PRELIMINARY ANALYSIS OF YIELD AND COMPOSITION OF LATEX FROM *ALSTONIA ANGUSTILOBA*

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HALIMAHTON MANSOR & MORRIS, M.D. 1989. Preliminary analysis of yield and composition of latex from *Alstonia angustiloba*. Seven trees of *Alstonia angustiloba* from four different locations were examined using a double tapping system with two days recovery between tappings. The yield of latex from each tree varied greatly between 6 - 740 *ml*. The latex composition varied little between trees. It consisted of 5 - 8% rubber, 18 - 24% resins and 2 - 4% insoluble material, the remainder being water. The rubber portion was identified as 1,4-polyisoprene of > 99% *cis* content.

Key words: *Alstonia angustiloba* - latex - rubber - resins - tapping - polyisoprene

Introduction

Alstonia angustiloba (pulai), is found throughout the Malay Peninsula and in the Asian tropics. This species, along with the smaller *Alstonia scholaris* which is not distinguished by the Malays or the Javanese, has long been known for its medicinal value as well as for its soft, easily shaped timber. Use of the bark in medicine has been recorded in India as far back as 600 A.D. whilst its medicinal use in Malaysia dates from before records exist. The tree contains a latex which can be extracted by tapping, and it is used by various tribes in native medicines, being commonly applied to sores and skin diseases (Browne 1955).

Little is known about the composition or properties of pulai latex. Early examination of the latex at the Botanic Gardens, Singapore found it similar to jelutong (*Dyera costulata*) but inferior to it in strength, in readiness to coagulate and in tendency to turn yellow (Eaton 1910). Hooper (1905) stated that the latex does not yield rubber but nearly half of it consists of resins. Browne (1952) determined the yields of latex from a number of *A. angustiloba* trees over three consecutive days but no analysis of the latex was carried out. Quantitative

analysis of rubber hydrocarbon in one sample of latex from *A. scholaris* by Ullah *et al.* (1980) found it to contain 22.55% solids and 2.43% rubber hydrocarbon, but no chemical identification was carried out.

In this study, the yield and composition of latex obtained from uncultivated *A.angustiloba* trees were examined. We also discuss the commercial potential of the species for latex production.

Materials and methods

Selection of the trees for this study was based primarily on ease of access. Seven trees were selected from four locations in the Kuala Lumpur area, Malaysia. Girths were measured at 1.3 *m* from the ground. Tapping was carried out in the mid to late morning using a full spiral cut which penetrated the bark and cambium. Latex was collected until flow naturally ceased at which point the flow time and quantity of latex were measured. All of the trees were tapped twice with an interval of two days between tappings. The latex pH was determined immediately using narrow-range indicator paper and confirmed by pH meter in the laboratory. Centrifugation was performed at 19,000 r.p.m. (g_{max} = 43,500) for one hour at 0-2°*C*. Total solids content (TSC) was determined by drying weighed samples of the latex at 100°*C* in air to constant weight.

Acetone extractables were determined by coagulating a weighed sample of latex with excess acetone, decanting the serum and hot soxhlet extracting the coagulum with acetone for 16h. The soxhlet extracts were combined with the serum from coagulation and evaporated to dryness under reduced pressure to give the acetone extractables.

Thin-layer chromatography (TLC) was carried out using analytical silica TLC plates, eluting with hexane : ethyl acetate (7:3). Plates were developed by spraying with 20% sulphuric acid followed by heating at 105°*C*.

Nuclear magnetic resonance (NMR) spectroscopy was carried out using a Varian VXR-400 spectrometer at 400 *MHz* for proton and 100 *MHz* for ^{13}C spectra. Solutions (2%) in deuterated chloroform were used with TMS as an external standard.

Gel permeation chromatography (GPC) was performed using 0.02% tetrahydrofuran solutions on mixed-bed Micrel columns with UV detection at 220 *nm*. Calibration was by external polystyrene standards.

Differential scanning calorimetry (DSC) was carried out on a Perkin Elmer 7 series TAS using a heating rate of 20°*C* min^{-1}.

Results and discussion

Yield characteristics

The yields obtained from the trees in this study varied widely from 6-740 *ml*

cumulative total for two tappings (Table 1). With the exception of tree 7, which was observed to be suffering from some trunk damage, increasing tree girth generally gave increasing yields of latex. Although there appears a correlation between tree girth and yield for trees in one environment (location A), the trees from other locations fail to conform to this correlation.

Table 1. Girth and yield data of trees involved in the study

Tree	Location*	Girth (*cm*)	Latex volume collected (*ml*)		
			First tapping	Second tapping	Total
1	A	147	70	80	150
2	A	63	7	1	8
3	A	58	5	1	6
4	A	98	29	10	39
5	B	196	40	50	90
6	C	260	350	390	740
7	D	246	35	50	85

* Locations A : Off Jalan Damansara, Kampong Sungai Pencala
B : Off Jalan Damansara, Taman Tun Dr Ismail
C : Off Jalan Kuching, Segambut
D : Muslim Cemetery, Kampong Batu Lima, Kepong

Latex flow times (Table 2) varied between 30 and 80 *min* but there seems to be no relationship between flow time and yield. There also appears to be no relationship between the TSC of the latex and yield (Table 2); the variation in TSC from 29.0 to 35.9% is relatively minor compared to the variation in yield. These results confirm that yield in terms of latex volume collected gives a good reflection of the real productivity of the tree.

Table 2. Flow times and total solids contents (TSC) of latex samples collected

Tree	First tapping		Second tapping	
	Flow time (*min*)	TSC (%)	Flow time (*min*)	TSC (%)
1	80	29.0	70	28.7
2	77	31.4	70	-[1]
3	70	35.9	70	-[1]
4	30	32.1	45	28.5
5	36	35.8	45	34.5
6	40	30.0	60	29.6
7	45	30.3	45	29.9

[1]Sample insufficient for TSC determination

Comparing overall solids yields between the first and second tappings (Table 3) it is observed that trees 2, 3 and 4 suffer from a substantial drop in yield

whereas the others show yields enhanced by 10-40%. The overall low yields and large decrease in yields of trees 2, 3 and 4 indicate that these trees are probably not mature as far as latex production is concerned. Trees 1, 5, 6 and 7 on the other hand are not only capable of regenerating the extracted latex within a reasonable period of two days, but they are in fact stimulated by tapping to produce higher yields for the subsequent tapping. This phenomenon is common to other mature, latex producing species, notably *Hevea brasiliensis* (Mann *et al.* 1934). The girth size at which *A. angustiloba* reaches maturity can therefore be estimated at between 100 and 140 *cm.* The largest tree examined by Browne (1952) in his study on latex yields was 36 *in* (90 *cm*), indicating that all of the trees studied were immature by the criterion outlined above. This would explain why poor and decreasing yields over a period of three days were obtained in his work.

Table 3. Comparison of dry yields between first and second tappings

Tree	Dry yield first tapping (*g*)	Dry yield second tapping (*g*)	% Change
1	20.3	23.0	+13
2	2.2	0.3*	-86
3	1.8	0.3*	-83
4	9.3	2.9	-69
5	14.3	17.3	+21
6	105.0	115.4	+10
7	10.6	14.9	+40

* Estimated yield based on 30.0% TSC

Latex characterisation

The latex obtained during this study had pH in the range of 4.5 - 5.3 and was stable indefinitely at 0 - 5°*C* without the need for preservatives. Under storage at 25 - 30°*C*, the latex developed a foul odour and dark colouration due to microbial attack, however it was not destabilised. Centrifugation of the latex separated it into four clearly distinguishable zones. The fairly minor top fraction which was creamy in nature appeared to contain rubbery material. The main fraction was milky and below it was a substantial bottom fraction consisting of heavy material. A thin layer of black residue coated the bottom of the centrifuge tube. It can be deduced that at least two major types of particles are present in the latex; one less dense than the serum, apparently containing rubber and the other of higher density than the serum.

The latex could not be coagulated with excessive amounts of acetic or formic acid, but treatment with ethanol, methanol or acetone induced separation of the latex into a moderately coherent coagulum and a clear serum. When

dried, the white coagulum was not rubbery, but rather resinous in nature. Hot acetone extraction of the coagulum left a residue which was rubbery and which darkened on exposure to air and light. The level of acetone extractable material varied between 17.7 and 24.3% of the total latex from the high-yielding trees (Table 4), and the level of the rubber-containing residue was in the range 9.3 to 11.9%. Reprecipitation of the residue from toluene, chloroform or tetrahydrofuran into ethanol yielded a translucent rubber which was essentially stable to air and light. This purified sol rubber constituted 60-70% by weight of the residue from acetone extraction, corresponding to around 6.5% of the total latex or 22% of the total solids.

Table 4. Composition of latices from high-yielding trees

Tree	TSC (%)	Water content (%)	Acetone extractables (%)	Residue (%)
1	28.7	71.3	19.4	9.3
5	34.5	65.5	24.3	10.2
6	29.6	70.4	17.7	11.9
7	29.9	70.1	20.0	9.9

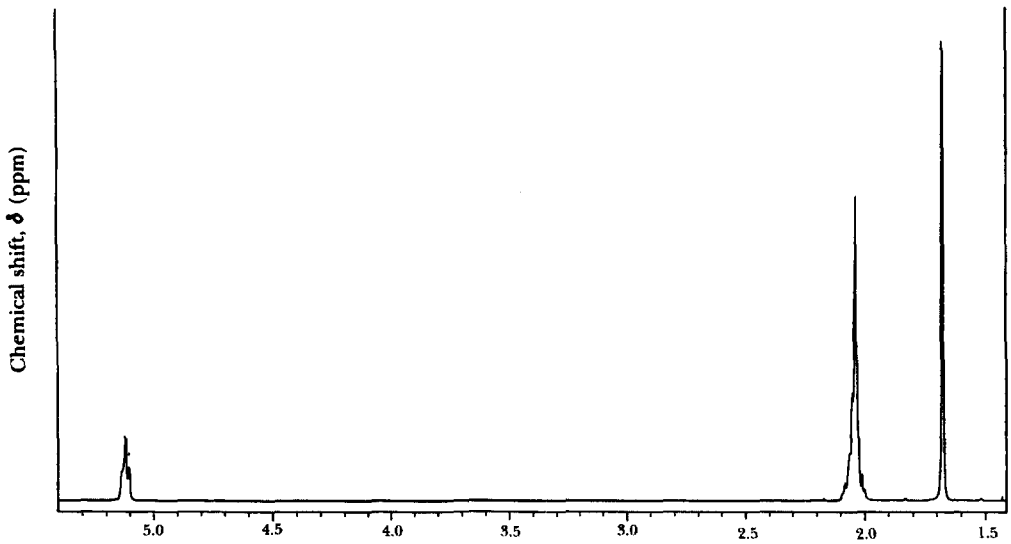

Figure 1. ^{1}H NMR spectrum of the rubber fraction of *Alstonia angustiloba* latex

Analysis of the purified rubber fraction by ^{1}H and ^{13}C NMR spectroscopy (Figures 1 & 2) enabled identification of the polymer as *cis*-1,4-polyisoprene, the same polymer as in natural rubber from *Hevea brasiliensis*. Both spectra are fully consistent with the spectra of purified natural rubber. There is no evidence for 3,4-addition groups or for the *trans*-1,4-configuration. At the level of

sensitivity used, it can be concluded that the polymer is greater than 99% *cis*-1,4-polyisoprene. The impurity resonances observed in the spectra can be attributed mainly to products of polymer autoxidation. Confirmation of the polymer type was obtained from DSC analysis which indicated a glass transition onset temperature, T_g of -64°C compared to a reference temperature of -67°C for a standard sample of natural rubber. The small difference in T_g may be attributable to differences in abnormal functional groups present in the two polymers.

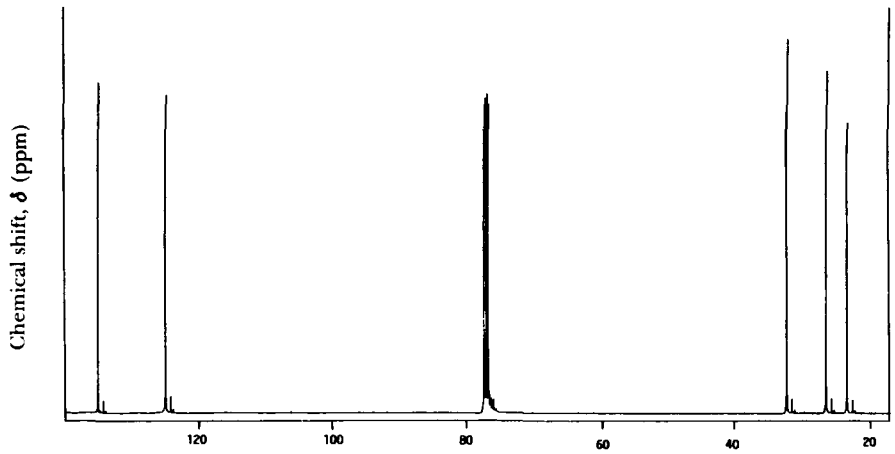

Figure 2. ^{13}C NMR spectrum of the rubber fraction of *Alstonia angustiloba* latex

The molecular weight distribution of *cis*-polyisoprene from *A. angustiloba* as determined by high pressure GPC (Figure 3) is bimodal with number-average molecular weight, M_n around 130,000 and weight-average molecular weight, M_w around 720,000. These are comparable to those found in low molecular weight clones of *Hevea* natural rubber. The bimodal nature of the molecular weight distribution is also observed to a varying extent in *Hevea* rubber, depending on the clone (Subramaniam 1976).

Preliminary examination of the acetone-extractable portion of the latex by analytical TLC revealed three major and two minor components. Although it has been reported that *A. angustiloba* contains alkaloids in its bark (Browne 1952), tests on the acetone-extractable portion of its latex using Meyers and Dragendorff reagents proved negative. Preparative-scale separation of the components is required before analysis of this fraction of the latex can be completed.

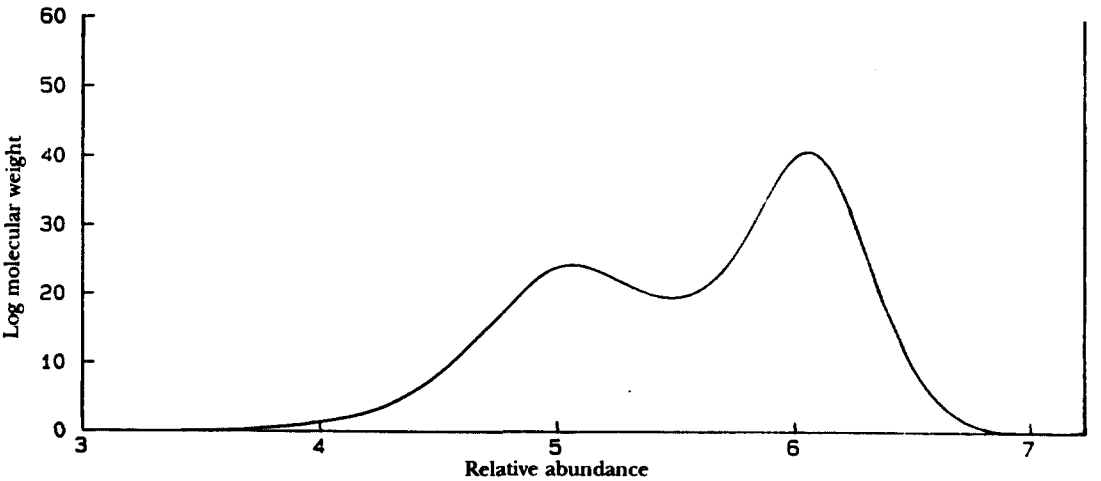

Figure 3. GPC trace of the rubber fraction of *Alstonia angustiloba* latex

Conclusions

A. angustiloba has the potential to produce large quantities of latex at a high rate although since only two tappings per tree were carried out it is not known whether the yields are sustainable. The productivity of the tree seems to depend primarily on size; trees < 100 *cm* girth produced very little latex whereas trees > 140 *cm* girth produced substantial quantities and were stimulated by tapping. Environmental and genetic factors may have a significant effect on yield but the relative importance of the many environmental influences cannot be commented upon. More extensive tapping trials are required to confirm these tentative conclusions.

The latex obtained from *A. angustiloba* comprised about 5-8% rubber, 18-24% resinous, acetone-soluble material, and 2-4% acetone-insoluble solids, the remainder being water. The rubber content of the latex has been identified as 1,4-polyisoprene of greater than 99% *cis* content. The resinous material which forms the bulk of the latex solids has been shown to be a separable mixture of compounds which require further analysis and identification.

Due to the low rubber content of the latex compared to that of *H. brasiliensis*, which generally contains 30 - 40% rubber, it would not be commercially viable to exploit *A. angustiloba* solely for its rubber. If commercially valuable products were to be found in or derived from the other latex components, the possibility exists that cultivation of *A. angustiloba* could be worthwhile. With proper selection and crop management, the yields of latex obtainable could be very high though the immature period required before tapping could commence is as yet unknown.

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