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Antibacterial, Cytotoxic and Antioxidant Potential of Different Extracts from Leaf, Bark and Wood of *Tectona grandis*

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ABSTRACT
The timber value of *Tectona grandis* has been well known from decades. Teak is a major exotic species found in tropical regions. The present study was meant to characterize pharmacological potential of different extracts from leaf, bark and wood of teak. Antibacterial activity of all extracts from *Tectona grandis* were checked against *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), hospital strains of *Salmonella paratyphi* and *Proteus mirabilis* by disc diffusion assay. Chloroform extract of leaf showed inhibition to growth of *S. aureus* (14 mm) and *K. pneumoniae* (8 mm). Cytotoxic potential of extracts were checked by MTT assay and chloroform extract of bark exhibited very high activity against chick embryo fibroblast (CEF) and human embryonic kidney (HEK 293) cells with 87 % and 95.3 % inhibition respectively. Antioxidant activity of extracts was checked with DPPH and $ABTS^+$ free radical. Ethyl acetate extract of wood showed very high activity with 98.6 % inhibition against DPPH and $ABTS^+$ free radicals. The value was higher than standard compounds used for the study.

Keywords: *Tectona grandis*, antibacterial, cytotoxicity, antioxidant, disc diffusion assay, MTT assay, DPPH, $ABTS^+$, free radical scavenging.

INTRODUCTION

Tectona grandis Linn. (Common name – Teak; Family - Lamiaceae) is one of the most famous timbers in the world and is renowned for its dimensional stability, extreme durability and hard which also resists decay even when unprotected by paints and preservatives. Timber value of teak has been well known from decades. Teak is a major exotic species found in tropical regions. It is commonly found in India and other South-East Asian countries. [1-2] Teak is also considered as a major constituent in many folklore medicines. Extracts from various parts of teak shows expectorant, anti-inflammatory, anthelmintic properties and is also used against bronchitis, biliousness, bronchitis, hyperacidity, dysentery, diabetes, leprosy, astringent, anthelmintic and dysentery. In traditional medicine, a wood powder paste has been used against bilious headache and swellings. They are also used for treating inflammatory swelling. [3-4] The present study is to screen different extracts from leaves, bark and wood of *Tectona grandis* for antibacterial, cytotoxic and antioxidant properties. Teak is well known for durability and insect resistance from olden times.

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It is confirmed that accumulation of certain quinones and quinone derivatives are mainly responsible for its quality. [5] Eventhough some works on pharmacological potential of teak has carried out earlier, a systematic approach on characterization is lacking which can bring out a clear picture on medicinal value of the plant. [6-9] The present work was designed to give a detailed picture of antibacterial, cytotoxic and antioxidant potential of extracts from different parts of *Tectona grandis*.

MATERIALS AND METHODS

Plant material extraction: Leaf, bark and wood of *Tectona grandis* was collected from the campus, University of Kerala, Kerala, India during the period July-August, 2007. Plant materials were kindly compared with deposited specimen (No. 5616, 5617) at Institute herbarium, Kerala Forest Research Institute (KFRI), Kerala, India by Dr. Valsala kumari (Reader and Curator, Dept. of Botany, University of Kerala). All parts were extracted with hexane, chloroform, ethyl acetate and methanol using soxhlet apparatus. After removal of solvents under reduced pressure, extracts were stored at -20°C until use.

Antibacterial activity screening: Antibacterial activity of all extracts from Tectona grandis were checked against *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), hospital strains of *Salmonella*

paratyphi and *Proteus mirabilis* by disc diffusion assay. [10] Samples were tested at 25, 50, 100, 250 and 500 µg concentration per disc of 5mm diameter (Whatman No.1). Carrier soaked discs were also kept as negative control. Result expressed as diameter of inhibition zone and compared with standard antibiotic ciprofloxacin.

Cytotoxic potential: Ability of crude extracts to reduce the viability of chick embryo fibroblast (CEF) and human embryonic kidney (HEK 293) cells were checked. Cells were seeded at log phase in 24 well plates with a count of 5×10^4/ml. Cell viability was also confirmed by counting with trypan blue stain. After 16 h of incubation or till confluent growth, media was removed and fresh media added with extracts at different concentration (0.5, 5, 12.5 and 50 µg/ml) or DMSO as control. Present media was serum omitted and cells were incubated for 24 h. Volume of DMSO with sample was restricted to 0.1 % to avoid solvent-induced cytotoxicity. All samples were given in triplicate and MTT assay was carried out as previously described protocol [11] and absorbance measured at 570 nm using UV-Vis. spectrophotometer (UV-1700, Shimadzu, Japan). A graph with concentration of extracts against cell viability was also plotted for calculating percentage of cytotoxicity. Effect of toxicity was also visualised by Ethidium bromide-Acridine orange which distinctly stains live and dead cells. [12]

Antioxidant activity: Leaf, bark and wood extracts of *Tectona grandis* were taken and subjected to antioxidant screening by chemical methods at different concentration.

***DPPH assay*:** The free radical scavenging property of extracts were analyzed by 1, 2-diphenyl 1-picryl hydrazil (DPPH) assay. [13-14] Hexane, chloroform, ethyl acetate and methanol extract were checked at different concentrations from 5-1000 µg/ml and activities compared with quercetin as standard. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the Eq.1. Antioxidant activities of test compounds or extracts were expressed as SC_{50}, defined as the concentration of the test material required to scavenge a 50 % of initial DPPH concentration.

$$\text{Inhibition (\%)} = 100(A_{blank} - A_{sample})/A_{blank} \qquad (1)$$

***ABTS assay*:** $ABTS^+$ radical scavenging method were carried with a slight variation from previously defined procedure. Time and concentration dependent methods of assay were done to express the speed, stability and efficiency of extracts to react with free radical $ABTS^+$. [15] The radical cation was prepared by reacting 7 mM aqueous $ABTS^+$ with 2.45 mM potassium persulfate. The mixture was kept in dark for 16 h by which the $ABTS^+$ turned blue green. The solution was diluted with methanol to a final absorbance of 0.7±0.02 at 734 nm. The extracts were dissolved in 30 µl methanol and added to 2950 µl $ABTS^+$ solution just before measuring the change in absorbance. Results were expressed as in comparison with standard trolox. Change in absorbance was measured by time scan method at every one minute interval for six minutes after addition and mixing of 1mg sample. Concentration dependent activity was measured at 10, 100 and 1000 µg/ml concentration.

RESULTS AND DISCUSSION

Antibacterial activity: Preliminary study on antibacterial activity of crude extract from leaf, bark and wood showed chloroform extract of leaf to be most promising. Out of the four cultures tested, it showed good activity against *S. aureus* (14 mm) and *K. pneumoniae* (8 mm) at the highest concentration checked (500 µg). Methanol extract of leaf and ethyl acetate extract of wood was also able to show fairly good activity against gram positive and negative species. On comparison, only chloroform extract of leaf was able to produce activity even at least concentration tested. The result supports previously reported data on antimicrobial activity of aqueous extract of teak against *S. aureus* and *K. Pneumonia.* [16] But a detailed study on different extracts of leaf has not been carried out so far. Antifungal and antibacterial activity of saw dust, wood and bark of teak along with the compounds identified has been reported earlier. [17-18] As plant leaves are site of synthesis for different class of compounds, the chance of finding bioactive novel compounds will also be high. According to reviews, extracts or phytochemicals showing activity against gram positive and negative organisms are rare. So the presence of a compound(s) with broad spectrum activity against both types of organisms has to be explored by further purification.

Cytotoxic potential: Cytotoxicity of teak extracts were checked against HEK293 and CEF cells. Different extracts showed high toxicity against both cell lines even at lowest concentration tested. Against chick embryo fibroblast cells, chloroform extract of bark showed 87 % inhibition at 10µg/ml concentration with methanol extract of wood (61%) and hexane extract of leaf (54 %) in subsequent positions (Fig 1). Chloroform extract of bark was able to exhibited high toxicity (95.3 %) against HEK293 cells also. Methanol extracts of wood (76.6 %) and bark (73.2 %) were next in position showing toxicity against the later (Fig 2). A concentration dependent difference was found when cells are stained with ethidium bromide-acridine orange mix. More cells were found as stained with ethidium bromide with increase in extract concentration, which shows cell death. No reports are available so far on toxicity evaluation of extracts from different teak parts. Compound isolated from teak wood showing cytotoxicity against brine shrimp larvae has been reported by Khan and Mlungwana, 1999. Lapachol, dehydro-α-lapachone, betulinic acid etc. are some other compounds with reported toxicity and has been present in teak. [19-20] Teak is very famous of its timber value and decay resistance. This implies the presence of highly toxic compounds in wood. The result of our study on cytotoxicity of teak is of much importance because the cell death at low extract concentration points out the chance of active compounds to be present in bark (Fig 3). As it is showing very high activity against HEK293 cells, its ability to be used as anti-cancer drug after suitable chemical modifications to reduce normal cell death is also under consideration of future studies.

Antioxidant activity: Antioxidant status of all extracts was checked by DPPH and $ABTS^+$ free radical. Its ability to scavenge those free radicals at different concentrations was analyzed. Ethyl acetate extract of wood showed maximum activity against DPPH and $ABTS^+$ and was higher than quercetin and trolox, which were the respective standards (Table I & II). It was also effective in scavenging $ABTS^+$ when assayed at regular time intervals. Extracts was able to reduce colour more than 98 % from the first minute of assay. The standard compound, Trolox, was not able to exhibit that much activity and the result shows the speed and effectiveness of ethyl acetate extract of teak wood to be used

Table I: Effect of extracts from leaf, bark and wood of *Tectona grandis* on DPPH free radical scavenging. Each experiment was performed at least 3 times and data are expressed as average percent change from control ± S.D.

Plant part/ compound	Solvent	Concentration of extract/compound (µg/ml)							
		5	10	25	50	100	250	500	1000
Leaf	Hexane	1.4±0.3	2.6±0.47	7.4±0.35	14.6±0.3	22.9±0.41	43±0.61	58.3±0.45	81.1±0.66
	Chloroform	9.5±0.3	22.1±0.26	33.6±0.26	48.3±0.2	75.5±0.17	90±0.75	92.1±0.41	93.6±0.41
	EtOAc*	15.9±0.26	22.6±0.5	33.5±0.66	43.6±0.57	61±0.4	73±0.75	82.2±0.76	95±0.35
	MeOH**	12.5±0.76	22±0.15	35.1±0.26	57.9±0.76	91.6±0.83	96.5±0.35	97.3±0.2	97.6±0.1
Bark	Hexane	0	0.7±0.05	2±0.38	2.8±0.2	3.4±0.44	5.3±0.5	7±0.2	8.2±0.35
	Chloroform	3.4±0.25	5±0.64	5.9±0.17	6.8±0.17	9.1±0.26	12.3±0.4	22.7±0.44	40.9±0.21
	EtOAc	14.9±0.71	22.6±0.4	30.3±0.75	37.6±0.2	48.2±0.6	60.1±0.8	71.7±0.75	84.9±0.95
	MeOH	1.6±0.5	3.2±0.2	9±0.32	12.2±0.32	19±0.35	27.3±0.26	34.3±0.5	45.1±0.36
Wood	Hexane	0	0	0.36±0.15	1.3±0.36	2.1±0.25	2.8±0.25	4.2±1.0	4.5±0.31
	Chloroform	2.9±0.4	3.5±0.15	6.2±0.26	9±0.57	17.4±0.3	36.7±0.85	58±0.46	82.9±0.15
	EtOAc	16.6±0.4	34.1±0.56	63.6±0.25	89.5±0.36	96.8±0.32	96.7±0.52	97.7±0.2	98.6±0.12
	MeOH	11.2±0.6	17.3±0.56	27.4±0.9	36.3±1.2	47.5±0.92	60±0.47	76.8±0.5	89±0.67
Quercetin		17.8±0.46	26.1±0.46	58.8±0.71	78.4±0.45	84.6±0.38	91.4±0.26	92.2±0.41	93.1±0.5

*EtOAc – Ethyl acetate; **MeOH – Methanol

Table II: Concentration dependent $ABTS^+$ radical scavenging activity of extracts from leaf, bark and wood of *Tectona grandis*. Each experiment was performed at least 3 times and data are expressed as average percent change from control ± S.D.

Plant part/ compound	Extract	Concentration of extract/compound (µg/ml)		
		10	100	1000
Leaf	Hexane	4.2±0.62	8.4±0.25	39.4±0.45
	Chloroform	4.2±0.26	24.9±0.8	66.1±0.5
	EtOAc*	26±1.18	55.4±0.76	81.1±0.65
	MeOH**	7.7±1.3	87.9±0.6	95.9±0.15
Bark	Hexane	3.07±0.83	4.3±0.7	8.2±0.92
	Chloroform	2.3±0.61	7±0.55	30.2±0.86
	EtOAc	21.87±0.72	40.47±0.5	66.8±0.67
	MeOH	4±1.12	11.1±1.06	25.07±1.86
Wood	Hexane	4.27±0.31	5.37±0.56	6.9±0.75
	Chloroform	3.13±0.45	10±0.6	47.5±0.46
	EtOAc	56.9±0.97	98.2±0.12	98.6±1.91
	MeOH	26.03±0.5	44.9±1.5	78.37±2.25
Trolox		90.63±0.06	97.23±0.12	98.07±0.06

*EtOAc – Ethyl acetate; **MeOH – Methanol.

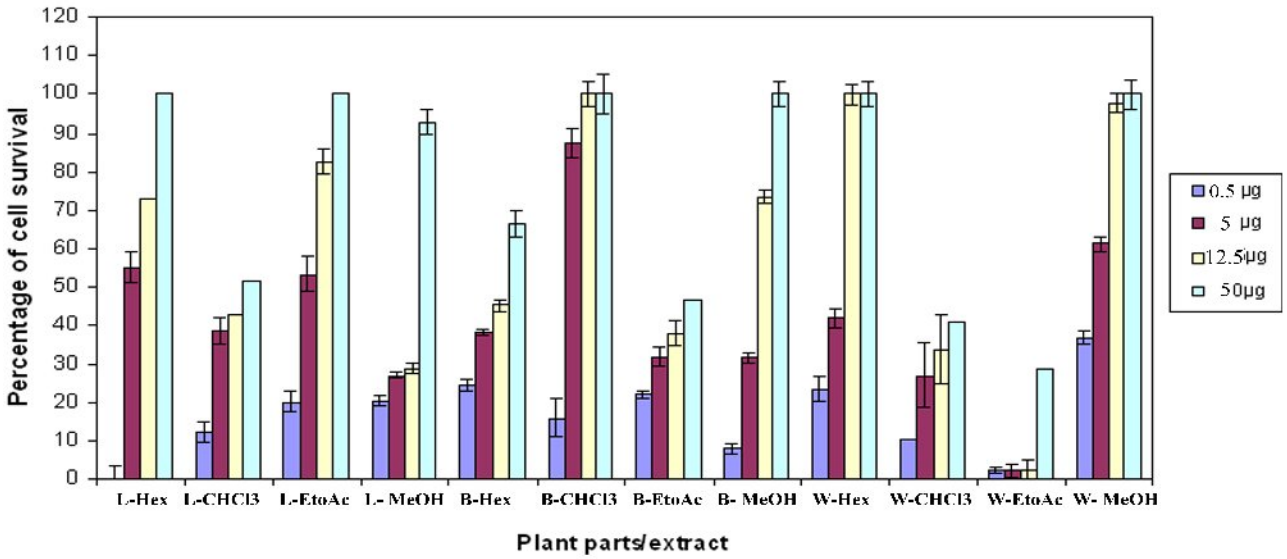

Fig 1: Bar diagram showing cytotoxic effect of different extracts of plant parts from teak against chick embryo fibroblast cells. L- leaf, B- bark, W- wood, Hex- hexane, CHCl3- chloroform, EtoAc- ethyl acetate, MeOH- methanol.

as a drug source. Eventhough plants under Lamiaceae family are biologically active and rich in biologically active compounds; antioxidant status of different parts from teak has not been analyzed in detail previously. The only study on antioxidant activity of teak with its crude ethanol extracts by H_2O_2 scavenging activity, DPPH and FRAP assay proved its potential. But the activity mentioned was not appreciable as the results available in the present study. [9]

Concluding the study, the importance and value of teak on pharmacological basis is also highly notable along with its timber value. With the present study, we were able to highlight some of biological activities on various parts of teak. More studies on isolation of active fractions responsible are under process and will be a boon to mankind concerning the present scenario of emerging diseases.

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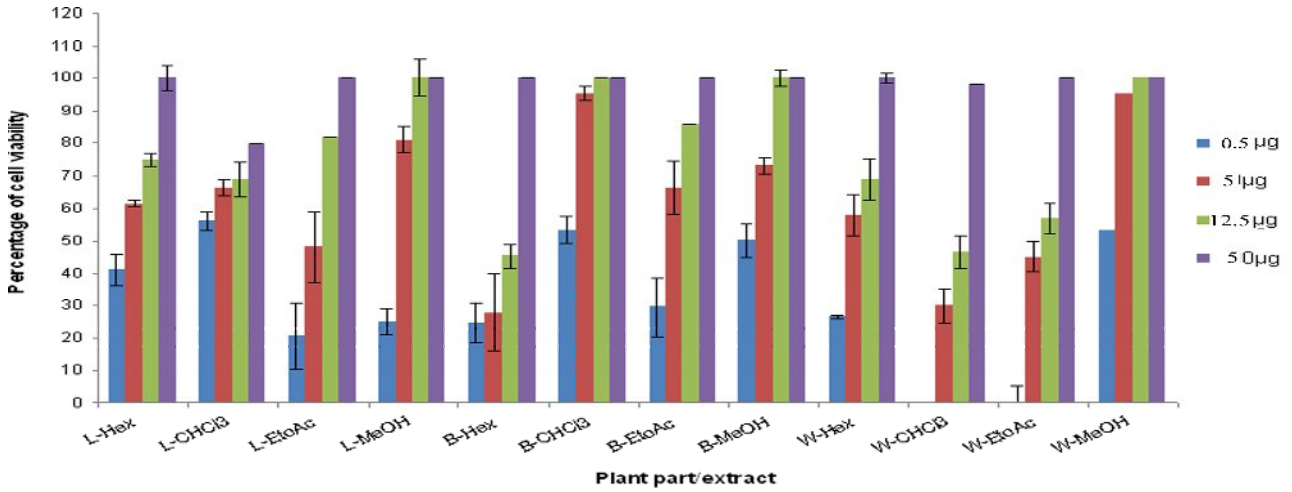

Fig 2: Bar diagram showing cytotoxic effect of different extracts of plant parts from teak against HEK293 cells. L- leaf B- bark, W- wood, Hex- hexane, CHCl3- chloroform, EtoAc- ethyl acetate, MeOH- methanol

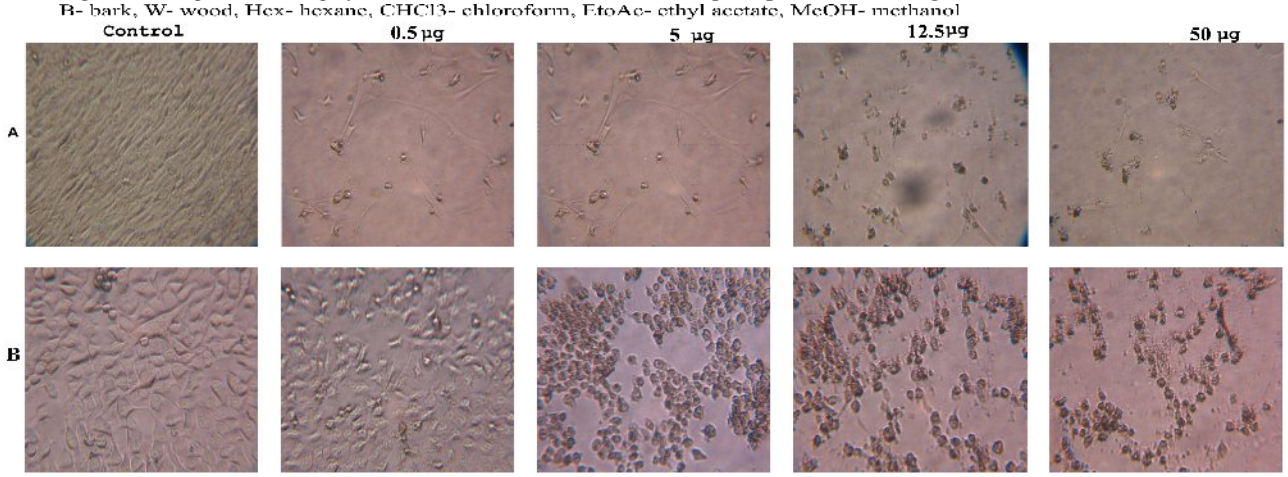

Fig 3: Cytotoxicity of chloroform extract of teak leaf against A) Chick embryo fibroblast B) HEK293 cells

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