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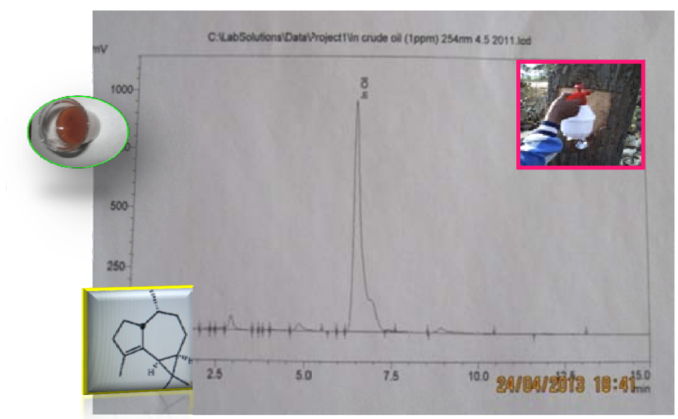
Study on the Production Methods of In-Resin (*Dipterocarpustuberculatus*) and their Chemical properties



Thida Cho, Research Assistant-2

Khin May Lwin, Research Officer

AungSoe, Assistant Research Officer

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အင်ဆီထုတ်နိုင်မှုယူနည်းစနစ်နှင့် ၎င်း၏ ဓါတုဂုဏ်သတ္တိများကိုလေ့လာခြင်း

သီတာချို၊သုတေသနလက်ထောက်−၂

ခင်မေလွင်၊ သုတေသနအရာရှိ

အောင်စိုး၊ လက်ထောက်သုတေသနအရာရှိ

စာတမ်းအကျဉ်း

သစ်တောအတွင်းနေထိုင်သူများသည် အင်ဆီကိုမိရိုးဖလာနည်းများအတိုင်း ထုတ်ယူကြပါသည်။ လက်ရှိအသုံးပြုနေသောမြန်မာနိုင်ငံတွင် အင်ဆီထုတ်ယူနည်းများသည် အထွက်နှုန်းနည်းခြင်း၊ အရည်အသွေးညံ့ခြင်းနှင့် အခန့်မသင့်ပါကအပင်၏ အသားကိုထိခိုက်ပျက်ဆီးစေခြင်းများ ရှိတတ်ပါသည်။ သို့ပါ၍ ဓာတုလှုံ့ဆော်ပစ္စည်း အသုံးပြုသော ပိုမိုကောင်းမွန်သောအစေးထုတ်ယူနည်း(bark-chipped method)ကို သုတေသနတွင် အသုံးပြုထားပါသည်။ ပိုမိုကောင်းမွန်သော အစေးထုတ်ယူနည်းတွင် ဆာလဖျူရစ်အက်ဆစ်ပြင်းအား၁၀%သည် အပင်ကိုထိခိုက်ပျက်ဆီးစေမှု အနည်းဆုံးနှင့် အထွက်နှုန်းများ၍ အရည်အသွေးကောင်းမွန်သော အင်ဆီကိုထုတ်ယူနိုင်ကြောင်း စမ်းသပ်တွေ့ရှိပါသည်။ ထိုအပြင် အစေးထုတ်ယူနည်းနှစ်နည်းဖြင့် ထုတ်ယူထားသော အင်ဆီများ၏ ဓါတုဂုဏ်သတ္တိများကိုstandard wood oil များ၏ ဓါတုဂုဏ်သတ္တိများနှင့်လည်း နှိုင်းယှဉ်ခဲ့ပါသည်။ အင်ဆီများ၏ ဓါတုဂုဏ်သတ္တိများသည်standard Copaiba oil ၏ ဓါတုဂုဏ်သတ္တိများနှင့် တူညီလုနီးပါးရှိသဖြင့် ဆေးဝါးဖော်စပ်ရာတွင်Copaiba oil နေရာတွင် ၎င်းအင်ဆီကို အစားထိုးအသုံးပြုနိုင်ပါသည်။ထို့အပြင်bark-chipped method ဖြင့်ထုတ်ယူထားသော (၁၀%အက်ဆစ်ပြင်းအားနှင့် အက်ဆစ်မပါ)အင်ဆီများတွင် ဓါတုဂုဏ်ဒြပ်ပေါင်းများ ပါဝင်မှုကို HPLC ကိရိယာဖြင့် ဓါတ်ခွဲစမ်းသပ်ခဲ့ပါသည်။ အင်ဆီတွင် အဓိကဒြပ်ပေါင်းနှစ်မျိုးဖြစ်သည့် α-gurjunene နှင့် allo-aromadendrene များပါဝင်မှုကို HPLC နည်းပညာ အားဖြင့် ခွဲခြားနိုင်ခဲ့ပါသည်။ ၎င်း sesquiterpenesဒြပ်ပေါင်းများသည်အင်းဆက်ပိုးမွှားများတိုက်ဖျက်ရာတွင် အရေးပါသော အခန်းကဏ္ဍမှ ပါဝင်နေရုံမျှမက ခြစားမှုကို ကာကွယ်နိုင်ခြင်းနှင့် မှိုဖြစ်ပွါးမှုမှကာကွယ်နိုင်ခြင်း စသည့်ဂုဏ်သတ္တိများကိုလည်းပိုင်ဆိုင်ကြောင်း လေ့လာမှုအများအပြားမှ ဖော်ပြထားပါသည်။

Study on the Production Methods of In-Resin(*Dipterocarpustuberculatus*) and their Chemical properties

Thida Cho
KhinMayLwin
AungSoe

Abstract

In Myanmar, resin (In resin) is harvested traditionally by forest- dependent communities. The present system of resin exploitation by rural people is lower yield, poor quality and sometime destruction of wood. Thus, improved tapping method, the bark-chipped method with chemical stimulant was determined from this research. This method proved 10% sulphuric concentration could be used to produce optimum yield and better quality resin with less damage inflicted on the tree. Then,physio-chemical properties of the crude oil samplesof the two methods of tapping were compared with the standard values of ordinary wood oil. The properties of the oil samples are nearly identical with those of Copaiba oil and suggestion was made that it might be used in medicine as a substitute for Copaiba oil. Moreover, the crude oil harvested by the bark-clipped method (no stimulant and 10% acid concentration) was investigated by High Performance Liquid Chromatography (HPLC). The two major components of the oil, α-gurjunene, allo-aromadendrene were isolated by HPLC.Many investigations suggest that these sesquiterpenes, which occur in many dipterocarps, play an important role in defense against insects and also possesstermiticidal and antifungal properties.

Keyworks: bark-chipped method, physio-chemical properties, standard values, phyto- chemistry, HPLC, sesquiterpenes

Contents

1. Introduction

Resin is a non-timber forest product (NTFP) which is extracted mainly from the *Dipterocarpus*species. The resin from *dipterocarps*may be harvested into two forms: liquid resin which contains resinous fraction and essential fraction (oleoresin), and solid resin (dammar) which is collected from *Hopea*, *Shorea* and *Vatica*.

In tree(*Dipterocarpustuberculatus*)is the principal source of resin known as "In resin" in Myanmar. Commercial productionis through artificial wounding. It is obtained by cutting various shape (depend on collectors) into the trunk. Although it can flows freely from the wound, the tappers required the aid of fire.The fireset for five to ten minutes to stimulate the resin flow. Then, the fire is extinguished. After a few days the resin gathered in the bottom of the hole is collected. This process can be repeated 4 to 9 times per month, mainly in the dry season.TheIntree yields resin throughout the year. Resin exudates simultaneously from several niches on a tree. At the end of the season the dried resin is scraped off and used to make torches.Traditionally it is used for caulking the inside of boats, torches, coating wood as a protection against weather, for water-proofing bamboo baskets, for well bucket, for medicinal and industrial, and other minor purposes. It is also used in paints, lacquers and varnishs (Foppes and Kethpanh 1997) Moreover; it is used in water proofing oil cloth for Myanmar umbrella.

Due to their properties and chemical constituents, resin have gained commercial importance in industry and trade of other countries like Cambodia, Malasia, Vietnam, Laos, Thailand, etc. In Myanmar, In-resin is harvested traditionally by forest- dependent communities. They sell to traders, either at the forest, the tapper's village, or along the road. But, the price is limited depend on the quality of resin. The present system of In-resin exploitation by local people is lower yield, poor quality and environmentally destructive due to the risk of over- tapping. Thus, environmentally friendly improved harvesting techniques, which are sustained production, optimum resin yield, better quality and less destructive to the trees are needed not only to improve socio-economic benefits and income of rural communities but also to conserve environment.

A less brutal method known as the bark-chipped method is less destructive and the yield and oleoresin quality better (Ibrahim et.al 1990).To increase resin production, the bark-chipped method accompanied by application of chemical stimulants has now been used on a large scale in many countries.

The present study employed the bark-chipped method using aqueous sulpuric acid as the chemical stimulant to determine the suitable extraction methods of tapping In (D.tuberculatus)for production of better quality and optimum resin yield.

Further, as the preliminary investigation ofdipterocarps resin, thephysio-chemical properties and novel active chemical principals of resin (In-oil) extracted from *D.tuberculatus*by the bark-chipped method with aqueous sulpuric acid as the chemical stimulant are reported here.

2. Objectives

To evaluate more effective method which is less destructive and the yield and resin quality better

To investigate the chemical properties of"In resin".

To search the active chemical principal from dipterocarpusspecies

3. LiteratureReview

The resin is obtained by a process of tapping.The collection season is November-May and a tree of 2 m girth canyield 9 kg of resin in one season. This resin comparesfavourably with balsam of copaiba (Balfour 1985).Traditionally in Myanmar, resin was obtained bycutting 2-3 deep pyramidal hollows, (the apex pointingtowards the interior of the stem), near the base of thetree and by applying fire to the upper cut surface. The oilwas collected at the bottom of the hollow which wasemptied at 3 or 4 day intervals. Fire was applied everytime the oil was removed and the upper surfaces of thehollow were rechipped 3 or 4 times in a season. About180 kg of resin oil was collected from 20 trees in aseason. The oil was marketed locally in the form oftorches and also exported. Later, tree tapping wasprohibited owing to the heavy damage to the trees (SimmathiriAppanah, Jennifer M. Turnbull.1998).

In Bangladesh, the practice was to cut a deep hollow,(transverse hole pointing downwards), in the tree andplace fired charcoal in it during the night. The oil wasremoved in the morning and the charcoal replaced. Theprocess was repeated until the oil ceased to low. Three,four or more such hollows were made which often killedthe tree. In Burma the charcoal practice was not adopted (SimmathiriAppanah, Jennifer M. Turnbull.1998).

In India, theoil was collected by cutting a hole into the centre of thetree. It is also reported that a large notch was cut into thetrunk of the tree about 75 cm above the ground level, inwhich fire was maintained until the wound was charredand the liquid began to ooze out. A small gutter was cutinto the wood to a vessel attached to receive the oil. Theaverage yield from the best trees was 180 litresperseason. At 3 or 4 week intervals the old charred surfacewas cut off and burnt afresh. Tapping occurred fromNovember to February and sick trees were rested for 1or 2 years (SimmathiriAppanah, Jennifer M. Turnbull.1998).

Studies suggest this approach is similar across Cambodia, Vietnnum, Laos and Thailand (Evans et.al 2003, Juiprik and Kijtwachakul 2001, Ankarfjard and Kegl 1998).Literature related to tapping oleoresin unanimously claim that it can go on for a very long time (50-80)years, without killing the trees (Crevost 1926,Enfield 1998, Foppes and Kethpanh 1997, Gianno 1990, Smitinand 1989). Foston (1935), on the basis of having examined two*D.Kerrii*, concluded that the tapping may not be as damaging as originally thought.Only a handful of references have been found describing this activity (Crevost 1926, Gianno 1990, Howes 1949, Smitinand el.al 1990).

But, Renee Ankarfjard(2000),concluded that many authors seem to rely on information obtained from local people. Foxworthy (1922) and Burkill (1935) criticizes the technique used as crude which resulted in the destruction of some wood. The burnt wood around the 18 deep tapped areas would certainly affect the growth of the tree. Renee Ankarfjard (2000) assessed the impact of tapping on the physical condition of the tapped trees. Tapping holes can cause a reduce strength in the trunk against the wind.Moir (1981) indicated that the traditional procedure of chopping a hole in the trunk where the oleoresin will accumulate and the use of firing to stimulate further oleoresin flow could be improved. Also the yield and quality of oleoresin is poor. Environmentally friendly improved collection techniques especially for stimulating oleoresin exudation are needed to provide sustained production and income.

Thus, the present system of resin exploitation by local people is generally unsustainable and damaging to the trees. The old method of tappingis by notching a hole in the trunk and blazing to stimulatefurther resin flow. This is repeated at about weeklyintervals and the yield per tree is 150 to 280 ml pertapping (Gianno 1986). A less brutal method has beendeveloped, known as the barkchipped methodaccompanied by application of chemical stimulants,which is less destructive and the yield and resinquality better (Ibrahim *et al.* 1990).

Among the chemicals, aqueous sulphuric acid is themost widely used.Riyanto (1980),concluded that the stimulation might be due to:

- the hydrolysation of the sap channel and substantial drop in the wallpressure, resulting in a sap secretion above normal;

- the hydrolysation of the parenchymal cells, resulting in the secretion ofcell fluids which will be absorbed by the sap; the quantity of watery sapincreases more and more and is secreted above normal;
- the buffering effect of the acids, preventing the sap to form its cyclic chain,thus maintaining its form as an aldehyde; so the sap continues to be wateryand is secreted continuously above normal.

However, other researchers believe that ethylene produced endogenously by plant tissues in response to injury (acid treatment) plays a dominant role in the formation of exudates (Abeles 1973, Hillis 1975).

The oleoresin isprocessed to separate the essential oil from the resin.The essential oil, known commercially as gurjan balsam,is used as a fixative or a base in perfume preparationsand occasionally as an adulterant of patchouli and copaibabalsam oils. Traditionally the oleoresin is used forcaulking the inside of boats, coating wood as a protectionagainst weather, in torches, and for medicinal purposes.The oil is also used to make varnishes in backyardindustries (Burkill 1935).

There is some industrial use for ink, paints and wax production, and in producing balsam oil as a perfume base. It is also used in traditional medicine to protect feet against the effects of working long hours in rice paddy water. Locally this oleoresin is mixed with sawdust from soft woods or rotten wood and used to produce torches(www. nafri.orgla/document/ntfphandbook/gng/76-349-325pdf).

While the biggest suppliersof gurjan balsam oil are Indonesia, Malaysia and Thailand,limited quantities are produced in India and thePhilippines. Sumatra is the biggest producer of all, andin 1984 it produced about 20 tonnes of the oil (Lawrence1985). The oil is now becoming scarce with anincreasing demand, resulting in increased prices. Theprice is currently over US $30 per four gallon tin. Theoleoresin is mainly collected by natives and aboriginesand has a ready market in Singapore where it is exported to Europe (SimmathiriAppanah, Jennifer M. Turnbull.1998).

Literature survey suggested that dipterocarp resins might containbiologically active molecules.Dipterocarp resins contain a large and complex array ofchemical constituents (Hegnauer, 1966; Bisset et al., 1971; Ashton, 1982; Diaz et al., 1966),many of which are terpenes. wood resins are thought to be important defensiveagents (Langenheim, 1990). Chemical factors in resins may also protect dipterocarps from microbialattack. Though they contain defensive chemicals, which presumably protect against biological attack, (Richardson et al.,1989), massive defoliations of dipterocarp trees by unidentified Lepidoptera have been reported(Anderson, 1961).

Toxic and inhibitory effects of some of the chemicals found in dipterocarp resins (β-caryophyllene, and caryophyllene oxide for example) have been demonstrated for the generalist herbivores Spodopteraexiaua (Lepidoptera: Noctuidae) (Langenheim et a 1980) and Heliothisvirescen$ (Gunasenaetal., 1988), as well as for leafcutting ants (Hubbell ., 1983; Howard etaj.., 1988). Such toxic chemicals might have been a factor in causing the mortality of. **,** cerigoides larvae in laboratory culture; other lymantriids cultured on toxic host plants (castor, Ricinuscommunis L.) in the laboratory showed substantial (>60%) mortality by the fifth instar (Islam &Joarder, 1983).Dipterocarp timbers themselveshave been shown resistant to biological attack (Sen-Sarma, 1963; Bakshi et al.,1967; Sen-

Sarma and Chatterjee, 1968; Balasundaran and Gnanharan, 1986).Untreated dipterocarp timbers are reported to be highly resistant to fungalinvasion (Bakshi et al., 1967).

4. Materials and Methods

TwentyIn (*D.tuberculatus)*trees, located inthe Nga Oh reserved forest, MabainTownship, northern Shan State were chosen for resin production. Before starting the resin production, measurement of girth at breast height was taken and weeding out around the tree in a six foot circle was done in preparation for the tapping. Among twenty In(*D.tuberculatus*)trees, ten trees at least 40-50cmin diameterat breast height were selected for two methods of tapping. Then, resin of In (*D.tuberculatus*) was collected by using the traditional method and the bark-chipped method with various concentration of aqueous sulfuric acid.

3.1 Traditional tapping method

Five trees were selected from the Nga Oh reserved forestMabainTownship, northern Shan State, for the traditional tapping method. Firstly, a hole was made in the trunkof the tree by using axe as shown in figure (1).After it had been made, the injury stimulated a slow flow of resin which was collected in the bottom of the hole for about five days. Then, the surface of the hole was burned for five to ten minutes after which the resin runs for five days. This process was repeated three times; briefly burning the tap and collecting the resin. At the end of the fifteen days the surface of the niche was cut afresh in order to clear away the charcoal produced by burning and the niche enlarged. After the resin had run for five days, the surface burned briefly again and the original process repeated. Moreover, the amount of resin collected per tree per tapping was recorded to compare with the bark-chipped method.

3.2 Bark-chipped method

Like traditional tapping method, other five trees (I,II,III,IV and V) were chosen from the Nya Oh reserved forest for In-resin production. In the bark-chipped method with a chemical spray as a stimulant, the tapping area was marked near the foot of the tree. Then, to get a smooth surface all the barksof the tree including inner barkswere removed using a tapping knife. At the bottom of the tapped area, a plastic container was attachedas the collector by using half inch nails. Various concentrations of aqueoussulphuric acid (2.5, 5,10,15 and25)were sprayed on the exposed wood surface using a sprayer, shown in figure (2). For each concentration of acid, the experiment was done in triplicates. A control experiment was done by following the similar tapping method but without chemical stimulant. The acid was sprayed on the freshly cut surface. After resin had run for five days, it was collected and the amount was recorded to compare with the traditional tapping method.

3.3 Separation of crude oil

The crude oil fractionsof In-resin obtained by two methods of tapping were separated from the resinous fractions by dilution with petroleum ether and centrifugation. The upper layer was evaporated to give clear oil. Upon standing at room temperature for a few days, the crude oil obtained by bark-chipped method turned lightbrownish yellowand the crude oil obtained by traditional method turned thickviscid red dishbrown. Due to the appearance of color, chemical spray method gives better quality. Thus, only In-resin, which is harvested by the bark-clipped method investigated emphatically for this research.

3.4 Determination of physico-chemical properties of crude oil

The physico-chemical properties such as color, specific gravity, saponification valueandacid value of the crude oil samples that obtained from the above two methods of tapping were tested as highlighted below. After the testing the physio-chemical properties of each were compared with the standard wood oil, garjam and copaiba balsam.

3.4.1 Determination of the Saponification Value

For the determination of the Saponification Values of the crude oil samples, An American Standard for Testing Material (ASTM) method was used. 3g of the crude oil was weighed into a200ml conical flask. 50ml of 0.5M ethanolic KOH solution was added. The mixture in the flask was heated on a water bath for 30 minutes. The resulting solution was titrated against 0.5N standardHCl, using phenolphthalein as indicator. The end point was obtained when the pink color changed into colorless. The same procedure was used for the blank determination.

Calculation;

Saponification value = 56.1N (X-V)/ W

Where, X– ml of HCl required by blank

V – ml of HCl required by sample

W – Weight of sample

N – Normality of HCl

56.1– Molar mass of KOH

3.4.2Determination of acid value

Acid values of the oils were determined by ASTM method. 10gs of sample was weighed into 250ml conical flask. 50ml of neutralized ethyl alcohol was added. The mixture was heated on a water bath to dissolve the sample. The solution was titrated against 0.1M KOH using phenolphthalein as indicator.

Calculation;

Acid Value = V x M x 56.1/W

Where, V = ml of 0.1M KOH consumed by sample

M = Morality of KOH

W = weight in grams of the sample

3.4.3 Determination of Specific Gravity

The sample is filtered with filter paper to remove any impurities. The sample is poured into the specific gravity bottle until overflowing. Then, the stopper is inserted. The bottle containing the sample is allowed to remain in a water bath at 60□± 0.2□C for 30 minutes. Any oil which has come through from the capillary opening is wiped off and removed from the bath, cleaned and dried thoroughly. The bottle and its contents are weighted and specific gravity is calculated.

$$\text{Specific gravity at } 60\square\ /\ 30\square\ C = \frac{wt.of\ bottle\ and\ sample - wt.of\ bottle}{wt.of\ water\ at\ 30°C}$$

3.5 Analysis of the crude oil extract by HPLC

3.5.1 Preparation of standards

Each of the stock solutions, α-gurjunene andallo-aromadendrenewere dissolved in mobile phase as 0.5mg/ml concentrations. The solutions were filtered through a 0.45 μm membrane filter. Standards are prepared freshly and immediately injected to HPLC column.

3.5.2 Preparation of Sample Solution

The 5g crude oilsample harvested by the bark-clipped method (10% acid concentration) wasextracted withdichloromethane. Then, 5 mg of the concentratedextractwas weighed and transferred into 10 ml volumetric flask anddissolved in 10 ml mobile phase, to prepare0.5 mg/ml sample solution. Then, it was filtered with 0.45 μmmembrane filter paper. Like that 5g crude oil sample harvested by the bark-clipped method without chemical stimulant was taken from that 10ml of samplesolution was prepared.Then, it was filtered with 0.45 μmmembrane filter paper.

3.5.3 Separation of compounds

High Performance Liquid chromatography (HPLC) analysis of the *D.tuberculatus* crude oil which is harvested by the bark-clipped method was carried outShimadzu HPLC model LC-20A Series equipped with a LC-20AT solved delivery module, DGU-20A on-line Degasser and shimadzu column oven CTO-20A using LC system column. The column was eluted with water-methanol at a flow rate of 0.8 ml/min. Ultraviolet detection was performed at 254mn with a shimadzu multi-function ultra-violet visible spectrophotometroric detector SPD-20A. The amount of the sample injected into the column is 20µl.All separations wereperformed at the temperature of 25°C. Identification of thesesquiterpenes was carried out by comparingtheirretention times with known standards (α-gurjunene and allo-aromadendrene).

4. Results and Discussion

The average In-resin yield obtained using the two harvesting methods are described in table1.

It is found that the average amount of In-resin yield obtainedusing the bark chipped method with sulphuric acid as stimulant is greaterthan that obtained using the traditional method. Many investigations have shown that the bark chipped method accompanied by application of chemical stimulant produce higherresin yield.

In-resin yield per tapping of chemical stimulation methodare described in table 2.

The results show that acid treatment stimulated resin exudation fromIn tree(*D.tuberculatus*). Application of aqueoussulphuric acid solution of various concentrations gave variety of resin yields. Without the stimulant, the resin flow was negligible. The resin yield increased sharply as the concentration of acid was increased up to 10 % concentration. At this concentration the yield reached its maximum level. The resin yield started to decrease at acid concentration greater than 10 % (15 or 25 % acid concentration). According to the bar graph, as illustrated in figure (5), there were relationships between resin yield and different strength of aqueous sulphuric acid for all trees. Thus, the results might indicate that the use of 10 % concentration was the best.

5.1 The physio-chemical properties

For comparison the standard values of ordinary wood oils and the values of the crude oil samplesof the two methods of tapping are described in table 3.

From the results it is clear that the properties of the crude oil samples are nearly identical with those of Copaiba oil. Thus, the crude oil might be used in medicine as a substitute for Copaiba oil.

5.2 Analysis of the crude oil extract

Qualitative analysis of the In crude oildichloromethane extract obtained by the bark chipped method with sulphuric acid were carried out using HPLC and the chromatographic profiles were compared with the retention times of reference standards. HPLC chromatogram of the crude oil sample obtained by using the bark chipped method with sulphuric acid (10%concentration) showed the presence of (4) compounds. Major compounds isolated by HPLC were identified as α-gurjunene andallo-aromadendrene respectively by comparing with those of standard samples. In addition there were very small amount of two other unidentified compounds. HPLC chromatogram of the crude oil sample obtained by using the bark chipped method is shown in figure (7).

Moreover, that chromatogram was nearly similar to the chromatogram of the crude oil obtained when no stimulant was applied. Thus, there is no change in composition of the crude oil when 10% aqueous sulfuric acid concentration is used. HPLC chromatogram of the crude oil without stimulant is exhibited in figure (6).

5. Conclusion

The present study demonstrated that the bark-chipped method with aqueous sulphuric acid solution as a stimulant give optimum yield, better quality and less damage inflicted on the tree than traditional tapping method. Among the different strength of acid, the use of 10% acid concentration is the best. Thus, bark-chipped method is a suitable harvesting technique for environmental conservation.This improved harvesting method will aid socioeconomic development of forest-relient communities and will also provide useful information for commercial exploitation of resin and sustainable harvesting. In addition some other aspects of the studies which should be looked into the influence of diameter size of tree, the influence of weather, the influence of age and site on resin yield and the use of other chemical stimulant.

According to the physio-chemical properties, the crude oil of *D.tuberculatus* may possibly be used in medicine as substitutes for Copaiba oil..HPLC chromatogram of the crude oil sample obtained by using the bark chipped method with sulphuric acid (10%concentration) showed the presence of α-gurjunene as major component, small quantities allo-aromadendrene and two other unidentified sesquiterpenes. Moreover, that chromatogram was nearly similar to the chromatogram of the crude oil obtained when no stimulant was applied. Thus, there is no change in composition of the crude oil when 10% aqueous sulfuric acid concentration is used.Many investigations suggest that these sesquiterpenes are responsible for the resin's termiticidal and antifungal activity and play an important role in defense against insects.

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Appendix 1

Table1. Comparing the average In-resin yield

Sr.No	Methods	Average amount of resin (collected per tree per tapping)
1	Traditional	50- 160g
2	Bark-chipped (10%Acid Con.)	74 - 241 g

Table3. Comparing the standard values of ordinary wood oils with the values of the crude oil samples obtained two methods of tapping.

Properties	bark-chipped method	traditional tapping method	Garjan	Copaiba
Acid value	17.67	17.62	5.0-10.0	33.1-81.5
Saponification value	43.95	45.18	10.0-20.0	49.0-94.3
Specific gravity	0.9587	0.9985	0.915 - 0.931	0.916 - 0.993
Color	brownish yellow	viscid reddish brown	Pale white - black	brownish-yellow

Table2.Resin yield per tapping of chemical stimulation method.

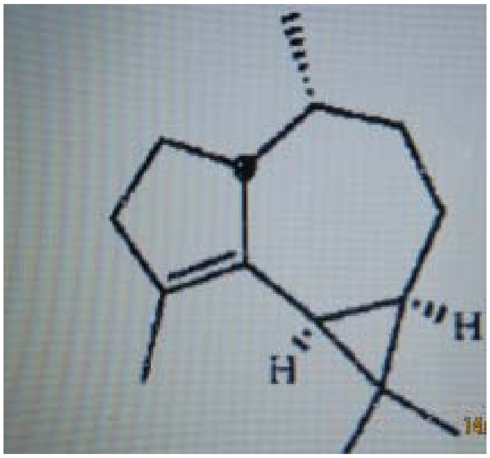
Trees	Acid concentration (%)	Production (g)			Average (g)
		1st time	2nd time	3rd time	
I	0	4.3	2.1	1.5	1.98
	2.5	101	102.5	117	80.75
	5	210	224.5	227	166.63
	10	308	260	271	212.25
	15	175	220	202	153.00
	25	160	160	180.5	131.38
II	0	3.5	4.5	4.5	3.13
	2.5	90.2	110	98.5	75.30
	5	210	200	184.8	149.95

	10	340	300	317.5	241.88
	15	200	193.2	213.4	155.40
	25	150.5	155	132.8	115.83
III	0	1.7	3.5	6.2	2.85
	2.5	42	50	40	33.63
	5	92	92	85	68.50
	10	200	180	165.5	138.88
	15	114.5	120	140	97.38
	25	190	170.7	165	137.68
IV	0	1.5	2.5	0.7	1.18
	2.5	30.5	25	25	20.75
	5	45	50.5	55	38.88
	10	90.5	98	95.2	73.43
	15	50.5	65	45	43.88
	25	65	75.5	80.6	61.53
V	0	0.5	5.4	6	2.98
	2.5	80.5	112	95.5	72.63
	5	212	190	200	151.75
	10	299	310	305.6	231.15
	15	210	210	199.5	158.63

Appendix 2

Figure1. Tapping of resin from In (*D.tuberculatus*) using Traditional tapping method

Figure 2.Tapping of resin from In (*Dipterocarpuskerrii*) using the bark-chipped method withaqueous sulphuric acid solution as stimulant



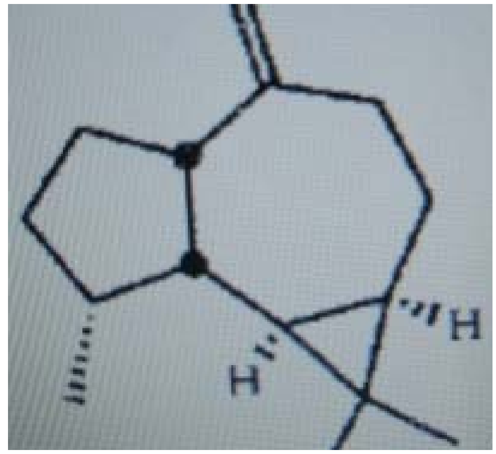


Figure 3. α -guriurene

Figure4. allo-aromadenrene

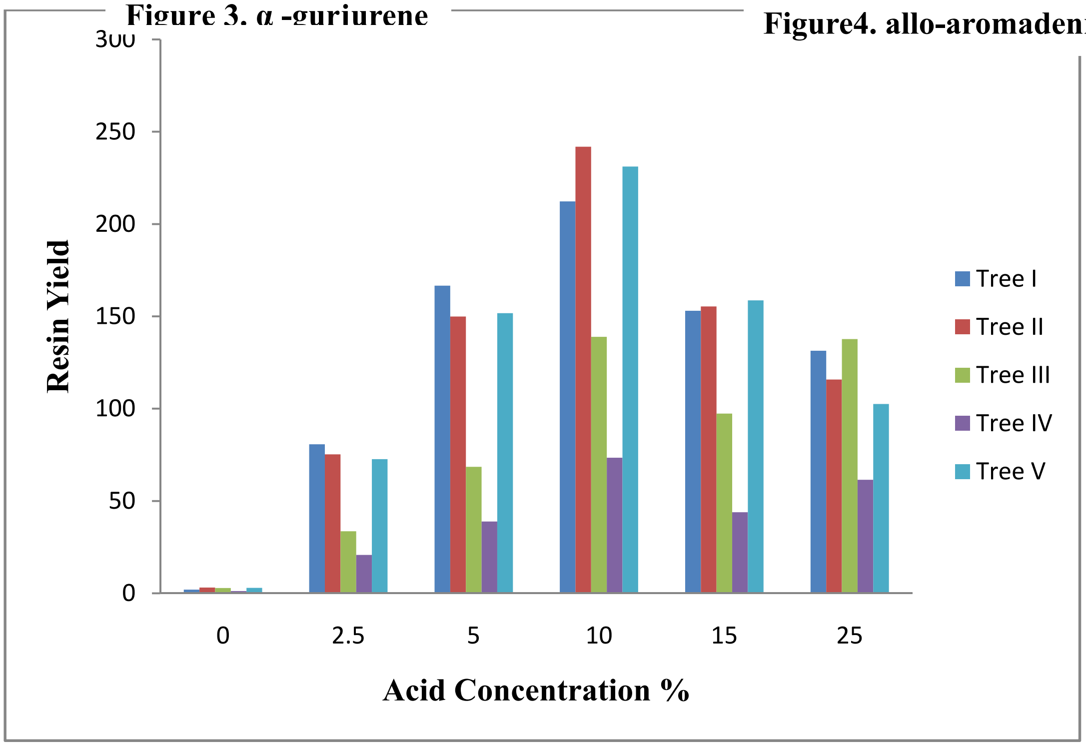


Figure5. Comparism of acid concentration and resin yield

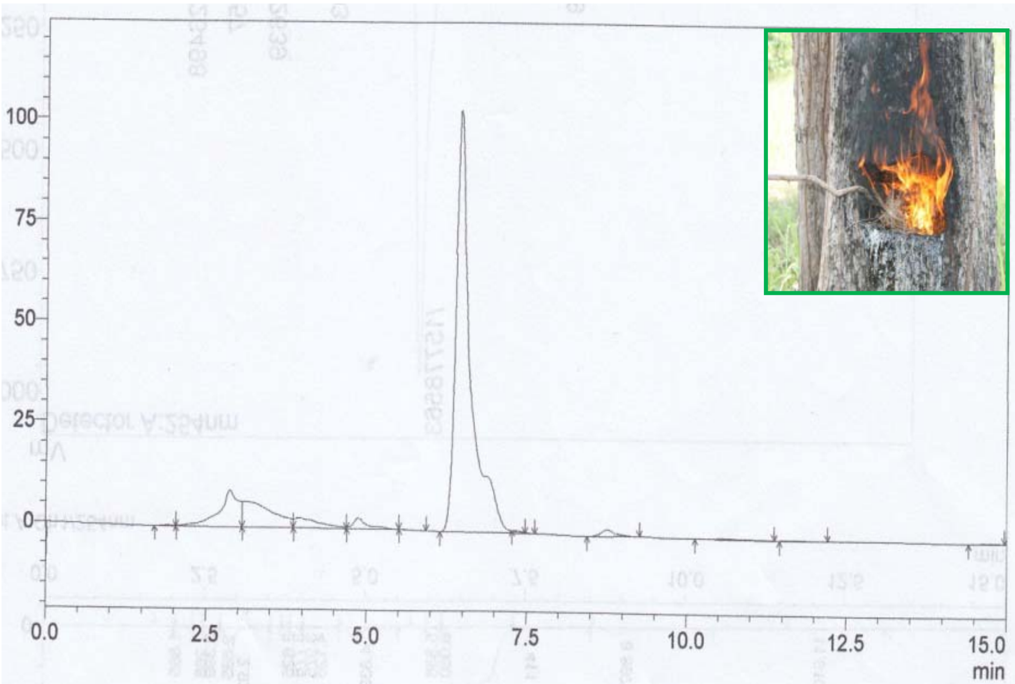


Fig (6) Chromatogram of the crude oil obtained when no stimulant was applied

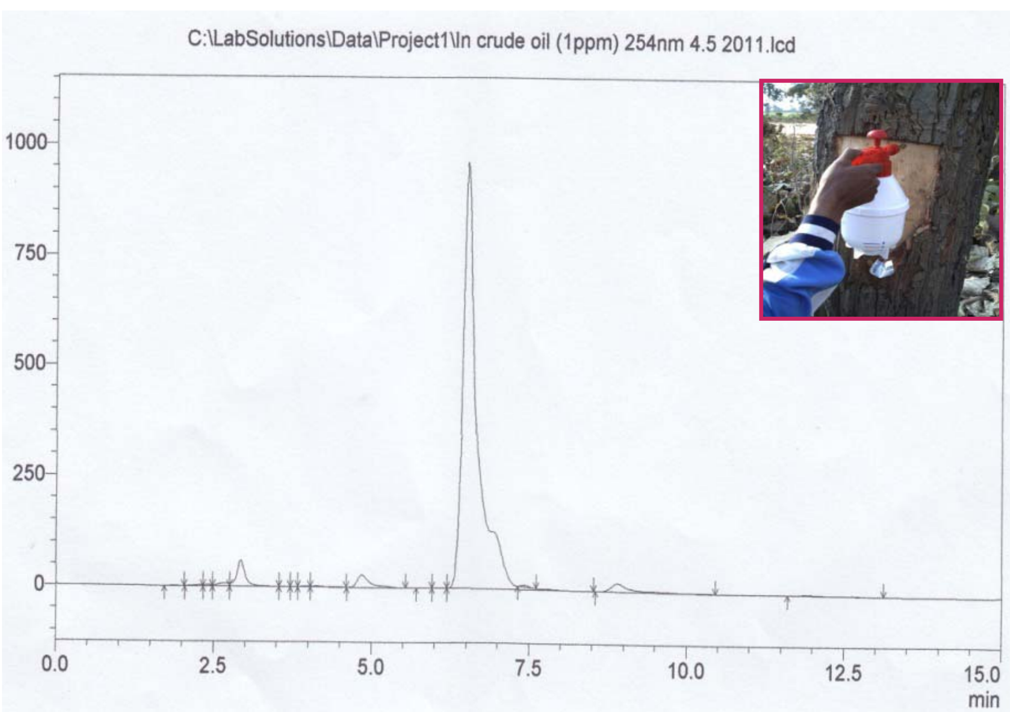


Fig (7) Chromatogram of the crude oil obtained by using the bark chipped method