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INVESTIGATION OF PHYTO-CONSTITUENTS & TLC OF *CHLOROXYLON SWIETENIA DC.* LEAVES

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Abstract

In the present study we carried out investigation of phytochemical constituents & thin layer chromatographic (TLC) of *C. swietenia* leaves at standard protocol. The qualitative phytochemical analysis of different solvent in leaves extracts and presence a number of constituents. The maximum constituents are presence in ethanolic & ethyl acetate extract. TLC studies of *C. swietenia* plant leaves constituted different colored phytochemical compounds with different Rf values. Rf value (0.62) it was confirmed the presence of Quercetin as flavonoids compound & Rf (0.15) value it was confirmed the presence of Gallic acid as phenol compound in the ethanol, ethyl acetate & aqueous extract.

Keywords: Phytoconstituents, Thin layer chromatographic, Flavonoids & Phenols.

INTRODUCTION:

C. swietenia, a member of *Rutaceae* family is a medicinal, tropical and aromatic tree of dry deciduous forests.[1] Its common name is Bherul in Sanskrit and Satinwood in English.[2] *C. swietenia* is a moderate-size tree of 9-15 meters in height and 1.0-1.2 meterin girth, with short straight, clear bole up to 3 meter, and spreading crown, common indry, deciduous forests throughout peninsular India. The bark is thick, corky, rough, furrowed, pale yellow or light grey. Leaves are pinnate, 12.5-22.5 cm long, aromatic and leaflets up to 10-20 pairs, oblong, gland-dotted, 2.5 cm long. Flowers are small, white or cream and terminal or axillary paniclulated, calyx short 5 lobed, petals 5, clawed imbricate. [3] *C. swietenia* is considered as a folklore medicinal plant having different types of medicinal uses in the folklore remedies.[4,5] The Malasar tribes Coimbatore (Tamil Nadu) uses leaf paste on wounds, cuts, burns and skin diseases for quick relief & treat worm infested wound of animals, fungal infection of skin and rheumatism. [6-8] Phytochemicals are responsible for medicinal activity of plants the most important of these bioactive compounds of plants are alkaloids, flavonoid and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants Phytochemicals are not considered to be essential to the human diet, but are believed to be beneficial to human health. According to phytochemicals info, there are more than a thousand known preventive chemicals in many plants that ward off diseases. The present

study was to investigate the phytochemical properties & TLC of the plant *C. sweitenia* which has medicinal properties.

MATERIAL AND METHODS

Identification and collection of plant

C. *swietenia* plant fresh leaves free from disease were collected from forest of Hoshangabad month of July 2016. Subsequently identified by one of the authors Prof. Ravi Upadhyay HOD in Botany Govt. P.G. College Pipariya, Hoshangabad.

Preparation of plant extract-

Leaves were washed thoroughly 2-3 times with running tap water and dried in room temp. After one week dried plant material was blended to make homogenous powder and used for successive soxhlet extraction. [9-11] 65 g of the powder was filled in the thimble and extracted successively with various solvents like Pet. Ether, Chloform, Ethyl acetate, Ethanol, and Aqueous (in the ratio of 1:2). [12] Soxhlet was kept running for 72 hours at 30-40 C, until the solvent color appears in the collection tube.

Preliminary phytochemical screening

Successively extract of plant material was subjected to preliminary phytochemical analysis to test the presence or absence of phytochemical constituents by the method of according to the standard procedures. [13,14] Such as steroids, alkaloids and phenols [15]; fatty acids, glycosides & saponins [16]; tannins[17]; reducing sugars [18]; flavonoids.[19]

Thin layer chromatography

TLC is a very effective technique for the separation of chemical constituents of an extract and for their identification. On the basis of result found in phytochemical screening the ethyl acetate, ethanol and aqueous extract was showed the presence of phenols and flavonoids. Prepared TLC plates (TLC silica gel 60 F_{254}, Merck, Germany) were used for the thin layer chromatographic study and solvent system used for study (i)- Toluene: ethyl acetate: Formic acid (7:5:1) for Gallic acid and (ii)- Toluene: ethyl acetate: Formic acid, (5:4:1) for quercetin. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

RESULT AND DISCUSSION

Determination of Percentage Yield

Yield of Extraction: The crude extract so obtained after the soxhlet's extraction process, extract was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, leaves of plant or different solvents used. The yield of extracts obtained from different samples using Pet. ether, chloroform, ethyl acetate, ethanol, aqueous as solvents are depicted in the table 1.

Table 1: % Yield of plant material

S. No.	Solvent	% Yield (W/W)
1.	Pet. ether	1.90
2.	Chloroform	4.25
3.	Ethyl acetate	2.60
4.	Ethanol	11.25
5.	Aqueous	9.45

Phytochemical screening of extract

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the **table 2.**

Table 2: Phytochemical Screening of *C. swietenia in* different extract

S. No.	Constituents	Test name	Pet. Eather	Chloroform	Ethyle acetate	Ethanol	Aqueous
1	Alkaloids	Wagner's test	-ve	+ve	-ve	+ve	-ve
		Hager's test	-ve	-ve	-ve	+ve	-ve
2	Glycosides	Legal's test	-ve	+ve	+ve	-ve	-ve
3	Flavonoids	Alkaline test	-ve	-ve	+ve	+ve	+ve
		Lead acetate	-ve	-ve	+ve	+ve	+ve
4	Phenolic	$fecl_{3\ test}$	-ve	-ve	+ve	+ve	+ve
5	Amino acids	Ninhydrin test	-ve	+ve	+ve	+ve	+ve
6	Carbohydrates	Fehling's test	-ve	-ve	+ve	+ve	+ve
7	Diterpenes	Copper acetate	-ve	-ve	+ve	+ve	+ve
8	Proteins	Xanthoproteic test	-ve	+ve	+ve	+ve	+ve
9	Saponins	Froth test	-ve	-ve	+ve	+ve	+ve

Thin layer chromatography of extracts

1- From the Rf value it was confirmed the presence of Quercetin as flavonoids compound in the extract.

Table 3: Calculation of R_f Value

S. No.	Compound	Extract	R_f Value (Std) (Std)
1.	Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.62

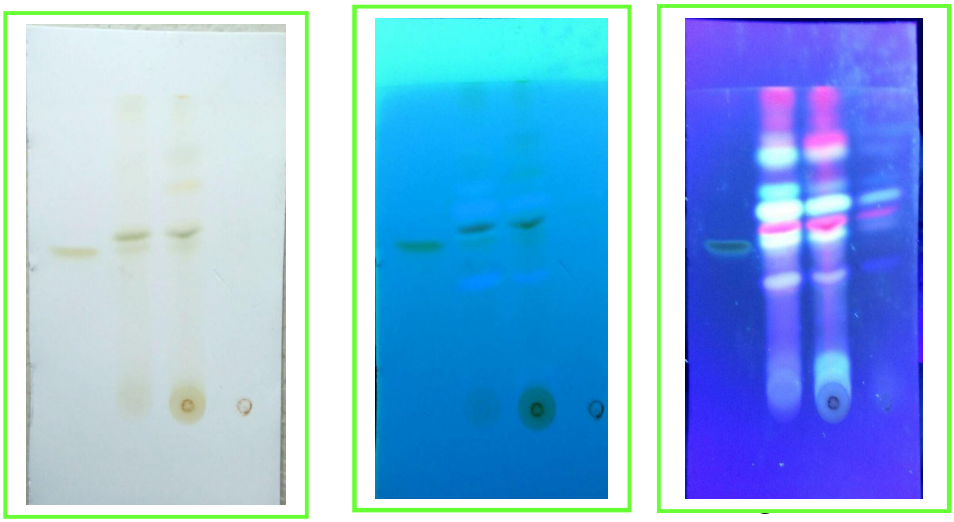


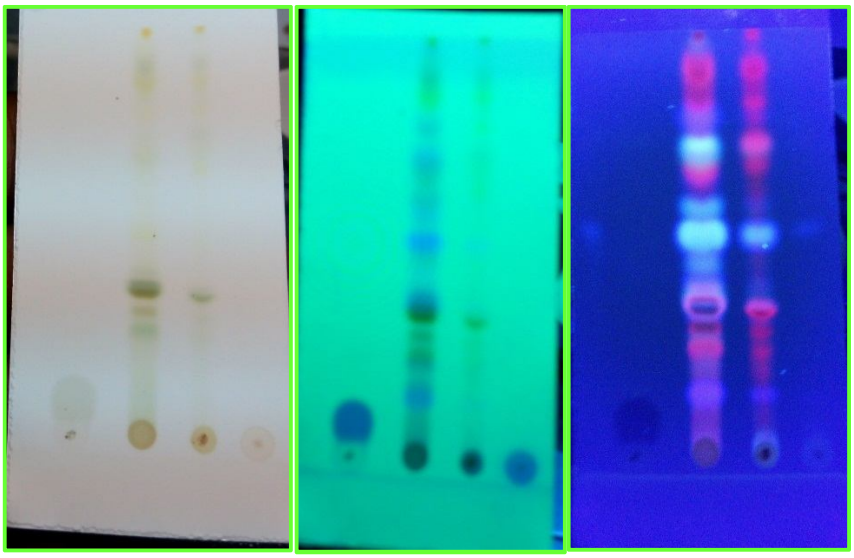
Figure 1: Photograph of T.L.C. (Quercetin)

{1st spot - Standard (Quercetin), 2nd spot- Ethyl acetate, 3rd spot- Ethanol, 4th spot- Aqueous}

2-From the Rf value it was confirmed the presence of Gallic acid as phenol compound in the extract.

Table 4: Calculation of R_f Value

S. No.	Compound	Extract	R_f Value(Std)
1.	Gallic acid	Toluene: Ethyl acetate: Formic acid (7:3:.2)	0.15



Normal Light **Short U.V** **Long U.V**

Figure 2: Photograph of T.L.C. (Gallic acid)

{1st spot - Standard (Gallic acid), 2nd spot- Ethyl acetate, 3rd spot- Ethanol, 4th spot- Aqueous}

CONCLUSION

In conclusion, the result of the present investigation of *C. swietenia* shows that the higher phytoconstituents such as alkaloids, flavonoids & phenol are present in ethanol & ethyl acetate extract. Quercetin as flavonoids & Gallic acid as phenol compound conformed by TLC method. These results suggest compounds have excellent scope for further development of pharmacological properties & different types of biological activity suggested for the further studied.

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