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ACUTE TOXICITY AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY STUDY OF *CASSIA AURICULATA* FLOWERS

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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.

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 systems¹. 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 physician².

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 laboratories⁵.

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 alcohol⁹.

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.

Materials and Methods

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; $25 \pm 3^\circ\text{C}$; 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)

Plant collection and identification

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.

Preparation of the plant extract

Flowers of *Cassia auriculata* were dried in an oven below 60°C for 2 hrs. They were finely powdered and extracted with 80% 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.

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.

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.

Results

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)

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.D₅₀ of MECA could not be determined. (Table - 2)

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. Quantitation 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).

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 LD₅₀ 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.

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 especially in inflammatory disorders, oxidative stress induced pathogenesis and carcinogenesis needs to be evaluated in details and on larger scale.

Acknowledgements

We wish to thank Agharkar Research Institute, Pune for the authentication of the sample, Dr. S.R.Naik of Bharat Serum for supplying us animals for the study and Anchrom laboratories, Mumbai for supporting to carry out HPTLC studies.

Analysis of the extract**Preliminary Phytochemicals present in the extract:****TABLE 1:**

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 MECA extract

Gross observation	1000mg/k g	2000mg/k g	5000mg/k g
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

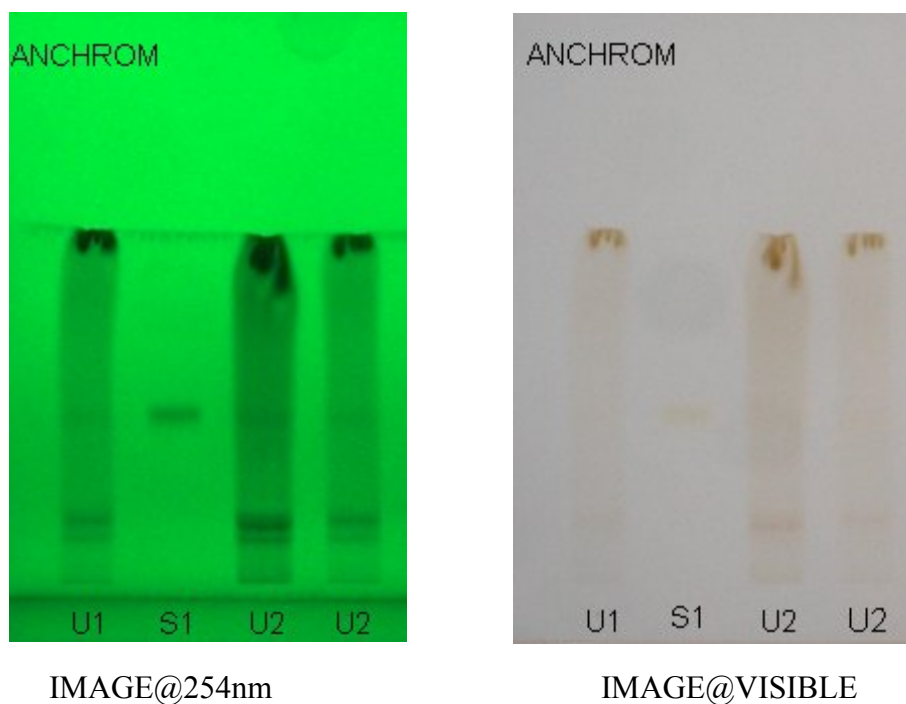
U1 and U2 – MECA extract at different concentrations.

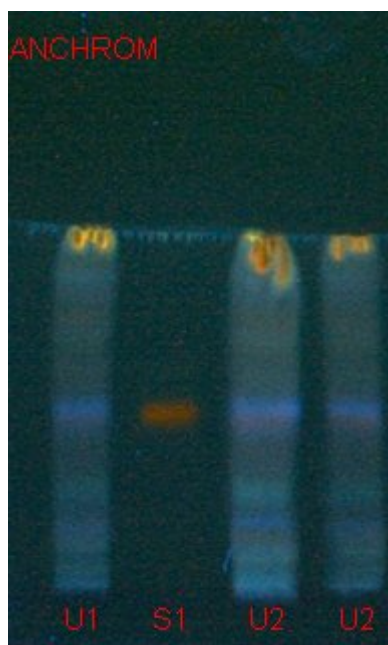
Sr.no	Sample initials as shown in the figure	Sample identification	Concentration of the sample
1.	U1	<i>Cassia auriculata</i>	250 μ g
2.	S1	Rutin (Standard)	0.1 μ g
3.	U2	<i>Cassia auriculata</i>	500 μ g
4.	U2	<i>Cassia auriculata</i>	500 μ g

S1- Rutin standard at 0.1 μ g concentration

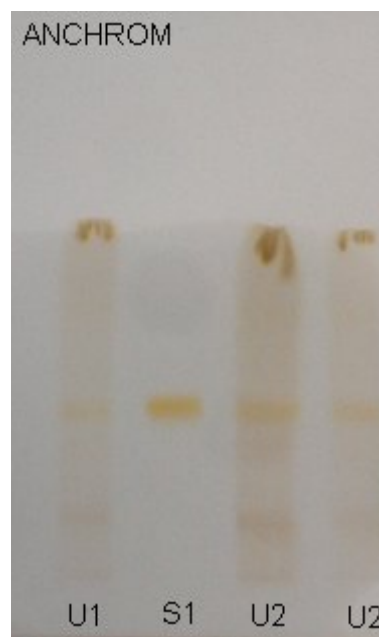
CASSIA AURICULATA HPTLC IMAGES

FIGURE 1: Before and after deivatization:





IMAGE@366nm



IMAGE@VISIBLE

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