



ISOLATION AND CHARACTERIZATION OF ACTIVE PHYTOCONSTITUENTS / CHEMICAL COMPONENTS OF *CUSCUTA REFLEXA* ROXB AND *CASSYTHA FILIFORMIS* LINN

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ABSTRACT

The present study was undertaken to isolate and characterize the bioactive phytoconstituents present in *Cuscuta reflexa* Roxb. and *Cassythia filiformis* Linn. The plant materials were subjected to suitable extraction procedures, followed by bioactivity-guided fractionation to identify fractions containing potentially active chemical constituents. The active fractions were further separated and purified using column chromatography to obtain individual compounds. Thin-layer chromatography (TLC) was employed to monitor the separation process and assess the purity of the isolated compounds based on their characteristic chromatographic profiles. The isolated phytoconstituents were subsequently subjected to spectroscopic characterization using ultraviolet-visible (UV-Vis), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometric (MS) techniques. The combined spectral data were used to determine the structural characteristics of the isolated compounds. The study provides useful information regarding the bioactive chemical constituents of *C. reflexa* and *C. filiformis* and supports further investigation of their pharmacological potential.

KEYWORDS: *Cuscuta reflexa*; *Cassythia filiformis*; Bioactivity-guided fractionation; Column chromatography; Phytoconstituents.

INTRODUCTION

Medicinal plants have been an important source of therapeutic agents and bioactive molecules since ancient times. The diverse chemical constituents present in plants, including alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, glycosides, and other secondary metabolites, contribute to their biological and pharmacological activities. Natural products research plays an important role in the discovery and characterization of such bioactive molecules. Therefore, isolation and structural characterization of individual phytoconstituents from medicinal plants are essential steps in establishing their chemical profile and exploring their potential therapeutic applications.

Cuscuta reflexa Roxb. is a parasitic medicinal plant belonging to the family Convolvulaceae. It is characterized by its slender, twining, yellowish-green to

orange stems and reduced leaves. The plant has been traditionally used in various systems of medicine for several therapeutic purposes, particularly in preparations associated with hair and skin care and inflammatory conditions. Previous phytochemical investigations have indicated the presence of diverse classes of secondary metabolites in *C. reflexa*, suggesting that the plant may serve as a source of biologically active natural compounds.^[1]

Cassythia filiformis Linn. belongs to the family Lauraceae and is a slender, parasitic or semi-parasitic plant commonly found in tropical and subtropical regions. The plant possesses long, thread-like stems and highly reduced leaves. Different parts of *C. filiformis* have been traditionally utilized for various medicinal purposes. Its reported biological activities and phytochemical diversity have generated interest in the

isolation and characterization of its active chemical constituents. However, the biological activity of a crude plant extract represents the combined effects of numerous compounds, and identification of the individual constituents responsible for such activity requires systematic fractionation and purification.^[2]

Bioactivity-guided fractionation is an effective approach for the discovery of active natural products. In this method, a crude extract is fractionated into different portions, and each fraction is evaluated for biological activity. Fractions exhibiting significant activity are subsequently subjected to further separation and purification. This process is repeated until individual active compounds are obtained. Such an approach helps establish a direct relationship between the observed biological activity and the chemical constituents present in the plant material.^[3,4]

Chromatographic techniques are fundamental tools in natural product isolation. Column chromatography enables the separation and purification of individual compounds based on differences in their physicochemical properties and affinity toward stationary and mobile phases. Thin-layer chromatography (TLC) is commonly employed to monitor the progress of separation, identify similar fractions, assess compound purity, and determine characteristic chromatographic profiles. The combined use of column chromatography and TLC provides an efficient preliminary strategy for the isolation and purification of phytoconstituents.

Following isolation, structural characterization is essential for determining the chemical identity of the purified compounds. Spectroscopic techniques such as ultraviolet-visible (UV-Vis) spectroscopy provide information related to chromophoric systems, while infrared (IR) spectroscopy assists in identifying characteristic functional groups. Nuclear magnetic resonance (NMR) spectroscopy provides detailed information regarding the molecular framework, proton and carbon environments, and connectivity of atoms. Mass spectrometry (MS) provides information about molecular mass and fragmentation patterns, which are valuable for establishing molecular formulae and structural features. Interpretation of these complementary spectral data enables reliable identification and characterization of isolated natural products.

Although *C. reflexa* and *C. filiformis* have considerable traditional medicinal importance, systematic isolation and characterization of their bioactivity-associated phytoconstituents remain important areas of natural product research. Identification of individual active compounds can provide a chemical basis for the reported medicinal properties of these plants and may facilitate their further pharmacological and pharmaceutical investigation.

Therefore, the present study was designed to isolate and characterize active phytoconstituents from *Cuscuta reflexa* Roxb. and *Cassytha filiformis* Linn. using a bioactivity-guided fractionation approach. Active fractions were separated by column chromatography, monitored by TLC, and the isolated compounds were subjected to UV, IR, NMR, and mass spectral studies for structural characterization. The study aims to contribute to the chemical standardization and scientific evaluation of these medicinally important plants.

MATERIALS AND METHODS

(a) Column chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible. As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity.

Column chromatography of aqueous extract of *Cuscuta reflexa*

The aqueous extract of *Cuscuta reflexa* (8 g) was subjected to chromatography over silica gel 60-120 mesh (130 g) of column length 100 cm diameter of 3 cm. Elution was carried out with solvents and solvent mixtures of increasing polarities. The fractions were collected in 5 ml portions and monitored on TLC (silica gel G as adsorbent with suitable solvent system). The fractions that showed similar spots were mixed and the chromatographic details are given in Table 1.

The fractions 260-300 yielded a yellowish coloured solid mass and the TLC profile showed two spots. This yellowish solid was designated as fraction-I. The fraction 400-480 yielded dark greenish coloured solid and the TLC profile showed three spots and it was designated as fraction-II.

Separation and purification of fraction-I

A cream coloured precipitate was separated when fraction-I was dissolved in methanol, which was then separated by filtration and the solid residue was treated with acetone. The precipitate was separated by filtration and washed with acetone to recrystallize. A colourless solid weighing 10 mg was obtained. It was designated as compound-I. The isolated compound-I was subjected to TLC physical, chemical and spectroscopic studies for its characterization.

Separation and purification of fraction-II

The fraction was washed with methanol, and the dark green coloured solid turned to light yellow colour and gave a single spot in TLC analysis. The light yellow colour solid 30 mg was subjected to recrystallization

with acetone. The solid precipitate was designated as compound-II and was subjected to TLC physical, chemical and spectroscopic studies for its characterization.

Fractionation of the ethanol extract of *Cassia filiformis*

The ethanol extract (50 g) was suspended in distilled water (100 ml) and was extracted successively in a separating funnel with n-hexane petroleum ether (60-80°C), chloroform, ethyl acetate and Methanol. The fraction was washed with distilled water, dried over anhydrous sodium sulphate and the solvent was removed by distillation under reduced pressure.

Yield of the various fractions

n-Hexane fraction	:	10 gms
Petroleum ether fraction	:	10 gms
Chloroform fraction	:	8 gms
Ethyl acetate fraction	:	9 gms
Methanol fraction	:	5 gms

Ethyl acetate fraction

The dark greenish residue of ethyl acetate fraction (8 g) was dissolved in methanol and was then loaded on a silica gel column (20 g). The column was eluted with 100% ethyl acetate followed by graded mixtures of 1%, 2%, 3%, 5% methanol in ethyl acetate. Elution of the different fraction was monitored by TLC Silica-Gel (Ethyl acetate : Methanol : water; 100 : 16.5 : 13.5) and visualized with UV/NH₃.

The eluate obtained with 100% ethyl acetate and 1% methanol in ethyl acetate showed the presence of a single spot on the TLC. The eluate on concentration deposited a yellowish brown colour compound (8 mg). The compound was designated as compound-III, which was further purified and recrystallized with acetone. The solid precipitate was subjected to spectroscopic studies for its characterization.

(b) Thin Layer Chromatography Analysis

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

TLC is used for the separation of simple mixtures where speed, low cost, simplicity are required.^[5,6]

Preparation of plates

30 g of silica gel GF was weighed out and made in to a homogenous suspension with 60 ml of distilled water to form a slurry. The suspension was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on a flat glass plate (20 x 10 cm and 20 x 5 cm). The coated plates were allowed to dry in air, followed by heating at 100–105°C for 1 h, cooled and protected from moisture. The plates were stored in a dry atmosphere. Whenever required the plates were dried in a hot air oven at 100°C for 30 min for activation.

Application

The extracts were dissolved in methanol (0.1-1%w/v). They were sucked within capillary tubes and spotted on the prepared TLC plate 2 cm above its bottom end. The spots were equally sized as far as possible and had a diameter ranging from 2-5 mm.

Selection of mobile phase

The solvent systems were selected based on the chemical constituents identified in the qualitative phytochemical analysis. The solvent or mobile phase used depends upon various factors mentioned below

- Nature of the substance to be separated
- Nature of the stationary phase
- Mode of chromatography (i.e., normal phase and reversed phase)
- Separation to be achieved (i.e., analytical or preparative)

Pure solvents or mixture of solvents were used as mobile phase. The selection of solvent system for mobile phase is important in TLC, because the same solvent system can be used in HPTLC.

Several solvent systems were used as mobile phases for separation of various plant constituents from respective plants. The better solvent system, which gave good separation and more number of spots was selected for each extract. The R_f values were noted for each of the selected plant extracts, using different detecting agents viz., daylight, UV light, iodine vapour, with Dragendorff's reagent, Vanillin sulphuric acid and ninhydrin reagents.

(c) High Performance Thin Layer Chromatography (HPTLC)

HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughput and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC the various steps involved are.

Application of sample

An automatic applicator (Linomat) is used for sample application. A known quantity of sample is dissolved in a known volume of solvent and the sample applied on precoated TLC plate either in the form of a spot or a band. However a band form is preferred because:

- Larger quantities of sample can be handled for application.
- Better separation because of rectangular area in which compounds are present on the plate
- Response of densitometry is better due to variable concentration of substances in a spot.

Chromatographic development (separation)

Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

Detection of spots

For densitometric scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.

Quantification and documentation

Densitometry is *in situ* instrumental measurement of visible, UV absorbance and fluorescence quenching

directly. The scanner converts the spot/band on the layer into a chromatogram consisting of peaks similar in appearance to HPLC. The portion of the scanned peaks on the recorder chart is related to R_f values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

(i) Application of sample

Commercially available pre-coated plates of silica gel GF₂₅₄ were used for the study. The different fractions were applied on plates with bandwidth of 6mm. Application rate was maintained at 10 μ l/min, using Linomat IV applicator (automatic TLC applicator, Camag, Switzerland). A sample volume of 10 μ l was applied.

(ii) Chromatogram development

The plates were developed in twin-trough chamber using the solvent system as used in the TLC of different fractions. After developing, the plates were air-dried and observed under UV light chamber (Camag UV chamber-3, model no: 022.9120).

(iii) Densitometric Scanning

The developed plates were scanned using densitometer at 254 and 366 nm (Camag TLC Scanner – 3, model no: 0227.6480, combined with integration software, CATS 4.06 (Switzerland)).

Table 1: Column chromatography of aqueous extract of *Cuscuta reflex*.

No. of Fraction	Eluent	Residue on Evaporation
1-20	n-Hexane - 100%	No residue
21-36	n-Hexane : petroleum ether (75:25)	No residue
37-54	n-Hexane : petroleum ether (50:50)	No residue
55-70	n-Hexane : petroleum ether (25:75)	White residue
71-92	Petroleum ether - 100%	No residue
93-118	Petroleum ether : Chloroform (90:10)	Colourless residue
119-135	Petroleum ether : Chloroform (80:20)	White residue
136-153	Petroleum ether : Chloroform (70:30)	No residue
154-161	Petroleum ether : Chloroform (60:40)	Colourless residue
162-183	Petroleum ether : Chloroform (50:50)	Brownish yellow residue
184-198	Petroleum ether : Chloroform (40:60)	No residue
199-210	Petroleum ether : Chloroform (30:70)	Cream residue
211-220	Petroleum ether : Chloroform (20:80)	Dark yellow residue
221-231	Petroleum ether : Chloroform (10:90)	Creamish white residue
232-245	Chloroform - 100%	No residue
246-259	Chloroform : Ethyl acetate (90:10)	No residue
260-274	Chloroform : Ethyl acetate (80:20)	Yellow residue
275-292	Chloroform : Ethyl acetate (70:30)	Yellowish cream residue
293-300	Chloroform : Ethyl acetate (60:40)	Yellow residue
301-325	Chloroform : Ethyl acetate (50:50)	Colourless residue
326-353	Chloroform : Ethyl acetate (40:60)	Green residue
354-387	Chloroform : Ethyl acetate (30:70)	Creamish yellow residue
388-399	Chloroform : Ethyl acetate (20:80)	Green residue
400-421	Chloroform : Ethyl acetate (10:90)	Dark green residue
422-439	Ethyl acetate -100%	Creamish green residue
440-457	Ethyl acetate : Methanol (90:10)	Light green residue
458-468	Ethyl acetate : Methanol (80:20)	Light brownish residue

469-480	Ethyl acetate : Methanol (70:30)	Dark green residue
481-501	Ethyl acetate : Methanol (60:40)	Brown Residue
502-535	Ethyl acetate : Methanol (50:50)	No residue
536-560	Ethyl acetate : Methanol (40:60)	No residue
561-579	Ethyl acetate : Methanol (30:70)	Light brown residue
580-599	Ethyl acetate : Methanol (20:80)	Light brown residue
600-699	Ethyl acetate : Methanol (10:90)	No residue
700-730	Methanol -100%	Dark brown residue

RESULTS AND DISCUSSION

Characterization of compound-I

The aqueous extract of *Cuscuta reflexa* was subjected to column chromatography on silica gel. Elution was carried out with solvents and solvent mixtures of increasing polarities. The fractions were collected in 5 ml portions and monitored on normal TLC with appropriate solvent system. The chemically similar fractions that showed similar spots were pooled together. The fractions 260-300 yielded a yellowish coloured solid mass and the TLC profile showed two spots. This yellowish solid was designated as fraction-I. The fraction-I was further

purified by solvent extraction and recrystallization technique and yielded a colourless solid. It was designated as compound-I. The isolated compound-I was subjected to TLC physical, chemical and spectroscopic studies for its characterization.

The homogeneity of compound-I was proved by melting point and a single spot in TLC and HPTLC using pre-coated plates of silica gel GF₂₅₄ as adsorbent and solvent mixtures of different polarities as developers. The solvent systems used and their corresponding R_f values are shown below.

Table 2: The solvent systems used and their corresponding R_f values.

S. No.	Mobile phase	No. of spots	R _f value
1	Ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27)	Single	0.48
2	n-Butanol:glacial acetic acid:water(40:10:50)	Single	0.49
3	n-Butanol:ethyl acetate:water (4:1:2.2)	Single	0.32

The compound-I exhibited a positive test for phenols. It gave a green colour with ferric chloride.

The melting point of the isolated compound-I was found to be in between 207-209°C (uncorrected). The isolated

compound-I was subjected to UV, IR, ¹H NMR, ¹³C NMR, and mass spectroscopic analysis to find out the structure.^[7-9]

Spectral studies of compound-I

(i) UV λ_{max} MeOH (nm): 255, 354 (Fig. 1).

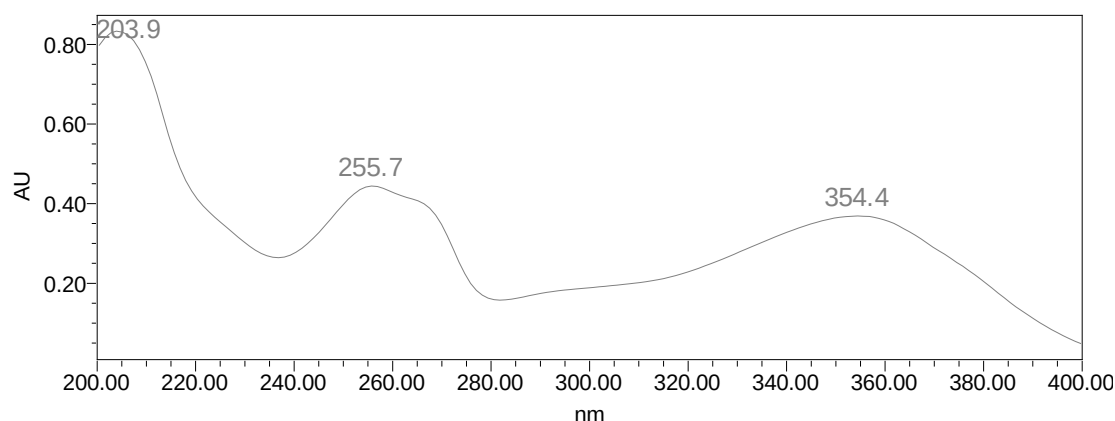


Fig. 1 UV data of compound – I.

(ii) IR $\nu_{\max}$ cm: 671.25, 790.84, 1207.48, 1222.91, 1602.90, 2357.09, 2854.74, 3016.77, 3607.01, 3695.73 (Fig. 2).

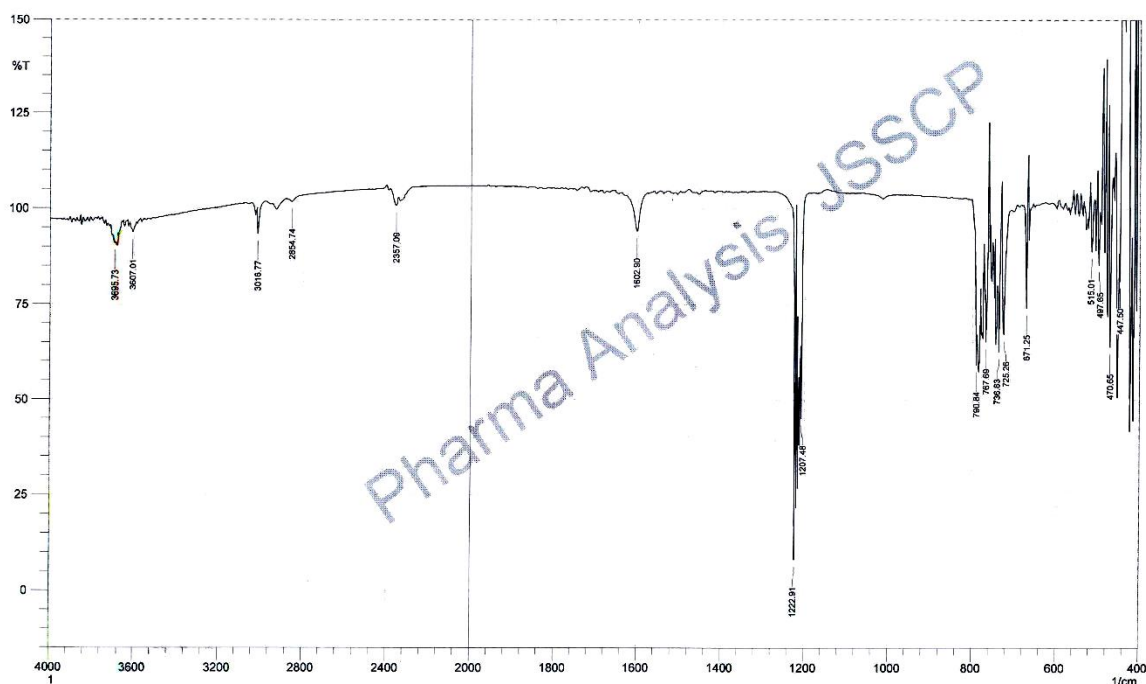


Fig. 2 IR of compound – I.

(iii) $^1\text{H NMR}$: δ 7.43 (d, J = 15.8 Hz, C-7'H), 7.07 (d, J = 1.5 Hz, C-2'H), 6.91 (dd, J = 8.2 Hz, C-6'H), 6.85 (d, J = 8.2 Hz, C-5'H), 6.27 (d, J = 15.8 Hz, C-8'H), 5.11 (dd, J = 8.5 Hz, C-3H), 4.21 (dd, C-5H), 3.67 (dd, C-4H), 2.04 (dd, J = 11.9, J = 2.4 Hz, C-6H α), 1.98 (2H, d, J = 8.5 Hz, C-2H $_2$), 1.42 (dd, J = 11.9, J = 1.5 Hz, C-6H β) (Fig. 3)

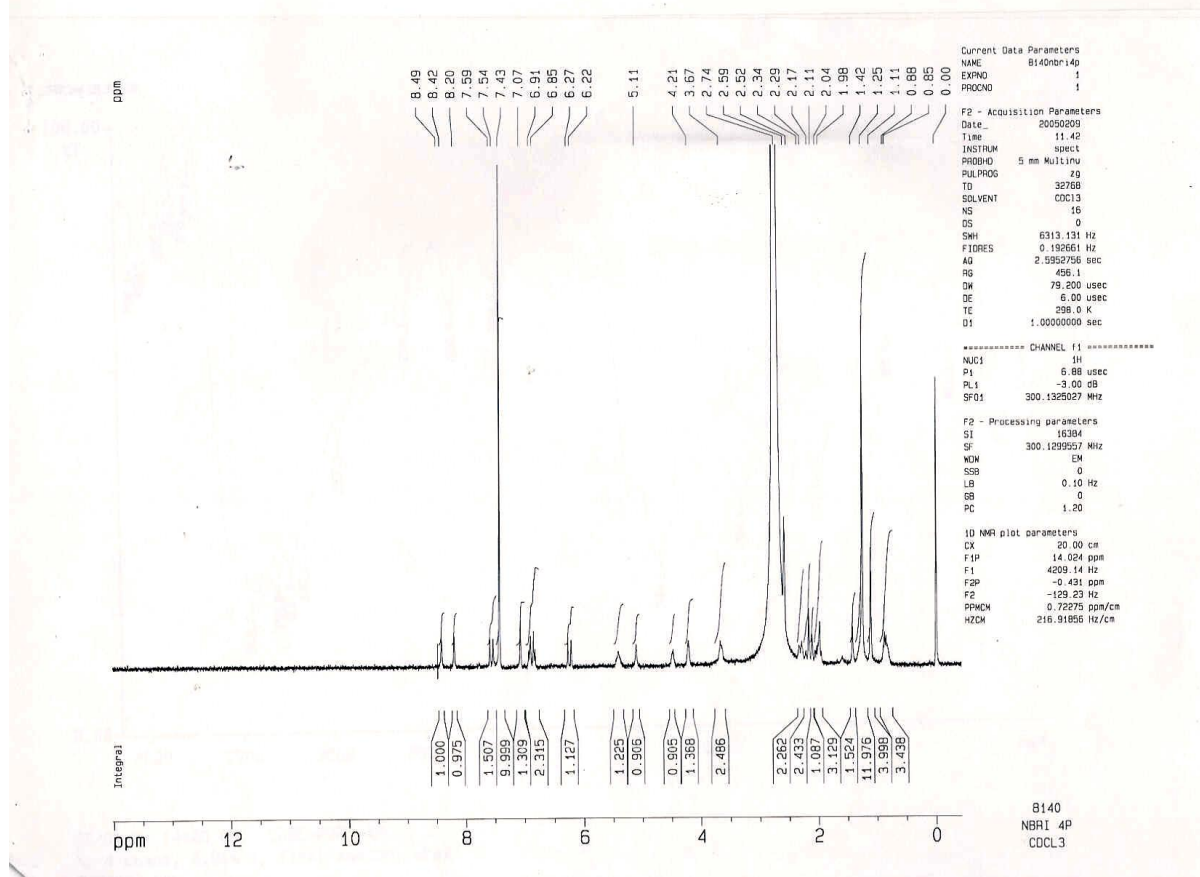


Fig. 3 $^1\text{H NMR}$ of compound – I.

(iv) LC-MS m/z (re lint): 355.2 $[M]^+$. (Fig. 4).

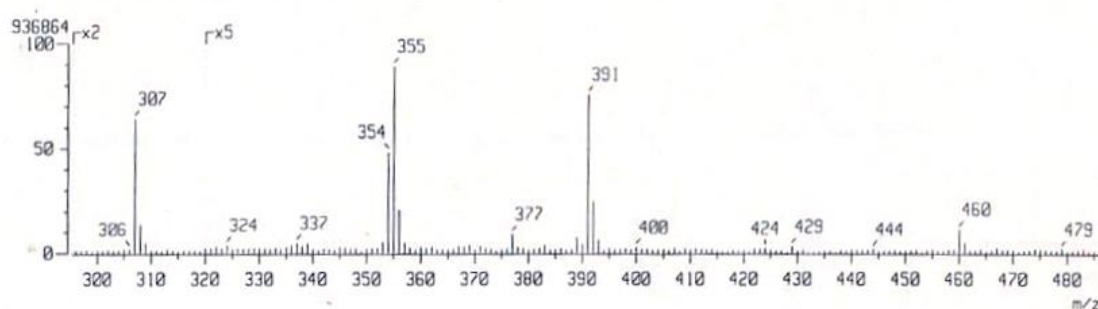


Fig. 4 Mass Spectrum of compound – I.

(v) ^{13}C NMR: δ 168.4 (s, C-9'), 149.2 (s, C-4'), 146.6 (s, C-3'), 146.4 (d, C-7'), 127.8 (s, C-1'), 122.9 (s, C-6'), 116.6 (d, C-5'), 115.4 (d, C-8'), 115.1 (d, C-2'), 76.7 (s, C-1), 74.4 (d, C-4), 71.7 (d, C-3), 70.8 (d, C-5), 49.0 (t, C-2), 38.2 (t, C-6) (Fig. 5).

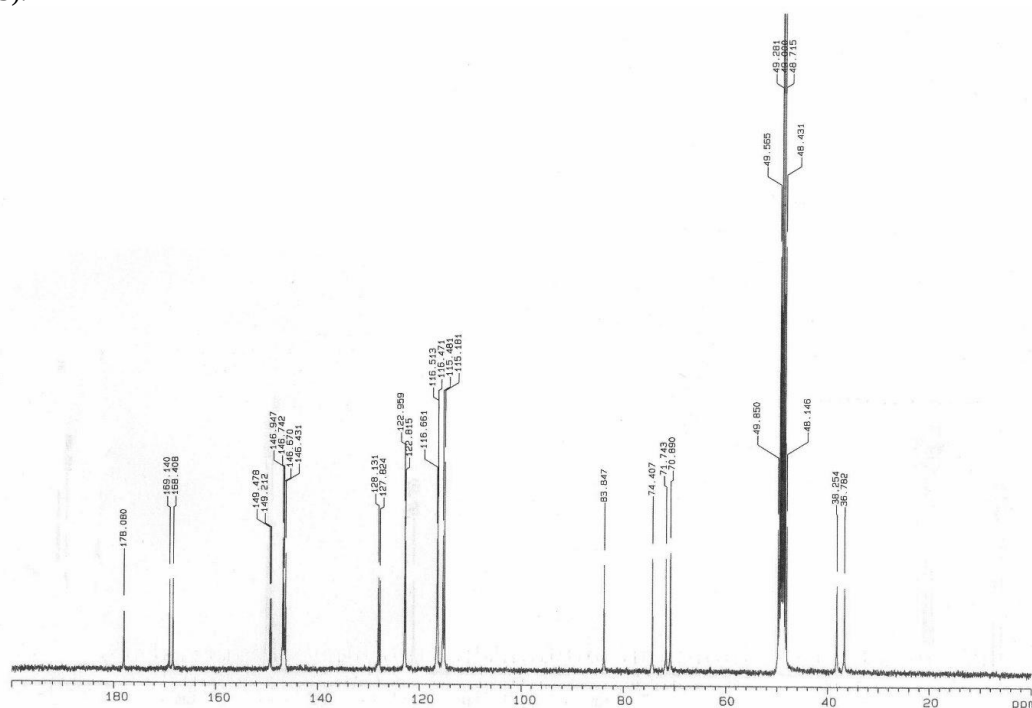
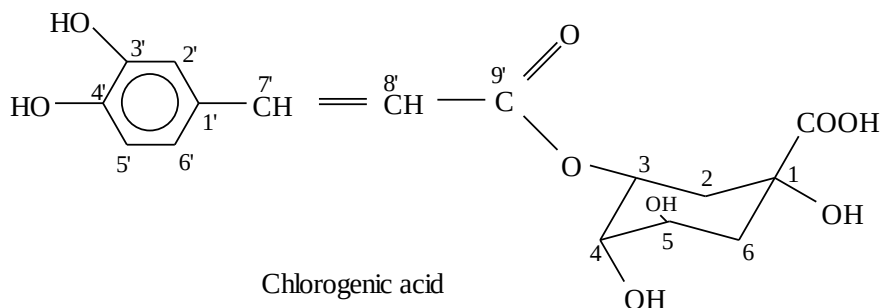


Fig. 5 ^{13}C NMR of compound – I.

The spectral data matched with that of the chlorogenic acid¹³⁴, thus confirming the structure of chlorogenic acid.



Characterization of compound-II

The fraction 400-480 yielded dark greenish coloured solid and its TLC profile showed three spots which was designated as fraction-II. The fraction-II was purified further by solvent extraction and recrystallization

technique and yielded a light yellow colour solid. It was designated as compound-II. The isolated compound-II was subjected to TLC physical, chemical and spectroscopic studies for its characterization.

The homogeneity of compound-II was proved by melting point and a single spot in TLC and HPTLC using pre-coated plates of silica gel GF₂₅₄ as adsorbent and solvent mixtures of different polarities as developers. The

solvent systems used and their corresponding R_f values are shown below.

Table 3: solvent systems used and their corresponding R_f values.

S. No.	Mobile phase	No. of spots	R _f value
1	Ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27)	Single	0.63
2	n-Butanol:glacial acetic acid:water(40:10:50)	Single	0.62
3	n-Butanol:ethyl acetate:water (4:1:2.2)	Single	0.48

The compound-II exhibited a positive test for flavanoid and phenols. It gave an olive green colour with ferric chloride, a reddish pink colour in Shinoda's test and a yellow colour with ammonia. It gave positive colour reaction in Molish's test indicating the presence of a

glycoside. The melting point of isolated compound-II was found to be in between 216-218°C (uncorrected). The isolated compound-II was subjected to UV, IR, Proton NMR, ¹³C NMR, and Mass Spectroscopic analysis to find out the structure.

Spectral studies of compound-II

(i) UV λ_{max} MeOH (nm): 253, 368 (Fig. 6).

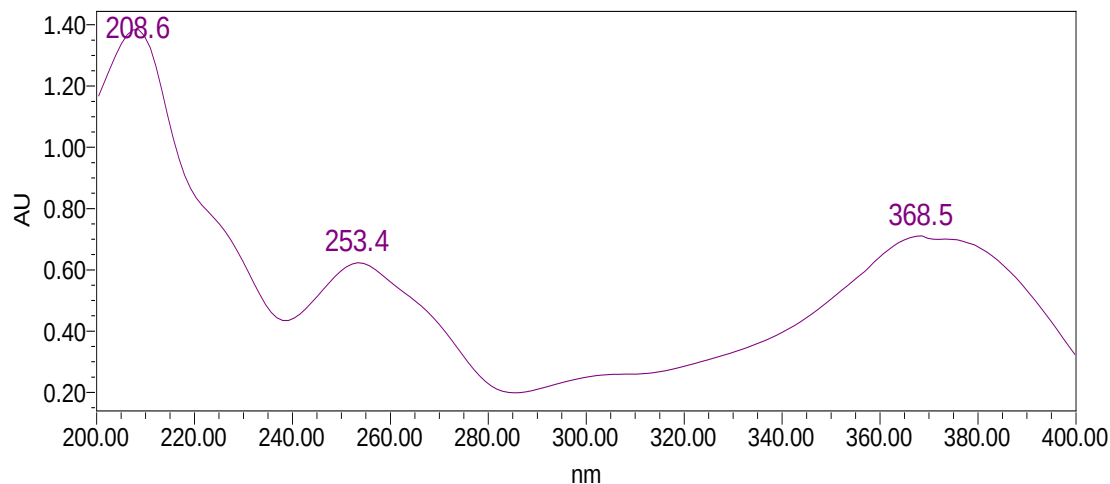


Fig. 6 UV data of compound – II.

(ii) IR ν_{max} cm⁻¹: 3392, 3369, 1654, 1609, 1558, 1508, 1458, 1429 (Fig. 7).

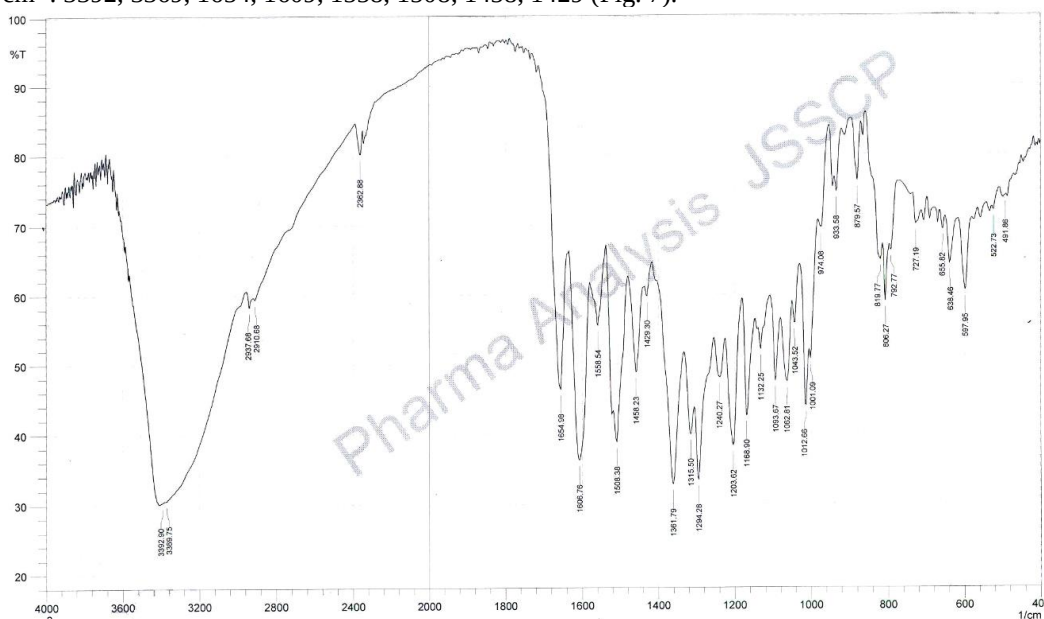


Fig. 7 IR of compound – II.

(iii) $^1\text{H NMR}$: δ 7.15 (CH), 6.93(CH), 6.72(CH), 6.25(CH), 5.94(CH), 11.85(OH), 10.68(OH), 10.29(OH), 9.48(OH) (Fig. 8).

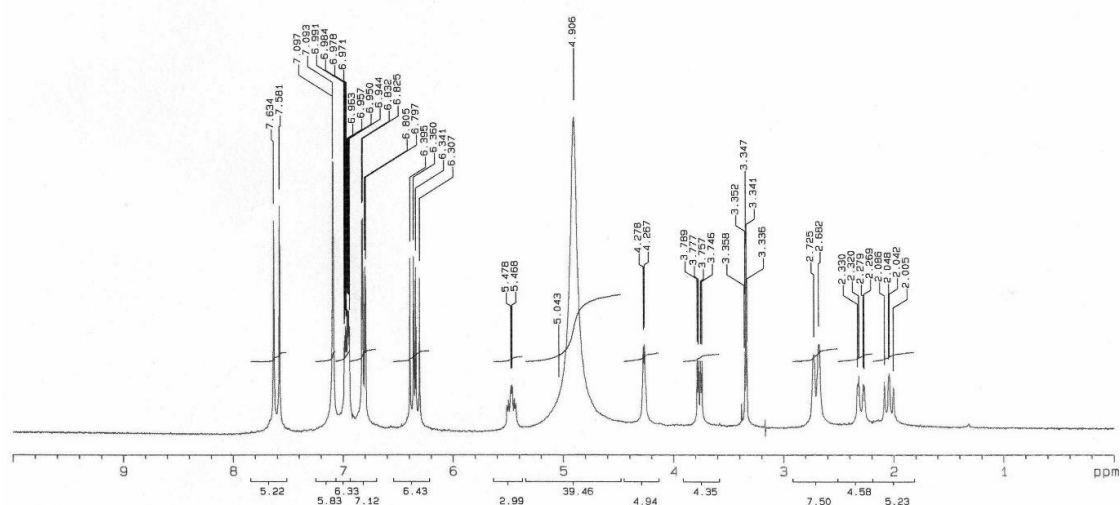


Fig. 8 $^1\text{H NMR}$ of compound – II.

(iv) $^{13}\text{C NMR}$: δ 136.5 (C), 146.9 (C), 146.5(C), 145.9(C), 158.8 (C), 161.8(C), 166.4(C), 122.8(C), 115.3 (CH), 104.5 (C), 117.2 (CH), 176.1 (C), 98.3(CH), 94.0(CH), 121.8(CH) (Fig 9).

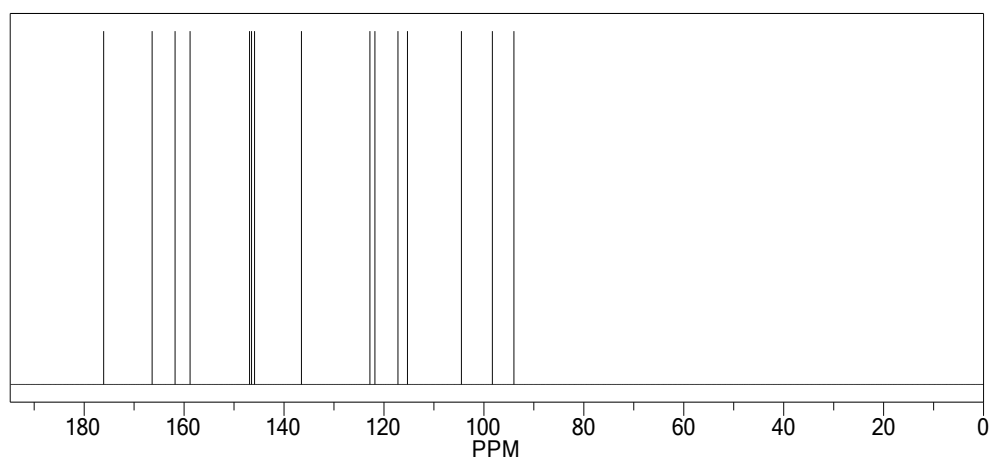


Fig. 9 $^{13}\text{C NMR}$ of compound – II.

(v) FAB-MS: pso. Ions 303 [M - H] (Fig. 10).

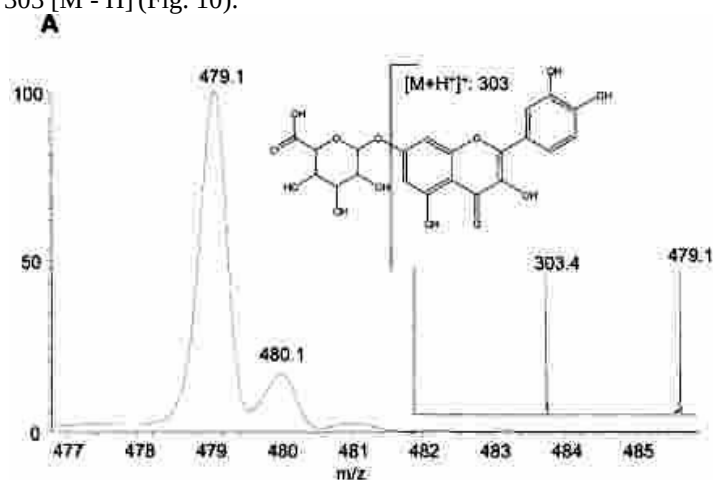
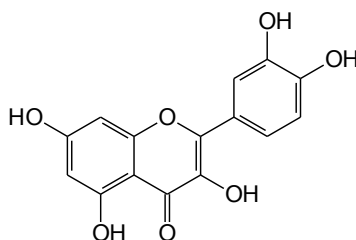


Fig. 10 Mass Spectrum of compound – II.

The spectral data matched with that of the quercetin, thus confirming the structure of quercetin. Quercetin (3,3',4',5,7-pentahydroxy flavone)



Characterization of compound-III

The ethanol extract of *Cassytha filiformis* was suspended in distilled water (100 ml) and was fractionated successively in a separating funnel with n-hexane petroleum ether (60°-80° C), chloroform, ethyl acetate and methanol in that order. The fraction was washed with distilled water, dried over anhydrous sodium sulphate and the solvent was removed by distillation under reduced pressure.

The dark greenish residue of ethyl acetate fraction was dissolved in methanol and was then loaded on a silica gel column. The column was eluted with 100% ethyl acetate followed by graded mixtures of 1%, 2%, 3%, 5% methanol in ethyl acetate. Elution of the different fraction was monitored by TLC silica-gel (ethyl acetate :

methanol : water; 100 : 16.5 : 13.5) and visualized with UV/NH₃.

The eluate obtained with 100% ethyl acetate and 1% methanol in ethyl acetate showed the presence of a single spot on the TLC. The eluate on concentration deposited a yellowish brown colour compound. The compound was designated as compound-III, which was further purified and recrystallized with acetone. The solid precipitate was subjected to spectroscopic studies for its characterization.

The homogeneity of compound-III was proved by melting point and a single spot in TLC and HPTLC using pre-coated plates of silica gel GF₂₅₄ as adsorbent and solvent mixtures of different polarities as developers. The solvent systems used and their corresponding R_f values are shown below.

Table 4: solvent systems used and their corresponding R_f values.

S. No.	Mobile Phase	No. of spots	R _f value
1	Ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:27)	Single	0.65
2	n-Butanol:glacial acetic acid:water(40:10:50)	Single	0.62
3	n-Butanol:ethyl acetate:water (4:1:2.2)	Single	0.59

The melting point of isolated compound-III was found to be in between 217-219°C (uncorrected). The isolated compound-III was subjected to UV, IR, ¹H NMR, ¹³C NMR, and mass spectroscopic analysis to find out the structure. From the spectral studies of compound-III it was confirmed as quercetin as that of compound-II of *Cuscuta reflexa*.

CONCLUSION

The aqueous extract of *Cuscuta reflexa* and ethanol extract of *Cassytha filiformis* were subjected to column chromatography and fractionation for isolation of its active constituents. The aqueous extract of *Cuscuta reflexa* was chromatographed on silica gel column. Elution was carried out with solvents and solvent mixtures of increasing polarity. Fractions were collected in 5 ml portions and monitored by TLC and the fractions showing similar spots were combined. Three compounds were isolated and their purity was confirmed as single spot by TLC using three different solvent systems. The melting points of compound-I was 207-209°C and its R_f was 0.48. The melting points of compound-II and compound-III was 216-218°C and. 217-219°C and its R_f value was 0.61 and 0.63 respectively. These compounds

were subjected to spectral analysis viz., UV, IR, NMR and mass spectrum. From the spectral data compound-I was characterized as chlorogenic acid. The compound-II and compound-III was characterized as quercetin.

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