

# Chemotaxonomic Study in the Heartwood of Javanese Teak: Analysis of Quinones and Other Related Components

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## Abstract

Ethanol-benzene soluble extracts from the heartwood collected from 87 individual teak trees grown in the island of Java were analyzed using GC and GC-MS. The variations of quinones (tectoquinone, deoxylapachol, isodeoxylapachol, lapachol, tectol) and other components (palmitic acid, squalene, and two unknown compounds) were investigated for a chemotaxonomical study. There were wide variations in the contents of the constituents among individuals from three habitats, Purwakarta (plantation forest), Randublatung (plantation forest) and Gunungkidul (community forest) regions. Cluster and discriminant analysis results showed that teak trees can be classified into three clusters based on the composition of quinones and squalene. Cluster I was distinguished by relatively high amount of squalene and low amount of quinones. In contrast, high amount of tectoquinone and low amount of squalene was observed in cluster III. Further, comparatively high amount of naphtaquinones (lapachol, deoxylapachol and its isomer) and tectol was found in cluster II. Based on geographical distribution, Purwakarta, Randublatung, and Gunungkidul regions mostly produce cluster II, I, and III type individuals, respectively.

**Keywords:** *Tectona grandis*; chemotaxonomy; extractives, quinones, Java.

## Introduction

Teak that often appears in Java is one of the species most associated with the Javanese since ancient times. Teak is generally classified as a fancy wood and has also been used as a general purpose timber. This tree grows throughout the island of Java beginning in easternmost Java (Banyuwangi, East Java Province) and ending in Sukabumi, West Java Province to the west.

Past research has confirmed that many quinones are present in teak heartwood (Sandermann and Simatupang 1966; Windeisen *et al.* 2003; Lukmandaru and Takahashi 2009). It is also proved that the quinone and its derivatives exhibit antitermitic activities (Rudman and Gay 1961; Sandermann and Simatupang 1966). It is reported that the composition of these quinones vary with tree age (Lukmandaru and Takahashi 2009; Lukmandaru 2009), site and tree type (Sandermann and Simatupang 1966; Windeisen *et al.* 2003). This study investigated the

variations in quinone constituents of teak (tectoquinone, deoxylapachol, isodeoxylapachol, lapachol, and tectol) and chemotaxonomic study based on the quinones and other related components.

## Materials and Methods

### Sample Preparation

The tree samples were collected from three provinces, West Java (Purwakarta region, 32 trees), Jogjakarta (Gunungkidul region, 13 trees) and Central Java (Randublatung region, 42 trees). Those three regions have been known to produce teak trees annually in considerable amount. The condition of the sites and tree characteristics are described in Table 1. The wood from the base part from two opposite radii of the outer heartwood were converted into wood powder (40~60 mesh) and were then combined to form a single sample for further analysis.

Table 1. Description of the sampling and sites.

Factor	Purwakarta	Randublatung	Gunungkidul
Origin/type	Plantation forest	Plantation forest	Community forest
Province	West Java	Central Java	Jogjakarta
Altitude (m)	110	140	270
Soil type	Latosol, loamy sand	Humous marginalitic, loamy sand	Black calcareous, loam
Annual rainfall range (mm)	1200 - 1800	1300 - 2000	1400 - 1800
Temperature range (°C)	22 - 28	20 - 34	22 - 36
Relative humidity range (%)	76 - 80	70 - 74	68 - 72
Number of samples	32	42	13
Diameter breast height range (cm)	39 - 137	23 - 83	25 - 38

### Analysis of Extractive Components

Wood powder (about 2 g) was extracted using a soxhlet apparatus with ethanol-benzene (1:2, v/v) for 8 h. After evaporation of the solvent, the extracts (concentration of 100 mg/mL) were analyzed using GC (Hitachi Model G-3 500), NB-1 bonded capillary 30m. Operation temperature was 120–300°C with a heating rate of 4°C/min and held at 300°C for 15 min. Injector and detector temperatures were set at 250°C. Helium was used as the carrier gas, the split ratio was 80:1, and the injected volume was 1.0 µL.

The identification of components was based on a comparison between authentic components, references (Perry *et al.* 1991; Lemos *et al.* 1999; Windeisen *et al.* 2003), and GC–MS analysis results. The following authentic components were used: tectoquinone (25753-31 Kanto Chemical), lapachol (142905 Sigma-Aldrich), 2-hydroxymethyl anthraquinone (17241-59-7 Acros Organics), squalene (37309-30 Kanto Chemical), and palmitic acid (32016-30 Kanto Chemical). Mass spectrometry measurements were obtained from GC-MS analysis on a Shimadzu QP-5000 with operation conditions being similar to GC analysis. The MS operating parameters were temperature ionization voltage of 70 eV, transfer line temperature at 250°C, and scan range of 50–500 atomic mass unit.

### Statistical Analysis

SPSS 10.0 version with Windows was used for cluster and discriminant analysis. For cluster analysis, agglomerative hierarchical clustering (AHC) using average linkage with square Euclidean distance measure was applied to examine the inter-relationships between populations and its chemical constituents. Discriminant analysis was based on Mahalanobis' generalized distance.

### Results and Discussion

Figure 1 shows the content of the ethanol-benzene extracts in the outer heartwood collected from the 87 teak trees. The content varied from 4.15 to 16.24% and showed a normal distribution with a mean value of 9.19% and standard deviation of 2.69%. The average levels of those from Purwakarta region slightly higher (10.80%) than those of Randublatung region (8.17%) and Gunungkidul region (7.01 %).

Figure 2 shows the gas chromatogram of the ethanol-benzene extracts in outer heartwood. Nine constituents, namely, deoxylapachol, palmitic acid, lapachol, isodeoxylapachol, tectoquinone, unknown compound 1 (UN1), unknown compound 2 (UN2), squalene, and tectol were detected. The peaks 1 and 4 were provisionally assigned as deoxylapachol and its isomer (Perry *et al.* 1991; Windeisen *et al.* 2003) due to the almost identical mass spectra of the two compounds (Lukmandaru and Takahashi 2009). The molecular masses of unidentified compound 1 (UN1) and 2 (UN2) were found to be m/e (base

peak) = 244 and 242, respectively. On the basis of their chemical structures, the quinones were from naphtaquinone (lapachol, desoxylapachol and its isomer), anthraquinone (tectoquinone), as well as naphtaquinone dimer (tectol). Table 2 summarizes the composition of components determined by capillary gas chromatography and relative contents of the constituents for 87 typical individuals of teak from 3 habitats. The contents of these nine constituents showed wide variations among individuals and habitats. Triterpene squalene was generally the most abundant component in ethanol-benzene soluble extracts (varied from 6 to 65 %) while the quinone fraction ranged from 15 to 64%.

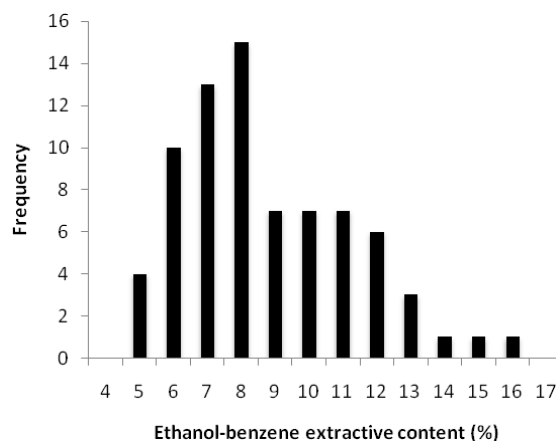


Figure 1. Histogram of ethanol-benzene extract contents in the heartwood of teak.

Cluster analysis was applied to all individual data, using the Euclidean distance between individuals as an index of their similarity and clustering was performed according to the weighed average linkage method. The dendrogram obtained differentiated three groups as shown in Figure 3. Cluster I assigned as squalene-type as it showed substantial percentages of squalene (average 52.5%) but low percentage of quinones such as tectoquinone, deoxylapachol and isodeoxylapachol (average 7.1, 3.3 and 4.4%; respectively of the samples analyzed). On the other hand, cluster III was characterized by high percentage of tectoquinone (average 15.4%); but relatively low amount in squalene (22.5%), thus, this cluster is assigned as tectoquinone-type. Cluster II had tectol and tectoquinone as major compound (average 11.8 and 12.9%, respectively; of the samples analyzed) but relatively low in squalene percentage (39.7%). It is observed that naphtaquinones (lapachol, deoxylapachol and its isomer) contents of this cluster comparatively higher than those of cluster I and III, therefore this cluster is assigned as naphtaquinone-type. The mean chemical composition of the teak heartwood extracts of each cluster is presented in Figures. 4a-c.

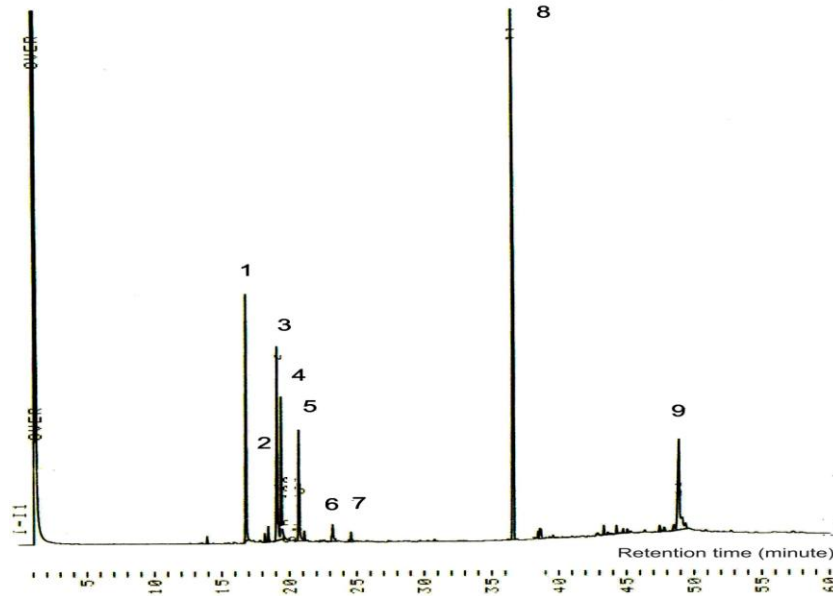


Figure 2. Gas chromatogram of the ethanol-benzene extract from the teak outer heartwood. Nine major compounds are detected: peak 1 ( $R_t$  16.9) and 4 ( $R_t$  19.7) = desoxylapachol and its isomer; peak 2 ( $R_t$  18.6) = palmitic acid; peak 3 ( $R_t$  19.0) = lapachol; peak 5 ( $R_t$  20.9) = tectoquinone; peak 6 ( $R_t$  23.1) = unidentified compound 1; peak 7 ( $R_t$  24.6) = unidentified compound 2; peak 8 ( $R_t$  36.7) = squalene; and peak 9 ( $R_t$  49.0) = tectol.

Table 2. The relative contents of ethanol-benzene soluble components in the outer heartwood of teak in three habitats.

Components	Purwakarta ( $n=32$ )			Randublatung ( $n=42$ )			Gunungkidul ( $n=13$ )		
	Min.	Max	Average (St. dev.)	Min.	Max.	Average (St. dev.)	Min.	Max.	Average (St. dev.)
Deoxylapachol	0	26.79	6.65 (7.95)	0	17.09	3.44 (3.97)	0	23.05	2.90 (6.60)
Palmitic acid	0.49	3.34	1.34 (0.60)	0	10.45	2.03 (1.67)	0	13.98	3.35 (3.41)
Lapachol	0	12.92	4.25 (4.06)	0	17.42	3.37 (4.10)	0	14.03	4.39 (3.87)
Isodeoxylapachol	0	13.44	5.17 (3.08)	2.15	13.78	5.71 (2.37)	0	25.94	6.05 (6.66)
Tectoquinone	4.07	51.71	14.61 (10.46)	2.16	21.18	8.05 (5.28)	3.17	23.92	11.31 (6.24)
UN1	0	4.00	0.48 (0.88)	0	11.24	1.75 (2.63)	0	7.28	1.81 (1.96)
UN2	0	9.80	1.90 (2.20)	0	3.82	1.10 (0.80)	0	11.32	2.16 (3.02)
Squalene	7.59	62.60	41.89 (12.03)	12.45	65.23	42.75 (14.66)	6.86	54.02	26.29 (15.10)
Tectol	2.25	28.91	10.71 (5.92)	3.34	17.99	9.71 (2.86)	0	20.34	6.75 (6.22)

Note: UN: unknown compound.

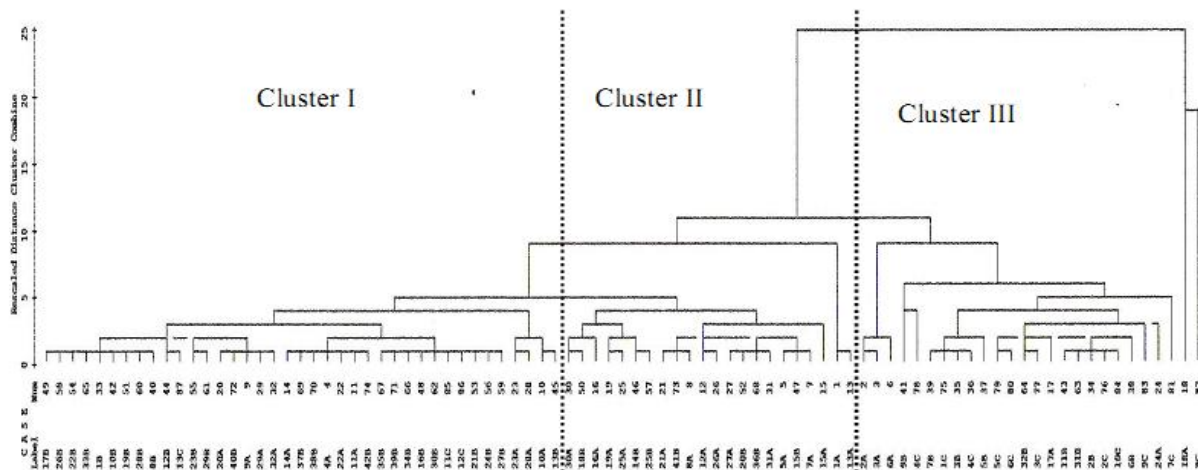
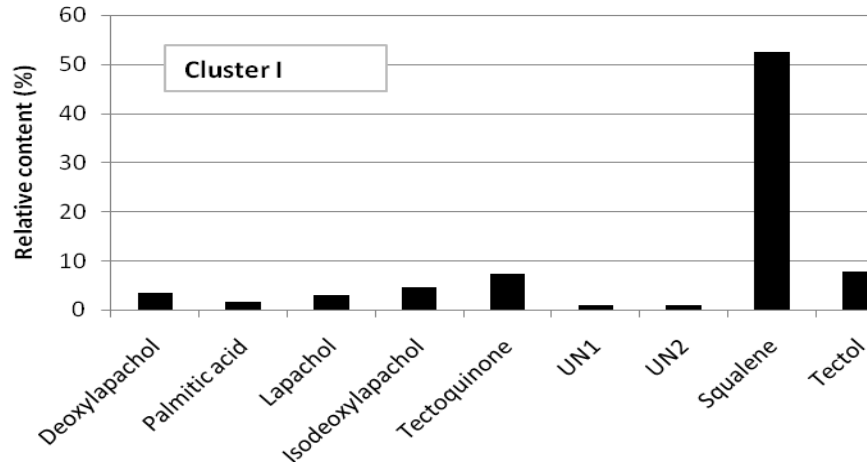
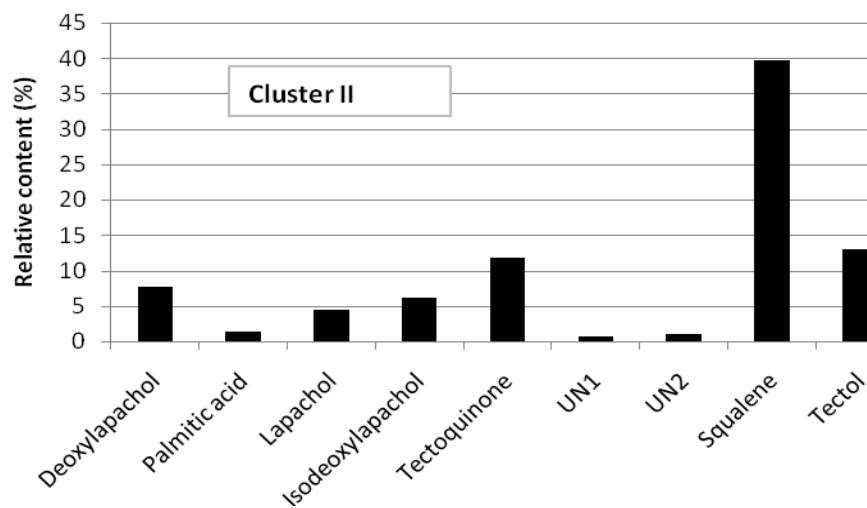


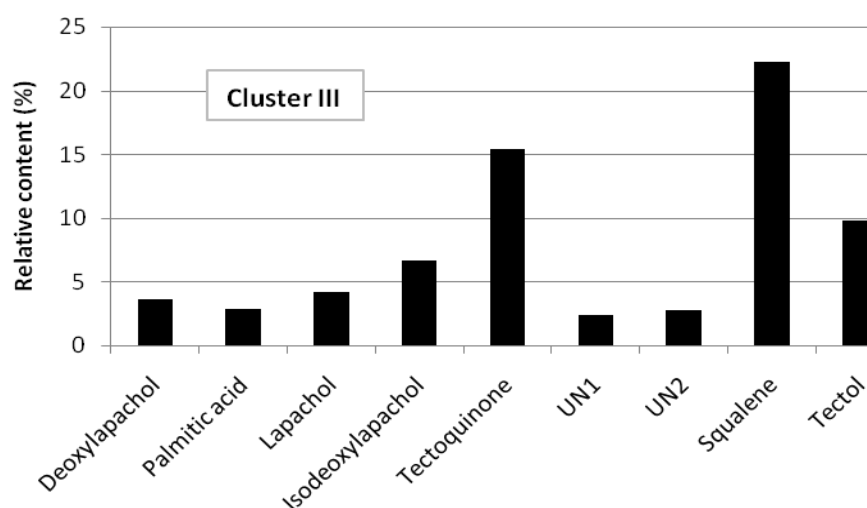
Figure 3. Dendrogram obtained from the cluster analysis of 87 samples of teak from Java. Horizontal: samples analysed (A = Purwakarta, B = Randublatung, C = Gunungkidul), vertical: similarity levels between samples.



(a)



(b)



(c)

Figure 4a-c. Mean chemical composition of ethanol-benzene extracts of cluster I-III of teak.

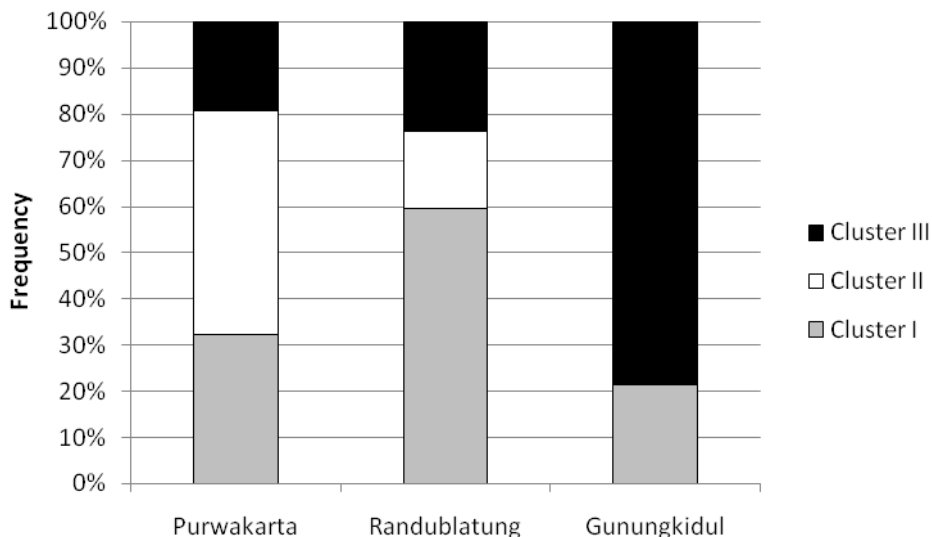


Figure 5. Proportion of three clusters of ethanol-benzene extracts of teak from three habitats.

On the basis of geographical distribution (Figure 5), cluster I accounted for 43.6% (38/87) of the population and mostly distributed in Randublatung regions (59.53%). Cluster II accounting for 25.2% (22/87) of the populations was distributed mostly in Purwakarta region (46.2%). Cluster III which can produce high amounts of tectoquinone, was the most common type in Gunungkidul region and accounted for 31.0% (27/87) of the population. It is also noted that cluster II was absent in Gunungkidul samples.

As the final step, discriminant analysis was used. The discriminant ratio was 94.3% (82/87), only 3 individuals of cluster I was discriminated incorrectly as cluster II, while 1 individual of each cluster II and cluster III were discriminated incorrectly as cluster I. Teak individuals can be thus classified into three clusters on the basis of the contents of quinone constituents and squalene. Further, their distribution corresponds to variations in the contents. For more comprehensive investigations, the subsequent works should be conducted with larger samples including plantation teak trees from East Java as well as from community forests that scattered in the island of Java.

### Conclusions

This is the first report on the chemotaxonomical study of teak heartwood. The tree samples were collected from three regions, Purwakarta, Gunungkidul, and Randublatung, for a total of 87 individuals. Based on the cluster analysis, three types can be obtained from the relative content of quinones and squalene. Cluster I (squalene-type), II (naphtaquinone-type) and III (tectoquinone-type) refers to heartwood with relatively high amount of squalene but low amount of quinones, high amount of naphtaquinones (lapachol, deoxylapachol and its isomer), and high amount of tectoquinone but low amount of squalene relative contents, consecutively. Based on geographical distribution,

it is found that squalene-type is the major type in Randublatung, while tectoquinone-type and naphtaquinone-type are the most abundant in Randublatung and Purwakarta, respectively.

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