Acute toxicity and High Performance Thin Layer Chromotography study of *Cassia auriculata* flowers methanolic extract.

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ABSTRACT

Cassia auriculata plant belonging to Fabaceae grows on black cotton soil and dry stony ground throughout the savannah and thorn forests of the Deccan and Guzerat, also in the Southern Mahratta country and the Latteriate region along the sea coast. It has great importance to tanner and workers. Tea and compound syrup prepared from flowers is being prescribed for diabetes, nocturnal emissions and inflammation. The methanolic extract of *Cassia auriculata* flowers (MECA) was screened for acute toxicity at three different doses levels and it did not show any toxic or deleterious effects for any of the symptoms as per OECD guidelines indicating low toxicity of the extract at high doses. High Performance Thin Layer Chromatographic studies of the extract revealed the presence of rutin in minute amounts proving its antioxidant and anti-inflammatory potential. Thus, the studies enlighten the flowers seems to high content of flavanoids and bioflavonoids.

KEY WORDS: HPTLC, Kalpa Herbal Tea, Avarai panchaga choornam, Tanners *Cassia* Avaram.

1. INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[1]. The history of drugs is intimately related to plants from the earliest times and even today plant products have extensive use in ethno medicine, traditional system of medicine as well as in the armamentarium of the modern physician[2].

Plant drugs have been used since ancient times in treatment of various diseases in both man and animals. In an agricultural country like India, where medicinal plants are abundantly and easily available at relatively low cost, there is every virtue in exploiting such local and traditional medicine of vegetable origin for human and animal use [3,4]. The Herbal renaissance has produced a profound effect on Western medicine , which is trying to acknowledge methods of healing that have existed for millennia in the traditional system of medicine throughout the world especially , Asia. The surge in research on drugs from natural sources is now moving

from the herbalists' shop away from the core texts into drug research laboratories[5]. *Cassia auriculata* native of India has meaningful explanation such as Cassia from the name **Kasian** and auriculata means **small eared**[6]. An evergreen, xerophitic, branched and gregarious shrub with 1-3 meters height, reddish brown branches, flowers bright yellow in sub-terminal- axillary corymb and fruit pods, flat, thin, papery, pale brown, deeply depressed between the seeds grows wild in the Central Provinces, Western Coast, and South India and Ceylon[7,8]. *Cassia auriculata* contains several active constituents such as flavonoids, β-sitosterol-β-D-glucoside, polysaccharides, anthracene, dimeric procyanidins and myristyl alcohol[9].

The present studies of the flowers were undertaken to enlighten their role in any of the In-*vivo* and analytical evaluation indicating their use as beneficial in treatment of oxidative stress as antioxidant.

2. MATERIALS AND METHODS

2.1 *Experimental animals*

Albino Wistar rats of either sex (150-200g body weight) purchased from Bharat Serum and Vaccines, Thane were used in the experiments. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light/12 h dark cycle; 25 ± 3°C; humidity 35-60 %).They were fed with Amrut brand pelleted standard diet manufactured by Nav Maharashtra Chakan oils, Ltd., Maharashtra and drinking water *ad libitum*. The animals had free access to water all the time. They were allowed to adapt to our animal house conditions by keeping them for 8-10 days period prior to the experiments. The study was conducted following local animal ethical committee clearance (Animal house reg. no. 25/ 1999/ CPCSEA).

2.2. *Plant collection and identification*

Fresh flowers of *Cassia auriculata* were collected from Nyeveli, Cuddalore district, Tamil-Nadu, India in month of August-September. The plant material was identified and authenticated at Agarkar Research Institute, Pune. A voucher specimen (No.3/386/2006/Adm.7646) was deposited in the botany department of Agarkar Research Institute, Pune. Preliminary phytochemical analysis of MECA was carried out as described by C. Kokate[10]. The tests studied for the evaluation of the extract were carbohydrates, reducing sugars,saponin glycosides,flavonoids, alkaloids and tannins and phenolic compounds which were found to be positive.

2.3 *Preparation of the plant extract*

Fresh flowers of *Cassia auriculata* were dried in an oven below 60°C for 2 hrs. They were finely powdered and extracted with 70% aqueous methanol using Soxhlet apparatus at 55°C. The soluble part was concentrated over water bath maintained below 60°C and dried in a vacuum oven to obtain free flowing powder.

2.4 *Acute toxicity studies*

18 Albino wistar rats weighing 100-150 gm were divided into three groups of (3 females + 3 males each) as 1000mg/kg p.o, 2000mg/kg p. o, 5000mg/kg p.o. They were critically observed for clinical signs, gross behavioral changes and mortality if any following the administration of the test formulation at different time intervals like 30min, 1hr, 2hr, 4hr, 24hr and then 48 hr up to 72 hrs period as per OECD guidelines.

2.5 *High Performance Thin Layer Chromatography (HPTLC)*

HPTLC analysis was performed at the Anchrom laboratories, Mumbai. The analysis was carried out by application of the sample (dissolved in methanol) on Silica gel GF254 TLC plate using CAMAG LINOMAT V APPLICATION device.
The plate was impregnated with 0.5%w|v of Sodium dihydrogen phosphate -1 hydrate. Ascending chromatographic technique in twin chamber was then developed after the presaturation of the chamber for 15min. A suitable solvent system (ethyl acetate: n-butanol: formic acid: Water, 5:3:1:1 was used.) Scanning of the developed plates was carried out at 254 nm and 366nm.

3. RESULTS

3.1 *Yield of Plant extract*
The yield of dried powdered plant material was 18.2 %. Preliminary phytochemical screening revealed presence of saponins, carbohydrates, flavanoids, steroids and tannins. (Table - 1)

3.2 *Acute toxicity studies*
The MECA when orally administered in the dose levels of 1000mg/kg, 2000 mg/kg and 5000 mg/kg body weight did not produce any significant changes in the autonomic or behavioral responses, including no mortality during the observation period. Since no mortality was observed up to the dose of 5000mg/kg, the L.D_{50} of MECA could not be determined. The methods observed were stimulants effects(hyperactivity, irritability, tremor, convulsions, piloerection) depressant effects (sedation,anesthesia, ataxia, loss of righting reflex, analgesia, straub's tail, rotating rod test, loss of pinal reflex), autonomic effects (labored respiration , blenching, reddening ,abnormal secretion), motor activity and mortality (Table - 2).

3.3 *High Performance Thin Layer Chromatography (HPTLC)*

The MECA has shown three well resolved spots on the HPTLC plate. The images were obtained at 254nm UV visible wavelength but well resolved spots were obtained only on 366 nm that is after derivatization. Quantiation by HPTLC confirms that out of three fractions, one spot that is resolved was rutin identified by comparison with standard which was found to be in minute amounts i.e. 0.43 µg in total of 500 µg of the MECA (Table-3, figure -1).

4. DISCUSSION

Cassia auriculata plant has wide range of medicinal properties. There is evidence of its safety in human beings. In the present study we have tried to evaluate its probable role in inflammation and as antioxidant by evaluating its acute toxicity study and HPTLC analysis.
Since acute toxicity testing is necessary to evaluate the toxic effects after administration of a single large dose of the drug, MECA was evaluated and did not show any toxic or deleterious effects upto 5000mg/ kg oral dose indicating low toxicity of the extract at high doses. As the rats were administered upto maximal possible dose, the LD_{50} value of the MECA could not be determined.
We have performed HPTLC analysis of MECA and found rutin in minute amounts by comparing it with standard rutin which might have a role in exerting anti-inflammatory and antioxidant activity.

5. CONCLUSION

Cassia auriculata plant seems to have a high potential for anti-inflammatory and antioxidant activity, thereby proving its wide utility in treatment of liver disorders and inflammation.

Clinical use of *Cassia auriculata* flowers extract as well as different plant parts extracts with wide range of solvents especially in inflammatory disorders, oxidative stress induced pathogenesis and carcinogenesis needs to be evaluated in details and on larger frontier scale.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

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TABLE 1: Preliminary Phytochemicals present in the extract

Sr. No.	Test / Reagent	Positive (+ ve) or Negative (-ve)
1.	For Carbohydrates- Molish's test	+ ve
2.	Reducing sugars -Fehling's test -Benedict's test	+ve
3.	Tests for Saponin glycosides- Foam test	+ve
4.	Flavonoids - Shinoda test	+ve
5.	Alkaloids -Dragendorff's test - Hager's test - Wagner's test - Mayer's test	+ve
6.	Tests for Tannins and Phenolic compounds -5% $fecl_3$ solution - Bromine water -Dilute iodine solution	+ve

TABLE 2: Acute Toxicity Studies for Methanolic extract of *Cassia auriculata*

Gross observation	**1000mg/kg**	**2000mg/kg**	**5000mg/kg**
Stimulant effects			
Hyperactivity	-	-	-
Irritability	-	-	-
Tremor	-	-	-
Convulsions	-	-	-
Piloerection	-	-	-
Depressant effects			
Sedation	-	-	-
Anesthesia	-	-	-
Ataxia	-	-	-
Loss of righting reflex	-	-	-
Analgesia	-	-	-
Straub's tail	-	-	-
Rotating rod test	-	-	-
Loss of pineal reflex	-	-	-
Autonomic effects			
Labored respiration	-	-	-
Blenching	-	-	-
Reddening	-	-	-
Abnormal secretion	-	-	-
Motor activity in rats	-	-	-
Mortality	-	-	-

Absent, + Mild, ++ Moderate and +++ Marked

TABLE 3: HPTLC analysis of MECA extract

U1 and U2 – MECA extract at different concentrations.

Sr.no	Sample initials as shown in the figure	Sample identification	Concentration of the sample
1.	U1	*Cassia auriculata*	250µg
2.	S1	Rutin (Standard)	0.1µg
3.	U2	*Cassia auriculata*	500µg
4.	U2	*Cassia auriculata*	500µg

S1- Rutin standard at 0.1µg concentration procured from Hi-media laboratories.

CASSIA AURICULATA HPTLC IMAGES

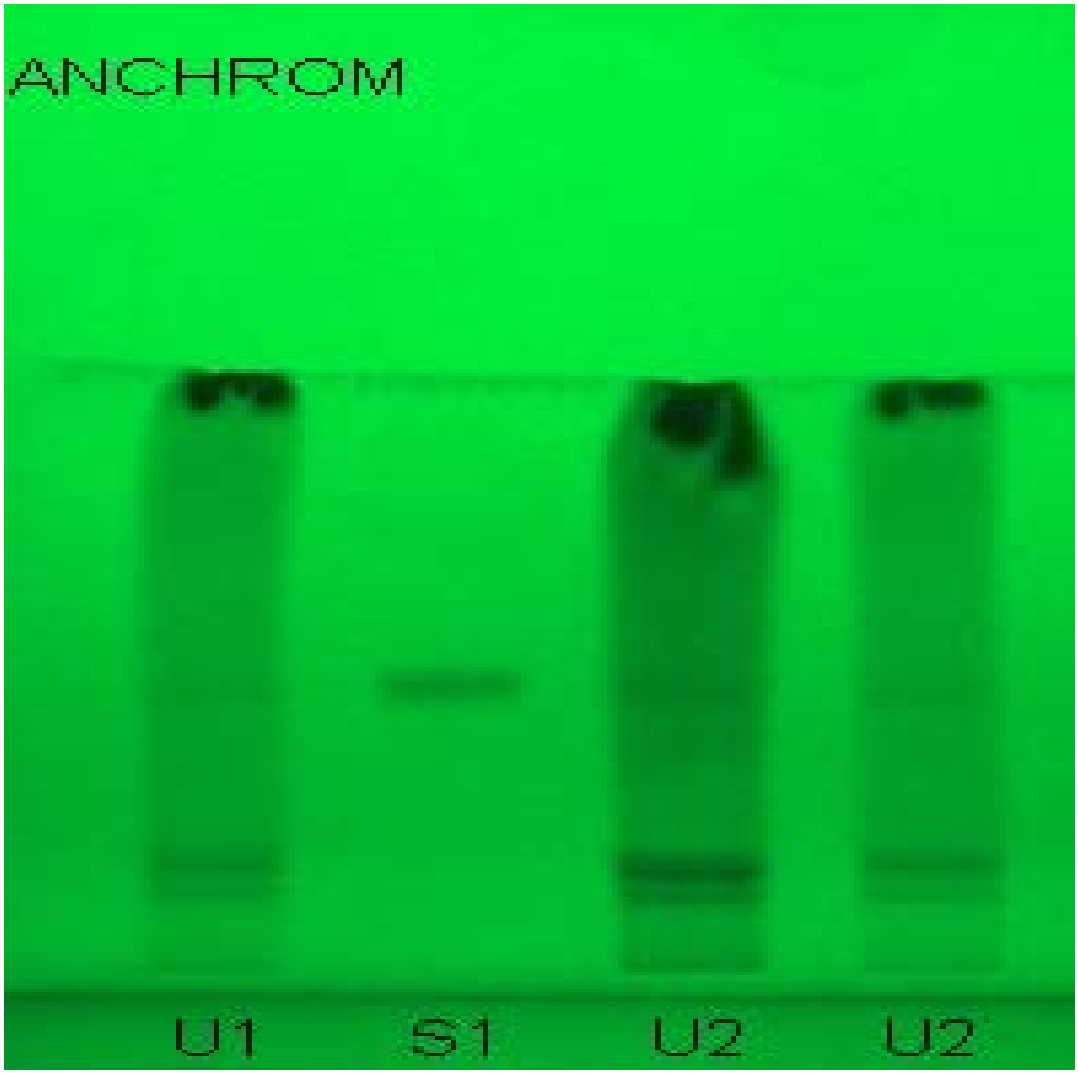

FIGURE 1A: Before deivatization (IMAGE@254nm)

FIGURE 1B: Before deivatization (IMAGE@VISIBLE)

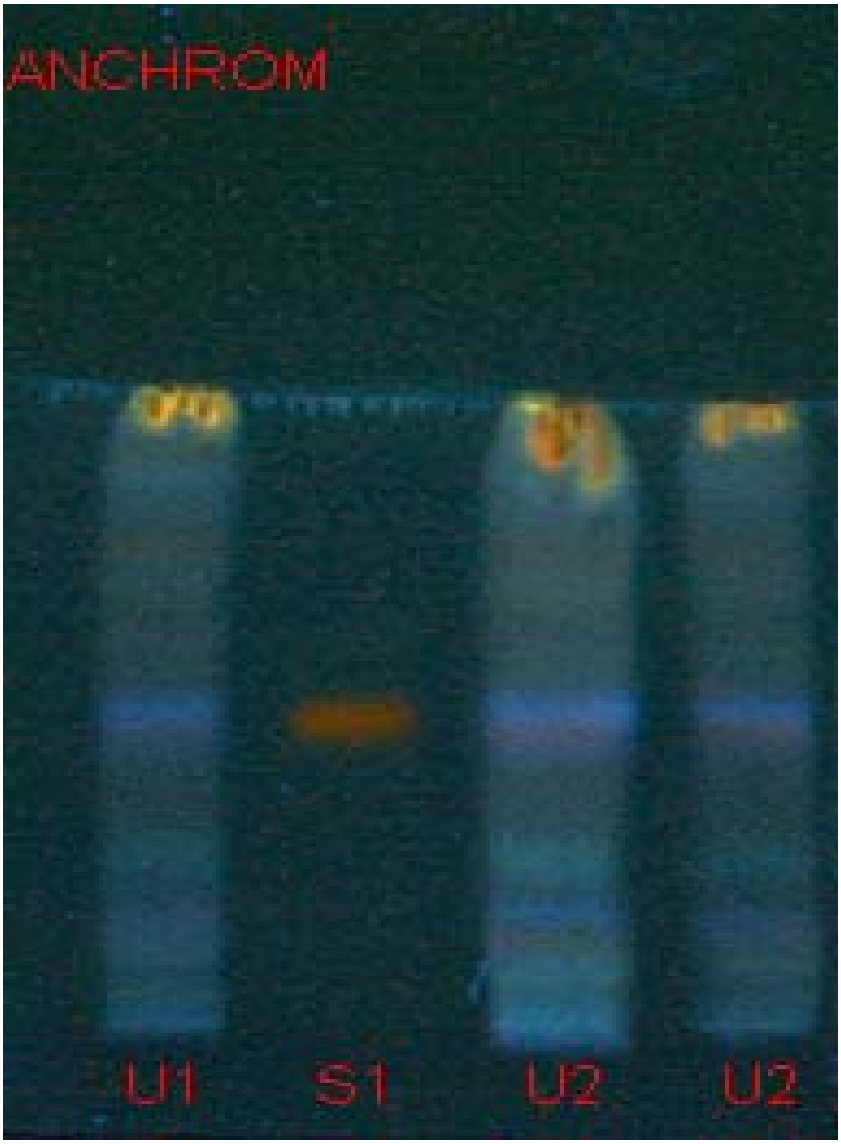

FIGURE 1 C: After deivatization (IMAGE@366nm)

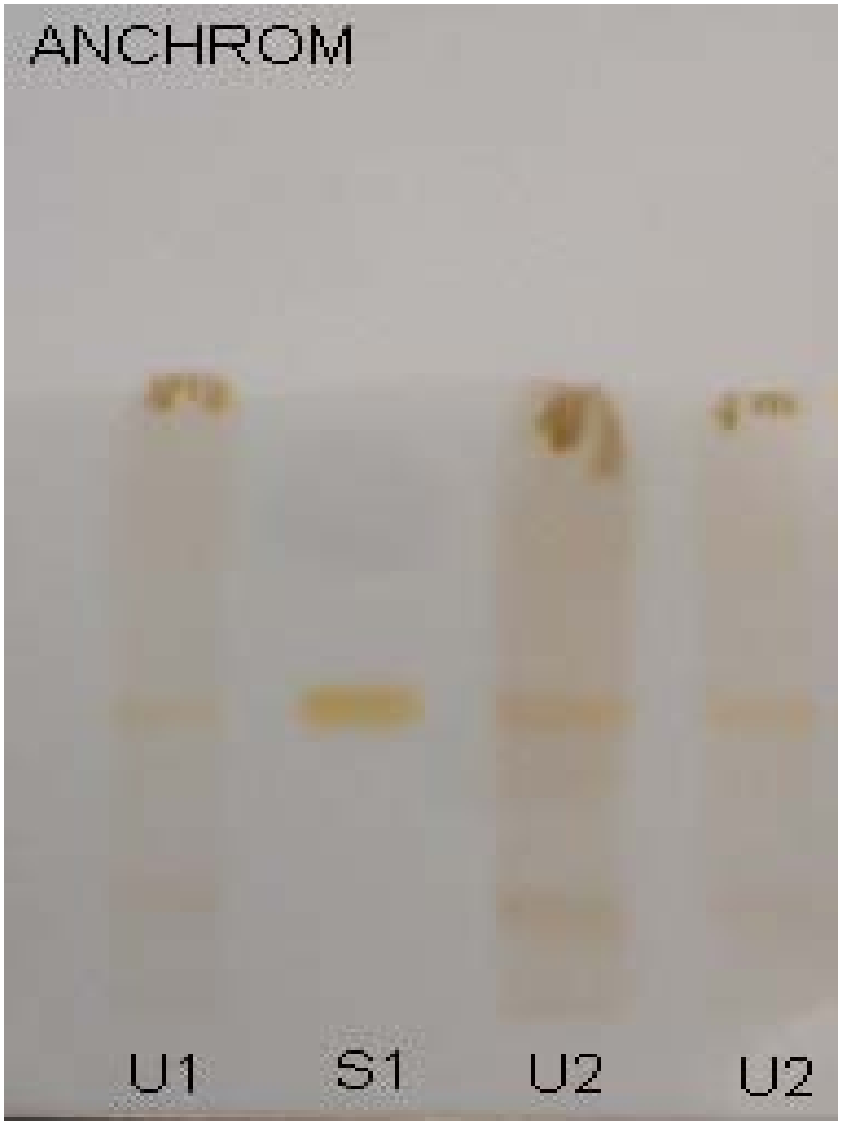

FIGURE 1D: After deivatization (IMAGE@VISIBLE)