

## Identification of phytochemical compounds in ethanol and n-hexane leaf extracts of *Piper retrofractum* vahl. by gas chromatography mass spectrometry

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### Abstract

*Piper retrofractum* or Javanese chili is one of the *Piperaceae* family species which has the potential to be used as herbal medicine. The purpose of this research was to investigate bioactive compounds of ethanol and n-hexane extracts in the *P. retrofractum* leaves. *P. retrofractum* leaves were first washed and dried, crushed into powder, before being extracted with ethanol and n-hexane. Chromatogram profile showed that ethanol and n-hexane extracts contained 21 compounds and 16 compounds respectively. The ethanol extract contained three main components, which were piperine (41.71%), 5,6-Dihydro-2,10,11-trimethoxy-3H-phenanthro (9,10,1-only) (1) pyridine (10.90% ), and 3,3a, 4,5,6,7-hexahydro-2H-indene (9,03%), while n-hexane extract of *P. retrofractum* leaves contained three main components, which were phytol isomer (23.95%), germacrene (16.66%), and 13-acetyl-2,3,3a, 5,6,7,8,13, 13a, 13b-decahydro-11,12-dimethoxy-2,6-dimethyl-.

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## 1. Introduction

Piperaceae is a tropical plant found to contain various secondary metabolites and has been known to be used as raw material for various medicines. Piperaceae consists of up to 1000 species all around the world [1,2]. Piperaceae species found in Indonesia including *P. betle*, *P. nigrum*, *P. retrofractum*, *P. crocatum*, *P. cubeba*, and numerous others. *Piper retrofractum* is one member of Piperaceae family. This plant is originated from Indonesia, commonly grown as garden plant and widely sprouted in lowland forest. This plant grows as vine, having rotund or oval leaves with heart- or round-shaped base and pointed tip. Its young fruit color is gray that then turn into green before finally ripen into yellow- or red-colored fruit. *P. retrofractum* is commonly used for spices in cooking and medicine material. The taste is spicy, sharp, and aromatic [3]. In general the retrofractum piper is an epiphytic plant [4] Essential oil isolated from *P. retrofractum* leaves collected from Research Center for Spice and Medicinal Plant, Cimanggu, Bogor, found the main content to be germakren D (24.20%), tetrametilsiklo153.1.0(4,11)-undek 8-ena (17.73%), ar-turmeron (11.55%), and benzil benzoat (6.28%) [5]. Methanol extract of *P. retrofractum* fruit found to be contained piperine, oleic acid, N-isobutyl-2E,4E, 14Z-eicosatrienamide, and methyl piperate [6]. *Piper retrofractum* has potential as anti-microbe, aphrodisiac, and anti-cancer. Methil chloride extract of Javanese chili previously also found could be used as insecticide against *Nezara viridula* [7]. *P. retrofractum* can be used as a medicine for fever, abdominal pain, hypotension, and cholera in Indonesia. This plant can be found on the island of Java, especially in East Java and Central Java [7]. *P. retrofractum* was found to contain alkaloids, piperin, and essential oil which could be used as aphrodisiac due to its various effects, such as androgenic, anabolic, and reinforcing blood circulation thus it has potential to treat erectile dysfunction [9]. Ethanol extract of *P. retrofractum* and *Zingiber officinale* was previously found could be applied as anti-cancer against He-La and T 47D cells [7]. Generally, community uses *P. retrofractum* fruit as material for medicine, while the other plant organ has yet to be utilized properly, thus information of chemical content in the ethanol and n-hexane extracts of *P. retrofractum* leaves were not available yet. This research was aimed to explore bioactive content of ethanol and n-hexane extracts of *P. retrofractum* leaves.

## 2. Experimental

### 2.1. Plant collection

Plant sample was obtained from flower market in Bratang, Surabaya, East Java, Indonesia. Plant specimen was identified to ascertain the species and a few were made into herbarium saved in Department of Biology, Faculty of Science and Technology, University of Airlangga.

### 2.2. Plant extraction process

About 4 kg *P. retrofractum* leaves were first washed under tap water before air-dried. Dry weight of leaf obtained was 700 g, which was then crushed into powder. Extraction method used was maceration with ethanol and n-hexane solvent. Volume of respective solvent used was 3,5 litres. Result of ethanol extract evaporation was about 57.3 g, while n-hexane extract was about 19.2 g. Ethanol and n-hexane extracts were then analyzed its chemical content using Gass Chromatography Mass Spectrometry (GC-MS) method.

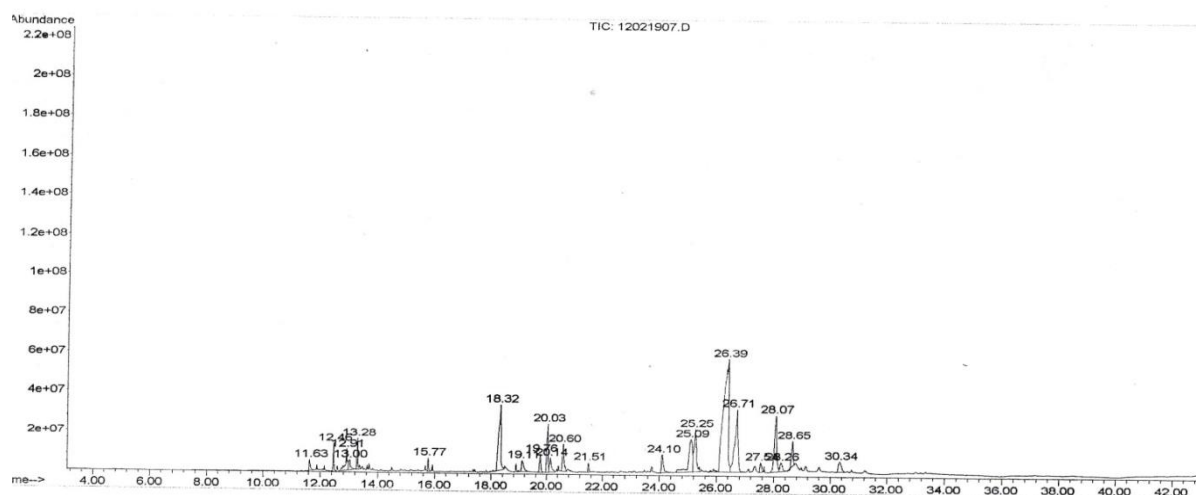
### 2.3. GC-MS Analysis

Extract sample was filtered using 0.45µl nylon filter membrane. About 1 µl ethanol extract was injected into GC-MS instrument, while n-hexane extract injected was about 0.4 µl. Condition of GC-MS was as the following: instrument used was Agilent 6980 N Network GC System using autosampler with Agilent 5973 inert MSD detector. Column used was J and W Scientific, HP-5MS, sized 0.25mm x 30m x 0.25 µm. Inlet: split 1/10, 300°C. Oven was programmed at

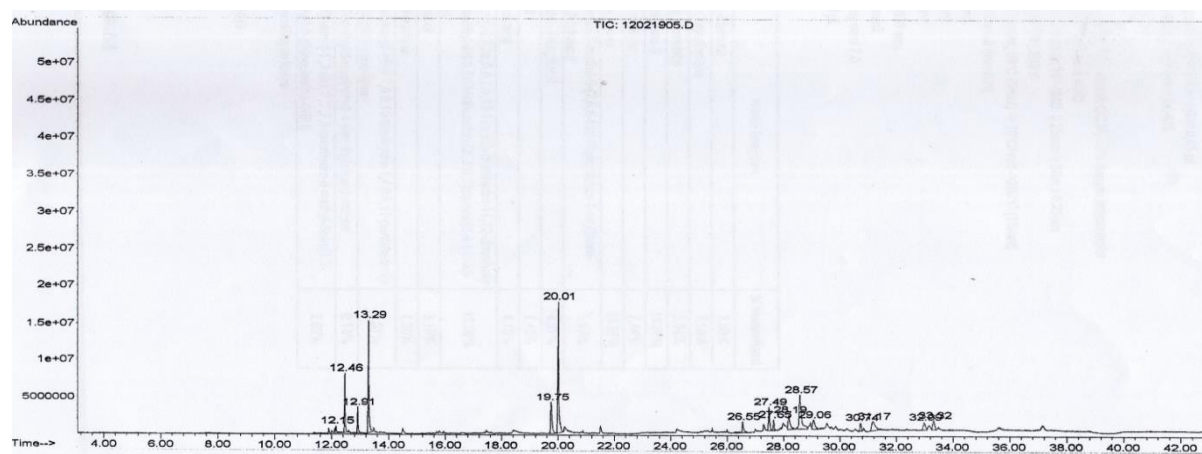
50<sup>0</sup>C (5 minutes), 10<sup>0</sup>C/minute, 280<sup>0</sup>C (15 minute). Flow inside column was about 1 ml/minute (constant). Aux 300<sup>0</sup>C, MS Quad 150<sup>0</sup>C, Scan Mode 20-600 amu, with solvent delay of 0 minute. Reference used was Wiley ver. 7.0.

### 3. Result and discussion

Based on the result of Gas Chromatography, ethanol extract contained 21 compounds while n-hexane extract contained 16 compounds. Chromatogram profile of ethanol and n-hexane extract of *P. retrofractum* leaves is presented in Figure 1 and 3.



**Figure 1:** Chromatogram profile of ethanol extract of *P. retrofractum* leaf



**Figure 2:** Chromatogram profile of n-hexane extract of *P. retrofractum* leaf

Based on the result of GC-MS analysis, bioactive contents of ethanol and n-hexane extract of *P. retrofractum* leaf are presented in Table 1 and 2. *Piperaceae* contains a variety of secondary metabolites such as phenylalanine, sesquiterpen, monoterpenes and phenylpropane derivatives [9]. Three main compounds found in the ethanol extract of Javanese chili leaf including piperin, 5,6-dihydro-2,10,11-trimethoxy-3H-phenanthro[9,10,1-ija]quinolizin-3-one, and 3,3a,4,5,6,7-hexahydro-2H-indene, at concentration of 41.71%, 10.90%, and 9.03%. Piperin possessed the widest area at 41.71%. Piperine was the main compound found in black pepper. Piperine had chemical formula of C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub> and had biological activity as anti-oxidant, anti-tumor, anti-asthmatic, anti-pyretic, analgesic, anti-inflammatory, anti-diarrhea, anxiolytic, anti-depressant, hepatoprotective, anti-bacterial, anti-fungal, anti-metastatic, and anti-thyroid. Piperin content contained in black pepper was about 2.5%, while piperin content in the ethanol extract of *P. retrofractum* leaf was 41.71%. This showed positive result due to its higher content compared to extract from other

species [10]. Methanol extract of *P. nigrum* in addition to contain piperin also had piperidine. This compound was found to possess biological activity as anti-bacteria, analgesic, and anti-inflammatory [11]. Ethanol and n-hexane extract of *Piper retrofractum* leaves contain Caryophyllene, namely 1.12% and 7.10%. This amount is relatively less when compared with Caryophyllene in essential oil of *Piper nigrum* which amount to 26.2 % [12].

**Table 1:** Chemical content of ethanol extract of *P. retrofractum* leaf

| Peak | Retention time | Chemical compound   | Area (%) |
|------|----------------|---|----------|
| 1    | 11.63          | Benzenepropanoic acid, ethyl ester  | 0.69%    |
| 2    | 12.46          | trans-Caryophyllene   | 1.12%    |
| 3    | 12.91          | $\alpha$ -Humulene  | 2.04%    |
| 4    | 13.00          | Benzenepropanoic acid   | 1.01%    |
| 5    | 13.28          | Germacrene-D  | 1.58%    |
| 6    | 15.77          | 8-heptadecene   | 0.58%    |
| 7    | 18.32          | 3,3a,4,5,6,7-hexahydro-2H-indene  | 9.03%    |
| 8    | 19.11          | 2-Furanmethanol   | 1.40%    |
| 9    | 19.76, 21.51   | 1-Octadecene  | 1.80%    |
| 10   | 20.04          | Phytol isomer   | 3.82%    |
| 11   | 20.13          | 2-oxo-4-phenyl-3-butenic acid   | 1.56%    |
| 12   | 20.60          | Ethyl 3-carboxybenzyllevulinate   | 2.30%    |
| 13   | 24.11          | Benzyl 2,3,5-trianisoyl- $\alpha$ -D-arabinofuranoside  | 1.91%    |
| 14   | 25.09, 26.39   | Piperine  | 41.71%   |
| 15   | 25.25          | 2,4-bis(methylthio)-1,9-dimethyl-5,6-dihydrobenzothiepine[4,5-c][1]pyridine   | 5.76%    |
| 16   | 26.71          | 5,6-Dihydro-2,10,11-trimethoxy-3H-phenanthro[9,10,1-ij]quinolin-3-one   | 10.90%   |
| 17   | 27.46          | D, $\alpha$ -Tocopherol   | 0.75%    |
| 18   | 28.07          | (3 $\alpha$ , 3 $\alpha$ $\beta$ , 7 $\alpha$ $\beta$ )-(+)-3-[(1,2-dihydro-1-methoxy-2-oxo-3H-indole-3-ylidene)methyl]hexahydro-6-oxopyrano[3,4-c]pyrrole-2(3H)-carboxylic acid methyl ester | 7.27%    |
| 19   | 28.26          | Stigmasterol  | 1.01%    |
| 20   | 28.66          | (23S)-ethylcholest-5-en-3 $\beta$ -ol   | 2.32%    |
| 21   | 30.34          | Propanoic acid, 2-ethoxy-1-methyl-2-oxoethyl-2-formylamino-3-phenyl   | 1.42%    |

Several species from *Piperaceae* family did not contain piperine and piperidine, but contained other compound instead. *P. cernuum* consisted of main component bicyclogermacrene at 21.88%, while the main component of *P. regnelli* was myrcene at level of 52.6% [9]. *P. hispidum* found to have  $\alpha$ -pinene at 15.3% [13]. On the other hand, water extract of *P. betle* possessed main component of 4-chromanol at 27.81%, while its methanol extract had phenol 2-methoxy 4-(2-propenyl)acetate at 61.15% [14]. Ethanol extract of *P. nigrum* collected from Karumandhuri, Salem found to contain alkaloids, flavonoids, glycoside, sterole, and saponin [12]. Essential oil of *P. guinense* fruit composed

predominantly of linalool at 52.2% [15]. Essential oil extracted from *P. longum* leaf collected from Vietnam possessed main component fonenole at 40.5% [16].

**Table 2:** Chemical content of n-hexane extract of *P. retrofractum* leaf

| Peak | Retention time | Chemical compound   | Area (%) |
|------|----------------|---|----------|
| 1    | 12.16          | B-elemene   | 1.50%    |
| 2    | 12.46          | Trans-Caryophyllene   | 7.10%    |
| 3    | 12.91          | $\alpha$ -Humulene  | 3.54%    |
| 4    | 13.29          | Germacrene-D  | 16.66%   |
| 5    | 19.75          | 1-Octadecene  | 7.44%    |
| 6    | 20.01          | Phytol isomer   | 23.95%   |
| 7    | 26.55          | 3-n-Heptyl-7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenal  | 2.87%    |
| 8    | 27.49          | D- $\alpha$ -Tocopherol   | 4.31%    |
| 9    | 27.65          | Phytol  | 1.45%    |
| 10   | 28.19          | Stigmasterol  | 4.15%    |
| 11   | 28.57          | 13-acetyl-2,3,3a,5,6,7,8,13,13a,13b-decahydro-11,12-dimethoxy-2,6-dimethyl-4H-3,8a-Methanofuro[2',3':6,7]azonino[5,4-b]indol-14-one | 13.06%   |
| 12   | 29.06          | Handianol   | 3.16%    |
| 13   | 30.74          | Neophytadiene   | 1.58%    |
| 14   | 31.17          | 7-(acetyloxy)8-ethyl-7,8,9,10-tetrahydro-1,4,6,8,11-pentahydroxy-5,12-Naphthacenedione  | 4.51%    |
| 15   | 32.99          | 1-(1-Trimethylsiloxyethenyl)-4-trimethylsiloxy-benzene  | 2.13%    |
| 16   | 33.32          | 2'-[2H]indene]-1'3'diol,1',3',7,8-tetrahydro-6-methyl-spiro[1,3-dioxolo[4,5-g]isoquinoline-5(6H)                                    | 2.62%    |

In the case of n-hexane extract of Javanese chili leaf, three of its predominant components were phytol isomer, germacrene D, and 13-acetyl-2,3,3a,5,6,7,8,13,13a,13b-decahydro-11,12-dimethoxy-2,6-dimethyl-4H-3,8a-Methanofuro[2',3':6,7]azonino[5,4-b]indol-14-one, at concentration of 23.95%, 16.66%, and 13.06%, respectively. Phytol is a diterpene alcohol which is found able to inhibit inflammatory [17]. Phytol contained in the methanol extract of *P. nigrum* had biological activity as anti-oxidant [13]. Phytol was found in the 95% ethanol extract of *Pergularia daemia* leaf and known to possess anti-microbial and anti-asthmatic activities [18]. The second predominant component, germacrene, is a type of hydrocarbon sesquiterpene also found in other *Piperaceae* species. Essential oil isolated from the leaves of *P. betle* var Bangladesi and *P. betle* var Deswari were found to contain germacrene B at 0.12% and 0.42% respectively [19]. Dichloromethane extract of *Bucerra copallifera* leaf had germacrene D at 56.2% [20]. Essential oil from *Stachys cretica* also possessed germacrene D, at level of 32.8%. This

compound was found to have biological activity as anti-microbe [21]. *Kundmannia sicula* herb from *Apiaceae* family is another plant that also have germacrene D content, at concentration of 81.2% and found to be able to be applied as anti-oxidant and anti-tumor [22]. Essential oil from *Artemisia vulgaris* also found to possess germacrene D [23]. Aqueous extract and ethanol piper betle contain chavibetol and hydroxychavicol compounds which are eugenol isomers while in this study, ethanol extract and n-hexane retrofractum piper leaves did not have these compounds [24].

## 4. Conclusion

*Piper retrofractum* leaves contained predominantly of piperine and phytol, both compounds could be developed into herbal medicine as they were found to have various biological activity for the means of people's health.

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