



Research article

Geographical variations and correlation among some chemical and thermal properties of Almaciga (*Agathis philippinensis* Warb.) resins from selected commercial sites in the PhilippinesRamon A. Razal^a, Aileen A. Jara^a, Genne Patt O. Samar^{a,*}, Ralph Lauren M. Alomia^b,
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ABSTRACT

Almaciga (*Agathis philippinensis* Warb.), a tropical conifer that is tapped for its resin commercially known as Manila copal, grows in many parts of the Philippines, but resin buyers prefer and pay a better price for resin from southern Palawan. The study was done to characterize almaciga resins obtained from commercial sites in Davao Oriental and Palawan (Brooke's Point and Marufinas) to explain the quality and price differences. Fresh and aged resin samples were subjected to wet chemical, thermal, and spectral analyses. Generally, the resin samples were found to be soluble in the more polar solvents although significant variations were observed for resins from various sites. Titrimetric determination revealed no significant variation in acid and saponification values, although the Brooke's Point resin had significantly lower unsaponifiable components. Predominant Fourier transform infrared spectroscopy (FTIR) absorption peaks for fresh resin include strong C=O stretch and weak =C-H stretch. Aged Palawan resins, which showed increased solubility in polar solvents, exhibited increased intensity of prominent FTIR peaks such as O-H stretch and C=O stretch. The weak peak at 1719 cm⁻¹ seen in fresh Palawan resins was no longer observed in aged resins. DSC revealed the semi-crystalline nature of almaciga resin and melting temperatures similar to diterpenoid resin acids. This is attributed to the presence of increasing amounts of oxidized abietic acids as shown by gas chromatography-mass spectrometry (GCMS). For the Davao Oriental resins, significant amount of agatholic acid was shown, while resin acids of the abietane and pimarane type were conspicuously absent in the GC-MS spectrograms. The study found evidence that almaciga resins from Davao and Palawan differ in chemical composition and physical properties, which could explain the quality and price differences.

1. Introduction

Manila copal, the resin from the tropical conifer almaciga (*Agathis philippinensis* Warb.), has been a valuable income source for indigenous peoples in the Philippines (Connelly, 1985; Langenheim, 2003). Almaciga trees are found growing at an altitude of 150–2000 m above sea level, although the best development is attained on well-drained slopes at 600–1500 m above sea level (Gonzales and Abejo, 1978). The resinous exudate can be obtained by tapping a living almaciga tree of a certain size, or by digging the solidified resin from under the ground where an almaciga tree once stood (Gonzales and Abejo, 1978; Coppen, 1995). Good tapping practices include diameter-based selection of trees, tapping

without damage to the cambium, spraying the cut portion with sulfuric acid to stimulate resin flow, and periodic tapping for sustained resin production (Ella and Tongacan, 1992). The soft, resinous exudate from fresh injuries on the almaciga bark hardens over time (Gonzales and Abejo, 1978) as reactive and volatile components escape or combine to form stable species and higher molecular mass compounds (Pagacz et al., 2019).

According to Coppen (1995), the Philippines is the second largest producer of copal next to Indonesia, with an average export of 350 tons per annum from 1988–93. But in 2018, only 204 thousand kilograms of almaciga resin was reported, the second lowest production volume over a 20-year period (FMB-DENR, 2018). Competition with petroleum-based

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synthetics had caused a decline in the demand for natural resin (Whitmore, 1980; Coppin, 1995; Langenheim, 2003). High reliance on imported materials by processing industries also limited the development of raw almaciga resin into various products (Quintos et al., 2012). However, increasing domestic demand for almaciga resins and rising global consumption of green products could lead to considerable industry growth (Razal et al., 2013).

The chemistry of almaciga resin has not been studied much except by a few investigators. Lassak and Brophy (2013) found that limonene comprised at most 72.4% of the steam volatile components of almaciga resin from the Philippines. Other components found in significant amounts were α -pinene and α -thujene (10.4%), α -terpineol (6.2%), and β -pinene (1.5%). In Australia, fewer components were found in a related species, *Agathis atropurpurea* (Garrison et al., 2016). Limonene is the main essential oil in *A. atropurpurea* resin, accounting for almost 90% of the total volatiles present. While the volatile fraction of almaciga resin had been characterized (Lassak and Brophy, 2013), the composition of the larger, non-volatile fraction remains largely unexplored.

Fourier transform infrared spectroscopy (FTIR) was recently utilized to study resin ageing, which showed the transformation of abietic acid to its oxidized forms with time (Beltran et al., 2016). Likewise, FTIR analysis revealed significant absorption patterns, including a broad O–H stretch, strong C=O stretch, and weak C=C vibrations that could be used to distinguish resins from oils, carbohydrates, and waxes (Edwards and Farwell, 1996; Katsibiri and Howe, 2010; Quintos et al., 2012; Martin-Ramos et al., 2018). Meanwhile, differential scanning calorimetry was used to illustrate the transformation of resins between two different states, which is dependent on their composition and structure (Zhang et al., 2012). Some studies that used this method showed the softening point of copal resin to vary from 79 to 117 °C (Fiebach and Grimm, 2000; Morkhade et al., 2006; Umekar and Yeole, 2008; Adalina and Sawitri, 2020) and a melting point range of 115–135 °C for a variety of *Agathis* resins (Boer and Ella, 2000; Adalina and Sawitri, 2020).

Almaciga resin traders in the Philippines prefer those from southern Palawan, which command a higher price than from other parts of the country, including central Palawan. Manufacturers consider Southern Palawan resins to be of superior quality and easier to process. However, it is not exactly known why the Southern Palawan resin is more amenable to processing than the almaciga resin from other locations. According to Gonzales and Abejo (1978), the resin source could be a factor affecting the resin quality. This was supported by Coppin (1995) who stated that the quality of copal is highly variable as it depends on the source as well as the manner of collection. Likewise, Tavita and Palanginan (1998) concurred that resin quality can be influenced by the physical properties and chemical composition, which could vary according to the source and the type of resin. Thus, this study was done to characterize almaciga resin obtained from known commercial resin tapping sites in the country. The results will help establish the scientific basis for the quality and price differences. Furthermore, elucidation of the chemical characteristics of the resin will lead to improved downstream processing and product development.

2. Methodology

2.1. Sample collection and preparation

The almaciga resin tapping was done in three commercial resin tapping sites in the Philippines: a) Davao Oriental in June 2019, b) Brooke's Point, in southern Palawan in September 2019, and from c) Marufinas, Puerto Princesa City, central Palawan in November 2019. The fresh resins were collected within two days from tapping, while the aged resins were taken three months from the time of tapping. The sites are geographically distant and remote, so the resin samples could not be collected at exactly the same time. This is one limitation of the study.

The fresh samples were kept in a sealed vial and immediately stored in a refrigerator to maintain their freshness and integrity. The fresh resin remained sticky even after refrigeration and was not amenable to any physical manipulation prior to analysis. The aged resin, which hardened within three months of exposure to the environment, was kept at room temperature after collection, and then pulverized for easier homogenization for the various chemical tests.

2.2. Analysis of resin quality

The following physicochemical properties of the resin were determined: solubility in 95% ethanol, solubility in other organic solvents, acid value and saponification value. In addition, resin analysis via Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) were also performed.

2.2.1. Solubility in 95% ethanol

The method by Gonzales and Abejo (1978) with modification, was employed in triplicate measurement. About 0.1 g of fresh/hardened resin sample was placed in a screw-capped vial to which 2 mL of 95% ethanol was added. The suspension was shaken for 4 h at room temperature and was allowed to stand overnight. The resulting mixture was filtered through a tared fritted crucible and was oven-dried at 105 °C for 2 h. The material insoluble in 95% ethanol was the residue retained in the crucible, which was taken as the difference in mass before and after filtration. The percent ethanol-insoluble material (EIM) and percent ethanol-soluble material (ESM) were calculated using Eqs. (1) and (2), respectively:

$$\%EIM = \frac{m_{\text{crucible+residue}} - m_{\text{crucible}}}{m_{\text{sample}}} \times 100 \quad (1)$$

$$\%ESM = 100 - \%EIM \quad (2)$$

2.2.2. Solubility in other organic solvents

The solubility of the resin in the other organic solvents such as hexanes, dichloromethane, ethyl acetate, isopropanol, and lacquer thinner, was determined by employing the same method in determining the ethanol-soluble material. Determination of solubility in each of the solvents was done in triplicate measurement for each resin sample.

2.2.3. Acid value determination

The method reported by Gonzales and Abejo (1978) with some modification, was employed. About 0.10 g of sample was dissolved in 10 mL of 4:1 (v/v) 95% ethanol:hexanes in a 50 mL Erlenmeyer flask and was allowed to stand for a few minutes. The resulting solution was titrated with standardized 0.1 N solution of ethanolic KOH. The acid value was calculated using Eq. (3) below:

$$\text{Acid Value} \left(\frac{\text{mg KOH}}{\text{g resin}} \right) = \frac{\text{mL(KOH)} \times \text{N(KOH)} \times 56.1}{\text{g resin sample}} \quad (3)$$

The determination was done in triplicate measurement for each resin sample.

2.2.4. Determination of saponification value

The method reported by Gonzales and Abejo (1978) was also employed. About 0.25 g of sample was dissolved in 25 mL of 4:1 (v/v) 95% ethanol:hexanes in a 50 mL Erlenmeyer flask. Ten mL of 0.5 N ethanolic KOH was added to the resulting solution, which was refluxed in a boiling water bath for one hour. The solution was cooled and titrated with standardized 0.3 N H₂SO₄ solution. The saponification value was calculated using Eq. (4) below:

$$\text{Saponification Value} \left(\frac{\text{mg KOH}}{\text{g resin}} \right) = \frac{(\text{mL } H_2SO_4(\text{blank}) - \text{mL } H_2SO_4(\text{sample})) \times N(H_2SO_4) \times 56.1}{\text{g resin sample}} \quad (4)$$

The determination was done in triplicate measurement for each resin sample.

2.2.5. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrum of each resin sample was obtained using Nicolet 6700 Fourier Transform Infrared Spectrometer with wavenumber ranging from 4000 to 500 cm^{-1} to determine chemical differences among the resins from different sites as well as to compare fresh and hardened almaciga resin samples. The functional groups present in each resin sample were determined using this technique.

2.2.6. Differential scanning calorimetry (DSC)

The melting profile of 10 mg resin samples were determined using TA Instruments Q20 differential scanning calorimeter in the UPLB Nanotechnology Laboratory. The heat flow (W/g) against temperature ranging from 30 - 250 $^{\circ}\text{C}$ was plotted for each resin sample. The determination was done in duplicate measurement for each resin sample.

2.3. Analysis of the components of almaciga resin

The chemical composition of fresh almaciga resin was analyzed through tandem gas chromatography-mass spectrometry (GC-MS) of the esterified resin components using a QP2010ULTRA instrument (Shimadzu; Kyoto, Japan) with RTX-5 column of 30 m \times 0.25 mm ID \times 0.25 μm . About 25 mg of resin was weighed into a 50 mL round bottom flask and 2.5 mL BF_3 in methanol solution was added. The mixture was then refluxed on a steam bath for 3 h. Thereafter, water and 1 mL of isoctane were added, and the resulting mixture was swirled. This was transferred to a 50 mL volumetric flask, and more water was added until the mixture reached the neck of the flask. The isoctane layer was transferred to a vial and was subjected to GC-MS analysis. The parameters used for GC-MS were as follows: electron ionization (EI) source at 70 eV, column oven temperature of 80.0 $^{\circ}\text{C}$, injection temperature of 280.0 $^{\circ}\text{C}$, split injection mode, linear velocity flow control mode, pressure of 93.5 kPa, total flow of 71.8 mL/min, column flow of 1.37 mL/min, linear velocity of 43.1 cm/s, purge flow of 2.0 mL/s, split ratio of 50.0.

2.4. Statistical analysis

Data obtained from solubility tests, acid and saponification values were analyzed via statistical means using GraphPad Prism v8.0 (GraphPad Software Inc., CA, USA) where one-way ANOVA followed by Tukey's multiple range test at 95% confidence level ($p < 0.05$) was performed. Data were expressed as mean \pm standard deviation.

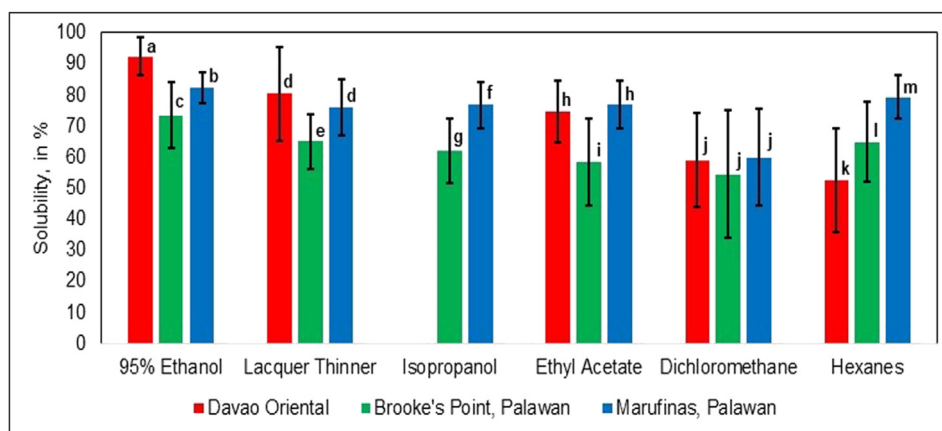
3. Results and discussion

3.1. Solubility in various organic solvents

Both fresh and aged resins were subjected to solubility tests in 95% ethanol, which is a routine test used for the quick determination of the quality of almaciga resin, and in other organic solvents such as ethyl acetate, dichloromethane, isopropanol, hexanes, and lacquer thinner. We found the resins to be most soluble in ethanol, followed by isopropanol and lacquer thinner. They were least soluble in dichloromethane and hexanes, as shown in Figures 1 and 2.

Significant variations in the solubility of the fresh and aged resin samples from the various sites were observed. Among the fresh resin samples, the Davao Oriental samples were the most soluble in 95% ethanol. The Marufinas samples were the most soluble in slightly polar to nonpolar solvents like ethyl acetate, dichloromethane, and hexanes. Among the aged resin samples, the resin from Marufinas had the highest solubility in all solvents except ethyl acetate. Overall, no significant difference was observed in the solubility in dichloromethane of fresh almaciga resin samples and in ethyl acetate for aged samples from the various sites. Increase in solubility in 95% ethanol upon ageing was observed in most samples except for the aged resin from Davao Oriental. The fresh resin samples from this site had higher solubility in 95% ethanol and lacquer thinner than the aged resin samples.

For the resin samples from Brooke's Point, Palawan, both the fresh and aged samples were most soluble in 95% ethanol and in lacquer thinner, while fresh and aged samples from Marufinas, Puerto Princesa City were most soluble in 95% ethanol only. Other solvents that dissolved the fresh samples from Marufinas were hexanes and ethyl acetate, but the



*Mean values with different letters indicate significant differences at $p < 0.05$.

Figure 1. Percent solubility of fresh resin samples of *A. philippinensis* collected from various sites. Mean values with different letters indicate significant differences at $p < 0.05$. Solubility of fresh resin from Davao Oriental in isopropanol was not performed due to low 2-day-old resin yield.

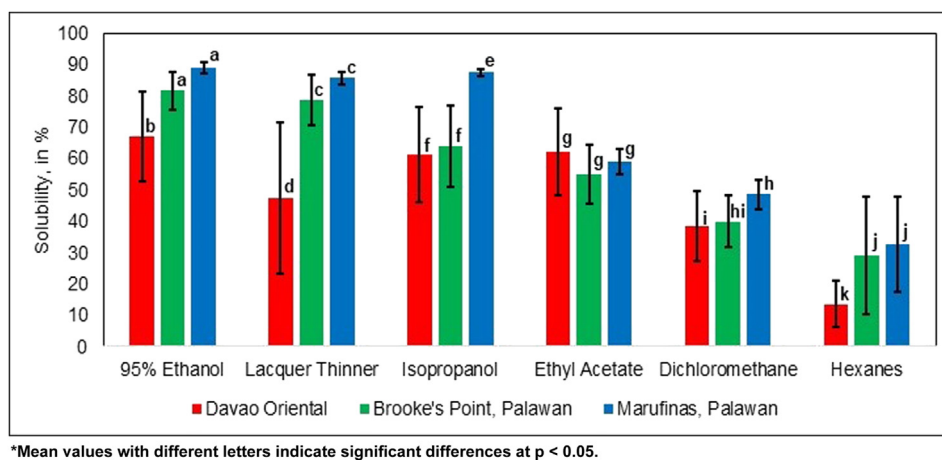


Figure 2. Percent solubility of aged resin samples of *A. philippinensis* collected from various sites. Mean values with different letters indicate significant differences at $p < 0.05$.

aged resin was better dissolved in isopropanol and lacquer thinner. Ethanol, isopropanol, and lacquer thinner undergo hydrogen bonding and have higher polarity compared to ethyl acetate, dichloromethane, and hexanes which are more nonpolar and less prone to hydrogen bonding. Fresh resin samples contain more nonpolar components; hence they were more soluble in hexanes and ethyl acetate. However, oxidation of the components accompanies the ageing of the resin, which increased solubility in the more polar solvents. In a subsequent section, both the FTIR and GC-MS data provide supporting evidence for the presence of more nonpolar components in fresh samples and larger presence of polar components in the aged samples.

Differences in almaciga resin solubility with collection site was previously reported by Gonzales and Abejo (1978). In their study, almaciga resin samples from Luzon and Palawan islands had higher solubility in 95% ethanol than resin samples from Mindanao. Likewise, they found that resin from Davao Oriental had the lowest solubility, differing substantially from resins collected from the other sites. A separate study by Boer and Ella (2000) found that *A. philippinensis* resin samples were deemed soluble in ethanol and acetone, but were partly soluble in turpentine, petrol, and chloroform. Solubility tests by Umekar and Yeole (2008) showed that copal resin was most soluble in ethanol, followed by ethyl acetate, isopropanol, acetone, and chloroform. Similarly, gum copal from *Bursera bipinnata*, another resin-exuding tree species, was soluble in

chloroform and dichloromethane, but had lower solubility in slightly polar to polar solvents such as acetone, isopropanol, and ethanol (Mor-khade et al., 2006). In contrast, almaciga resin had higher solubility in polar solvents. Boer and Ella (2000) contended that resin solubility in alcohol is dependent on the copal type and not on the period between exudation and harvesting.

Solubility tests in 95% ethanol entails the dissolution of pulverized resin in the solvent, which had been customarily used as a basis in the commercial grading of aged almaciga resin (Boer and Ella, 2000; Lassak and Brophy, 2013). High solubility in 95% ethanol means low insoluble content, hence, high recovery of desirable components (Tavita and Pal-anginan, 1998). Low solubility in 95% ethanol can be attributed to the presence of gummy substances in the resin (Boer and Ella, 2000).

Almaciga resin mainly goes to the production of varnishes and paints used by small furniture makers and cottage industries in the Philippines (Boer and Ella, 2000). Boer and Ella (2000) also suggested subjecting Manila copal resin to a 2-hour thermal processing at around 315–360 °C to increase solubility in drying oils, and esterification of the resin with the aid of glycerol to increase solubility in drying oils used in varnishes.

3.2. Acid and saponification value

Acid and saponification values measure the reactivity of carboxyl groups in secondary metabolites targeted for utilization. Acid number indicates the free acids present while saponification value measures the amount of alkali needed to saponify resin or similar substances (Gonzales and Abejo, 1978; Lassak and Brophy, 2013). The acid value of hardened resins, which were collected 3 months after tapping, ranged from 110.68 for Marufinas resin to 119.40 for samples from Brooke's Point, Palawan (Figure 3). However, no statistically significant differences in the acid values of samples from various localities were found.

Despite the lower solubility in polar organic solvents, hardened resin samples from Davao Oriental were found to have high acid value. This indicates that acid number does not correlate with resin solubility in polar organic solvents (Gonzales and Abejo, 1978; Lassak and Brophy, 2013). Variation among Palawan resin samples was also observed, with samples from Brooke's Point having higher acid value compared to the samples from Marufinas. Copal resin studied by Umekar and Yeole (2008) gave an acid value of 129.82. Mantell and Rubenkoenig (1937) obtained an acid number of 107–114 for Manila copal, whereas Boer and Ella (2000) mentioned a wider range of 81–170. Boer and Ella (2000) observed a higher acid value for darker resin than lighter-colored ones, possibly due to increased oxidation upon ageing. Resins with high acid number are considered suitable for production of paper sizing agents (Boer and Ella, 2000; Lassak and Brophy, 2013). When combined with

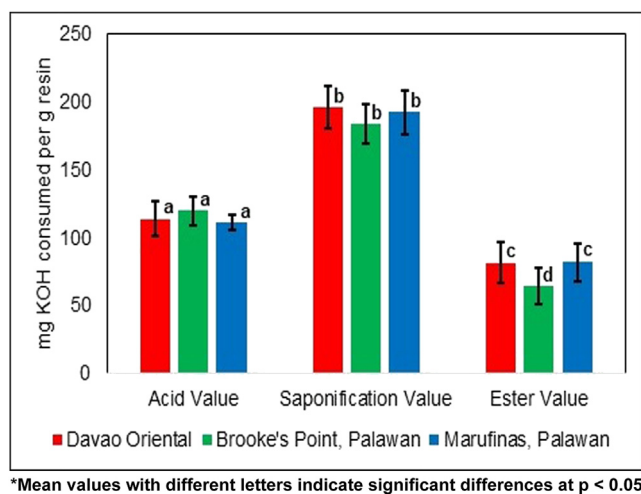


Figure 3. Acid, saponification, and ester values of aged resin samples of *A. philippinensis* collected from various sites. Mean values with different letters indicate significant differences at $p < 0.05$.

basic pigments, resins with high acid number were unsuitable as varnishes and paints due to stiffening (Boer and Ella, 2000). Chemical or thermal modification can reduce the acid number as the resin is converted to an oil-soluble product suitable for paint production (Tavita and Palanginan, 1998).

Saponification value is a measure of total fatty acid content, whether free or combined with glycerin. The saponification values of aged, hardened resins ranged from 183.36 for Brooke's Point resin to 195.79 for samples from Davao Oriental (Figure 3). There were no significant differences in the saponification values among the samples from Palawan and Davao Oriental. The present study gave higher values than previous studies where saponification values ranged from 171 to 176 (Gonzales and Abejo, 1978; Umekar and Yeole, 2008; Lassak and Brophy, 2013). But a 1994 study by Fernandez on purified almaciga resin from Palawan resulted in acid and saponification values of 128.20 and 189.68, respectively. Saponification numbers of 136–157 were obtained by Mantell and Rubenkoenig (1937) for Manila copal. High saponification numbers ranging from 147–204 and higher acid values were observed for darker resin specimens (Boer and Ella, 2000).

The unsaponifiable resins or ester value is computed as the difference between saponification and acid values (Gonzales and Abejo, 1978). The ester values of the samples varied significantly with the source. As shown in Figure 3, the ester values of hardened almaciga resin range from 63.96 for samples from Brooke's Point to 81.42 for samples from Marufinas. The value for the Brooke's Point sample was comparable to those previously reported by Tavita and Palanginan (1998). These authors contend that an

ester value higher than 20 would indicate the presence of considerable amounts of unsaponifiable substances in the resin.

3.3. Fourier transform infrared spectroscopy

The FTIR spectroscopic analyses of fresh and aged almaciga resin are shown in Figure 4, where the major IR absorption peaks can be seen. For fresh samples, the predominant absorption peak present is a sharp, strong peak at near 1700 cm^{-1} , which corresponds to carbonyl group stretch. In addition, a weak C-H stretch is present, indicating unsaturation. The presence of a broad peak at around 3400 cm^{-1} , which is indicative of O-H stretching, is evident in the aged samples, suggesting oxidation in the hardened resins. Peak broadening at around 1700 cm^{-1} was also observed in the aged resin samples, which according to Martin-Ramos and co-workers (2018) was more noticeable with aged and oxidized resins, although the band maximum remained in this region. Despite the absence of substantial variation in the dominant bands, there is a noticeable increment in their relative intensities with age. Shriner et al. (1997) attributed the more intense bands to certain vibrations arising from a substantial change in the molecule's dipole moment. Thus, oxidation of almaciga resin with ageing also appeared as increasing intensities of the prominent FTIR absorption peaks, such as carbonyl and C=C stretching (Beltran et al., 2016).

A weak peak at 1719 cm^{-1} is present in fresh resin samples obtained from Palawan. This weak shoulder peak due to C=O stretching was absent in aged resin samples as a result of masking caused by band broadening. The weak shoulder stretch denotes the presence of carboxylic acids or esters that have higher bond order as compared to aldehydes and ketones. According to Shriner et al. (1997), bond order or ring strain could influence the wavenumber of a stretching vibration of a bond. Moreover, there was a decrease in the wavenumber of C-H stretch and an increase in the wavenumber of C=O stretch in aged almaciga resin, regardless of site. There was also weakening of the peak due to C-H stretch at around 3080 cm^{-1} , possibly arising from conjugation with a C=C or aromatic ring unit, while ring strain could shift a particular band to a higher wavenumber (Shriner et al., 1997). This suggests oxidation and transformation via conjugation or ring formation with ageing, as reactive and volatile components are converted to stable species and higher molecular mass compounds (Pagacz et al., 2019). The presence of strong carbonyl stretch and broad hydroxyl stretch upon ageing reveals the presence of polar groups in the chemical components of the resin. These polar groups were likely responsible for the increased solubility of resin samples in polar solvents such as ethanol, isopropanol, and lacquer thinner.

In the literature, FTIR analysis of natural resins featured broad hydroxyl stretch at around 3400 cm^{-1} , strong carbonyl stretch at $1690\text{--}1740\text{ cm}^{-1}$, weak signal of C=C vibrations at about 1610 cm^{-1} , methyl and methylene stretch at 2860 and 2920 cm^{-1} , respectively, and C-O stretch at around 1230 , 1160 , and 1090 cm^{-1} (Edwards and Farwell, 1996; Katsibiri and Howe, 2010; Quintos et al., 2012; Martin-Ramos et al., 2018). Despite variations among various resinous materials, these prominent IR characteristics could distinguish them from carbohydrates, oils, and waxes (Martin-Ramos et al., 2018).

3.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) can describe the thermodynamic variation in resin and can explain transformation between two different physical states. It is useful in resin identification as these thermodynamic properties are sensitive towards resin composition and structure (Zhang et al., 2012). As such, DSC was utilized to determine the melting nature of hardened almaciga resins.

The thermal profile of aged almaciga resin samples from the various sites is shown in Figure 5. The DSC spectra appears to conform with that of a semi-crystalline material (Knapczyk and Simon, 1992). Cold crystallization, an exothermic reaction that could occur when previously

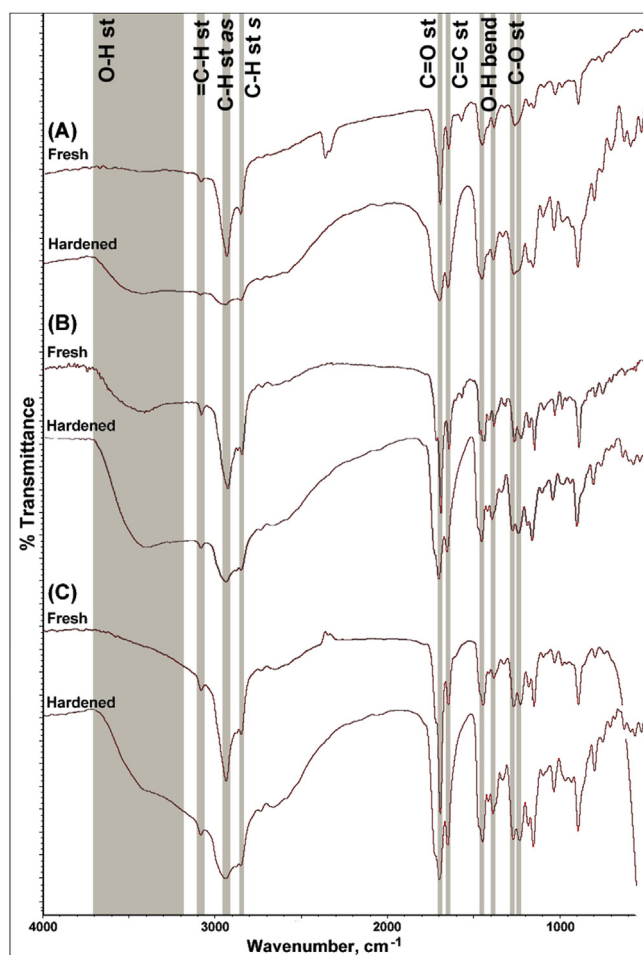


Figure 4. FTIR spectra of almaciga resin samples from (A) Davao Oriental, (B) Brooke's Point, Palawan and (C) Marufinas, Puerto Princesa City, Palawan, Philippines.

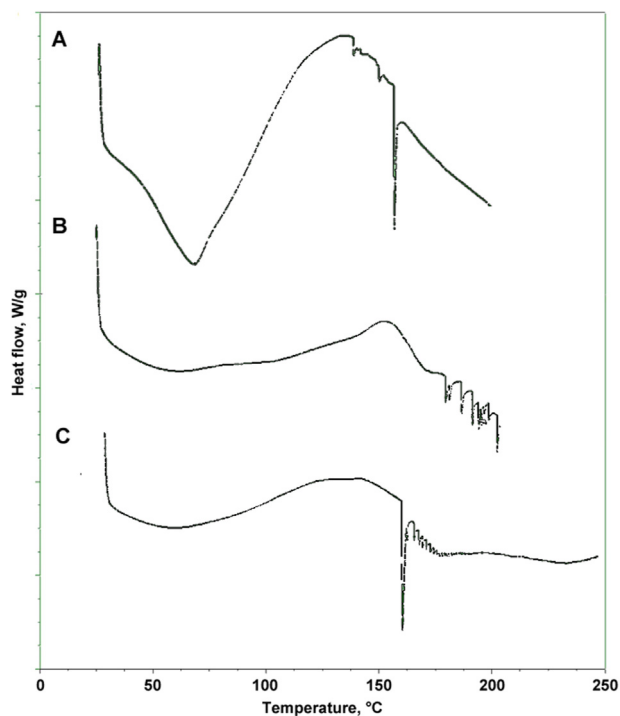


Figure 5. DSC thermograms of hardened almaciga resin samples from (A) Davao Oriental, (B) Brooke's Point, Palawan and (C) Marufinas, Puerto Princesa City, Palawan, Philippines.

heated resin cooled rapidly with no time to crystallize during cooling, yielded a temperature of 62.0 °C for Brooke's Point to 66.8 °C for Davao Oriental almaciga resins. As for the melting point, Knapczyk and Simon (1992) mentioned that amorphous polymers do not exhibit well-defined melting points above glass transition temperature (T_g) in view of their disorderly structure. The amorphous nature of resin is further indicated by the absence of sharp melting point, while its soft nature is characterized by low T_g and softening point values (Morkhade et al., 2006). For the brown gum copal from *Bursera bipinnata*, Morkhade et al. (2006) reported a T_g value of 38.79 °C. On the other hand, semi-crystalline polymers have a more rigid structure above T_g and would require a high temperature to melt the crystalline component (Knapczyk and Simon, 1992). In the current work on aged almaciga resin, T_g was not detected within the temperature test range of 30–250 °C. This seems to be apt for the almaciga resins which were almost rubbery at room temperature, with low or no apparent T_g value above 30 °C. This affirms the complex nature and unorganized assembly of the chemical components present.

A melting temperature of 127.35 °C was observed for samples coming from Davao Oriental while Palawan samples gave average melting points of around 140 °C. High melting point indicates the presence of polar components which require larger energy to break the intermolecular forces present in the material. The DSC thermogram of samples from Davao Oriental showed a sharp dip from 30 to 110 °C while the rest of the samples had broad dips ranging from 30–160 °C. This suggests the lower heat capacity of samples from Davao Oriental as compared to resin samples from the other sites.

Knapczyk and Simon (1992) stated that melting and degradation of organic materials can happen at adequately high temperatures as fragmentation, rearrangement, or oxidation exists between bonds. Likewise, oxidation or other changes caused by acids, moisture, or photochemical reactions could occur at ambient conditions. Ageing is also characterized by cross-linking and color change in resin polymers (Knapczyk and Simon, 1992). When heated at temperature around 300–310 °C, the dibasic acids of Manila copal resin turned monobasic, thus reducing its

acidity (Manalo and West, 1941). Mass loss of up to 20% was also observed with the melting of Manila copal (Fiebach and Grimm, 2000).

Several authors (Mantell and Rubenkoenig, 1937; Fiebach and Grimm, 2000; Morkhade et al., 2006; Umekar and Yeole, 2008; Adalina and Sawitri, 2020) revealed different values of the softening point of copal resin, which ranged from 79 to 117 °C. Mantell and Rubenkoenig (1937) recorded a melting point range of 123–132 °C, which depended on the classification of Manila copal. *Agathis dammara* from West Java, Indonesia had a melting point of 118–123 °C (Adalina and Sawitri, 2020). Stickiness of *Agathis alba* resin was observed with thermal resistance test at 110 °C for 30 min (Mulyono and Adrianus, 2012). Dielectric behavior studies by Goswami and Bhattacharya (1977) on Manila copal revealed two transformation points at around 70 and at 100 °C. This could indicate disparity in the resin molecule conformation upon transitioning from solid to liquid states. Boer and Ella (2000) reported melting points of the resin of various *Agathis* species that ranged from 115 to 135 °C, with increments marked by increasing hardness of the samples. But the melting point of soft and hard copal of *A. labillardieri* was about 70–80 °C and 100 °C, respectively. According to Li and co-workers (2012), Indonesian and Colombian copals yielded endothermic peaks at about 174–178 °C, consistent with their melting characteristics.

3.5. Gas chromatography - mass spectroscopy of the almaciga resin

GC-MS analyses of the nonvolatile fraction of fresh (Figure 6) and aged (Figure 7) almaciga resins revealed strikingly different chromatograms for the Davao Oriental resin (6A and 7A) from the Palawan resins (6B&C and 7B&C). Many compounds eluted at shorter retention times for the Palawan resins as opposed to the Davao Oriental resin where retention times were much longer. This indicates that there are less polar compounds in the fresh and aged Davao Oriental resins than their counterpart from Palawan. Sandaracopimaric (A) and abietic acids (D) were present in the resin from Brooke's Point (Figure 6B), while for the Marufinas resin (Figure 6C), pimaric acid (B) was found in addition to compounds A and D. On the other hand, only agatholic acid (G), a labdane diterpenoid, could be matched with the library, which was present in both fresh and aged resins from Davao Oriental (Figure 6A). The abietane and pimarane types were not detected in the chromatogram for Davao Oriental resin. For the aged resins from Palawan, three other compounds, that are more oxidized forms of abietic acid, were detected. These were 15-methyldehydroabietate (C) and 7-oxodehydroabietic acid (F), which were present in both the Brooke's Point and Marufinas resins, and 15-methoxydehydroabietic acid (E) which was seen in only the resin from Marufinas. Sandaracopimaric acid (A) and abietic acids (D) remained present in the aged Marufinas resin, albeit in smaller amounts. For the Brooke's Point aged resin, these two compounds were undetected. Other changes in the eluant profile were observed with ageing, but regrettably, matching with the NIST library led to the identification of only 7 plausible compounds (see Figure 8). Work is on-going on the identification of the other components.

As discussed above, the observed increased solubility in polar solvents of Palawan resins was corroborated by the increased intensity of O–H stretch and C=O stretch in the FTIR spectra. We could now attribute these polar functional groups to the presence of oxidized abietanes in the resins. For the aged Davao Oriental resin, we attribute the decline in solubility in polar solvents partly to decreases in agatholic acid content with time.

There had only been a few studies on the solid resin residue of *A. philippinensis*, and investigators tended to associate the almaciga resin with the diterpenoid compounds reported in pine and other copal resins. Manh et al. (1979, 1983) reported the presence of 19-noranticopalic acid, agatholic acid, sandaracopimaradienol, and methyl sandaracopimarate in *Agathis lanceolata* resin. In the resins of *A. macrophylla* from Fiji and *A. microstachya* from Australia, communic, sandaracopimaric, abietic and agathic acids were found (Carman and Marty, 1970; Carman and Marty, 1970; Smith et al., 1981; Frezza et al., 2020). A study on raw and

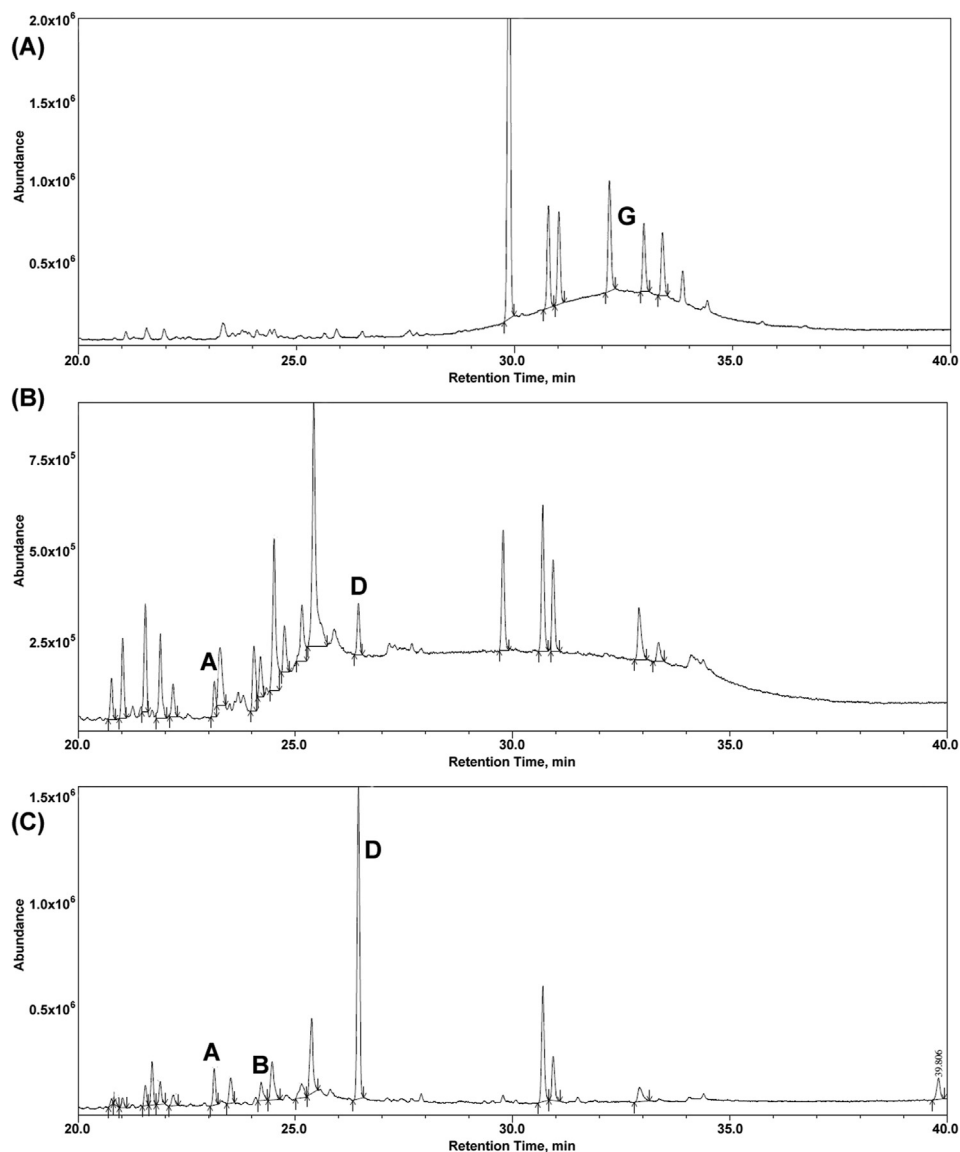


Figure 6. Gas chromatogram of fresh resin samples from (A) Davao Oriental, (B) Brooke's Point, Palawan, and (C) Marufinas, Puerto Princesa City, Palawan, Philippines.

artificially aged Manila copal resin showed the presence of sandaracopimaric acid, patchoulane, and agathic acid diethyl ester in both resins (Cartoni et al., 2004).

Other studies that determined the composition of various copal resins were those of Fiebach and Grimm (2000), who found agathene dicarboxylic acid ($C_{20}H_{30}O_4$) to be the main diterpene present in kauri copal. Boer and Ella (2000) mentioned the presence of agathol, agathal, and agathic-dicarbon acids in Manila copal at around 6.8% and 13.5% in soft and hard copal resin including resin acids in the ethanol-soluble fraction such as agathal, sandaracopimaric, and acetoxyagathol acids, with proportions of about 38%, 9%, and 8%, respectively. Another study (Reeves et al., 2013) revealed the presence of a variety of terpenoid products, such as methylated forms of *trans*-communic, sandaracopimaric, isopimaric, and agatholic acids. Lu et al. (2013) showed that labdanes and abietanes dominate the bi- and tricyclic terpenoids in various *Agathis* species. *A. robusta* and *A. moorei* had high amounts of aromatic abietanes, while saturated abietane were predominant in *A. robusta* only (Lu et al., 2013). On the other hand, labdane diterpenoids were found to be

abundant in Manila copal (Scalarone et al., 2003; Osete-Cortina and Domenech-Carbo, 2005).

Resins from *Pinaceae*, which is related to the *Araucariaceae* family, are mainly composed of abietane and pimarane diterpenoids (Osete-Cortina and Domenech-Carbo, 2005; Beltran et al., 2016). Isomerization of carbon-carbon double bonds is responsible for variability in composition of various abietane diterpenoid acids (Osete-Cortina and Domenech-Carbo, 2005). Beltran et al. (2016) also showed that abietic acid transforms into dehydroabietic acid upon ageing. Further oxidation could lead to the formation of 7-oxodehydroabietic acid and 15-hydroxydehydroabietic acid (Osete-Cortina and Domenech-Carbo, 2005; Beltran et al., 2016). According to Scalarone et al. (2003), the formation of oxygenated diterpenoids could be attributed to the methylene radical reacting with atmospheric oxygen to form alkoxy radicals, which in turn could lead to the formation of alcohols and ketones. They also likened copal ageing with polyabdanoid maturation in fossil resins.

Variations in diterpenoid composition in resins is also related to the genus or family of tree species (Seigler, 1998). Furthermore, Kononenko and co-workers (2017) claimed that the period of resin storage variation

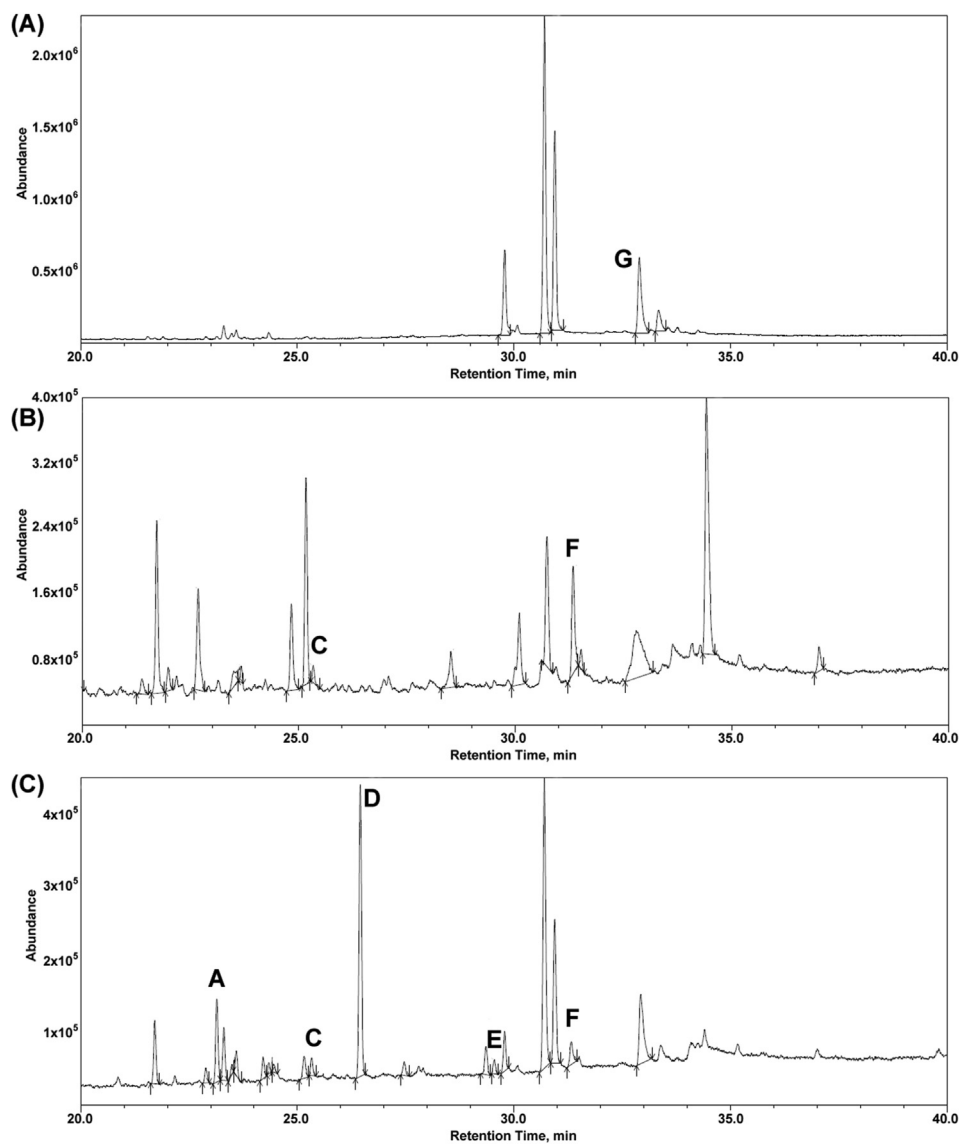


Figure 7. Gas chromatogram of aged resin samples from (A) Davao Oriental and (B) Brooke's Point, Palawan, and (C) Marufinas, Puerto Princesa City, Palawan, Philippines.

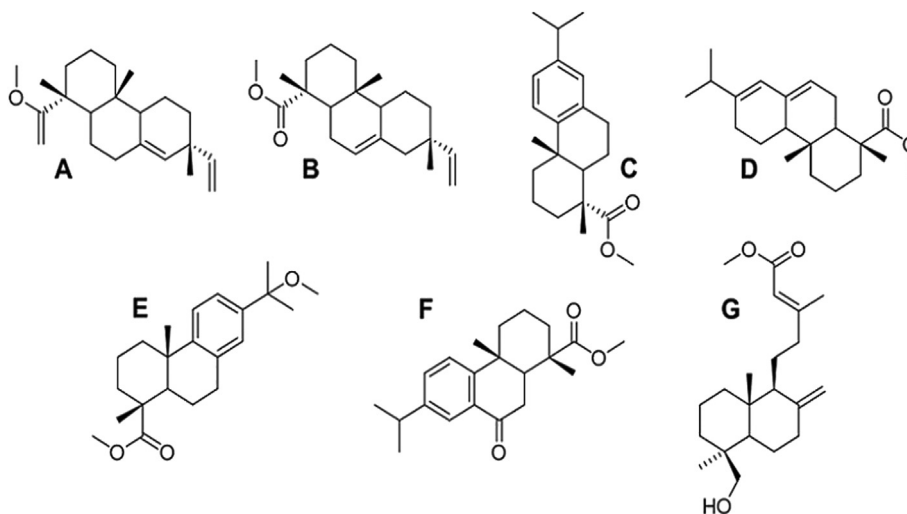


Figure 8. Resin acids identified via GC-MS using NIST library: (A) methyl sandaracopimarate, (B) methyl isopimarate, (C) methyl dehydroabietate, (D) methyl abietate, (E) methyl 15-methoxydehydroabietate, (F) methyl 7-oxodehydroabietate, and (G) methyl agatholate.

could lead to changes in resin composition, thus uniformity could not be assured even if the resin originated from the same tree species.

4. Conclusion

Wet chemical, spectroscopic, and thermal characterization tests of almaciga resin from different geographic sites in the Philippines were performed to assess and compare their properties. Significant variation for solubility in 95% ethanol upon ageing was observed. Palawan samples had increased solubility while solubility was decidedly lower for Davao Oriental samples. FTIR revealed the presence of carbonyl groups with band broadening upon ageing. A distinctive feature in the FTIR spectra from Palawan resins was the presence of an additional peak at around 1719 cm^{-1} , which could be due to carboxylic acids or esters that are of higher bond order than aldehydes and ketones. These carboxyl-containing compounds were determined to be diterpenoid resin acids with labdane, abietane, and pimarane skeleton, as affirmed by GC-MS. Thus, in terms of composition, abietic and pimaric acids were present in Palawan *Agathis philippinensis* resins, while agathic acid was present in Davao Oriental resin with no resin acids of the abietane or pimarane type. Thermal analysis showed that the aged resin samples had melting points close to the known diterpenoid resin acids present in them. For Palawan resins, the higher melting point is due to the tricyclic nature of the diterpenoid resin acids present, which required a larger transition energy as compared to agathic acid, the bicyclic resin acid present in Davao Oriental resin. These resin acids have been reported in pine resins that are used in functional coating, adhesives, paper sizing, etc. There are significant differences in almaciga resins obtained from Davao and Palawan in terms of chemical composition and physical properties, which could explain the quality and price differences.

Declarations

Author contribution statement

Ramon A. Razal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Aileen A. Jara and Ralph Lauren M. Alomia: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Genne Patt O. Samar: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kem M. Taghap: Analyzed and interpreted the data; Wrote the paper.

Marinelle R. Agonia and Ma. Diana DM. Rendon: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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