Analyzing Gas Chromatography-Mass Spectrometry (GC-MS) of the Bioactive Compound of Javanese Long Pepper (Piper retrofractum Vahl.) Essential Oils

Sulifah A. Hariani¹, Siti Zubaidah*², AD Corebima³

¹Malang State University, Jl. Cakrawala No.5, Sumbersari, Kec. Lowokwaru, Malang, 65145 East Java Indonesia
²Department of Biology FMIPA, Malang State University, Jl. Cakrawala No.5, Sumbersari, Kec. Lowokwaru, Malang, 65145 East Java, Indonesia
³Malang Kanjuruhan University, Jl. S. Supriadi No.48, Bandungrejosari, Kec. Sukun, Malang, 65148 East Java, Indonesia

Article History:
Received on: 25 Aug 2020
Revised on: 27 Sep 2020
Accepted on: 28 Sep 2020

ABSTRACT

Piper has an economically high commercial value because it is an aromatic compound that is widely used as a spice, an essential raw material for medicine, aromatic, antifungal, antiviral, insecticide and others. One species of Piper is Piper retrofractum Vahl. This herb is useful for treating fever, hypotension, abdominal pain, beriberi, cholera, non-perspiration, and anthelmintic. The study of bioactive compounds from essential oils of P. retrofractum is crucial to be revealed, especially regarding variations in chemical compounds present in these oils because of the enormous benefits in human life. The purpose of this study was to characterize the composition of bioactive compounds from essential oils P. retrofractum from Kediri, East Java Province, Indonesia. The essential oil component of P. retrofractum distilled-fruit was then analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) with GC-MS SHIMADZU QP2010 Plus specifications. The results of the GC-MS analysis showed that there were 28 compounds present in the fruit of P. retrofractum. The compounds consisted of terpenoids (monoterpenes, sesquiterpenes), fatty alcohols, alkene hydrocarbons, cyclic hydrocarbons, carbon alkene, and others. The research results of several researchers indicated that these compounds have great potential as bioactive compounds and useful in the pharmaceutical, health, cosmetics industries, and can utilize to increase the added value of food products.

INTRODUCTION

Piper has members of about 700-1000 species whose distribution is mainly in the tropics and some in subtropical regions (Chansang et al., 2005; Parmar et al., 1997). Piper is widely cultivated in Asian regions, such as India, Malaysia, the Philippines, Indonesia, Thailand, Vietnam, and several other countries in South and Southeast Asia, and Tropical America (Le, 2017). Piper has high commercial value economically because it is an aromatic compound that is widely used as a spice, an essential raw material for medicine, aroma, antifungal, antiviral, insecticide and others (Menon et al., 2000; Scott...
et al., 2005; Sumathykutty et al., 1999).

One of the species of the Piper genus is Piper retrofractum Vahl, which is widely cultivated in Indonesia, especially in Kediri, East Java Province. This plant is in the form of bushes, creepers, round stems, woody, broad and brownish, with wild branches: single leaf, pinnate leaf reinforcement. Flowers in the form of compound flowers, with the type of spadix, the fruits are attached shaped like a club on the stalk when they were young green after dark red. The taste is spicy and sharp, typical of aromatic plants. Breeches are round or ovate brownish white (Khare, 2003). P. retrofractum is useful as a medicinal plant to cure fever, hypotension, abdominal pain, beriberi, corela, non-perspiration, anthelmintic, anti-malaria (Mgbeahuruike et al., 2017), rheumatiz, illness after childbirth, hypertension, and influenza (Haryanto, 2009; Chakraborty and Shah, 2011). Extract from the fruit of P. retrofractum Vahl., can be used as larvicides (Chansang et al., 2005), aphrodisiacs (Rahmawati and Bachri, 2012), insecticides, antifungal, treating and protecting gastrointestinal organs (Muhrini et al., 2015), antioxidants (Luyen et al., 2014).

Piper retrofractum is an aromatic plant that contains essential oils as a secondary metabolite of the plant (Zahira and Thamilmani, 2016; Jong-Woong et al., 1992). Essential oils are natural products produced by plants as nutrients, protection, or decoy. Essential oils are mixtures of complex organic compounds that give plants unique odours and flavours (Agusta, 2000). Oil of P. retrofractum can be used for medicinal purposes, such as antioxidants, controlling fungi or antifungal, antileishmanial, anti-bacteria and other microbes (Luyen et al., 2014).

Typical compounds in essential oils can be used as chemical markers to distinguish species and subspecies (Purnomo and Asmaryani, 2005; Wartono et al., 2014). Chemical compounds from medicinal plants can also be used as important therapeutic agents in biomedical and studies of natural products from plants (Mgbeahuruik et al., 2017; Ortega and Campos, 2019). The purpose of this study was to characterize the composition of bioactive compounds from essential oils P. retrofractum from Kediri, East Java Province, Indonesia, using GC-MS.

**MATERIALS AND METHODS**

**Plant Samples**

The material used was dried fruit or simplicia from

*P. retrofractum* fruit originating from Kediri district, East Java Province, Indonesia. The determination was carried out at the Botanical Laboratory of Biology Education Study Program at FKIP University of Jember.

**Distillation**

The process of drying *P. retrofractum* is done by blanching process, is the harvested fruit is boiled for about 3 minutes then dried in the sun for three days. The dried fruit is then weighed as much as 150 grams and blended, and the dried powder is then distilled Stahl with 2L equates at 100°C for 3 hours. The distillation results are then added with *n*-hexane 50 ml which serves to dissolve the essential oil. This oil still contains water then is added to anhydrous sodium until saturated. The resulting oil is weighed to measure its yield and then analyzed by GC-MS.

**Analysis of GC-MS**

The essential oils component of *P. retrofractum* distilled-fruit was then analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) with the GC-MS SHIMADZU QP2010 Plus specification using the Rest Rxcolumn ®-50(Crossbond® 5% phenyl-50% phenyl-50% methyl polysiloxane) 30 meters, 0.25 mmID, 0.25μm df, Paint, # 10523 serial # 679017. Max programmable temp 320°C, minimum bleed at 300°C. The carrier gas used is Helium with a pressure of 102.5 kPa, Program Oven temperature 80.0°C, Holding time 1.00 minutes and injection temperature 250°C holding time 5.00 minutes. Total Flow 155.8 mL / min, column flow 1.50 mL / min, linear velocity 45.1 cm / sec, purge flow 3.0 mL / min. The mass spectrum of each compound peak detected on the chromatogram is compared with the compound already known in the data bank of Wiley8.LIB. The quantity of the chemical compound is shown as the percentage of the peak area shown on the chromatogram. GC-MS analysis was carried out at the Laboratory Central Mineral and Advanced Materials, Faculty of Mathematics and Natural of Sciences, Malang State University.

**RESULTS AND DISCUSSION**

*Piper retrofractum* is a medicinal plant that can be a source of new drugs. Many modern medicines are produced directly from medicinal plants. Chemical extraction and analysis of medicinal plants have an important role in the development of herbal medicines. Chemical compounds are bioactive compounds that play a role in medicine. Bioactive compounds present in *P. retrofractum* by molecular formula, molecular weight (MW), retention time (RT), and concentration (peak area %) are presented in
Table 1: GC-MS Analysis of Essential Oils *Piper Retrofractum Piper* Vahl

| No. | Name of the Compound | Molecular formula | Molecular Weight | Retention time | Peak Area (%) |
|-----|-----------------------|-------------------|------------------|----------------|---------------|
| 1.  | 1.6-Octadien-3-ol, 3,7-Dimethyl- | C_{10}H_{18}O | 154 | 6.811 | 0.29 |
| 2.  | Undecane | C_{11}H_{24} | 156 | 11.668 | 1.25 |
| 3.  | 3-Cyclohexene-1-Methanol, Alpha., Alpha., 4-Trimethyl-, Propanoate | C_{13}H_{22}O_2 | 210 | 12.848 | 0.17 |
| 4.  | Cubenol | C_{15}H_{26}O | 222 | 13.901 | 0.25 |
| 5.  | Cyclodecadiene, 1- Methyl-5-Methylene-8-(1-Methylethyl) -, [S- (E, E)] -N-Phenyl-3-Ureido-1-Diazo-Propan-2- One | C_{10}H_{10}N_{4}O_2 | 218 | 14.862 | 0.22 |
| 6.  | Bicyclo [7.2.0] Undec-4-Ene, 4,11,11-Trimethyl-8-Methylene-, 2- Norpinene, 2,6-Dimethyl-6- (4-Methyl-3-Pentenyl) -, Trans- | C_{15}H_{24}O_4 | 278 | 16.042 | 0.38 |
| 7.  | Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{11}H_{24}O | 172 | 16.363 | 2.03 |
| 8.  | 1-Pentadeca zero | C_{15}H_{32}O | 226 | 16.511 | 1.91 |
| 9.  | Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{15}H_{32}O | 222 | 15.819 | 1.58 |
| 10. | 1,4,8-Cycloundecatriene, 2,6,6,9-Tetramethyl-, (E, E, E) -1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{15}H_{24}O_4 | 204 | 15.963 | 3.37 |
| 11. | O- ((1s) -1,5-Dimethylbicyclo [2.2.1] Hept-5-En-2-Endo-Carbonyl) -D-Pantolactone | C_{15}H_{24}O_4 | 204 | 16.663 | 21.79 |
| 12. | 1-Undecanol | C_{11}H_{24}O | 172 | 16.363 | 2.03 |
| 13. | 1-Pentadeca zero | C_{15}H_{32}O | 226 | 16.511 | 1.91 |
| 14. | Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{15}H_{32}O | 222 | 15.819 | 1.58 |
| 15. | 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{15}H_{32}O | 204 | 16.663 | 21.79 |
| 16. | Alpha-Selinene | C_{15}H_{24}O_4 | 204 | 17.027 | 4.88 |
| 17. | Cyclohexene, 1-Methyl-4- (5-Methyl-1-Methylene-4-Hexenyl) -, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, Alpha-Selinene | C_{15}H_{24}O_4 | 204 | 17.210 | 5.32 |
| 18. | (-) - Alpha-Panasinsen | C_{15}H_{24}O_4 | 204 | 17.601 | 3.10 |
| 19. | 2-Norpinene, 2,6-Dimethyl-6- (4-Methyl-3-Pentenyl) -, Trans- | C_{15}H_{24}O_4 | 204 | 17.604 | 0.39 |
| 20. | Cyclohexene, 1-Ethenyl-1-Methyl-2,4-Bis (1-Methylene) -, [1s- (1.Alpha., 2.Beta., 4.Beta.)] -1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, 1,6-Cyclodecadiene, 1-Methyl -5-Methylene-8- (1-Methylethyl) -, 1-Undecanol | C_{15}H_{24}O_4 | 204 | 19.279 | 0.28 |
| 21. | 1-Heptadecanol | C_{17}H_{36}O | 256 | 20.992 | 1.32 |
| 22. | 1-Heptadecanol | C_{17}H_{36}O | 256 | 20.992 | 1.32 |
| 23. | 1-Heptadecene | C_{17}H_{36}O | 238 | 21.323 | 7.52 |
| 24. | Hexane, 3,3-Dimethyl- | C_{8}H_{21}O_{3} | 214 | 21.136 | 2.43 |
| 25. | Hexadecane | C_{16}H_{32}O_{4} | 226 | 21.589 | 0.35 |
| 26. | 1-Hexadecanol, Acetate | C_{18}H_{36}O_{25.512} | 284 | 25.512 | 0.21 |
| 27. | 1-Heptadecanol | C_{17}H_{36}O | 256 | 25.711 | 0.70 |
| 28. | Pentadecane | C_{15}H_{32}O | 212 | 25.928 | 0.22 |
Figure 1: GC-MS Chromatogram of Essential Oils *Piper Retrofractum* Vahl

Table 1.

Figure 1 shows a chromatogram of chemical compounds in fruit essential oils of *P. retrofractum*. The results of GC-MS analysis showed that there were 28 active compounds present in the essential oils of *P. retrofractum* (Figure 2 and Figure 3).

GC-MS analysis results of essential oils of *Piper retrofractum* shows various complex compounds consisting of monoterpenoids, sesquiterpene, fatty alcohols, alkene hydrocarbons, cyclic hydrocarbons, carbon alkene, and others. There are 28 chemical compounds with different percentages of peak areas, ranging from large to smallest (Table 1). Fifteen main components of the twenty-eight compounds present in the essential oils are 1,6-Cyclodecadiene, 1-Methyl-5-Methylene-8-(1-Methylethyl) - (21.79%); Bicyclo [7.2.0] Undec-4-Ene, 4,11,11-Trimethyl-8-Methylene- (11.36%); 1-Heptadecene (7.87%); 1-Tetradecanol (7.52%); Hexadecane (6.35%); Cyclohexene, 1-Methyl-4-(5-Methyl-1-Methylene-4-Hexenyl) - (5.32%); Alpha.-Selinene (4.88%); 1,4,8-Cycloundecatriene, 2,6,6,9-Tetramethyl-,- (E, E, E) - (3.37%); (-) - Alpha-Panasinsen (3.1%); Hexane, 3,3-Dimethyl-(2.43%); 1-Undecanol (2.03%); 1-Pentadecanol (1.91%); Benzene, 1- (1,5-Dimethyl-4-Hexenyl) -4-Methyl-, (1.78%); 1,6,10-Dodecatrien-3-Ol, 3,7,11-Trimethyl- (1.58%); and 1-Heptadecanol (1.32%).

Each of these chemical compounds has great benefits in human life. 1,6-Cyclodecadiene, 1-Methyl-5-Methylene-8- (1-Methylethyl) or Germacrone D, are organic hydrocarbons, especially sesquiterpene. Germacrone D has antimicrobial and insecticidal properties (*He and Cane, 2004*). Germacrone D is also a component of essential oils from *P. cubeba* (*Mustaparta and Stranden, 2005*).

Bicyclo [7.2.0] Undec-4-Ene, 4,11,11-Trimethyl-8-Methylene- is a sesquiterpene with other names cis-Caryophyllene, β-cis-Caryophyllene, and is caryophyllene. Isocaryophyllene is also found in the plants of *Syzygium aromaticum* (*Ghelardini et al., 2001*), *Cannabis sativa* and *Salvia rosmanirus* (*Ormeño et al., 2008*). These compounds can act as local anaesthetics (*Ghelardini et al., 2001*). Other research results showed that the concentration of non-cytotoxic beta-caryophyllene significantly increased the anticancer activity of alpha-humulene and isocaryophyllene in MCF-7 cells that cause cancer in humans (*Legault and Pichette, 2007*). Greenhouse and field conditions indicate that isocaryophyllene and trans-2-dodecanol provide a significant attraction for *Curvorthinus lividipennis*. Isocaryophyllene and trans-2-dodecenol have the potential to be effective attractors to help *C. lividipennis* in locating rice fields where plant hopper pests exist (*Liu et al., 2019*).

1-Heptadecene is a seventeen unbranched alkene hydrocarbon with one double bond between C-1 and C-2. Hydrocarbons can be used as an energy source or fuel source (*Silberberg, 2004*). 1-Tetradecanol is a long chain of primary fatty alcohol. This compound is also found in *Myristica fragrans* and can be used as an ingredient in cosmetics such as cold creams for their emollient properties. It is also used as an intermediate in chemical syntheses, such as surfactants (*Kreutzer, 1984*). Hexadecane or cetane is an alkane hydrocarbon. This compound can be found in all spice plants as well as in *Piper longum* (*Yannai, 2007*). Cyclohexene, 1-Methyl-4- (5-Methyl-1-Methylene-4-Hexenyl) - or beta-bisabolene are sesquiterpenes that are also found in *Piper cubeba*, *Citrus limon*, *Origanum vul-
Figure 2: Active Compounds of Essential Oils of *Piper retrofractum* fruit

gare, *Commiphora Guidotti* (Yeo et al., 2016) and some fungi (Spakowicz and Strobel, 2015). β-bisabolene is also effective in reducing the growth of 4T1 breast cancer tumours that are transplanted in vivo so that it has the potential to treat breast cancer (Yeo et al., 2016). Alpha-Selinene is a sesquiterpene group that is also found in seeds *Apium graveolens* (Attokaran, 2011). Alpha-selinenine has bioactivity as antimalarial, anti-plasmodial, and can be used as a perfume (Duke, 2004).

1,4,8-Cycloundecatriene, 2,6,6,9-Tetramethyl-, (E, E, E) - with the synonyms of the names Humulene, Alpha-Humulene, and Alpha-Caryophyllene. In experimental animals, humulene has a strong anti-inflammatory activity similar to dexamethasone (Fernandes et al., 2007). Humulene also has topical and systemic anti-inflammatory properties (Chaves et al., 2008) and is an effective analgesic when consumed topically, orally, or aerosolically. Humule can also be found in Cannabis, sage, and ginseng plants (Hartsel et al., 2016). (-) - Alpha-Panasinsen is also found in tea, ginseng oil. Hexane, 3,3-Dimethyl- can be found in herbs and spices, as well as constituents of oil *Osmanthus fragrans* and
1-Undecanol belongs to the class of organic compounds known as a fatty alcohol. Undecanol can act as a fungicide against Saccharomyces cerevisiae (Kubo et al., 2003). 1-Pentadecanol is very-long-chain alcohol that can reduce plasma cholesterol in humans. Benzene, 1-(1,5-Dimethyl-4-Hexenyl) -4-Methyl or alpha-curcumin are sesquiterpenes. Alpha-curcumin has antimicrobial activity (Govinden-Soulange et al., 2004). The compound has hydrophobicity activity that can distort lipids from microbial cell membranes, disrupt cell structure and make it more permeable (Sikkema et al., 1994). Extensive leakage from microbial cells or the release of molecules and critical ions can cause death (Prabuseenivasan et al., 2006).

1-Heptadecanol is a long-chain fatty alcohol which is a heptadecane where a hydroxy group replaces one of the methyl hydrogen terminals. This compound is a plant metabolite. Fat alcohol can be useful in the production of detergents and surfactants; it is also a thickening component of cosmetics, food, and industrial solvents (Noweck and Grafahrend, 2016).

Bioactive compounds from essential oils P.
*P. retrofractum* in large quantities have great potential to be developed. The development can be done in the pharmaceutical, health, cosmetics, and can be utilized to increase the added value of food products.

**CONCLUSION**

Medicinal plants *Piper retrofractum* is a medicinal plant that is widely used by the people of Indonesia and India; have potentially used in the pharmaceutical industry. Identification of bioactive compounds in *P. retrofractum* was carried out by GC-MS analysis to determine the composition and presence of certain compounds. The results of the GC-MS analysis showed that there were 28 compounds present in the fruit of *P. retrofractum*. The compounds consisted of terpenoids (monoterpenes, sesquiterpenes), fatty alcohols, alkene hydrocarbons, cyclic hydrocarbons, carbon alkene, and others. The research results of several researchers indicated that these compounds have great potential as bioactive compounds that useful in the pharmaceutical, health, cosmetics, and to increase the added value of food products.

**ACKNOWLEDGEMENTS**

This research was supported by BPPDN scholarship from Directorate General of Higher Education of the Ministry of Research and Technology, Republic of Indonesia.

**Conflict of Interest Statement**

We declare that we have no conflict of interest for this study.

**Funding Support**

The authors declare that they have no funding support for this study.

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