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Pharmacognostic Standardization, Phytochemical Evaluation and Anti-Pyretic Activity of *Tectona Grandis* Leaf Extracts

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ABSTRACT

This study evaluates the pharmacognostic, physicochemical, phytochemical, and pharmacological properties of *Tectona grandis* (Teak) leaves. Quantitative microscopy established key plant constants including vein-islet number (8/sq mm), vein-termination number (6/sq mm), palisade ratio (7), stomatal numbers, and stomatal indices. Extractive values varied across solvents, with ethanol yielding the highest percentage (12.45% w/w). Proximate parameters showed a loss on drying of 4.55%, total ash of 6.85%, and foreign organic matter of 1.2%. Quantitative phytochemical analysis revealed a Total Phenol Content (TPC) of 24.7 mg/g GAE and Total Flavonoid Content (TFC) of 10.45 mg/g Quercetin equivalents, with qualitative tests confirming high concentrations of terpenoids and flavonoids in the ethanol extract. Furthermore, the ethanolic extract of Tectona grandis (EETG) demonstrated significant, dose-dependent anti-pyretic activity in Brewer's yeast-induced pyrexia in rats, particularly at doses of 200 mg/kg and 400 mg/kg, performing comparably to the reference drug paracetamol (30mg/kg). These effects are likely mediated via the inhibition of prostaglandin synthesis.

1. Introduction:

Herbal care or traditional system of medicine are used throughout the world and from century's herbs have been the original source for most of the drugs. Medicinal plants contain so many chemical compounds which are the major source of therapeutic agents to cure human diseases. [1,2] Recent discovery and advancement in medicinal and aromatic plants have lead to the enhancement of health care of mankind. Various medicinal plants like Teak (Tectona grandis), Neem, Arjuna, Aswagandha, Tulsi, etc. traditionally used for treating fever.[3] The extract prepared from the heartwood of Acacia catechu, stem bark and leaves of Bauhinia racemosus, Cleome viscosa etc. reported to have antipyretic activity in rats. Standardization and quality control of crude drugs are vital preliminary steps in validating the authenticity, purity, and therapeutic efficacy of herbal medicines, as well as detecting potential adulterants.[4,5]

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Tectona grandis (commonly known as teak) is a well-known plant traditionally used in various medicinal applications.[6] It contains many phytoconstituents, including phenolic compounds, flavonoids, tannins, terpenoids, and alkaloids. Teak is also used in Cameroon to treat typhoid fever and other diseases.[7,8] However, there are no studies on the potentially toxic effects of teak. *Tectona grandis* leaves, also known as teak leaves, have antipyretic properties and can help reduce fever. [9]

This study aims to establish comprehensive pharmacognostic standards, determine physicochemical profiles, identify key phytoconstituents, and evaluate the anti-pyretic potential of Tectona grandis leaf extracts using standard experimental models.

2. Materials and Methods:

2.1. Quantitative Microscopy and Physical Constants

Quantitative microscopic parameters of the leaf including vein-islet number, vein-terminal number, palisade ratio, stomatal number, and stomatal index—were determined using standard microscopy. Additionally, foaming index and swelling index evaluations were performed.

2.2. Preparation of Extracts and Extractive Values

Powdered leaf material of *Tectona grandis* was subjected to successive solvent extraction using petroleum ether, hexane, acetone, ethanol, and aqueous/water media. Percentage yields and physical colors of the respective extracts were recorded.

2.3. Physico-chemical (Proximate) Parameters

Proximate parameters including loss on drying, total ash, acid-insoluble ash, water-soluble ash, and foreign organic matter were analyzed on air-dried powder. The pH of the ethanol extract and melting point of the methanol extract were also measured. [10]

2.4. Phytochemical Screening (Qualitative and Quantitative)

- **Qualitative Analysis:** Preliminary screening for sterols, terpenoids, carbohydrates, flavonoids, proteins, alkaloids, glycosides, saponins, tannins, mucilages, and volatile oils across different solvent extracts was performed using standard qualitative tests. [11]
- **Quantitative Analysis:** Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of the ethanol extract were estimated spectrophotometrically using Gallic Acid Equivalents (GAE) and Quercetin Equivalents, respectively.[12]

2.5. Chromatographic Separation (TLC)

Thin Layer Chromatography (TLC) profiling of the extracts was carried out using Silica gel G as the stationary phase for the identification of flavonoids and terpenoids under UV and visible light, calculating respective Rf values. [13]

2.6. Pharmacological Screening: Acute Oral Toxicity and Anti-Pyretic Activity

- **Acute Oral Toxicity:** Carried out according to OECD guidelines (Draft Guideline 423) to determine the LD50 of the extracts up to 2000 mg/kg b.w.
- **Brewer's Yeast-Induced Pyrexia:** Rats (150–200 g) were divided into five groups (n=6). Pyrexia was induced via subcutaneous injection of 10 ml/kg body weight of yeast suspension. Group I served as the normal control (0.5% CMC), Groups II, III, and IV received EETG at doses of 100, 200, and

400 mg/kg (p.o.), respectively, and Group V received paracetamol (30 mg/kg p.o.). Rectal temperatures were monitored via telethermometer at regular intervals.[14]

3. Results

3.1. Quantitative Microscopy and Physical Evaluation:

The quantitative leaf constants and foaming/swelling characteristics of *Tectona grandis* are summarized below:

Table 1 A: Quantitative evaluation of the leaf of *Tectona Grandis*

S.No.	Plant constants	Values
1	Vein islet no.	8/sq mm
2	Vein termination no.	6/sq mm
3	Palisade Ratio	7
4	Stomatal number (upper)	110/mm^2
5	Stomatal number (lower)	85/mm^2
6	Stomatal index (upper)	16
7	Stomatal index (lower)	13

Table 1 B: Quantitative studies of *Tectona Grandis* leaf

S.No.	Estimation	Observation
1.	Foaming Index	< 100
2.	Swelling Index	>1

3.2. Extractive Values and Physicochemical Parameters:

Extractive values across various solvents indicated that ethanol yields the maximum amount of phytoconstituents (12.45% w/w).

Table 3: Extractive values and color of extract in different solvent

Extract	Color of Extract	Extractive value (% w/w)
Petroleum ether (60-80°C)	Pale green	2.5
Hexane	Green	1.32
Acetone	Greenish Brown	4.65
Ethanol	Greenish Brown	12.45%
Aqueous/Water	Brownish green	10.52%

Physico-chemical parameters:

The loss on drying was 4.55%. Total ash was 6.85%, acid insoluble ash was 1.45% and water soluble ash was 3.28%. Foreign Organic Matter was 1.2 % which is in standard range and pH and melting point of methanol extract was 3.8 and 107°C respectively.

Table 4: Physico-chemical parameter of crude powder of *T. Grandis*

Parameters	Value (w/w)
Loss on drying	4.55%
Total ash	6.85%
Acid insoluble ash	1.45%
Water soluble ash	3.28%
pH of Ethanol extract	3.8%
Foreign Organic Matter	1.2%
Melting point of methanol extract	107^0C

3.3. Phytochemical Analysis:

Table 5: Quantitative phytochemical analysis of ethanol extract of leaf of *Tectona Grandis*

Total Phenol (mg/g)	Flavonoid (mg/g)
24.7 mg/g GAE	10.45 mg/g Quercetin

Table 6: Preliminary qualitative phytochemical analysis of *Tectona Grandis*

S. No	Test	Drug powder	Pet-ether extract	Benzene extract	Ethanol extract	Aqueous extract
1.	Sterols	-	+	-	-	-
2.	Terpenoids	++	-	-	+++	+
3.	Carbohydrate	++	-	-	++	++
4.	Flavanoids	++	-	-	+++	+
5.	Proteins	-	-	-	-	-
6.	Alkaloids	++	-	-	++	-
7.	Glycosides	+	-	-	++	+
8.	Saponins	+	-	-	++	++
9.	Tannins	+	-	-	+	+
10.	Mucilages	-	-	-	++	+
11.	Volatile oil	+	-	-	++	+

Result and discussion:

In crude powder and ethanol extract maximum amount of Terpenoids and Flavanoids were present. Alkaloid, phlobotannins, saponins and triterpenes were present in moderate amount. Mucilages and steroids were absent in crude powder as well as in ethanol extract. Terpenoids and Flavanoids are mainly responsible for anti-pyretic potential of herbal medicine and leaf of *Tectona Grandis* contain higher amount of these constituents.

3.4. Chromatographic Separation (TLC):

Flavonoids TLC: Stationary phase: Silica gel G; Mobile phase: n-Hexane: ethyl acetate (3:7); Detection: 10% Alcoholic $FeCl_3$. Showed a reddish-pink spot at Rf = 0.41 under visible light.

Terpenoids TLC: Stationary phase: Silica gel G; Mobile phase: Ethyl acetate: methanol: water (100:13.5:10); Detection: 10% Alcoholic KOH. Showed a blue-black spot at Rf = 0.65 under visible light.

3.5. Acute Toxicity Studies:

Acute oral toxicity testing demonstrated no mortality up to 2000 mg/kg b.w. across all extracts (Ethanol, Chloroform, Petroleum Ether, and Aqueous). Hence, the LD50 was established at > 2000mg/kg b.w.

3.6. Anti-Pyretic Activity (Brewer's Yeast-Induced Pyrexia):

Table 7: Grouping and dose of drug for anti-pyretic activity

Group	Drug and Doses
Brewer's yeast-induced pyrexia	
Group I	Normal Control (Received 0.5% CMC)
Group II	Ethanol Extract of *Tectona Grandis* 100mg (i.p.)
Group III	*Tectona Grandis* 200mg (i.p.)
Group IV	*Tectona Grandis* 400mg (i.p.)
Group V	*Paracetamol* 30 mg/kg (oral)

Drug administration:

After 18 h of yeast injection 0.5% CMC was administered orally to the control Group I. The extracts of *Tectona Grandis* were administered at doses of (100, 200 and 400 mg/kg b.w. p.o) to groups II, III, IV animals respectively. Group V animals received the standard drug Paracetamol (30 mg/kg b.w. p.o) and Rats were restrained for recording rectal temp by at the 18 h, immediately before extracts, normal saline or paracetamol administration, & again at one hr intervals upto the 21 h after yeast injection.

Result:

The subcutaneous injection of yeast suspension (Nape of neck) markedly elevated the rectal temperature after 18 h of administration. Treatment with the *T. Grandis* at doses 200 and 400 mg/kg significantly ($p<0.01$) decreased the rectal temperature of the rats in dose dependent manner up to 3 h after pyrexia when compared with control (Table7.15). Effect of 100mg/kg extract was negligible. The antipyretic effect was sustained throughout the remaining period of the experiment in a manner similar to the reference drug Paracetamol 30mg/kg, b.w. p.o.

Table 8: Anti-pyretic Effect of Ethanolic extract of *Tectona Grandis* on yeast induced pyrexia in rats.

Treatment	Dose mg/kg	Rectal temp. ºC before and after treatment						
		Normal	18h	30 min after treatment	60 min after treatment	90 min after treatment	120 min after treatment	180 min after treatment
Control	-	37.6±0.22	38.8±0.18	38.7±0.14	38.7±0.08	38.7±0.22	38.6±0.36	38.5±0.16
Paracetamol	30	37.6±0.12	38.8±0.08	38.1±0.08	37.8±0.14	37.7±0.08	37.6±0.18	37.64±0.08
EETG	100	37.7±0.18	38.8±0.24	38.4±0.18	38.12±0.17	37.98±0.16	37.90±0.12	37.90±0.14
EETG	200	37.6±0.25	38.8±0.08	38.3±0.2	37.9±0.20	37.82±0.04	37.8±0.3	37.8±0.18
EETG	400	37.6±0.45	38.8±0.1	38.2±0.12**	37.8±0.18**	37.76±0.08**	37.7±0.12**	37.7±0.16

Values are expressed as mean ± SEM (n=6). **P<0.01 Statistically

Values are expressed as mean ± SEM (n=6). **P<0.01 statistically significant as compared to control

In antipyretic study the experimental rats showed a mean increase of about 1.15 °C in rectal temperature 18 h after Brewer's yeast injection. Ethanol extracts showed significant ($p < 0.05$) antipyretic activity and reduction of the elevated rectal temperature after 3 hr of treatment (Table 7.15) in a dose dependent manner. The initial and final rectal temperatures (°C) in the group treated with ethanol extract (200 mg/kg body weight) and ethanol extract (400 mg/kg body weight) were found to be 37.76±0.04 and 37.70±0.07 respectively, compared to 37.64±0.12 in paracetamol (reference drug) treated group. At the dose of 200 and 400 mg/kg body weight the ethanol extract reduced 81% and 83.2% respectively of the elevated rectal temperature as compared to reference drug paracetamol (97.8 %) after 3 h. Hence, the ethanol extract in dose of 200mg/kg and 400mg/kg was found to be effective to treat fever compare to paracetamol.

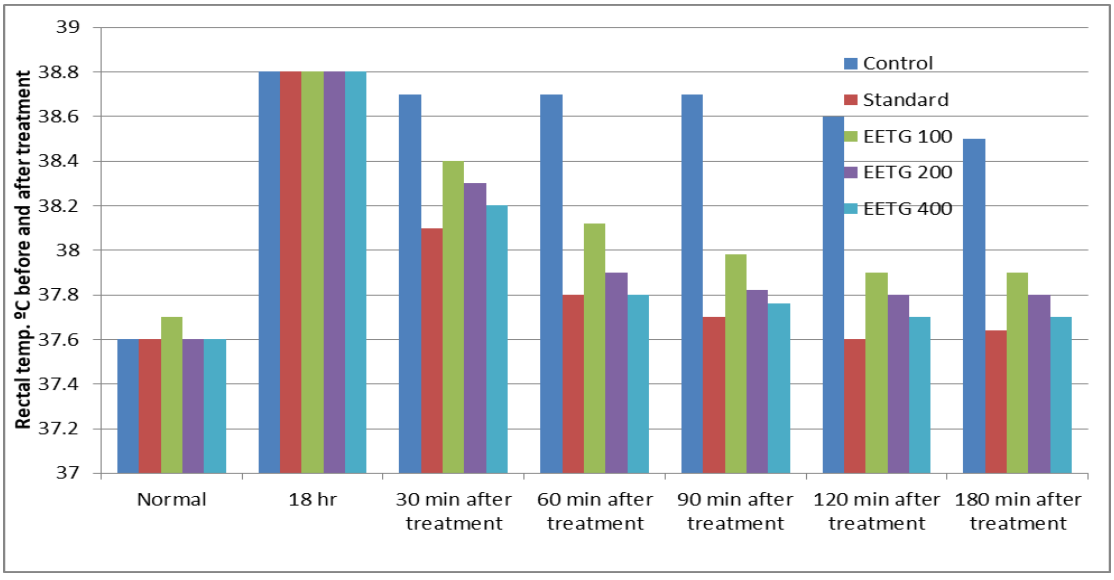


Figure 1: Anti-pyretic Effect of Ethanol extract of *T. Grandis* on yeast induced pyrexia in rats

4. Discussion

Standardization results validate the identity and purity of *Tectona grandis* leaves. Extractive values confirm that polar solvents (especially ethanol) are ideal for extracting bioactive principles due to maximum yield (12.45%) and high solubility of secondary metabolites.[15] Qualitative and quantitative analyses revealed rich concentrations of terpenoids and flavonoids, which are primarily implicated in mediating pharmacological activities such as anti-pyretic action. [16]

In the pharmacological evaluation, the yeast-induced pyrexia model successfully demonstrated the anti-pyretic efficacy of EETG. Pyrexia induced by Brewer's yeast is characterized by elevated prostaglandin levels in the hypothalamus. The anti-pyretic mechanisms of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and paracetamol typically involve the suppression of prostaglandin synthesis or the inhibition of cyclooxygenase (COX) iso-enzymes in the central nervous system. Given that EETG exhibited significant dose-dependent temperature reductions comparable to paracetamol, it is reasonable to conclude that the flavonoids and terpenoids present in *Tectona grandis* exert their anti-pyretic effects via similar mechanisms specifically by preventing prostaglandin biosynthesis in the hypothalamus. [17-18]

5. Conclusion

The comprehensive pharmacognostic, physicochemical, and chromatographic data generated in this study establish reliable benchmarks for the standardization and quality control of *Tectona grandis* leaves. Furthermore, the ethanolic extract exhibits an excellent safety profile LD50 > 2000mg/kg and potent, dose-dependent anti-pyretic activity comparable to standard reference drugs, likely due to its high concentration of active flavonoids and terpenoids.

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