ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

PRELIMINARY PHYTOCHEMICAL SCREENING AND HPTLC METHOD FOR QUALITATIVE DETERMINATION OF PHYTOCONSTITUENTS IN EXTRACT OF ALYSICARPUS RUGOSUS (WILD.)

Santosh R Tarke[1], Bharat V. Dhokchawle[2], Pravin G Morankar[3], Vishal G Sakhare[4]

[1, 4]B S P M's B.Pharmacy College, Ambajogai, Maharashtra

[2,3] St John Institute of Pharmacy and Research, Vevoor, Manor Road, Palghar (E), Dist-Palghar-401404, Maharashtra, India.

tarkesantosh@gmail.com

Abstract

The plant kingdom for its therapeutic use has been inadequately explored and there is a tremendous scope for the drug discovery for the human health problems. India has rich in plant wealth and also having excellent usage of medicinal plants with Ayurvedic science. The objective of this study was to carry compound class detection present in extract of Alysicarpus rugosus (Wild.) using chromatographic and by Phytochemical screening methods. Preliminary phytochemical screening was done, physical constants were evaluated and HPTLC studies were carried out. Preliminary phytochemical screening and HPTLC analysis of the extract showed the presence of Tannins, Phenolic compounds and flavonoids. It can be concluded that HPTLC analysis of extract can be used as an authentic diagnostic tool for the correct identification of secondary metabolites and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: Antioxidant, Alysicarpus rugosus, HPTLC, phytochemical screening, Flavonoids, Tannins

Introduction: Qualitative determination of phytochemical compounds is important for maintaining the standard of phytoconstituents. Most Pharmacopoeias only Physicochemical parameters are mentioned which will not justify the quality standard of plant extract. Phytochemical constituents are responsible for the medicinal activity of plant species. Phytochemical screening is an important step in identifying bioactive compounds present in particular medicinal plants. Most pharmacological activities like antidiabetic, antioxidant, anti-allergic, anticancer, immunomodulatory, fungicidal were observed with flavonoids, saponins, steroids and tannins. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters [1, 2]. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [3, 4]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time. Alysicarpus rugosus (Wild.) is one of such plants which being used in Indian traditional medicine for its nutritional value and in livestock as feed. Alysicarpus rugosus (Wild.) an Indian medicinal plant [5, 8]. The native range of this species is India, Tropical & S. Africa, Madagascar, N.

Oman, China (Yunnan) to Tropical Asia, N. Australia. It is an annual or perennial and grows primarily in the seasonally dry tropical biome. Erect, prostrate or ascending annual or perennial herb or subshrub to 60 (150) cm. Stem glabrous or pilose. Leaves stipulate, mostly unifoliolate (simple), rarely trifoliolate, to 1.5–7.5 (–10) cm long and 0.2–2 (–2.5) cm broad, usually oblong, rarely orbicular or linear-lanceolate, obtuse, apiculate, glabrous above, appressed hairy below, obtuse to broadly rounded or subcordate at the base; stipules scarious, almost encircling the stem, 6–11 mm long; petiole c. 3–6 (–20) mm long [9, 11]. Crude protein concentrations range from 15 to 22% for leaf, and 8 to 10% for stem. Tends to become stemmy in mixed pastures. In Zambia, stem comprised 60% of total yield. [12, 14].
The present study was undertaken to Preparation of hydro alcoholic extract by Soxhlet extraction method and its sub fraction by successive extraction in n-Hexane, Ethyl acetate, and aqueous solvents. Phytochemical screening and quantitative estimation of phenolic and flavonoid content for hydro alcoholic extract, its fractions [15, 16]. Primary screening of phytochemical and qualitative analysis of secondary metabolites like Glycosides, Tannins, Saponins, Phenols, Triterpenoids, Flavonoids, Alkaloids and Steroids were carried out by HPTLC method [17, 18]

Materials:
Plant Material: Whole plant of Alysicarpus rugosus (Willd.) DC., collected during the month of February 2018 from the Ambajogai, district Beed of Maharashtra state. Authenticated from Botanical Survey of India [Authentication number: No. BSI/WRC/100- 2/Tech./2018/37], Ministry of Environment, Forest and Climate Change, Western regional center, Pune (Maharashtra) India.

Methods

Extraction: The plant material of *Alysicarpus rugosus* (Willd.) DC were dried at room temperature for fifteen days and then reduced to a coarse powder. This powder was used for the preparation of hydro alcoholic extract. The plant powder was extracted with 50% ethanol for 12 h at 50˚ C. The obtained extract was concentrated under reduced pressure on the rotary evaporator at 40º C to obtain a brownish residue. The above extract was dissolved in distilled water and partitioned sequentially with n-hexane, ethyl acetate, methanol and aqueous to obtain n-hexane, ethyl acetate, methanol and aqueous fractions. All these fractions were concentrated using a rotary evaporator. The yield of hydro alcoholic extract obtained by reflux method was found to be 23.25% w/w. The yield of n-hexane, ethyl acetate, methanol and aqueous fractions obtained by successive solvent-solvent extraction of hydro alcoholic extract was found to be 0.75, 2.85, 17.10 and 24.96% w/w, respectively.

Quantification of total phenolic content: The total phenolic content of the extracts were determined with Folin-Ciocalteu (FC reagent).Reagents used are Folin-Ciocalteu (FC reagent), 20% Sodium carbonate solution and Gallic acid. Stock solution of Standard Gallic acid was prepared by 10mg of gallic acid dissolved in 10 ml of distilled water to obtain the concentration of 1mg/ml. Working solutions were prepared 50, 75, 100, 125, 150, 175, 200, 225, 250µg/ml. of the standard gallic acid were taken from the stock solution and the volume made up to 1 ml with distilled water to obtain the final concentration of 50 250µg/ml. 1

mg/ml of the test plant extracts were prepared. The volume is made up to 45 ml with distilled water in a volumetric flask. 1 ml of FC reagent (diluted 1:2 with distilled water) was then added and the content of the flask mixed properly. After three minutes, 3 ml of 20% sodium carbonate was added to the mixture and it was allowed to stand for 2 hours with occasional shaking. The absorbance of the blue the colour that developed was read at 760nm in the spectrophotometer.

Quantification of total flavonoid content: The total flavonoid content was determined by following the Aluminium chloride colorimetric methods described by Lobo et al, 2011. Reagents used are 5% Sodium nitrite solution, 10% Aluminium chloride solution and 1 M Sodium hydroxide. Stock solution of Standard Quercetin was prepared. Working solution of 10, 25, 50, 75, 100, 125, 150,175, 200, 225, 250µlof the standard Quercetin solution were taken from the stock solution and the volume made up to 1 ml with distilled water to obtain the final concentration of 10- 250µg/ml.1 mg/ml concentration of the test plant extracts were prepared.2 ml of distilled water was added in to the 1ml of the extracts and mixed properly. After 5 minutes 3ml of 5% sodium nitrite and 0.3ml of 10% aluminium chloride were added and stand for 6 minutes. After 6 minutes, 2 ml of 1M sodium hydroxide was added to the solution and the volume was made up to 10 ml with distilled water. The red colored complex formed was measured at 510 nm in a spectrophotometer.

Phytochemical Screening: The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analyses were done using the procedures of (Harborne, 1998) and (Khandelwal, 2005).The following tests were performed on extracts to detect various Phytoconstituents present in them. The detection of various compounds in plant extracts involves a series of chemical tests. For flavonoids, the alkaline reagent test is conducted by adding 1 N NaOH solution to the extract, resulting in a yellow colour that vanishes after adding dilute acid, indicating the presence of flavonoids. Alkaloids are detected by stirring a solvent-free extract with dilute hydrochloric acid and filtering the mixture, followed by testing the filtrate with reagents like Dragendorff's reagent. A prominent yellow precipitate forms if alkaloids are present. For carbohydrates, the filtrate is tested with Benedict's reagent, and upon heating, a characteristic colored precipitate appears, confirming the presence of sugars. Glycosides are detected by hydrolyzing the extract with concentrated hydrochloric acid and filtering it, then subjecting the hydrolysate to the Keller-Killiani test. The appearance of a reddish-brown lower layer and a bluish-green upper layer indicates glycosides. Saponins are identified through the foam test, where shaking an extract diluted with distilled water results in a 2 cm foam layer if saponins are present. To test for proteins and amino acids, the filtrate is treated with copper sulfate, ethanol, and potassium hydroxide in the Biuret test, and the appearance of a pink colour in the ethanolic layer suggests the presence of proteins. Phenolic compounds and tannins are detected by adding Ferric chloride to the extract, which produces a dark green color in their presence. Lastly, phytosterols are identified using the Salkowski test, where the addition of chloroform and concentrated sulfuric acid results in a red-colored lower layer if sterols are

present, while a yellow color suggests the presence of terpenoids. These tests help to identify and characterize the bioactive compounds in plant extracts.

HPTLC Screening:

Table 01: Plate layout of Phytoconstituents for HPTLC screening.

Parameters →	Stationary phase	Plate format	Application	Track
Fingerprint	Merck, Aluminium TLC Plates silica gel 60 F 254	100 x 100 mm	Position Y: 8.0 mm, Length: 8.0 mm, width:0.0	First position X: 20.0 mm, distance:17.4 mm
Flavonoids	Merck, Aluminium TLC Plates silica gel 60 F 254	100 x 100 mm	Position Y: 8.0 mm, Length: 8.0 mm, width:0.0	First position X: 23.9 mm, distance:17.4 mm
Steroids	Merck, Aluminium TLC Plates silica gel 60 F 254	100 x 100 mm	Position Y: 8.0 mm, Length: 8.0 mm, width:0.0	First position X: 23.9 mm, distance:17.4 mm
Saponins	Merck, Aluminium TLC Plates silica gel 60 F 254	100 x 100 mm	Position Y: 8.0 mm, Length: 8.0 mm, width:0.0	First position X: 23.9 mm, distance:17.4 mm
Tannins	Merck, Aluminium TLC Plates silica gel 60 F 254	100 x 100 mm	Position Y: 8.0 mm, Length: 8.0 mm, width:0.0	First position X: 23.9 mm, distance:17.4 mm

Solvent front position 70mm.

Table 02: Development Chamber for Phytoconstituents

Parameters →	Mobile phase	Saturation time	Volume front through	Volume rear through	Drying time
Fingerprint	Toluene: Chloroform: Ethanol (4:4:1 v/v/v)	20 min	5 ml	5 ml	5 min
Flavonoids	Ethyl acetate: Water: Formic acid: Glacial Acetic acid(100:26:11:11 v/v/v/v)	20 min	5 ml	5 ml	5 min

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

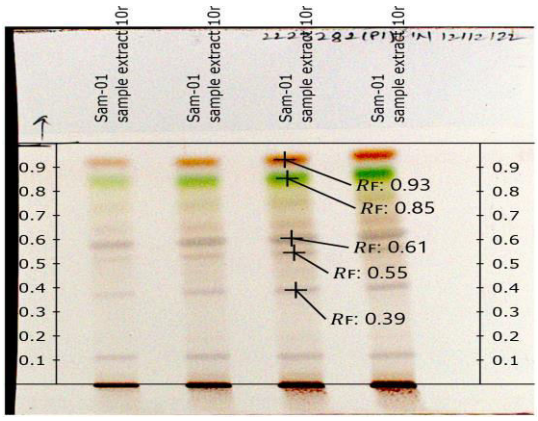
Steroids	n-butanol: Methanol: water (3:1:1 v/v/v)	20 min	5 ml	5 ml	5 min
Saponins	Chloroform: Acetic acid: methanol: water (6:3.2:1.2:0.8 v/v/v/v)	20 min	5 ml	5 ml	5 min
Tannins	Toluene: Ethyl acetate: Formic acid(6:4:0.3 v/v/v)	20 min	5 ml	5 ml	5 min

Drying temperature: Room temperature

Table 03: Derivatization of Phytoconstituents

Parameters	Reagent name	Dipping speed	Dipping time	Heating
Fingerprint	10 % Sulphuric acid in methanol	5 ml	0 s	100 ˚C for 3 min, heated after
Flavonoids	10 % Sulphuric acid in methanol	5 ml	0 s	100 ˚C for 3 min, heated after
Steroids	Anisaldehyde Sulphuric acid	5 ml	0 s	100 ˚C for 3 min, heated after
Saponins	Anisaldehyde Sulphuric acid	5 ml	0 s	100 ˚C for 3 min, heated after
Tannins	$FeCl_3$	5 ml	0 s	None

R white



R366

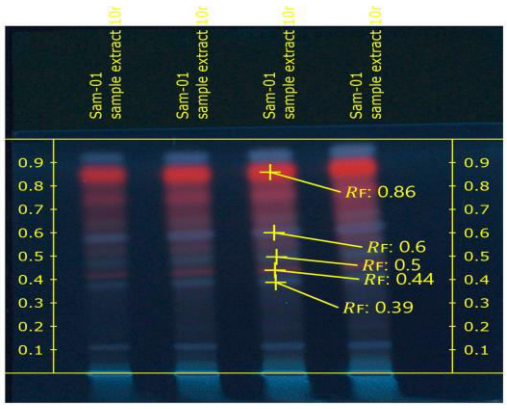


Fig 1: HPTLC profile of Fingerprint

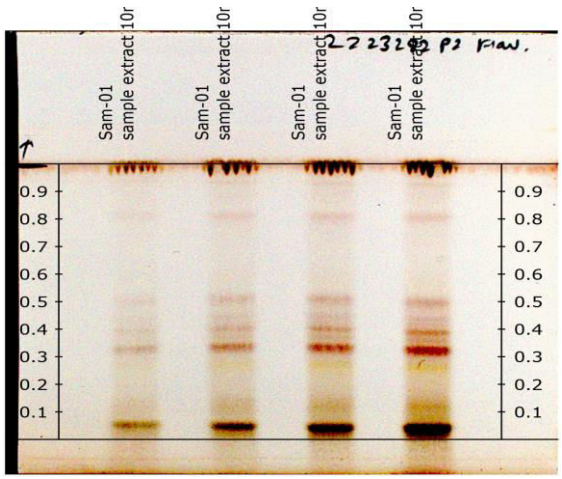
Table 4: HPTLC profile of Fingerprint

Peak	Start	Max	End	Area

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.048	0.0000	0.081	0.0478	7.66	0.126	0.0000	0.00144	4.33
2	0.135	0.0000	0.163	0.0125	2.01	0.190	0.0002	0.00033	1.01
3	0.324	0.0118	0.371	0.0679	10.89	0.405	0.0222	0.00288	8.67
4	0.477	0.0314	0.529	0.0776	12.44	0.553	0.0500	0.00412	12.42
5	0.555	0.0497	0.582	0.1001	16.05	0.624	0.0505	0.00526	15.84
6	0.624	0.0505	0.647	0.0618	9.91	0.698	0.0351	0.00374	11.25
7	0.700	0.0350	0.735	0.0535	8.58	0.776	0.0361	0.00340	10.23
8	0.777	0.0361	0.842	0.0794	12.74	0.877	0.0489	0.00579	17.43
9	0.879	0.0487	0.918	0.1229	19.71	0.981	0.0007	0.00625	18.82

R white



R366

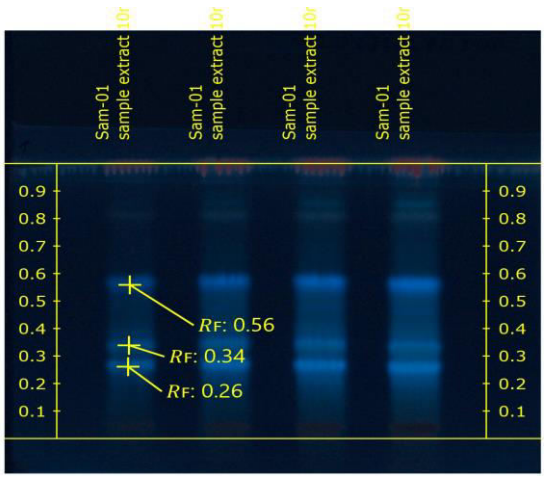


Fig 2: HPTLC profile of Flavonoids

Table 5: HPTLC profile of Flavonoids

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.11	0.005	0.22	0.185	12.8	0.24	0.082	0.0095	13.9
2	0.25	0.082	0.26	0.224	15.5	0.32	0.094	0.0114	16.6
3	0.32	0.094	0.34	0.123	8.54	0.38	0.053	0.0049	7.18
4	0.41	0.033	0.45	0.084	5.83	0.47	0.060	0.0039	5.75
5	0.47	0.060	0.56	0.158	10.9	0.58	0.000	0.0078	11.3

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

6	0.70	0.001	0.76	0.087	6.02	0.79	0.014	0.0032	4.76

R white R366

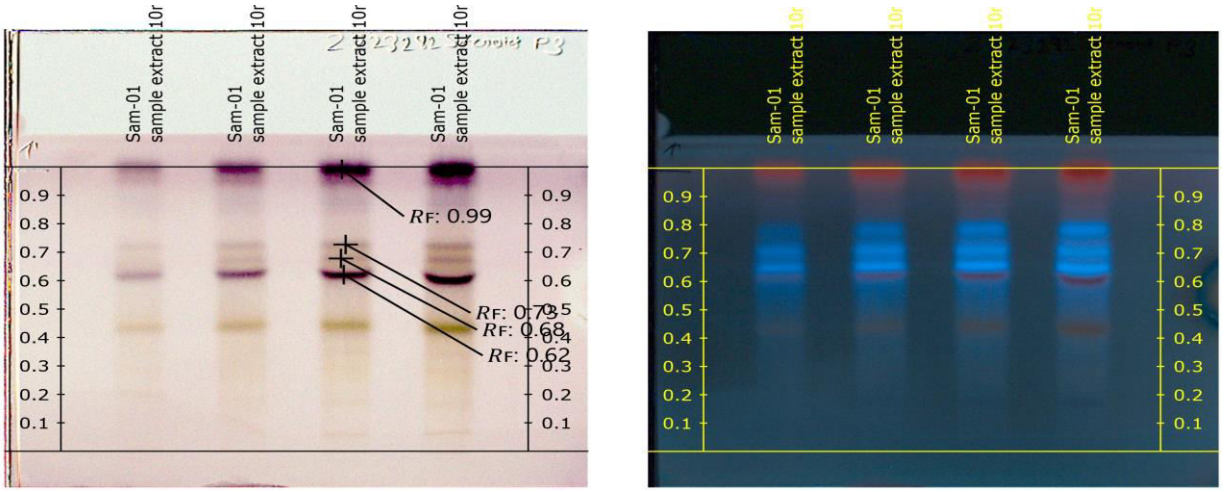


Fig 3: HPTLC profile of Steroids

R white R366

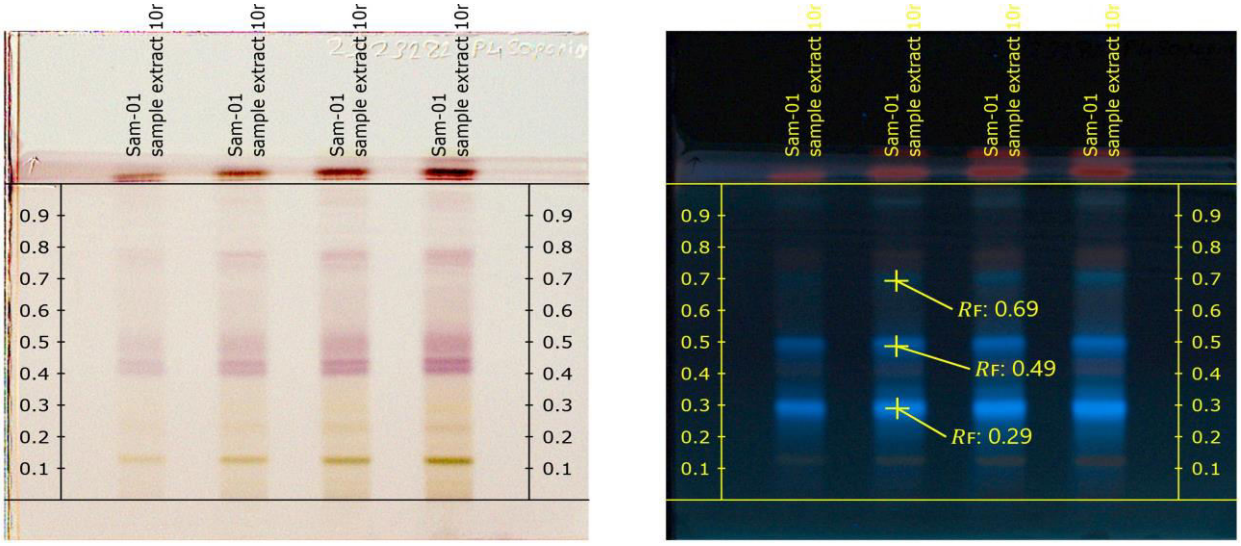


Fig 4: HPTLC profile of Saponins

Table 5: HPTLC profile of Saponins

Peak #	Start		Max			End		Area	
	*R*F	H	*R*F	H	%	*R*F	H	A	%
1	0.173	0.0156	0.234	0.0597	13.43	0.277	0.0190	0.00349	33.10
2	0.390	0.0159	0.435	0.0372	8.37	0.473	0.0141	0.00203	18.70
3	0.915	0.0126	0.963	0.3476	78.20	0.995	0.0001	0.00535	49.20
Peak	Start		Max			End		Area	

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.181	0.0206	0.232	0.0994	17.41	0.274	0.0250	0.00503	33.55
2	0.381	0.0203	0.437	0.0624	10.93	0.482	0.0152	0.00353	23.58
3	0.916	0.0146	0.966	0.4092	71.65	0.997	0.0000	0.00642	42.87
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.166	0.0222	0.232	0.1361	19.94	0.277	0.0346	0.00729	35.93
2	0.381	0.0290	0.440	0.0895	13.12	0.495	0.0210	0.00537	26.43
3	0.913	0.0186	0.968	0.4568	66.94	0.994	0.0012	0.00764	37.64

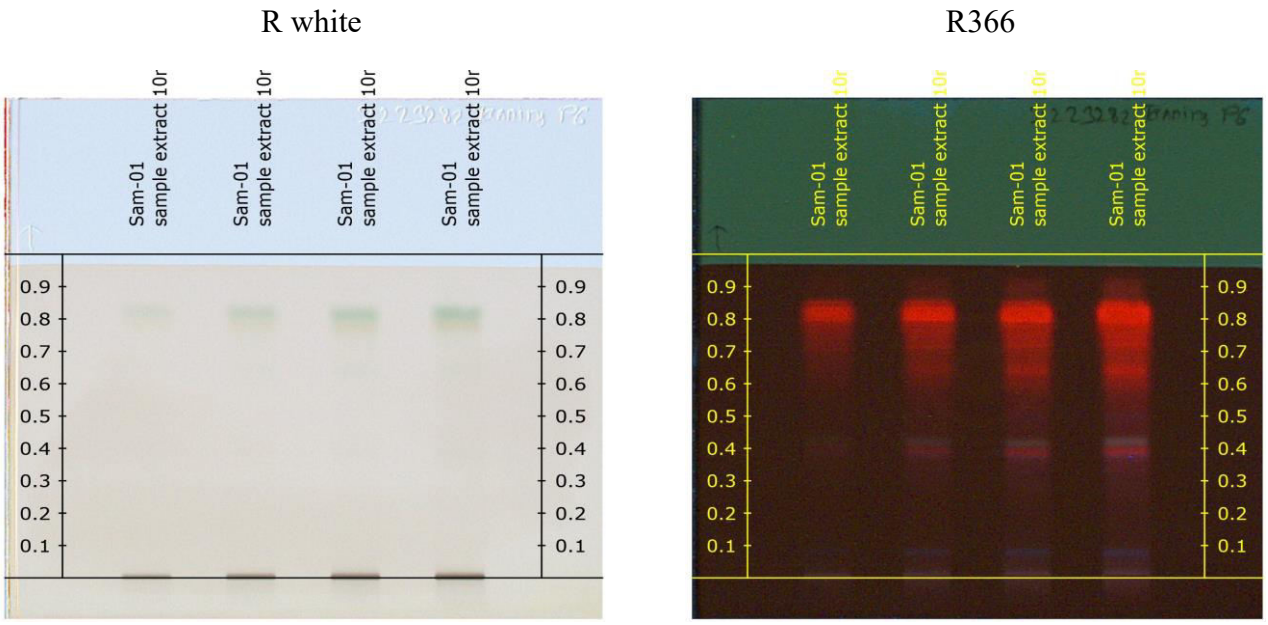


Fig 5: HPTLC profile of Tannins

Result and Discussion:

Hydroalcoholic extract and fractions: The yield of hydroalcoholic extract obtained by reflux method was found to be 23.25% w/w. The yield of n-hexane, ethyl acetate, methanol and aqueous fractions obtained by successive solvent-solvent extraction of hydro alcoholic extract was found to be 0.75, 2.85, 17.10 and 24.96% w/w, respectively.

Table 6: Solvent extraction of Hydroalcoholic extract

Name of extract	Description	Polarity	Yeild
Hydroalcoholic extract	Brown	50% ethanol	23.25% w/w
N-hexane fraction	Green	100% Nhexane	0.75% w/w

Ethyl acetate	Army	100% Ethyl acetate	2.85% w/w
Methanol extract	-	100% Methanol	17.10% w/w
Aqueous fraction	Fluorescent brown	100% water	24.96% w/w

Quantification of total phenolic content: The concentration of total phenols was expressed as Gallic acid equivalents in mg/gm of dry extracts.

Table 7: UV absorbance of various extracts for estimation of Gallic acid

Sample	Abs.at 760 nm		µg/mg
Blank	0.028	-	-
Aqueous Extract	0.449	0.421	43.8
Ethanolic Extract	0.653	0.625	64.2
Methanolic Extract	0.157	0.129	14.6
Ethyl Acetate extract	0.24	0.212	22.9
n-Hexane extract	0.104	0.076	9.3

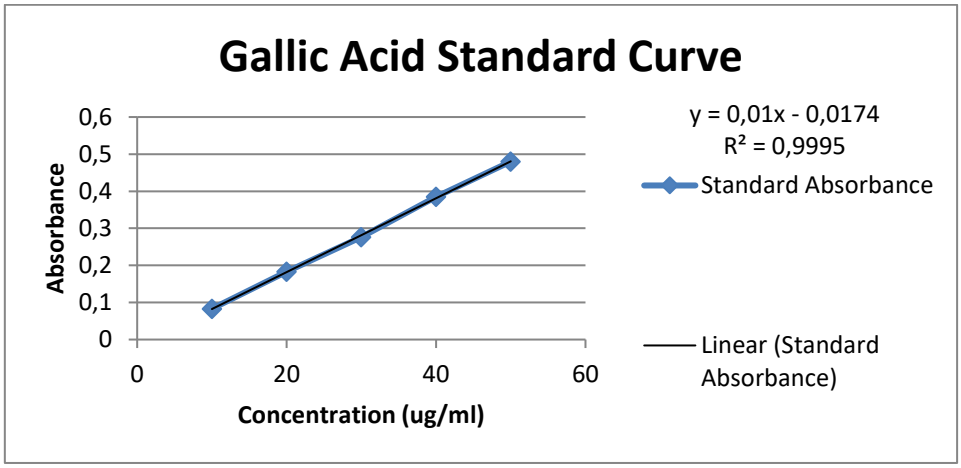


Fig 6: Standard calibration curve of Gallic acid

Quantification of total flavonoid content: The percentages of total flavonoids were calculated from the standard calibration curve of Quercetin (10-250µg/ml) and total flavonoids were expressed as Quercetin equivalents in milligrams per gram sample.

Table 8: UV absorbance of various extracts for estimation of Quercetin

Sample	**Abs. at 510 nm**		**µg/mg**
Blank	0.008	-	-
Aqueous Extract	0.301	0.293	291
Ethanolic Extract	0.903	0.895	893
Methanolic Extract	0.222	0.214	212
Ethyl Acetate extract	3(TURBID)	-	-

n-Hexane extract	0.002	-	-

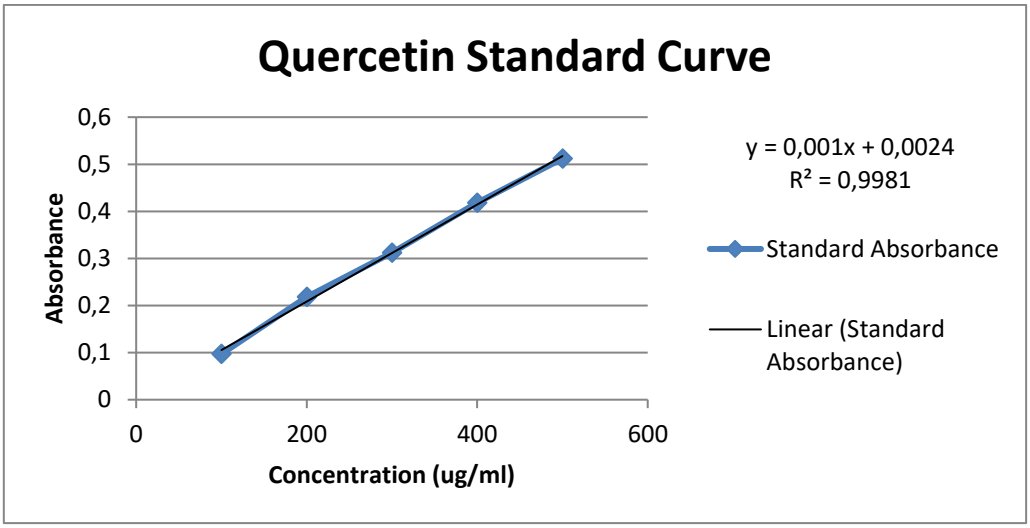


Fig 7: Standard calibration curve of Quercetin

Phytochemical Screening: The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The tests show following results.

Table 9: Phytochemical Screening of various extracts of

Test for	Aqueous Extract	Ethanolic Extract	Methanolic Extract	Ethyl acetate extract	n-Hexane Extract
Flavonoids	+	+	+	+	-
Alkaloids	+	+	-	-	-
Carbohydrates	++	+	-	-	-
Glycosides	-	++	+	+	+
Saponins	++	+	-	-	-
Proteins	-	-	-	-	-
Phenolics	+	+	+	-	-
Terpenoids	+	-	-	+	-
Sterols	-	+	-	+	+

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

HPTLC Screening Analysis: Alkaloids are detected by adding 1–2 ml of Dragendorff's reagent to a few ml of filtrate, which results in a prominent yellow precipitate, indicating a positive test for alkaloids. Flavonoids are identified through derivatization, where fluorescent bands are observed at Rf values of 0.26, 0.34, and 0.56, showing blue bands under UV light at 366 nm after treatment with 10% methanolic sulfuric acid. The presence of phenolic compounds is confirmed by the appearance of a dark green color when the extract is treated with ferric chloride. Glycosides are detected by adding glacial acetic acid, 5% FeCl3, and concentrated sulfuric acid to the test solution, resulting in a reddish-brown lower layer and a bluish-green upper layer. For saponins, after derivatization with Anisaldehyde Sulfuric Acid Reagent (ASR), pinkish and greenish bands are observed at Rf values of 0.29, 0.49, and 0.69 under UV light at 366 nm, indicating the presence of saponins. Steroids are identified by the appearance of violet bands after derivatization with ASR, and the detection of steroids occurs at Rf values of 0.62, 0.68, 0.73, and 0.99 under white light. Tannins, however, are not detected in the sample, as no blue color bands appear after derivatization with FeCl3 solution. Lastly, triterpenoids are identified by adding chloroform and concentrated sulfuric acid to the test extract, where the lower layer turns red, indicating sterols, and the yellow color in the upper layer indicates terpenoids.

Discussion: A vital stage for both research and commercial preparations is the chemical and genetic authentication of therapeutic plants. These days, in addition to morphological markers, the organisms are also classified using anatomical, biochemical, cytological, and molecular markers. To identify or assess the quality of the herbs under investigation, the whole chromatographic profiles are typically utilized. Doing a safety risk evaluation requires a detailed grasp of their chemical compositions. Due to various growth conditions, including climate, soil fertility, harvest season, leaf age, drying process, etc., the chemical contents and their levels in herbs might vary. According to studies, HPTLC is more inventive than standard TLC techniques since the spots are well-resolved. The ability to analyze a large number of compounds effectively and economically makes it a valuable tool for evaluating the quality of botanical materials. In addition to being a useful phytochemical marker, it is also a reliable indicator of genetic variability in plant populations. The unique feature of the HPTLC picture-like image combined with the digital scanning profile makes it increasingly appealing for herbal analysis to create a herbal chromatographic fingerprint. The HPTLC profile of Alysicarpus rugosus (Willd.) DC produced determined the total amount of bioactive components. The chromatographic finger prints that are produced can be accurately stored as electronic images and modified for future use.

Conclusion

Preliminary phytochemical screening was done, physical constants were evaluated and HPTLC studies were carried out. Preliminary phytochemical screening and HPTLC analysis of the extract showed the presence of Tannins, Phenolic compounds and flavonoids. Phytochemical screening can be used to identify secondary metabolites, and the HPTLC method can be used to verify the results. HPTLC analysis has shown the presence of flavonoids, saponins, steroids in the chosen plant. Therefore, the study can be continued to investigate this therapeutic plant in greater depth.

Conflict of Interest: The authors declare that they have no conflict of interest.

References:

1) Philipson JD, Charlwood BV, Rhodes MJ. Plants as sources of Valuable products, Secondary Products from Plant tissue Culture, Oxford, Clarendon Press, 1990, 1- 22.
2) Admuthe NB, Nalwade AR. Micropropagation for Ehretia laevis Roxb. A rare Indian medicinal plant. International Journal of Advanced Scientific and Technical Research. 2016; 6(3):411-422.
3) Ulyana A, Daniel E, Michel H, Edward J, Kennelly S. Antioxidant activity of browning reaction prepared from glucosamine, Phytotherapy Research. 2002; 16(3):63-65.
4) Kamble GS, Upadhye SV, Deshpande NR. In-Vitro Antioxidative Activity of Phenolic and Flavonoid Contents of Leaves of Medicinal Plant – Ehretia laevis. International Journal of Pharma and Bio Sciences. 2011; 2(1):810-815.
5) Jyothirmai N, Nagaraju B, Yasodeepika M, Suresh Kumar J, Rani SG. Evaluation of Anti-Inflammatory and Anti-Bacterial Activities of Different Solvent Extracts of Ehretia laevis Roxb. Journal of Pharmaceutical Sciences and Research. 2016; 8(8):715-720.
6) Torane RC, Kamble GS, Khatiwora E, Ghayal NA, Deshpande NR. Antioxidant Capacity of Leaves and Stem of Ehretia laevis. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2):233-236.
7) Ahmad VU, Rashid MA, Abbasi MA, Rasool N, Zubair M. New salirepin derivatives from Symplocos racemosa. Journal of Asian Natural Products Research. 2007; 9(3):209-215.
8) Pimpliskar MR, Jadhav RN, Jadhav BL. Study on antimicrobial principles of Rhizophora Species along Mumbai Coast. Journal of Aquatic Bio technology. 2011; 26(1):6-11.
9) Sharma B, Gupta S, Sharma K. Natural folk remedy for menorrhagia. Journal of information, knowledge and research in humanities and social service. 2011; 2(1):86-88.
10) Shetty P, Palve A, Jadhav RN, Shinde P, Pimpliskar M. In-Silico docking analysis of S. lychnophora compounds against protein causing Alzheimer's disease. International Journal of Engineering Science and Innovative technology. 2014; 3(4):158-164.
11) Bhutani KK, Jadhav AN, Kalia V. Effect of Symplocos racemose Roxb. On gonadotropin release in immature female rats and ovarian histology. Journal of Ethno pharmacology. 2004; 94(1):197-200.
12) Ahmad UV, Abbasi MA, Hussain H, Akhtar MN, Farooq U, Fatima N et al. Phenolic glycosides from Symplocos racemosa: natural inhibitors of phosphodiesterase –I. Phytochemistry. 2003; 63(2):217-220.
13) Sathiavelu M, Arunachalam S. High performance thin layer chromatography profile of Cassytha filiformis. Asian Pacific Journal of Tropical Biomedicine, 2012, S1431-S1435.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 01, 2022

14) Goodarzi M, Russel PJ, Heyden YV. Similarity analyses of chromatographic herbal fingerprints: A review. Analytica Chimica Acta. 2013; 804:16-28.
15) Khandelwal KR. Practical Pharmacognosy. 12th ed. Pune: Nirali prakashan, 2004, 149-160.
16) Dinesh Kumar C. Pharmacognosy can help minimize accidental misuse of herbal medicine. Current Science. 2007; 93:1356-1358.
17) Elangovan M, Dhanarajan MS, Rajalakshmi A, Jayachitra A, Pardhasaradhi M, Narasimharao B. Analysis of Phytochemicals, Antibacterial and Antioxidant activities of Moringa oleifera Lam. Leaf extract- an in vitro stud. International Journal of Drug Development and Research. 2014; 6(4):280-285.
18) Tarke Santosh Rangnathrao and Dr. P. Shanmugasundaram. Preliminary phytochemical screening and HPTLC method for qualitative determination of phytochemical compounds in extract of Ehretia laevis Roxb. J Pharmacogn Phytochem 2018;7(6):867-874.