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ARTICLE

CHROMATOGRAPHIC ISOLATION OF TERPENES AND GLYCOSIDES FROM THE LEAF EXTRACTS OF *SIMAROUBA GLAUCA*

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Abstract

Simarouba glauca DC. commonly known as Lakshmi Taru in India is an exotic plant popular for its medicinal values like antiparasitic, anticancerous and antipyretic. Phytochemicals like terpenes and glycosides not only act as antiseptic, antimicrobial, antibiotic but also acts as plant growth regulators. The purpose of the present study was to detect presence of terpenes and glycosides in the leaf extracts of *Simarouba glauca* using TLC followed by the isolation of the fractions contains terpenes and glycosides using column chromatography. Salkowski test and Keller-Killiani test were used for the detection of terpenes and glycosides. The dried leaves of *Simarouba glauca* were extracted with ethyl acetate using Soxhlet. TLC profiling was done using the solvent system hexane: ethyl acetate (8.5:1.5, 5:5, 1.5:8.5). As different phytochemicals have specific Rf values. The presence of terpenes and glycosides were deduced from the Rf values of the band obtained in the chromatogram. It was further confirmed as these bands turned purple upon spraying with H_2SO_4 and subsequent heating at 110°C. column chromatography was carried out to isolate the fractions containing terpenoids and glycosides with different solvent systems using silica gel (60-120 mesh size). The fractions were eluted using hexane (100%), hexane: ethyl acetate (8.5:1.5, 5:5 and 1.5:8.5) and ethyl acetate 100%. TLC profiling revealed the presence of nine (C1–C9) different terpenoid compounds based on the Rf values and characteristic colour produced in UV and visible light and on anisaldehyde spray. Column chromatography yielded 15 fractions, out of which ten fractions tested positive for glycosides and eleven fractions showed positive for terpenoids. These fractions can be further analysed for isolation and identification of different classes of terpenoid compounds and glycosides.

Keywords: *Simarouba glauca,* terpenes, TLC, Column chromatography

Introduction

Simarouba glauca DC belongs to the family Simaroubaceae and is commonly known as 'The Paradise Tree or Lakshmi Taru tree'. Recent researches have provided enough evidence that *Simarouba glauca* DC. is an economically important multipurpose evergreen tree. It is a poly gamo dioecious tree which attains a height of 7-15m and has a tap root system. *Simarouba glauca* is one of the important medicinal plant used against dysentery hence its bark is also known as dysentery bark. The bark and leaf extracts of *S. glauca* is well known for its different types of pharmacological properties such as haemostatic, antihelminthic, antiparasitic, antidysentery, antipyretic and anticancerous. In addition to its medicinal properties, *S. glauca* is also a potential source of biodiesel. Its seeds are known to produce edible oils. The pharmacological importance of *Simarouba glauca* can be attributed to the phytochemical constituents of the leaves. Four alkaloids derivatives isolated from *Simarouba glauca* showed cytotoxic activity against human colon cancer, human oral epidermoid cancer, human hormone dependent prostate cancer and human lung cancer cells.

Terpenoids are a class of hydrocarbons which are one of the pharmacologically active phytochemicals. Terpenes have gained much attention because of their importance as hormones, membrane anchors, pharmaceutical and industrial applications (Jiang et al., 2016). The isolation and characterisation of pharmacologically important terpenoids is very important. The present work deals with the isolation and detection of the terpene and glycoside from the ethyl acetate extract of leaves of *Simarouba glauca* DC. using TLC and column chromatography.

Materials and Methods

Plant material

Fresh leaves were collected from the Kollam district during November 2018. Botanical identification was authenticated by P. Nousaifa Beevi, Assistant Professor, Department of Botany, Ikbal College, Thiruvananthapuram. The leaves of *Simarouba glauca* were separated, washed under the running tap water followed by distilled water and dried at 45°C

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in the oven. The dried leaves were then homogenized to fine powder and stored in air tight containers at room temperatures for further use.

Extraction

Powdered leaves were then packed in Soxhlet apparatus. 30gm of dry powder was subjected Soxhlet extraction with 300ml of ethyl acetate. Extraction was carried out for three hours 9 cycles and temperature were maintained at 65^{0}C. The colour of the extract was dark green. The extract was collected and cooled at room temperature, filtered and poured in glass Petri dishes and then evaporated at 40^{0}C using hot air oven dried extract were kept in desiccator for two days and stored at 5^{0}C in air tight containers for their future use.

Preparation of TLC plates

The TLC plates were prepared by using silica gel G. 30 gm of silica gel was weighed and made to a homogeneous suspension with 60ml distilled water for 2 minutes this suspension was distributed over the plates which was air dried until the transparency of layer disappeared. The plates were dried in hot air oven at 110^{0}C for 30 minutes and saved for further use. The plates were activated at 60^{o}C for 5 min before use. Samples were prepared by diluting the crude extracts with ethyl acetate solvent and then applied 1-10ul volumes to the origin of a TLC plate 2cm above its bottom with the help of capillary tube. The sample loaded plates were kept in TLC glass chambers (saturated with solvent). The plates were developed in respective mobile phase up to 3/4th of the plate. N-hexane: ethyl acetate proportions (8.5:1.5, 5:5, 1.5:8.5) were used as mobile phase for the isolation of terpenoid and glycoside containing compounds followed by their confirmatory tests were done. The movement of the compounds was expressed by its retention factor (Rf) values, calculated as

Rf = Distance travelled by the solute/Distance travelled by the solvent front

Detection- The plates were sprayed with freshly prepared 0.5 % p-anisaldehyde-sulphuric acid reagent. P-anisaldehyde in 50 ml glacial acetic acid and 1ml 97% sulphuric acid and heated to 105^{o}C until maximum visualisation of spots (Biradi & Hullattti, 2017).

Column chromatography

The column with a length of 20 cm length and a diameter of 1.5cm was used for the packing silica gel of mesh size (60-120 mesh size) as an adsorbent. At the bottom of the column cotton plug was kept and wet slurry of silica gel with hexane was made for packing of the column and above it a filter paper was kept. 1gm of ethyl acetate extract and silica gel was ground in mortar and the mixture was placed on top of the column. The eluting solvent was allowed to run through the column to get the fractions. Gradient elution was used to isolate different fractions from the extract by using different ratio of solvent from non-polar to polar (hexane and ethyl acetate) as an eluent. The isolated fractions were tested for the presence of terpenes and glycosides.

Detection of terpenoids and glycosides

Salkowski test -2ml of the extract was dissolved in 1ml chloroform and then equal volumes of concentrated sulphuric acid (1ml) was mixed a reddish-brown coloured interface indicates presence of terpenoids (Gul et al., 2017).

Keller-Killiaini – To 2ml of the extract 2-3 drops of ferric chloride was added. To this solution 2ml conc. H_2SO_4 was added carefully along the walls of the test tube, bluish green colour indicates the presence of glycosides (Singh et al., 2016).

Results and Discussion

Thin Layer Chromatography

TLC profiling of ethyl acetate extracts produced distinct bands. The chromatogram obtained using the solvent system hexane: ethyl acetate (8.5:1.5, 5:5, 1.5:8.5) are shown in figure.1a & 1b. Maximum of seven bands were obtained in Hexane: ethyl acetate 8.5:1.5 solvent system the corresponding Rf values of the bands obtained are given in the Table.1. Out of the seven bands five bands with rf values 0.15, 0.48, 0.57, 0.63 and 0.76 tested positive for the presence of terpenes and glycosides when sprayed with anisaldehyde. Band 3 with Rf value 0.48 can be seen in UV with red fluorescence and grey in visible light while it turned blue when sprayed with anisaldehyde. It was further confirmed as these bands turned purple upon spraying with H_2SO_4 and subsequent heating at 110^{o}C. Three bands having Rf value 0.052, 0.315 and 0.705 were obtained in solvent system with proportion hexane: ethyl acetate 5:5 and tested positive for terpenes and glycosides with anisaldehyde spray. The Rf value of the corresponding bands and their colours in UV long range 364 nm and visible light are given in the Table.2. Two band were obtained in hexane: ethyl acetate 1.5: 8.5 mixture with Rf values 0.052 and 0.810 both showed the presence of terpenes and glycosides (Table 3).

The results revealed that hexane: ethyl acetate mixture in the proportion 8.5: 1.5 shows maximum separation of terpenes or glyosidic bands. The six different Rf values indicate different terpenoid compounds. Jiang et al., 2016 have also pointed out the importance of hexane ethyl acetate mixture in the same proportion is a preferable solvent system for terpenoid compounds. The selection of appropriate solvent system for the isolation of particular phytochemical from a plant extract can be obtained by analysing the Rf values in different solvent systems (Gujjeti and Mamidala, 2013). Kumar et al., 2016 have reported the presence of terpenes and glycosides in *Simarouba glauca* in the phyto-chemical screening using TLC and preliminary screening but a different solvent system was used, the present study shows the potential of hexane: ethyl acetate solvent mixture in isolation and separation of terpenes and glycosides from other

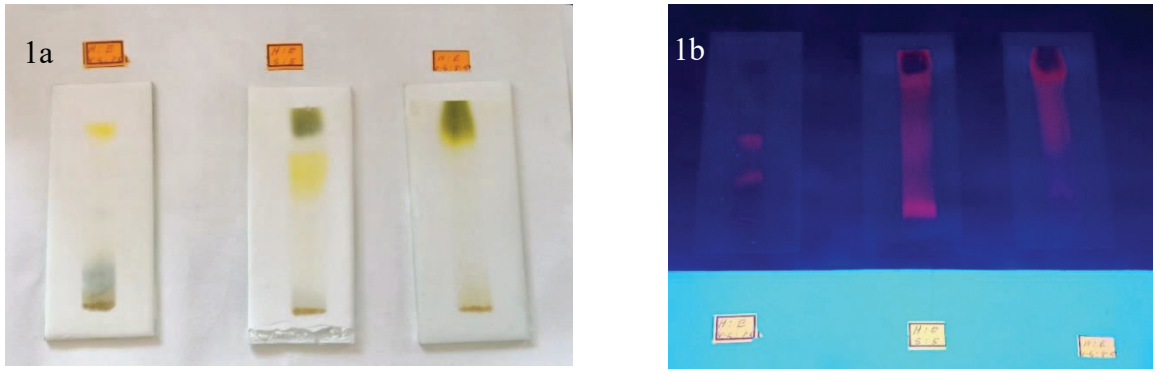

Figure 1 - TLC chromatograms developed using solvent systems n-hexane: ethyl acetate 8.5:1.5 5:5, 1.5:8.5 for ethyl acetate extract of *Simarouba glauca*. 1a TLC chromatograms under visible light 1b. TLC chromatograms under UV long 364nm.

Table 1. Summary of Rf values and the colours of the bands in UV 364nm visible light and after anisaldehyde spray obtained in TLC using Hexane: ethyl acetate mixture (8.5:1.5)

Band no	Rf value	Band colour in UV	Band colour in visible light	Band colour after anisaldehyde spray	Compound identity
1	0.15	-	Grey	Greenish blue	C1
2	0.2	Red	-	-	
3	0.48	Red	Light grey	Light blue	C2
4	0.57	-	-	Light blue	C3
5	0.63	-	Straw	Brown	C4
6	0.76	-	-	Blue	C5
7	0.85	-	Yellow	Blue	C6

Table 2. Summary of Rf values and the colours of the bands in UV 364nm visible light and after anisaldehyde spray obtained in TLC using Hexane ethyl acetate mixture (5:5)

Band no	Rf value	Band colour in UV	Band colour in visible light	Band colour after anisaldehyde spray	Compound identity
1	0.052	Red	Light Grey	Light Blue	C2
2	0.315	-	-	Straw	C7
3	0.705	Brown	Yellow	Yellow	C8

phytochemical constituents using TLC.

The study also sheds light on the colours of terpenes and glyosidic compounds in UV and visible light. Most of the terpenoid and glyosidic bands were not detected in UV in 8.5:1.5 solvent system while most of the bands in the other two solvent systems showed fluorescence quenching in UV long 364 nm. By detecting and tracing the colours of the bands the identity of bands with similar compounds can be done and their Rf values in different solvent systems can be detected. The bands with the terpenoid fractions in different proportions of the hexane: ethyl acetate mixture gives us important information regarding the polarity of different terpenoid and glyosidic compounds in the ethyl acetate extract of *Simarouba glauca*. The results indicate that most of the terpenes in the extract are nonpolar and maximum bands were obtained in 8.5 :1.5 Hexane: ethyl acetate. The

Table 3. Summary of Rf values and the colours of the bands in UV 364nm visible light and after anisaldehyde spray obtained in TLC using Hexane ethyl acetate mixture (1.5:8.5)

Band no	Rf value	Band colour in UV	Band colour in visible light	Band colour after anisaldehyde spray	Compound identity
1	0.052	-	Brown	Grey	C9
2	0.810	Red	Yellow	Yellow	C8

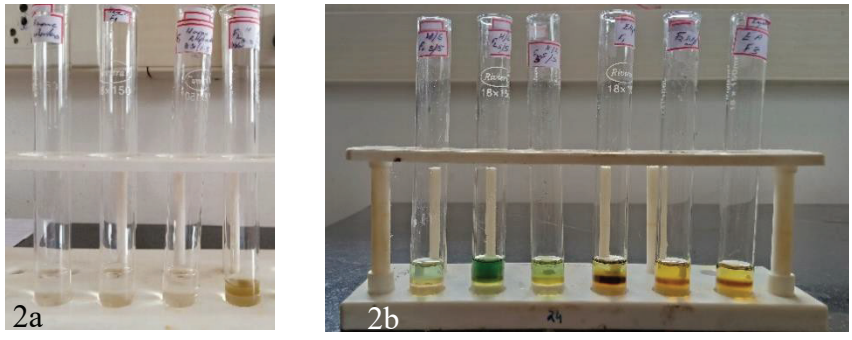

Figure 2 - Results of Salkowski test for presence of terpenoids in different fractions yielded from column chromatography. 2a fractions form 100% hexane and H: EA 8.5:1.5. 2b fractions yielded from H: EA 5:5 and 100% Ethyl acetate.

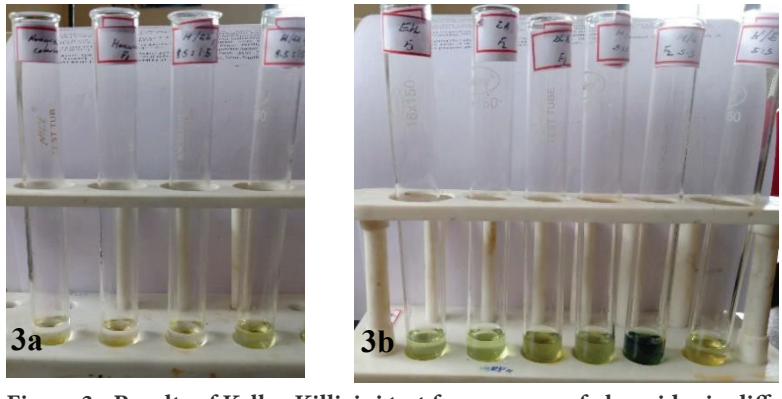

Figure 3 - Results of Keller-Killiaini test for presence of glycosides in different fractions yielded from column chromatography. 3a fractions form 100% hexane and H: EA 8.5:1.5. 3b fractions yielded from H: EA 5:5 and 100% Ethyl acetate.

band with the red fluorescence quenching in UV represented by C2 had lower Rf value in nonpolar solvents system but showed slightly higher Rf vale in 5:5 solvent system. We conclude that the terpenoid compound in band 3 in

H: EA 8.5 :1.5 and band 1 of 5:5 proportion is same as in both chromatogram it showed the same colour pattern i.e., Red in UV 364nm, Light grey in visible light and Light blue after spraying with anisaldehyde spraying. TLC profiling revealed the presence of nine (C1 – C9) different terpenoid compounds based on the Rf values and characteristic the colour produced in UV and visible light and on anisaldehyde spray.

Column chromatography

Column chromatography was carried out to isolate the fractions containing terpenoids and glycosides using gradient elution of increasing polarity with hexane (100%), hexane: ethyl acetate (8.5:1.5, 5:5 and 1.5:8.5) and ethyl acetate 100% were used. Out of the different proportions of the solvent systems used the fractions yielded from 100% ethyl acetate and hexane: ethyl acetate (5:5 and 8.5:1.5) tested positive for the presence of terpenes and glycosides. All fractions obtained from the 100% hexane elusion tested negative for terpenoids and glycosides.

Salkowski test (Figure 2) indicated traces of terpenoids in F2 and F3 of H: E (8.5:1.5), F1 and F2 of H: E (5:5) and F1 and F2 of H: E (1.5:8.5). F1 of 100% Ethyl acetate showed abundance of terpenes while F3 of H: E (5:5) and

H: E (1.5:8.5) showed moderate amounts of terpenoids. Keller-Killiaini test (Figure 3) revealed that glycosides were abundant in F2 fraction of H: E 5:5. Moderate amounts of glycosides were detected in F1 of 100% ethyl acetate and F3 of H: E (5:5). Glycosides were absent in F1 and F2 fractions yielded from of H: E (8.5:1.5) mixture. The results of the quantitative screening of the fractions yielded from column chromatography are summarized in Table 4. The fractions can be used for the isolation and characterisation of terpenoid compounds and further analysis of their biological activities can be carried out.

The present study revealed that pharmacologically valuable terpenoids can be isolated from *Simarouba glauca* leaf extracts by using proportions of hexane: ethyl acetate solvent systems. TLC results revealed the presence of nine different terpenoid compounds. Column chromatography yielded fractions can be further investigated and analysed for the isolation and identification of different classes of terpenoid compounds and glycosides. To conclude the proposed solvent systems and screening procedure is efficient in detection and isolation of terpenoids and glycosides. This can be further harnessed if combined with spectroscopic techniques.

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