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Characterization
of the Fragrant Resin from *Shorea roxburghii*, a Lac Insect Host Tree of Tropical Deciduous Forest in South India

The natural resin exudate from the bark of Shorea roxburghii G. Don possess a unique fragrance. In this study, we have characterized the S. roxburghii resin for its physicochemical properties and chemical composition. The physicochemical properties such as melting point, ash content, volatile matter and acid number of S. roxburghii resin were determined and compared with those of S. robusta resin. The chemical compounds and groups responsible for the fragrance of this resin were determined by gas chromatography mass spectrometry (GCMS). The results revealed that S. roxburghii resin has higher volatile matter (3.21%) and lower melting point (64-65 ºC), ash content (0.14%) and acid number (16.88) as compared to S. robusta resin. A total of 37 compounds were identified in the S. roxburghii resin sample by GCMS. These included monoterpenoids, sesquiterpenoids and triterpenoids. The sesquiterpene hydrocarbons comprising β Elemene, β Caryophylene, β Farensene, Selinene, α Panasinsen etc., was the largest group. It was followed by triterpenoids which included Lup-20(29)-en-3-one, β Amyrin, α- Amyrin, Urs-12-en-28-al and others. The oxygenated compounds such as terpinyl acetate and caryophyllene oxide were also identified in the resin. The chemical components of the resin can be further isolated and characterized for the development of various industrial applications.

Key words: Natural resin, Sesquiterpenes, Triterpenoids, Chemical profiling, GCMS

The fragrant resin of Shorea roxburghii possess distinct physicochemical properties and terpenoid profile which may be explored for various applications.

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Introduction

Shorea genus of the Dipterocarpaceae family comprises of 196 species which are found in the region of India and Southeast Asia. *Shorea roxburghii* G. Don (syn. *Shorea talura*) is a lac insect host tree widely distributed in India, Myanmar, Thailand, Malaysia, Cambodia, Laos and Vietnam (Morikawa *et al.*, 2012a). In India, it is present mainly in the states of Tamil Nadu and Karnataka. The resinous exudate from *S. roxburghii* tree has a pleasing fragrance and it is traditionally used as dhoop incense, locally known as *Sambarani*, *Jalari, Talura* or *Kungiliyam*. *S. roxburghii* is classified as a Vulnerable species in the IUCN red list. As a conservation measure, the pruning of *S. roxburghii* trees is not practiced in the southern part of India as its flowers are widely offered in worship of Lord Shiva (Mohanasundaram *et al.*, 2018).

Natural resins as minor forest products are an important source of livelihood of tribal populations. There has been increasing interest in the documentation and characterization of natural resin exudates of different trees (Thombare *et al.*, 2018). The physicochemical characterization and chemical composition of these resins can lead to the development of value-added products for perfumery, cosmetics, health and industrial applications. However, there is a lack of information on the chemical characteristics of *S. roxburghii* resin.

The exudate resins are generally composed of terpenoids, a diverse group of chemical compounds. The composition of different monoterpenes, sesquiterpenes and higher terpenoids in different plant

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parts is characteristics of the species. This determines the various biological functions such as plant defence as well as the quality characteristics of the plant products, for example, the fragrance of the exudate resin. A wide range of species of *Shorea* and *Hopea* genus in the Dipterocarpaceae family such as *S. robusta, S. javanica, S. laeviforia, H. pubescens* etc. have been studied for their resin content (Chan, 1969; Brackman *et al*., 1984; Ukiya *et al*., 2010; Mulyono *et al*., 2013). The dammar resins from these trees have triterpenes like dammarane, oleanane, and oleanonic acid as the major constituents.The dammar resins find industrial applications such as weighting agents and as materials for the extraction of fragrant essential oils (Mulyono and Apriyantono, 2004). During the period of millions of years, the resin exudates from the forests of dipterocarp trees have matured into copal and ultimately to amber (Lambert, 2013).

There have been studies on the chemical composition of stem/bark, leaf and inflorescence extracts of *S. roxburghii* and related species (Patcharamun *et al*., 2011; Chainukool *et al*., 2014; Ragasa *et al*., 2014; Guo *et al*., 2020). These studies are mainly focused on polar components such as stilbenoids and other polyphenols. Some of the studies on the chemical composition of resin from the related species *Shorea robusta* have revealed a prevalence of sesquiterpenoid and triterpenoid groups (Misra and Ahmad, 1997; Sushma *et al*., 2017). However, reports on the chemical composition of *S. roxburghii* resin are lacking. In this study, we are reporting for the first time the physicochemical properties and gas chromatography mass spectrometry (GC-MS) based chemical composition of *S. roxburghii* resin.

Material and Methods

Material

Shorea roxburghii trees were identified during a survey of lac host plants in Thalamalai area of Satyamangalam forest, Erode district, Tamil Nadu, India. The trees exudated a resin from open cracks in the bark (Fig. 1). The resinous exudates (500 g) was carefully collected and stored in polybags for further analysis. For comparison of physicochemical properties, resin samples of *S. robusta* (Sal tree) were procured from Bahubali Udyog, Bilaspur, India. All solvents and chemicals used in the study were of analytical grade (SISCO Research Laboratories Pvt. Ltd., India).

Physicochemical properties

The samples were analysed for melting point, acid number, ash content and volatile matter. The melting point was determined by a laboratory melting point apparatus. BIS procedure IS 7437: 1974 was followed for Ash content, volatile matter and acid number.

Differential scanning calorimetry

Thermal stability of the resin was studied by differential scanning calorimetry (DSC). The DSC analysis was done using a Perkin Elmer DSC-7 instrument in the Nitrogen atmosphere. The output was produced in terms of heat flow versus temperature curves, denoting the endothermic exchange on the upside. The heating rate was kept at 20 °C min^{-1}. The same parameters were determined for the resin of *S. robusta*.

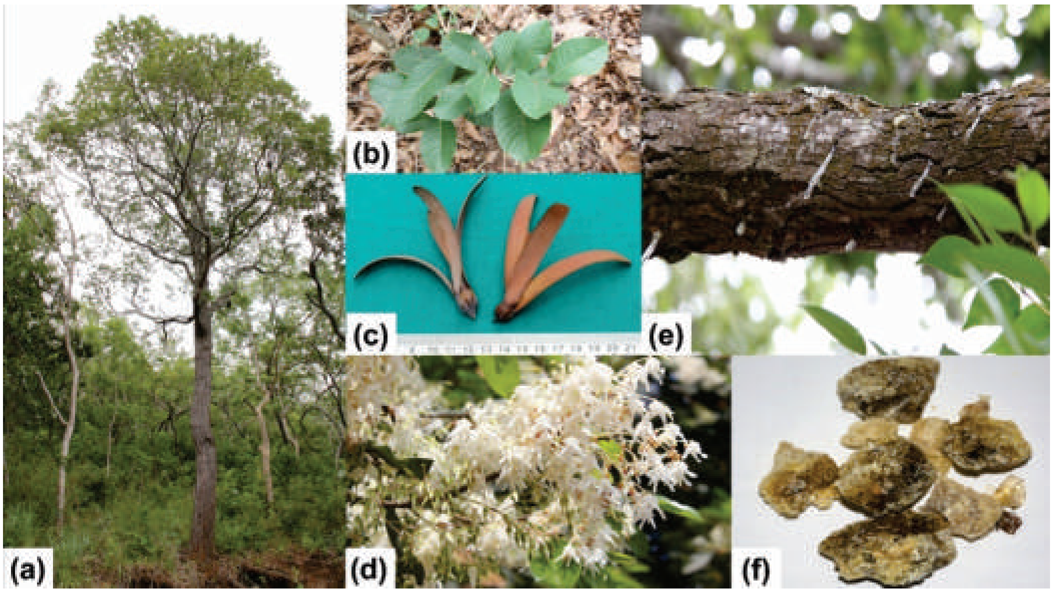

Fig. 1: Photographs of *Shorea roxburghii* (a) tree, (b) leaves, (c) seeds, (d) inflorescence, (e) resin exudate from tree bark and (f) collected resin

Extraction of S. roxburghii resin

5 g *S. roxburghii* resin was ground in pestle and mortar to obtain a creamy-white coloured powder. Extractions of *S. roxburghii* resin samples were done using different solvents. The resin sample was taken in a conical flask and 25 mL of extraction solvent was added to it. Extraction was done thrice at room temperature using an ultrasonic bath for 10 minutes. Petroleum ether extraction of this powder using ultrasonic bath yielded 4.5 g crude extract. The residue was extracted successively with dichloromethane followed by ethyl acetate. The filtrate was evaporated on a vacuum evaporator at 40 ^{0}C. Thin layer chromatography (TLC) of the crude extracts were run. The solvent system used was 20:80 ethyl acetate in petroleum ether. Column chromatography fractionation was done for petroleum ether crude extract of *S. roxburghii* resin. Fractions of 50 ml each were obtained. The fractions were compared with crude by thin layer chromatography. Fractions that gave the same spots on TLC were pooled and evaporated under a rotary evaporator. GC-MS analysis of pooled fractions was done to compare with the crude extract. However, no single compound was obtained by the column fractionation.

Column chromatography

A glass column (length 450 mm, bore 30 mm) was used for the column chromatography. Silica gel of mesh size 60-120 mesh was used as the stationary phase and petroleum ether and ethyl acetate solvent system was used for eluting the compounds through the column. Silica gel slurry was prepared in petroleum ether and poured into the column. Then, the sample mixture was placed over the silica gel pack and allowed to elute through the different solvent systems. The solvent systems used were 100% petroleum ether, 2%, 4%, 8%, 15%, and 50% ethyl acetate in petroleum ether. Thin layer chromatography of fractions and crude was run using 20% ethyl acetate in petroleum ether solvent systems. Anisaldehyde charring solution was used to visualize the spots. The crude extracts and the fractions were subjected to GC-MS analysis.

GC-MS analysis

GC-MS analysis was done using an Agilent HP 6890 Gas chromatograph mass spectrometer having an HP-5MS column (30 m x 250 µm x 0.25 µm). Helium was used as a carrier gas. Petroleum ether extract of *S. roxburghii* resin was analysed using oven conditions as described in Table 1. The mass spectrometer transfer line temperature was 310 °C. Samples were prepared in ethyl acetate and 1µl was injected manually. The mass analysis was done under full scan mode and the mass fragmentations obtained were compared with the NSIT library for probable matches.

Table 1: Gas chromatography mass spectrometry (GC-MS) oven temperature gradient conditions for the analysis of *Shorea roxburghii* resin samples

Rate	Oven conditions		
	Temp(^{0}C)	Hold (min)	Run time (min)
	40	2	2
4 ^{0}C/min	200	0	42
10 ^{0}C/min	300	15	67

Note: Temp denotes the temperature achieved in the gradient,
Hold is the time for which the temperature is kept constant at the achieved temperature,
Rate is the increase in temperature per minute to achieve the next temperature ramp,
Run time is the cumulative time at the end of a particular temperature ramp

Results and Discussion

Physicochemical properties

Shorea roxburghii resin was having shiny crystal-like appearance, yellowish-white colour with some dark patches. The resin quality was better in terms of fragrance and physical appearance as compared to the *S. robusta* resin which was cylindrical in shape and darker in colour. The physicochemical properties of *S. roxburghii* resin as compared to *S. robusta* resin are presented in Table 2. The melting point range of *S. roxburghii* resin was found to be much less than that of *S. robusta* resin. However, the *S. roxburghii* resin contained comparatively higher volatile matter and less ash content than *S. robusta* resin. The acid number is defined as the quantity of potassium hydroxide (KOH) in milligrams required for the neutralization of one gram of the test substance. The acid number is thus correlated to the number of carboxylic acid groups in a chemical substance. The lower acid number of *S. roxburghii* resin than *S. robusta* resin may denote the comparatively lower number of acidic groups in *S. roxburghii* resin such as ursolic acid, oleanolic acid and other terpenoid acids. The values of ash content, acid number and volatile content of *S. robusta* resin were found comparable to those reported in previous studies (Poornima, 2009; Vashisht *et al*., 2017).

Table 2: Melting point, acid number, volatile matter, ash content and DSC glass transition temperature of *Shorea roxburghii* and *Shorea robusta* resins

S.No.	Properties	*S. roxburghii* resin	*S. robusta* resin
1.	Melting point (ºC)	64 - 65	184 - 185
2.	Acid number	16.88	28.53
3.	Volatile matter (%)	3.21	2.06
4.	Ash content (%)	0.14	0.22
5.	DCS glass transition temp. (ºC)	55.6	74.8

Differential scanning calorimetry

DSC curves of *S. roxburghii* and *S. robusta* resins are shown in Fig. 2. The DSC experiments were run up to the temperature of 250°C. Both the samples showed endothermic behaviour initially. This is because of the desorption of the moisture adsorbed in the samples. The endothermic behaviour of the samples during the latter part of the curves *i.e.*, above 160 °C for *S. roxburghii*and 180 °C for *S. robusta* denote the deformation of the polymeric chains of the resins. Differential scanning calorimetry results in the dehydration, depolymerization and decomposition of biopolymer material at higher temperatures. The behaviour of the material depends on its structure and functional groups (Singh and Bothara, 2014). The presence of both endothermic and exothermic effects in DSC profiles of natural resins correspond to their softening, partial melting, release of volatiles, relaxation and post-curing reactions (Pagacz *et al*., 2019). The thermal transitions of the material throughout the temperature range are thus studied. In the heating curves, the glass transition temperature of *S. roxburghii* and *S. robusta* appeared at 55.6 and 74.8 °C respectively. The glass transition temperature of a material denotes its working temperature range. Depending upon the glass transition temperature the substance can be in a glassy or rubbery state at the given application temperature (Schilling, 1989). The lower glass transition temperature of *S. roxburghii* resin as compared to that of *S. robusta* resin denotes that it is less brittle than *S. robusta* resin. The midpoint glass transition for different dammar resin samples was reported by Schilling (1989) to range from 60 to 64 °C.

Chemical composition

The chemical composition of the non-polar constituents of the S. *roxburghii* resin was determined by GC-MS analysis of its petroleum ether extract. The total ion chromatogram (TIC) has been presented in Fig.3. The analysis of mass fragmentation of various peaks and comparing with the NSIT library resulted in the identification of compounds present in the resin (Table 3).

Overall, 37 compounds were identified in the *S. roxburghii* resin sample accounting for a total of 94.74% of the peak area. β Caryophylene (Rt- 25.93, MW – 204), Isopropenyl dimethyl octahydronapthalene (Selinene) (Rt – 27.99, MW – 204), α Panasinsen (Rt – 28.99, MW – 204), Lup-20(29)-en-3-one (Rt – 55.59, MW – 424), Octamethyl octadecahydro picen-3-one (Rt -55.81, MW – 424), β Amyrin (Rt –56.12, MW – 426), α Amyrin (Rt – 56.69, MW – 426), Urs-12-en-28-al,3-(acetoxy)-3β- (Rt – 59.05, MW – 482) and Urs-12-en-28-al (60.04, MW – 424) were the major compounds found in *S. roxburghii* resin.

Sesquiterpenes were the largest group of compounds identified in the resin, followed by triterpenoids and the oxygenated compounds were the lowest. However, the oxygenated compounds generally have more impact on the aroma than the terpene hydrocarbons and thus the overall fragrance is a result of the unique combinations of all the components (Yinggam *et al*., 2021).

While studies on the characterization of *S. roxburghii* resin are limited, previous studies of this

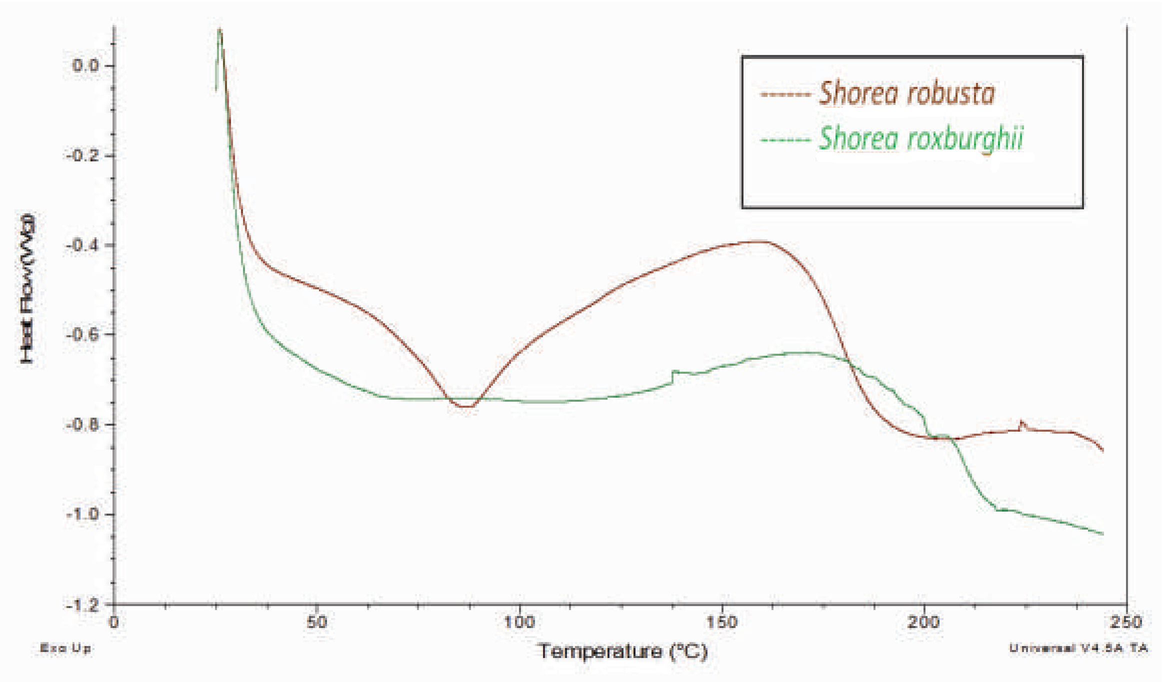

Fig. 2: Differential ccanning calorimetry (DSC) curves of *Shorea robusta* and *Shorea roxburghii* resin

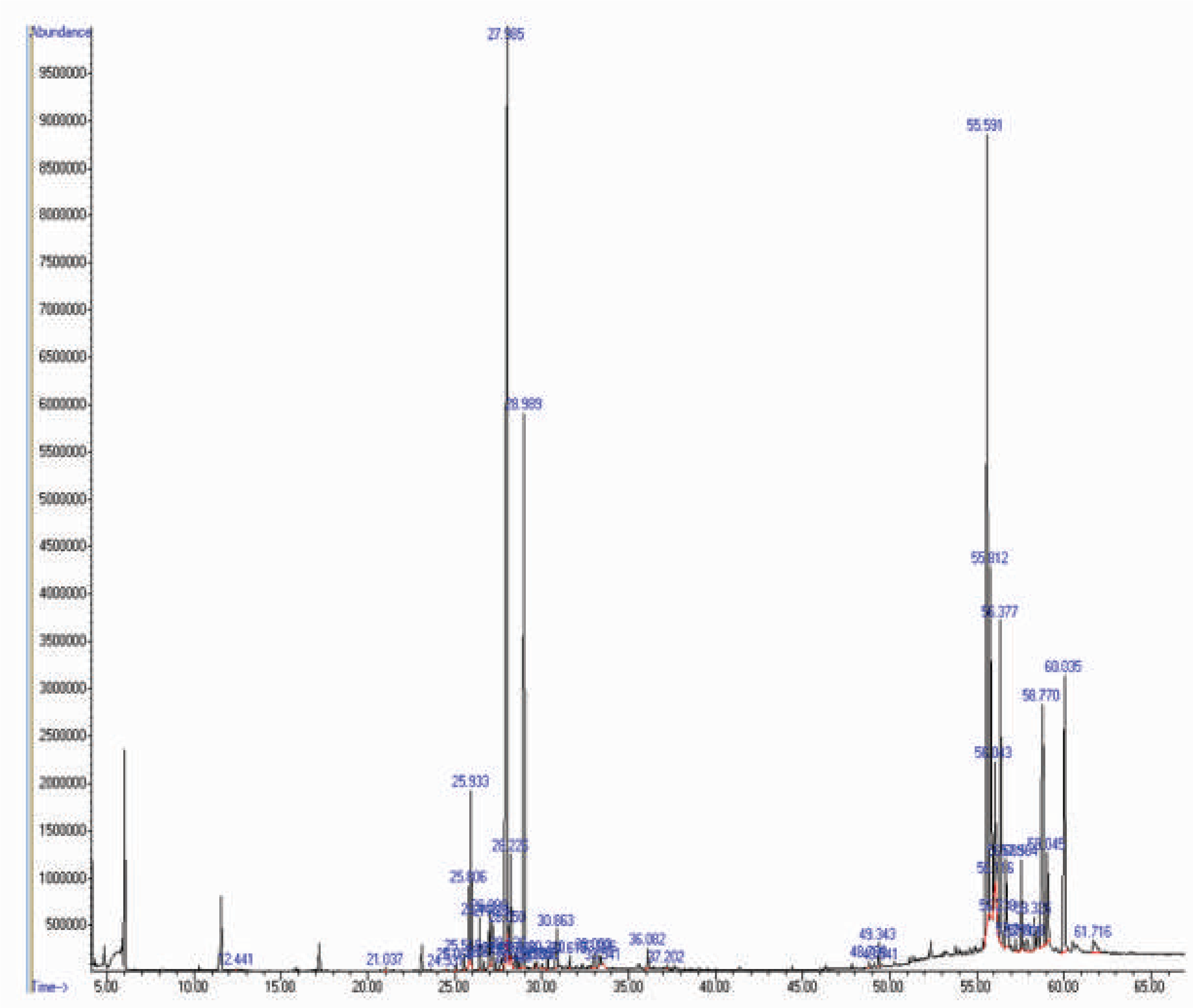

Fig. 3: GC-MS TIC of *Shorea roxburghii* resin petroleum ether extract

species have focussed mainly on the chemical constituents from the stem/bark and roots. The main groups reported are stilbenoids, oligostilbenoids and dihydroisocoumarins. Resveratrol dimer roxburghiol A was reported from the roots of *S. roxburghii* by Patcharamun *et al*. (2011). Morikawa *et al*. (2012b) isolated three new dihydroisocoumarins from the methanolic extract of *S. roxburghii* bark in addition to 24 already known compounds including stilbenoids and oligostilbenoids. Hopeaphenol, a tetramer resveratrol was isolated from the crude extract of the *S. roxburghii* stem bark (Nor *et al*., 2013). Trans-resveratrol along with its glycosylated form were extracted from the stem bark of *S. roxburghii* by subcritical water extraction method (Chainukool *et al*., 2014). Makinde *et al*. (2020) identified 15 compounds in the n-hexane fraction of *S. roxburghii* stem which included triterpenoids, sterols and fatty acids such as methyl isopalmitate, hexadecenoic acid, stearic acid, ergost-5-en-3-ol, stigmasterol, β-sitosterol, olean-12-en-3-one, lupeol etc. In the current study also, non-polar constituents such as sesquiterpenoids and triterpenoids have been identified for the first time in the resinous exudate of *S. roxburghii.*

A study on the chemical composition of the essential oil extracted from the *S. roxburghii* inflorescence has been recently reported (Yingngam *et al*., 2021). A total of 51 compounds of different classes of terpenes were identified. A comparison of this report with the current study would reveal that sesquiterpenoids cubebene, caryophyllenes, selinene, α-panasinsen, btuse-3,7(11)-diene, caryophyllene oxide, etc. and monoterpenoid terpinyl acetate are common in both. However, the inflorescences essential oil contained monoterpenes and diterpenes whereas the resin samples had triterpenoids. Since, the studies involved different plant components of *S. roxburghii*, the composition of higher terpenes varied.

Relatively more similarity in terpenoid class is observed when the chemical composition of *S.*

Table 3: List of putative compounds identified in *Shorea roxburghii* resin by GC-MS

S.No.	Rt	Compound	MW	Molecular formula	% Peak area
1	12.44	β-Terpinyl acetate	196	$C_{12}H_{20}O_2$	0.032
2	21.036	Megastigma-4,6,8-triene	176	$C_{13}H_{20}$	0.051
3	24.531	α Cubebene	204	$C_{15}H_{24}$	0.057
4	25.066	β Elemene	204	$C_{15}H_{24}$	0.151
5	25.809	α Bergamotene	204	$C_{15}H_{24}$	1.116
6	25.931	β Caryophylene	204	$C_{15}H_{24}$	2.85
7	26.671	Eremophilene	204	$C_{15}H_{24}$	0.142
8	26.988	α Caryophyllene	204	$C_{15}H_{24}$	0.768
9	27.128	β Farnesene	204	$C_{15}H_{24}$	0.953
10	27.365	Bicyclodecenol dimethyl isopropenyl	262	$C_{17}H_{26}O_2$	0.172
11	27.985	Isopropenyl dimethyl octahydronapthelene (Selinene)	204	$C_{15}H_{24}$	22.453
12	28.05	Eudesmadiene (selina-3,7(11)-dien)	204	$C_{15}H_{24}$	0.139
13	28.99	α Panasinsen	204	$C_{15}H_{24}$	11.237
14	29.536	β Maaliene	204	$C_{15}H_{24}$	0.104
15	29.694	Cis-α-Bisabolene	204	$C_{15}H_{24}$	0.4
16	30.03	α Patchaulene	204	$C_{15}H_{24}$	0.158
17	30.34	Isoaromadendrene epoxide	220	$C_{15}H_{24}O$	0.252
18	30.862	Caryophyllene oxide	220	$C_{15}H_{24}O$	0.709
19	31.617	Tetramethyloxabicyclododecadiene	220	$C_{15}H_{24}O$	0.181
20	33.003	Juniper camphor	222	$C_{15}H_{26}O$	0.382
21	33.286	Humuladienol	222	$C_{15}H_{26}O$	0.115
22	36.082	7 Isopropenyl 1, 4a dimethyl hexahydronapthelen-one	218	$C_{15}H_{22}O$	0.334
23	55.592	Lup-20(29)-en-3-one	424	$C_{30}H_{48}O$	15.241
24	55.812	Octamethyloctadecahydro picen-3-one	424	$C_{30}H_{48}O$	6.951
25	56.043	Olean-18-ene	410	$C_{30}H_{50}$	1.646
26	56.116	β Amyrin	426	$C_{30}H_{50}O$	0.172
27	56.238	Cycloursan-3-one	424	$C_{30}H_{48}O$	0.248
28	56.377	Octamethyloctadecahydro picen-3-one	424	$C_{30}H_{48}O$	5.69
29	56.689	α Amyrin	426	$C_{30}H_{50}O$	2.25
30	57.241	D:C-Friedours-7-en-3-one	424	$C_{30}H_{48}O$	0.231
31	57.918	Lupeol	426	$C_{30}H_{50}O$	0.367
32	58.32	Nor dioxo β amyrindidehydrodehydroxy	408	$C_{28}H_{40}O_2$	0.702
33	58.77	Lup-20(29)-en-3-one	424	$C_{30}H_{48}O$	8.156
34	59.045	Urs-12-en-28-al,3-(acetyloxy)-,(3β)-(Oxoursenyl acetate)	482	$C_{32}H_{50}O_3$	1.839
36	60.037	Urs-12-en-28-al	424	$C_{30}H_{48}O$	7.629
37	61.717	3-β-Myristoyl olean-12-en-28-ol	652	$C_{44}H_{76}O_3$	0.866

roxburghii resin in the present study is compared with that of resin samples of *S. robusta* species (Yusuf and Srinivasan, 2015). A range of triterpenoids such as ursolic acid, dihydroxyursenoic acid, dihydroxyoleanenoic acid, trihydroxyursenoic acid, 28-nor-urs-12-en-3β-ol, ursaldehyde, ursaldehyde acetate and 3β-acetoxy-28-nor-urs-12ene in *S. robusta* resin were reported by Hota and Bapuji (1994). In another study, triterpenoids such as ursolic acid, α and β amyrin, mangiferonic acid, benthamic acid, btuse acid, α -amyrenone and uvaol in *S. robusta* resin were reported (Misra and Ahmad, 1997). Similarly, β-amyrin, α-amyrin, Humulane-1, 6-dien-3-ol and Lupeol were identified in *S. robusta* resin apart from caryophyllene and other sesquiterpenes by Sushma *et al*. (2017). The resins of both *S. roxburghii* and *S. robusta* have common compounds in the sesquiterpenoid group as well. Cubebene, elemene, α and β caryophyllenes and caryophyllene oxide among other sesquiterpenes were also reported in the *S. robusta* resin essential oil sample (Kaur *et al*., 2001). However, sesquiterpenes α Panasinsen and Isopropenyl dimethyl octahydro-napthalene (selinene) are unique to *S. roxburghii* resin.

It is worth noting that the complete characterization of the samples and absolute assignment of the identity of the components is not possible with GC-MS analysis alone. Further isolation and characterization of individual compounds by other chromatographic and spectroscopic techniques is required. The current study reports the unique terpenoid profile of *S. roxburghii* resin based on GC-MS analysis. Some of these compounds can be isolated and explored for various applications.

Terpenoids and other plant secondary metabolites possess varied biological activities. Extracts from different parts of *S. roxburghii* have been evaluated for their health applications. Hypoglycemic and hypolipidemic effects of *S. roxburghii* stem extracts on rats have been reported by different studies (Makinde *et al*., 2020; Zhang *et al*., 2020). Similarly, the antimelanoma effect and hepato protective effect of *S. roxburghii* stem extract and protective effect of *S. roxburghii* leaves extract on diabetes-induced testicular damage in rats have also been reported (Moriyama *et al*., 2018; Ninomiya *et al*., 2017; Zhao *et al*., 2020). In this way, different crude extracts from *S. roxburghii* resin and their purified constituents can also be evaluated for their therapeutic effects. Natural antioxidant components of the resin may find use in nutraceutical formulations. Antioxidant activity has been reported from different extracts and Hopeaphenol from *S. roxburghii* using in-vitro assays (Subramanian *et al*., 2013, 2015). Further studies can be done on the isolation of individual components from *S. roxburghii* resin, their chemical characterization and evaluation of bioactivity.

Conclusion

S. roxburghii resin differed significantly in physicochemical properties from *S. robusta* resin. The presence of sesquiterpenes such as α Panasinsen and Isopropenyl dimethyl octahydronapthalene (Selinene) separates *S. roxburghii* from resins of other related species. Thus, owing to its distinct physicochemical properties and unique terpenoid profile, the *S. roxburghii* resin may find use in various industrial applications. Further studies are required for the development of new products and applications of *S. roxburghii* resin to promote its commercial tapping.

दक्षिण भारत के उष्ण कटिबंधीय पर्णपाती वन के *शोरिया रॉक्सबर्गी* - लाख कीट मेजबान वृक्ष के सुगंधित राल का विश्लेषण

सौरभ स्वामी, अरुमुगम मोहनसुन्दरम और वैभव डी. लोहोत

सारांश

तालुरा वृक्ष की छाल से निकलने वाले प्राकृतिक रेजिन (राल) में एक अनोखी खुशबू होती है। इस अध्ययन में हमने तालुरा रेजिन के भौतिक-रासायनिक गुणों और रासायनिक संघटन का विश्लेषण किया है। तालुरा रेजिन के भौतिक-रासायनिक गुण जैसे गलनांक, राख सामग्री, वाष्पशील पदार्थ और एसिड संख्या ज्ञात कर उसकी साल वृक्ष के रेजिन के साथ तुलना की गई। इस राल की सुगंध से संबंधित रासायनिक यौगिकों और समूहों का निर्धारण गैस क्रोमैटोग्राफी मासस्पेक्ट्रोमेट्री (जीसीएमएस) द्वारा किया गया था। परिणामों से पता चला कि साल रेजिन की तुलना में तालुरा रेजिन में उच्च वाष्पशील पदार्थ (3.21%) और कम गलनांक (64-65^{0}C), राख सामग्री (0.14%) और एसिड संख्या (16.88) है। जीसीएमएस द्वारा तालुरा रेजिन में कुल 37 यौगिकों की पहचान की गई। इनमें मोनोटेरेपेनोइड्स, सेस्क्यूटरपेनोइड्स और ट्राइटरपेनोइड्स शामिल थे। इनमें सेस्क्यूटरपीन हाइड्रो कार्बन समूह प्रमुख है जिसमें β एलीमीन, β कैरियोफिलीन, β फारेनसीन, सेलिनेन, पैनासिनसेन आदि शामिल है। तत्पश्चात ट्राइटरपेनोइड्स समूह में ल्यूप-20(29)-एन-3-वन, β एमिरिन, α-एमिरिन, उर्स-12-एन-28-अल और अन्य शामिल है। तालुरा रेजिन में टेरपिनिल एसीटेट और कैरियोफिलीन ऑक्साइड जैसे ऑक्सीजन युक्त यौगिकों की भी पहचान की गई। विभिन्न औद्योगिक अनुप्रयोगों के लिए तालुरा रेजिन के रासायनिक घटकों को और भी पृथक करके विश्लेषित किया जा सकता है।

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