

Comprehensive Study on Extractable Aleuritic Acid Variability in Lac Resins: Species, Host Plant, and Seasonal Effects with Storage Impact for Optimum and Sustainable Utilization of Lac Resins

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ABSTRACT

Lac is non wood forest produce secreted by different species and strains of Lac insects grown on different host plants during different seasons. However, the diversity in extractable aleuritic acid content in various lac were not yet studied. Therefore, the study developed an improved method for extracting aleuritic acid and studied their variations and the influence of storage on its content in different lac to identify the most appropriate lac and optimum storage period for their efficient utilization. The developed method produces 25-30% more aleuritic acid and saves 10-15% of lac and 5-7 L of water per kilogram of scrapedlac. The study found a 13-28% variation in extractable aleuritic acid in the different scrapedlacs. However, the scrapedlac of *Kerria Chinensis* contains the most extractable aleuritic acid (28%), while *Kerria Sharda* contains the least (13%). Further, Even though the aleuritic acid was extracted by hydrolysis of scrapedlac, however the study found that the extractable aleuritic acid content in scrapedlac's decreases during storage due to the extensive alkali stable polymerization of their constituent molecules. Therefore, the study concluded that the production of aleuritic acid from any or old scrapedlac is not economical. However, the fresh scrapedlac produced by *K. chinensis* species grown on *F. macrophylla* is the most suitable raw material for the profitable production of aleuritic acid. Furthermore, the study investigated the thermal behaviour of the scrapedlac using DSC and found that the technique might be used as a tool for the quality assessment of scrapedlac.

Keywords: *K. Chinensis*, *K. Lacca*, Non wood forest produce, Lac, Aleuritic acid, DSC

INTRODUCTION

Throughout the world, 99 species of Lac insect have been recorded from 9 genera, out of which 26 species belonging to two genera are found in India, representing 26.3 % diversity of the known Lac insect species (Sharma, 2017). However, among the 26 species, only a few species, particularly *Kerria Chinensis*, *Kerria sharda* and *Kerria lacca*, based farming is carried out for commercial purposes in various parts of India depending on the abundance of host trees and favourable climate (Sharma, 2016). Additionally, among this commercially grown specie, *k. lacca* is the principal lac-producing insect in India's primary lac-growing states. The *K. chinensis* species is primarily grown in northeastern states, and the *K.sharda* species is the predominant lac-producing insect in the coastal states of India.

Lac insect farming for Lac production depends on the life cycles of the strain or species of lac insects and their respective host plants. The *rangeeni* and *kusmi* strains of *k. lacca* species

complete their life cycle twice annually and produce two harvests a year, in August- September and December-January (Mohanta *et al.*, 2014). However, the tri-voltine insect, *K. Sharda*, produces three harvests annually. However, in each case, the period of the life cycle and the seasons of maturity differ; likewise, *baisakhi* (summer rangeeni crop) inoculated during October-November and harvested during June-July, and another crop, *katki* (rainy rangeeni crop), inoculated in June-July and harvested in October-November (Mohanasundaram *et al.*, 2020). Similarly, *aghani* (winter kusmi crop) is inoculated in June-July but harvested in January-February, and *jethwi* (summer kusmi crop) is inoculated in January-February and harvested in June-July (Singh, 2015). *K. chinensis* also produce two harvests annually, i.e., a summer crop from October to April and a rainy crop from April to October (Rahman *et al.*, 2022).

The Lac resin is polyester of polyhydroxy aliphatic acid and

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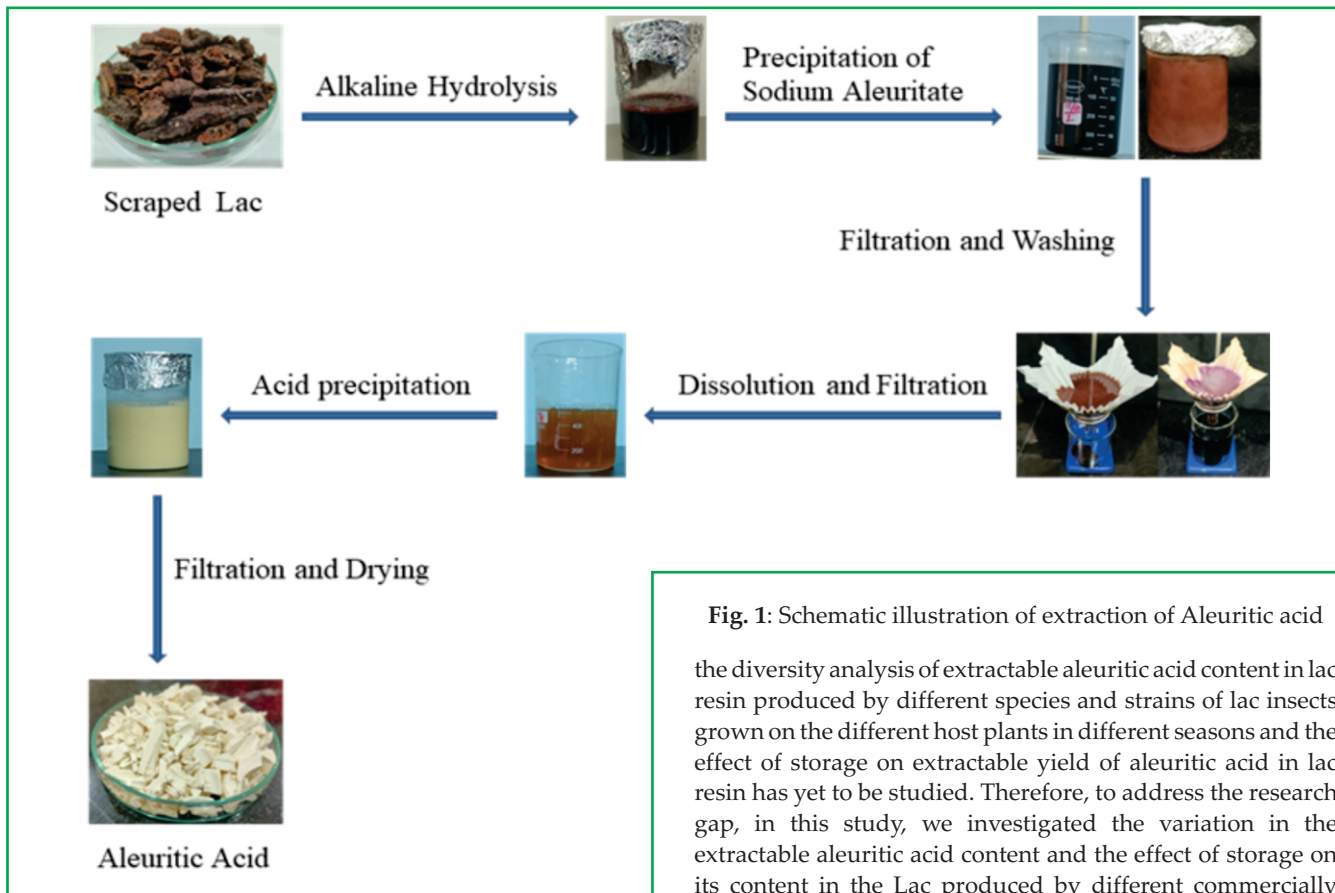


Fig. 1: Schematic illustration of extraction of Aleuritic acid

the diversity analysis of extractable aleuritic acid content in lac resin produced by different species and strains of lac insects grown on the different host plants in different seasons and the effect of storage on extractable yield of aleuritic acid in lac resin has yet to be studied. Therefore, to address the research gap, in this study, we investigated the variation in the extractable aleuritic acid content and the effect of storage on its content in the Lac produced by different commercially important lac insect species and strains grown on the different host plant during different seasons. The significance of the study is three-fold; first, the developed method might be used as an alternative method for the profitable production of aleuritic acid using the existing facilities of the Lac industry with minimal investment, and second, for decision or strategy making for the collection of suitable Lac exclusively for aleuritic acid production and for preparation of other semi- and refined product of Lac for optimum resource utilization and profit maximizing; and third is the application of DSC as a tool for quality evaluation of Lac sample during the procurement and for monitoring the quality of Lac or other refined product of Lac during storage to minimize the resource wastage by their timely utilization before their quality deterioration.

MATERIALS AND METHODS

Scraped lacs were obtained from ICAR-National Institute of Secondary Agriculture (Formerly ICAR-Indian Institute of Natural Resins and Gums), Ranchi, India, sodium hydroxide and sulfuric acid (99%) was purchased from Rankam India, Student Grade filter paper Grade: 615 from Himedia, India, NaCl from local market, Ranchi, India.

Extraction of Aleuritic acid from different Lac for its diversity analysis

The below-described method was developed by improving our previously reported method (Ali *et al.*, 2022). Therefore

sesquiterpenic acid (Singh *et al.*, 1974). Lac, due to its versatile properties utilized in surface coatings, textiles, cosmetics and medications, adhesives, the electrical sector, etc. (Yuan *et al.*, 2021). However, besides this, Lac is the only natural source of aleuritic acid, which is in high demand in the perfumery and pharmaceutical industries for the preparation of "macrocylic lactones such as civetone, ambrettolide, isoambrettolide, and various bioactive compounds like prostaglandins, cyclic ureides, and pheromones. The compounds like diketones, dilactones, triesters, and substituted coumarin derivatives" were also prepared from it (Nagappayya and Gaikar, 2010). Therefore, to meet the high demand in the international market, the lac industries collect the Lac from different regions of India, secreted by different species and strains of lac insects grown on the different host plants in different seasons for production of aleuritic acid. However, the variations in extractable aleuritic acid content in this Lac are not yet studied.

Further, the lac industries are continuously concerned about two things: first, obtaining the differences in the yield of aleuritic acid from lac collected in different seasons from different parts of India, and the second is the reduction in the aleuritic acid yield on storage. However, studies suggested that the quality of Lac depends on the host plant and climatic conditions (Sharma, 2016). Further, Saha 1992, Goswami *et al.*, 2009 and Giri *et al.*, 2010 studied the effect of storage on lac resin's physico-chemical properties. They reported that its physico-chemical properties deteriorate over time on storage (Saha, 1992), (Goswami *et al.*, 2009), (Giri *et al.*, 2010). However,

100 g of scraped lac was dissolved in a 10% sodium hydroxide solution with heating as required and left undisturbed at room temperature for ten days. The hydrolyzed mixture was then heated to boiling and left at room temperature. The ice-chilled cold water was added to the hot mixture solution and the condensed wax was removed from the mixture by filtration using a common sieve, and the mother liquid mixture was collected. The wax-free mixture solution was saturated with sodium chloride and incubated for 24 hr at 8-10 °C. The precipitated sodium aleuritate was separated by filtration using filter paper under gravity, followed by washing with cold brine water. The washed sodium aleuritate was redissolved, heated to boil for a few minutes, and then suction filtered immediately. Finally, the aleuritic acid was precipitated from the filtered mother liquid mixture by adding sulphuric acid and filtered and rinsed off under suction with cold water. The obtained aleuritic acid was dried at 45 °C in a hot air oven until it reached a constant weight. The dried aleuritic acid was kept in the refrigerator for further studies. The process produces a maximum yield of 21% (w/w) of aleuritic acid with 96-98% purity. The modified process is illustrated in Figure 2. below.

Diversity analysis of aleuritic acid

The diversity or variability of extractable aleuritic acid content in different lac resins produced by different species and strains of lac insects on different host plants in different seasons was determined using the method described in section 2.1 by extracting the aleuritic acid from their respective scraped lac samples (Table 1) and determining the quantity and quality of extracted aleuritic acid in accordance with Indian Standard 13160: 1991.

Effect of storage period on quality of lac resin in respect of aleuritic acid content.

The scraped lac produced by different species and strains of lac insects grown on the different host plants in different seasons (table. 1) was collected from our institute farm. Scraped lac of pre-weight 100 grams each were separately packed in a muslin cloth bag in three replications for each experiment and were stored for 6, 12 and 18 months at room temperature as the lac industry people did. The extractable aleuritic acid quantity and quality were determined in the stored lac resin after every six months for one and a half years following the method described in section 2.2. Each experiment was carried out in triplicate. FT-IR and DSC characterized the quality of lac resin.

Characterization

The FT-IR spectra of the three Lac samples were recorded in the mid-IR region (4000–400 cm^{-1}) at a resolution of 0.4 cm^{-1} using a Perkin Elmer, USA. The thermal behaviours of the three samples were recorded from room temperature to 450 °C at a heating rate of 10 °C per minute using TA Instruments, USA, Q10 Model, Differential Scanning Calorimetry (DSC). The quality and quantity of the extracted aleuritic acid were determined according to the Indian standard (IS-13160-1991) method.

RESULTS AND DISCUSSIONS

Extraction of Aleuritic acid

Lac resin is mainly polyester of polyhydroxy aliphatic acid and sesquiterpenic acid. However, scraped lac contains approximately 68% lac resin, 10% lac dye, 6% lac wax, 5.5% gluten, 6.5% foreign bodies and 4% other impurities (Sharma *et al.*, 2020). The aleuritic acid was extracted from the scraped lac resin by dissolving it in sodium hydroxide solution and left for ten days for hydrolysis of the ester bond, like the previously reported studies (Zhou *et al.*, 1993), (Chawla, 2008), (Nagappayya and Gaikar, 2010). The aleuritic acid on alkaline hydrolysis of lac resin liberates as sodium aleuritate, which is partially soluble in water. As a result, some portion of it precipitated from the mother liquid in due process during the extended hydrolysis period. As mentioned above, the scraped lac along resin contains about 6% wax. Therefore, to remove the lac wax, the hydrolyzed mixture was heated to boil to melt the condensed lac wax and kept at room temperature for a minute to accumulate the low denser lac wax at the surface of the hot solution. Ice-chilled cold water was added to the hot mixture to condense the wax (Ali *et al.*, 2022(b)). The condensed wax was removed by filtration. Following this step, the sodium aleuritate was precipitated from the mother liquid using NaCl, by salting out mechanism. Therefore, to precipitate the sodium aleuritate, the mixture solution was saturated with NaCl and then left at 8-10 °C for 24hr to precipitate. As a result, sodium aleuritate precipitates, leaving other soluble impurities in the mother liquid solution, like the precipitation of proteins from an aqueous solution on salting out (Green and Hughes, 1955), (Scopes, 1982), (Duong-Ly and Gabelli, 2014).

The scraped lac contains approximately 10% lac dye (Sharma *et al.*, 2020); even though it is soluble in alkaline water, some of it also comes in the precipitated sodium aleuritates. Therefore, after the separation of sodium aleuritate precipitates from the mother liquid by filtration, it was washed with NaCl-saturated water to remove the lac dye while minimizing the elution of partially soluble sodium aleuritate. After washing, the sodium aleuritate was redissolved in alkaline water and heated to boil to degrade the still leftover lac dye as the dye degraded on heating above 150 °C (Nacowong and Saikrasun, 2016), (Ali *et al.*, 2022(b)). The hot solution was filtered to remove the insoluble impurities, and on cooling, the collected filtrate was treated with 10% sulphuric acid to yield the aleuritic acid.

In all the known and available methods, aleuritic acid is mainly extracted from seed lac (semirefined product of scraped lac) and produces yields 10-18 g/100 g of seed lac (9, 14-15); however, Lac resins contain 30-35% of Aleuritic acids (Nagappayya and Gaikar, 2010). The scraped lac contains about 70% lac resin; however, the seed lac contains 88% lac resin (Sharma *et al.*, 2020). The seed lac was prepared by crushing, sieving, and washing with alkaline water, and in due process, approximately 10-15% of the lac resin goes waste with washing water. Further, for washing One kilogram of scraped lac, consume 5-7 liters of 1-5% alkali water which is later discharged from the industry as wastewater. Therefore, in the present study, an improved method was developed and

efficiently extracted the aleuritic acid from the scraped lac. It produces a maximum yield of 19.81 g/100 g of scraped lac, which in terms of per cent yield to lac resins is 28.3% as the scraped lac contains approximately 70% lac resin. Further, extracting the aleuritic acid from scraped lac, the improved process saves 10-15% of lac resin and 5-7 liters of water per kg of scraped lac, unlike the commercially available technology. Additionally, the purity of aleuritic acid obtained through the commercially known process is 70-85%, which is not considered a suitable purity level for consumption, particularly in the perfumery industry. Therefore, it is required to purify further by chromatography or multiple times recrystallization or repeated alkali dissolution and reprecipitation with mineral acid and causing losses of 40-50% of extracted aleuritic acid, extra processing costs, and ultimately causing environmental pollution (Agarwal *et al.*, 1988), (Rao *et al.*, 1997). However, the developed process produces an aleuritic acid of a maximum purity of 90-98% in a single process without using charcoal, bleaching agents, other toxic organic solvent and subsequent purification requirement, unlike the known processes as the impurities like lac wax, dye and other insoluble and soluble impurities were removed during the process as discussed above. Therefore, the improved method is more efficient, cost-effective and environmentally friendly than known and available methods (9, 14-15)

Diversity analysis of aleuritic acid and storage effect of on its content in the different lac resin

The extractable aleuritic acid content diversity or variability in the scraped lac produced by the four different commercially important strains of lac insect belonging to three different species grown on the different host plants in the different seasons was determined by extracting it using the method described in section 2.2. Further, according to Indian Standard (IS-13160-1991), the quality and quantity of the extracted aleuritic acid was determined (table 1). The study result (Figure 2 and Table 1) reveals that the extractable aleuritic acid content in the studied scraped lacs is not the same but has a variation ranging from 9.23-19.81g/100g. However, the least amount of 9.23 g/100g in *K. Sharda*, while the maximum content of 19.81g/100g in *K. chinensis* scraped lac's recorded. Further, among the two strains of *K. Lacca* species, the scraped lac (Yellow colour) produced by the *kusmi* strain grown on the Ber plant in the winter season shows the highest content of aleuritic acid of 17.07%. In contrast, the scraped lac (Crimson colour) produced *kusmi* strain grown on Kusum trees in the summer season shows the lowest content of 13.09%.

The study result showed that the extractable aleuritic acid content is highest in the scraped lac of *K. Chinensis* species. However, in India, the two strains of *K. lacca* species are the principal lac-producing insect in the primary lac-grown states. Further, *K.chinensis* is the primary lac-producing insect in the northeastern states of India and other Southeast Asian countries. Therefore the *k. chinensis*-based lac cultivation would be needed to be explored and extended to the other part of India for more profitable production of aleuritic acid, or at

the present moment; the lac industry can exclusively use the lac procured from the northeastern region of India for profitable aleuritic acid production. Additionally, the lac industry can utilize the finding of the study for strategic decision-making and judicious resource utilization to make the awareness of farmers for the separate collection of their produced scraped lac obtained from different species and strains of lac insects on the different host plants in different seasons so that the industry can procure and utilize the scraped lac as per the demand of the Lac product, like utilization of scraped lac produced by *k. chinensis* exclusively for aleuritic acid production and the other sources scraped lac for production of other their semirefined and refined product for their sustainable growth.

The lac resin contains approximately 30-35% of aleuritic acid (9); however, the lac industry uses the semirefined product (seedlac) of scraped lac and produces a maximum yield of 16% with 70-85 % purity. Additionally, in the preparation of seedlac from scraped lac, almost 10-15 % of lac resin went waste with the washing water. However, the present study demonstrated that the extraction of aleuritic acid directly from scraped lac and that of *k.chinensis* by the process described in section 2.2 produces about 25-30% more yield of aleuritic acid with 97-98% purity in a more eco-friendly way.

Additionally, in this study, we further studied the effect of storage period on the content of aleuritic acid in scraped lac produced by different species and strains of lac insects grown on the different host plants in different seasons over 18 months (table 1) to find the ideal scraped lac for prolong storage with minimal aleuritic acid losses. The study result shows that the extractable aleuritic acid gradually decreases over time in all the studied scraped lac [Fig. 2 (A) and (B)]. The study's results further revealed that the reduction in the extractable aleuritic acid is lowest in the lac resin secreted by *K. lacca* species, followed by *K. Sharda* and *k. chinensis*. However, among the different strains of *k. lacca* species, the loss of valuable acid is lowest in scraped lac produced by the *kusmi* strain, followed by the *rangeeni* strain [Fig. 2 (A) and (B)].

Additionally, among the 6, 12 and 18 months old scraped lac, the extractable aleuritic was found lowest in WBK(Y), which is 0.79% after six months; SKK(Cr) shows 4.66% and 21.12% after 12 and 18 months. However, the highest loss of 30.69%, 54.2% and 66.63 % were recorded in lac resin produced by RTF (Cr) on 6, 12, and 18 months of storage. The study result reveals that the reduction rate in extractable aleuritic acid was maximum in the first six months of storage and then decreased as storage time increased (Figure 2. C). It may be due to occurring of maximum polymerization during the first six months of storage, as Saha 1992, Goswami *et al.*, 2009 and Giri *et al.*, 2010 in their study, reported that lac resin undergoes polymerization during storage (Saha, 1992), (Goswami *et al.*, 2009), (Giri *et al.*, 2010). Saha *et al.* postulated that the deterioration in the physic-chemical properties of lac resin is due to photo-induced and self-polymerization of constituent lac molecules and that the self-polymerization happened between the two hydroxyl groups of two or more lac

molecules (Saha, 1992). The DSC thermograph (figure. 4) of fresh, one and two-year-old samples further evidenced the occurrence of polymerization from shifting their maximum melting point toward higher temperatures with increasing

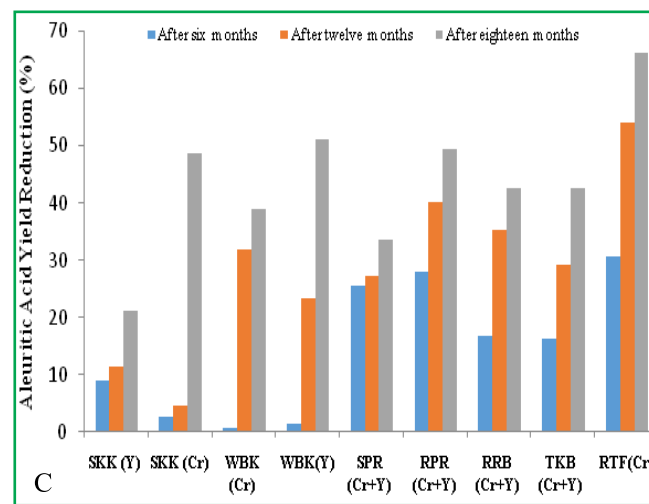
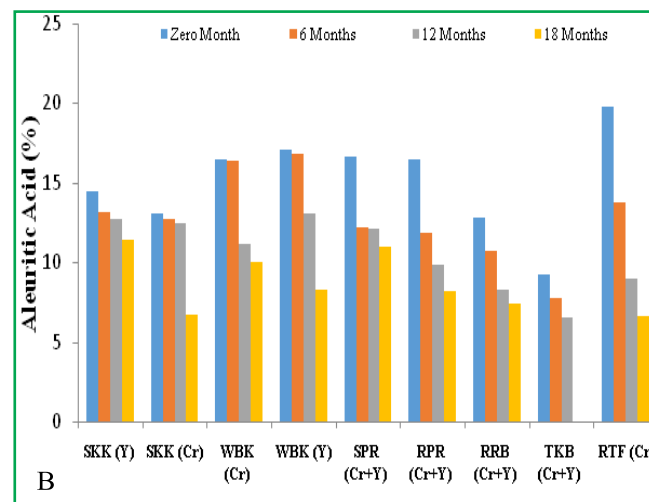
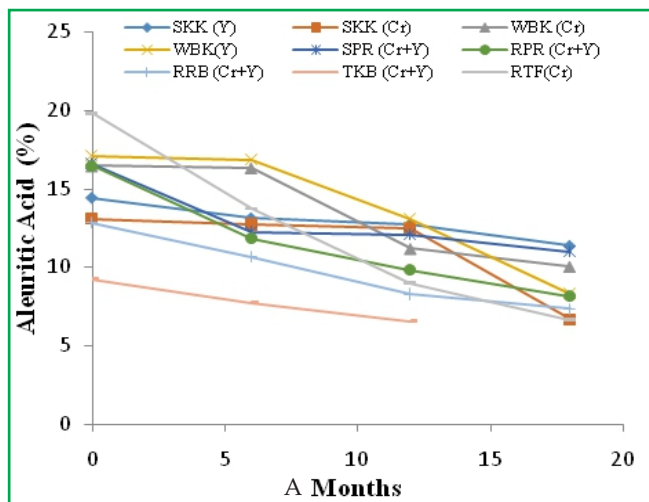


Fig. 2: Reduction in extractable Aleuritic acid in lac resin storage for over a period of 18 month

Table 1: Diversity analysis of extractable Aleuritic acid contents in scrapedlac's produced by different commercially important Lac insect species and strains grown on different host plants in different seasons

Abbreviations	Host Plant	Strain	Growing season	Species	Colour of insect	After harvesting			6 months after harvesting			12 months after harvesting			18 months after harvesting		
						Aleuritic Acid (%)	MP (°C)	Purity (%)	Aleuritic Acid (%)	MP (°C)	Purity (%)	Aleuritic Acid (%)	MP (°C)	Purity (%)	Aleuritic Acid (%)	MP (°C)	Purity (%)
SKK(Y)	Kusum	Kusmi	Summer	<i>K. lacca</i>	Yellow	14.42	92.5	95.0	13.13	95.2	96.35	12.76	94.5	92.81	11.375	94	95.71
SKK(Cr)	Kusum	Kusmi	Summer	<i>K. lacca</i>	Crimson	13.09	92.5	97.2	12.74	93.5	96.16	12.48	94.5	87.03	6.69	94.5	96.15
WBK(Cr)	Ber	Kusmi	Winter	<i>K. lacca</i>	Crimson	16.47	92.5	94.09	16.34	91.1	96.50	11.19	95.5	95.00	10.04	96	91.36
WBK(Y)	Ber	Kusmi	Winter	<i>K. lacca</i>	Yellow	17.07	92.5	93.71	16.84	92.2	98.63	13.06	94.5	92.78	8.31	93.5	92.78
SPR(Cr+Y)	Palas	Rangeeni	Summer	<i>K. lacca</i>	Crimson + Yellow	16.60	94.5	92.05	12.22	91.5	97.14	12.08	93.5	97.07	11	94.5	96.3
RPR(Cr+Y)	Palas	Rangeeni	Rainy	<i>K. lacca</i>	Crimson + Yellow	16.46	94.5	97.54	11.84	91.5	97.09	9.85	93	97.15	8.15	92.5	96.04
RRB(Cr+Y)	Ber	Rangeeni	Rainy	<i>K. lacca</i>	Crimson + Yellow	15.84	93.5	97.41	10.685	91.5	97.10	8.3	93	97.78	7.37	91.5	97.37
TKB(Cr+Y)	Ber	Trivoltine Kusmi	Rainy	<i>K. lacca</i>	Crimson	9.23	92.5	93.90	7.72	91.5	90.61	6.56	91.5	98.55	-	-	-
RTF (Cr)	<i>E. macrophylla</i>	Thailand	Rainy	<i>K. chinensis</i>	Crimson	19.81	92.5	98.78	13.732	89.5	98.74	9.01	89.5	98.55	6.65	96.5	96.08

storage time. Further, the maximum polymerization in the fresh sample occurs as it contains a maximum number of free hydroxyl groups for polymerization. As a result, the maximum reduction in the yield of aleuritic acid's observed within six months of the storage.

Characterization

FT-IR

The fresh, one-year-old, and two-year-old samples of scrapedlac were characterized by FT-IR to identify a discernible spectral shift; however, all three samples exhibited the same spectral features, except for an increase in the intensity of peaks as the storage period increased. "The broad and prominent peak at around 3397 cm^{-1} of the FT-IR spectra (Fig. 3) signifies the presence of intermolecular hydrogen-bonded hydroxyl groups. The peaks at 2916 cm^{-1} represented the asymmetric C-H stretching band of aliphatic $-\text{CH}_2-$, and the peak at around $2,849\text{ cm}^{-1}$ attributed to symmetric C-H stretching band of aliphatic $-\text{CH}_2-$. The peak at around $1,708\text{ cm}^{-1}$ signified the C=O stretching band $-\text{COOH}$ overlapped with the C=O stretch of α, β -unsaturated ester. The peak at around 1461 and 1368 cm^{-1} shows the asymmetric bending of the $-\text{CH}_2-$ and $-\text{CH}_3$ groups. The overlapped C-O stretching peak of both carboxylic acid and ester appeared at around 1233 cm^{-1} . The stretching bands at 1147 cm^{-1} denoted the ester linkage's C-O-C band. The band at 1001 cm^{-1} revealed primary alcohol's C-O stretching. The absorption band at 720 cm^{-1} denoted the bending motion associated with four or more $-\text{CH}_2-$ in an open chain (long-chain band). The peak at 781 cm^{-1} corresponded to the $-\text{C}-\text{H}$ of the Cedrene skeleton's tri substituted carbon" (Ali *et al.*, 2023(c)).

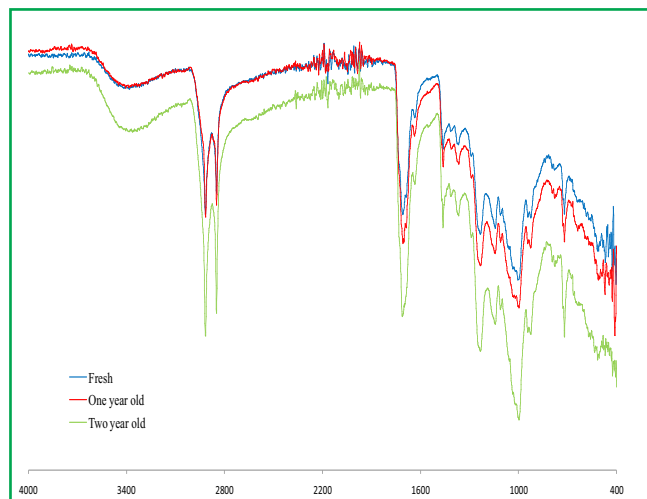


Fig. 3: FT-IR Spectra of different aged Lac samples

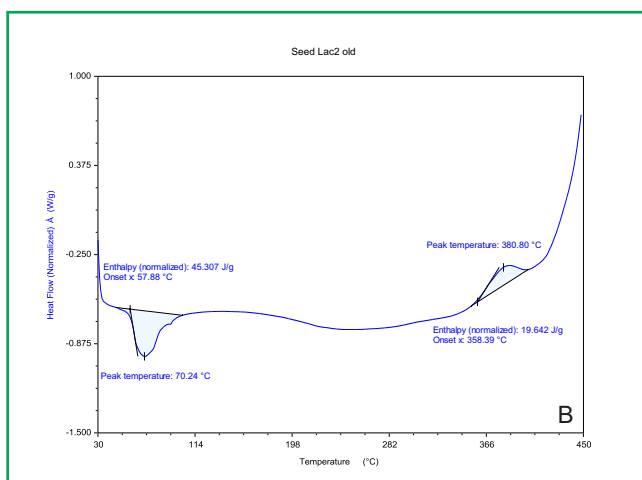
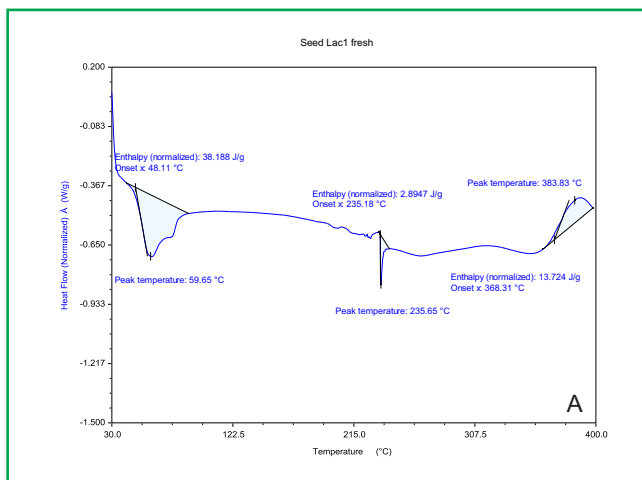
The result of the present study (Figure 2) explicitly reveals that the extractable aleuritic acids content in the scrapedlac resins decreases during storage with deterioration in its physico-chemical properties (Saha, 1992), (Goswami *et al.*, 2009), (Giri *et al.*, 2010). Therefore, we investigated the relationship between the thermal behaviour and quality deterioration of lac resin using Differential Scanning Calorimetry. The result of the study (Fig. 4) shows that the onset and maximum melting

point of scrapedlac changes by shifting towards higher temperatures with increasing the storage period.

The thermograph of the fresh scrapedlac [Figure 4. (A)] shows that it started to melt at 48.11°C and reached a maximum melting point of 59.66°C . However, the one-year-old sample has shown the shifted onset and maximum melting point at 57.88°C and 70.24°C [Fig. 4(B)]. Likewise, the two-year-old sample's onset and maximum melting point shift to 58.11°C and 181.70°C [Fig. 4(C)].

The scrapedlac resin mainly consists of oligomeric and monomeric compounds (Al-Gousous *et al.*, 2015), (Yan *et al.*, 2021). Therefore the shifting in the onset and maximum melting point of scraped lac resin on storage could be because of an increase in their crystallinity due to the polymerization of their constituents, monomeric and oligomeric compounds ((Saha, 1992), (Goswami *et al.*, 2009), (Giri *et al.*, 2010), (Al-Gousous *et al.*, 2015), (Yan *et al.*, 2021).

Further, as the thermal behaviour of scraped lac changes with its quality deterioration, the DSC technique could be used as a tool for the quality analysis of scrapedlac and other semirefined and refined products of scrapedlac during its procurement and also for monitoring their quality during storage to minimize the resource wastage by timely utilization.



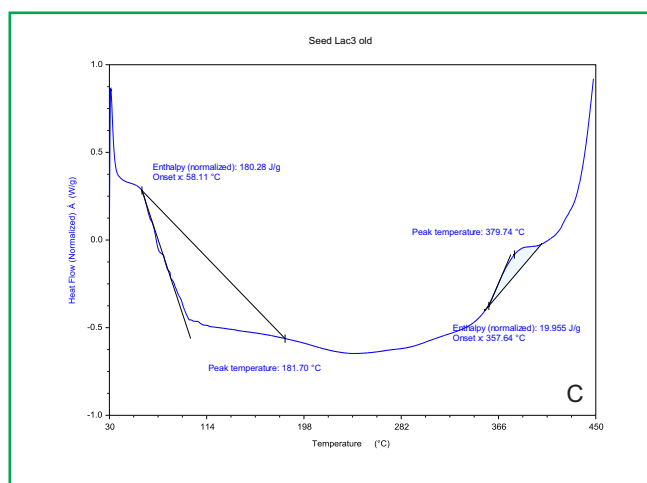


Fig. 1: DSC thermograph of Fresh (A), One year old (B) and Two Year old (C) lac samples

CONCLUSION

In this study, an improved method for extracting aleuritic acid was developed and studied the diversity and influence of storage effect on aleuritic acid content in scrapedlac produced by different commercially important species and strains of lac insects on the different host plants in different seasons. The studies found that the extraction of aleuritic acid using the fresh scrapedlac produced by *K. chinensis* species grown on *F.*

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