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HPTLC CHARACTERISATION OF CAKRAMARDA BIJA - (SEED OF *CASSIA TORA* LINN.)

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ABSTRACT

Background: *Cassia tora* Linn. is commonly known as Foetid Cassia, a well-known Plant to treat Dadru Kushtha and several diseases explained in Ayurvedic texts is noted to be one of the principle ingredients on many of the formulations like Maharaja prasarini tailam, Trnatailam and Dadruvidravahana malhara, Pancanimbadi Curna etc. The drug is found to be a Laxative, Febrifuge and known to exhibit different Pharmacological activities. The Powder extract which is taken for the study must have authentication and scientific evaluation for the safety usage and application in Clinical trials for therapeutic efficacy. HPTLC method is the accurate and rapid screening technique for the evaluation of Phyto-Constituents present in Powder extract of the seed can be useful for the standardisation of the drug. An attempt is made to find the chemical constituents in the drug Cakramarda Bija (*Cassia tora* Linn.). The presence of bio active compounds in plants makes them Pharmacologically valuable due to qualitative and quantitative analysis and is essential as the constituents of the extracts identified will be useful in various diseases. Therefore, in the present study, the high performance thin layer chromatography (HPTLC) analysis of *Cassia tora* (Seed) was performed. The densitometric scans, Photo documentation and retention factor values were all recorded. The data given in this paper will provide the parameters required for the quality control of the drug and standardisation purposes.

KEYWORDS: Densitometric scan, *Cassia tora* Linn., Cakramarda, HPTLC, Seed, Di ethyl ether, Toluene, Acetic acid, Rf Value.

RESULTS

The HPTLC densitometric scan at 620nm shows 2 spots, the area percentage 95.50% with the Rf value 0.00 at 366nm shows 11 spots, area percentage of 29.84% with Rf value of 0.00 and densitometric scan at 254 nm shows 15 spots with maximum area percentage 23.52% at the Rf value of 0.00 was more prominent.

CONCLUSION

HPTLC is one of the most important instrumental technique for the identification and quantitative determination of the main Phytochemical constituents present in the plants, so to study the quantitative analysis and identification of Seed *Cassia tora* Linn., the present study was carried out which also ensures the quality of the drug.

1. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Aim: To study the identification and quantitative determination of Phytochemical constituents of seeds of Cakramarda.

The HPTLC Study of the powdered drug of Cakramarda seed was performed at S.D.M. Centre for research in Ayurveda and allied sciences, UDIPI (Karnataka).

Particulars of sample: Cakramarda Bija Curna for the given sample (*Cassia tora* Linn.). Later HPTLC was performed, Photo documentation, Rf values and densitograms were documented.

2. METHODOLOGY

A. Sample Details

1gm of Cakramarda Bija Curna

B. Test Solution

1g of *Cakramarda Bija (Cassia tora seeds)* sample was kept in 20ml of ethanol macerated at room temperature with intermittent soaking. After 24hrs it was filtered through filter paper and filtrate (extract) was further used for HPTLC.

C. Stationary Phase

3, 6, 9µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator.

D. Mobile phase

The plate was developed in **Di ethyl ether: Toluene: Acetic acid (10: 5: 10)**.

E. Development

The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm (Post derivatisation). R_f, colour of the spots and densitometric scan were recorded.

F. HPTLC Instrumentation

Densitometric scan

G. Derivatization

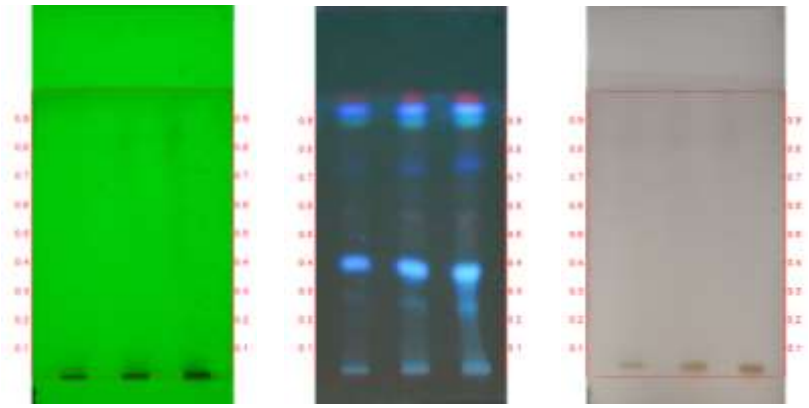
Derivatised with Vanillin Sulphuric Acid.

Interpretation of Sample Cakramarda Bija (*Cassia tora* Linn.) Curna

At long UV (366nm) shows 11 Spots having more area percentage of 33.35%, 29.84% with Rf values of 0.02,0.00. After post derivatisation (620nm) with vanillin sulphuric acid there were 2 spots observed with purple colour intensity at the Rf value of 0.00 and 0.79. Densitometric scan at 254nm shows 15spots with maximum area percentage of 23.5% at the Rf value of 0.00.

RESULTS

Results of **Cakramarda Bija (*Cassia tora* Linn.),** HPTLC Photo documentation, Rf values, densitometric scan are given in respective tables and figures.



Short UV **Long UV** **Post derivatisation**

Fig. 1: HPTLC Photo documentation of ethanol extract of Cakramarda Bija (*Cassia tora* Linn.)

Track 1 - **Cakramarda Bīja (Cassia tora)**– 3µl

Track 2 - **Cakramarda Bīja (Cassia tora)**– 6µl

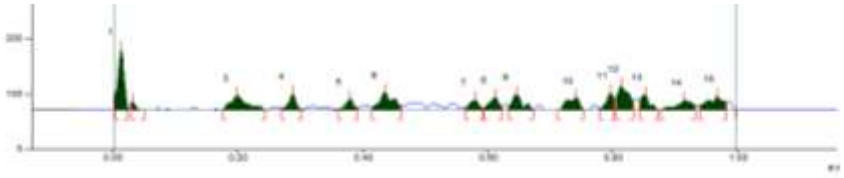
Track 3 - **Cakramarda Bīja (Cassia tora)**– 9µl

Solvent system – Di ethyl ether: Toluene: Acetic acid (10: 5: 10)

Table No. 1: - Rf values of sample of Cakramarda Bija (*Cassia tora* Linn.)

Short UV	Long UV	Post derivatisation
-	0.25 (F. blue)	-
-	0.36 (F. blue)	-
-	0.45 (F. blue)	-
-	0.50 (F. blue)	-
-	0.55 (F. red)	-
-	0.75 (F. blue)	-
-		0.85 (Purple)
-	0.90 (F. blue)	-
-	0.94 (F. blue)	-

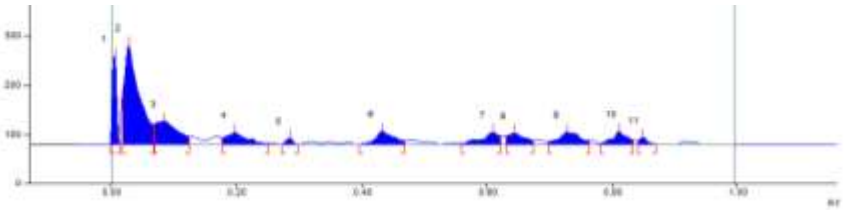
*F – Fluorescent; L –Light; D – Dark



Track 3, ID: Cakramarda beeja (Cassia tora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	27.3 AU	0.01 Rf	110.2 AU	23.52 %	0.02 Rf	0.8 AU	766.9 AU	14.75 %
2	0.03 Rf	13.2 AU	0.03 Rf	14.9 AU	3.19 %	0.05 Rf	0.0 AU	72.1 AU	1.39 %
3	0.18 Rf	0.1 AU	0.20 Rf	26.8 AU	5.73 %	0.25 Rf	0.2 AU	482.2 AU	9.28 %
4	0.27 Rf	3.0 AU	0.29 Rf	29.1 AU	6.22 %	0.30 Rf	1.0 AU	245.9 AU	4.73 %
5	0.36 Rf	1.9 AU	0.38 Rf	20.1 AU	4.28 %	0.39 Rf	4.3 AU	186.6 AU	3.59 %
6	0.42 Rf	4.0 AU	0.44 Rf	31.4 AU	6.70 %	0.46 Rf	8.0 AU	490.6 AU	9.44 %
7	0.56 Rf	3.0 AU	0.58 Rf	17.7 AU	3.77 %	0.59 Rf	3.5 AU	196.8 AU	3.79 %
8	0.60 Rf	3.6 AU	0.61 Rf	23.7 AU	5.06 %	0.62 Rf	7.3 AU	253.4 AU	4.87 %
9	0.64 Rf	7.3 AU	0.65 Rf	28.0 AU	5.98 %	0.67 Rf	2.0 AU	328.1 AU	6.31 %
10	0.71 Rf	0.1 AU	0.74 Rf	22.0 AU	4.70 %	0.76 Rf	0.5 AU	318.7 AU	6.13 %
11	0.78 Rf	2.7 AU	0.80 Rf	31.4 AU	6.71 %	0.80 Rf	20.9 AU	269.1 AU	5.18 %
12	0.81 Rf	21.4 AU	0.82 Rf	42.5 AU	9.08 %	0.84 Rf	16.5 AU	583.5 AU	11.22 %
13	0.85 Rf	16.3 AU	0.85 Rf	28.0 AU	5.98 %	0.87 Rf	0.6 AU	265.0 AU	5.10 %
14	0.88 Rf	0.4 AU	0.92 Rf	18.4 AU	3.92 %	0.93 Rf	7.7 AU	311.7 AU	6.00 %
15	0.94 Rf	8.3 AU	0.97 Rf	24.1 AU	5.14 %	0.98 Rf	14.2 AU	428.3 AU	8.24 %

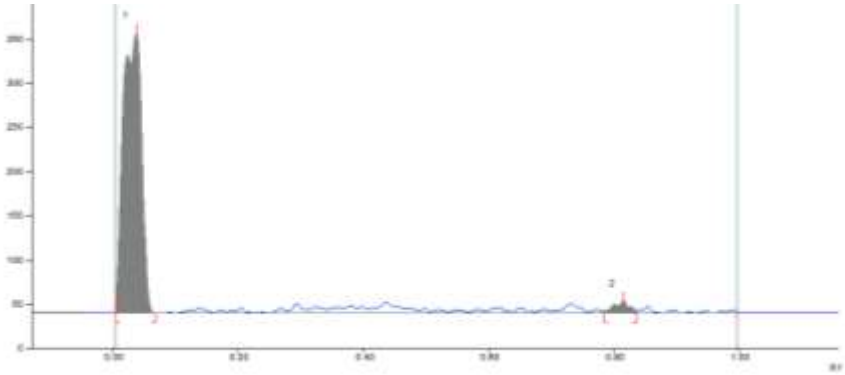
Fig. 2:- Densitometric scan of Cakramarda Bīja (*Cassia tora*), At 254nm.



Track 3, ID: Cakramarda beeja (Cassia tora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	179.1 AU	29.84 %	0.01 Rf	18.9 AU	764.9 AU	8.83 %
2	0.02 Rf	105.4 AU	0.03 Rf	200.1 AU	33.35 %	0.07 Rf	41.2 AU	3407.7 AU	39.36 %
3	0.07 Rf	41.6 AU	0.09 Rf	47.4 AU	7.89 %	0.13 Rf	15.8 AU	1214.4 AU	14.03 %
4	0.18 Rf	13.7 AU	0.20 Rf	24.0 AU	4.00 %	0.25 Rf	1.7 AU	567.8 AU	6.56 %
5	0.27 Rf	0.2 AU	0.29 Rf	11.9 AU	1.99 %	0.30 Rf	0.1 AU	91.7 AU	1.06 %
6	0.40 Rf	0.1 AU	0.43 Rf	26.2 AU	4.37 %	0.47 Rf	7.7 AU	541.5 AU	6.25 %
7	0.56 Rf	1.1 AU	0.61 Rf	24.5 AU	4.08 %	0.62 Rf	17.4 AU	475.4 AU	5.49 %
8	0.63 Rf	16.1 AU	0.65 Rf	22.6 AU	3.77 %	0.68 Rf	8.0 AU	439.1 AU	5.07 %
9	0.70 Rf	6.2 AU	0.73 Rf	23.9 AU	3.98 %	0.76 Rf	7.0 AU	590.3 AU	6.82 %
10	0.78 Rf	1.6 AU	0.81 Rf	26.4 AU	4.40 %	0.84 Rf	8.6 AU	432.4 AU	4.99 %
11	0.84 Rf	8.1 AU	0.85 Rf	13.8 AU	2.31 %	0.87 Rf	0.3 AU	133.0 AU	1.54 %

Fig 3. At 366nm, flu.



Track 3, ID: Cakramarda beeja (Cassia tora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	17.0 AU	0.04 Rf	316.7 AU	95.50 %	0.07 Rf	0.1 AU	6791.3 AU	96.52 %
2	0.79 Rf	2.2 AU	0.82 Rf	14.9 AU	4.50 %	0.84 Rf	2.5 AU	244.7 AU	3.48 %

Fig. 4: At 620nm (Post derivatisation with VSA).

DISCUSSION

- At long UV(366nm) spots were observed with 8 bands at Rf values of 0.25, 0.36, 0.45, 0.50, 0.55, 0.75, 0.90 ,0.94 with Blue colour intensity.
- Densitometric scan at 254nm shows 15 spots, with area percentage of 23.52%, 3.19%, 5.73%, 6.22%, 4.28%, 6.70%, 3.77%, 5.06%, 5.98%, 4.70%, 6.71%, 9.08%, 5.98%, 3.92%, 5.14% with the Rf values of 0.00, 0.03, 0.18, 0.27, 0.36, 0.42, 0.56, 0.60, 0.64, 0.71, 0.78, 0.81, 0.85, 0.88, 0.94 respectively.
- Densitometric scan at 366nm shows 11 spots, area percentage of 29.84%, 33.35%, 7.89%, 4.00%, 1.99%, 4.37%, 4.08%, 3.77%, 3.98%, 4.40%, 2.31% with the Rf values of 0.00, 0.02, 0.07, 0.18, 0.27, 0.40, 0.56, 0.63, 0.70,0.78, 0.84 respectively.

- Densitometric scan at 620nm shows 2 spots, the area percentage 95.50%, 4.50% with the Rf values 0.00,0.79. respectively.
- After Post derivatisation with Anisaldehyde sulphuric acid reagent at 620nm. one band is observed at Rf values of 0.85 with Purple colour intensity.
- The important pharmacological active components of Cakramarda seed contain **Oleic Acid, Palmitic Acid, Linoleic Acid, Chrysophanic Acid, Lignoceric Acid, and Sitosterol.**
- The drug has been reported to show Anti-fungal activity, Anti-microbial activity, Laxative property, Hypolipidemic activity, Anti-inflammatory activity, Anti- helminthic, Anti-oxidant, Anti-Cancerous, Hypo Lipidemic and Hypotensive action.

CONCLUSION

There is no doubt that Cakramarda plant is a reservoir of potential chemical compounds which can be noted from the 8 bands at long UV(366nm),15 spots at 254nm, densitometric scan at 620nm shows 2 spots and one band after Post derivatisation with Anisaldehyde sulphuric acid this can serve as new lead and clue for modern drug design by synthesis of single chemical constituent. The HPTLC studies of Seed *Cassia tora* Linn. can be a source for many new researches in other disciplines like Bio-chemistry, Bio technology, Pharmacology, Ethno-botany, Medicines etc. for development of new drug in various diseases mentioned in the study. The fantastic drug *Cassia tora* Linn. is magical wonder found in the Indian Traditional Medicine Ayurveda and its literature.

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