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Comparison of Chemicals in *Lagerstroemia speciosa* (L.) Pers. at Growing Stage Levels by GC-MS

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ABSTRACT Banaba, *Lagerstroemia speciosa* (L.) Pers. (Lythraceae) is a tree that grows in the tropic islands of the Pacific. This plants are used for medical purposes in the world. The components of *L. speciosa* were analyzed for the contents according to growing stages at leaves. The distributions of the corosolic acid (2 α, 3 β-dihydroxyurs-12-en-28-oic acid), phytol, campestrol, and vitamin E were rich among samples in this study. These were contained much fatty acids. The mean content of palmitic acid was from 2.4% across all growing stages, varying from 2.15% for young leaves with the lowest content and 2.86% for fallen leaves with the highest content. Oleic acid, linoleic acid, and linolenic acid were contained nearly mean 2.0% in all leaves of banaba. Our results have shown that the phytochemical profile of young *L. speciosa* leaves differs quite radically from that of old *L. speciosa* leaves. In addition, these subdividing results according to plant growth should allow future researches to conduct targeted experimental studies and use of particular medical components of interest, examining chemical variation on the inter-developmental levels.

Keywords : banaba, *Lagerstroemia speciosa*, components, GC-MS, growing stage

Banaba (*Lagerstroemia speciosa* (L.) Pers.) is a tree that grows in the Philippines and tropic islands of the Pacific (Philippine Medicinal Plants, 2004). The plant bears purple flowers during the rainy season. Banaba is a deciduous, tropical, flowering tree that can grow to 18 m in height, with a 9 to 12 m spread (Philippine Medicinal Plants, 2004). The large, oblong, dark-green, leathery leaves measure 5 to 10 cm wide by 12 to 30 cm long. The leaves turn an orange-red color in the fall. The flowers are pink to purple in color, giving way to oval, nut-like fruits. The bark of the tree is thin, mottled, and peeling.

It has been known in the country since ancient times as a natural diuretic and as a cure for kidney and bladder problems (Philippine Medicinal Plants, 2004). Banaba is a common name of *L. speciosa* and also has been called queens crape myrtle, queen's flower, and pride of India. Recently the tree is gaining attention from the rest of the world because of its medicinal properties (Carew and Chen, 1961; Kakuda *et al*., 1996). There have been many studies done on this remarkable herb. Research conducted by Kim *et al*. (2008) have shown that the banaba contains corosolic acid, which has insulin-like properties. The studies indicate that corosolic acid activates the transport of glucose across cell membranes. Kim *et al*. (2008) have also shown that anti-diabetic effects of banaba are not single chemical, but several compounds. The herb, therefore serves as a glucose transporter which helps reduce blood sugar levels.

Banaba leaves contain ellagic acid derivatives (Hayashi *et al*., 2002). A later report confirms ellagitannins, lagerstroemin, flosin B, and reginin A, which are all possible glucose transport enhancers (Murakami, 1993; Hayashi *et al*., 2002). Lagertannins, beta-sitosterol, stigmasterol, campesterol, and some olefins also have been found in banaba leaves and extracts (Unno *et al*., 2004). Lageracetal (1, 1-Dibutoxybutane), 1-pentanol, ellagic acid, and corosolic acid (a triterpene) have been isolated from leaves (Kim *et al*., 2008). Another study reports 16 amino acids, pyrogallol tannins, and lipids also present in banaba leaf (Hayashi *et al*., 2002). From the neutral fraction of hot ethanol extracts of banaba leaves, nonacosane, hentriacontane, tritriacontane, olefins, and esters of palmitic, daturic, stearic, arachinic, and behenic acids were identified (French *et al*., 2002).

Banaba bark was found to contain similar constituents to its leaves. One report finds ellagic acids, beta-sitosterols, and

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colosolic acids from bark extracts (Murakami *et al*., 1993).

Seeds of banaba contain caprylic, lauric, myristic, palmitic, steric, arachidic, behenic, lignoceric, oleic, and linoleic acids in the oil (Sinhababu *et al*., 1999). 9-keotoctadec-cis-11-enoic acid has been isolated from seed oil as well. Chemical investigation of amino acid components in banaba seed oil has been performed. Components nonanedioic acid, 12-acetyloxy-9-octadecenoic acid, and 16-methyl-heptadecandic acids present in seed extracts have been identified as having antibacterial activity (Klein *et al*., 2007).

Although banaba has great potential as a medicinal species, further research is still needed. Some import traders have advertise extensively like cure-all in the world. As plants have grown, they are accumulated some secondary metabolites or lose chemicals. It is very important to use exact medicinal stuffs for a medical prescription slip. Thus, the detailed composition of the general fractions at the growing stages have not unknown in banaba.

The aim of this paper was to analyze for the content according to growth stages at leaves and were compared to those of the stem.

MATERIALS AND METHODS

Plant materials and Extraction

Leaves and stems were collected from natural (wild) populations of *L. speciosa* at the Banaba Island and Butaritari Island in the Philippines.

Seven mature trees ($\geq$ 2 yr) were randomly collected from each populations. The fresh young leaves were collected from the labelled trees on May 17, 2008, matured leaves sampled same trees after two months, and the old leaves were also sampled from same trees on September 17, as soon as the falling of leaves were fallen from trees to soil. Thus, we repeated seven experiments because we were used different samples from each labelled tree. 50 g of *L. speciosa* leaves were added to 50 ml distilled water and homogenized in homogenizer with 3,000 rpm for 2 minutes. Samples were treated in an ultrasonic bath for 30 min. Samples were dried and ground hydro-distilled for 2.5 h using a Clevenger-type apparatus. The components were extracted from the distillate with ether, and then dried with anhydrous Na_2SO_4. The solvent was removed by distillation at atmosphere pressure, and the pure oil was kept at 4℃ until analysis.

Analysis by GC–MS

A gas chromatography was equipped with a fused silica capillary column (30 m × 0.25 mm), with a 0.25 um film thickness of PTE-5 and FID was used for GC measurements (Petersen *et al*., 2006). The injection volume was 5 ml. The operating conditions were as follows: temperature programme was 260℃ at 4.3 min for injector. Detector temperature was 280℃ and chromatographic elution was carried out at a flow rate 1.0 ml / min using the carrier gas (He). GC-MS analyses were performed on a Hewlett Packard (model 6860GC / 5972MSD) equipped with fused silica HP-5MS capillary column (30 m × 0.25 mm) of film thickness 0.25 um. Constituents were identified by comparison of their mass spectra to those from MS libraries (Adams89, Nist92, and Amdis32) and the results obtained were correlated with calculated and Adam's retention indices. Area percent was obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

Analysis by oils

Dried leaves (4g) were subjected to hydrodistillation and solvent extraction using a Likens-Nikerson apparatus for 2 h from which a yield of 0.15% of oil was obtained. The solvent was peroxide free diethyl ether. The solvent was removed by distillation at atmosphere pressure, and the pure oil was kept at 4℃ until analysis. A gas chromatography equipped with a 60 m × 0.32 mm fused silica capillary column, with a 0.32 um film thickness of PTE-5 and FID was used for GC measurements. The opening conditions were as fellows: temperature programme 60-285℃ at 4.3℃ min and an injector and detector temperature of 250℃; the carrier gas He (2 ml/min). GC-MS analyses and constituent identifications were same as the previous methods.

Seven experiments were conducted from each growing stage. The main component with constituent (above 1%) and high quality (above 70) were selected. Homogeneity of variance among data was tested by Bartlett's statistics (Zar, 1984).

RESULTS AND DISCUSSION

The applicability of gas chromatography to analysis of

compounds that were resolved on packed columns was investigated. Figs. 1~3 showed the mass spectra of GC-MS fractions in the banaba. As shown in Figs. 1~3, all analysed samples showed broad diversity in both the number and concentration of these compounds. Leaves of banaba at the developmental stages (early, matured, and old) were analysed for compounds with the high percentages (1%>) and high quantity among groups (Table 1).

Compounds were mostly present (old leaves), partially present or entirely absent (e.g. young and matured leaves). For example, eight compounds, unidentified peaks were not detected in young and matured leaves, providing unique GC-MS profiles for old leaves. When same contents of three stages were compared, the relative rates for minimum / maximum showing differences with above 50% had shown in Fig. 4. Young leaves had the eight most contents among three stages, matured leaves were five, and old leaves were ten. Seven repeated data between same stages can be calculated and compared for those of different stage groups. Many main compounds of them were significantly different from those of the other groups ($p < 0.01$) (Table 1). For example, twenty-seven main compounds of 35 (77.1%) were significantly different from other groups. Whereas, only eight compounds of 35 (22.9%) did not show significantly different from other groups.

He distributions of the corosolic acid (2 α, 3 β - dihydroxyurs - 12 - en - 28 - oic acid), phytol, campestrol, and vitamin E were rich among samples in this study.

Phytol was most rich in all banaba samples. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is not surprised that phytol is an extremely common terpenoid, found in all plants esterified to chlorophyll to confer lipid solubility. The content of phytol was from 29.97% for matured leaves to 10.22% for fallen leaves. The contents of linolic acid and vitamin E were same trend. Overall, secondary metabolites such as the terpenes (made from mevalonic acid, composed almost entirely of carbon and hydrogen), phenolics (made from simple sugars, containing benzene rings, hydrogen, and oxygen), and nitrogen-containing compounds (extremely diverse, may also contain sulfur) were more rich in fallen leaves than young and matured leaves. It is correspond with general concept that

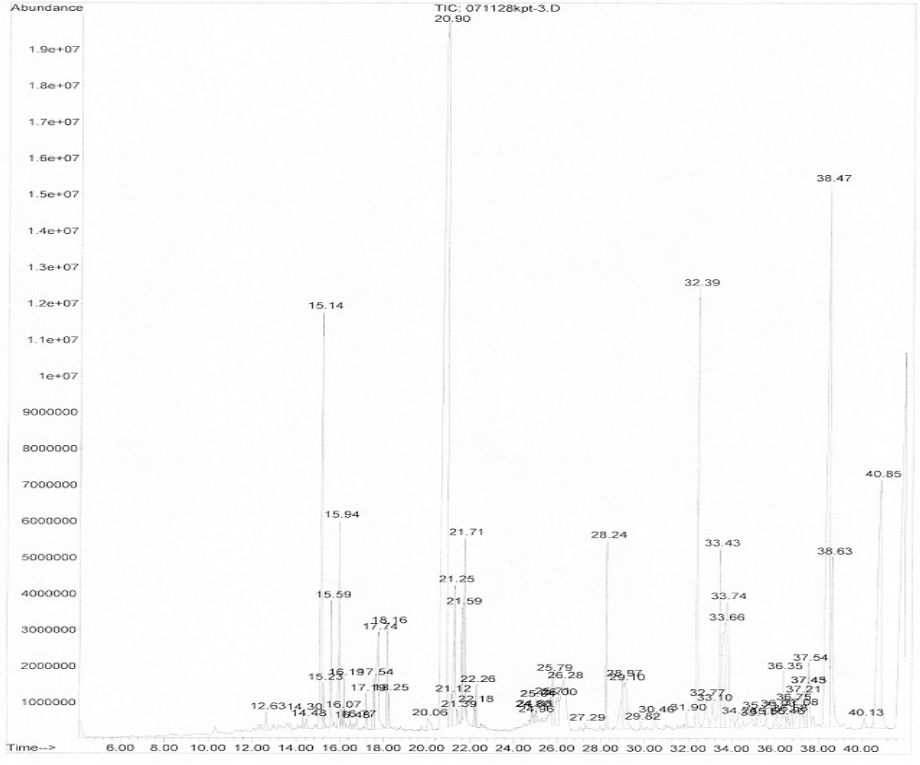

Fig. 1. Chemical composition of the young leaves of banaba.

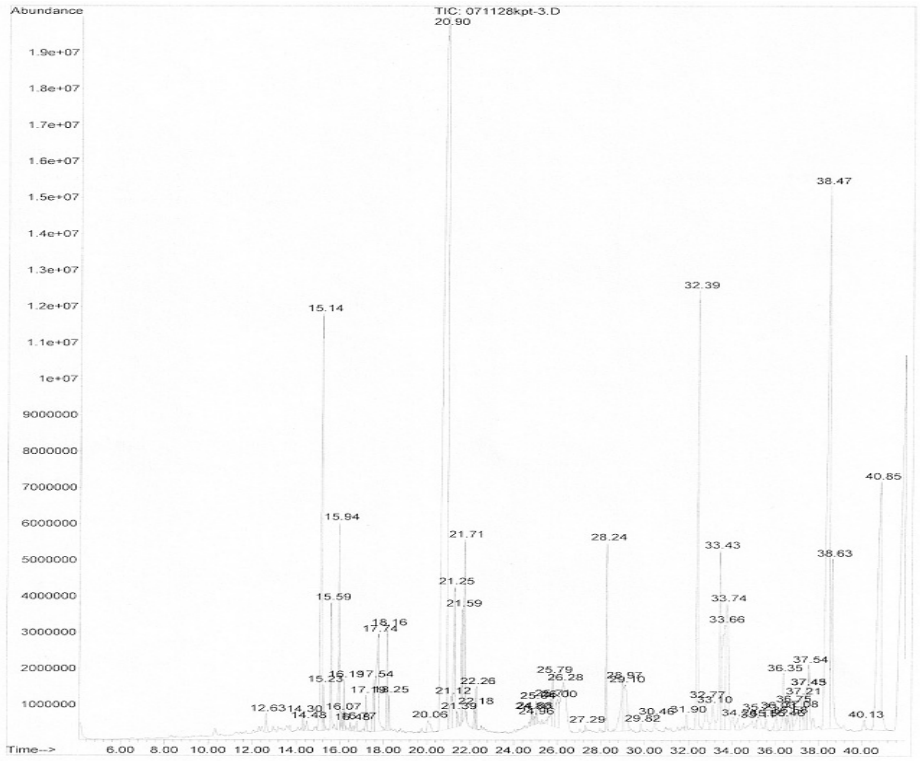

Fig. 2. Chemical composition of the matured leaves of banaba.

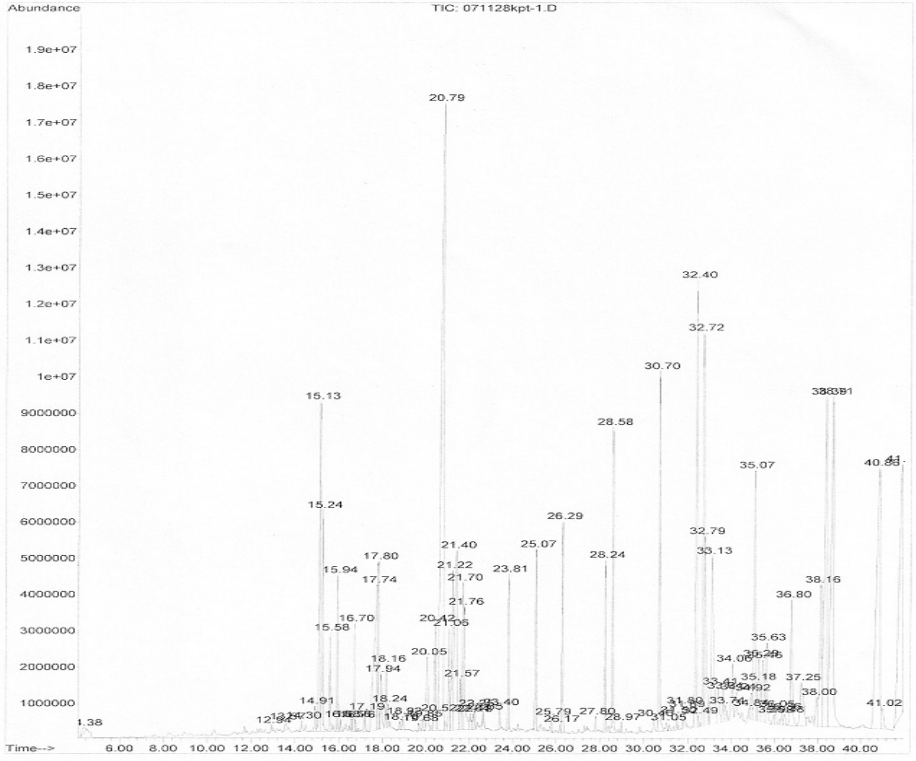

Fig. 3. Chemical composition of the old leaves of banaba.

Table 1. Qualitative determination in the leaves of banaba (*Lagerstroemia speciosa*).

RT	Code	Compounds	Y.L/QL	M.L/QL	F.L/QL	F-test	P
15.14		Neophytadiene	4.48/99	4.57/87	2.56/76	16.78	**
15.24	A	2-Pentadecanone	-	1.74/76	1.31/93	1.59	ns
15.44		Heptadecane	1.01/90	1.43/90	1.31/91	0.13	ns
17.74		n-Hexadecanoic acid	2.15/99	2.19/99	2.86/99	1.45	ns
	B	Palmitic acid	2.15/96	2.19/98	2.86/99	0.13	ns
20.90		Phytol isomer, phytol	23.43/95	29.97/93	10.22/94	218.10	***
21.45	C	9-Octadecanonic acid, Oleic acid	1.72/99	0.88/95	2.87/93	208.46	***
21.61	D	Cycloonanone	-	2.09/69	-		
21.71	E	9, 12-Octadecatrienoic acid, Ethyl ester	1.84/83	2.05/99	0.41/95	390.70	***
		Ethyl linolic acid	1.84/38	2.05/99	0.41/99	390.70	***
21.78		Octadecanoic acid, Ethyl oleate	0.18/98	0.88/95	1.28/86	96.87	***
22.07		Linolenic acid	1.96/99	2.29/99	2.87/81	0.16	ns
		Ethyl linolenate	1.96/99	2.29/99	2.87/78	0.16	ns
25.07	F	2, 4, 12, 16-Tetramethylheptadecan-4-olide, 2, 5-hexanedione, Diacetonyl	-	0.29/96	1.11/98	2.79	ns
26.29	G	Stigmastan-3, 5-dien	2.23/93	-	1.25/91	127.83	***
		2, 8-diisopropyl-peri-xanthenoxanthene-4, 10-quionone. 2, 8-diisopropyl-oeri-xanthenoxanthene-4, 10-puionone	2.23/83	0.13/97	1.25/78	3.96	*
28.24	H	Bis(2-ethylhexyl) phthalate, Diamyl phthalate, Dicyclohexyl phthalate	1.19/91	0.99/74	0.93/91	0.03	ns
28.58	I	Cyclononasiloxane	-	0.07/63	1.78/72	64.77	***
32.39	J	2, 6, 10, 14, 18, 22-Tetracosahexaene	4.44/98	0.98/97	3.96/97	23.48	**
		Squalene	4.44/94	0.98/95	3.96/95	23.48	**
32.72	K	4-(3, 4-Dimethoxybenzylidene)-1-(4-nitrophenyl-2-pyrazolin-5-one	-	0.21/63	3.43/90	150.49	***
33.12	L	(+)-(P, 1R, 3S)-5-(4, 5-dimethoxy-2-methyl-1-naphthyl)-6, 8-dimethoxy-1, 2, 3-trimethy-1, 2, 3, 4-tetrahydroisoquinoline	-	-	2.64/83	336.62	***
33.43	M	Cyclotetracosane	2.29/95	0.15/95	0.42/89	120.50	***
	N	17-Pentatriacontene	2.29/91	-	-	204.55	***
33.74	O	9,19-Cyclolanostan-3-ol	2.60/98	1.42/47	-	372.88	***
	P	(4'R, 6'R)-4-(4', 6'-dimethyl-1', 3'-dioxan-2'-yl)-5-methoxy-2, 2-dimethy-2, 3-dihydroanthra[1, 2-b]furan-6, 11-dione	2.60/91	0.52/47	-	215.66	***
34.25	Q	Z-5-Nonadecene	-	1.63/95	-	68.76	***
		1-Heneicosyl formate	-	1.63/94	-	68.76	***
		1-Hexacosanol	-	1.63/91	-	68.76	***
35.07	R	monocarbonyl-(1, 3-butadiene-1, 4-dicarbonic acid	-	-	2.66/86	191.13	***
35.46	S	2, 6, 10, 14, 18-Pentamethyl-2, 6, 10, 14, 18-eicosapentaene	-	-	1.03/98	26.73	**
36.80	T	Preussometrin I	-	-	1.60/74	17.89	**
38.47	U	Vitamin E, 2H-1-Benzopyran-6-ol	13.45/98	10.22/98	5.76/98	21.74	**
38.63	V	2-methoxy-3-methyl-5-(1-pyrrolidinyl)-1, 4-benzoquinone	3.25/64	1.07/38	6.76/64	16.47	**
40.85		Ergost-5-en-3-ol, Campestrol	7.55/99	13.06/99	7.14/99	6.83	*
41.88	W	Stigmasta-5	-	-	5.61/99	82.46	***

RT: Rooting time, Y.L: Young leaves, M.L: Matured leaves, F.L: Fallen leaves, QL: Quality.
ns: non-significnat at the 0.05 level, *: $p < 0.05$, **: $p < 0.01$ ***: $p < 0.001$.

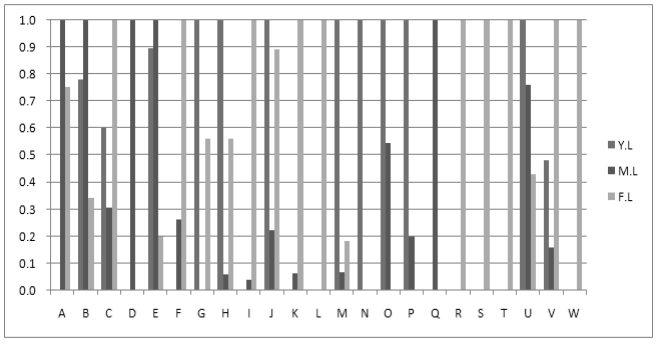

Fig. 4. The relative rate of chemical composition of the *Lagerstroemia speciosa* leaves at growing stages. Codes are same as Table 1. Y.L: Young leaves, M.L: Matured leaves, F.L: Fallen leaves.

secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses (Stamp, 2003).

These were contained much fatty acids. The mean content of palmitic acid was from 2.4% across all species, varying from 2.15% for young leaves with the lowest content and 2.86% for fallen leaves with the highest content. Oleic acid, linoleic acid, and linolenic acid were contained nearly mean 2.0% in all leaves of banaba.

Components nonanedioic acid, 12-acetyloxy-9-octadecenoic acid, and 16-methyl-heptadecandic acids present in seed extracts have been identified as having antibacterial activity (Unno *et al*., 2004). Although The their contents were low, they were detected in all growing leaves.

Banaba leaves contain ellagic acid derivatives (Suzuki *et al*., 2001; Hayashi *et al*., 2002). Lagertannins, beta-sitosterol, stigmasterol, campesterol, and some olefins also have been found in banaba leaves and extracts. Lageracetal (1, 1-Dibutoxybutane), 1-pentanol, ellagic acid, and corosolic acid (a triterpene) have been isolated from leaves (Judy *et al*., 2003). They also presented in banaba leaf. From the neutral fraction of hot ethanol extracts of banaba leaves, nonacosane, hentriacontane, tritriacontane, olefins, and esters of palmitic, daturic, stearic, arachinic, and behenic acids were also identified.

The results of growing stages will be provided a valuable forecast of the quantitative analysis of these *L. speciosa* species, few of which had been previously examined. In addition, these subdividing results according to plant growth should allow future researches to conduct targeted experimental studies of particular *Lagerstroemia* species of interest, examining chemical variation on the inter-developmental levels. Finally, species with diverse chemical profiles have been identified that may be of interest for further large-scale bioassay-guided fractionation studies.

Within this mind, we must stress that, while several screening of plant constituents have been made on variability within *L. speciosa* at the developmental stages. Our results have shown that the phytochemical profile of young *L. speciosa* leaves differs quite radically from that of old *L. speciosa* leaves, suggesting that further research on chemical variation among an organs (root, bark, and flower) is needed.

MS detection was only completed to evaluate assay specificity. Assay validation (accuracy and reproducibility and quantitative analysis) was not completed with MS detection. LC-fluorescence was also more specific than LC-UV at either wavelength and may actually be as specific direct probe MS-MS. Furthermore these studies are needed in identifying new found banaba.

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