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The Cause of Differential Resistance of *Pterocarpus* Wood to some Tropical Polypores

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The Cause of Differential Resistance of *Pterocarpus* Wood to some Tropical Polypores

Summary

An attempt was made to ascertain the probable cause of differential decay resistance of wood of *Pterocarpus marsupium* to five tropical polypores. Heartwood saw dust was extracted separately with methanol, ether and petroleum ether and the efficacies of these extractives were tested on corresponding extractive free sapwood. Petroleum ether extractive appeared to be most effective in conferring resistance to wood. Resistance of sapwood decreased considerably after removal of petroleum ether solubles but partial resistance was regained when solubles were added to it. Again, the resistance of extractive free sapwood increased proportionately when treated with increased concentrations of petroleum ether extractive up to a 3% level. Three phenolic compounds were detected in the said extractive by TLC but none of these could be accounted for the differential resistance of wood. Of the three, catechol was found to be most fungitoxic.

Schlüsselwörter (Sachgebiete)
Pilzresistenz
Holzbefall
Phenolische Extraktstoffe
Splintholz
Kernholz
Padauk
Polyporus Arten

Ursache der unterschiedlichen Resistenz von Padauk gegenüber tropischen Polyporus-Arten

Zusammenfassung

Die Ursache der unterschiedlichen Widerstandsfähigkeit von Padauk bei Pilzbefall durch tropische Polyporus-Arten wurde untersucht. Holzmehl aus dem Kernholz wurde mit Methanol, Äther und Petroläther extrahiert und die Wirksamkeit dieser Auszüge wurden an entsprechenden extraktfreiem Splintholz geprüft. Petrolätherauszüge erwiesen sich am wirksamsten. Wenn die petrolätherlöslichen Stoffe entfernt wurden, nahm die Resistenz des Splintholzes beträchtlich ab, beim Hinzufügen dieser Stoffe wurde eine Teilresistenz wieder hergestellt. Die Resistenz von extraktfreiem Splintholz nimmt proportional der Konzentration von Petroläther bis 3% zu. Drei phenolische Verbindungen konnten durch TLC isoliert werden, aber keine davon konnte für die unterschiedliche Widerstandsfähigkeit des Holzes verantwortlich gemacht werden. Katechol besaß von den 3 Verbindungen die größte fungizide Wirksamkeit.

Introduction

There is evidence that sometimes the natural durability of timber species depends on the concentration of toxic extractable substances of wood formed during the formation of heartwood. This concept was first put forward by Hawley et al. (1924) and later by others (Karrer 1958; Rudman 1962; 1965). Hot water extractives of African mahogany (Findley 1957), methanol extractives of tallow wood (Da Costa and Rudman 1958), ether and methanol extractives of teak (Rudman and Da Costa 1959; Rudman 1961) are known to contain substances with antifungal activities.

The purpose of this study is to ascertain the probable cause underlying the durability of wood of *Pterocarpus marsupium* to decay caused by *Daedalea flavida* Lev., *Pycnoporus sanguineus* L. ex Fries, *Coriolopsis* sp., *Trametes cingulata* Berk and *Flavodon flavus* KL. Ryv. *P. marsupium* is an important timber yielding plant in India.

Materials and Methods

For the preparation of wood extractives wood sample was prepared following the method abstracted by Browning (1967) from "TAPPI Standard T 11 m".

Preparation of wood extractives

A sample (8 g) of air-dried, finer saw dust (heartwood) of *P. marsupium* was extracted in a soxhlet with a known volume of methanol for a period of 8 hr. as suggested by Browning (1967). Similarly, test samples were also extracted separately with ethanol, ether and petroleum ether. The combined extractives in each case were separately concentrated in a rotary film evaporator at room temperature (25°C). The residues were dried at 60°C, weighed and dissolved in a known volume of desired organic solvent and tested for its antifungal activity.

Bioassay of Wood extractives (Fig. 1)

To test the antifungal activity of heartwood extractives, the saw dust dish soil jar technique of Da Costa and Rudman (1958) was followed with modifications.

Four gramme sample of extracted sapwood meal (extracted with the desired organic solvent) was mixed thoroughly with 7.5 ml of heartwood extractives and packed tightly in a weighing bottle, left for 2 days for uniform distribution of extractives throughout the sapwood meal and also for reacting with the wood components. The treated sapwood meal was dried at 40°C with frequent stirring. Finally, 0.4 g of dried sapwood meal was transferred to perforated aluminium foil cups (2.5 cm diameter, 2 cm high), and sterilized by fumigation with propylene oxide. Prior to sterilization, the moisture content of the sapwood meal was raised to about 15% for stimulating fungal growth. These cups were exposed to each test fungus growing

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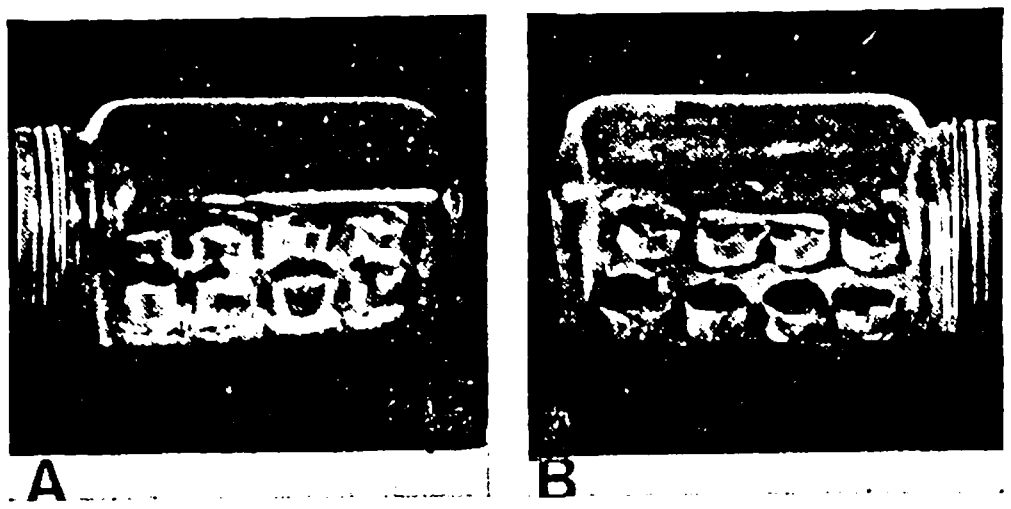

Fig. 1. Bioassay test of Wood extractives using saw-dust — soil jar technique.
A — showing mycelial growth of *D. flavida* on untreated sapwood dust of *P. marsupium* in aluminium foil cups in the soil jar.
B — untreated, non-inoculated sapwood dust in the aluminium foil cups in the soil jar.

on thick filter paper discs (3.5 cm diameter) in soil jars. The filter paper discs were initially soaked in a sterilized nutrient medium and mounted on moist soil (350 g soil/glass jar) in glass jars (21 × 10 × 10.5 cm) and inoculated with the test fungus. The soil jars were incubated at 28°—30° C for 5 weeks. The humidity within the soil jar was approximately 90—100% during incubation. At the end of the experimental period, the aluminium foil cups were taken out carefully, dried at 40° C and weighed. The amount of decay was expressed as the percentage of loss in oven dry weight.

Detection of Wood phenolics by thin layer chromatography (TLC)

Eight grammes of air dried heartwood saw dust were treated with 5% aqueous Na_2CO_3 solution (100 ml) for 4 hr. with frequent stirring. The extractives were filtered and the filtrate was acidified with 5% HCl. The acidified extractive (pH 5) was extracted thrice with an equal volume of solvent ether (b. p. 35° C). The ether fraction was evaporated to complete dryness in a rotary film evaporator at room temperature (25° C), the residue being dissolved in 1 ml of ethanol. Aliquots of the solution (30 μl) were applied to a thin layer chromatogram of silica-gel G (according to Stahl-type 60) on glass plates and developed two dimensionally using methanol:benzene:glacial acetic acid (90:60:8) as the first solvent and petroleum ether: chloroform:anhydrous ethanol (47.5:47.5:5) as the second (Stahl 1965). After drying, the chromatograms were sprayed with 1% aqueous $FeCl_3$ solution and dried. Some of the phenolics were identified by co-chromatography with authentic samples.

Table 2

Significance test of the petroleum ether extracted sapwood dust in relation to control

Fungus	Computed 't' for the difference of means	Whether significant 5%	Whether significant 1%	Remarks
D. flavida . . .	8.908	Significant	Significant	t = 2.776 (P = 0.05) t = 4.604 (P = 0.01)
P. sanguineus . .	1.830	Notsignificant	Notsignificant	
Coriolopsis sp. . .	2.770	-do-	-do-	
T. cingulata . .	1.252	-do-	-do-	
F. flavus	4.938	Significant	Significant	

Results

Effect of methanol, ether and petroleum ether extracts of heartwood on the decay resistance of sapwood

Both sapwood and heartwood dusts were extracted separately with methanol, ether and petroleum ether as described earlier. Methanol extracted sapwood was treated with the methanol extract of heartwood. Similarly, sapwood extracted with peroxide free diethyl ether (b. p. 34°—35° C) or petroleum ether (b. p. 40° to 60° C) was treated with the corresponding ether or petroleum ether extract of heartwood. Finally the resistance of both treated and untreated sapwood dusts to fungi was tested. It appears from the Table 1 that the resistance of sapwood decreased considerably after ether and petroleum ether extractives. But when petroleum ether extractive of heartwood was added to the petroleum ether extracted sapwood dust it conferred partial resistance to the test fungi. This extractive was comparatively more effective than both methanol and ether extractives and most and least toxic to *F. flavus*, *D. flavida* respectively. It is worthwhile to

Table 1

Effect of heartwood extractives on the decay resistance of sapwood of *P. marsupium*

Fungus	Average amount of decay as percentage weight loss with Standard Error: Methanol extracted sapwood dust — Untreated (Control)	Methanol extracted sapwood dust — Treated*)	Ether extracted sapwood dust — Untreated (Control)	Ether extracted sapwood dust — Treated**)	Petroleum ether extracted sapwood dust — Untreated (Control)	Petroleum ether extracted sapwood dust — Treated***)
D. flavida	32.73 ± 0.44	32.03 ± 1.36	32.27 ± 5.14	30.17 ± 2.93	40.30 ± 0.26	35.70 ± 0.85
P. sanguineus . . .	27.83 ± 5.99	27.20 ± 0.17	26.33 ± 1.02	25.60 ± 0.92	37.30 ± 4.13	30.80 ± 4.56
Coriolopsis sp. . . .	28.37 ± 3.78	27.00 ± 1.10	25.87 ± 1.52	29.17 ± 4.01	34.07 ± 4.76	28.57 ± 3.09
T. cingulata	30.07 ± 2.38	23.87 ± 1.46	25.03 ± 4.74	18.77 ± 4.04	27.60 ± 3.59	24.23 ± 2.27
F. flavus	33.60 ± 3.99	30.17 ± 1.97	29.60 ± 1.85	26.30 ± 4.33	22.50 ± 1.00	18.30 ± 1.08

*) Treated with 1% methanol extract of heartwood
**) Treated with 1% ether extract of heartwood
***) Treated with 1% petroleum ether extract of heartwood

Table 3

Effect of aqueous heartwood extractives before and after removal of petroleum ether soluble components on the mycelial growth of the test fungi

Fungus	Average diameter of mycelial mat (mm)								
	Agar + 1% heartwood extractives			Agar + heartwood extractives — Petrol ether soluble			Agar medium (Control)		
	4 days	8 days	11 days	4 days	8 days	11 days	4 days	8 days	11 days
D. flavida	25	46	60	30	60	75	38	60	88
P. sanguineus . . .	20	43	56	28	55	82	40	60	90
Coriolopsis sp. . . .	20	42	55	25	50	80	35	55	85
T. cingulata	16	28	38	20	40	65	27	45	70
F. flavus	15	25	28	40	65	85	45	75	95

Temperature 25 ± 1°C — 3 replicates/treatment

mention here that *D. flavida* caused maximum damage to the sapwood of *P. marsupium* while *F. flavus* was responsible for a minimum amount of decay. Methanol and ether extracts were also more or less toxic to *T. cingulata* and *F. flavus*.

The data in Table 2 show that the absolute differences in the averages are of similar magnitude but the nature of variability (S. E.) in *D. flavida* and *F. flavus* are markedly different from those of *Coriolopsis* sp., *P. sanguineus* and *T. cingulata*. This has been reflected in the computation of 't'. In case of *D. flavida* and *F. flavus* the control and treatment means differ significantly while in other cases (e. g., *Coriolopsis* sp., *P. sanguineus* and *T. cingulata*) these differences are not significant.

Effect of aqueous heartwood extractives before and after removal of petroleum ether soluble components

In order to determine whether the fungitoxic substance is more soluble in water or petroleum ether, the following experiment was carried out with one percent heartwood extractive which was obtained following the technique described under materials and methods. Fifty percent of the total volume of aqueous heartwood extractive was re-extracted thrice with similar volumes of petroleum ether. The petroleum ether and water fractions were then separated. One percent heartwood extract and water fractions of the same (after removal of ether solubles) were supplemented separately with 2% agar medium. Agar medium without any extract served as a control. The pH of each of the three media was adjusted to 5.5 prior to sterilization. Petridishes (20 ml/Petri dish) containing sterilized media were inoculated as usual and incubated at 25 ± 1°C. The results are given in Table 3.

All the test fungi grew well in the control agar medium where maximum and minimum growth were respectively exhibited by *F. flavus* and *T. cingulata*. The growth rate of all the organisms, however, markedly declined when the agar medium was supplemented with 1% aqueous heartwood extract of *P. marsupium*. The maximum inhibition of growth was observed in *F. flavus* while it was minimum in *D. flavida*.

It is interesting to note that the test fungi grew well (almost as in the control) in the agar medium containing 1% aqueous heartwood extract without petroleum ether solubles. In this case also *F. flavus* and *T. cingulata* showed maximum and minimum growth, respectively, like the control. It suggests that the antifungal substance is more soluble in petroleum ether.

Table 4

Effect of petroleum soluble components of aqueous heartwood extractive of *P. marsupium*

Fungus	% Weight Loss				
	Unextracted sapwood dust (Control)	Petroleum ether extracted sapwood dust (Untreated control)	Petroleum ether extracted sapwood dust treated with heartwood extractive*)		
			'X'	'X/2'	'X/3'
D. flavida	27.90	42.40	12.10	25.30	31.35
P. sanguineus	22.05	37.55	5.15	13.25	21.05
Coriolopsis sp.	21.55	34.40	5.05	12.40	20.10
T. cingulata	13.10	27.60	3.20	10.50	15.20
F. flavus	10.35	23.05	3.00	9.30	15.00

*) Petroleum extracted sapwood dust treated with different concentration of petroleum ether soluble components of aqueous heartwood extractive of *P. marsupium*

Effect of petroleum ether soluble components of aqueous heartwood extract of *P. marsupium* on the decay resistance of sapwood

One hundred ml of 1% aqueous heartwood extractive of *P. marsupium* were extracted thrice with equal volumes of petroleum ether (b. p. 40°—60° C), combined, evaporated to dryness in a rotary film evaporator and the residue dissolved in 5 ml petroleum ether. The quantity of residue dissolved in 5 ml of petroleum ether was considered as the 'X' concentration. Two dilution grades (X/2, X/3) were also prepared by adding appropriate volume of petroleum ether and tested for their antifungal activities. The results are embodied in Table 4.

The 'X' concentration was certainly more effective than the other two (Table 4). Fungi-toxicity gradually declined with dilution of the extractive. The results also confirm that petroleum ether extractive confers resistance to decay caused by test fungi as the extractive was most and least inhibitory to *F. flavus* and *D. flavida*, respectively.

Effect of different concentrations of petroleum ether extract of heartwood on the decay resistance of sapwood of *P. marsupium*

Four concentrations of petroleum ether extract of heartwood were prepared and supplemented separately to sapwood meal and their resistance tested against fungi as described earlier. The results are shown in Fig. 2. The percentage weight loss of sapwood was minimum when treated with a 3% extract but maximum in 0.5% irrespective of the fungi used.

Chromatographic separation of wood phenolics

For wood phenolics, heartwood dust was extracted and the phenolics were detected by thin layer chromatography (TLC) as described.

Three phenolics namely, gallic acid, phloroglucinol and catechol were identified by co-chromatography with authentic samples. Although only three phenolics were identified in the heartwood extract of *P. marsupium*, it is not unlikely that other phenolics could also be present. It is, however, often difficult to detect these compounds if they occur in very low concentrations. Besides, similar solvent systems may not always be effective for the detection of all compounds.

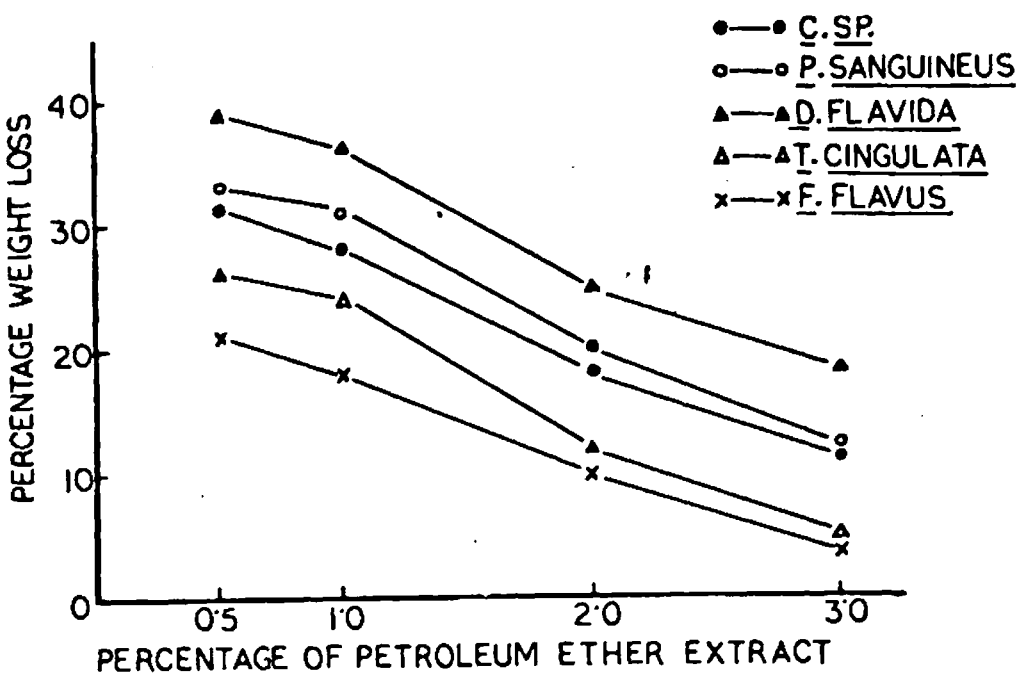

Fig. 2. Decay resistance of sapwood after treatment with different concentrations of petroleum ether extract of heartwood of *Pterocarpus marsupium* (Polyporous mycelia).

Effect of some wood phenolics on the mycelial growth of wood rotting fungi

Three phenolic compounds (viz., gallic acid, phloroglucinol and catechol) detected in *Pterocarpus* wood were tested on the growth of five wood rotting fungi: catechol (0.5%), phloroglucinol (0.5%) and gallic acid (0.5%) were separately sterilized and added to 2% malt agar medium before use.

Each Petri dish (100 mm. diam.) containing 20 ml of medium was inoculated with the test fungus and incubated at 25 ± 1° C in the dark for 4 days. The results are given in Table 5. Of the compounds tested, catechol appeared to be most fungitoxic. Total inhibition of mycelial growth of *F. flavus*, *C.* sp. and *P. sanguineus* occurred at the 0.5% level. For *D. flavida* and *T. cingulata* growth was very poor. Phloroglucinol was also inhibitory for the mycelial growth of *T. cingulata* and *C.* sp. while gallic acid was highly and moderately inhibitory to *F. flavus* and *Coriolopsis* sp., respectively. Although the aforesaid phenolic compounds markedly inhibited the growth of the five test fungi, these compounds could not be correlated with the differential resistance of wood to fungal decay.

Table 5

Effect of detected wood phenolics on the mycelial growth of wood rotting fungi Polyporous mycelia

Fungus	Average diameter of mycelial mat (mm)							
	Control		0.5% gallic acid		0.5% catechol		0.5% phloroglucinol	
	24 hr.	96 hr.	24 hr.	96 hr.	24 hr.	96 hr.	24 hr.	96 hr.
D. flavida	16	64	12	35	12	13	8	30
P. sanguineus	18	70	10	30	No growth	No growth	8	28
Coriolopsis sp.	15	60	10	28	No growth	No growth	8	20
T. cingulata	12	48	8	25	9	10	7	16
F. flavus	28	78	No growth	15	No growth	No growth	10	45

Temperature 26°—28° C — 3 replicates/treatment

Discussion

The durability of wood or its natural resistance to decay is always variable. The factors responsible for such variation are, however, numerous.

In the present investigation petroleum ether extractive of heartwood of *P. marsupium* conferred partial resistance to the wood against test fungi. The ether and methanol extracts also conferred little resistance to *T. cingulata*, but they failed to resist the other test fungi. It is significant to note that the sapwood extracted with methanol showed more or less uniform resistance against the test fungi, but when the sapwood was extracted with petroleum ether it exhibited different degrees of resistance to the test organisms as observed in the decay resistance test. This is not surprising since the major wood components including colouring matters are soluble in methanol while relatively few compounds are soluble in petroleum ether. Besides, the sapwood dust may not be entirely free from toxic compounds even after extraction with petroleum ether. The more important point here is that when methanol extract of heartwood was added to methanol extracted sapwood dust it gave little or no resistance against all test fungi excepting for *T. cingulata*. Similarly, when the ether extract of heartwood was mixed with the ether extracted sapwood dust, the result was more or less similar to that of the control. The failure of methanol or ether extract to resist decay may be due to a very low concentration of the toxic substance (resistance factor) in the heartwood extractive. Of the three extractives tested, the petroleum ether one appeared to be more encouraging and therefore, a number of experiments were carried out with this extractive only.

Hart and Hillis (1974) reported that growth inhibition of wood rotting fungi occurred with stilbenes and polyphenols in *Eucalyptus sideroxylon*. They found that the heartwood of *E. sideroxylon* was not decayed in vitro by *Polyporus versicolor* and *Poria monticola*. Methanol extracts of heartwood diluted 1000 times in 3% malt extract were toxic to *P. monticola* but not to *P. versicolor*. Again, heartwood blocks extracted with methanol retained their capacities to resist decay. The ether solubles rich in stilbenes and water soluble fractions (rich in ellagitannins) were inhibitory to both fungi in 3% malt extract at concentrations below those for both classes of compounds occurring in the original wood. These findings are very similar to those of the present investigation because certain substances which were toxic to both *P. versicolor* and *P. monticola* were absent in the methanol extracts but occurred in the ether as well as in water extractives. The substances toxic to all the test fungi in the present case, were present in sufficient amounts in water as well as in the petroleum ether extractives. The concentration of the inhibitory substance in the wood and that required for the growth inhibition of the invading fungi may not necessarily be similar in all cases. Hart and Hillis (1974) also emphasized that the concentrations of stilbenes and ellagitannins were much higher in the wood than those required for the suppression of the test organisms.

Hanover and Hoff (1966) compared the phenolic constituents of *Pinus monticola* either resistant or susceptible to *Cronartium ribicola*. They isolated a large number of phenolics from both the susceptible and resistant plants but one polyphenol possibly a guaiacyl derivative was found to be present in higher concentrations in the resistant ones.

In the present study 3 phenolic compounds namely, gallic acid, phloroglucinol, and catechol were isolated and identified. These compounds inhibited the growth of the test fungi significantly but their decaying capacities could not be correlated with their rates of growth in media containing any of the aforesaid phenolics (percentage reduction in growth was compared in relation to the control).

The different activities of the test fungi on *Pterocarpus* wood may perhaps, be due to the presence of other compounds besides, gallic acid, phloroglucinol and catechol.

King et al. (1953) studied the chemistry of the heartwood extractives of *Pterocarpus* spp. namely those of *P. dalbergioides*, *P. macrocarpus*, *P. soyanxii* and *P. tinctorius* and detected pterocarpin, homopterocarpin and pterostilbene compounds. These compounds were absent in *P. angolensis*. It was reported by Akisanya et al. (1959) that pterostilbene was strongly toxic to the brown rot fungus, *Coniophora cerebella*.

But Rudman (1963) held that pterostilbene was not the sole or even the major cause of decay resistance in those *Pterocarpus* spp. from which this compound was isolated.

In the present study no stilbene compound was detected in the extractive of *P. marsupium*. Hence no attempt was made to test the effect of this compound on the growth of the test fungi. It is also apparent from the results of Rudman (1963) that pterostilbene is non-toxic to a majority of the test wood-rotting fungi. However, the role of stilbenes in disease resistance of different plant species, remains unexplained (Ingham 1972). Ptetroleum ether extractive of *P. marsupium* heartwood contains a factor which is perhaps responsible for the differential resistance of wood to fungal decay. Further work is necessary for the characterization and identification of this active principle before any definite conclusion can be drawn.

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