PHYTOCHEMICAL SCREENING BY HPLC ANALYSIS AND FTIR SPECTROSCOPIC TECHNIQUES OF CASSIA TORA LEAVES EXTRACT IN DIFFERENT SOLVENTS

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Abstract:
The present study is aimed to analyse the chemical constituents of the chloroform, ethanol and petroleum ether extracts of leaves of *Cassia tora* through preliminary phytochemical investigation, HPLC and FTIR spectroscopy method. The results of the phytochemical study showed that flavonoids, alkaloids, saponins, glycosides, terpenoids, steroids, and tannins were present. With a C18-150 × 4.6 mm column, a 10 µinjection volume, and a 70:30 methanol: water mobile phase at 30°C, a reversed-phase HPLC analysis was carried out. The UV-detector at 254 nm was used to record the detection. The FTIR spectroscopic analyses of the extracts under study revealed varied characteristic stretching frequencies and peak values with various functional components. Preliminary phytochemical investigation of ethanol and petroleum ether leaves extract of *Cassia tora* confirmed the presence of emodin, anthraquinone, glycoside, tannin and alkaloids. The existence of primary amines, alcohols, amide, aldehydes, ethers, aromatics, alkanes, alkyl halides, alkenes, and aliphatic amines compounds, which display prominent peaks, was confirmed by FTIR analysis of leaf extracts in ethanol, chloroform, and petroleum ether. The phytochemical analysis, HPLC spectra, and FTIR spectrum profiles for these medicinally significant plants were produced as a consequence of the current study and can be applied in the field.

Keywords: *Cassia tora*, phytochemical investigation, HPLC, FTIR

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INTRODUCTION:

The phytochemicals found in the therapeutic plant extract are non-toxic, cost-effective at low doses, and environmentally friendly. Plant scientists have been interested in phytochemical research as a result of the advancement of cutting-edge methods. These methods were very important in the hunt for additional raw material sources for the pharmaceutical sector [1]. The primary stage in recovering and isolating bioactive phytoconstituents from plant sources is plant leaf extraction [2]. In the past 20 years, High Performance Liquid Chromatography (HPLC) analysis has been the most popular and easily adaptable technology for the measurement of flavonoids [3]. According to Wosch et al. [4], analytical methods like Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) can be used to determine the quality of raw materials and related products for therapeutic application. One of the most popular spectroscopic methods for examining functional groups in plants is infrared spectroscopy. The different functional groups responsible for the therapeutic characteristics of the medicinal plants will be revealed by identifying the chemical composition of the phytochemical substances found in them [5].

One of them is the well-known, tiny annual plant known as a weed in Asian nations, *Cassia tora* [6]. It grows in Asian countries on hills and tropical countries plains and waste places at low elevations of 1800 m. Different parts of the plant are useful against various ailments in indigenous medicines [7]. In India, it is found as the weed in wild areas in Orissa, Himachal Pradesh and Bihar. It is an important content of Ayurvedic Preparation 'Dadhungnavati' which is an important antifungal formulation [8, 9]. The leaves are acrid, antiperiodic, thermogenic laxative, depurative, liver tonic, and useful in dyspepsia, fever, flatulence, bronchitis, leprosy, helminthiasis, cardiac disorders, pruritus, and hemorrhoids [10, 11]. In folklore practice, leaves have been reported to possess antirheumatic activity. The seeds are used in Chinese medicine as aperient, hypolipidemic, for diuretic, improving vision, and atherosclerosis [12]. Various chemical compounds have been isolated from *Cassia tora* such as Anthraquinones, sennosides, emodin and their extract possesses hepatoprotective, anti-inflammatory [13, 14]. This study aims to identify the different chemical constituent, isolation of compound and functional groups of isolated compound in chloroform, ethanol and petroleum ether extract of *Cassia tora* by using HPLC and FT-IR techniques.

MATERIALS AND METHODS:

Collection and authentication of plant: The leaf of *Cassia tora* was used for present analysis. The plants were collected from the Rohilkhand Region, Bareilly (U.P.) and taxonomically authenticated from Department of Plant Science, Dr. Alok Srivastava, MJP Rohilkhand University, Bareilly (Voucher specimen no. PS/2019/19). The leaves were shade dried and grounded to fine powder.

Extraction of Plant leaves: After the leaves dried in shadow, it was finely powdered with a grinder. Dried powdered leaves were extracted with each Soxhlet apparatus using various solvent system i, e. from non-polar to polar to organic solvents. In study organic solvent such as petroleum ether (43.7g), chloroform (41.4g), ethanol (40.8g) and Aqueous (39.8g) are non-polar to polar respectively. Each round bottom flask had 500 ml of petroleum ether, 400 ml of chloroform, 500 ml of ethanol, and 500 ml of aqueous solvent poured to it. Each flask was connected to a Soxhlet extractor and condenser. Warm solvent progressively fills the compartment containing the solid substance. In the heated solvent, some of the desired chemical dissolves. When the solvent level in the Soxhlet chamber reaches the syphon, it spills back into the distillation flask when the chamber is almost full. We allowed this cycle to occur several times for 48–72 hours. till the colour cycle is understood. The extract solution was then placed on a water bath for a few days at a temperature of 40–50°C to remove the solvents and produce the drug extract. [15, 16]

Phytochemical Analysis: Phytochemical analysis was carried out for identification of tannins, Glycosides, flavonoid, alkaloid, phenol, phytosterol, and saponins according to standard methods. [17, 18]

HPLC Analysis: Column C18 (150 ×4.6 mm), a universal loop injector (Rheodyne 7725) with an injection capacity of 10 µL, a UV-Vis spectrophotometer detector, and HPLC (Shimadzu Prominence HPLC apparatus) were used for the HPLC analysis. Shimadzu Lab Solution 6.43 SPI was the programme utilised for the HPLC analysis. Before inserting the injection of the solution of chloroform, ethanol, and petroleum ether extract, the column was equilibrated by passing the pure solvent through the column for one and a half hours.

FTIR Spectroscopic Analysis: All of the FTIR spectra were captured within the spectrum range

(4000 to 650 cm^{-1}) using an FTIR spectrophotometer (Agilent Cary 630 FTIR, Agilent Technologies, Inc. California). Using a diamond attenuated total reflectance (ATR), characteristic peaks of several natural and functional groups were measured, and IR values were calculated by comparing the readings to the IR frequencies. [19-24]

RESULTS AND DISCUSSION:

Extraction: Percentage yield value of different extract of Cassia leaves were mentioned in table 1.

Table 1: Percentage yield *Cassia tora* leaves extract.

S. No.	Solvent	Weight of Drug (gm)	Yield	% Yield
1	Ethanol	40.8	8.56	20.98
2	Petroleum ether	43.7	7.92	18.12
3	Chloroform	41.4	7.45	17.99
4	Aqueous	39.8	5.32	13.36

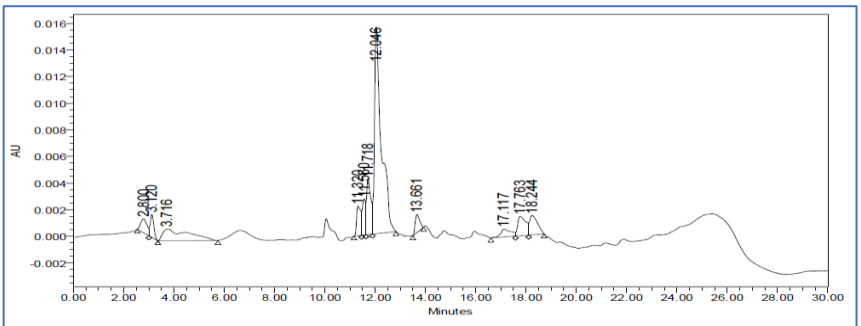
Phytochemical Analysis: Table 2 contains the findings of a phytochemical investigation of several extracts. The *Cassia tora* plant is used locally to treat a variety of ailments because its leaves contain phytochemicals. Secondary metabolites found in plants including carbohydrates, saponins, glycosides, alkaloids, tannins, and flavonoids are thought to be active and are primarily in charge of pharmacological action. The plant contains flavonoids, anthraquinone, saponins, glycosides, alkaloids, and terpenoids. Pet ether was shown to be a relatively ineffective solvent for extracting the phytochemicals under study. The polarity of the ethanol solvent may be reflected in the ethanol extract with the highest concentration of metabolites.

Table 2: Preliminary phytochemical investigation of different extracts of Cassia tora

S. No.	Phytoconstituents	Chloroform	Ethanol	Pet. Ether	Aqueous
1	Carbohydrate	-	+	-	+
2	Flavonoids	+	+	+	+
3	Alkaloids	+	+	-	-
4	Tannin	+	+	+	+
5	Glycosides	+	+	+	+
6	Saponin	-	-	-	+
7	Anthraquinone	+	+	+	-
8	Sterols	-	-	-	-
9	Protein	-	-	-	+
10	Terpenoids	+	+	-	-

Present- (+) Absent- (-)

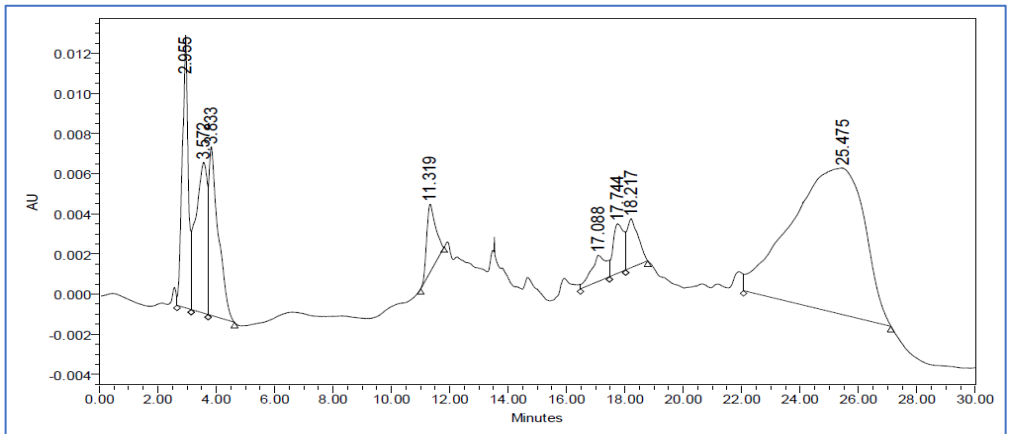
HPLC: HPLC analysis of chloroform, ethanol and petroleum ether extracts were used for estimating compounds in the extract. HPLC analysis of chloroform, ethanol and petroleum ether extracts of leaves of *Cassia tora* was found to contain several glycosides compounds like quercetin, emodin, anthraquinone in varying amounts. The presence of secondary metabolites was confirmed by phytochemical analysis and HPLC analysis, both of which had distinct peaks and retention times.



	RT	Area	% Area	Height
1	2.800	18409	3.11	1057
2	3.120	17776	3.00	1734
3	3.716	62833	10.62	887
4	11.320	25686	4.34	2229
5	11.560	24578	4.15	2702
6	11.718	48974	8.28	4158

	RT	Area	% Area	Height
7	12.046	295274	49.91	15536
8	13.661	16554	2.80	1356
9	17.117	16622	2.81	599
10	17.763	33131	5.60	1455
11	18.244	31781	5.37	1464

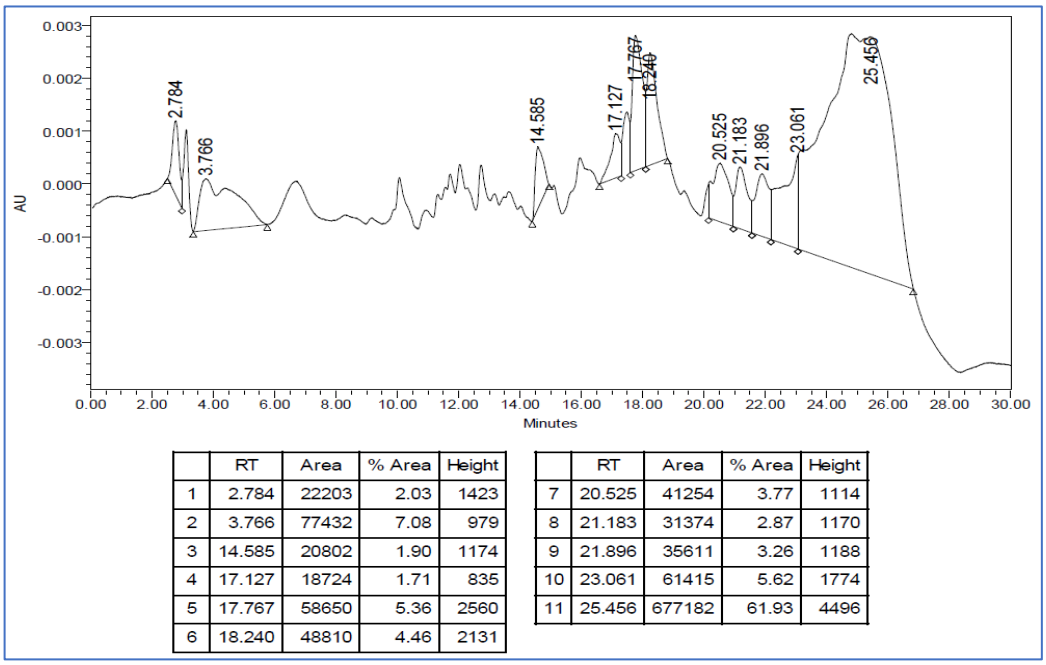
Figure 1: HPLC Analysis of chloroform extract



	RT	Area	% Area	Height
1	2.955	189511	9.10	13588
2	3.572	204409	9.82	7517
3	3.833	190935	9.17	8407
4	11.319	70840	3.40	3399
5	17.088	45958	2.21	1301
6	17.744	63533	3.05	2464

	RT	Area	% Area	Height
7	18.217	64942	3.12	2439
8	25.475	1251627	60.12	7302

Figure 2: HPLC analysis of ethanol extract



	RT	Area	% Area	Height
1	2.784	22203	2.03	1423
2	3.766	77432	7.08	979
3	14.585	20802	1.90	1174
4	17.127	18724	1.71	835
5	17.767	58650	5.36	2560
6	18.240	48810	4.46	2131

	RT	Area	% Area	Height
7	20.525	41254	3.77	1114
8	21.183	31374	2.87	1170
9	21.896	35611	3.26	1188
10	23.061	61415	5.62	1774
11	25.456	677182	61.93	4496

Figure 3: HPLC Spectra of petroleum ether extract

FTIR Spectroscopy: The FTIR peaks revealed a variety of functional groups that were very important to *Cassia tora's* therapeutic effects. The identification (authentication), assessment, and standardisation of the plant are the initial uses for this data. It is possible to determine the origin of different extracts and the identity of medicinal ingredients using the macroscopic fingerprint characteristics of the FTIR spectrum. The existence of distinct phytochemical substances extracted using different solvents was shown by the bands between wavenumbers (4000-650 cm^{-1}). The interpretation of infrared absorption spectra can also be used to identify bonds in diverse substances. The plant included functional groups of several phytochemicals, including phenol, alkanes, alkenes, aldehydes, alcohols, amines, and ethers, according to FTIR data. These functional groups may be responsible for a variety of pharmacological actions. These phytochemicals have various functional groups like C-O, O-H, C-H, CH_3 C=C, and N-H. No bond was found in the region of 3600 to 600 cm-1 indicating that no cyanide group was present in the samples.

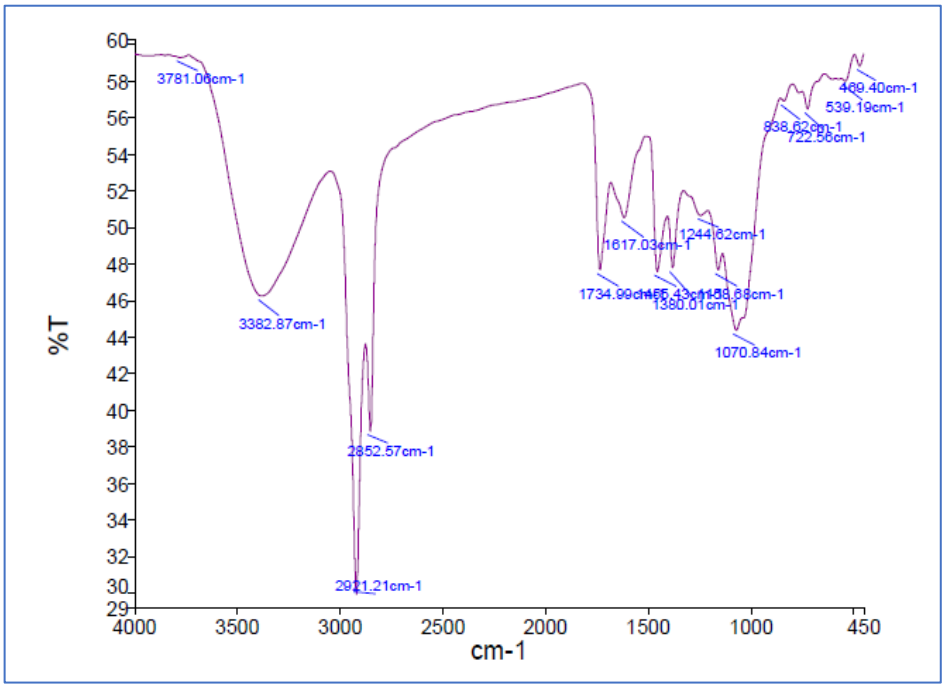


Figure 4: FTIR Spectra of Chloroform extract

Table 3: Interpretation of chloroform extract

Characteristic Absorption (cm^{-1})	Group Frequency (cm^{-1})	Vibrational Mode	Functional Group
3382.87	3400-3200 (s)	-OH Stretch Broad	Alcohol
2921.21	2950-2700 (w)	-CH Stretch usually two bands	Ac-H
2852.57	2865-2845 (s)	Sym stretch	Alkanes, -CH2-
1734.99	1740-1730 (s)	C=O stretch	Aldehyde saturated
1617.33	1650-1620 (s)	NH def	Amide II Primary
1455.43	1470-1430 (m)	-CH3 antisym def	Alkanes
1380.01	1410-1310 (s)	CH3, Sym def	Alkanes
1244.62	1250-1150 (s)	Ar-O stretch	Ether, alkyl aryl
1158.68	1200-1000 (m)	C-O stretch	Ethers, diaryl
1070.84	1100-1000 (s)	C-N stretch	Aliphatic amines
838.62	900-860 (w)	C-H out of plane, def	Aromatic compounds m-disub
722.56	750-700 (s)	C-Cl stretch	Halogen, chloroform

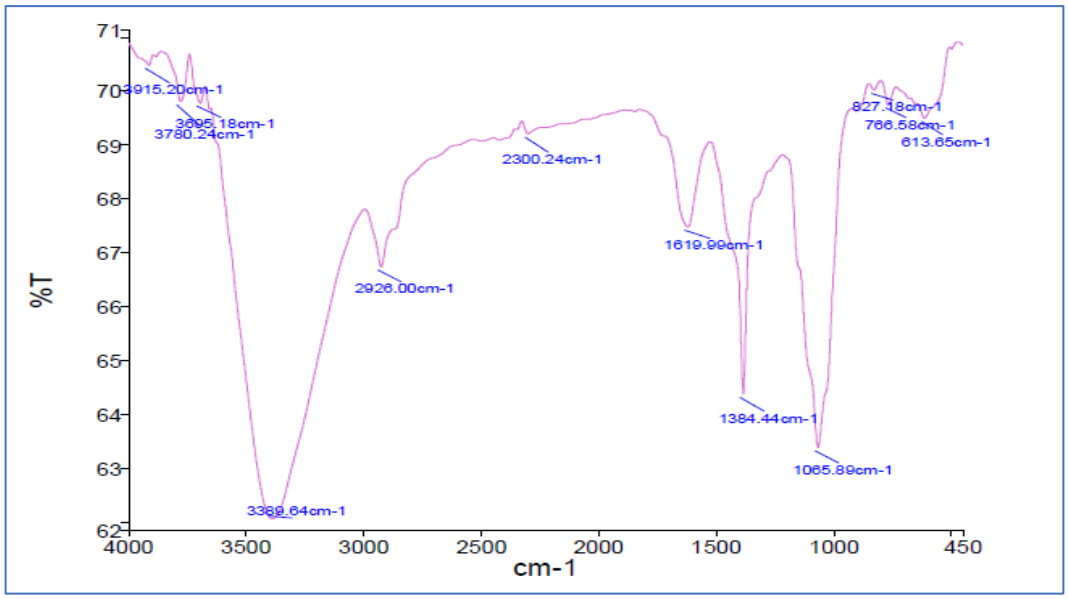


Figure 5: FTIR Spectra of ethanol extract

Table 4: Interpretation of ethanol extract

Characteristic Absorption (cm^{-1})	Group Frequency (cm^{-1})	Vibrational Mode	Functional Group
3389.64	3400-3200 (s)	-OH Stretch Broad	Alcohol
2926	2950-2700 (w)	-CH Stretch usually two bands	Ac-H
2300.24	3200-2500(w)	O-H stretch, very broad	Alcohol and Phenol
1619.99	1650-1550 (m)	NH def	Amines Secondary
1384.44	1410-1310 (s)	CH3, Sym def	Alkanes
1065.89	1100-1000 (s)	C-N stretch	Aliphatic amines
827.18	900-860 (w)	C-H out of plane, def	Aromatic compounds m-disub
613.65	690-515 (s)	C-Br stretch	Halogen, Bromo

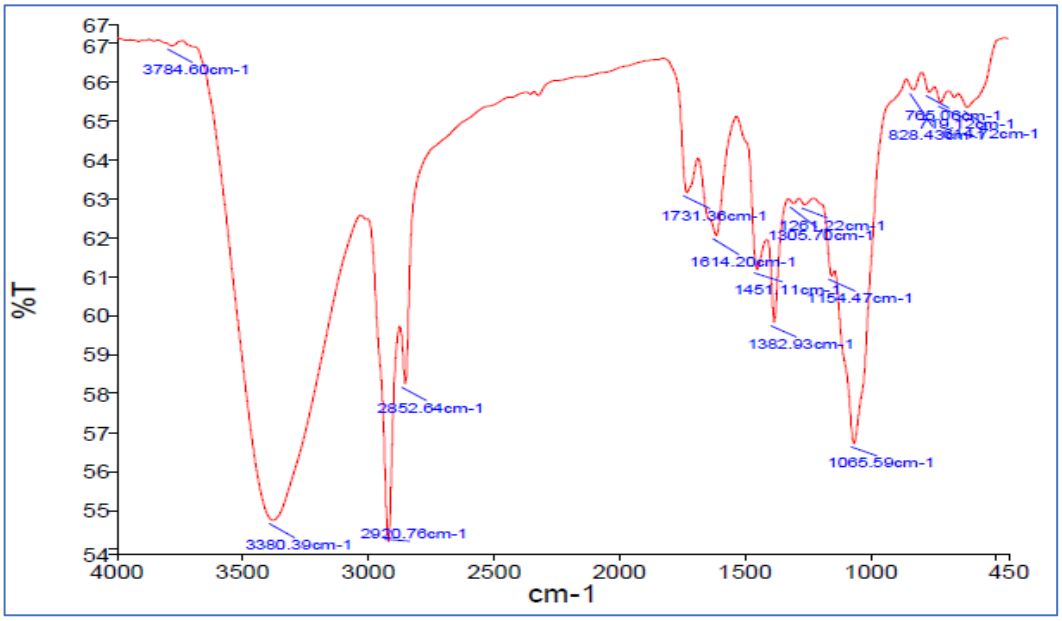


Figure 6: FTIR Spectra of Petroleum ether

Table 5: Interpretation of petroleum ether extract

Characteristic Absorption (cm^{-1})	Group Frequency (cm^{-1})	Vibrational Mode	Functional Group
3380.39	3400-3200 (s)	-OH Stretch Broad	Alcohol
2920.76	2950-2700 (w)	-CH Stretch usually two bands	Ac-H
2852.64	2865-2845 (s)	Sym stretch	Alkanes, -CH2-
1731.36	1740-1730 (s)	C=O stretch	Aldehyde saturated
1614.02	1650-1620 (s)	NH def	Amide II Primary
1451.11	1470-1430 (m)	-CH3 antisym def	Alkanes
1382.93	1410-1310 (s)	CH3, Sym def	Alkanes
1305.07	1380-1370 (m)	-CH3 sym def	Alkanes
1261.22	1270-1230 (s)	OH in-plane def	Alcohol, secondary
1154.47	1200-1000 (m)	C-O stretch	Ethers, diaryl
1065.39	1100-1000 (s)	C-N stretch	Aliphatic amines
832.43	900-860 (w)	C-H out of plane, def	Aromatic compounds m-disub
719.12	750-700 (s)	C-Cl stretch	Halogen, chloroform

CONCLUSION:

From the above results obtained in this study, it is concluded that the leaf extracts (chloroform, ethanol, petroleum ether) of plants *Cassia tora* extracts with their phytoconstituents may act as an effective source for the treatment of diseases. The presence of phytoconstituents such flavonoids, carbohydrates, alkaloids, glycosides, and saponin is likely indicated by the various plant extracts. The -CH_3 functional group was discovered to be the most frequently detected among the various functional groups in these plant extracts. Therefore, additional research is required to isolate, purify, and characterise these compounds in order to use them as lead compounds for creating medications with a variety of biological effects. It is necessary to do additional research on bioactive substances to assess their effectiveness through in vivo investigations and to demonstrate their effectiveness and safety in clinical trials.

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CONFLICT OF INTEREST:
The authors declare that there are no conflicts of interest.

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