

Research Article



Formulation, Evaluation and HPTLC Fingerprinting of Topical Ointments Containing *Cassia tora* Linn. Leaf Extracts

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Accepted on: 26-04-2014; Finalized on: 30-06-2014.

ABSTRACT

Cassia tora Linn. (Caesalpinaceae) is a well known oriental herb or under shrub, found as a rainy season weed throughout India. Its different parts have a variety of pharmacological activities such as hepatoprotective, anti-inflammatory, hypolipidemic, anti proliferative, immuno stimulatory, purgative, antioxidant, antibacterial activity etc. The aqueous, alcoholic and hydro alcoholic extracts of *Cassia tora* Linn were prepared and used to formulate topical ointments. The ointments were subjected to qualitative analysis to identify the presence of active phytoconstituents. All the three extracts were used as standards for TLC and HPTLC fingerprinting of topical ointments. HPTLC studies showed the presence of glycosides and flavonoids in all the three topical ointments with respect to the extracts. Thus HPTLC fingerprinting emerged as an important tool for identification, qualitative analysis and phytochemical analysis of ointments of *Cassia tora* Linn. leaf extracts.

Keywords: Anti-inflammatory activity, *Cassia tora* leaves, Flavonoids, Glycosides, HPTLC fingerprinting, Topical preparation.

INTRODUCTION

Many medicinal plants have been traditionally used for thousands of years and are part of herbal preparations of the Indian traditional health care system. They are proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations, some have been thoroughly investigated for their therapeutic action while others are still to be explored. Monographs of plant materials in various pharmacopoeias mainly specify only the physicochemical parameters. Hence the modern methods used for identification and quantification of active constituents in the plant material may be useful for proper standardization of herbal extracts and their preparations. The WHO has also emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards.^{1,2} HPTLC fingerprint has better resolution and the estimation of active constituents is done with reasonable accuracy in a shorter time. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment.³

Cassia tora Linn. is a well known oriental herb used in traditional medicine which grows up to 1-2 m in height and is found as a weed throughout India.⁴ It is commonly known as Chakunda in hindi and bengali, Chakramarda in Sanskrit and Foetid cassia in English. In Unani, it is known as Sanjisboya. It is found up to a height of 1400 m in Himachal Pradesh and mainly in wastelands, on roadsides, field borders etc. It grows abundantly during rainy season, in dry soil throughout the tropical parts. Flowering and fruiting of this plant occurs in months of August-October.⁵ The leaves of *Cassia tora* Linn contain several anthraquinone glycosides which are well known

for their therapeutic value. The leaf extracts of this plant are reported to have significant antifungal and hepatoprotective effect against the toxicity of carbon tetrachloride in rats and the plant has been used as laxative, purgative and in treatment of skin disorder.⁶

The proposed work was aimed to study the HPTLC fingerprinting of the extracts and their respective topical ointment preparation.

MATERIALS AND METHODS

Collection and authentication of plant material⁷

The fresh leaves of *Cassia tora* Linn. were collected in the month of August-September from Marunje village, Tal.-Mulshi, Dist. Pune, Maharashtra, India. The plant was identified, confirmed and authenticated by Dr. P.G. Diwakar, Joint Director of Botanical Survey of India, Pune. The authentication No. for the plant is BSI/WRC/Tech/2011/667. Collected fresh leaves were washed, dried, pulverized and used for further studies.

Extraction of *Cassia tora* Linn. leaves^{8,9}

The dried leaves powder was extracted by continuous hot extraction method by using Soxhlet apparatus. The dried material (20g) extracted with water, ethanol and water ethanol mixture (1:1) in a Soxhlet apparatus for 3 hours. The extracts collected were evaporated on water bath and dried in desiccator. The extracts were labeled as AQE, ALE and HAE for Aqueous, Alcoholic and Hydro alcoholic extract respectively. All the extracts were studied for appearance, pH and their percentage yield. The results are reported in Table 1.



Table 1: Extracts obtained from different solvents

Type of Extract	Appearance	pH (10%)	% Yield of Extract
Aqueous Extract	Brown (Thick)	5.8	12.5% w/w
Alcoholic Extract	Dark Green (Thick)	6.5	09% w/w
Hydro-alcoholic Extract	Green (Thick)	6.0	9.5% w/w

Preliminary Phytochemical screening of Extracts^{10,11}

The plants are considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, Saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. All the extracts were subjected to various qualitative chemical tests. They showed the presence of carbohydrates, anthraquinone glycosides, flavonoids and phenolic compounds etc.

Preparation of Topical ointment¹²⁻¹⁴

Extracts (Aqueous, Alcoholic, Hydro-alcoholic), Emulsifying Wax (Fine Chemicals) Liquid Paraffin White (Fine Chemicals), Soft Paraffin (Fine Chemicals) were used to prepare the topical ointment.

Preparation method of topical Ointments

Herbal topical Ointments of all three extracts were prepared separately; Trial batches were prepared by using aqueous, alcoholic and hydro alcoholic extracts of *Cassia tora* Linn leaves. The ointments were prepared by fusion method, in this method the constituents of the base were placed together for melting, on a water bath heated at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled to 45°C and required quantity of extracts were added and stirred well. The prepared ointments were put in aluminium tubes, labeled, stored at room temperature and were used for further studies. The formula for the preparation of ointment is given in Table 2. The preparations were labeled as AQF, ALF and HAF for Aqueous, Alcoholic and Hydro alcoholic preparation respectively.

Table 2: Herbal Topical Ointment Preparations

Ingredients	Quantity
Extract (Aqueous, Alcoholic, Hydro-alcoholic)	1 g
Emulsifying Wax	30 g
Liquid Paraffin	20g
White soft Paraffin q.s	100g

Thin layer chromatography for all the extracts¹⁵

All three ointments of *Cassia tora* Linn. Leaf extracts were subjected to thin layer chromatographic studies using leaf extracts as standard, to find out the number of compounds present in formulation. The Alcoholic and

Hydro alcoholic extract ointments were found to show significant no of spots for glycosides and flavonoids in specified mobile phases where as aqueous extract ointment did not show any significant spots; hence the alcoholic and hydro alcoholic ointments were selected for HPTLC studies.

HPTLC fingerprinting for Topical ointment preparation^{16, 17}

HPTLC studies of ointments were carried out to ensure the presence of active constituents present with respective extracts used as standard.

Chromatographic Conditions

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttentz, Switzerland).

Syringe: 100µl Hamilton (Bonadug, Switzerland)

TLC chamber: AMD II Densitometer, TLC scanner III with Win cats 4 software.

Stationary Phase : Silica gel G 60 F₂₅₄ (Merk, Germany)

Size : 10 x 10 cm

Development mode : Ascending mode

Mode of application : Band

Band Size : 5 mm

Scanning Wavelength : 254 nm/366nm

Applied volume : 10 µl

Saturation time : 30 min

Mobile phase for Glycosides: Ethyl acetate: Méthanol: Water (100:13.5:10) and

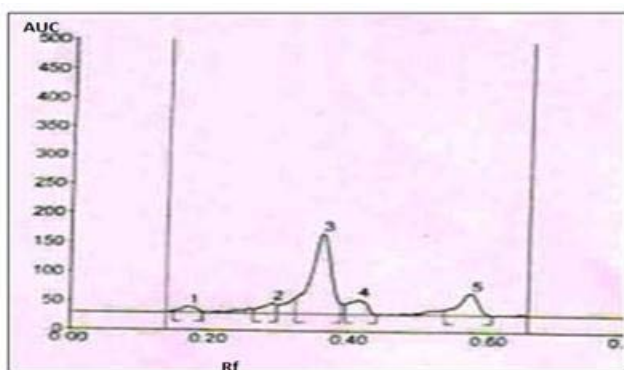
Mobile phase for flavonoids: Ethyl acetate: Formic Acid: Glacial Acetic Acid: Water (100: 12:12:24).

The chromatogram observed for detection of glycosides and flavonoids are shown in Figure 1(a) & 1(b), 2(a) & 2(b) and 3(a), 3(b), 4(a) & 4(b) respectively. And the R_f values found were reported in Table 3.

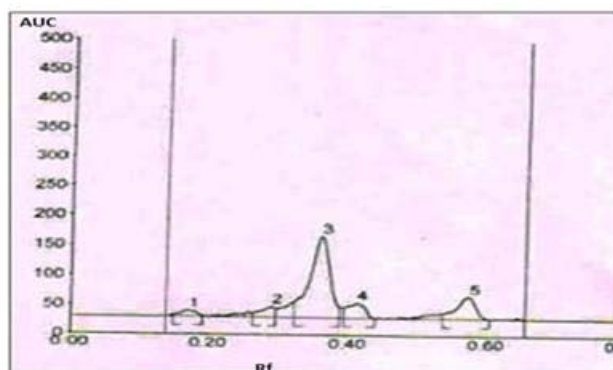
Table 3: R_f values for Glycosides and flavonoids

ALE	ALF	HAE	HAF
R_f values of HPTLC fingerprinting of Glycosides			
0.19	0.19	0.30	0.30
0.32	0.34	0.44	0.44
0.38	0.38	0.58	0.58
0.44	0.44	---	---
0.58	0.58	---	---
R_f values of HPTLC fingerprinting of Flavonoids			
0.44	0.44	0.44	0.44
0.52	0.52	0.50	0.50
0.61	0.61	0.57	0.57
0.68	0.68	0.68	0.68
0.77	0.77	0.73	0.73



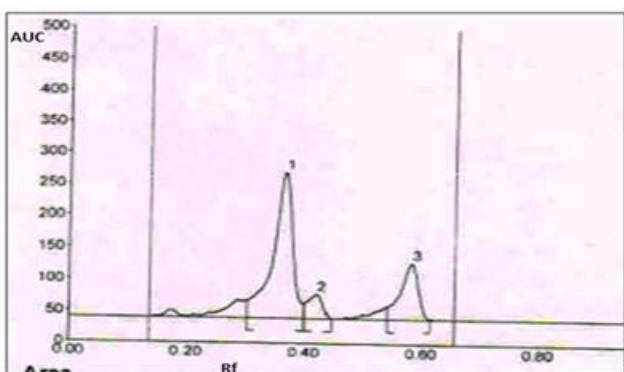


1(a)

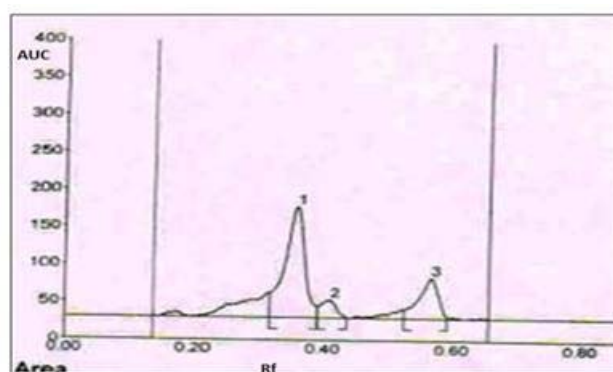


1(b)

Figure 1: HPTLC chromatogram of Glycosides for ALE 1(a) and ALF 1(b)

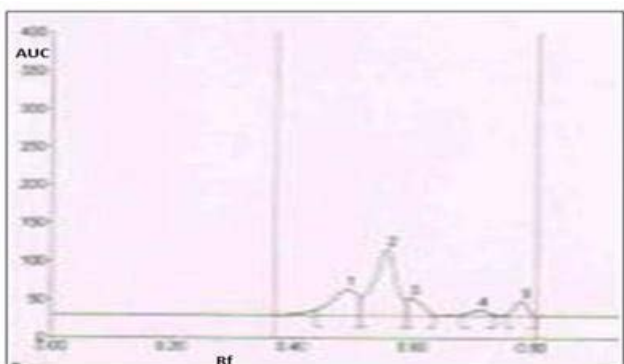


2(a)

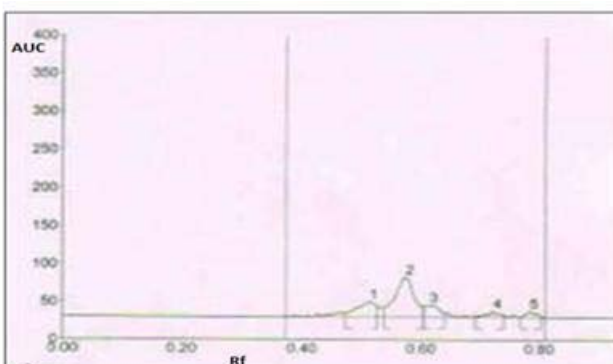


2(b)

Figure 2: HPTLC chromatogram of Glycosides for HAE (a) and HAF (b)



3(a)



3(b)

Figure 3: HPTLC chromatogram of flavonoids for ALF 3(a) and ALF 3(b)

RESULTS AND DISCUSSION

Extraction of *Cassia tora* Linn leaves

The percentage yield obtained for AQE, ALE and HAE were found to be 12.5% w/w (Brown), 9% w/w (Dark Green) and 9.5% w/w (Green) respectively. The pH observed were 5.8, 6.5 and 6.0 for AQE, ALE and HAE respectively.

Preliminary Phytochemical screening of Extracts

The three extracts showed the presence of carbohydrates, anthraquinone glycosides, flavonoids and phenolic compounds. The presence of these phytoconstituents indicates therapeutic activity like

hepatoprotective, anti-inflammatory, antioxidant and in treatment of skin disorder.

HPTLC fingerprinting for topical preparation

HPTLC for ALE, ALF, HAE and HAF were carried out and it was observed that both ointments and their respective standards show same number of spots. The R_f values for ALE and ALF were found to be 0.19, 0.32, 0.38, 0.44, 0.58 and for HAE and HAF were found to be 0.30, 0.44, 0.58 for Glycosides. The R_f values for ALE and ALF were found to be 0.44, 0.52, 0.61, 0.68, 0.77 and The R_f values for HAE and HAF were found to be 0.44, 0.50, 0.57, 0.68, 0.73 for flavonoids.

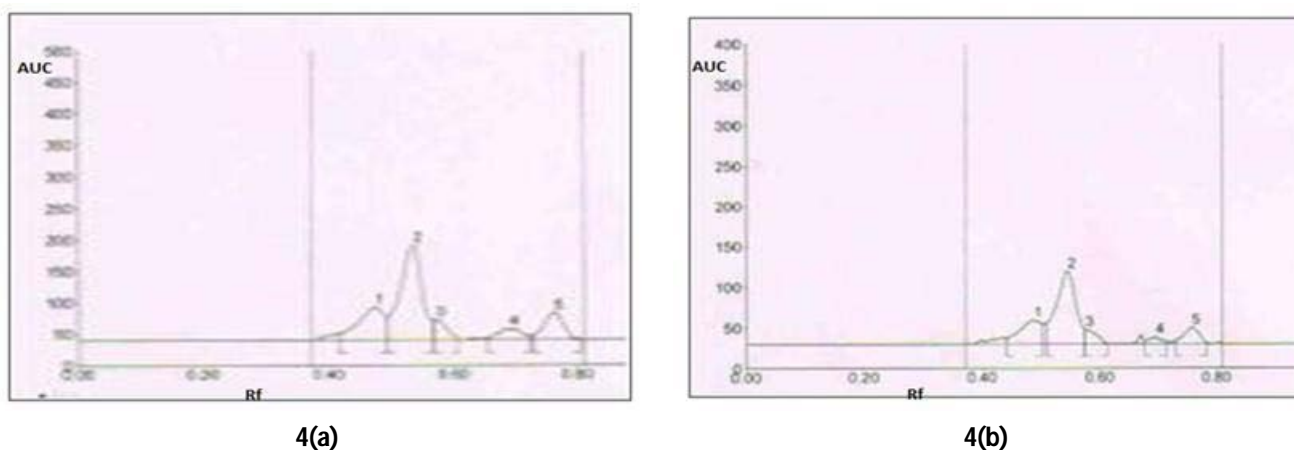


Figure 4: HPTLC chromatogram of flavonoids for HAE 4(a) and HAF 4(b)

CONCLUSION

The phytochemical analysis using HPTLC fingerprinting is helpful for standardizing ointment preparations containing leaf extracts of *Cassia tora* Linn. The preliminary phytochemical screening of alcoholic and hydro alcoholic extracts shows presence of glycosides and flavonoids. HPTLC fingerprinting was carried out to identify the presence of Glycosides and flavonoids in both ointment and respective extracts. The presence of phytoconstituents like glycosides and flavonoids confirms therapeutic efficacy in leaf extract ointments. Herbs and herbal preparations are complex mixtures with numerous natural compounds; however HPTLC provides sufficient information about therapeutic efficacy of the extracts, their preparations drug and also in the identification, standardization and quality control of medicinal plant.

REFERENCES

- Chaudhay R, Herbal Medicine for Human Health, Regional Publication, SEARO, No. 20, W.T.O, New Delhi, 1992,1-80.
- Quality Control Method for Medicinal Plant Materials; W.H.O, Geneva, 1989, 1-15.
- Gunalan G, Saraswathy A, Vijayalakshmi V, HPTLC fingerprint profile of *Bauhinia variegata* Linn. Leaves, Asian Pacific Journal of Tropical Disease, 2012, S21-S25.
- Sethi PD, High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Preparations, CBS Publishers and Distributors, New Delhi, 1996, 10-60.
- Choudhary M, Gulia Y, *Cassia tora*: Its chemistry, medicinal uses and Pharmacology, Pharmacology online, 3, 2011, 78-96.
- Maity T, Mandal S, Shah BP, Studies on some Pharmacognostic profile of *Cassia tora* Linn Leaves, Ancient Science of Life, Vol. No XIX (3&4), 2000, 113-115.
- Lans C, Harper T, Georges K, Bridgewater E, Medicinal and ethnoveterinary of hunters in Trinidad, BMC Complementary and Alternative Medicine, 2001, 10.
- Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy, Nirali Prakashan, 40, 2008, 16.12-16-14.
- Agarkar SV, Jadge DR, Phytochemical and pharmacological investigation of Genus Cassia: A review, Asian journal of chemistry, V.11 (2), 1999, 295-299.
- Khandelwal KR, Practical Pharmacognosy, Techniques and Experiments, Nirali Prakashan, New Delhi, 20, 2006, 149.
- Indian Pharmacopeia, Government of India, Ministry of Health and Family Welfare, Published by, The Indian Pharmacopoeial Commission, Ghaziabad, 2007, 134, 191.
- Rajasree PH, Vishwanad V, Cherian M, Jincy E, Singh R, Preparation and evaluation of antiseptic polyherbal ointment, International Journal of Pharmacy and Life sciences, 3(10), 2012, 2021-2031.
- Sangram KP, Narayan CD, Standardization of Vatari Guggulu – An Ayurvedic polyherbal preparation, International Journal of Pharma World Research, 2, 2011, 1–13.
- Kharbadi SS, Deore SL, Baviskar BA, Experimental Phyto pharmacognosy, A Comprehensive Guide, Nirali Prakashan, 2011, A 2.1 – A 2.8.
- Avalaskar AN, Itankar PR, Joshi VS, Agrawal M, Vyas J, Phytochemical and TLC Studies of Ethanolic Extract of *Sesbania grandiflora* (Fabaceae), International Journal of Pharm Tech Research, 3(3), 2011, 1346-1349.
- Patel NA, Patel M, Patel RP, Formulation and Evaluation of Polyherbal Gel for Wound Healing, International Research Journal of Pharmaceuticals, 01(01), 2011, 15-20.
- Sheikh S, Asghar S, Ahmad S, Development of HPTLC Qualitative Finger Printing Profile of Almond Oil in Marketed Herbal Cream, International Journal of Research in Pharmacy and Science, 3(1), 2013, 85-92.

Source of Support: Nil, **Conflict of Interest:** None.