

## Cuticular Wax of *Tectona grandis* L. Leaves – A Resistance Marker against Plant Pathogens

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### Abstract

The leaf surface of higher plants is composed of varied profile of hydrocarbons which play a vital role in chemical recognition and potentially involved in plant-pathogen interactions and communication with nature. There is little evidence to the direction that plant cuticle play a major factor in plant defense against pathogens besides imparting physical barrier to the plant. Teak (*Tectona grandis* L) is one such tree found with diverse geographical distribution. In the present work, our objective was to study the cuticular composition of the fresh leaves of teak which is responsible for its wide adaptation and its antimicrobial activity. Cuticular compounds from the hexane fraction of the teak leaves were isolated, purified and spectral analyses revealed a long chain unbranched heneicosane (C<sub>21</sub>) with a mol. wt. of 296. This saturated hydrocarbon forms a continuous layer on the leaf surfaces which act as physical barrier to the micro-organisms and also possesses a strong defending activity against a number of pathogens which implies its role in defensive mechanisms of the plant. The antifungal activity of heneicosane on each of the microbial species was statistically significant at P≤0.001 level. Its abundant recovery from teak leaves may justify its putative role as a resistance marker.

**Keywords:** Heneicosane; Plant cuticle; Plant-pathogen interactions; Resistance marker; Teak (*Tectona grandis* L).

**Abbreviations:** FAs: Fatty Acids; HFTk: Hexane Fraction of *Tectona grandis* leaves; TLC: Thin Layer Chromatography; MS: Mass Spectrometry, NMR: Nuclear Magnetic Resonance; FTIR: Fourier Infra-red

### Introduction

Plant cuticles are extracellular hydrophobic layers that cover leaves and many stems and fruits. It is deposited at the outermost extracellular matrix of the epidermis in primary tissues of terrestrial plants. Besides forming a protective shield against the environment, the cuticle influences plant-insect interactions, helps to prevent germination of pathogenic microbes, causes shedding of water droplets and dust particles as well as spores. It is also a source of signals used by invading fungi to activate pathogenic responses or by plants to induce defense mechanisms [1-3]. Recent observations have been made to reveal complex inter-relationships between cuticular lipids and plant defense [4].

Cuticular lipids consist of fatty acids (FAs). They are an important source of reserve energy and essential components of membrane lipids in all living organisms [5]. In plants, FA metabolic pathways play significant roles in pathogen defense. FAs were only assigned passive roles in plant defense such as biosynthetic precursors for cuticular components or the phyto-hormone jasmonic acid [6].

Cuticular wax serves the essential function of limiting nonstomatal water loss, and is therefore one of the key adaptations in the evolution of terrestrial plants. Epicuticular wax, because it is exposed at the outermost surface of plant organs, also plays important roles in the interactions of the plant with its environment. The wax surface influences plant-insect interactions, helps to prevent germination of pathogenic microbes, and causes shedding of water droplets and dust particles as well as spores [7,8].

Most plant-pathogenic fungi gain access into their host by penetration of unwounded tissue. The cuticle thus serves as the first surface barrier that the pathogen has to breach [9]. There is little evidence for the mere

physical strength of the plant cuticle as a major factor in plant defense against pathogens. In this context, teak was chosen for our work taking into consideration of its wide geographical distribution with changing topologies.

Teak (Family Verbenaceae) is a large deciduous tree 30-35-metre-tall with light brown bark [10], leaves simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots. Leaves are rough on the upper surface and hairy at the lower surface. Flowers are white in colour, small and have a pleasant smell.

It is one of the most important natural and manipulated forest tree in India [11] and also the source of one of the most valuable wood. It is native to India, Indonesia, Malaysia, Myanmar, northern Thailand, and north western Laos [12]. It is found in a variety of habitats and climatic conditions from arid areas with only 500 mm of rain per year to very moist forests with up to 5,000 mm of rain per year (Figure 1). Very little is known about chemical composition of cuticle of teak wax leaves and its role as defending plant pathogens despite its many traditional applications. Hence it is important to elucidate the compositions of the components of the cuticle wax leaves.

Our objectives were to i) examine the cuticular composition of fresh teak leaves, ii) isolate and identify the major component from the cuticular layer of teak leaves collected from varied environmental conditions and iii) examine pathogen defense or pathogen resistance activity.

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## Materials and Methods

### Collection of leaf samples

Teak is widely distributed all over the world with varied climatic condition. For our work, shedded teak leaves are collected from different location of India in the month of January to February.

### Isolation and characterization of active allelochemical

Shedded teak (*Tectona grandis* L.) leaves (200 gm) were air-dried and ground using Sample Miller Machine (Cyclotec 1093. Sample Mill, TECATOR) and then soaked in 500 ml of methanol. The entire mixture was then vortexed in high speed (3000 rpm) using Mechanical Stirrer (Model No. DC Stirrer NZ-1000s AC220V, EYELA) for 2 h and then filtered through sintered disc funnel. The brown coloured extract was collected and concentrated in a rotary vacuum evaporator (EYELA, Model No. N1-NW) and subsequently extracted with hexane, ethyl acetate, acetone and methanol, respectively. Finally, the compounds were purified by column chromatography and thin layer chromatography. Hydrocarbons are highly soluble in hexane so we put emphasis on Hexane fraction of Teak (henceforth referred to as HFTk) for biological activity. Amount of cuticular wax was found in higher (20% to 22%) in teak leaves that are occurred in polluted and stress condition than the leaves that are collected mainly forest area (16% to 19%).

**Column chromatography:** Columns (38 × 4 cm) were tightly packed with silica gel (mesh size 60 -120) up to 25 cm. After that material coated silica gel was poured. Column was then run with different proportionate ratio of highly non-polar solvent and different fractions were collected at different time intervals. These fractions were subjected to TLC analysis.

**Thin layer chromatography:** TLC plates (20 × 20 cm) were used for this study. Silica gel G of TLC grade was used as a coating material and plates were coated uniformly with 0.5 mm thick layer of silica gel. A solvent system [13] in the ratio of 95:5. Pet. Ether (40-60)°C : Hexane was used. Plates were loaded with 20 µl solution (500 ppm of HFTk compound) and developed up to a height of 18 cm in glass chamber pre-saturated with desired solvent system. After that TLC plates were then taken out and dried under a stream of hot air. Finally compounds were detected by exposing the plates under iodine vapour or under UV light (365 nm).

### Spectral analysis

**Mass spectrometer analysis:** Mass Spectrometric analysis (Waters GCMS) was done at Indian Institute of Chemical Biology (IICB), Jadavpur, Kolkata 700032. Purified HFTk was subjected to MS analysis for detecting the exact molecular weight of the compound. Mass Spectra (ESI) was recorded on a Micro mass Q-TOF Micro TM Spectrometer using positive ion mode and the sample run time is 20 minutes.

**<sup>1</sup>H NMR analysis:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) available at Indian Institute of Chemical Biology, Jadavpur, Kolkata 700032, was used for analysis of extra-purified HFTk or fraction-1 of *Tectona grandis*. For <sup>1</sup>H, Pulse Programme is Zg and number of scan is 64. <sup>1</sup>H NMR spectra were observed on δ ppm (0-10) scale with end sweep at 0 ppm. Samples were analyzed at ambient temperature. CDCl<sub>3</sub> was used as solvent for HFTk compound for <sup>1</sup>H NMR.

**<sup>13</sup>C NMR analysis:** <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) available at Indian Institute of Chemical Biology, Jadavpur, Kolkata 700032, was used for analysis of extra-purified HFTk. For <sup>13</sup>C NMR, Pulse Programme is Zgdc

and number of scan is IK (1024). <sup>13</sup>C NMR spectra were observed on δ ppm (0-10) scale with end sweep at 0 ppm. Samples were analyzed at ambient temperature. CDCl<sub>3</sub> was used as solvent for HFTk compound for <sup>13</sup>C NMR analysis.

**FTIR analysis:** IR analysis was used to confirm the important functional group in the extracted and purified HFTk with the help of IR Spectrometer (JASCO-SP-Model No. 410). Solid-state spectrum was obtained by mixing the required quantity of sample in KBr plate.

### Effect of HFTk against aspergillosis causing fungi

The impact of extracted HFTk compound against three different fungal species viz., *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus favus* was performed by agar diffusion method using Muller Hinton Agar Medium. *Aspergillus* is a member of the Deuteromycetes fungi, causing Aspergillosis. The most common pathogenic species are *Aspergillus fumigatus* and *Aspergillus favus* which produces aflatoxin which is both a toxin and carcinogen. Few fungal spores of above *Aspergillus* species were transferred to Potato Dextrose Agar Media (PDA) slants and incubated for one week for colony growth. After one week, one loop full of fungal spore of each species was added separately to the sterile saline water and mixed well. Fungal spore suspension (1 ml) in water was then poured in a sterile Petri dish containing molten PDA and allowed to solidify the plates. Four cups (25mm<sup>2</sup> dia) were cut at equidistant positions and in these cups 0.5 ml solution of HFTk at 500, 1000, 1500 and 2000 ppm was added. 40 mg of purified HFTK were dissolved in 20 ml of dimethyl sulfoxide (DMSO). This constituted the stock solution of 2000 ppm. For this stock solution, further dilutions were made. Treated plates were incubated at 28 ± 1°C for 24-48 hrs. After 48 hrs plates were taken out and observations were recorded for colony growth inhibition. Area of inhibition zone was calculated as:

$$\left[ \text{Area of inhibition at } x \text{ ppm} = 3.14 \left\{ (TRx)^2 - (r)^2 \right\} \right]$$

Where: x = concentration used, r = radius of the cup area, TR = Total radius of the inhibition zone at specific concentration [14].

### Effect of HFTk against multi drug resistance bacteria

Antibacterial activities of HFTk compound was also detected by agar diffusion method using Muller Hinton Agar Medium against two Gram-positive bacteria (*Staphylococcus aureus* and *Sarcina lutea*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). Of which *Staphylococcus aureus* and *Pseudomonas aeruginosa* are multidrug resistant. Multidrug-resistant organisms are bacteria that have become resistant to certain antibiotics, and these antibiotics can no longer be used to control or kill the bacteria. *Escherichia coli* are Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. *Pseudomonas aeruginosa* is a common bacterium that can cause disease in animals, including humans. *Staphylococcus aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. *Sarcina* is a genus of Gram-positive cocci bacteria in the family Clostridiaceae. The bacterium *Sarcina lutea* is found in various parts of the body and is one of the causes of body odour.

Firstly the strains of test bacteria were transferred to Nutrient Agar Media (NA) slants and incubated at 37 ± 1°C. After 24 hrs, 1 loop full of bacterial spore of each test species was added separately to sterile

nutrient broth and mixed well and incubated at 37°C for two and half hr. Bacterial spore suspension (1 ml) in sterile nutrient broth was then added to sterile petri dish containing molten NA medium and allowed to solidify. After complete solidification, similar procedure as that of anti-fungal assay was followed. Treated plates were incubated at 37 ± 1°C for 24 hrs and the inhibition zones were measured using the formula cited above.

### Exploratory statistical analysis

Exploratory data analysis was done by using Statistical Software (SPSS Statistics 20). Calculation of Descriptive Statistics such as number, mean, standard deviation, have been done on the complete dataset. Univariate Tests were worked out to find the effect of allelochemicals at different concentrations on different fungal and bacterial species assuming the reserve as null hypothesis that there is no effect. The results were interpreted graphically by mean plots. In our experiment significance level of 0.001 was considered.

## Results

### Isolation and characterization of HFTk

Following the proposed scheme of fractionation by liquid-liquid extraction, Fraction-1 of *Tectona grandis* or HFTk was isolated and purified by column chromatography and thin layer chromatography. At least eight repetitions of the procedure was necessary to achieve a single pure compound (purity of >99%).

### Thin layer chromatography analysis

After purification HFTk was run on TLC which showed single silver fluorescent spot under UV light with R<sub>f</sub> value 0.64. Physical state of purified HFTk was white lipid like compound at room temperature.

### Spectral analysis

**Mass spectrometer analysis:** Mass Spectra (MS) of the purified compound of HFTk showed a base peak at m/z 297.33 and another small peak at 319.33 [15] corresponding to [M+H]<sup>+</sup> and [M+Na]<sup>+</sup> respectively, suggesting a molecular weight of 296 (Figure 2).

**FTIR analysis:** FTIR Spectra of purified compound showed five important absorption at 2919.7 cm<sup>-1</sup>, 2852.2 cm<sup>-1</sup>, 1463.71 cm<sup>-1</sup>, 1374.03 cm<sup>-1</sup> and 723.175 cm<sup>-1</sup>, while the first two peaks correspond probably to asymmetrical and symmetrical stretching of methylene groups. Peaks at 1463.71 cm<sup>-1</sup> and 1374.03 cm<sup>-1</sup> correspond to symmetrical and asymmetrical C-H bending vibrations [16]. The spectrum is transparent in the 1480-1850 cm<sup>-1</sup> region and 4000-3000 cm<sup>-1</sup> region indicating absence of C=C and C-heteroatom single/double/triple bond [17]. The FTIR spectra therefore indicate the compound to be of alkane type (Figure 3).

**<sup>1</sup>H NMR analysis:** In the <sup>1</sup>H NMR Spectra, there is a board multiplet centered at δ 0.88 for methyl carbons and a second very board signal centered at δ 1.255 for methylene carbons. An extra sharp peak appear at δ 1.545 is due to the HCl, associated with CDCl<sub>3</sub> solvent. <sup>1</sup>H NMR spectra are not very revealing but it indicates again for the sample to be an alkane (Figure 4).

**<sup>13</sup>C NMR analysis:** The <sup>13</sup>C NMR Spectra is very simple in appearance. It shows no peak after δ C 37.2. The board band decoupling (BB), DEPT-135 and DEPT-90 spectra together indicates absence of one CH<sub>3</sub>-type can be at δ C 14.12, a very strong peak of CH<sub>2</sub>-carbon at δ C 29.73 and three peaks for CH<sub>2</sub>-carbons at δ C 31.96, 29.39 and 22.71 of exactly equal intensities; no peaks for quaternary carbons or methane-carbon have appeared in the spectra (Figure 5).

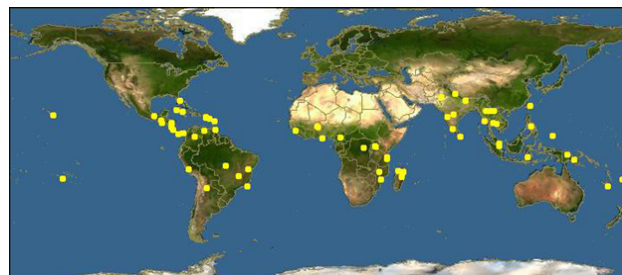


Figure 1: Distribution map of *Tectona grandis*, L.

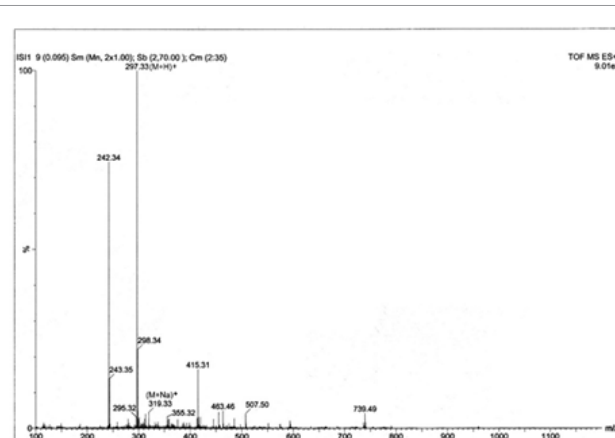


Figure 2: Mass spectra of purified HFTk or fraction-1 of *Tectona grandis*, L.

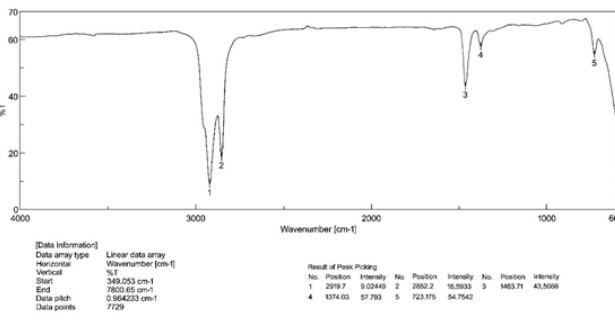


Figure 3: IR spectra of purified HFTk or Fraction-1 of *Tectona grandis*, L.

The molecular weight of 296 therefore indicates the formula to be C<sub>21</sub>H<sub>44</sub> and because of absence of methane and quaternary carbons in the structure of the compound appears to be as shown in Figure 6.

The assignment is as follows: δ C at 14.12 for CH<sub>3</sub> [(1) and (21)], δ C 22.71 for CH<sub>2</sub> [(2) and (20)], δ C 31.96 for CH<sub>2</sub> [(3) and (19)], δ C 29.39 for CH<sub>2</sub> [(4) and (18)], δ C 29.73 for CH<sub>2</sub> [(5) and (17)]; the last peaks corresponds to all the thirteen methylene carbons together. The assignment is in very good agreement with the guidelines for calculating δ C values of a long unbranched hydrocarbon [C<sub>21</sub>] and discrepancies always lie in the tolerable range of 0.1-0.7 δ. It is also apparent that the δ C values are always slightly lower than those of the model systems and is ascribed to the conformational aspects of the long C<sub>21</sub>H<sub>44</sub> unbranched alkanes.

### Effects of HFTk against aspergillosis causing fungi

HFTk showed strong antifungal activity against aspergillosis causing

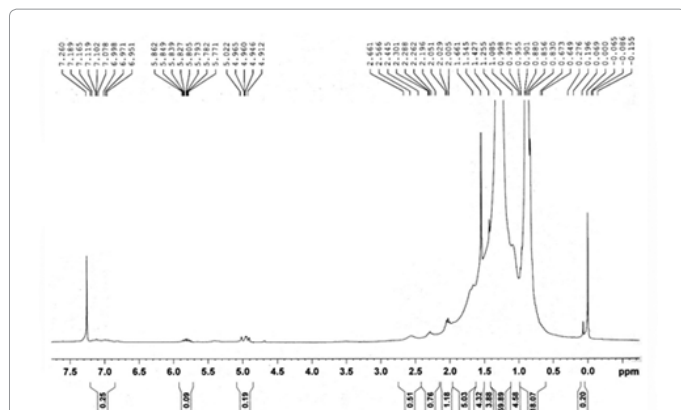


Figure 4: <sup>1</sup>H NMR spectra of purified HFTk or Fraction-1 of *Tectona grandis*, L.

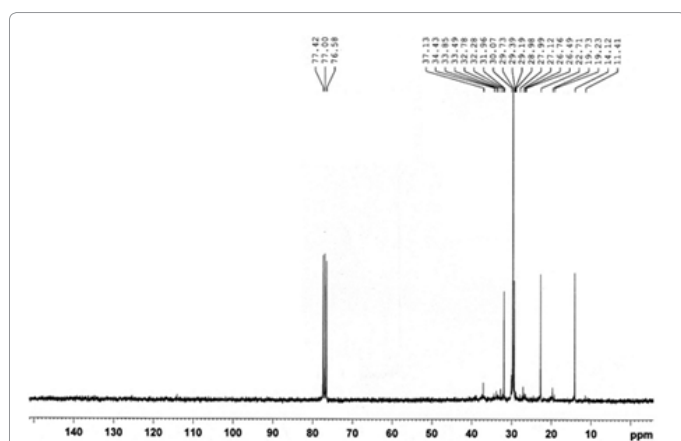


Figure 5: <sup>13</sup>C NMR spectra of purified HFTk or Fraction-1 of *Tectona grandis*, L.

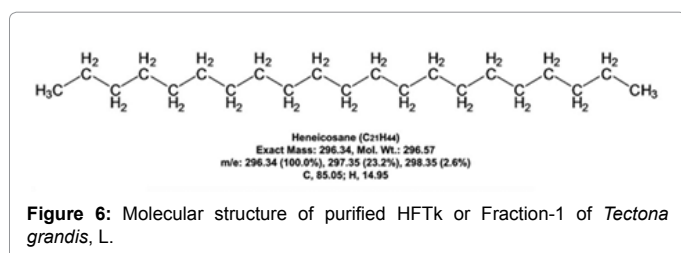


Figure 6: Molecular structure of purified HFTk or Fraction-1 of *Tectona grandis*, L.

fungi such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus favus* were much more inhibitory than *Aspergillus fumigatus* and *Aspergillus niger*. Both *Aspergillus favus* and *Aspergillus niger* exhibited maximum inhibition at 500 ppm whereas *Aspergillus fumigatus* revealed maximum inhibition at 2000 ppm (Figure 7).

#### Effects of HFTk against multi drug resistance bacteria

All the bacterial species are inhibited by all concentrations of heneicosane. The effect is much pronounced in *Pseudomonas aeruginosa* and *Sarcina lutea* than *Escherichia coli* and *Staphylococcus aureus*. In case of *Pseudomonas aeruginosa*, *Sarcina lutea* and *Escherichia coli*, maximum inhibition was noticed at 1500 ppm whereas *Staphylococcus aureus* exhibited maximum inhibition at 2000 ppm (Figure 8).

#### Descriptive statistical analysis

Descriptive statistics of all the variables was done to see the profile

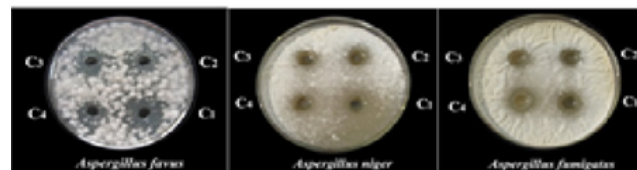


Figure 7: Effects of HFTk or Hexane fraction of *Tectona grandis*, L. at different concentrations (C1, C2, C3 and C4 indicates 500, 1000, 1500 and 200 ppm respectively) on *Aspergillus favus*, *Aspergillus niger* and *Aspergillus fumigatus*.

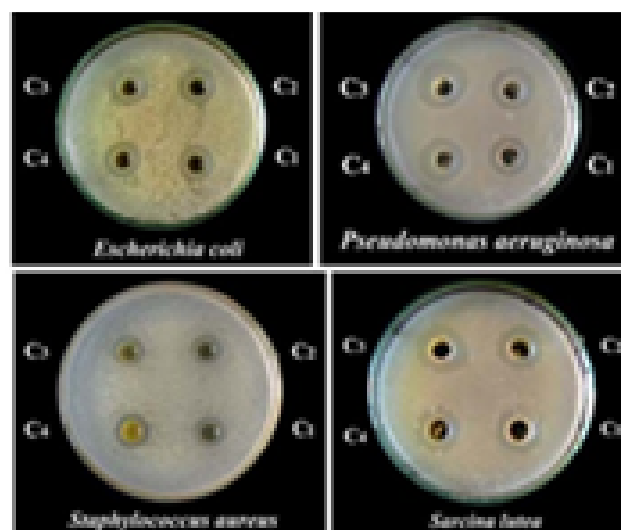


Figure 8: Effects of HFTk or Hexane fraction of *Tectona grandis*, L. at different concentrations (C1, C2, C3 and C4 indicates 500 ppm, 1000 ppm, 1500 ppm and 200 ppm respectively) on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Sarcina lutea*.

on the complete data set. Significant changes in mean of diameter of inhibition zone as exhibited by different fungal (Table 1) and bacterial (Table 2) species were observed at different concentrations of heneicosane.

Tables 3 and 4 shows the Univariate Tests<sup>c</sup> with diameter of the inhibition zone is taken as the dependent variable and concentration of HFTk and name of the species as fixed factors. Statistically significant difference observed in the mean of diameter of inhibition zone in terms of concentration and species taken individually or the added consideration of both the parameters.

Mean plots of both microbial species exhibited differential concentration-dependent inhibitory activity by HFTk (Figure 9).

#### Discussion

The leaf surface of higher plants is composed of a varied profile of hydrocarbons which play a vital role in chemical recognition and communication with nature. Cuticular hydrocarbons (CH) are also useful for identifying insect species and differentiating populations. The primary role of CH on aerial plant surfaces is to prevent of water loss, but their species-specific chemical composition and their variable crystal morphology suggest other ecological functions [18]. Among these, the most important role of CH is a mediator of interactions between plants and insects herbivores [19] The cuticular hydrocarbons act as chemical cues for nest-mate recognition in the invasive Argentine ant (*Linepithema humile*) and also reduces the susceptibility of host plants by preventing biological invasions [20].

Dependent variables: Diameter of inhibition zone			
Concentration of HFTk (ppm)	Name of the species	Mean (N=5)	Standard Deviation
500	<i>Aspergillus fumigatus</i>	11.9460	0.51772
	<i>Aspergillus niger</i>	9.7860	0.12522
	<i>Aspergillus favus</i>	21.8240	0.53092
1000	<i>Aspergillus fumigatus</i>	8.3760	0.56752
	<i>Aspergillus niger</i>	6.6240	0.16577
	<i>Aspergillus favus</i>	18.4640	0.21279
1500	<i>Aspergillus fumigatus</i>	9.7820	0.01483
	<i>Aspergillus niger</i>	6.6220	0.10257
	<i>Aspergillus favus</i>	13.7420	0.66424
2000	<i>Aspergillus fumigatus</i>	13.7460	0.99754
	<i>Aspergillus niger</i>	8.3800	0.21587
	<i>Aspergillus favus</i>	17.3260	0.44534

Table 1: Descriptive statistics of fungal species.

Dependent Variable: Diameter of inhibition zone			
Concentration of HFTk (ppm)	Name of the species	Mean (N=5)	Std. Deviation
500	<i>Pseudomonas aeruginosa</i>	13.7467	6.35624
	<i>Escherichia coli</i>	11.8475	0.22187
	<i>Staphylococcus aureus</i>	8.3800	0.19365
	<i>Sarcina lutea</i>	14.9640	0.20768
1000	<i>Pseudomonas aeruginosa</i>	17.3260	0.20948
	<i>Escherichia coli</i>	11.8120	0.18700
	<i>Staphylococcus aureus</i>	8.3860	0.17126
	<i>Sarcina lutea</i>	17.3220	0.20873
1500	<i>Pseudomonas aeruginosa</i>	20.7580	0.29115
	<i>Escherichia coli</i>	13.7400	0.24566
	<i>Staphylococcus aureus</i>	8.3640	0.19870
	<i>Sarcina lutea</i>	20.7000	0.21794
2000	<i>Pseudomonas aeruginosa</i>	17.3200	0.16171
	<i>Escherichia coli</i>	9.7880	0.20729
	<i>Staphylococcus aureus</i>	11.8760	0.18488
	<i>Sarcina lutea</i>	16.1420	0.17035

Table 2: Descriptive statistics of bacterial species.

Dependent Variable: Diameter of inhibition zone					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	1045.529 <sup>a</sup>	11	95.048	430.017	0.000
Intercept	22732.784	1	22732.784	102847.764	0.000
Concentration	571.722	3	190.574	862.195	0.000
Species	150.583	2	75.291	340.633	0.000
Concentration * Species	323.225	6	53.871	243.723	0.000
Error	10.610	48	0.221	--	--
Total	23788.923	60	--	--	--
Corrected Total	1056.139	59	--	--	--

a. R Squared = 0.990 (Adjusted R Squared = 0.988)

b. Name of the microbes = Fungi

Table 3: Tests of between-subjects effects<sup>b</sup>.

In this context, teak was considered for our work taking into consideration of its wide geographical distribution with changing topologies [21]. Water extract of teak leaves exhibited inhibitory activity on the growth of *Monilia* sp. and *Arthrinium phaeospermum*, the cause of wood decay [22,23] and also inhibited sporulation of *Alternaria cajani* and *Helminthosporium* species [24]. A number of pharmacological properties such as urinary discharge, bronchitis, cold and headache, in scabies, used as a laxative and sedative, as diuretic, anti-diabetic, analgesic and anti-inflammatory etc, of this plant have

been reported [25-28]. Various phytochemicals namely Juglone (5-Hydroxy-1,4-naphthalenedione) with anti-microbial activity [29], betulin [2-Hydroxy-3-(3-methylbut-2-enyl)-naphthalene-1,4-dione] aldehyde with anti-tumor activity [30] and lapachol [2-Hydroxy-3-(3-methylbut-2-enyl)-naphthalene-1,4-dione] with anti-ulcerogenic activity [31] have been isolated from *Tectona grandis*.

In our studies, this plant was evaluated for hydrocarbon profiling from leaf cuticle which revealed the presence of n-heneicosane (C<sub>21</sub>H<sub>44</sub>)

Dependent Variable: Diameter of inhibition zone					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3023.915 <sup>a</sup>	15	201.594	63.077	0.000
Intercept	39537.628	1	39537.628	12370.984	0.000
Concentration	97.271	3	32.424	10.145	0.000
Species	2643.141	3	881.047	275.672	0.000
Concentration * Species	283.450	9	31.494	9.854	0.000
Error	204.544	64	3.196	--	--
Total	43538.781	80	--	--	--
Corrected Total	3228.459	79	--	--	--

a. R Squared = 0.937 (Adjusted R Squared = 0.922)  
b. Name of the microbes = Bacteria

Table 4: Tests of between-subjects effects<sup>b</sup>.

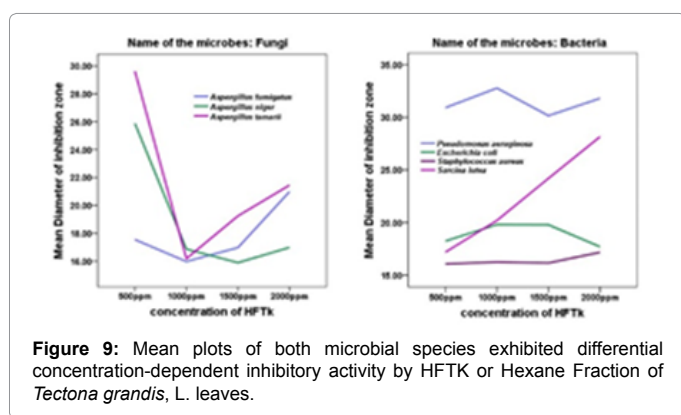


Figure 9: Mean plots of both microbial species exhibited differential concentration-dependent inhibitory activity by HFTK or Hexane Fraction of *Tectona grandis*, L. leaves.

- a long chain aliphatic hydrocarbon as the predominant component. Spectral analysis revealed a mol. wt. of 296 with terminal methyl groups. Their occurrence is also reported in shade-dried petals of *Rosa damascene* mill leaf extract of *Kigelia pinnata* [32], leaves of *Abie spindrow* [33] and *Temnopleurus alexandri* [34]. In spite of the fact that long-chain normal hydrocarbons (e.g. alkanes, alkenes and dienes) are rare biological molecules and their biosynthetic origins are obscure, the amount of heneicosane recovered from teak amounts to approx. 16-19% of the weight with <99% purity. This huge occurrence indicates its putative role in the plant. This compound showed a strong antimicrobial activity against aspergillosis causing fungi and a variety of multi drug resistance bacteria which implies its role in defensive mechanisms of the plant. The effect of heneicosane on each of the microbial species showed statistically significant results ( $P \leq 0.001$ ) which further confirms heneicosane as disease resistance marker. Presence of higher amount of cuticular wax in teak leaves that are collected in polluted and stress condition than the leaves of forest area also indicates its role in plant resistance. This may provide evidences that teak plant leaf hydrocarbons act as chemical communicators corresponding to other neighbouring population cuticle hydrocarbons and that n-heneicosane shares a vital role for diverse adaptation of teak plants. Thus, leaf cuticular composition of higher plants plays an important role in plants adaptation and protection against pathogens.

## Conclusion

Cuticular composition of Teak leaves collected from different parts of India revealed the presence of Heneicosane – a long chain unbranched aliphatic hydrocarbon – as a predominant compound. Higher amount of cuticular wax in teak leaves that occurred in polluted and stress condition than in forest area suggests its role in plant

adaptation and resistance. It forms a protective shield on the plant surface against the environment, thus limiting nonstomatal water loss, and is therefore one of the key adaptations in the evolution of teak tree. Moreover, this compound exhibited a strong defense activity against a number of pathogens such as aspergillosis causing fungi and a variety of multi drug resistance bacteria which implies its role in defensive mechanisms of the plant.

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