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Phytochemical Analysis and HPTLC Screening with Quantification of Bioactive Compounds in Cassia fistula for Pharmaceutical Applications

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ABSTRACT
Aim: The goal of this study involves thorough identification and measurement of bioactive compounds in Cassia fistula pods ethanolic extract through High-Performance Thin-Layer Chromatography (HPTLC).
Study Design: An experimental investigation of Cassia fistula pods ethanolic extract through phytochemical screening and HPTLC analysis took place.
Place and Duration of Study: These experiments were performed at the Department of Pharmacognosy within BharatiVidyapeeth's structures during the six-month duration.
Methodology: The preliminary phytochemical screening tested crude ethanolic extracts of Cassia fistula pods to identify alkaloids, flavonoids, phenols along with tannins as secondary metabolites. The research team used HPTLC to perform structured analysis for identifying main bioactive components in the investigated samples and to measure the levels of flavonoids along with phenolic acids specifically.
Results: The phytochemical analysis showed that the plant contains important secondary metabolites. The results from HPTLC analysis showed high contents of flavonoids and phenolic acids that have documented both antioxidant and anti-inflammatory properties.
Conclusion: The scientific study reveals that Cassia fistula contains abundant bioactive components which makes it interesting for usage in pharmaceutical research and biomedical applications. Scientists should continue studies regarding the individual determination and structural definition of compounds to establish new therapeutic entities.

Keywords: Phytochemical Analysis, HPTLC, Cassia fistula, Bioactive Compounds, Quantification, Pharmaceutical Applications

1. INTRODUCTION

Cassia fistula, commonly known as the golden shower tree [1], is a South Asian native that spreads throughout the tropical and subtropical regions of the globe. The plant holds a prime position as an ornamental due to the attractive, cascading yellow flowers and held firm in traditional medical systems such as Ayurveda, Unani, and Siddha due to its medicinal properties. Historically, various parts of the plant have been used to treat numerous diseases [2]. Among these are skin disorders, gastrointestinal conditions, respiratory fevers, and inflammatory diseases [3]. Investigations into bioactive compounds in Cassia Fistula are imperative because of its long history in traditional medicine that implies it has significant pharmacological potential. Its purgative, laxative, and anti-inflammatory properties have made it popular in propulsive traditional medical practices. While dermatitis and ringwormto are treated with leaves and bark respectively,the fruit pulp is generally administered as an ant constipation remedy[4]. Due to its established antibacterial properties, the plant can be employed for disease management. The potential of Cassia fistula to lower inflammation is one of its most noteworthy medicinal properties, and it has caught the interest of contemporary pharmacology. Natural products like Cassia fistula provide promise alternative or complementary therapy in light of the increasing frequency of chronic inflammatory illnesses including arthritis, cardiovascular disease, and inflammatory bowel problems [5]. The necessity to properly examine such traditionally used plants for their pharmacological advantages is further highlighted by the increased interest in plant-based chemicals for pharmaceutical and biological uses [4,6].

The benefits of Cassia fistula are linked to its high-quality phytochemical composition. Current scientific studies validate the medicinal properties of this plant. Preliminary phytochemical investigations on the plant indicate the presence of secondary metabolites which include flavonoids, tannins, phenolic acids, alkaloids, and glycosides. These have been established to possess anti-inflammatory, antioxidant, and antibacterial activities [7]. This review focuses particularly on the scavenging of free radicals, inhibition of pro-inflammatory enzymes, and modulation of immunological responses by flavonoids; they have particularly attracted attention and thus are excellent candidates for chronic inflammation management. The strong antioxidant properties of phenolic acids may protect against oxidative stress damage. More work is therefore required to define better bioactive compounds in Cassia fistula and their specific roles in physiological regulation [8]. HPTLC has worked well as an effective analytical method for the qualitative and quantitative determination of phytochemicals. Some advantages include high sensitivity, quick analysis time, and capability to analyze multiple samples simultaneously. It has become one of the major methods used in analyzing compounds derived from plants; this is especially true concerning quality control in herbal formulations [9]. The accurate identification and quantification of bioactive components such as flavonoids and phenolic acids contribute significantly toward establishing their role regarding anti-inflammatory characteristics as well as medicinal properties in Cassia fistula [10].

The present work aims to carry out an exhaustive phytochemical evaluation of Cassia fistula and utilize HPTLC screening for the identification and quantification of bioactive compounds [11]. This literature review addresses previous works concerning the analysis of pharmacological properties of the other compounds against inflammation, oxidative stress, and bacterial inhibition to emphasize their importance in secondary metabolite production. It is hoped that this study will partially contribute to the existing scientific body of Cassia fistula; asked for potential growth into pharmacological substances [12].

2. MATERIALS AND METHODS

Mature pods of Cassia fistula L. were picked from natural habitat during the month of August 2024 at Wai Forest area, Satara. Cleaning was done carefully, and air drying was done at room temperature to remove moisture followed by pulverizing into fine powder using a mechanical grinder for extraction; pod extracts were prepared from powdered plant material using Soxhlet apparatus and rotary evaporator. In a laboratory that was completely hygiene maintained with all necessary safety precautions, the experimental procedures were performed for accurate, dependable results. The chemicals and reagents selected of analytical grade for the phytochemical analysis as follows ethanol, distilled water, and various standard chemicals for qualitative tests. To avoid contamination, thoroughly cleaned all glass wares and equipment before use.

2.1. Hptlc Methodology

2.1.1. Sample Preparation

Dissolving 100 mg CF Extract in 10 ml volumetric flask, to which add 5 ml methanol, and sonicate for 15 mins at 25 °C. Then dilute up to the mark with methanol 10000 (100 mg/ml). Centrifuge this solution at 10,000 rpm for 10 minutes, and the supernatant should be collected in glass vial for HPTLC analysis. Mobile phase - Toluene: Ethyl acetate: Methanol:Water: Formic acid (6.0 : 12.0 : 1.0 : 1.0 : 2.0 v/v/v/v/v), b) Stationary phase - HPTLC silica gel 60 F254 TLC plate (Merck). Chamber Saturation Time: 20 Min Solvent Run: 80%

2.1.2. Quantification Of Scopoletin

Standards Solution preparation: Take 10 mg of Scopoletin in 10 mL volumetric flask. Add 5 mL of (70: 30/water : Methanol) Solution and sonicate for 15 minutes at 25 °C. After that dilute up to the mark with (70 : 30 / water : Methanol) Solution then take 1ml from this stock solution in a 10 mL volumetric flask and dilute upto the mark with (70 : 30/ water : Methanol) Solution.

3.0. RESULTS& DISCUSSION

Phytochemical screening C. fistula extracts indicated to harbour several substances in different solvents as shown in Table 1. Alkaloids were present in all extracts including petroleum ether, ethanol, ethyl acetate, and aqueous. Flavonoids were found in aqueous and petroleum ether extracts, as well as ethyl acetate. Tannins were present in aqueous, ethyl acetate, and ethanol extracts, but phenols were present in only the aqueous extract. Proteins were present in ethanol extract and aqueous extract, as well as ethyl acetate. The carbohydrates were present in all extracts, resins in all except aqueous extracts. Steroids were detected only in the petroleum ether extract, glycosides were present in the aqueous, ethyl acetate and ethanol extracts, quinones were found in the ethanol extract, and terpenoids were identified in the aqueous, ethyl acetate, and petroleum ether extracts. [13-15].

Table 1. Phytochemical screening of Cassia fistula pods, (+ present; - absent).

Sr. No.	Phytochemical Constituents	Phytochemical Tests	Inference			
			Aqueous	Ethyl acetate	Ethanol	Pet ether
1	Alkaloids	Hager's test	+	+	+	+
2	Flavonoids	Alkaline	+	-	+	+
		Zn-HCl acid reduction	+	+	-	-
3	Phenol	Ferric chloride test	+	-	-	-
4	Tannis	Ferric chloride test	+	+	+	-
5	Proteins	Xanthoproteic acid test	+	-	+	+
		Biuret test	+	+	+	-
6	Carbohydrates	Molish test	+	+	+	+
7	Resin	Acetone distilled water test	-	+	+	+
8	Steroids	Salkowski test	-	-	-	+
9	Glycosides	Legal test	+	+	+	-
10	Quinones	Alcoholic KOH test	-	-	+	-
11	Terpenoids	Salkowski test	+	+	-	+

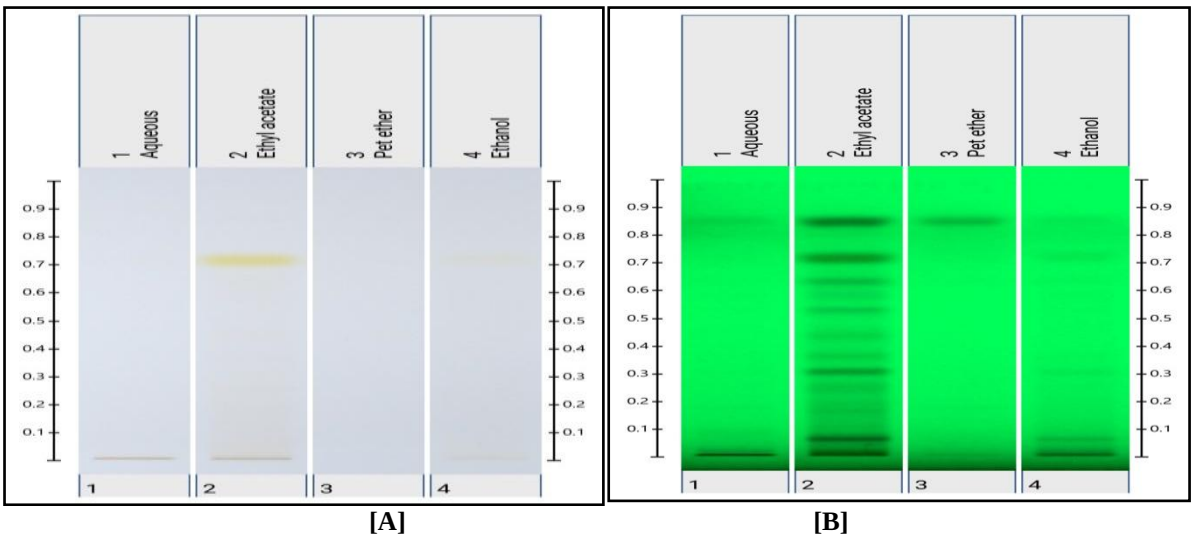
The samples and standards mentioned in table 2 have to be placed on a uniform thickness of 0.2 mm pre-coated silica gel $60F_{254}$ TLC plate (Merck). Development with the specified mobile phase will continue until a solvent front of around 80 mm is observed. The plates are then left to dry. The separated components were observed under white light and UV light at 254 and 366 nm. This execution will provide primordial information on the phytocomposition of the samples by identification and characterization of the compounds based on their absorbance and fluorescent properties.

Table 2 Sample and Standard Details for TLC Analysis

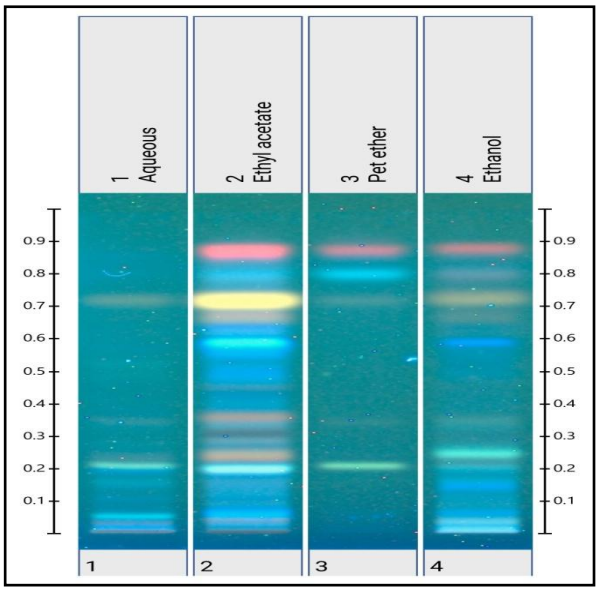
Sr.No	Track No.	Name of Sample/Standard	Batch and Lot No.	Concentration in PPM	Volume in µL
1	1	Aqueous Extract	---	10000	10
2	2	Ethyl acetate Extract	---	10000	10
3	3	Pet ether Extract	---	10000	10
4	4	Ethanol Extract	---	10000	10

The developed TLC plate was observed under white light and two UV wavelengths, 254 and 366 nm, as illustrated in figure 1. The examination indicated the presence of several prominent bands with different colors, which correspond to the presence of various compounds in the extract. The bands appeared in visible colors under white light, while 254 and 366 nm excited them to fluoresce in different patterns. The observations made at multiple wavelengths allow the identification and differentiation of the phytochemical constituents present in the samples, thus providing insights into their chemical composition and probable bioactive properties.

a)After development HPTLC Screening image at White light,254nm and 366nm



[A] **[B]**



[C]

Fig. 1 indicating the presence of different compounds in the extracts After development HPTLC Screening image at White light ,254nm and 366nm: A) White light, B) 254nm C) 366nm

Chromatographic analysis was conducted on extracts of Cassia fistula, and multi-wavelength scanning of the developed plates was achieved in white light, UV 254 nm, and UV 366 nm showing multiple peaks [17] displayed in table 3. These peaks refer to different possible phytoconstituents that have specific patterns of absorbance at different wavelengths. The findings under 254 nm light correspond to compounds that show strong absorbance in the UV region, and under 366 nm light, those compounds exhibiting fluorescence were highlighted. The observation in white light reveals visible bands that support the overall phytochemical characterization and identification of constituents in Cassia fistula. The ethyl acetate and ethanol extract shows maximum bands/ constituents (flavonoids) at 366nm.

Table 3 Peak observed after development, scan at White light at 254 & 366 nm observations are shown.

Sr. No	Name of extract	White light	At 254nm	At 366nm
		No. of Band observed	No. of Band observed	No. of Band observed
1	Aqueous	-	2	7
2	Ethyl acetate	1	10	11
3	Pet ether	-	4	4
4	Ethanol	1	12	12

In Table 4, the preparation of different Cassia fistula extracts for HPTLC analysis is described. For the aqueous extract, 109 mg of the extract was weighed and dissolved in a 10 ml volumetric flask using a 70:30 water solution, sonicated for 15 minutes at 25°C, then diluted to the mark with the same solution. After centrifugation at 10,000rpm for 10 minutes, the supernatant was collected, achieving a final concentration of 10,900 PPM. For the ethyl acetate extract, 260 mg was similarly processed, yielding a final concentration of 26,000 PPM. Lastly, the ethanol extract was prepared by weighing 129 mg and following the same protocol, resulting in a final concentration of 12,900 PPM. These prepared supernatants were used for HPTLC analysis. Apply the samples and standards as indicated in table 4 on a pre-coated silica gel $60F_{254}$ TLC plate (Merck) with a uniform thickness of 0.2 mm. developing the plate in the designated mobile phase to a distance of 80 mm. After development, allow the plate to dry. Visualize the developed plate under 254 nm, 366 nm, and white light as shown in Figures A to E to observe the separation of compounds. Finally, scan the plate at 345 nm for detailed analysis as depicted in Figure F to I.

Table 4: Quantitative Tracking of Scopoletin in Cassia Fistula Extract Using HPTLC Analysis

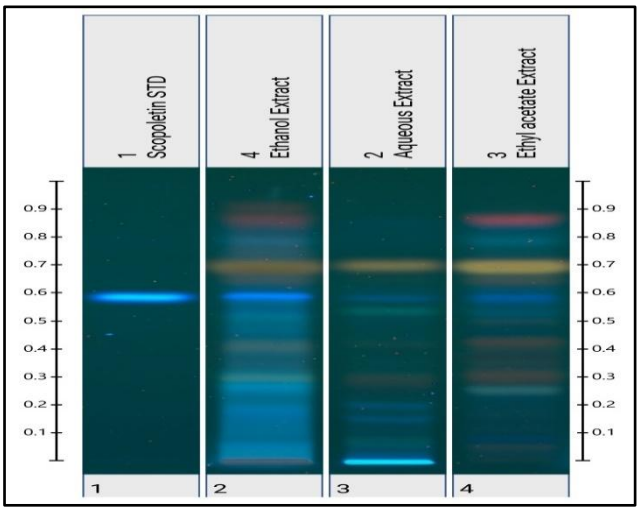
Sr.No.	Track No.	Name of Sample/Standard	Concentration in PPM	Volume in µL
1	1	Scopoletin	100	0.1
2	2	Scopoletin	100	0.3
3	3	Scopoletin	100	0.5
4	4	Scopoletin	100	1.0

5	5	Scopoletin	10000	3.0
6	9	Ethyl acetate extract	26000	20.0
7	10	Ethanol extract	12900	20.0
8	11	Aqueous extract	10900	40.0

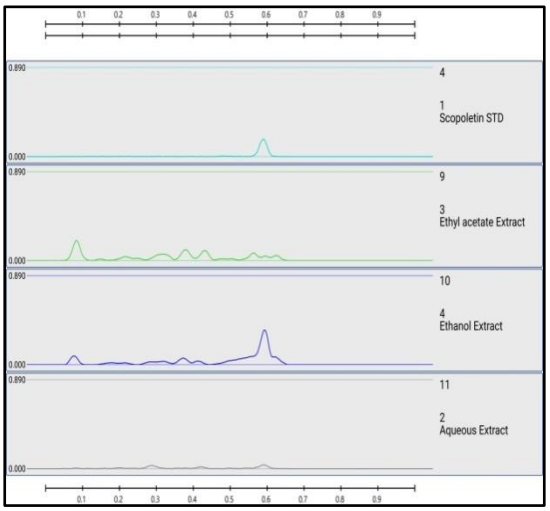
The results of the HPTLC analysis indicate the presence of scopoletin in various Cassia fistula extracts as displayed in table 5. 4.502 µg of scopoletin was noted for an otherwise unspecified amount of the aqueous extract weighing 109.0 mg. In the second assessed ethyl acetate extract, 2.917 µg of scopoletin were present in 260.0 mg of extract. In contrast, the ethanol extract contained a significantly high concentration with scopoletin present at 132.8 µg in only 129.0 mg of extract. This observed result, therefore, indicates varying levels of scopoletin within the various extracts.

Table 5 Quantitative Tracking of Scopoletin in Cassia Fistula Extract Using HPTLC Analysis at 345 nm after Development

Sr.No	Track No	Vial Id	Name of Sample/Extract	Scopoletin Observed in the Extract
1	11	2	Aqueous extract	4.502 µg in 109.0 mg
2	9	3	Ethyl acetate extract	2.917 µg in 260.0 mg
3	10	4	Ethanol extract	132.8 µg in 129.0 mg



[A]



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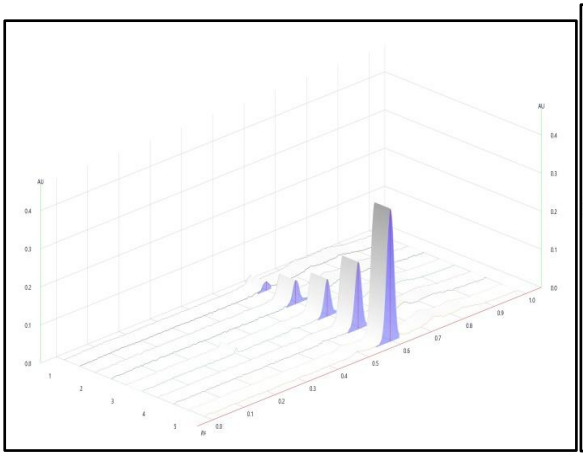

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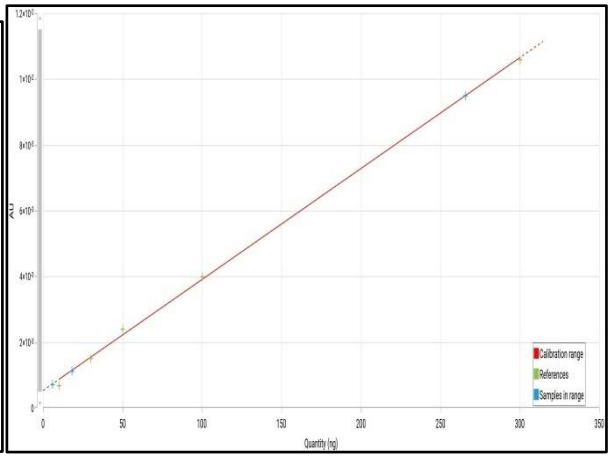
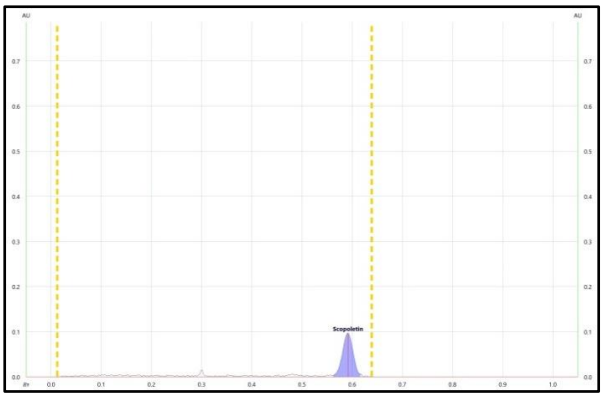
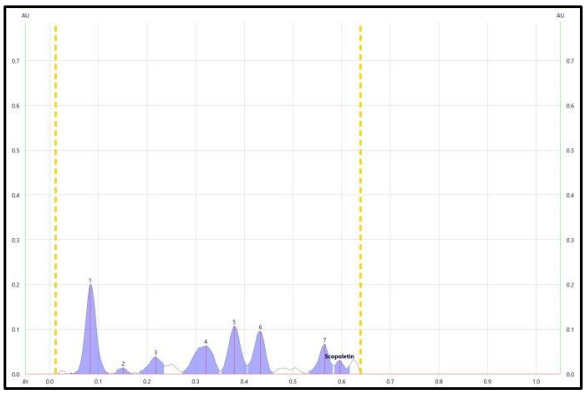


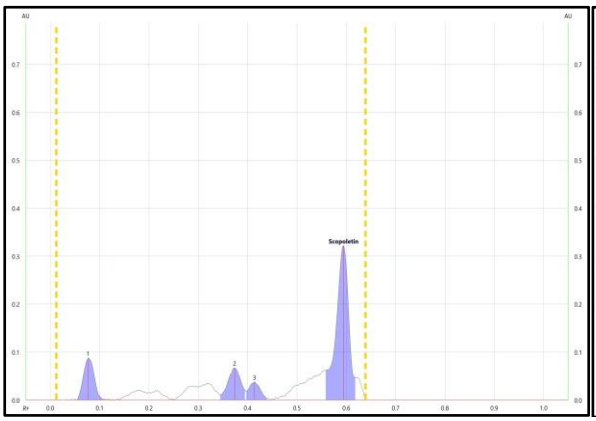
[D]



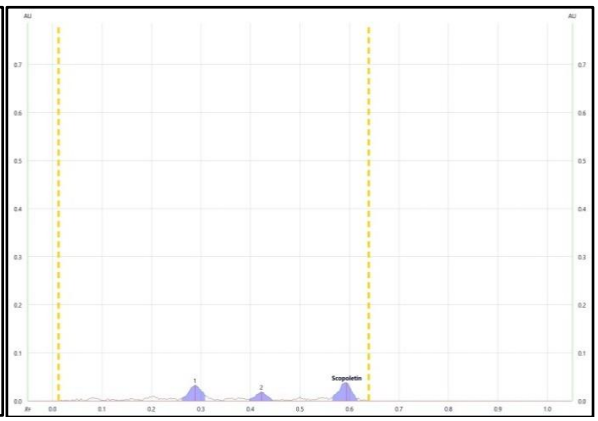
[E]



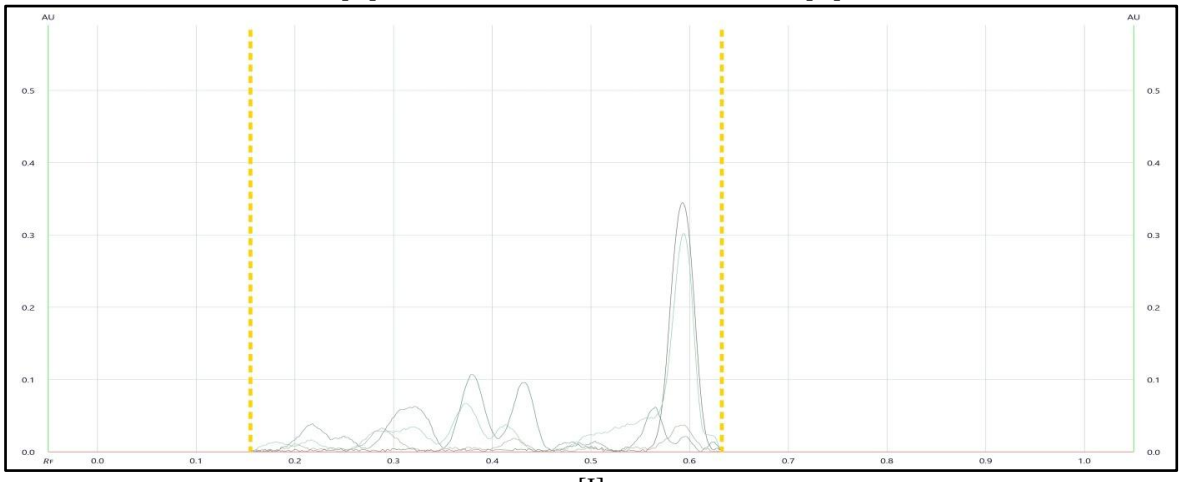
[F]



[G]



[H]



[I]

Note: Fig. 2 Quantitative Tracking of Scopoletin in Cassia Fistula Extract Using HPTLC Analysis at 345 nm After Development: A) Quantitative Tracking of Scopoletin in Cassia Fistula Extract Using HPTLC Analysis at 345 nm After Development, B) Overlay of All Three extract, C) Linearity, 3D Image of Scopoletin STD, D) Linearity of Scopoletin STD, E) Densitogram of Scopoletin STD, F) Densitogram of Ethyl acetate extract, G) Densitogram of Ethanol extract, H) 11Densitogram of Aqueous extract, & I) Overlay Image of Scopoletin STD with three extract.

4.0. CONCLUSION

According to HPTLC analysis, the extracts from the pulp of Cassia fistula pods exhibit specific phytochemical profiles in varying solvents. Scopoletin was observed in ethanol extracts in substantially higher concentrations as compared to ethyl acetate and aqueous extracts. Phytochemical screening revealed a presence of alkaloids, flavonoids, phenols, tannins, proteins, carbohydrates, resins, steroids, glycosides, quinones, and terpenoids, depending upon the solvent used which extracted differential sets of compounds. Visualization with varying wavelengths (254 nm, 366 nm, and white light) showed separation into distinct bands, indicating a mixture of

different phytochemicals. Thus, a complete profile provides an indispensable insight into the chemical composition of extracts derived from Cassia fistula along with their prospects in pharmaceuticals and other biological sciences.

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