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PHYTOCHEMICAL AND GC-MS ANALYSIS OF OLEORESIN OF *DIPTEROCARPUS GRACILIS* BLUME: AS A BASIC CONSIDERATION FOR HUMAN REMEDY

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ABSTRACT: *Dipterocarpus gracilis* Blume is one tropical species of Dipterocarps which is included in the red list IUCN as a rare and endangered tree species. In consideration of *Dipterocarpus gracilis* Blume conservation and sustainability of its oleoresin, this present study aimed to determine specific characteristics of oleoresin origin of *Dipterocarpus gracilis* Blume as an establishing basis for appropriate product diversification of human remedy. Here, is also evaluated the presence of different phytochemical along with GC-MS investigations of water and ethanol soluble crude extracts, and anti-oxidant obtained in oleoresin. The results showed that oleoresin of *Dipterocarpus gracilis* Blume revealed the presence of alkaloid and triterpenoid, whereas antioxidant activity test had low in 95% ethanol soluble. While, GC-MS analysis of organic compound of oleoresin of *Dipterocarpus gracilis* Blume identified that the most organic compound such as Caryophyllene, Caryophyllene oxide, Cyclohexanepropanal, 2,2-dimethyl-6-methylene-, 2, 6, 10, 14, 18, 22-, Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-E)-, 4-Pregnen-21-ol-3,20-dione glucoside, 1,4-Methanophthalazine, 1, 4, 4a, 7, 8, 8a-hexahydro-9,9-dimethyl-, (1α, 4α, 4a α, 8a α)-, and 9, 10-Secoergosta-7, 10(19), 22-triene-3, 5, 6-triol, (3β)- had an effective anti-arthritic agent, anti-inflammatory, anti-cancer, anti microbial, anti-bacterial as well as contain vitamin D which will affect health of human liver. Thus, the present study has proved the utilization of *Diptero-carpus gracilis* Blume for potential sources of active drugs for a human being.

INTRODUCTION: Dipterocarp is the most important tribe dominates tropical lowland forest area in Kalimantan, Indonesia consisting of several genera which have great utilizing for a human being. One important species can be utilized *Dipterocarpus gracilis* Blume of *Dipterocarpus* genus.

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D. gracilis Blume is one of 23 species in *Dipterocarpus* genus which is included in the red list as a rare and endangered tree species [1]. Besides producing commercially valuable timber, this species also produces Oleoresin which is one of Non-Timber Forest Products (NTFPs) that has high economic value in the market. Oleoresin is a liquid resin, good odor, sticky and oily.

As a natural essential oil, local communities have recognized oleoresin as "keruing" oil, but also known as balsam, oil resin or lagan oil has high economic value [2]. Oleoresins are used by local communities for lighting (torches), putty on wooden vessels and wood coatings to improve water resistance.

Further, Oleoresin also used as room varnish and medicinal materials such as disinfectants, laxative, diuretic, light stimulant, and analgesic liniments. In Ratanakiri province, Cambodia was determined that there are two kinds of oleoresin tapping of *Dipterocarpus spp. i.e.,* individual tapping and communal tapping [3]. Combustion effects of stimulating oleoresin discharge in communal tapping are more controlled than individual tapping. In Indonesia, Oleoresin tapping has been conducted in Sumatra. However, Oleoresin is used as mixed solution material of patchouli oil, thereby reducing the quality of Indonesian patchouli oil industries [4]. Therefore, to support the conservation program of *Dipterocarpus* and also provide an alternative product diversification, it is necessary to research specific characteristics of its oleoresin. Thus, this study aims to determine specific characteristics of oleoresin origin of *D. gracilis* Blume as an establishing basis for appropriate product diversification of herbal remedy for human health interest.

MATERIALS AND METHODS:

Research Location: This research was conducted in Labanan Forest Research, Labanan Village, Teluk Bayur, Berau, East Kalimantan, Indonesia for sample collection (N 01º56'39.4" E $117^0$13'20.5", 135 m above the sea level). The plant sample was identified by PROSEA database Protologue: Bidjr.fl.Ned.Ind. 5: 224 (1825).

Thus, Phytochemical and GC-MS analysis was conducted in Laboratory Wood Chemistry (Mulawarman University and Gadjah Mada University, Indonesia).

Tapping Oleoresin: In this research, activity was conducted by identification and recording of 3 trees of *D. gracilis* Blume along with the explored location. *D. gracilis* Blume trees recorded at least 40 cm dbh. The tapping procedure is as follows:

a. Preparation of tapping hole (snag) with an axe and carved, at 1.30 m above ground. The hole is made with a width of 25 cm, the height of 20 cm and a depth of 12 cm with 45° angle of tapping hole/snag.

b. Oleoresin flowed into the bottle using a hose. Harvesting and renewal of tapping hole done once every 4 days as much as 3 times.

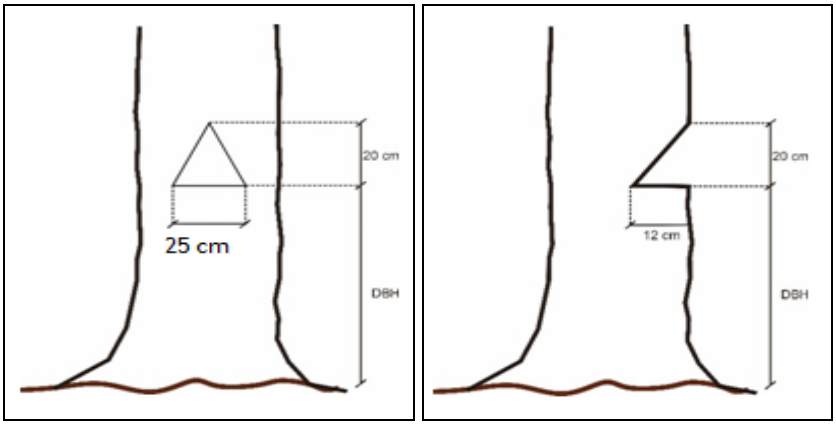

FIG. 1: TAPPING PROCESS OF *DIPTEROCARPUS GRACILIS* BLUME: (A). SNAG MAKING WITH 25 cm WIDTH, AND 20 cm HEIGHT, AND (B). SNAG MAKING WITH 20 cm HEIGHT, AND 12 cm DEPTH

Chemical Analysis: Taken 100 ml of oleoresin *D. gracilis* Blume, further diluted in 100 ml 95% ethanol and filtered. The results of this filter were tested phytochemicals, antioxidant, and GC-MS.

Phytochemical Test: Extracts were tested for the presence of active principles such as flavonoids, saponins, steroids, tannins, terpenoids, alkaloids, and carbohydrate by using some following standard procedures [5, 6].

Flavonoids Determination: About 1 ml of ethanolic extract was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate, and the yellow color in the ammonical layer (bottom layer) indicates the presence of flavonoids.

Saponins Determination: 5 ml of the filtrate was diluted with 20 ml of water and shaken vigorously (15 min). A stable froth (foam) upon standing indicates the presence of saponins.

Steroids Determination: 1 ml of ethanolic extract of each sample is boiled with 10 ml chloroform, cooled, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids.

Tannins Determination: Test solution (5 ml ethanolic extract) with sodium hydroxide solution (1%) gives yellow to red precipitate within short time indicates the presence of tannins.

Triterpenoid Determination: 1 ml of ethanolic extract of each sample is boiled with 10 ml chloroform, cooled 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake well and allow standing for some time, reddish purple color appears at the lower layer indicates the presence of triterpenoids.

Alkaloids Determination: 5 ml ethanolic extract was reacted with 2 drops Potassium bismuth iodide solution reagents in test-tubes. Development of creamy and an orange color respectively indicated a positive result.

Carbohydrate Determination: Extract hydrolyzed with HCl in the water heater. Then, it was added with 1 ml of pyridine and a few drops of a solution of sodium nitroprusside into the hydrolyzate, after it was etched with an alkaline solution of sodium hydroxide. The formation of a pink to red color indicates the presence of glycosides.

Antioxidant Assay: In this test used 100% of 5 concentrated sample with 50, 100, 200, and 400 times of dilution, respectively. 1mg of vitamin C was weighed, then dissolved in 5000 µl of distilled water and regarded as a positive control. Negative control was used in its solvent (distilled water). 100 µl sample was mixed in the cuvette with 400 µl of distilled water was added, and 500 µl of 2,2-diphenyl- 1- picrylhydrazyl (DPPH) radical scavenging activity. Mixing was stopped when the sample volume has reached 1000 µl (1 ml). Samples were incubated for 20 min in indoor with minimum light. Antioxidant Activity (AA) was determined by decolorization of DPPH with a wavelength of 518 nm using a spectrophotometer [7]. The scavenging activity was calculated as a percentage of DPPH decoloration relative to a negative control using the following equation [8]:

Free-radical scavenging activity (%) = ((Control-Sample) / Control) × 100

GC-MS Analysis: Gas Chromatography combined with mass spectrometry (GC–MS) was used for identification of component [9]. The analysis was performed according to the GC-MS equipment's by Shimadzu QP 2010: RTX - column type is 5 ms, Restek Corp (30 m length). The injector and detector temperatures were both maintained at 250 ºC, while operation temperature at 50-300 ºC. The column temperature was programmed at 50-120 ºC, with 4 ºC increase per min which was maintained for 1 min. Then it was programmed at 120-300 ºC, with 6 ºC increase per min and held on for 5 min, with retention time (Rt) totaled 60 min.

Helium was used as a carrier gas is 50-500 atomic mass unit (amu). The identification of the compounds and structure determination were based on the comparison of mass spectra and their fragmentation profiles using published data, Wiley, NIST library search.

RESULTS: Based on tapped resin or oil yield obtained in solid form rather clumped, milky white color. Harvested by using a tablespoon every three days, then put in a plastic bag, and it was obtained 358.91 ml **Table 1**. Further, 100 ml oleoresin was diluted in 100 ml 95% ethanol and then filtered. The results of this filter were tested phytochemicals, antioxidant, and GC-MS analysis.

TABLE 1: THE YIELD OF OLEORESIN TAPPED ON *DIPTEROCARPUS GRACILIS*

Day	Yield of oleoresin tapped on *Dipterocarpus gracilis* Blume (ml)			
to	First tree	Second Tree	Third Tree	Average
3	97.05	29.12	73.22	66.46
6	27.13	26.70	15.68	23.17
9	18.17	23.64	14.15	18.65
12	7.95	20.65	5.45	11.35
Total	150.30	100.11	108.50	119.64

TABLE 2: PHYTOCHEMICAL ANALYSIS OF *DIPTEROCARPUS GRACILIS* OLEORESIN IN ETHANOL SOLUBLE (95%)

Phytochemical parameters	Results
Alkaloid	+
Triterpenoid	+
Steroid	-
Tannin	-
Carbohydrate	-
Saponin	-

Note: All the value expressed in the table is the mean of three replication. (+: detected, -: not detected)

TABLE 3: ANTIOXIDANT TEST OF *DIPTEROCARPUS GRACILIS* OLEORESIN IN ETHANOL SOLUBLE (95%)

	100 ppm	50 ppm	25 ppm
D. gracilis	55.0%	33.0%	19.0%
Vitamin C	96.8%	97.0%	

Note: All value expressed in the table is mean of three replication

TABLE 4: CHEMICAL COMPOUNDS OF GC-MS ANALYSIS OF *DIPTEROCARPUS GRACILIS* OLEORESIN IN ETHANOL SOLUBLE (95%)

Retention time (min)	Area (%)	Name
22.77	2.74	Copaene
24.36	51.21	Caryophyllene
25.51	2.82	1,4-Methanophthalazine, 1,4,4a,7,8,8a-hexahydro-9,9-dimethyl-, (1α,4α,4a α,8a α)-
25.53	4.37	α -Caryophyllene
30.19	17.84	Caryophyllene oxide
37.49	2.35	9,10-Secoergosta-7,10(19),22-triene-3,5,6-triol, (3β)-
55.82	0.85	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane
56.28	7.87	Cyclohexanepropanal, 2,2-dimethyl-6-methylene-
56.30	6.98	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
56.37	2.97	4-Pregnen-21-ol-3,20-dione glucoside

DISCUSSION: Phytochemical test of *D. gracilis* Blume oleoresin soluble ethanol 95% contained alkaloid and triterpenoid. Alkaloid extracts also displayed as well as the anti-bacterial and anti-fungal activities [10]. While triterpenoids are widely distributed throughout the vegetable kingdom and are the major components of many medicinal plants from Asia.

As an active component in the plants, triterpenoids have to contribute to the production of several biological and pharmacological effects such as anti-inflammatory, anti-cancer, anti-diabetic, and hepatoprotective effects [11]. Further, mainly of Oleoresin from *Dipterocarpus* family contained sesquiterpene, triterpenoids and coumarin derivatives [12].

Antioxidant test of *D. gracilis* Blume oleoresin soluble ethanol 95% showed at 100 ppm 55%, 50 ppm 33%, and 25 ppm 19.7%. While, vitamin C as control 100 ppm 97.1%, 50 ppm 96.81%, and 25 ppm 97.01%. Orange peels oleoresin has antioxidant activity from 54.20% to 73.0% [13]. *D. gracillis* oleoresin soluble ethanol 95% had low antioxidant activity. Antioxidant activity of ethanolic extract of oleoresin of *Shorea robusta* by total antioxidant method ranged between 44.4% in 100 µg/ml concentration and 97.3% in 500 µg/ml concentration [14]. These findings have led to increased interest in the antioxidant as well as in the plants as potential sources of naturally occurring antioxidants [15].

Based on GC-MS analysis, *D. gracilis* Blume oleoresin soluble in ethanol 95% contains 51.21% caryophyllene. Caryophyllene and caryophyllene oxide compounds are a volatile sesquiterpene compound [16]. Caryophyllene is also known as β-caryophyllene. β- caryophyllene caused a significant reduction in cyst size and produced apoptosis in endometrial explants without interfering with pregnancy or ovulation [17]. β-caryophyllene is an effective anti-arthritic agent experimentally and holds prospect in future rheumatoid arthritis treatment [18]. While 4.37% of α - Caryophyllene (α - humulene) in this study belonging to sesquiterpene compounds that act as anti-inflammatory [19].

D. gracilis Blume oleoresin soluble ethanol 95% identified 17.84% of caryophyllene oxide compounds. Caryophyllene oxide compounds have the function of gastroprotective activity [20]. Caryophyllene oxide exerts strong anti-cancer effects against MG-63 human osteosarcoma cells by inhibiting cancer cell migration tendency and including apoptosis characterized by cellular shrinkage, membrane blebbing, chromatin condensation, and apoptotic body formation [21].

Another chemical compound identified was Cyclohexanepropanal, 2, 2-dimethyl-6-methylene- (7.87%) belongs to serissaserissoides essential oil which serves as a raw material of active drug [22].

Moreover, it was identified 2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl compound (6.98%). These compounds belong to triterpenoids with a straight chain and act as antioxidant, anticancer, pesticide, sunscreen, perfumery, chemopreventive [23], antimicrobial and anti-inflammatory [24], immunostimulant and lipoxygenase-inhibitors [25]. This study provided 2.97% of 4-Pregnen-21-ol-3, 20-dione glucoside compound. Pregnen compound belong to the steroid group [26]. Steroids are commonly used for anti-inflammatory and increasing autoimmune [27]. 4-Pregnen-21-ol-3, 20-dione is the main member of a group of hormonal steroids called progestins that are responsible mainly for the maintenance of pregnancy in mammalians [28].

The phthalazine derivatives were identified as 2.82% of 1, 4-Methanophthalazine, 1, 4, 4a, 7, 8, 8a-hexahydro-9, 9-dimethyl-, (1α, 4α, 4a α, 8a α) compound. Like the other members of the isomeric benzothiazine series, have been widely applied as therapeutic agents due to their anticonvulsant, cardiotonic, vasorelaxant and anti-inflammatory properties [29]. Copaene compound (2.74%) is similar to one component in *Euphorbia macrorrhiza* oil which serves as an anti-microorganism and anti-tumor [30].

Even though identified in small amount, 9, 10-Secoergosta-7, 10 (19), 22-triene-3, 5, 6-triol, (3β) compound was predicted impact for human health's. 9, 10-Secoergosta-7, 10 (19), 22-triene-3, 5, 6-triol is one form of dehydrocalciferol from vitamin D [31]. Hydroxyvitamin D form in the body will affect the health of a human's liver [32].

CONCLUSION: *Dipterocarpus gracilis* Blume is Dipterocarpaceae species that potential ability for producing oleoresin with general characteristics such as white milk colored that clumps or crystallize, sticky and good odor. This applied oleoresin tapping technique was introduced by making snag as well as tapping process with simple tools. Oleoresin yielded of *Dipterocarpus gracilis* Blume in this study was contained alkaloid and triterpenoid which are bioactive components revealed significant activities such as anti-bacterial, anti-fungal activities, anti-inflammatory, anti-cancer, anti-diabetic, and hepatoprotective effects have antioxidant as a part of their activities.

In the current study, *Dipterocarpus gracilis* Blume had low antioxidant activity in 95% ethanol soluble, even better than other antioxidant sources. Furthermore, some important chemical compounds of GC-MS analysis determined that Oleoresin of *Dipterocarpus gracilis* Blume had an effective anti-arthritic agent, anti-inflammatory, anti-cancer, antimicrobial, anti-bacterial as well as contain vitamin D which will affect the health of the human liver. As mentioned in the above studies, oleoresin could be considered a good alternative for human remedy.

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CONFLICT OF INTEREST: Authors would like to declare that they have no conflicts of interest.

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