A Qualitative and Quantitative Evaluation of Terpenoid and Alkaloid in Root and Stem of Pasak Bumi (*Eurycoma longifolia* Jack)

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Abstract

*Herbal, as health solution, regained its popularity because many studies showed advantages of using it. One of the most industrially needed herbal materials is the extract of pasak bumi (*Eurycoma longifolia *Jack), which is popular for its alleged sexual-enhancing properties.* E. longifolia *was known to produce secondary metabolites, especially terpenoid and alkaloid. The presence of major alkaloids and terpenoids in root and stem of* E. longifolia *was evaluated quantitatively by analyzing the ethanol extract of root and stem of* E. longifolia *using GC-MS. Qualitative analysis was conducted through colorimetric test on both extracts by using Dragendorff and Lieberman-Burchad reagents. Colorimetric test showed that there was a difference in both terpenoid and alkaloid amounts on ethanol extract of both root and stem. GC-MS analysis showed that the ethanol extract of* E. longifolia *from the root contained 14.631% terpenoid and that from the stem was only 7.781%. The ethanol extract of* E. longifolia *from the stem contained 1.785 % of alkaloid, while that from the root was 5.117 % from total compounds. Major alkaloids found on both organs were 3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10] phenanthroline, which showed higher concentration in root. Major terpenoid in root included 2H-1-Benzopyran-2-one, 3-phenyl- (coumarin derivate), whilst that in stem was Stigmasterol. These substances have different activities in target cells. Therefore, the knowledge of active compounds in organ parts of pasak bumi will lead to a more efficient production process.*

Keywords : Alkaloid, Eurycoma longifolia, GC-MS, Histochemistry, Terpenoid

Abstrak

Herbal merupakan solusi kesehatan alternatif yang kembali marak digunakan karena manfaatnya yang beragam. Salah satu bahan herbal yang banyak diperlukan di industri adalah ekstrak pasak bumi (Eurycoma longifolia Jack) yang terkenal karena kemampuannya meningkatkan aktivitas seksual. E. longifolia *dikenal mengandung terpenoid dan alkaloid. Kehadiran dan kadar alkaloid dan terpenoid utama pada akar dan batang* E. longifolia *dievaluasi secara kualitatif dengan analisis GC-MS ekstrak etanol dari akar dan batang, sedangkan secara kuantitatif dilakukan melalui analisis colorimetrik menggunakan reagen Dragendorff dan Lieberman-Burchard. Hasil uji colorimetrik menunjukkan adanya perbedaan jumlah alkaloid dan terpenoid baik di ekstrak etanol akar maupun batang. Hasil analisis GC-MS menunjukan bahwa ekstrak etanol akar mengandung 14,631% terpenoid, sedangkan batangnya mengandung 7,781% terpenoid. Ekstrak etanol batang* E. Longifolia *mengandung 1,785% alkaloid, sementara akarnya mengandung 5,117% alkaloid dari total senyawa. Alkaloid utama yang ditemukan pada kedua organ adalah senyawa 3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10] phenanthroline, dengan konsentrasi lebih tinggi pada akar. Terpenoid utama yang ditemukan di akar adalah 2H-1-Benzopyran-2-one, 3-phenyl- (turunan coumarin), sementara pada akar adalah Stigmasterol. Senyawa-senyawa tersebut memiliki aktivitas berbeda pada setiap sel. Oleh karena itu, pengetahuan mengenai senyawa aktif yang terkandung dalam organ pasak bumi dapat meningkatkan efisiensi proses industri sesuai dengan tujuan produksinya.*

Kata kunci : Alkaloid, Eurycoma longifolia, GC-MS, Histokimia, Terpenoid

1. Introduction

Health is considered as one of the main issues today. Therefore, some people consumed herbal drug for health maintenance. Herbal drug acts like any other drug, either curing or acting as supplement that support body system to operate. Such fact leads to vast development of herbal industry and demanding the industry to increase quality and quantity of their material. One of the most industrially needed herbal materials is *Eurycoma longifolia*, commonly known as pasak bumi. Pasak bumi is popular for its alleged sexual-enhancing properties as observed by Ang and Cheang (1999). Therefore, in recent years, the demand for pasak bumi in Indonesia is increasing.

Pasak bumi has several active compounds in a group of terpenoid and alkaloid. These compounds are believed to have potential, such as on sexual activity as suggested by Baker *et al*. (1999), anticancer (Wang *et al.*, 2002) and antimalaria (Kuo *et al*., 2004). Several researchers, including Bedir *et*

al. (2003), reported that terpenoid could be obtained from pasak bumi ethanol extract. Pasak bumi-derived aphrodisiac compound is commonly obtained from its root, but it may also be possible to obtain the compound from other organ parts of pasak bumi as suggested by Ang and Sim (1997). However, most researches focused on pasak bumi root extract, while the stem organ was rarely used. Therefore, the study of chemical compound in stem is necessary.

Identification of terpenoid and alkaloid on root and stem of pasak bumi can be performed both qualitatively or quantitatively. The presence of alkaloids in pasak bumi can be tested histochemically by direct application of Dragendorff and Jefferey reagents onto pasak bumi fresh-cut of root and stem. However, further analysis is needed to confirm detail content of the extract.

Effectivity and efficiency of raw material usage are important in herbal industry, that is by only utilizing parts of pasak bumi that specifically has more yet specific alkaloid and terpenoid in it. Therefore, this research was conducted to study the presence of major terpenoid and alkaloid on pasak bumi stem and root, both qualitatively and quantitatively (by GC-MS analysis of ethanol extract of pasak bumi).

2. Methods

2.1 Extraction

Plant materials used in this experiment were the root and stem of pasak bumi (*Eurycoma longifolia* Jack) collected from West Kalimantan in Juli 2009. Five hundreds gram of simplisia were placed on different glass-jars, and submerged in 5 L of 96% ethanol for 24 hours. The materials were then filtrated and evaporated by distillation process until only 500 mL remained. The filtrates were then analyzed with GC-MS and colorimetric method.

2.2 Qualitative Analysis

Colorimetric analysis of terpenoid was conducted by using Lieberman Burchard method according to Goddel (2010). Positive result of terpenoid was shown by color shift from extract color to brown color. Alkaloid colorimetric-test was performed using Dragendorff method as described by Goddel (2010). Positive test-result was shown by color change, from extract-color to deep orange color.

2.3 Quantitative Analysis

GC-MS analysis of ethanol extracts was then conducted by using HP-5MS fused phenyl methyl silox capillary column. GC-MS analysis resulting in chromatogram was compared to complete library. Percent of total compound was calculated according to the equation below:

$$\text{Percent of total} = \frac{\text{Core area compound}}{\text{Total core area of all desired compound}} \times 100\%$$

3. Results and Discussion

Qualitative analysis was conducted by using colorimetric test. The results showed that the color of the stem extract was slightly lighter than that of the root extract. Verification of alkaloid presence in the ethanol extract of root and stem of pasak bumi (Figure 1) showed that the ethanol extract of both root and stem treated with the Dragendorf turned into orange with similar color intensity. Lieberman-Burchard (terpenoid) test showed deep-brown color on root extract test (top-row of Figure 1A), while the stem extract showed transparent brownish color (bottom row Figure 1B). Result of colorimetric test could suggest that the level of terpenoid on root ethanol extract was higher than that on stem ethanol extract, but alkaloid level showed no difference on both extracts. In order to reconfirm the colorimetric test result, root and stem ethanol extracts were analyzed by gas-chromatography mass-spectrofotometric (GC-MS).

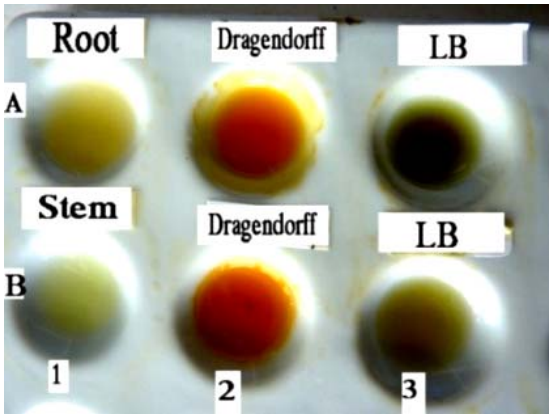

Figure 1. Colorimetric tests show the ethanol extract of root (A) and stem (B) without treatment, with Dragendorff treatment and with Lieberman-Burchard treatment, respectively.

GC-MS analysis of pasak bumi root ethanol extract showed 52 different compounds. Each peak has a different retention time and abundance. The retention time showed the character of the compound. Compound with higher polarity could be detected earlier than less polar compound, because it has different interaction with the immobile phase of Gas-Chromatography-column used. Moreover, compound with lower boiling point was detected earlier on GC-MS than the compound with higher boiling point (Schwender, 2009).

The retention time of root extract ranged from 10 to 30 minutes. The first peak was detected on minute 11,723 read as 2-Furancarboxaldehyde, 5-(hydroxymethyl)- a member of terpenoid group, while the last peak was on minute 26.601 and read as 2-Methyl-Z,Z-3,13-octadecadienol, which was also a member of terpenoid group.

Result of root ethanol extract showed seven major compounds of terpenoids and five major

compounds of alkaloids (Table 1). The highest terpenoid content in root ethanol extracts was 2H-1-Benzopyran-2-one, 3-phenyl-, it comprised about 21.44 % among all compounds. The highest alkaloid content in root ethanol extract was 3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3c] [1,10] phenanthroline, it made up to 27.83 % of major terpenoid and alkaloid in the root ethanol extract.

The highest terpenoid compound in the root ethanol extract was 2H-1-Benzopyran-2-one, 3-phenyl-, a derivate of coumarin. Coumarin was known as agent to enhance sperm activity, and gave hormonal effects on mammals. It was observed by Al-Qarawi (2005), that coumarin in *Ruta chalepensis* extract could increase sperm density and motility in mice epididimys. Another function of coumarin was to inhibit leukemia cell proliferation and coumarin could regulate the factor on G1-phase of cell cycle on leukemia cell (Wang *et al.,* 2002).

GC-MS analysis of stem ethanol extract of pasak bumi resulted in 60 peaks. The retention-time of compounds in the stem ethanol extract of pasak bumi ranged from 10 to 30 minutes. First peak emerged at minute 11.134 read as 2-Cyclohexen-1-one,2-methyl-5-(1methylethenyl)-,(S)-, from terpenoid group. Last peak was at minute 29.927 read as 4,22 Stigmastadiene-3-one, which was also a member of terpenoid group.

The stem ethanol extract of pasak bumi consisted of ten major terpenoids and three alkaloids (Table 2). The highest constituent of terpenoid in stem ethanol extracts was stigmasterol, while that of alkaloid was 3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10]phenanthroline.

Percentage of total compound was shown in Table 3.2, it represented volume domination of each compound among those 13 compounds only, but not the percentage of total volume domination of the whole total extract. Terpenoid comprised about 7.781 % of total chemical constituent detected in chromatogram GC-MS analysis, while the alkaloid group comprised 1.785 % of the total.

Table 1. Alkaloids and terpenoids found in the ethanol extract of the root of pasak bumi

	No	Compound	Total %
Terpenoid	1	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	3.04%
	2	Vanillin	2.57%
	3	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	5.79%
	4	Benzaldehyde, 3-ethoxy-	4.63%
	5	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	5.12%
	6	2H-1-Benzopyran-2-one, 3-phenyl- (coumarin derivate)	21.44%
	7	2-Methyl-Z,Z-3,13-octadecadienol	4.64%
Alkaloid	1	6-Tert.butyl-2,3-dicyanonaphthalen	3.59%
	2	6,7-Dimethoxy-1,4-dihydro-2,3-quinoxalinedione	10.90%
	3	1-Amino-9-fluorenone	2.50%
	4	3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10]phenanthroline	27.83%
	5	Oxazole, 2,2'-(1,4-phenylene)bis[4-methyl-5-phenyl-	2.31%

Table 2. Alkaloids and terpenoids found in the ethanol extract of the stem of pasak bumi

	No	Compound	Total %
Terpenoid	1	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)-	0.94%
	2	2,7-Octadien-4-ol, 2-methyl-6-methylene-, (S)-	1.15%
	3	Dibutyl phthalate	6.73%
	4	trans-3,4-Dimethoxy-2-ethoxy-.beta.-methylstyrene	1.64%
	5	1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ester	2.63%
	6	Campesterol	4.85%
	7	Stigmasterol	50.54%
	8	D-Homoandrosta-4,17-dien-3-one,,17a-dihydroxy-	1.76%
	9	Stigmasterol, 22,23-dihydro-	7.25%
	10	4,22Stigmastadiene-3-one	8.58%
Alkaloid	1	9H-Pyrido[3,4-b]indole-1-carboxylic acid, methyl ester	2.37%
	2	6H-Indolo[3,2,1-de][1,5]naphthyridn-6-one	1.79%
	3	3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10]phenanthroline	9.77%

Fifty percent of stem ethanol extract was stigmasterol. Stigmasterol is a member of phytosterol. Stigmasterol is a derivate of phytosterol, and Bouick *et al.* (1999) suggested that certain concentration of some plant sterol could influence the cellular proliferation of T-lymphosites, a cell responsible in immune system for killing pathogens. Stigmasterol in *E. longifolia* extract had also been tested and proven to be potential against lung cancer (Kuo *et al.*, 2004).

Another application of stigmasterol was studied by Barbosa *et al*. (1999). It showed that stem extract of M*ucuna aterrima* contained stigmasterol and sitosterol, which were widely known as nemacidal, and they were used to increase the mortality level of *Meloidogyne incognita*, a round worm nematode.

Terpenoid was a major compound found in both ethanol extracts of pasak bumi. The root extract contained 14.631% terpenoid and the stem extract contained only 7.781% terpenoid. This result confirmed previous colorimetric observation by Lieberman-Burchard method that showed greater color change in the root extract compared to that in the stem extract. The stem ethanol extract of pasak bumi contained 1.785 % of alkaloids and the root ethanol extract contained 5.117 % of alkaloid from total compounds.

Conclusion

The ethanol extract of the root of *Eurycoma longifolia* accumulates more alkaloids and terpenoids than that of the stem of *Eurycoma longifolia*, where more terpenoids were found on both extracts. Major terpenoid in root extract was 2H-1-Benzopyran-2-one, 3-phenyl- (coumarin derivate), whilst in stem was Stigmasterol. Major alkaloid in both organ was 3-Methyl-1-oxo-2,3-dihydro-1H-pyrazolo[4,3-c][1,10] phenanthroline.

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