Research Article

Preliminary Phytochemical Screening and HPTLC Analysis of *Cassia senna* l. Leaves

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ABSTRACT
Herbal remedies have been used throughout the world over 4000 years ago for treatment of various diseases due to the presence of beneficial chemical elements in them and their medicinal potential lie in their phytochemical constituents that produce a definite pharmacological action when applied to the human body. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Hence the present study is aimed at investigating the qualitative phytochemical and HPTLC analysis of various solvent extracts of *Cassia senna* L. leaves. The different solvent extracts namely petroleum ether, benzene, chloroform, ethyl acetate, successive ethanolic(SEE), direct ethanlic(DEE) and aqueous extracts were subjected to phytochemical screening and HPTLC analysis. Among the various solvent extracts analyzed, Direct ethanolic extract(DEE) followed by Successive ethanolic extract(SEE) of *Cassia senna* L. leaves were found to contain maximum number of phytoconstituents and DEE was found to segregate more number of alkaloids than SEE in HPTLC.

Keywords: *Cassia senna* L. leaves(CSL), Direct Ethanolic Extract(DEE).

1. INTRODUCTION

Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Vijayalakshmi *et al.*, 2012).

The phytochemical studies of the medicinal plants have provided some biochemical basis for their ethnopharmacological uses in the treatment and prevention of various diseases and disorders(Okigbo *et al.*, 2009).

All *Cassia* species are rich source of secondary metabolites and have been used in Chinese and Ayurvedic preparations. There are about 580 species of this genus scattered all around the world(Deshpande and Bhalsing, 2013). A clinical research on *Cassia* species indicated it as a source of effective liver tonic, antibiotic, antiinflammatory and antifungal agents(Farswan *et al.,* 2009).

Plants belonging to *Cassia* species are used extensively in various parts of the world against a wide range of ailments, the synergistic action of the metabolites being probably responsible for the plants beneficial effects(Verma *et al*., 2013). Species of *Cassia* are rich sources of flavonoids, polyphenols, anthraquinone derivatives and polysaccharides (Ayo, 2010).

Cassia senna L. leaves have been investigated for the presence of secondary metabolites and evaluated for the biological activities of the crude extracts with special emphasis to the antimicrobial, cytotoxic and thrombolytic activities(Hossain *et al.,* 2012). The ethanolic extract of *Cassia senna* L. leaves was found to suppress prostate tumor growth in experimental animals induced with prostate carcinogenesis using Testosterone and N-methyl N-nitrosourea(Kumar *et al*., 2012).

Although many scientific works have been carried out on the various species of *Cassia* belonging to the family Fabaceae with respect

to their biological activities, not much work has been done on the phytochemical analysis of *Cassia senna* L. Hence the present study was focused on "**Preliminary phytochmical screening and HPTLC analysis of *"Cassia senna* L. leaves"**.

2. EXPERIMENTAL PROCEDURE

2.1 Collection of the plant material

The fresh plant of *Cassia senna* L. was collected from Madurai district, Tamilnadu. The plant was identified and authenticated in Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2012). The plant material was cleaned. The leaves of the plant were shade dried and were coarsely powdered using a mechanical grinder. The powdered samples were stored in air tight and light resistant containers to be used for further analyses.

2.2. Preparation of the different solvent extracts from the leaf powder of *Cassia senna* L.

2.2.1 Preparation of organic solvent extracts by successive solvent extraction using Soxhlet apparatus

The leaf powder of *Cassia senna* L. was subjected to successive solvent extraction from non-polar to polar solvents namely petroleum ether, benzene, chloroform, ethyl acetate and ethanol. 15gms of each of the powdered samples of leaf and pod was subjected to soxhlet extraction for 8 hrs with 250ml of the selected solvents successively. The extracts obtained were then evaporated to remove the excess solvents. The residues were stored in a cool dry place and were used for the analyses(Vadivel *et al*., 2012).

2.2.2 Preparation of Direct Ethanolic Extract(DEE)

Leaf powder of *Cassia senna* L. (10 gm) was taken in 100 ml of ethanol and macerated in stopper flask for 48 hours, shaking frequently at room temperature. Next day the mixture was filtered by using Whattmann no.1 filter paper and it was dried on water bath until the constant weight with dry mass was obtained(Kokate, 2005).

2.2.3 Preparation of the aqueous extract

10 g of each of the powdered samples of leaf and pod of *Cassia senna* L. was mixed with distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 h, the supernatant collected at an interval of every 2 h, was pooled together and concentrated to make the final volume to be one-fourth of the original volume (Parekh *et al*., 2005).

2.2.4. Preliminary phytochemical screening of different solvent extracts of leaf of *Cassia senna* L.

Detection of Carbohydrates(Palanisamy *et al*., 2012)

A small quantity of various extracts of leaf was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Fehling's Test(Red sugar)

The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

Detection of glycosides(Harborne, 1984)

Keller-Killani Test(Cardiac glycoside)

5ml of extract was treated with 1ml of glacial acetic acid containing 1 drop of ferric chloride solution .This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates the presence of deoxysugar characteristic of cardenolides.

Modified Borntrager's Test(Tiwari *et al*., 2011)

Extracts were hydrolysed with dil. HCl, and then treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of proteins and aminoacids(Tiwari *et al*., 2011)

Xanthoproteic Test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes.

Formation of blue colour indicates the presence of amino acid.

Detection of alkaloids(Tiwari *et al*., 2011)
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test
Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test
Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test
Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of Flavonoids(Palanisamy *et al*., 2012)
Alkaline reagent Test
Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide.Apperance of yellow color indicates the presence of flavonoids.

Sulphuric acid Test
To small portion of each extract concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

Shinoda's Test
Small quantities of the extract were dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

Detection of Terpinoids and Steroids(Siddiqui and Ali, 1997)
Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

Detection of Diterpenes(Palanisamy *et al*., 2012)
Copper acetate test
Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of Triterpinoids (Harborne, 1998 and Kokate, 2001)
10mg of the extract was dissolved in 1ml of chloroform; 1ml of anhydride was added following the addition of 2ml of Conc.H_2SO_4. formation of reddish violet colour indicates the presence of diterpenes.

Detection of phytosterols (Palanisamy *et al*., 2012)
Small quantities of various extracts were dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following test to detect the presence of phytosterols.

Salkowski test
To 1 ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

Libermann Burchard test
The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid , 3ml of acetic anhydride. A blush green color appeared indicates the presence of phytosterols.

Test for Tannins(Harborne, 1998 and Kokate, 2001)
5ml of the extract and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

Detection of saponins(Tiwari *et al*., 2011)
Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of phenols(Tiwari *et al*., 2011)
Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.2.5. High Performance Thin Layer Chromatography (HPTLC)
A. HPTLC Profile for alkaloids
HPTLC studies were carried out by following the methods.

B. Sample Preparation

25mg of each of the solvent extracts of *Cassia senna* L. leaves was weighed accurately, dissolved in 312.5µl of respective solvents and centrifuged at 3000rpm for 5min. These solutions were used as sample solutions.

Sample application

2 µl of each of sample solutions and 2 µl of standard solution(Colchicine) were loaded as 5mm band length in the 3 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

C. Developing Solvent System

The samples loaded plate was kept in TLC twin trough developing chamber with respective mobile phase for Alkaloid : Ethyl acetate-Methanol-Water (10:1.35:1).

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV366nm.

Derivatization

The developed plate was sprayed with respective spray reagent for Alkaloid : Dragendorff's reagent followed by 10% Ethanolic sulphuric acid reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. The Rf values and finger print data were recorded by WIN CATS software.

Scanning

Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical screening of different solvent extracts of leaf of *Cassia senna* L.

Preliminary phytochemical screening(Table 1) of different solvent extracts of leaf of *Cassia senna* L. showed the presence of various phytochemicals such as carbohydrates, proteins, glycosides, alkaloids, flavonoids, terpenoids, steroids, phytosterols, tannins, saponins and phenols.

As evident from the Table 1, among the various solvent extracts analyzed, direct ethanolic extract(DEE) followed by successive ethanolic extract(SEE) of *Cassia senna* L. leaves were found to contain maximum number of phytoconstituents. Alkaloids of certain plant species are known to decrease blood pressure and balance the nervous system in case of mental illness. Tannins are known for their wound healing and anti-parasitic properties. The presence of terpenes suggests its possible use as anti-tumor and anti-viral agent as some terpenes are known to be cytotoxic to tumor cells (Ronan *et al*., 2009). Therefore the presence of terpenes, tannins and alkaloids in the leaf extracts of *Cassia senna* L. may preliminarily indicate their bioactivity especially antitumour activity.

Panda *et al*., (2011) reported that evaluation of phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids in *Cassia fistula* leaf extracts revealed the presence of most of constituents in polar extracts (ethanol, methanol, and aqueous) compared with nonpolar extracts (petroleum ether and chloroform) which is in agreement with our results.

Comparative studies of secondary metabolites using qualitative tests performed on ethyl acetate, methanol and ethanolic extracts of leaves of *Cassia spectabilis, Cassia siamea, Cassia fistula, Cassia biflora* and *Cassia hirsuta* revealed the presence of phytoconstituents like alkaloids, tannins, saponins, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins(Veerachari and Bopaiah, 2011).

Jose *et al*., (2012) have reported that phytochemical screening of fresh juice and ethanolic extract of leaves of *Cassia alata* showed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins. Preliminary phytochemical screening of the ethanol extract of *Cassia sophera* leaves revealed the presence of steroids, alkaloids, tannins, saponins, and flavonoids(Mondal *et al*., 2012).

3.2 HPTLC analysis

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved(Chavan *et al*., 2016).

The results of qualitative phytochemical analysis of solvent extracts of leaf of *Cassia senna* L. confirmed the presence of alkaloids. However, to ensure the presence of these phytocomponents and their derivatives HPTLC study was carried out with the various extracts of *Cassia senna* L. leaves along with the colchicine as alkaloid standard.

From the Peak Table(Table 2), it is clear that HPTLC analysis of leaf extracts confirmed segregation of different types of alkaloids and other unknown compounds in the chromatogram(Plate 1 and 2) and densitogram(Figures 1 to 8) with individual Rf values and peak area. The range of Rf values of these compounds was between 0.03 to 0.95(Table 2) and the Rf value of peak 7(unknown compound) of sample CSL7-DEE matched with standard colchicine. The leaf extracts showed good resolution at both 366nm and 254nm before and after derivatization(Plate 3 and 4). Yellow, Brownish-Yellow and Brownish blue coloured zones at visible light mode(Figure 17) were observed in the tracks from the chromatogram after derivatization, which confirmed the presence of different derivatives of alkaloids with individual Rf values in leaf extracts.

Peak densitogram recorded after scanning at 254nm displays varying number of peaks for both alkaloids and unknown compounds in each extracts of leaf(Figures 1 to 8). Among the seven extracts of leaf analysed, sample CSL6-SEE and sample CSL7-DEE were found to contain more number of alkaloid peaks(Table 2) seven and eight respectively as compared with other extracts which further confirming those ethanolic extracts as the good alkaloidal sources. This indicating its ability to extract more number of alkaloids.

Chavan *et al*., (2016) reported that The HPTLC analysis of methanolic extract of leaves by *Cassia fistula* L. confirmed that flavonoids and alkaloids are the major group of chemicals in leaf extract. HPTLC finger print profile of methanol and ethyl acetate extracts of leaves of *Cassia fistula* revealed presence of greenish, purple, pink and light yellowish orange bands showing the presence of steroids, terpenoids and saponins after spraying with anisaldehyde sulphuric acid reagent(Seasotiya *et al*., 2014).

In the HPTLC study reported by Dhandapani and Kadarkarai, (2011) four different types of flavonoids were detected in leaf ethanolic extract of *Cassia Occidentalis* using rutin as flavonoid standard.

From the HPTLC densitometric quantification of sennosides in leaves of *Cassia angustifolia*, it was noted that the percentage of active principles varied significantly in the samples procured from different solvents namely ethyl acetate, chloroform, ethanol and methanol and it was found to be best in polar solvents namely methanol followed by ethanol. (Upadhyay *et al*., 2011). Our findings are in agreement with above results in which ethanol was found to be best, as polar solvents have higher ability to extract the maximum phytoconstituents than nonpolar solvents. Ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang *et al*., 2010).

DEE was found to segregate more number of alkaloids than SEE, which is might be due to sequential extraction procedure, where SEE(successive ethanolic extract) is obtained after the extraction with petroleum ether, benzene, chloroform and ethyl acetate. This has been previously reported by Bhatt *et al*., 2012) who noted lower total phenolic content in methanol that is used after ethyl acetate and acetone.

4. CONCLUSION

In conclusion, it could be stated that, among the various solvent extracts of *Cassia senna* L. leaves analysed for preliminary phytochemical screening and HPTLC, direct ethanolic extract(DEE) followed by the sucessive ethanolic extract were found to contain more phytoconstituents than other solvent extracts which might be due to the polarity and extracting ability of ethanol. DEE was found to segregate more number of alkaloids than SEE, which is might be due to sequential extraction procedure applied to extract SEE.

From the investgation of qualitative phytochemical and HPTLC analysis, leaf of *Cassia senna* L. was found to be a good source of beneficial phytoconstituents which may have a novel therapeutic value against various degenerative diseases.

6. ACKNOWLEDGEMENT

If words are considered as symbols of approval and token of acknowledgement, then words play the role of thanks to exhibit the deeply embedded feelings of gratitude. At the outset I would thank the **Almighty**‖ for showering his blessings throughout this work. I extend my privilege to record my gratitude and sincere thanks to **Dr.(Tmt)S.Annapoorani**, Professor and Head and all the **staff members** of the Department for providing all necessary facilities and for their constant encouragement evinced throughout the course of this investigation.

Table 1: Qualitative analysis of phytochemicals in the leaf extracts of *Cassia senna* L.

S.No	Phytochemical Test	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Ethyl acetate Extract	Successive Ethanolic Extract(SEE)	Direct Ethanolic Extract (DEE)	Aqueous
I	**Test for Carbohydrates**							
	Molisch's Test	+	+	+	+	+	+	+
	Fehling's Test	+	+	+	+	+	+	+
II	**Test for Glycosides**							
	Keller-Killani Test	+	+	+	+	+	+	+
	Modified Borntrager's Test	+	+	+	+	+	+	+
III	**Test for Proteins and Aminoacids**							
	Xanthoproteic Test	-	+	+	-	+	+	+
	Ninhydrine Test	-	+	+	-	+	+	+
IV	**Test for Alkaloids**							
	Mayer's Test	+	+	+	+	+	+	+
	Wagner's Test	+	+	+	+	+	+	+
	Dragendroff's Test	+	+	+	+	+	+	+
V	**Test for Flavonoids**							
	Alkaline reagent Test	+	+	+	+	+	+	+
	Sulphuric Acid Test	-	-	-	-	+	+	-
	Shinoda's Test	-	-	-	-	+	+	-
VI	**Test for Terpenoids and Steroids**							
	Terpenoids	-	-	-	-	+	+	-
	Steroids	+	+	+	+	+	+	+
VII	**Test for Diterpenes**							
	Copper acetate Test	-	-	-	-	+	+	-
VIII	**Test for Triterpenoids**							
		-	-	-	-	+	+	-
IX	**Test for Phytosterols**							
	Salkowski's Test	+	+	+	+	+	+	+
	Libermann Burchard's Test	+	+	+	+	+	+	+
X	**Test for Tannins**							
	Lead acetate test	+	+	+	+	+	+	+
XI	**Test for Saponins**							
	Froth Test	-	-	-	-	+	+	+
XII	**Test for Phenols**							
	Ferric Chloride Test	+	+	+	+	+	+	+

Table 2: Peak Table

Track	Peak	Rf	Height	Area	Assigned substance
COL	1	0.44	499.2	11956.0	Colchicine standard
Sample 1	1	0.47	11.9	249.8	Alkaloid 1
Sample 1	2	0.90	528.6	39644.6	Alkaloid 2
Sample 2	1	0.23	14.7	203.7	Unknown
Sample 2	2	0.34	17.7	270.9	Alkaloid 1
Sample 2	3	0.37	17.9	480.6	Alkaloid 2
Sample 2	4	0.46	80.3	1824.5	Alkaloid 3
Sample 2	5	0.50	16.3	339.4	Unknown
Sample 2	6	0.58	14.5	149.0	Alkaloid 4
Sample 2	7	0.68	11.2	168.4	Unknown
Sample 2	8	0.81	60.7	1464.1	Unknown
Sample 2	9	0.85	87.4	1882.8	Unknown
Sample 2	10	0.93	268.4	13980.8	Alkaloid 5
Sample 3	1	0.30	11.9	168.7	Alkaloid 1
Sample 3	2	0.35	37.2	1113.6	Alkaloid 2
Sample 3	3	0.47	164.8	4323.4	Alkaloid 3
Sample 3	4	0.50	156.8	3595.9	Alkaloid 4
Sample 3	5	0.64	28.6	722.2	Alkaloid 5
Sample 3	6	0.67	20.9	371.7	Unknown
Sample 3	7	0.80	127.7	5406.6	Unknown
Sample 3	8	0.84	122.5	2554.6	Unknown
Sample 3	9	0.93	277.2	16808.1	Alkaloid 6
Sample 4	1	0.03	13.0	84.0	Unknown
Sample 4	2	0.14	79.4	2651.2	Unknown
Sample 4	3	0.22	25.4	689.0	Unknown
Sample 4	4	0.28	29.5	383.1	Unknown
Sample 4	5	0.32	37.2	963.7	Alkaloid 1
Sample 4	6	0.36	46.3	1272.2	Unknown
Sample 4	7	0.40	58.8	1346.3	Alkaloid 2
Sample 4	8	0.48	412.4	19238.7	Alkaloid 3
Sample 4	9	0.56	67.0	2559.7	Unknown
Sample 4	10	0.65	28.1	703.9	Unknown
Sample 4	11	0.67	35.8	1231.7	Unknown
Sample 4	12	0.89	520.7	48962.0	Alkaloid 4
Sample 5	1	0.03	41.2	735.7	Alkaloid 1
Sample 5	2	0.05	42.8	704.6	Alkaloid 2
Sample 5	3	0.09	14.1	168.0	Unknown
Sample 5	4	0.13	131.5	3654.2	Unknown
Sample 5	5	0.20	135.4	2664.9	Unknown
Sample 5	6	0.23	331.1	7943.6	Unknown
Sample 5	7	0.26	317.0	8543.3	Alkaloid 3
Sample 5	8	0.37	151.2	4861.5	Unknown
Sample 5	9	0.39	139.9	2412.8	Alkaloid 4
Sample 5	10	0.47	110.2	2345.3	Alkaloid 5
Sample 5	11	0.51	224.6	5605.7	Alkaloid 6
Sample 5	12	0.56	56.3	2139.3	Unknown
Sample 5	13	0.65	13.1	378.1	Unknown
Sample 5	14	0.90	245.6	15803.4	Alkaloid 7
Sample CSL 6	1	0.04	10.4	84.6	Alkaloid 1
Sample CSL 6	2	0.13	19.3	600.2	Unknown
Sample CSL 6	3	0.21	36.6	898.6	Unknown
Sample CSL 6	4	0.50	23.8	620.9	Unknown
Sample CSL 6	5	0.95	229.7	10787.6	Unknown
Sample CSL 7	1	0.03	29.7	386.8	Unknown
Sample CSL 7	2	0.07	21.6	485.1	Alkaloid 1
Sample CSL 7	3	0.13	86	2440	Alkaloid 2
Sample CSL 7	4	0.26	252.2	5244	Unknown
Sample CSL 7	5	0.29	268.5	5179.4	Alkaloid 3
Sample CSL 7	6	0.39	45.4	1300.9	Alkaloid 4
Sample CSL 7	7	0.44	30.4	572.4	Unknown
Sample CSL 7	8	0.50	155.9	4355.3	Alkaloid 5
Sample CSL 7	9	0.58	130.6	3035.5	Unknown
Sample CSL 7	10	0.63	29.7	1163.1	Alkaloid 6
Sample CSL 7	11	0.77	12.2	248.5	Alkaloid 7
Sample CSL 7	12	0.79	21.9	431	Alkaloid 8
Sample CSL 7	13	0.93	298.3	17099.2	Unknown

CSL-Cassia senna leaf, COL – Colchicine - alkaloid standard as reference marker,
Sample1 – Petroleum ether extract, Sample 2 –Benzene extract, Sample 3 –Chloroform extract
Sample 4 –Ethyl acetate extract, Sample 5 - Successive ethanolic extract(SEE),
Sample 6 - CSL6 Aqueous extract, Sample 7 – CSL7 Direct Ethanolic extract(DEE)

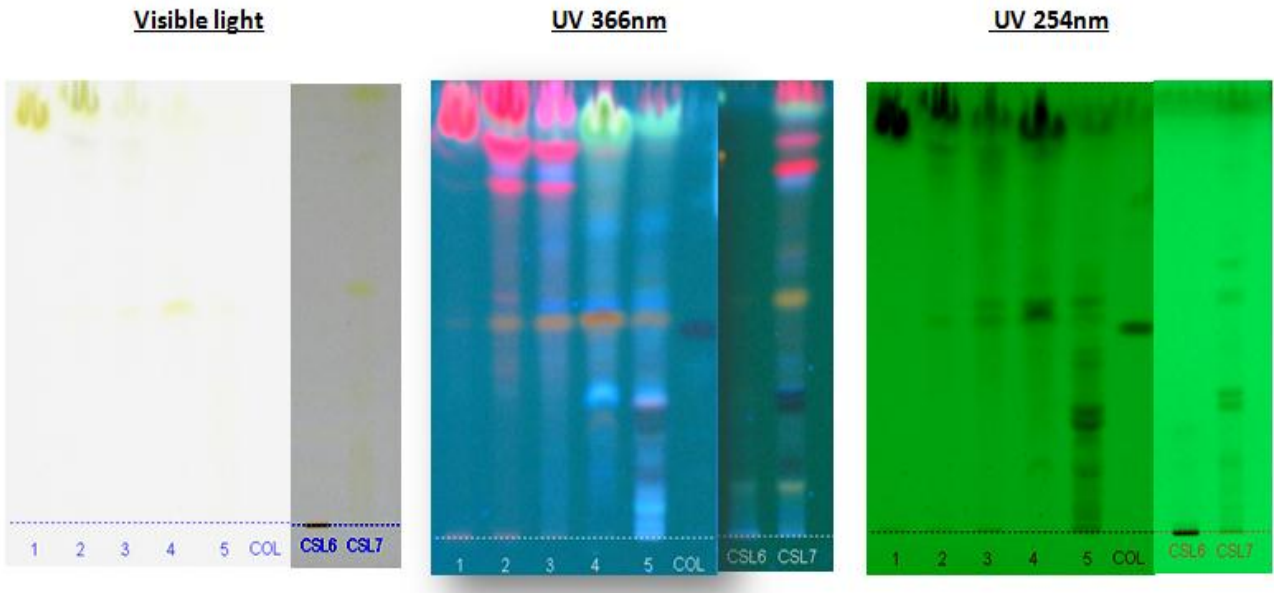

Plate. 1: Chromatogram before derivatization

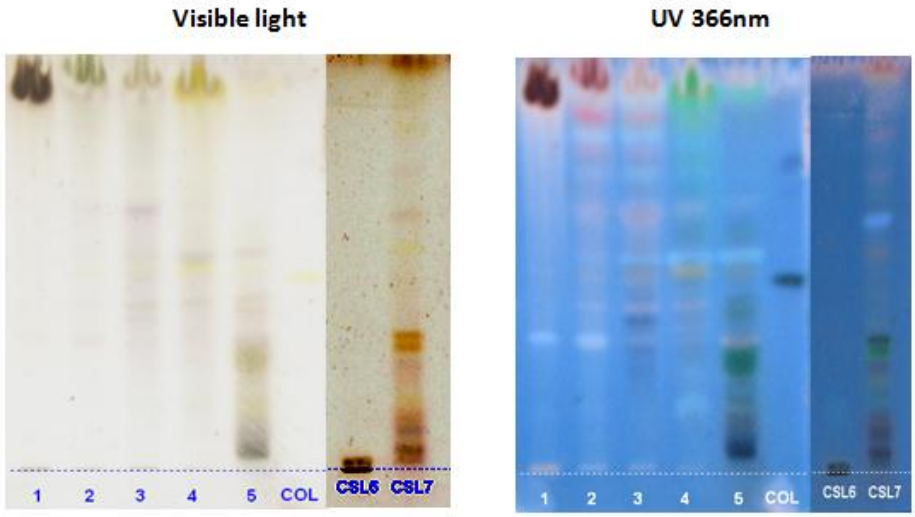

Plate. 2: Chromatogram after derivatization

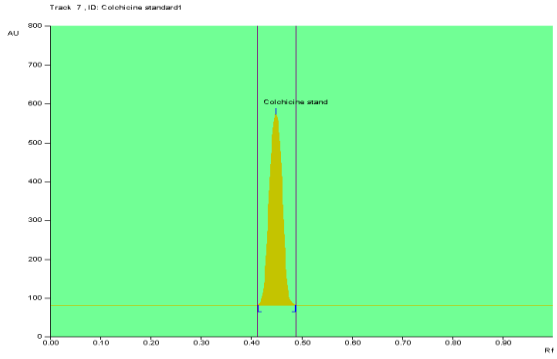

Fig. 1: Standard (Colchicine)

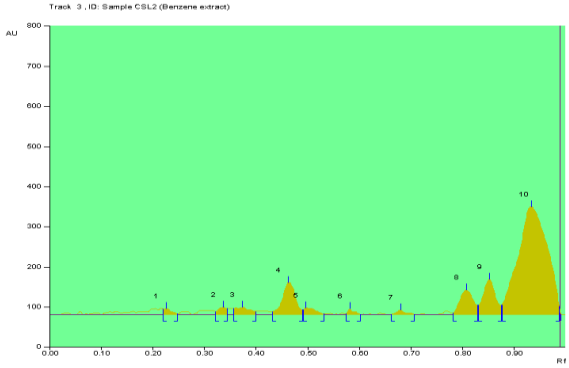

Fig. 2: Sample 1

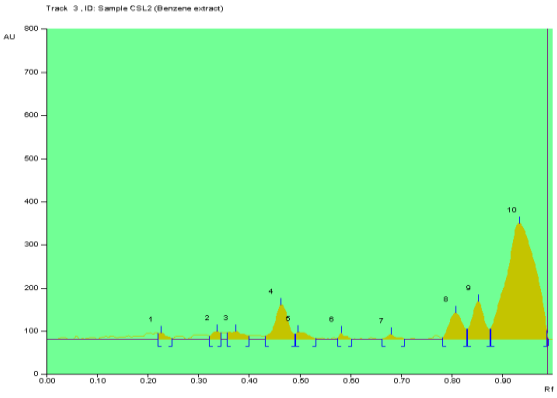

Fig. 3: Sample 2

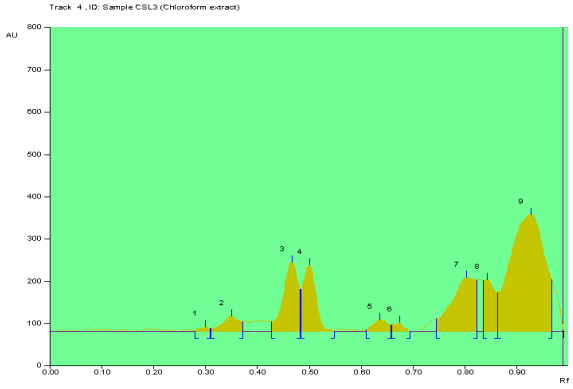

Fig. 4: Sample 3

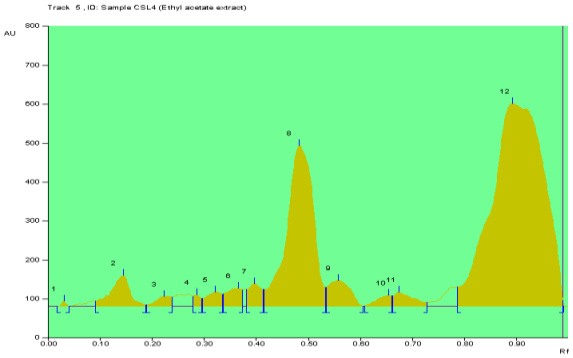

Fig. 5: Sample 4

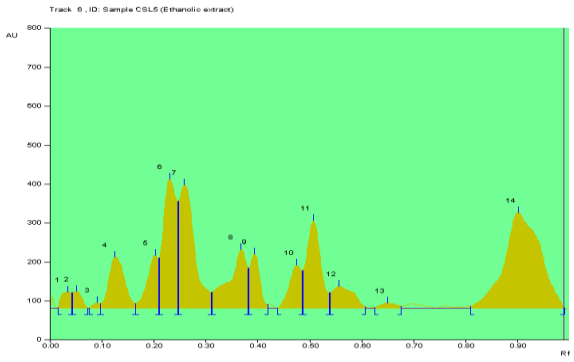

Fig. 6: Sample 5

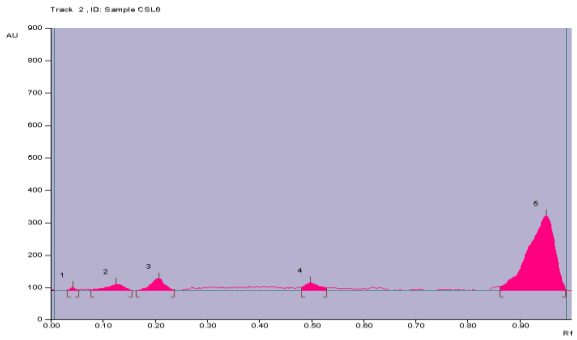

Fig. 7: Sample CSL 6

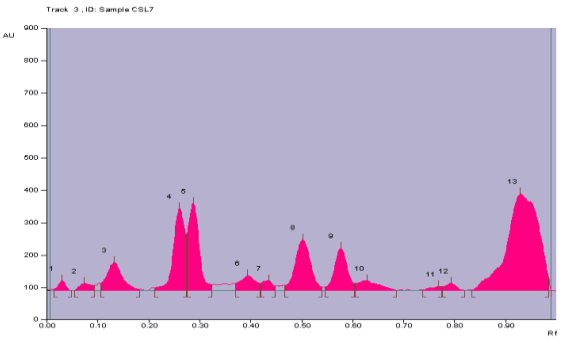

Fig. 8: Sample CSL 7

Peak densitogram display(Scanned at 254nm)

7
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