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RESEARCH ARTICLE

COMPARATIVE PHYTOCHEMICAL STUDY ON SOME *CASSIA SPECIES*

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ABSTRACT

Object: In this present study easily available four cassia species like *Cassia occidentalis*, *Cassia fistula*, *Cassia auriculata* and *Cassia alata* were selected and they were qualitatively and quantitatively evaluated for their anthroquinone glycosides like Sennoside A Sennoside B and compared with *Cassia angustifolia*. The presence of these phytoconstituents were confirmed by HPTLC technique. The evaluation of traditional drugs is primarily based on Pharmacognostical, Phytochemical, Pharmacological and allied approaches including various instrumental techniques such chromatography, microscopy, spectroscopy and others. Senna is commonly found medication traditionally used by Arabian physicians primarily as a Cathartic. The laxative effect exerted by Senna is mainly attributed to anthraquinones which include dianthrone glycosides, sennosides A and B, sennosides C and D. The genus *Cassia* in the family Leguminosae in the major group angiosperm. Anthraquinones are functionally diverse aromatic chemicals, structurally related to anthracene, with parent structure 9, 10-dioxoanthracene.

Results: The HPTLC spectrum shows the same R_f value in 4 species (*Cassia fistula, Cassia occidentalis, Cassia auriculata, Cassia alata*) and in *Cassia angustifolia* R_f value 0.45, 0.44, 0.46, 0.45, and 0.46 and R_f value 0.77, 0.76, 0.78, 0.79 and 0.79 respectively. Here, it was clearly verified the presence of Sennosides.

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INTRODUCTION

The genus *Cassia* in the family Leguminosae in the major group angiosperm. Species of the genera Senna and chamaecrista were previously included in cassia (Vijay and Meenakshi, 2010). Senna is commonly found medication traditionally used by Arabian physicians primarily as a Cathartic. In these easily available four cassia species like *Cassia occidentalis* (CO), *Cassia fistula* (CF), *Cassia auriculata* (C AU) and *Cassia alata* (C AL) were selected and they were qualitatively and quantitatively evaluated for their anthroquinone glycosides like sennoside A sennoside B and compared with *Cassia angustifolia* (CA). The evaluation of these drugs is primarily based on phytochemical, pharmacological and allied approaches including various instrumental techniques such chromatography, microscopy and others. The emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential (Gupta, 2010). The laxative effect exerted by senna is mainly attributed to anthraquinones which include dianthrone glycosides, sennosides A and B, sennosides C and D. The laxative effect is exerted by acting on the lower bowel and thereby increasing the peristaltic movements of the colon by irritating the colonic mucosa.

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Senna is suggest for "stimulant laxative" during pregnancy and lactation. Cassia now generally includes the largest species of the legume subtribe cassiinae, usually mid-sized trees senna is known as purgative action. Phytoconstituents principally responsible for its characteristic action are two anthroquinone glycoside namely sennoside A,sennoside B. This constituents are responsible upto 40-60% activity of crude senna. It contains small quantities of other anthroquinone glycosides such as sennoside C and sennoside D, rhein 8 glucoside, aloe emodin, rhein 8 diglucoside. Additionally it contains naphthale glucosides, phytosterol, myricyl alcohol, salicylic acid, mucilage, resin, chrysophenic acid, calcium oxalate. Sennosides are not restricted to leaflets only. Various vegetative, Reproductive structure (underground as well as aerial (Sandelbach, 1989). Anthraquinones are functionally diverse aromatic chemicals, structurally related to anthracene with parent structure 9,10-dioxoanthracene. It has the appearance of yellow or light gray to gray-green solid crystalline powder. Its other names are 9, 10-anthracenedione, anthradione 9,10-anthrachinon, anthracene 9,10-quinone and 9,10-dihydro-9,10-dioxoanthracene. Anthraquinone compound are used as laxatives mainly from their glycosidic derivatives and also used in the treatment of fungal skin diseases. It is frequently found in slimming agents and have been valued for their cathartic and presumed detoxifying action however may cause nausea, vomiting, abdominal cramps, diarrhea with over dose. Natural and synthetic anthroquinones have widespread applications throughout industry and medicine, thereby indirectly and directly exposing the human population (Hemen and Lalita, 2012). It is a valuable plant drug in ayurvedic and modern system of medicine

for the treatment of constipation. The leaves containing sennosides are efficient sources of health teas and are considered as astringent, cathartic, depurative, anthelmintic, cholagogue, expectorant and febrifuge, useful for leprosy, leukoderma, jaundice, typhoid, fever, tumors. Senna is an ornamental plant in landscaping. It is considered to be a bowel stimulant on the mysentric plexus of the colon to induce peristaltic contractions and decrease water absorption from inside the colon. Effects that would provide relief from constipation (Sushma and Sardana, 2014; Shabina *et al.,* 2016). Ayurveda has advised virechana (purgation therapy) in the condition of Hepatomegaly, splenomegaly and jaundice to relive excessive pitta from the body using the dried leaf or pod of senna plant. Senna leaf or pod in dried form is stimulates the liver for production of pitta. The usage of senna is contraindicated in people suffering from inflammatory colon diseases, severe dysentery (Theeshan *et al.,* 2005). The World Health Organization (WHO) estimates that about 80% of people living in developing countries rely exclusively on traditional medicines for their primary health care need. India is virtually a herbarium of the world, using plants and herbs as the basic source of medicine. Herbals which form a part of our nutrition and provide us an additional therapeutic effect are in demand and Cassia species is one of such plant (Jignasu, 2012; Aurapa and Wandee, 2009; Fakiha *et al.,* 2015).

MATERIALS AND METHODS

Collection and Authentication: This plant is collected from Theni, district and authentified in Botanical Survey of India, Coimbatore. Authentification number of *Cassia occidentalis* (BSI/SRC/5/23/2017/Tech/ 3300) *Cassia fistula* (BSI/SRC/5/23/2017/Tech/3301), *Cassia auriculata* and *Cassia alata* are (BSI/SRC/5/23/2017/Tech/ 3302).

Physiochemical Evaluation

Determination of Ash value (Kokate, 2014): Accurately weighed about 2g powdered drug was incinerated in a silica crucible at a temperature not exceeding 450^{o} C for 4 hours in a muffle furnace until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air – dried drug was calculated. The acid insoluble ash, water soluble ash and sulphated ash was done according to the standard procedure. Average of the triplicate values was calculated.

Determination of extractive value (Kokate, 2014): Macerated about 5g of accurately weighed coarsely powder air dried *Cassia angustifolia* with 100 ml of alcohol (90%) in a stoppered flask for 24 hours, shaked frequently during first 6 hours. Filtered rapidly through filter paper and evaporated the 25ml of alcohol extract to dryness in a tarred flat bottomed shallow dish, dry at 105 °C and weighed. The extract was kept it in desicator. The percentage w/w of alcohol 90% soluble extractive calculated with reference to the air dried drug. The same procedure was repeated with other drugs and different solvents like chloroform, Benzene, Ethanol, Acetone and water etc. The extractive values of the four drugs with different solvents were documented.

Determination of crude fiber content (Kokate, 2014): Weighed 3-4gm of finely powdered crude drug and extracted with petroleum ether at room temperature. Filter and dried the marc. Boiled 2g m of dried material with 200ml sulphuric acid for 30 minutes. Filtered the extracted material and washed with boiling water. Then boiled the material with 200ml of sodium hydroxide for 30 minutes. Filtered the solution again through muslin cloth and washed with 25ml of 1.25% H_2SO_4, 50ml of water and 25ml of alcohol successively. After washing the residue transfer to a silica crucible which is weighed previously (w1). Dried the residue for 2-3 hours (at 130cAfter washing the residue transfer to a silica crucible which is weighed previously (w1). Dry the residue for 2-3 hours (at 130c) and cool the crucible in a desiccators and weigh again (w2). Then incinerated the residue for 30 minutes at 600°C, cooled it to room temperature in a desiccators and weigh again (w3). The fiber content was calculated by using the following formula.

$$\text{Percentage of crude fiber in test sample} = \frac{(W2-W1)-(W3-W1)}{\text{Weight of test sample}} \times 100$$

Determination of Moisture content (Kokate, 2014): Weighed accurately 5g of the finely powdered drug in a flat bottomed dish. Dried in oven at 100-105°c for 3 hours then cool it in a desiccators over anhydrous silica gel. Weighed the drug again, calculated the weight difference after drying and calculate the percentage of loss on drying.

Preparation of extract (Kokate, 2014)

The extract is prepared by simple maceration process, in which 500gm of powdered drug is mixed well with distilled water separately and shaken well for 7 days and after which it was filtered and dried at below 50^{o}c temperature to obtain the final residual extract. Then the extract is packed in an air tight container and kept in a desiccators, these extract was taken for the further work.

Preliminary phytochemical analysis (Khadabadi *et al.,* 2013)

The methanolic and aqueous extracts of selected plant leaves were undertaken for the preliminary phytochemical analysis to identify the presence of photochemicals in that extraction.

Identification of anthraquinone glycosides: Boil 1 gm leaf powder with dilute sulphuric acid filter hot and to the cooled filterate organic solvent 5ml of Benzene was added, shaked well. The benzene layer was separated and equal volume of dilute ammonia solution was added, shaked well. The ammonical layer acquired rose pink colour.

Quantitative estimation

Estimation of sennoside: Anthraquinone glycosides are aglycone moieties of large number of glycosides containing plants like Senna, which is used for popular purgative action. Sennosides is a dimeric anthraquinone glycoside; it is a substance of anthraquinone type, were the first to be recognized, both in the free state and as glycoside. The derivatives of anthraquinone present in purgative drugs may be dihydroxy phenol such as chrysopanol, trihydroxy phenols such as emodine, tetrahydroxy phenols such as carminic acid. Anthraquinone derivation are often orange red compound, they are soluble in hot water and dilute alcohol.

Estimation of total anthraquinone glycoside (Khadabadi *et al.,* 2013): Accurately weighed 10gm quantity of powdered plant material was taken and refluxed the powder with water for 2 hours. Filter and concentrate to yield solid residue. Take 1gm of this residue and dissolved in 30ml with water. Mix and reflux on water bath for 15minutes. Cool, make up the volume to 30ml with water. Centrifuge this solution at 4000 rpm for 10 minute. Take 20ml of supernatant liquid and acidify with sufficient quantity of 2M hydrochloric acid. Extracted the acidic solution with 3 different portions of 15ml of chloroform layer. Combine the aqueous layer and add 0.10gm of sodium bicarbonate. Mix well, Shake for 3 minute and centrifuge at 4000rpm for 10 minute.

Table 1. Ash values of leaf powder of selected *Cassia species*

Parameters	*CA* %w/w	*CF* %w/w	*CO* %w/w	*C AU* %w/w	*C AL* %w/w
Total Ash	4.76%w/w	5.62%w/w	4.68%w/w	3.69% w/w	5.77%w/w
Water Soluble ash	1.52%w/w	1.35% w/w	1.37%w/w	1.29% w/w	0.92%w/w
Acid Insoluble ash	2.17%w/w	2.75%ww	2.26%w/w	2.48% w/w	2.31%w/w
Sulphated Ash	2.32%w/w	3.12% w/w	2.91%w/w	3.57% w/w	3.42%w/w

Table 2. Extractive values

Solvents	*CA*%w/w	*CF*%w/w	*CO*%w/w	*C AU*%w/w	*C AL*%w/w
Benzene	1.05%w/w	3.07%w/w	3.42%w/w	4.81%w/w	3.66%w/w
Acetone	1.73%w/w	2.52%w/w	1.97%w/w	2.92%w/w	2.58%w/w
Chloroform	2.42%w/w	3.48%w/w	1.23%w/w	3.69%w/w	2.27%w/w
Methanol	4.01%w/w	3.86%w/w	2.26%w/w	4.72%w/w	1.72%w/w
Water	7.42%w/w	5.92%w/w	6.27%w/w	5.85%w/w	6.32%w/w

Table 3. Crude fibre content (LOD)

Parameters	*C A* %w/w	*C F* %w/w	*C O*%w/w	*C AU*%w/w	*C AL*%w/w
Fibre content	2.23%w/w	2.31%w/w	2.51%w/w	2.16%w/w	2.26%w/w
Moisture content	2.02%w/w	2.21%w/w	2.56%w/w	2.33%w/w	2.01%w/w

Table 4. Preliminary phytochemicals in the leaf extract of *Cassia species*

S. No	Phytochemical Test	Methanolic leaf extract					aqueous leaf extract				
		C a	*C f*	*C o*	*C au*	*C al*	*C a*	*C f*	*C o*	*C au*	*C al*
1.	Saponins	-	-	-	-	-	+	+	+	+	+
2.	Tannins	+	+	+	+	+	+	-	+	+	-
3.	Flavonoids	+	+	+	+	+	+	+	+	+	+
4.	Alkaloids	+	+	+	-	-	+	+	+	-	-
5.	Anthraquinone glycoside	+	+	+	+	+	+	+	+	+	+

Present +; Absent –

Take 10ml of \supernatant and add 20ml of 10.5%w/v ferric chloride solution. The mixture was refluxed in water bath for 20minutes. Add 1ml of concentrated hydrochloric acid. Heat for 20 minutes, with shaking to obtain a clear solution. Cool and Shake with 25ml diethyl ether in a separating funnel. Repeat the step until anthraquinones are exhaustively extracted and tested by the borntragers reaction. Separate the diethyl ether extract and combine, the combined diethyl ether extract with 15ml distilled water twice. Take the diethyl ether extract in 100ml volumetric flask and adjust to volume with diethyl ether. Take 15ml of this solution and evaporate to dryness. Dissolve the residue in 10ml of 0.5%w/v folin denis-reagent in 10ml of sodium bicarbonate solution. Measure the absorbance at 515nm. Similarly prepare the standard calibration curve by using standard anthraquinone and calculate the percentage of anthraquinone present in the sample. The percentage of hydroxyanthraquinone glycosides present in the extracts of leaf *Cassia species* was calculated by using following formula.

Quantity in % = [Absorbance X 0.85] / Sample taken in gm.

Isolation of sennoside A and B (Khadabadi *et al.*, 2013): Accurately weighed 1gm quantity of a coarse powder of leaves. Extract the powder with a mixture of ethanol: chloroform (93:7) for half an hour. Filter and extract the dried marc with methanol containing a small quantity of oxalic acid. Filter and combine both the filtrates. Keep this mixture aside for 12 hours at room temperature. Precipitates of Sennoside A. Sennoside A was recrystallized using triethylamine. Solution that remains after separation of Sennoside A was used for isolation of calcium chloride to the above filtrate. Precipitate of Sennoside B was formed which can be purified by using 40% methanolic ammonia. Results are tabulated in Table 6.

Colorimetric estimation of sennoside *B* (Khadabadi *et al.*, 2013): Weighed accurately about 0.3gm of the powdered drug, Add 30ml of water, mix and weigh again place in a water bath and heat under a reflux condenser for 15 minutes. Cool, weigh and adjust the weight with water. Centrifuge and transfer 20ml of the supernatant liquid to a 150 ml separator add 0.1ml of dilute hydrochloric acid and shake with 3 quantities, each of 15.0ml of chloroform. Discard the $CHCl_3$ extracts. Add 0.1gm of sodium bicarbonate and shake for 3 minutes. Centrifuge the aqueous layer and transfer 10ml to a round bottom flask with a ground glass stopper. Add 20ml of ferric chloride solution and mix. Heat in water bath under a reflux condenser for 20 minutes, with frequent shaking, until the precipitate is dissolved. Cool, transfer to a separator and shake with 3 quantities, each of 25ml of solvent ether. Combine the ether extracts and wash with 2quantities, each of 15ml of water transfer the ether extracts to a 100ml volumetric flask and dilute to volume with solvent ether. Evaporate 10.0ml carefully to dryness and dissolve the residue in 10ml of N KOH. Filter, if necessary, through a sintered glass filter. Measure the extinction immediately of a 1cm layer of the resulting solution at500nm. Calculate the content of hydroxyanthracene derivatives as Sennoside B, taking 200as the value of E (1%, 1cm) at 500nm.Results are tabulated in Table 7.

HPTLC for sennoside A AND B (Indian Pharmacoepia, 2010): The presence of Sennosides were identified by chemical test, so to confirm the presence of Sennosides in the selected plants the HPTLC study has been conducted. It was performed by using CAMAG HPTLC system. Densitometry Scanning was performed by CAMAG TLC Scanner at 254 and 366nm, all measurements are operated by CAMAG WINCATS software.

Chemicals Equipments and Instruments: All chemicals and reagents are analytical grade. Stationary Phase used is Pre coated silica Gel aluminium plates 60F 254(20X 10) with 250µm thickness (E.Merk). The linear ascending development was carried out in 20cm X 10cm twin trough chamber using mobile phase N-butanol, Ethyl acetate, Glacial acetic acid and Water in 4:4:3:1 ratio v/v/v/v.

Preparation of the sample: Accurately weighed 1 gm of the leaf powder was treated with 10 ml of methanol and refluxed half an hour. Then it was filtered and the solution was transferred to the volumetric flask to make up to the volume. This solution was used for application.

Sample Spotting and development: The samples were spotted in the form of band with a Camag 100microlitre sample syringe. Plates were developed by ascending development. Results are tabulated in Table 8

RESULTS

Physiochemical evaluation

ASH Value: All the selected plants were subjected for physiochemical evaluation like determination of ash values, extractive values, crude fibre content and moisture content which is given in the Table 1, 2 & 3.

Total Anthraquinone Glycosides

Table 5. Total anthraquinone glycoside content of *Cassia species* leaves

S.no	Plants	Quantity (%w/w)
1	*C a*	2.25%
2	*C f*	2.14%
3	*C o*	2.01%
4	*C au*	2.6%
5	*C al*	3.6%

Phytochemical studies

Preliminary Phytochemical Study: The selected plant leaves methanolic and aqueous extracts were taken for the preliminary phytochemical analysis. The result shows the presence of anthraquinone glycoside in all plants. The results were given below.

Calorimetric estimation of sennoside B

Table 6. Estimation of Sennoside B in selected *Cassia species*.

S.no	Plants	% purity
1	*C a*	1.02%
2	*C f*	1.6%
3	*C o*	1.10%
4	*C au*	2.0%
5	*C al*	1.9%

Quantitative estimation of sennoside *A & B*

Table 7. Quantity of sennoside A and B in selected *Cassia species* leaves

S.No	Plants	Sennoside A	Sennoside B
1	*C a*	1.1%w/v	1.0%w/v
2	*C f*	2.0%w/v	1.5%w/v
3	*C o*	1.9%w/v	1.5%w/v
4	*C au*	2.2%w/v	1.9%w/v
5	*C al*	2.0%w/v	1.5%w/v

HPTLC Study

The selected plants leaf extracts were subjected for HPTLC study to isolate the Sennosides and to find out their R_f values. The results are given in the Table 8 and Figure 1 - 9.

HPTLC for isolated sennosides

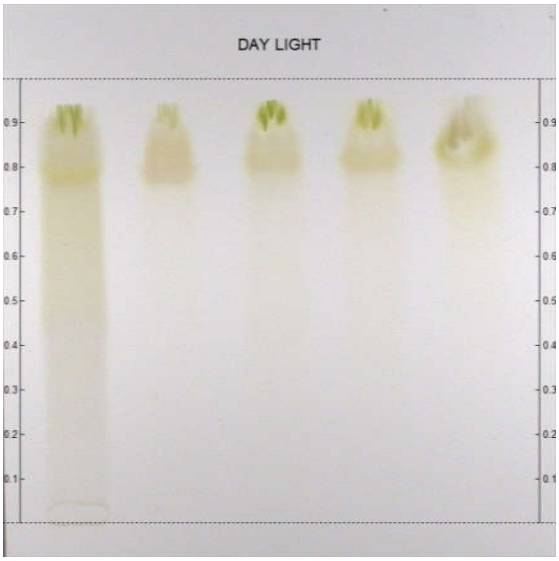

Figure 1. HPTLC spectrum of fluorescence at day light

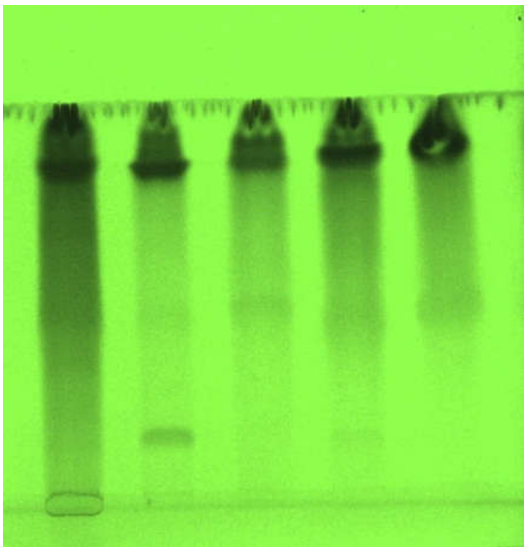

Figure 2. HPTLC spectrum at 254nm

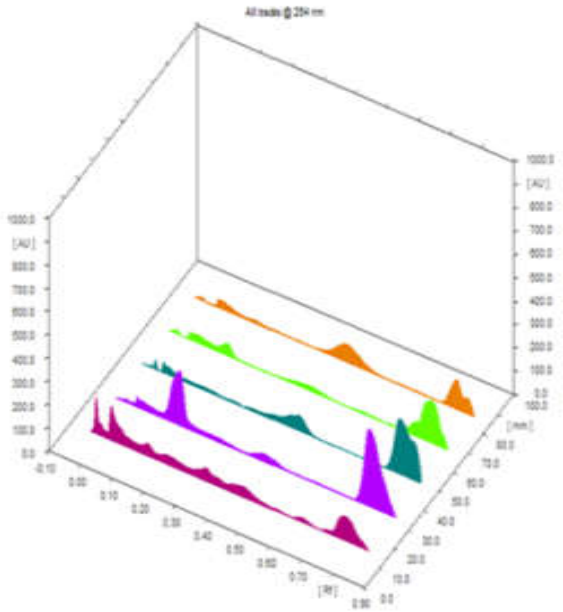

Figure 3. HPTLC spectrum 254 nm in 3D view

Table 8. Rf values of isolated compounds by HPTLC

z	*C a*		*C o*		*C f*		*C al*		*C au*	
1	R_f	Area	R_f	Area	R_f	Area	R_f	Area	R_f	Area
2	0.02	1724	0.01	144.9	0.01	69.2	0.00	220	0.00	2051
3	0.03	4326	0.03	554	0.00	244.2	0.03	363	0.13	1513.2
4	0.14	1582	0.05	884	0.03	1074.8	0.06	1161.6	0.18	199.9
5	0.21	2238	0.16	8392	0.16	582.7	0.15	1675.8	0.45	5018.0
6	0.33	3011	0.27	526	0.46	3583.9	0.41	1455.3	0.79	3798.5
7	0.45	2859	0.44	1485	0.78	10197.2	0.79	9360	0.83	1098.5
8	0.63	553	0.76	15385						
9	0.77	5459								

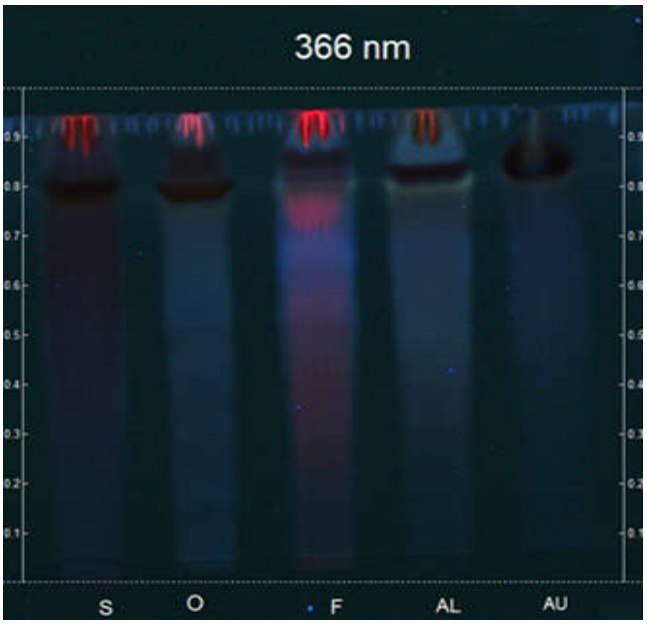

Figure 4. HPTLC spectrum at 366nm of the sample

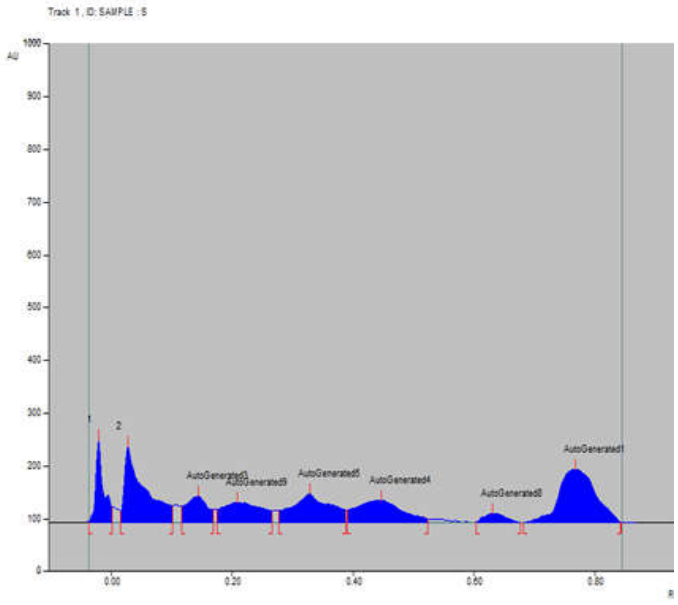

Figure 5. HPTLC spectrum of *C a*

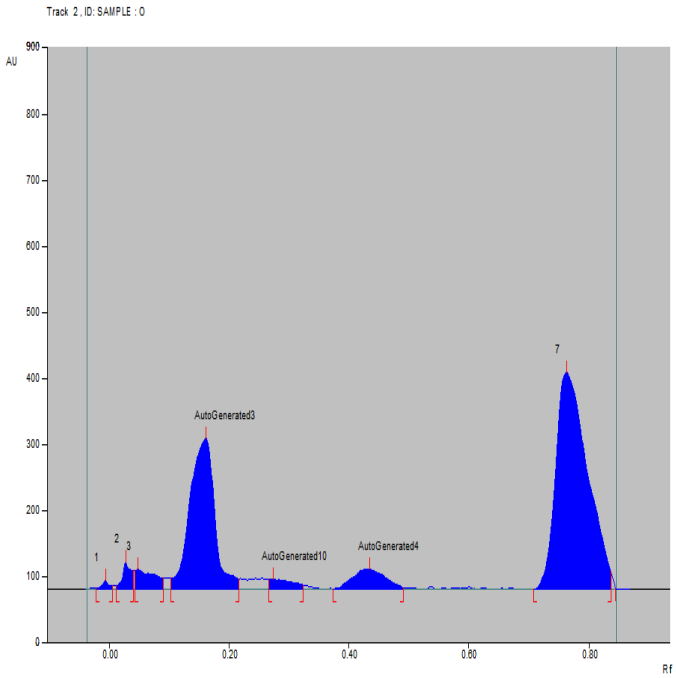

Figure 6: HPTLC spectrum of *C o*

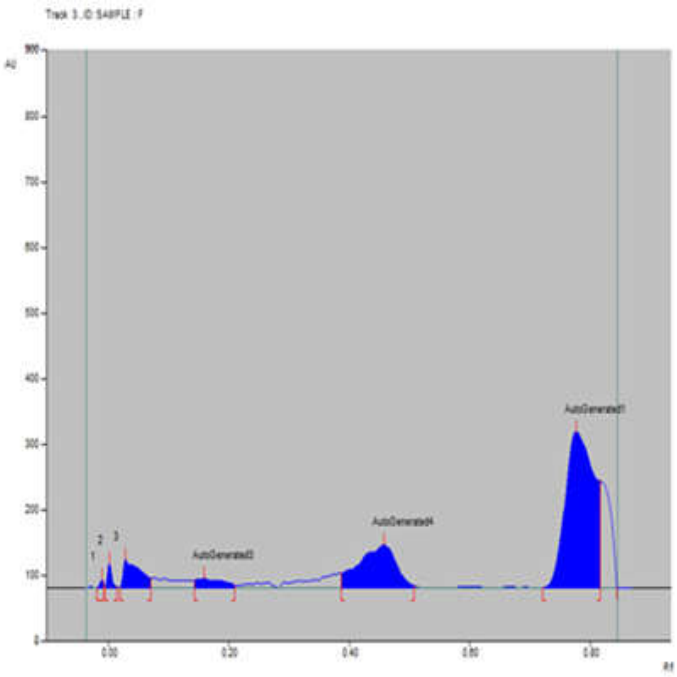

Figure 7. HPTLC spectrum of *C f*

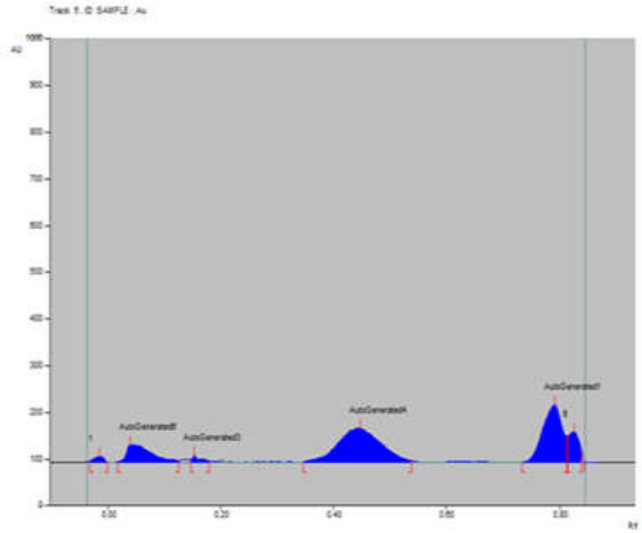

Figure 8. HPTLC spectrum of *C al*

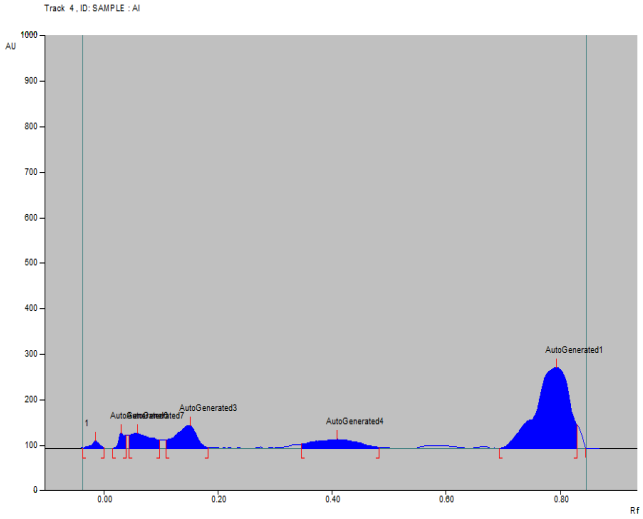

Figure 9. HPTLC spectrum of *C au*

DISCUSSION

Demands of traditional herbal medicines are increasing day by day not only by the developing countries but also by the developed countries throughout the world. The demand is due to the increased acceptance of ayurveda and traditional herbal medicines, because of having their safe therapeutic effect and no side effects, as such modern peoples relies more on drug resources of plant origin. Several chemical compounds such as Anthraquinone glycosides, Naphthopyrone glycosides, Phenolic compounds, Flavonoids etc. have been isolated from *Cassia species* plants (Vijay *et al.,* 2016; Saba *et al.,* 2012; Kheem, 2016; Monisha *et al.,* 2017; Joy *et al.,* 2012; Makinde *et al.,* 2007). These chemical compounds are responsible for pharmacological activities such as hepatoprotective, anti-inflammatory, antigenotoxic, hypolipidemic, spasmogenic and antinociceptive, antiproliferative, hypotensive, purgative, antidiabetic, estrogenic and antiestrogenic, antiulcer, antioxidant, antifungal, antishigellosis, anthelmintic, antimutagenic, antibacterial and antiplasmodial (Hemalata and Suraj, 1993; Nayak *et al.,* 2015; Adnan *et al.,* ; Senthilkumar *et al.,* 2011; Mahmood *et al.,* 2008; Bello *et al.,* 2010). There are 4 cassia plants were selected for this study. In this study the Pharmacognostical, Physiochemical and preliminary phytochemical study has been done. The phytochemical study confirms the presence of total anthraquinone glycoside and sennosides A and B were estimated from the selected plant leaf and documented and quantity was compared with that of the standard drug *Cassia angustifolia.* It shows the quantity of anthraquinone glycoside in the selected plants are also equivalent to that of the *Cassia angustifolia* and that the percentage content. Quantity of sennosides present in the selected plant leaves were estimated and the results are given in the table 6 and 7, which indicates the selected plants are also having Sennosides in equivalent quantity to that of the standard drug *Cassia angustifolia*. The HPTLC spectrum shows the same R_f value in 4 species (*Cassia fistula, Cassia occidentalis, Cassia auriculata, Cassia alata*) and in *Cassia angustifolia* R_f value 0.45, 0.44, 0.46, 0.45, and 0.46 and R_f value 0.77, 0.76, 0.78, 0.79 and 0.79 respectively , shown in the table 8 and figure 1-9. Here, it was clearly verified the presence of Sennosides.

Conclusion

The present study confirms the presence of anthraquinone glycosides in all the selected plants and also they are having Sennoside A and B in considerable quantity when comparing with the standard drug *Cassia angustifolia.* From this study we can suggested instead of *Cassia angustifolia* these selected 4 plants are also used for some medicinal purposes such as laxative, purgative, antimicrobial, cytotoxic, thrombolytic, antioxidant, antifungal, antianxiety etc.. Further study also can go for structural elucidation of the isolated compounds to confirm the compounds which are having the activity. We can develop a new herbal formulation instead of Senna leaf.

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