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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF RESINS OF *Boswellia serrata* Roxb., *Canarium strictum* Roxb., AND *Shorea roxburghii* G. Don

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Abstract

Resins are amorphous mixture of essential oil, oxygenated products of terpenes and carboxylic acids which are obtained as exudates from plants. They are considered as end products of metabolism. Terpenoids are secondary metabolic products which once were considered unimportant. Resins have many pharmaceutical applications and some of them are used as incense. The present study was aimed to study and reaffirm the phytochemical constituents and some of the pharmacological uses of some resin yielding plants. Collection of the resins from plants such as *Boswellia serrata* Roxb., *Canarium strictum* Roxb., and *Shorea roxburghii* G. Don., from Anchetty forest (Krishnagiri District)., Kolli hills (Namakkal District) and Sathyamangalam forest (Erode District). Phytochemicals (secondary metabolites) present in the resin complex of the 3 species were analyzed qualitatively. It was observed from the results that the resin of *Canarium strictum* showed more antioxidant activity (45 %) than the gum-resin of *Boswellia serrata* (20 %) and oleo-resin of *Shorea roxburghii* (26 %). The resin of *Boswellia serrata* and *Canarium strictum* showed antibacterial activity against the organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zone of growth inhibition (in mm) was not of much difference between the extracts of low concentration (25 µl) and high concentration (50 µl). The resin extract of *Shorea roxburghii* did not show any activity against the two pathogens (no zone of growth inhibition was formed).

Key words: *Canarium strictum, Boswellia serrata, shorea roxburghii,* Resins, Terpenoids, phytochemical, Antioxidant, Antimicrobial activity.

1. Introduction

Plants belonging to important families of flowering plants and the uses of plant parts and products in the manufacture of incense and essential oils. According to him, the members of the family Burseraceae are of little importance domestically. However, they are very rich in gums and resins of considerable value in world markets, notably frankincense and myrrh of biblical fame from *Boswellia* and *Commiphora* respectively. George and Lawrence (1967) and Pandey (1978) give a long list of plants which yield resins. The plants mainly belong to the phanerogamic families such as Anacardiaceae, Burseraceae, Dipterocarpaceae, Guttiferae, Hamamelidaceae, Leguminosae, Liliaceae, Styracaceae, Umbelliferae and Pinaceae. They are found in the stems of the trees and occur in abundance in special canals or ducts. Unlike gums, resins are insoluble in water, but more or less soluble in ether, alcohol and turpentine. They are brittle, amorphous and transparent. They possess lustre

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and burn with a smoky flame when ignited. The resin normally oozes out through bark and hardens on expose to the air. Usually tapping was necessary in order to obtain a large amount which has commercial value.

The resins of *Ailanthus malabarica* when burned gives out a fragrance. It was used as incense. The sweet scented gum- resin of *Boswellia serrata* known as salai gugul was burnt in religious ceremonies. The gum resin of *Commiphora mukul* known as Gugul or as an Indian Bdellium exudes from incisions made on the bark in cold season. The resin from *Liquidamber orientalis* was known as *Storax* and it was used as a fixative for heavy perfume, in the preparation of adhesives, lacquers and incense. The oleo- resin from *Shorea robusta* known as Sal Dammar or Bengal Dammar was widely used as an incense, especially as an ingredient of havan samagri which was burnt in religious ceremonies and cremation rites. The resin of *Styrax benzoin* was known as the true Benzoin or Gum Benjamin of commerce. It was burnt as an incense. The resin of *Vateria indica* was used as incense, commonly called as white dammar. The red gum of *Terminalia tomentosa* was used as incense and as a cosmetic.

Wallis (1985) has given a detailed account about resin formation. The resins are secreted by cells which may be external in the form of trichomes or in cells under the cuticle. It was secreted in internal cavities embedded in a mass of tissue. The secreting cells are unicellular or multicellular. The shape may be globular, ovoid. In some trees, the cells between the oleo-resin ducts breaks down and a lysigenous cavity disintegrate. The cell walls of the disintegrated cells are converted into resinous matter. They leave the secretion surrounded by a layer of secreting cells. Some remains of cell walls may be attached as in the glands of orange peel. phytochemical analysis, *in vitro* antioxidant and *in vitro* inflammatory activity of leaf extract of *Boswellia serrata.* The results revealed the presence of saponins, tannins, anthraquinones, terpenoids and flavonoids. His results were an indication of antioxidant potential of the leaf extract of *Boswellia serrata* (Afsar *et al.,* 2012).

2. Materials and Methods

Sample Collection

Resins are found as exudations from the trunk of the tree. It normally oozes out through the bark and hardens on exposure to the air. But to obtain a large amount, tapping was done. Incisions were made with the help of sharp knife on the bark (about 1 to 2 cm deep), after few days the resins were collected which oozed out from the bark incisions. The resins were collected and immediately packed in air tight plastic containers.

Solvent extraction method

Thirty grams of dried resins of *Boswellia serrata* and *Shorea roxburghii* were extracted with 70 % of methanol and the resin of *Canarium strictum* was extracted with acetone using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatman No.1 filter paper and used for estimation of phytochemicals and antibacterial activity.

Phytochemical Analysis

The phytochemicals in each sample was determined qualitatively by following the standard method (Odebiyi and Ramstard, 1978; Waterman 1993).

Test for Tannins

To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins

Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids

A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal was added and warmed. Then, 5

to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

Test for Steroids (Salkwoski's test)

Five drops of concentrated sulphuric acid (H_2SO_4) was added to 2 ml of each extract and observed for red coloration.

Test for Glycosides

To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Alkaloids

To 4 ml of extract filtrate, a drop of Mayer's reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones

One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl_4 then CCl_4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds

Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Quantitative Analysis

Tannins

A quantity of 500 mg of plant sample was weighed and transferred to 50 ml flask. Then, 50 ml of distilled water was added and stirred for 1 hrs. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm wavelength within 10 min (Harborne *et al.*, 1973; Tyler, 1994).

Saponins

Twenty gram of each ground plant samples were put into a conical flask and 100 ml of 20 % ethanol was added to the plant sample. The said sample is heated over a water bath for 4 hrs at about 55 °C with continuous stirring. The extracted mixture was then filtered and the residue was then re-extracted again with 200 ml of 20 % ethanol. The collective residues are reduced to 40 ml over a hot water bath. The concentration was then transferred to a separating funnel and 20 ml of diethyl ether was added to the plant extract and shaken vigorously. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. 60 ml of n- Butanol was added and combined n-Butanol extract were washed twice with 10 ml of 5 % sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.

Flavonoids

Ten gram of the plant sample was extracted with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight (Mattila and Hellstrom, 2007).

Alkaloids

Five grams of the plant sample was prepared in a beaker and 200 ml of 10 % Acetic acid (CH_3CO_2H) in ethanol (C_2H_5OH) was added to the plant sample. The mixture was covered and allowed to stand for 4 hrs. The mixture was then filtered and the extract was allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid, which was then dried and weighed.

Phenols

Plants sample was boiled for 15 min with 50 ml of Diethylether $(CH_3CH_2)_2O$. Five ml of the sample was pipette into 50 ml flask and 10 ml of distilled water was added. Then, 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol (CH_3 $(CH_2)_3CH_2OH$) was added to the mixture. The sample was made up to the mark and left to react for 30 min for colour development and measured for 505 nm wave length using a spectrophotometer (Tyler, 1994; Harborne *et al*., 1973).

Antioxidant activity (DPPH free radical scavenging activity)

The antioxidant activity of the fruit extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1 - 100 µg/ml solution. 0.002 % of DPPH was prepared in all the solvent and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Solvent like Methanol, Chloroform and Aqueous (1 ml) with DPPH solution (0.002 %, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below (Wong, *et al*., 2005).

$$\text{Percentage (\%) inhibition of DPPH activity} = A - B \times 10/A$$

Where, A = optical density of the blank and B = Optical Density of the sample.

Antimicrobial activity by Disc diffusion method

On the first day, the organisms to be tested (Gram negative bacteria, *Pseudomonas aeruginosa* and Gram positive bacteria, *Staphylococcus aureus*) were cultivated on a suitable agar medium under optimal incubation conditions to obtain a fresh overnight grown culture. Tubes containing 5 ml sterile suitable broth were prepared. The second day activities involved harvesting of a number of distinct colonies from the fresh grown plate culture. They were suspended in the tubes containing broth until turbidity (visually) corresponding to 1.0 Mc Farland standard was reached. Sterile cotton swabs were dipped into the adjusted cultured medium and squeezed. Lawn culture was made on Mueller Hinton agar media. The plates were allowed to dry for 15 minutes. Longer drying times allow pre-incubation of the cells which should be avoided. The plates were incubated soon after the application of the discs. Using sterile forceps, the discs (test compound loaded) were applied onto the agar surface. The plates were incubated under the optimal incubation conditions. On the third day, the diameter of the inhibition zones was measured to the nearest mm from the point of abrupt inhibition of growth using mm ruler.

Survey made to assess the use of incense by the people

The resins of the three plants *(Boswellia serrata, Canarium strictum* and *Shorea roxburghii)* are used as incense by the local people and also sold commercially, these are the natural incenses. But, in the market many synthetic types of incense are sold. A survey was made to study

the use of incense (natural or synthetic based) by the people. About 50 persons belonging to different age groups were interviewed on the basis of a Questionnaire. Ten persons belonging to the area around Anchetty reserve forest (Krishnagiri district), 10 persons belonging to the area around Sathyamangalam forest area (Erode district), 10 persons belonging to Salem, 10 persons of Kolli hills 10 persons of Velur (Namakkal district) were interviewed. The questions were related to the use of natural incense and health problems (if any) faced by the people on exposure to the smoke produced by the incense. The use of incense at home, functions, ceremonies and religious uses were studied.

3. Result and Discussion

The present study was made in the resins (gum resin/oleo resin) of three plant species namely *Boswellia serrata*, *Canarium strictum* and *Shorea roxburghii*.

Qualitative analysis

The Table - 1 represents the presence or absence of secondary metabolites in the resins of the three plant species chosen for the study. The results showed that out of the three species studies, *Canarium strictum* possessed more abundant tannins, saponins and glycosides in its resin. The phenolic compounds were found to be normal in quantity and alkaloids were quite less. The flavanoids, steroids and anthroquinones were totally absent in all the three species. Alkaloids were absent in *Boswellia serrata* and present in lesser quantity in *Shorea roxburghii*. The presence of tannins, saponins, glycosides and phenolic compounds were more or less same in both the species *Boswellia serrata* and *Shorea roxburghii.*

Quantitative analysis

The Table - 2 represents the amount of secondary metabolites (mg/g) present in the 3 species chosen for the study. From the results it was very clear that, *Canarium strictum* had a good amount of tannin, saponins, glycosides and phenolic compounds when compared to *Boswellia serrata* and *Shorea roxburghii* in their resins.

Antioxidant activity

The antioxidants were analyzed and the results of the antioxidant activity of the resins of the 3 species of the study (DPPH free radical scavenging activity) are shown in the Table - 3. It was observed from the results that the resin of *Canarium strictum* showed more antioxidant activity (45 %) than the gum-resin of *Boswellia serrata* (20 %) and oleo-resin of *Shorea roxburghii* (26 %).

Antimicrobial activity by disc diffusion method

The resin of *Boswellia serrata* and *Canarium strictum* showed antibacterial activity against the organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table - 4). The zone of growth inhibition (in mm) was not of much difference between the extracts of low concentration (25 µl) and high concentration (50 µl). The resin extract of *Shorea roxburghii* did not show any activity against the two pathogens (no zone of growth inhibition was formed).

Questionnaire on the usage of incense

Questionnaire conducted in Anchetty (Krishnagiri District)

Ten persons were questioned about the usage of incense. It was interesting to note that all of them used both natural (resin of *Boswellia serrata*) and synthetic incense at home to worship God and experience the aromatic odour of the fumes of resin when burnt. All of them were satisfied in using both the natural and commercial synthetic incense sold in the market but they found the natural resin had more fragrance and the fumes were more pleasing. Nobody faced any health problem in the form of irritation to eyes or breathing troubles. Most of them (7 persons) were using the incense for more than ten years. This shows that the use of incense was traditional and dates back to many years in the history. Of the 3 species chosen for the study, only *Boswellia serrata* plant was found in Anchetty reserve forests and that too few in numbers. The local people make incisions on the trees and collect the resins that exude in few weeks.

Questionnaire done in areas near by Sathyamangalam reserve forest (Erode District)

Out of the ten persons interviewed nine of them were using only natural incense (oleo-resin of *Shorea roxburghii*). Only one person used synthetic incense sold in the market. Out of the ten persons, three of them were using incense for more than ten years and one person for more than five years, three of them, two to five years and rest three persons were using the incense for one to two years. All the ten persons were satisfied with the use of incense (natural or synthetic) and faced no health problems with the use of incense.

The local people collected the oleo-resin from the incisions made on the bark of *Shorea roxburghii* growing in the slopes of Sathyamangalam reserve forests in groups. Though the plants are rare in occurrence, the local people collect the incense and sell them in the local market (Rs 300 per kg.).

Questionnaire put forth to ten persons of Kolli hills (Namakkal District)

The answers for the questions put forward to ten persons of Kolli hills area randomly revealed that all of them use incense at home with satisfaction and with no health problems faced. Most of them are using incense for more than ten years. All of them use both natural and synthetic incense. *Boswellia serrata* and *Canarium strictum* trees are the sources of natural incense in this area. They use synthetic incense when the natural incense was unavailable. The resins of *Boswellia serrata* and *Canarium strictum* are sold in the local market in the name of "kungilam" and "karuppu kungilam" respectively which are used as incense by the local people.

Questionnaire put forward to ten people of Velur (Namakkal District)

From the questionnaire, it was found that most of them were using only synthetic incense satisfactorily for more than ten years without facing any health problems. Few of them (three) use both natural and synthetic incense. The natural resin of *Boswellia serrata* was sold in the markets which are collected from Kolli hills.

Questionnaire made to persons of Salem District

From the questionnaire, it was clear that only synthetic incense (unnatural) was available in the market. Though, trees like *Boswellia serrata* are found in Yercaud, the nearby hill station of Salem, the natural resin was not collected from the tree and sold in the market for the use of incense as the trees are only few in numbers. All the ten persons were interviewed and use the synthetic incense for more than ten years satisfactorily without any health problems. From the questionnaire made to fifty persons of different areas, the overall results show that the use of incense (natural or synthetic) was satisfactory to all of them and they faced no health problems. Natural incense was commonly used in places where the plants (*Boswellia serrata*, *Canarium strictum* and *Shorea roxburghii*) which yield the resins are available. In places like Velur and Salem, the plants are unavailable and therefore they had to use only synthetic incense which is available in the local market.

Religious use of incense

In all the five places where the questionnaire was put forward to persons, mostly incenses are used during Poojas (by the Hindus) and Prayers (by the Christians). The people of all the three major religions (Hinduism, Christianity and Islam) use incense as a traditional practice and believed so spiritual. The resins of the 3 species of study are indirect burning resin also called as "non-combustible incense". Charcoal is used as the heat source which was placed in a censer. *Boswellia serrata* also sometimes referred to as Indian Frankincense (Gerbeth *et al*., 2011) and in accordance with this name it has sometimes been traditionally burnt at ceremonies (believed to contribute so spiritual exaltation) (Moussaieff *et al*., 2008). These statements were confirmed by the present study when 50 people of 5 different areas of Tamil Nadu were interviewed regarding the use of incense. The gum-resin of *Boswellia serrata* called "Sambirani" has been traditionally burnt

during Poojas and other religious ceremonies by the local people, nearby the forests.

Siddiqui (2011) has mentioned about the active compounds of *Boswellia serrata*. The oleo-gum resin was the exudates of the tree collected by making incision into the tree. The traditional preparation of the gum includes a month - long waiting period. The gum hardens when the water leaves which results in a final product of 30 % - 60 % resin, 5 – 10 % essential (and aromatic) oils (mostly monoterpenes such as E-beta-ocimene and Limonene), with the final 30 – 35 % consisting of polysaccharides. In the present study too, the collection of the resin from the tree was done by making incisions into the tree. This method is followed by the local people (Anchetty, Krishnagiri District) over long time. The resin exudates were collected few weeks after the incisions were made.

Augustine *et al*. (2006) have described about the resin of *Canarium strictum* which was called as "Sambrani" or "Dammar". This resin has medicinal as well as commercial uses which were used by tribal and folk people of different parts of India. This was explored through ethnobotanical studies. It was also used in Siddha system of medicine and also used as incense and in varnish industries. It was found from the present study too that the resin exudates of *Canarium strictum* were also used as incense and has commercial values and medicinal properties.

Varghese and Ticktin (2008) have described the three modes of resin collection from the trees of *Canarium strictum*. The collection of resin from the naturally formed fissures on the tree is the first type. The second type was largely employed by the harvesters. They make incisions with curved iron knives and collect the exuding resin. The third type involves setting a low fire at the base of the tree followed by incisions and resin collection. In the present study too, the resin was collected by making incisions into the trees of *Canarium strictum* and also collected from the natural flow through the fissures present on the trees. But, the third type was not followed by the local people (Kolli hills, Namakkal District) for collecting the resin.

In the previous studies, Suruse *et al*. (2010) have said about *Canarium strictum* Roxb., (Burseraceae) which was used in the traditional Ayurvedic medicine under the name Raladhupa and black dammar resin. Two compounds, A and B were isolated by counter current distribution method carried out from chloroform extract. The preliminary phytochemical screening of each compounds showed the presence of triterpenoid. The two compounds (A and B) were investigated for antibacterial and antifungal activity by using Cup plate method or Diffusion agar method. The results showed that both the compounds (A and B) possessed broad-spectrum antimicrobial activity at concentration of 100 µg/ml. The inhibitory effect of each compound was very close and identical for Gram positive, Gram negative bacteria and fungi. In the present study, the antimicrobial activity of the crude extract of the resin was screened against Gram negative (*Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus)* bacteria. The microbial growth inhibitory effects were proved positive.

In the previous studies, Solomon Raju *et al*. (2011) have described *Shorea roxburghii* as an Endangered semi evergreen tree species restricted to Peninsular India in the Eastern Ghats. The annual events include leaf shedding and flushing while flowering was not an annual event for this tree. When the tree flowers in March, it shows massive blooming. The study suggests that non-annual flowering, massive flowering for a short period, high bud/flower and fruit infestation rate, absence of seed dormancy and rocky habitat could attribute to the endangered status of *S. roxburghii*. In the present study, the resin was collected from the trees of *Shorea roxburghii* by making incisions into the tree. The trees are found in groups on the slopes of Sathyamangalam reserve forest (Erode District) in the Eastern Ghats. Throughout the period of study (one year), frequent visits were made to the study area but the plants were not found in flowering which confirms that the flowering of the tree was non-annual. The gregarious trees are not common, restricted to certain areas only.

Table - 1: Phytochemical analysis of medicinal plants

S. No	Phytochemicals	*Boswellia serrata*	*Shorea roxburghii*	*Canarium strictum*
1.	Tannins	+	+	+++
2.	Saponins	+	+	+++
3.	Flavonoids	-	-	-
4.	Steroids	-	-	-
5.	Glycosides	+	++	+++
6	Alkaloids	-	+	++
7	Anthroquinones	-	-	-
8	Phenolic compounds	+	+	++

+++ = Dark colour reaction, ++ = Medium colour reaction, + = Pale colour reaction, - = Absent

Table - 2: Quantitative analysis (phytochemical analysis)

S. No	Phytochemicals	*Boswellia serrata* (mg/g)	*Shorea roxburghii* (mg/g)	*Canarium strictum* (mg/g)
1.	Tannins	0.09	0.05	0.10
2.	Saponins	0.04	0.048	0.12
3.	Flavonoids	-	-	-
4.	Steroids	-	-	-
5.	Alkaloids	-	0.010	0.09
6	Glycosides	0.024	0.052	0.10
7	Phenolic compounds	0.012	0.020	0.85

Table - 3: Antioxidant activity

	Boswellia serrata	*Shorea roxburghii*	*Canarium strictum*	Ascorbic Acid
DPPH Assay	20 %	26 %	45 %	11 %

Table - 4: Antibacterial Activity

S. No	Sample Marking	Zone of inhibition (mm in dm)			
		S. aureus		*P. aeruginosa*	
		L (Low Conc.)	H (High Conc.)	L (Low Conc.)	H (High Conc.)
1	C (Control) (70 % Methanol)	NA	NA	NA	NA
2	*Boswellia serrata* Roxb. Burseraceae	4.5 mm	5.5 mm	4 mm	5.5 mm
3	*Shorea roxburghii* G. Don Dipterocarpaceae	NA	NA	NA	NA
4	*Canarium strictum* Roxb. Dipterocarpaceae	4.5 mm	6 mm	4.5 mm	5.5 mm

NA – No Activity

4. Conclusion

Resin, a secondary metabolite (terpenes), found as exudations from the trunk of various trees are useful to humankind in many ways. Resin is considered to be one of the important products of plants. Not all plants yield resin. Only few plants such as *Boswellia serrata*, *Canarium strictum*, *Commiphora* sp., *Pinus sylvestris* and *Shorea roxburghii* are sources of resins (gum-resin/oleo-resin). Most of the resins yielding plants were found in hills. The gregarious tree *Shorea roxburghii* found in the slopes of hills was considered to be an endangered species. So, it is essential to take steps for the multiplication of this species. Resins have commercial value, being used as incense (non-combustible, indirect-burning), have antioxidant, antimicrobial properties and many other medicinal uses. The pharmacological activities of the resins are supported by empirical results.

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