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Assessment of Genotoxicity of *Cassia auriculata* Flower Powder

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Abstract

The flower of *Cassia auriculata* commonly used for treatment, especially in Ayurvedic medicine in India was tested for mutagenicity by the Ames test using *Salmonella typhimurium* TA 98, TA 100 and TA 102 strains. The genotoxicity assessment of flower powder of *Cassia auriculata* was performed in aqueous extract 1:10. The flower powder of *Cassia auriculata* did not induce any frame shift mutation in the strain TA 98, which indicates its antimutagenic effect and did not cause any base pair substitution at the dose level tested 100µg/plate. The colony counts in all the three strains were found close to the spontaneous revertants frequency showing that flower powder of *Cassia auriculata* is antimutagenic at the dose level tested (100µg/plate).

Keywords: Aqueous Extract, *Cassia auriculata*, Mutagenicity, *Salmonella typhimurium*

1. Introduction

Cassia auriculata is a highly reputed ayurvedic medicinal plant and is used in the treatment of diabetes mellitus, skin diseases, leprosy, tumors, asthma and urethrorrhea since many years without any evidence of toxic side effects *Cassia auriculata*, a plant native to India and popularly known as 'avvarai' is distributed throughout central and south India. According to Bhavapriya *et al*," The flowers of *Cassia auriculata* is being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine"[1]. However, no scientific data with regard to the genotoxicity of this herb is available. *Cassia auriculata* has thus been chosen for the genotoxicity study.

As per the reports of Kelber *et al* , 'An assessment of genotoxicity is a precondition for marketing authorization respectively registration of Herbal Medicinal Products (HMPs), as well as for inclusion into the 'Community list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products'[2].

This paper provides data for using C*assia auriculata* as herbal medicine in the practical application of bracketing and matrixing concept, with the intention of facilitating its inclusion in the "Community list". The aqueous extract of Cassia *auriculata* was tested by Ames test.

According to EMEA, "Many herbal substances and preparations, used in well-established or traditional Herbal Medicinal Products (HMPs), a safety profile in accordance to modern standards is supported or at least partially substituted by their documented history of medicinal use. Thus, only if a safety concern is recognized or suspected, supporting non-clinical investigations may be needed".[3]

2. Materials and Methods

2.1 Sample Preparation

Fresh flowers of *Cassia auriculata* were collected and dried in shade. The dry flowers were finely powdered in a mill, sieved with a fine mesh and stored in an airtight container.

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2.2 Ames Test to Assess the Mutagenic/ Non-Mutagenic Effect of the Plant Material

The mutant bacterial strains of *Salmonella typhimurium* TA 98, TA 100 and TA 102, were obtained from Prof. B.N. Ames, University of California, Berkeley, U.S.A.

2.3 Preparation of Organic Extract

In order to check whether the components of the powder of *Cassia auriculata* flowers show mutagenic/ non-mutagenic effect, the aqueous extract was prepared using 100 g powdered *Cassia auriculata* .The extract was concentrated under reduced pressure and it was finally evaporated to dryness.

The extract was dissolved in sterile dimethyl sulfoxide (DMSO) to give the required concentration and used for mutagenicity assay. An arbitrary concentration of 100 µg in 10 µl DMSO / plate of the extract was used.

The co-mutagenecity/antimutagenicity of the sample extracts was tested as follows.

The standard mutagens namely sodium azide for the bacterial strain TA 98 and daunomycin for the strain TA 100 and TA 102 were plated along with the sample extract and the test was carried out.

The concentrations of standard mutagens used were as follows

Sodium azide – 1 µg / plate

Daunomycin – 6 µg / plate

2.4 Ames Bacterial Mutagenicity Assay (Maron and Ames, 1983)

A set of histidine requiring strains is used for mutagenicity testing. These strains are incapable of growth in the absence of histidine in the growth medium. When a mutagen is added to the culture, the strain is mutated back, thereby losing the histidine dependence for its growth. The number of revertant colonies resulting after the action of the mutagen depends on the potency of the compound.

The type of mutation in the histidine operon in the strains is different, thereby enabling the identification of frameshift mutagens (those which are mutagenic towards TA 98), base-pair substituting mutagens (those which are mutagenic towards TA 100) and oxidative mutagens (those which are mutagenic towards TA 102).

2.5 Reagents

2.5.1 Solution A

43.75 ml-distilled water (boiling)

8.3g citric acid monohydrate (MW 210.14)

54.6g $K_2HPO_4.3H_2O$ (MW 228.23)

14.6g $NaNH_4HPO_4.4H_2O$ (MW 209.07)

The substances were best soluble when added in the given order. Made sure that one was completely dissolved before adding the next. Checked if the volume was 50 ml and if not, added water to make up the volume. Transferred to a 100 ml conical flask, plugged and autoclaved. The solution was highly stable and could be stored for any length of time at room temperature. The risk of contamination by opening of the bottles was minimal, since bacteria cannot grow well in concentrated salt solutions.

2.5.2 Solution B

Dissolved 1 g of $MgSO_4.7H_2O$ (MW 246.48) in 50 ml water. Transferred to 100 ml conical flask, plugged and autoclaved. The solution can be kept indefinitely at room temperature. Opened bottles were discarded, since there was a risk of contamination.

2.5.3 Solution C

In a 500 ml conical flask, autoclaved 7.5 g of bactoagar and 375 ml of water containing a stirring rod.

2.5.4 Solution D

Autoclaved 10 g glucose and 125 ml water. Allowed the solution to cool to room temperature.

2.5.5 Basal Agar

Combined solutions C and D and added 10 ml of solution A and 5 ml of solution B. Mixed well and poured into sterile petriplates at ~25 ml per plate.

2.5.6 Nutrient Broth

Thirteen gram nutrient broth in 1 litre of water. Divided into 20–40 ml portions, autoclaved immediately and stored. The broth ould be stored for any length of time, without opening.

2.5.7 HB (Histidine – Biotin) Solution

This solution contained 0.5 mM biotin and histidine.

0.7758 mg L-histidine

1.2215 mg D-biotin

Dissolved in 20ml of sterile water in the laminar hood. Filtered and sterilized.

2.5.7.1 Top Agar

0.54g agar

0.45g NaCl
90ml water
Autoclaved the solution.

2.5.7.2 HB – Top Agar

Allowed the top agar to cool to 45°C and HB solution was added to it (10 ml HB solution to 90ml top agar) and distributed into 2 ml portions in sterile tubes when the solution cooled down to 45°C and held in a water bath at 45°C for the assay.

3. Procedure

Overnight grown cultures of TA 98, TA 100 and TA 102 of *Salmonella typhimurium* in nutrient broth were used for the mutagenicity test. Mutagenicity of the extract was tested using the method detailed by Maron and Ames (1983). 100 mg of the extract dissolved in 0.01 ml of DMSO was added along with 0.1 ml of the overnight grown culture of the bacterial strain to 2 ml molten top agar held at 45°C. The contents were mixed gently and thoroughly, poured over the basal agar and spread evenly. After the top agar had solidified, the plates were incubated inverted at 37°C for 48 hours. At the end of 48 hours, the number of histidine revertant colonies was counted.

After testing the mutagenicity of the extract alone, their (co- or antimutagenic) effect on a standard oxidizing mutagen was carried out in the same way. For this purpose, 0.1 ml of the overnight grown culture was plated with 0.01 ml of DMSO containing 100 mg of the extract and 1mg of sodium azide for TA 98 and 6mg of daunomycin for the strains TA 100 and TA 102.

- Each Assay included 5 Sets of Plates as follows:

1. The overnight grown cultures were serially diluted and known amounts of these cultures were plated on nutrient agar plate and were incubated at 37°C overnight and counted for number of colonies.
2. The spontaneous revertants in the inoculum were determined as above; with exception that 0.1 ml of the culture alone was added to the top agar before pouring onto the plate.
3. The number of revertants induced by the extract was determined by plating 0.1 ml of the culture and 0.01 ml of appropriate concentration (100 mg) of the test compound on minimal glucose agar plates supplemented with trace amounts of histidine.
4. The number of revertants induced by the standard mutagen was determined by plating 0.1 ml of the culture and 10 ml of standard mutagen containing 250 mg of phenyl hydrazine.
5. The effect of the extract on the mutagenicity of the standard mutagen was assayed by plating the microbes with the standard mutagen in the presence of the extracts in the same doses as above.

All the above groups were set up in four replicas of four plates per group. The plates were incubated inverted at 37°C in the dark for 48 hrs (24 hours for group one). At the end of the incubation period, the number of colonies in each plate was counted and recorded.

After calculating the number of viable bacteria in the inoculum (from group I plates), the number of induced revertants was finally converted per 2×10^8 bacterial cells and was referred to as revertants per plate. The results are expressed as mean ± standard deviation of 4 plates.

4. Results and Discussion

The aqueous extract of flower powder of *Cassia auriculata* were tested for direct-acting mutagenic effect of the sample in TA 98 (frame shift mutagens), TA 100 (base pair substituting mutagens) and TA 102 (oxidative mutagens). Further the influence of the sample on direct-acting mutagens added to TA 98, TA 100 and TA 102 was studied.

For this, the aqueous extract of flower powder of *Cassia auriculata* was added along with a standard mutagen characteristic of the strains to the top agar before pouring on to the basal agar plates.

The findings of the evaluation of the antimutagenicity of flower powder of *Cassia auriculata* in Ames *Salmonella* microsome assay are given in Tables- 1, 2 and 3

Table 1. Effect of flower powder of *Cassia auriculata* on the reversibility of standard tester strains and mutagenicity of standard mutagens of standard tester strain TA 98

Sample	Number of His^+ revertants in TA 98	
	Extract	Extract + SM
SR	40 ± 3.5	
SM	242 ± 15.2	
Flower powder	47 ± 2.4	40 ± 3.9

Values are mean of three plates, Normal range SR: 30-50, SR-Spontaneous Revertants, SM-Standard mutagen (sodium azide 1µg)

It is quite clear that the flower powder of *Cassia auriculata* did not induce any frame shift mutation in the strain TA 98, which indicates its antimutagenic effect.

Table 2. Effect of flower powder of *Cassia auriculata* on the reversibility of standard tester strains and mutagenicity of standard mutagens of standard tester strain TA 100

Sample	Number of His^+ revertants in TA 100	
	Extract	Extract + SM
SR	160 ± 17.8	
SM	364 ± 25.4	
Flower powder	137 ± 14.9	164 ± 17.5

Values are mean of three plates, Normal range SR: 120-200
SR- Spontaneous Revertants, SM- Standard mutagen (Daunomycin 6µg)

The values in the above table indicate that the flower powders of *Cassia auriculata* did not cause any base pair substitution at the dose level tested 100µg/plate.

Table 3. Effect of flower powder of *Cassia auriculata* on the reversibility of standard tester strains and mutagenicity of standard mutagens of standard tester strain TA 102

Sample	Number of His^+ revertants in TA 102	
	Extract	Extract + SM
SR	280 ± 28.4	
SM	532 ± 35.8	
Flower powder	244 ± 19.2	286 ± 20.6

Values are mean of three plates, Normal range SR:240-320, SR-Spontaneous Revertants, SM- Standard mutagen (Daunomycin 6 µg)

The colony counts in all the three strains were found close to the spontaneous revertants frequency showing that flower powder of *Cassia auriculata* is antimutagenic at the dose level tested (100µg/plate).

5. Conclusion

Ames *Salmonella* microsome assay indicated that both flower powder of *Cassia auriculata* is antimutagenic at the dose level tested (100 µg / plate).

6. Reference

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