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A Comparative Phytochemical Profiling of *Cassia tora* Linn.

SATISH A. BHALERAO
Environmental Sciences Research Laboratory, Department of Botany, Wilson College, Mumbai-400 007, India
E-mail: drsatishbhalerao@yahoo.com

Cassia tora Linn. (Family: Leguminosae, Sub-family: Caesalpinaceae) locally known as Takala, a herb that grows abundantly during the rainy season is an edible plant when in juvenile condition and is consumed as a vegetable. But the plant losses edibility after maturity *i.e.* when it bears flowers and fruits. It is also observed that cattle which usually eat this plant when it is young, avoid it after maturation. The possible reason for the loss of this edibility is the accumulation of metabolites that are toxic to human beings and work as anti-feedants or as agents of anti-herbivory in cattle. Present paper is based on the comparative estimation and profiling of the young and mature plants by HPTLC analysis method. The purpose of present study is to find out the possible anti-feedant agents if any, in the mature plant. It has been observed that total flavonoid content of the mature plant is remarkably high as compared to the young plants. These flavonoids might be responsible for the anti-feedant potential.

Key Words: *Cassia tora* Linn., Phyotochemical profiling, Anti-feedants, Anti-herbivory, HPTLC, Flavonoid.

INTRODUCTION

Cassia tora Linn. a herb that grows abundantly during the rainy season is an edible plant when in juvenile conditions and is consumed as a vegetable. But the plant losses edibility after maturity *i.e.* when it bears flowers and fruits. It is also observed that cattle which usually eat this plant when it is young, avoid it after maturation. The possible reason for the loss of this edibility is the accumulation of metabolites that are toxic to human beings and work as anti feedants or as agents of anti-herbivory in cattle. In the present study, a comparative estimation and profiling of the young and matured plants by HPTLC analysis method is performed. This is with the aim to find the possible anti feedant compounds.

Several compounds were found to be newly synthesized in the matured plants which are absent in the young plant. There is a remarkable increase in the total flavonoid content after maturation, indicating that flavonoids might be responsible for the anti feedant potential, as flavonoids from several plants have been identified till date to posses this activity.

Cassia tora, locally known as Takla is one of the seasonal vegetables found only in the rainy season. It is consumed as vegetable only during the early stage (vegetative stage) of its life cycle and it is known to loose edibility, after flowering.

According to local people it becomes bitter after flowering and hence is not consumed. Increase in the bitterness could be only one of the several possible reasons for its non edibility. It might be possible that these bitter compounds are toxic and hence aren't consumed.

Rationale: To investigate on these lines we aim to undertake profiling of different parts of *Cassia tora* during the above mentioned stages *viz.*, before and after flowering. This reports the phytochemical profiles in order to isolate the newly accumulated compounds after flowering. The biological activity studies can be taken up as a further extension of this project.

Plant description: Family: Leguminosae; Sub family: Caesalpinaceae; Botanical name: *Cassia tora* Linn.; Common name: Foetid cassia tora, sickle senna, sickle pod, coffee pod, tovara, chakvad; Parts used: Young leaves and seeds; Habitat: Grows in dry soil throughout tropical parts of India.

Morphological characteristics: It is an annual foetid herb, 30-90 cm high. **Leaves:** pinnate, up to 10 cm long rachis grooved, conical gland between each of two lowest pairs of leaflet, leaflets in 3 pairs, opposite, obovate, oblong and base oblique. **Flowers:** In pair in axils of leaves, petals five, pale yellow. **Fruit:** Pod, obliquely separate. **Seed:** 30-50 rhombhedral. **Flowering time:** After the monsoon rains (in Indian conditions).

Medicinal properties: The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders. It is aperient, germicide, mucilaginous and laxative. Useful in treating skin diseases like ring worm and itch and psoriasis. The decoction of the leaves is a laxative. It is useful when there is either chronic or acute constipation. It has been shown to be effective in lowering cholesterol and reducing blood pressure actions. Used as a poultice, warmed leaves reduce gout, sciatica and joint pains. The root is used in snakebite. In cultures, the leaf extracts of the plant showed antibacterial activity. Antiviral activity, particularly against Newcastle disease virus and Vaccinia virus is also exhibited by the plant.

Chemical constituents: Roots: 1,3,5-Trihydroxy-6-7-dimethoxy-2-methylanthroquinone and β-sitosterol. **Leaves:** Emodin, tricontan-1-0l, stigmasterol, α-sitosteral-β-D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin. **Seeds:** Naphtho-α-pyrone-toralactune, chrysophanol, physcion, emodin, rubrofusarin, chrysophonic acid-9-anthrone.

Biological activity studies: The chrysophonic acid-9-anthrone isolated from the seeds is shown to exhibit antifungal activity against *Trichophyton rubrum, T. mentagrophytes, Microsporum canis, M. gypseum* and *Geotrichum condidum*[1]. The leaf extracts of *Cassia tora* also exihibit antiinflammatory properties[2]. The water extracts of the plant posses antioxidant potential[3]. The anthraquinone aglycones and naphthopyrone glycosides isolated from the plant show *in vitro* antimutagenic properties[4].

EXPERIMENTAL

Collection of plant material: The material was collected from Karjat-a place near Mumbai on the Bombay to Pune route. Two different stages were collected. (a) June-July (early monsoon)-the plant germinates and is young. (b) August-Sept (late monsoon)-the plants starts flowering and later fruiting.

Processing: The plant material was segregated as root, stem and leaves of young and mature plants and fruits of matured plants. They were dried in shade and then powdered into a fine powder.

Extraction of plant material: The extraction procedures were followed[5] as mentioned: **Chemical fingerprinting:** A known and same amount of the dry powder of all the materials was exhaustively soxhlet extracted with methanol at 60 ºC. It was filtered and the filtrate was concentrated to obtain a final volume of 1 mL. Each of these methanolic extracts was used for fingerprinting. **Flavonoids:** A known amount of the dry powder of all the materials was exhaustively soxhlet extracted with methanol at 60 ºC. It was filtered and the filtrate was concentrated to obtain a residue. The residue was dissolved in demineralized water and was heated at 80 ºC in water bath for at least 0.5 h. This aqueous extract was then fractionated with ethyl acetate three times. The solvent phase was collected and concentrated dryness. The reside was weighted. This residue was re dissolved in ethyl acetate to a final volume of 1 mL. The weight of the ethyl acetate residue gives the total flavonoid content.

Chromatography: HPLTC analytical method was used for comparative profiling of these final extracts. Two runs were done: (i) All the methanolic extracts, (ii) All the flavonoid fractions; Instrument: Camag high performance thin layer chromatography; Software: WinCATS planar chromatography manager; Stationary phase: Silica Gel 60 F254 pre coated TLC plates (Merck, Germany).

Mobile phase: (1) Fingerprinting: toluene:ethyl acetate:formic acid (7:3:0.2); (2) Flavonoid: chloroform:aceton:formic acid (14:6:1); Detection: 254 nm (short UV) and 366 nm (long UV).

RESULTS AND DISCUSSION

Cassia tora, used as a vegetable when young, loses edibility as a vegetable after flowering and fruiting. It is known to become bitterer after maturation and is also known to be avoided by animals who normally consume this plant when young. This prompted us to work on comparing the chemical profiles of the plants at two different stages. The experimentation was focused on finding the new compounds synthesized in the plant, which impart anti-feedant potential to the plant.

Plants have a very strong defense mechanism comprising of chemical defense. They wisely protect themselves from adversities, the mechanism of which includes the synthesis or enhancement of several secondary metabolites. We tried to screen these newly synthesized compounds with relevance to the anti-feedant activity.

Flavonoids, a group of water soluble metabolites, one amongst the large amount of secondary compounds synthesized in plants, are known to exhibit anti-herbivory in plants[6]. Several flavonoids have been identified to exhibit this activity and are thought to be the key compounds in this kind of a protection. With these facts as a background, we also screened the flavonoid profiles of *Cassia tora*.

Chemical profiles: The methanolic extracts of mature and young plants show remarkable difference (Figs. 1a and 1b). The results of scanning at 366 nm (Fig. 1a) and 254 nm (Fig. 1b) when combined show that there were *ca.* 23 compounds detected in the young leaf (Fig. 1a u_2) and 17 compounds in the mature leaf (Fig. 1a and 1b u_1). Out of the 17 compounds in the profile of the matured leaf 4 were newly synthesized *viz.* one with R_f value 0.05 and the other two with 0.83 and 0.95 detected at 366 nm and the last one with 0.26 detected at 254 nm. Twelve compounds are found to be present in mature stem (Figs. 1a and 1b u_3) and 22 in young stem (Figs. 1a and 1b u_4), out of which one compound was found to be newly synthesized in the matured stem at R_f 0.43 detectable at 366 nm. Twenty three compounds were present in mature root (Figs. 1a and 1b u_5) and 25 in young roots (Figs. 1a and 1b u_6), out of which 6 compounds were found to be synthesized newly in the roots of the plant. These were with R_f 0.10 and 1.02 detectable at 366 nm and 4 compounds with R_f 0.27, 0.52, 0.74 and 0.96 detectable at 254 nm. Almost 20 compounds were detected in the seed (Figs. 1a and 1b u_8) and 19 in the seed pericarp (Figs. 1a and 1b u_9).

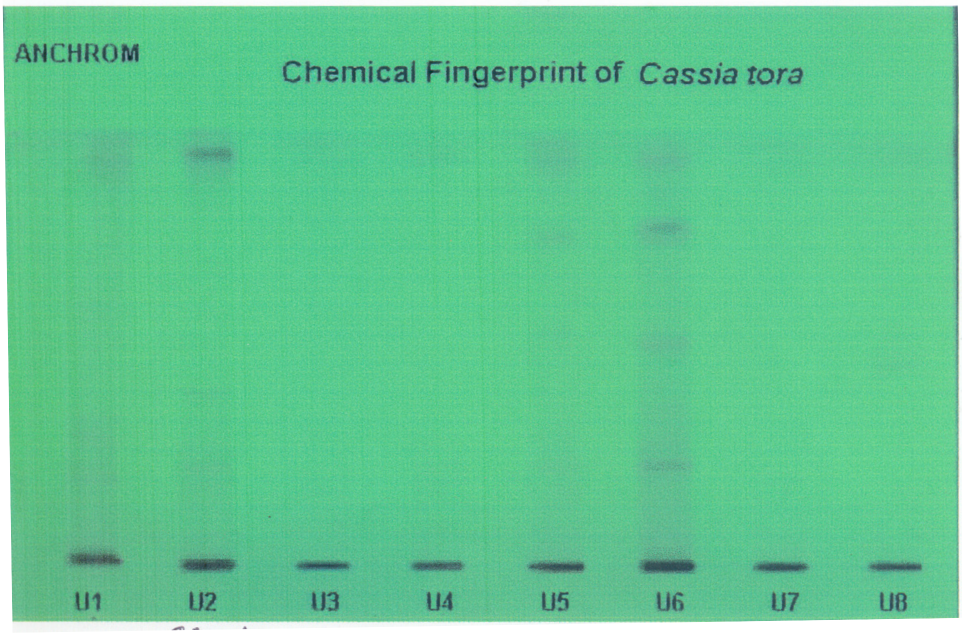

Fig. 1(a). Chemical fingerprinting of *Cassia tora* scanned at 254 nm

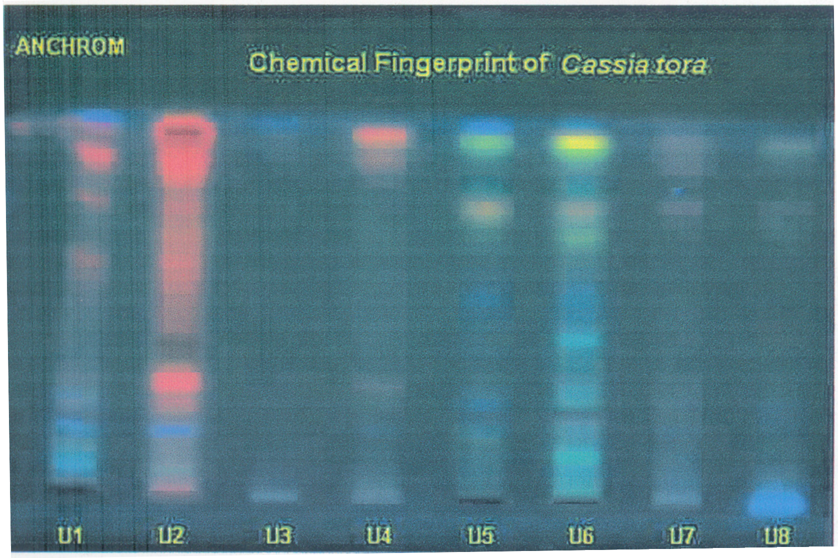

Fig. 1(b). Chemical fingerprinting of *Cassia tora* scanned at 366 nm

Together *ca.* 50 new compounds were present in the plant at the stage of maturation together or singly which might exhibit anti-feedant activity.

Although there were several new compounds that were observed in the matured plant, there was also a loss of certain metabolites in the plant after maturation. This loss does not support the assumption that only the new compounds are responsible for exhibiting the anti-feedant activity. The loss of some compounds might also play an important role. These compounds when present might exhibit protective properties like antioxidant effects or function in nullifying the effects of other toxic compounds produced by the plant.

New compounds in mature leaf (R_f): 366 nm: 0.05, 0.83, 0.95; 254 nm: 0.26; New compounds in matured stem (R_f): 366 nm: 0.43; New compounds in matured root (R_f): 366 nm: 0.10, 1.02; 254 nm: 0.27, 0.52, 0.74, 0.96

TABLE-1
TLC TRACKS AND THE SPECIFICATIONS OF THE EXTRACT

Track	Code	Material details
U_1	ML	Mature leaf
U_2	YL	Young leaf
U_3	MS	Mature stem
U_4	YS	Young stem
U_5	MR	Mature root
U_6	YR	Young root
U_7	MSe	Seed
U_8	MP	Fruit pericarp (pod pericarp)

Flavonoid profiles

Total flavonoid content: The amount of total flavonoids was also estimated using Wagner method and is expressed in terms of percentage dry weight (% DW). The results of these have been tabulated in Table-2. These indicate that there is a significant increase in the total flavonoids in matured plant parts. The exact values have been mentioned in the Table-2 and are graphically represented in Fig. 3.

TABLE-2
TOTAL FLAVONOID CONTENT IN DIFFERENT PARTS OF *Cassia tora* AT TWO DIFFERENT STAGES

Code	Material details	Flavonoid content (% DW of the plant material) (%)
ML_{Et}	Matured leaf	1.8
YL_{Et}	Young leaf	1.4
MS_{Et}	Matured stem	0.6
YS_{Et}	Young stem	0.5
MR_{Et}	Matured root	6.0
YR_{Et}	Young root	4.0
MSe_{Et}	Seed	3.8
MP_{Et}	Fruit pericarp (pod pericarp)	4.0

As mentioned earlier, flavonoids anti-feedant potential in plants and hence reported to be enhanced as a defense mechanism in plants. The results of the present experiment are in accordance with the same.

The comparative profiling of flavonoids of the two stages gave the following results when scanned at 366 nm (Fig. 2b) and 254 nm (Fig. 2a). (a) Eleven flavonoids were found to be present in the mature leaf (Figs. 2a and 2b u_1) and 20 flavonoids were present in the young leaf (Figs. 2a and 2b u_2). The mature leaf shows 7 flavonoids that were newly synthesized. Out of these, 3 compounds were detected at 366 nm with R_f values 0.05, 0.26 and 0.63 and 4 were detected at 254 nm with R_f values 0.16, 0.23, 0.57 and 0.66. (b) 6 Flavonoids were detected in the mature stem (Figs. 2a and 2b u_3) and 9 in the young stem (Figs. 2a and 2b u_4). The mature stem shows the presence of 2 new flavonoids, out of which 1 was detected at 366 nm with R_f 0.2 and the other detected at 254 nm with an R_f 0.94. (c) 10 Flavonoids were detected in the mature root (Figs. 2a and 2b u_5) and 8 in the young root (Figs. 2a and 2b u_6). The mature stem shows the presence of 3 new flavonoids, out of which 1 was detected at 366 nm with R_f 0.41 and the other 2 were detected at 254 nm with R_f values 0.57 and 0.64.

Eight flavonoids were detected in pericarp 13 flavonoids were detected in the seed combining the results scanned at 366 and 254 nm. Therefore in all, 33 new flavonoids were synthesized in the plant which contributes to a large extent in the anti-feedant potential property of the plant.

New flavonoids in matured leaf (R_f): 366 nm: 0.05, 0.26, 0.63; 254 nm: 0.16, 0.23, 0.57 and 0.66; New flavonoids in matured stem (R_f): 366 nm: 0.2; 254 nm: 0.94; New flavonoids in matured root (R_f): 366 nm: 0.41; 254 nm: 0.57 and 0.64.

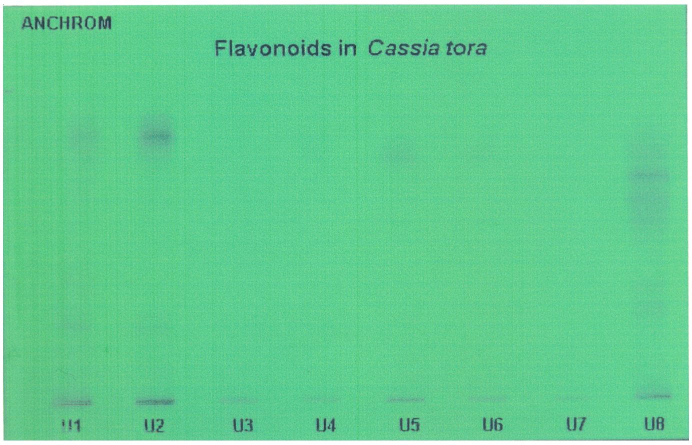

Fig. 2(a). Flavonoids in *Cassia tora* scanned at 254 nm

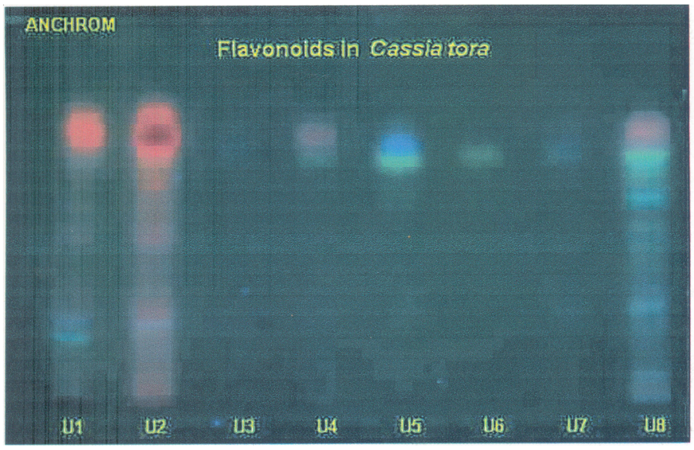

Fig. 2(b). Flavonoids in *Cassia tora* scanned at 366 nm

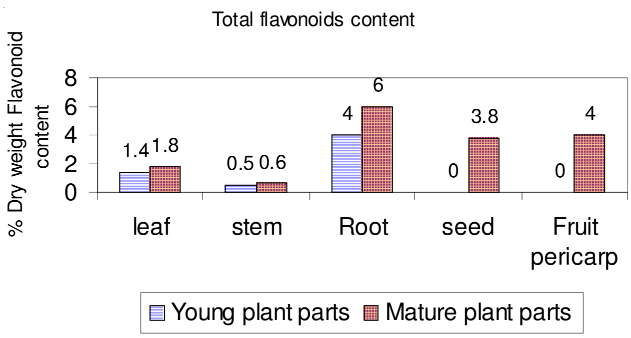

Fig. 3. Total flavonoid content in different parts of *Cassia tora* at two different stages

Conclusion

Many plants are well known for the defence mechanism like physical devices like thorns and prickles, anatomical like cork and rubber formation. Whereas many of them are famous for production of chemicals which act as anti feedant agents. *Cassia tora*, which is investigated for flavonoid contents which is acting as one of the anti feedant agent. Mature leaf shows presence of 7 newly synthesized flavonoids with respect to young leaf whereas this number goes to 2 in case of mature stem with respect to young stem. In case of root it goes to 3 new flavonoids. In pericarp and seed the flavonoids present are 8 and 13, respectively.

In all, 33 flavonoids which are all together newly synthesized, may contribute to the anti feedant property of mature plant over young plant, which is very common grazing material for herbivores.

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REFERENCES

1. T.K. Acharya and I.B. Chatterjee, *Lloydia*, **38**, 218 (1975).
2. T.K. Maity, S.C. Mandal, P.K. Mukherjee, K. Saha, J. Das, M. Pal and B.P. Saha, *Phytother. Res.*, **12**, 221 (1998).
3. G.C. Yen and D.Y. Chaung, *J. Agric. Food Chem.*, **48**, 2760 (2000).
4. J.S. Choi, H.J. Lee, K.Y. Park, J.O. Ha and S.S. Kang, *Planta Med.*, **63**, 11 (1997).
5. H. Wagner and S. Bladt, Plant Drug Analysis, Springer Verlag Berlin Hiedelberg, edn. 2004 (1996).
6. J.B. Harborne and C.A. Williams, *Phytochemistry*, **55**, 481 (2000).

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