Preliminary Phytochemical Screening and HPTLC Finger printing of Leaf Extracts of Tectona grandis Linn

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Abstract

Tectona grandis Linn. (T. grandis Linn.) (Family- Verbenaceae) is one of the most famous timber plant in the world the leaves of the plant Tectona grandis Linn. were collected, powdered and extracted successively with different solvents. The extracts were subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, and steroids. The TLC and HPTLC techniques were used for qualitative determination of possible number of components in the various extracts. Solvent systems for all the extracts were optimized in order to get maximum separation on plate. Presence of various phytochemicals was confirmed by the use of different spraying reagents.

Keywords: *Tectona grandis Linn, Phytoconstituents, Phytochemical screening, TLC , HPTLC.*

1.RODUCTION

Plants are indispensible sources of medicine since time immemorial. Studies on natural products are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential therapeutic agents[1]. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. In the developed countries, 25% of the medicinal drugs are based on plants and their derivatives. T. grandis Linn. (Family-Verbenaceae) 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[2]. This plant is commonly called as teak and locally known as sagon, sagwanIt is one of the most important heart wood of the world over. Timber value of teak has been well known from decades[3].

The application of green chemistry principles and practices renders regulation, control, clean-up, and remediation of the environment[4-7]. In year 1855 Robert Bunsen invented the burner acts as energy source for heating reaction vessel this was latter superseded by isomental, oil bath but the drawback of the heating though method remains the same[8-13]. Microwave Assisted Organic Synthesis had developed in now years which has been considered superior to traditional heating. Microwave assisted organic synthesis has as a new "lead" in the organic synthesis[14-21]. The technique offers clean, simple, efficient, fast and economic for the synthesis of a number of organic molecules such reaction has new tool in the organic synthesis[22-28]. Important advantage of this technology includes highly accelerated rate of the reaction time with an improvement in yield and quality of product. This technique is considered as important approach toward green chemistry because this technique is more environments friendly and this technology is used in the laboratory and has the potential to have a large impact on the fields of combinatorial chemistry, screening, medicinal chemistry and drug development. Conventional method of organic synthesis usually requires longer heating time, tedious apparatus setup which result in higher cost of process and the excessive use of solvents or reagents lead to environmental pollution[29-36]. Computational studies are the crucial steps in the drug designing. Docking study is the computational routine to determine probable binding manners of a ligand to the dynamic site of a receptor. It makes an image of the dynamic site with interaction points known as grid. Then it fits the ligand in the binding site either by grid search or energy search[37-44]. Due to failure of ADME so it necessary to perform docking studies before pharmacological activity. An outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) raises an unparalleled challenge in the discovery of appropriate drugs for prevention and treatment. Given the rapid pace of scientific research and clinical data produced by the large number of people quickly infected with SARS-CoV-2, clinicians need reliable proof of successful medical care for this infection as in intial stage with help of molecular docking software it is easy to do in-silico study. The chemical modification of drug delivery system for protein and peptide drugs is important in improving both enzymatic stability and membrane permeations can help to have good biological activity from any heterocyclic compound modification. Someday soon, you might be making your own medicines at home. That's because researchers have tailored a 3D printer to synthesize pharmaceuticals and other chemicals from simple, widely available starting compounds fed into a series[45-50].

2. MATERIALS AND METHODS

Collection of Plant Material

The Plant Tectona grandis Linn.were collected from Sangola region. It was authenticated from Department of Botany, Sangola.

Preparation of Extracts

Soxhlet Extraction:

The leaves of Tectona grandis Linn.were dried in shade under normal environmental condition and homogenized to coarse powder and stored in opaque screw tight jars until use. Powdered drug was charged into soxhlet apparatus and extraction was carried outwith following solvents successively.

1. Petroleum ether (40-60^{o}C)

2. Chloroform

3. Ethyl acetate

4. Acetone

5. Methanol

Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50^{o}C under vacuum and dried residue was collected in an opaque glass bottles for further studies. Percentage practical yield of petroleum ether (40-60^{o}C),chloroform, ethyl acetate, acetone, and Methanolic extracts were found to be 3.85, 2.33, 2.39, 7.07 % w/w respectively[51].

Microwave assisted extraction:

Shade drying was done for almost a month as to avoid chemical degradation due to sunlight. Grinding of the dried material was done, with the aid of a grinder and converted into coarse powder. Extraction of Tectona grandis Linn. was done by microwave extraction further filtered and excess solvent present was evaporated and dried extract were collected and subjected for further studies.

Chemicals:

Ferric chloride reagent for flavonoids, Dragendroff's reagent for alkaloids, Liebermann-Burchard reagent for steroids.

Preliminary Phytochemical Screening

The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins and flavonoids. These compounds are termed as secondary

metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the extracts were subjected to battery of chemical tests[52].

Thin Layer Chromatography

All the extracts of Tectona grandis Linn.were subjected to thin layer chromatographic studies, to determine the probable number of compounds present. The precoated TLC plates (Merk, Germany) made up of silica gel G as an adsorbent ,was activated in an oven for 30 minutes at 110°C. Test samples (1mg/ml of all extracts in respective solvents) were applied in the form of bands using Linomate IV applicator[53].

Development of Solvent System

A number of solvent systems were tried 8, 9, in order to get maximum separation on plate. After development of plates, they were air-dried and numbers of spots were noted & R_f values were calculated. Spots were visualized by spraying with various spraying reagents to find different cmpounds present in the extract[54].

3. RESULT AND DISCUSSION

Phytochemical Screening

Preliminary Phytochemical screening of various extracts revealed the presence of different primary and secondary metabolites. Tectona grandis Linn.leaves were found to contain steroids, flavonoids, carbohydrates, saponins, alkaloids and tannins (Table1.).

Table 1. Preliminary Phytochemical screening

Sr. no.	Plant Constituent	Test /Reagent	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract
1	Steroid	a. Salkowski reaction	+	+	+	-
		b. Liebermann-Burchard test	+	+	+	-
2	Alkaloid	a. Drangendroff's reagent	-	-	-	+
		b. Mayer's reagent	-	-	-	+
			-	-	-	+

		c. Hager's reagent d. Wagner's reagent	-	-	-	+
3	Tannin	a. Ferric chloride test b. Lead acetate test c. Potassium dichromate	- - -	- - -	- - -	+ - +
4	Flavonoid	Shinoda test	-	-	+	+
5	Carbohydrate	a. Molisch's test b. Barfoed's test	- -	- -	+ -	+ -
6	Protein	a. Biuret test b. Xanthoproteic test	- -	- -	- -	+ -
7	Saponin	Foam test	-	-	-	+

+ :- Found to be present

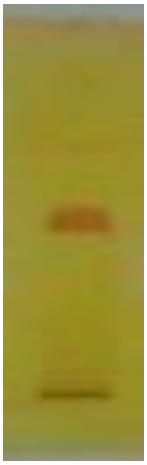
-:- Found to be absent

Thin Layer Chromatorgrpahy and HPTLC

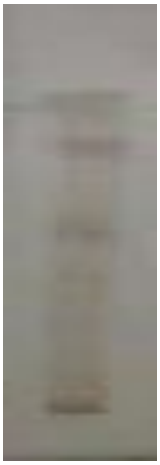
All the extracts were subjected to TLC and HPTLC studies to estimate number and type of Phytoconstituents present in it. Number of solvent systems were tried, however good resolution was obtained in the solvent system mentioned in table 2. In the optimized solvent system Pet ether extract showed-9 chloroform extract -5, ethyl acetate extract -6, Methanolic extract showed -11 bands (Fig. 2-5). Presence of Phytoconstituents in particular extract was confirmed by spraying TLC plates with different spraying reagents. Presence of steroid was detected visually by spraying with Liebermann-Burchard reagent (red color), flavonoids with ferric chloride solution (green cooler) alkaloids show orange color on spraying with Dragendroff's reagent (Fig. 1).

Table 2. Thin Layer Chromatography

Test extract	Solvent system	Number of spots	R_f values
Petrolium ether	Hexane : Ethyl acetate (8:2)	06	0.18, 0.30, 0.41, 0.45,0.59, 0.83
Chloroform	Chloroform : Methanol (9:1)	03	0.58, 0.75, 0.83
Ethyl Acetate	Chloroform : methanol : Formic acid (8:1:1)	04	0.02, 0.10, 0.18, 0.28
Methanol	Chloroform: Methanol (8:2)	04	0.05, 0.15, 0.22, 0.34



Dragendorff's
Orange color



Liebermann-Burchard
Red color

5% Ferric chloride
Green color

Figure 1. TLC

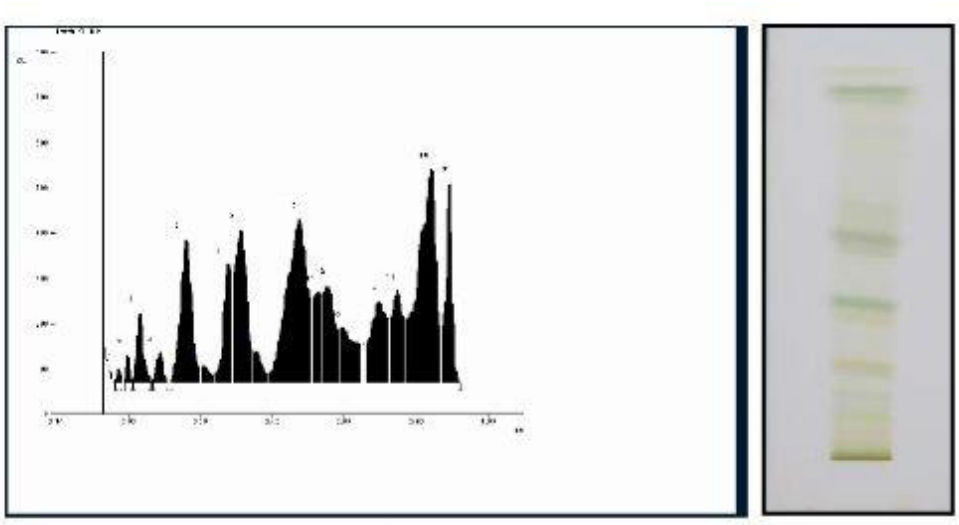


Figure 2. HPTLC of petrolum ether extract of Tectona grandisLinn.

9 compounds were separated having R_f values 0.02, 0.04, 0.07, 0.12, 0.18, 0.26, 0.30, 0.35, 0.41

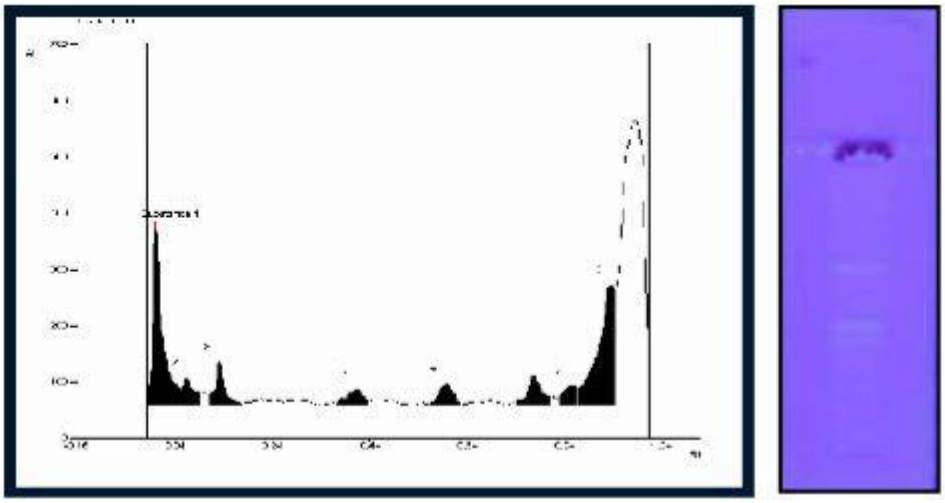


Figure 3. HPTLC of chloroform extract of Tectona grandis Linn.

5 compounds were separated having Rf values 0.58, 0.75, 0.83, 0.90, 0.96

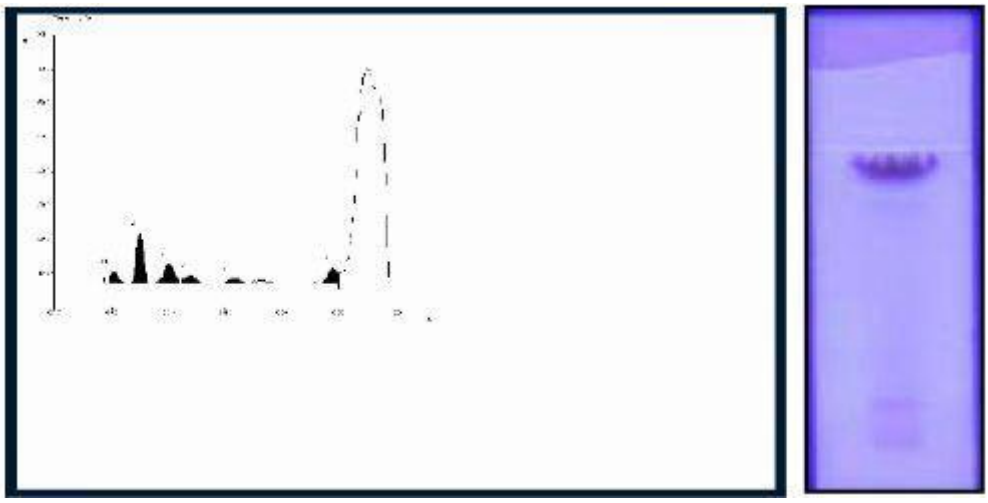


Figure 4. HPTLC of ethyl acetate extract of Tectona grandisLinn.

6 compounds were separated having R_f values 0.02, 0.10, 0.18, 0.28, 0.43, 0.77

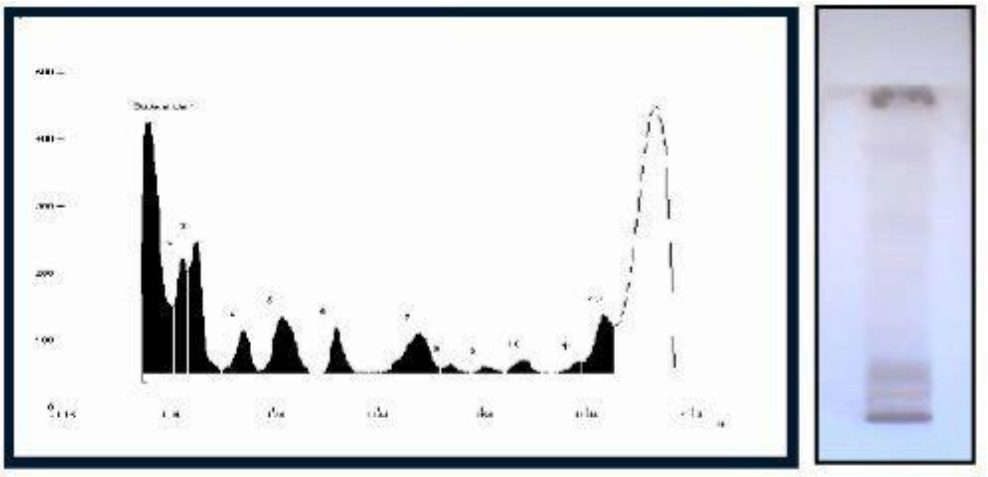


Figure 5. HPTLC of Methanolic extract of *Annona squamosa*

11 compounds were separated having R_f values 0.05, 0.08, 0.15, 0.22, 0.34, 0.45, 0.56, 0.62, 0.69, 0.79, 0.83.

4. CONCLUSION

The study suggests that the methanolic extracts of leaf of Tectona grandis Linn. have several secondary metabolites of medicinal importance and thus justifies medicinal usage. This may be a reason for its better Pharmacological activity.

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