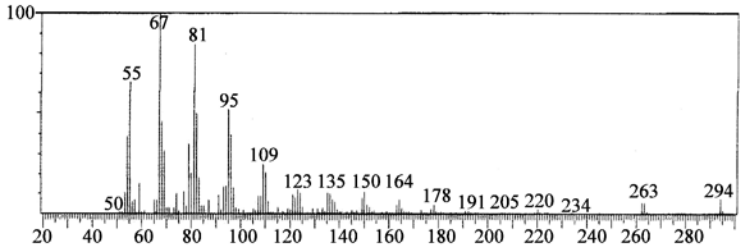

Fig. 4: Mass spectrum hexane-soluble extract of F27 from *C.tiglium*

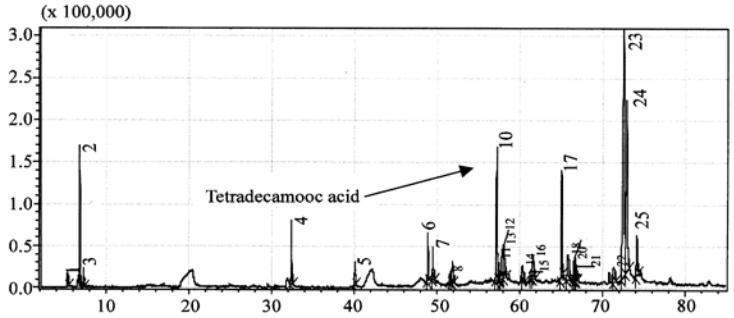

Fig. 5: GC-MS total ion chromatogram of ethanol-soluble extract

Table 2: Fatty acids content of *C.tiglium* (Hexane extract) and yield in the seeds

Component	Concentrating w/w in hexane extract (%)	Yield in the seed w/w, in (Ascumption as if all fatty acids were extracted by n-hexane) (%)
Caproic acid	1.09	0.59
Caprilic acid	0.52	0.28
Capric acid	3.24	1.78
Unknown	0.17	0.09
Unknown	0.19	0.10
Lauric acid	2.69	1.48
Unknown	2.24	1.23
Myristic acid	7.64	4.20
Unknown	5.02	2.76
Palmitic acid	6.86	3.77
Unknown	0.16	0.09
Unknown	0.58	0.32
Stearic acid	3.57	1.96
Oleic acid	19.98	10.99
Unknown	0.53	0.29
Linoleic acid	43.67	24.02
Linolenic acid	1.88	1.03

mass spectrum of F27 from hexane-soluble extract of *C. tiglium* seeds. The major compound according to MS data F29 is predicted as 9,12-octadecadienoic acid is suggested to be a essential fatty acid and use in cosmetic as emolient for dry skin (Dictionary of Natural Products, 1982).

GC-MS total ion chromatogram of the ethanol-soluble extract showed 25 major peaks, indicating its secondary metabolite constituents, as showed at Fig. 5. Some of trace components were also still containing 11,14-Eicosadienoic acid rt. 72.567, octadec-9-enoic acid rt. 72.952, tetradecanoic acid rt. 57.235, 11-eicosenoic acid rt. 58.059, the unknown, rts. 51.625 and 51.893, hexadecanoic acid rt. 65.077, 9,12-octadecadienoic acid, rt. 65.907 and the unknown rt. 60.391, 9-ocatadecenoic acid rt. 74.192 the unknown, rt. 66.858, eicosenoic acid, 61.657, the unknown rt. 57.400, dodecanoic acid rt. 48.911 and

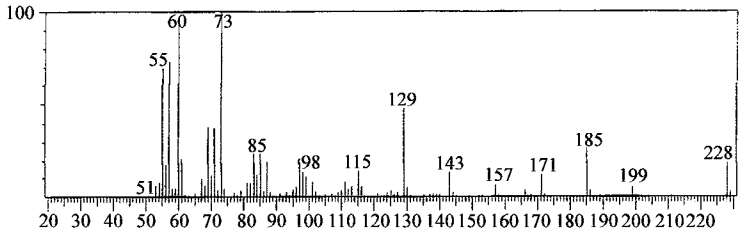

Fig. 6: Mas spectrum ethanol-soluble extract of F10 from *C. tiglium*

decanoic acid rt. 40.127 mins, respectively, indicating that the hexane extraction of the seeds of our experimental work, were not able completely to extract the fatty acid contents of the seeds.

The mass spectrums that gave the major compound with MW 228. Figure 6 show chromatogram mass spectrum of F10 from ethanol-soluble extract of *C. tiglium* seeds. According to mass spectrums data F10 is predicted as Tetradecanoic acid.

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