



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

Formulation And Evaluation of *Cassia Auriculata* Herbal Ointment For Anti-Microbial and Anti-Inflammatory Activity

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ARTICLE INFO

Published: 15 Feb. 2025

Keywords:

Cassia auriculata, ointment, anti-microbial, anti-inflammatory.

DOI:

10.5281/zenodo.14875435

ABSTRACT

The popularity of herbal medicine has surged in recent years, prompting their investigations into their therapeutic potentials. This study focused on the formulation and evaluation of herbal ointment containing *Cassia auriculata* for its anti-microbial and anti-inflammatory properties. A hydroalcoholic extract of *Cassia auriculata* was obtained through Soxhlet extraction method, and herbal extract was incorporated into the ointment base by levigation method. The resultant ointment was evaluated for various physicochemical properties including colour, odour, loss on drying, homogeneity, viscosity, spreadability and skin irritancy. The anti-microbial activity of herbal extract was tested against bacterial and fungal strains using agar disc diffusion method. The test organisms included two Gram- positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) two Gram-negative bacteria (*Escherichia coli*), and three fungal strains (*Aspergillus niger*, *Aspergillus clavatus* and *Candida albicans*). The results demonstrated notable antibacterial and antifungal activity, with inhibition zones comparable to those of standard antibiotics such as Ampicillin, Norfloxacin and Chloramphenicol as well as antifungal agents like Nystatin and Griseofulvin. Additionally, a rat paw edema model caused by carrageenan was used to evaluate the ointment's anti-inflammatory properties. Four groups of six rats each were treated with *Cassia auriculata* ointment at different concentrations with Diclofenac sodium serving as the standard. The results showed significant reduction in paw edema, indicating good anti-inflammatory potential. The present study confirmed that *Cassia auriculata* based herbal ointment exhibited promising antimicrobial and anti-inflammatory activity.

INTRODUCTION

Due to their therapeutic qualities, medicinal herbs have long been utilized in many cultures and are

crucial for overall health and well-being. These plants have been essential to traditional medical systems like Ayurveda, Siddha, and Unani. They

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



range from common kitchen herbs to rare and exotic kinds. Plants have been used for medical purposes for thousands of years; evidence suggests that herbal treatments have been used for over 4,000 years, as demonstrated in ancient Egypt, India, and Rome. *Cassia auriculata*, commonly known as Tanner's Cassia or Yellow Senna, is a plant species within the Fabaceae family that has been used in traditional medicine for a variety of ailments. Below is a summarized view of its classification, botanical description, medicinal uses, and phytoconstituents.

Ointment:

Ointments are semisolid preparation. They may or may not be containing medication used for the external application. Medicated ointments are intended to be applied externally in the body or to the mucous membrane. Non-medicated ointments commonly used as a base for the preparation of medicated ointments or used as such for lubricating or emollient effects. Plant drugs can also be formulated in the form of ointment. The effective ratio of active ingredients incorporating with ointment base by trituration and after completion of the formulation, the ointment quality is assessing in terms of diffusion, irritancy, stability, and Spreadability.

Antimicrobial Activity:

Antimicrobial agents are substances that help to combat or inhibit the growth of microorganisms. These microorganisms can include bacteria, viruses, fungi, protozoa, and certain parasites. The primary goal of antimicrobial agents is to either kill or prevent the growth of harmful microbes, thus preventing infections or treating existing ones. Antimicrobials can be classified into different categories based on their origin and chemical structure:

Anti-inflammatory Activity:

Anti-inflammatory substances are those that reduce or inhibit inflammation, which is the body's natural response to injury, infection, or harmful

stimuli. Inflammation can cause swelling, pain, redness, and heat in the affected area. Inflammation, while protective in some contexts, can become problematic if it is chronic or excessive. Anti-inflammatory agents are essential in reducing this inflammation, helping to alleviate pain, swelling, and potential tissue damage.

MATERIALS AND METHODS

Collection of plant material:

Fresh plant leaves of *Cassia auriculata*.L., was collected from local areas of Udumalapett shown in Table -1. The plant materials were thoroughly washed with deionized water to remove any impurities, then air-dried at room temperature under shade conditions to prevent direct sunlight exposure. Once dried the leaves were grounded into a coarse powder using an electric grinder.

Test Organisms:

Cultures of the microorganism, used in these studies were obtained from Department of Microbiology lab, RVS college of pharmaceutical sciences are: *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. Stock cultures of Bacteria and fungi were maintained on Nutrient agar and Potato Dextrose Agar slants, respectively. All cultures were sub cultured weekly and subsequently stored at 40C.

Preparation of herbal extract:

The dried leaves was finely ground into a coarse powder and then sieved through a 40 mesh sieve to obtain a uniform particle size. 30 g of the powdered herbal material was placed into the Soxhlet apparatus. The extraction solvent was a mixture of (ethanol and water 70:30). The extraction process was carried out by using Soxhlet method, which involves the repeated cycling of the solvent through the herbal powder to extract the desired compounds. After the extraction process was completed, the solvent was removed, leaving behind the herbal extract.

Formulation of ointment:



The ointment base is made using firstly weighed and finely crushed hard paraffin. After that, it is put over a water bath at 65°C in an evaporating dish. Once the hard paraffin had melted, the additional ingredients were added and vigorously stirred to help with melting and uniform mixing. The ointment base was then allowed to cool. To prepare the herbal ointment, precisely weighed *Cassia auriculata*.L extracts of leaves were blended with the ointment base using a levigation technique, an exact weight extract of leaves was combined with the ointment base to create the herbal ointment. As a result, a smooth paste that weighed two or three times the base was produced. More base was gradually added to the ointment once it was homogeneous before it was put in the appropriate container

RESULTS

Evaluation of ointments:

Physical evaluation: Preliminary evaluation of formulations at different concentrations was carried out as follows: Organoleptic parameters: Organoleptic parameters like colour, odour of the formulations were carried out by visual examination. Loss on drying: This is employed in IP and USP. Although the loss in drying, in the sample so tested, principally is due to water and small amount of other volatile material will be contribute the weight loss 1gm of ointment is placed digital moisture balance instrument set the temperature 105°C and run the instrument up to constant weight. Finally read out the percentage loss on drying automatically.

pH: The pH of various formulations was determined by using Digital pH meter (Digital pH meter 335, Systronics, Noroda, Ahmedabad). The 0.5 g of the weighed formulation was dispersed in 50 mL of distilled water and the pH was (Panigrahi et al., 1997) noted.

Homogeneity: All the developed ointments were tested for homogeneity by visual inspection. They

were tested for their appearance with no lumps (Panigrahi et al., 1997).

Viscosity: The measurement of viscosity of prepared ointments was carried out with Brookfield Viscometer (model LV-DV-II, Helipath spindle type S-96).

Spreadability: Spreadability of the formulation was determined by an apparatus suggested by Mutimer et al. (1956) which was suitably modified in the laboratory and used for the study. The experiment was performed as described by Wood et al. (1963). Spreadability was determined by using the formula $S = M \times L / T$. Where S = spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides completely from each other. In this present experiment, M = 80 g, L = 10 cm and T was recorded (Ehrlich and Hunt, 1968).

Acute skin irritation study: This test was performed on albino rats weighing between 150-200g. The animals were given standard animal feed and had free access to water ad libitum. The total mass was separated into four groups, each batch containing five animals. Dorsal hair at the back of the rats were removed one day prior to the commencement of the study and kept individually in cages to avoid contact with the other rats. Two groups of each were used for control and standard irritant. Other two groups were used as test. The 50mg of the each formulation were applied over one square centimetre area of whole and abraded skin to different animals. Aqueous solution of 0.8 % formalin was used as standard irritant. The animals were observed for seven days for any signs of oedema and erythema (Marzulli and Maibach, 1997).

Antifungal Activity Against A. Niger

Experimental Animals:

Healthy adult albino rats of both sexes between two to three months of age and weighing 180-240 g were screened for the study. Animals were allowed to be acquainted for a period of 15 days in



our laboratory environment prior to the experiment. Rats were housed in standard polypropylene cages (three animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark cycle; at an ambient temperature of $25 \pm 5^{\circ}\text{C}$; 35-60% of relative humidity); the animals were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and water ad libitum. The principles of Laboratory Animals' care 11.

Experimental Design:

In the experiment rats were divided into the following groups with six animals in each • Group I: Normal control received 1% w/v gum acacia 1ml/kg • Group II: Diabetic control received with Alloxan at a dose of 150 mg/kg b.w. • Group III: Diabetic rats treated with petroleum ether extract at 200mg/kg b.w • Group IV: Diabetic rats treated with chloroform extract at 300mg/kg b.w • Group V: Diabetic rats treated with ethyl acetate extract at 200mg/kg b.w • Group VI: Diabetic rats treated with ethanol extract at 200mg/kg • Group VII: Diabetic rats treated with aqueous extract at 100mg/kg • Group VIII: : Diabetic rats treated with Tolbutamide 250mg/kg b.w

Estimation of Glucose:

13 blood samples were obtained through tail vein by puncturing with hypodermic needle. A drop of blood so obtained was placed on the enzyme treated surface of the haemoglucostrip, which was kept in the glucometer. The glucometer was kept on, then after 2 minutes glucometer reading was recorded. Fasting blood glucose levels were estimated on Day 0, 1, 2, 4 and 7 with the help of single-touch glucometer (Ascensia Entrust, Bayer Health Care, USA).

Body weight measurement:

Body weight was measured totally four times during the course of study period 14 [i.e., before Alloxan induction (initial values), and on the first, fourth, and seventh days of the treatment period], using a digital weighing scale obtained from

KERN (EMB), Tischwaage, Germany. Measurement of serum Lipid, liver glycogen content and pancreatic Thiobarbituric acid reactive substances: Serum lipid profiles, liver glycogen profile 15 and pancreatic Thiobarbituric acid reactive substances 16 were measured after the animals were sacrificed after 12 days by decapitation shown in Table-4 and in fig-5.

Statistical analysis:

Results of the study were subjected to one way analysis of variance (ANOVA) followed by Dunnett's t test for multiple comparisons. Values with $p < 0.05$ acetate extract treated diabetic rats showed an significant increase in liver glycogen content ($p < 0.05$) shown in table-7.

Antimicrobial activity:

It was performed by agar diffusion method using a paper disc method and cup and plate method. Nutrient agar (purchased from Hi- Media) was used for bacterial and fungal strains respectively. The sterilized (autoclaved at 121°C for 20 min) medium (40- 500C) was inoculated (1ml/100ml of medium) with the suspension of microorganism. The plates were pre incubated for 1hr at room temperature and then incubated at 37°C for 24 and 300°C at 48hrs for antibacterial and antifungal activities respectively. Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Griseofulvin, Nystatin (5,25,50,100,250 μg /disc, respectively) was used as standard for antibacterial and antifungal activity respectively shown inTable-2,5&6 and figures are shown in fig-1,2,3&4.

Minimum inhibitory concentration:

The MIC was determined by the micro dilution method using liquid nutrient media with different aliquots of the test materials. 10 ml of sterilized double strength nutrient media was poured into sterilized test tube. From the stock solution (100 μg / ml in DMSO) different concentrations of extracts were added to double strength nutrient media, to all petri plate and 0.1 ml of bacterial



suspension was added and plates were incubated at required temperature. The growth was observed for inhibition and was determined by absence of growth shown in Table-3. MIC was determined by the lowest concentration of sample that inhibits the development of growth in plates.

DISCUSSION:

The Ethano botanical data including botanical name, local name, location and part used of selected plant species are summarized in Table 1. The plants are traditionally used in the treatment many diseases. The extractive yield of *Cassia auriculata* L was 11.5% w/w. Antimicrobial activities were performed by agar diffusion method using a paper disc and cup and plate method the plants leaves extract exhibited antibacterial activity against all bacterial strains and antifungal activity against all the fungal strains tested. The plant extract produced outstanding antibacterial activity against Gram positive with the greater zone of inhibition than the gram negative bacteria. Considering in this study the Gram positive bacteria are more susceptible than Gram negatives. Result showed that *Cassia auriculata* L had potential inhibitory action against fungal strains than bacterial strains tested and it also showed strong antifungal properties and, as it shown greater zone of inhibition against *Candida albicans*, and *Aspergillus niger*. The result is similar to that reported by K. Muthuthayaru [6]. The lowest concentration of the plant extract required for inhibiting the growth was considered as the MIC of the extracts against bacterial and fungal strains. The MIC values of each extract against the tested microorganisms were presented in Table 3. The anti-inflammatory activity was carried out by using rat as an animal, a comparative study is carried out between low dose and high dose of *Cassia auriculata* used in animals with standard as Diclofenac sodium and carragenin as inducing inflammation. It is carried out by 4 batches, each batch contains 6 animals

that showed a uniform and continuous anti-inflammatory activity.

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HOW TO CITE: M. Sujatha*, Akelesh T., W. D. Sam Solomon, Formulation and Evaluation of Cassia Auriculata Herbal Ointment for Anti-Microbial and Anti-Inflammatory Activity, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 2, 1196-1208. <https://doi.org/10.5281/zenodo.14875435>

Table 1: Ethnobotanical data for *Cassia auriculata* Linn

Botanical name	Family	Local name	Part of plant used	Area of collection
<i>Cassia auriculata</i> L	<i>Fabaceae</i>	Avaram	Leaves	Udumalapett

Table 2: Antimicrobial activities of *Cassia auriculata* Linn

Plant materials	Conc. (µg/ml)	Zone of inhibition (mm)			
		Bacterial strains		Fungal strains	
		<i>S. coccus</i>	<i>E. coli</i>	<i>c. albicans</i>	<i>A. niger</i>
<i>Cassia auriculata</i> L	10	11 ± 0.1	8	13 ± 0.4	10.4 ± 0.1
	30	12 ± 0.3	10.8 ± 0.4	15.7 ± 0.2	13.2 ± 0.5
	50	15.4 ± 0.2	13	17.2 ± 0.3	15.7 ± 0.3
	100	18	17.9 ± 0.1	24 ± 0.3	22.3 ± 0.1

Antibacterial and antifungal activity used as *cassia auriculata* Linn. (*E.coli* ATCC 8739, *S. aureus* ATCC 49342, *C. albicans* ATCC 10231, *A.niger* ATCC 16888).

Table 3: MIC values for *Cassia auriculata* Linn

Microorganism	MIC values (µL)
	<i>Cassia auriculata</i> L
<i>S. aureus</i>	65
<i>E. coli</i>	85



<i>C. albicans</i>	50
<i>A. niger</i>	55

Table 4: Effect of *Cassia auriculata* on Carrageenin -induced paw edema in rats (body weight)

Group	Only Carrageenin	Carrageenin + STD	Carrageenin + L.D 250 mg/kg	Carrageenin + H.D 500mg/kg
Initial bodyweight	156.9±1.333	142.5±28.48	161.4±4.973	166.3±2.813

Table- 5: Antibacterial activity of standard drugs against bacterial test organism

Drugs	Concentration	Zone Of Inhibition (mm)	
		<i>E. coli</i>	<i>S. coccus</i>
Ampicillin	5	14	11
	25	15	14
	50	16	16
	100	19	18
	250	20	19
Chloramphenicol	5	14	10
	25	17	13
	50	23	19
	100	23	20
	250	23	20
Ciprofloxacin	5	20	16
	25	23	19
	50	28	21
	100	28	21
	250	28	22
Norflaxcin	5	22	18
	25	25	19
	50	26	20
	100	27	21
	250	29	21

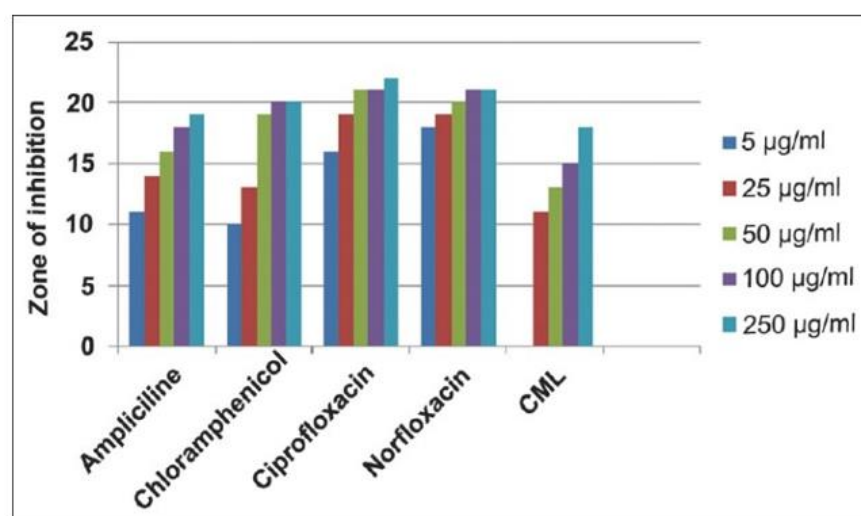
Table-6: Antifungal activity of standard drugs against fungal test organism

Drugs	Concentration	Zone Of Inhibition(mm)	
		<i>A.niger</i>	<i>c.albicans</i>
Griseofulvin	5	19	18
	25	23	21
	50	25	22

Nystatin	100	25	22
	250	28	24
	5	18	18
	25	19	21
	50	24	24
	100	29	25
	250	29	26

Table-7: Effect of *Cassia auriculata* on Carrageenan -Induced Paw edema in rats

Group	Mean paw volume before Carrageenan injection	Paw Volume after induction with Carrageenan injection (mean $\pm$ SEM)/Percent inhibition of edema						
	0 min	30 min	1h	2h	3h	4h	5h	6h
Control	4.10 $\pm$	7.42 $\pm$	7.48 $\pm$	8.38 $\pm$	8.39 $\pm$	7.86 $\pm$	8.28 $\pm$	7.91 $\pm$
	0.16	0.15	0.14	0.20	0.10	0.08	0.29	0.22
Standard	4.085 $\pm$	7.03 $\pm$	7.81 $\pm$	7.79 $\pm$	7.96 $\pm$	7.79 $\pm$	6.03 $\pm$	5.66 $\pm$
	0.16	0.10	0.19	0.25	0.20	0.12	0.15	0.27
L D 250 mg/kg	3.62 $\pm$	6.87 $\pm$	7.65 $\pm$	8.55 $\pm$	8.31 $\pm$	7.24 $\pm$	6.33 $\pm$	6.19 $\pm$
	0.58	0.96	0.57	0.21	0.26	0.50	0.81	0.96
H D 500 mg/kg	4.283 $\pm$	7.068 $\pm$	7.49 $\pm$	7.8 $\pm$	8.16 $\pm$	7.75 $\pm$	6.35 $\pm$	5.49 $\pm$
	0.094	0.10	0.22	0.15	0.10	0.037	0.15	0.18

Fig-1: Antibacterial activity against *S. coccus*

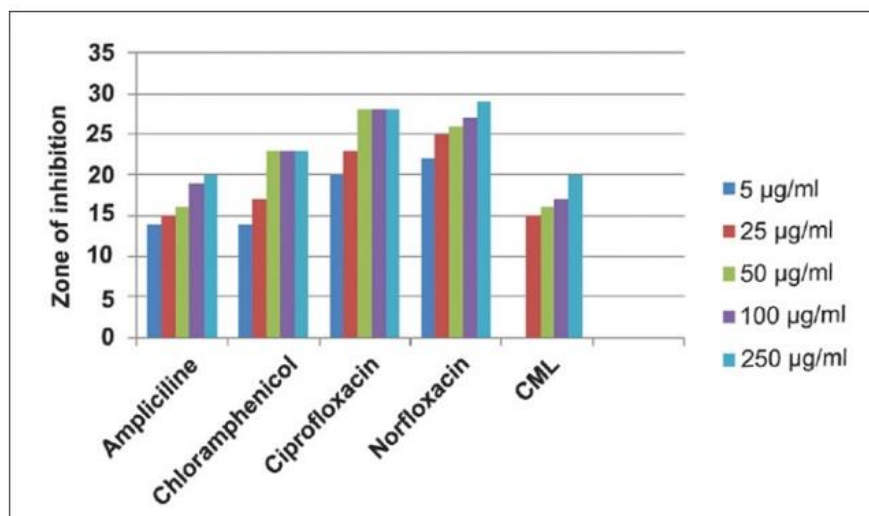


Fig-2: Anti-bacterial activity against *E. coli*

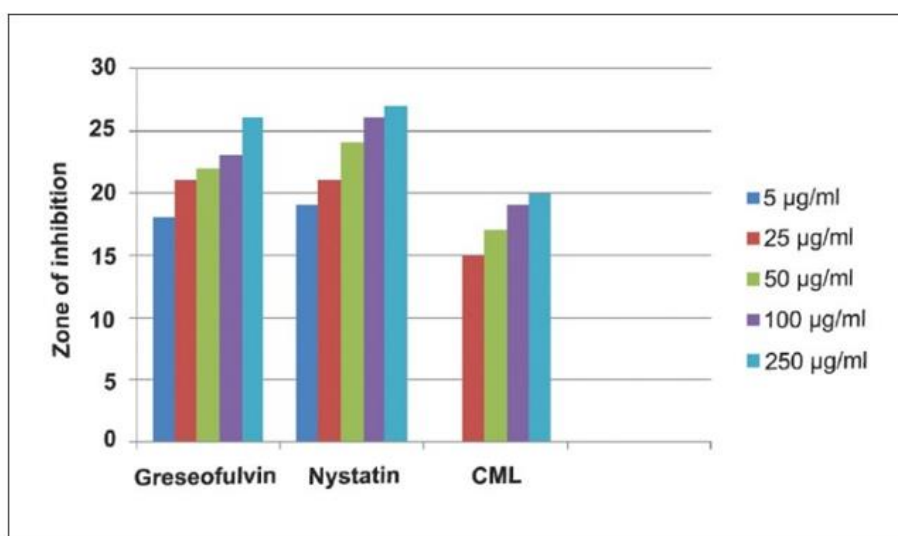


Fig-3: Antifungal activity against *C. albicans*

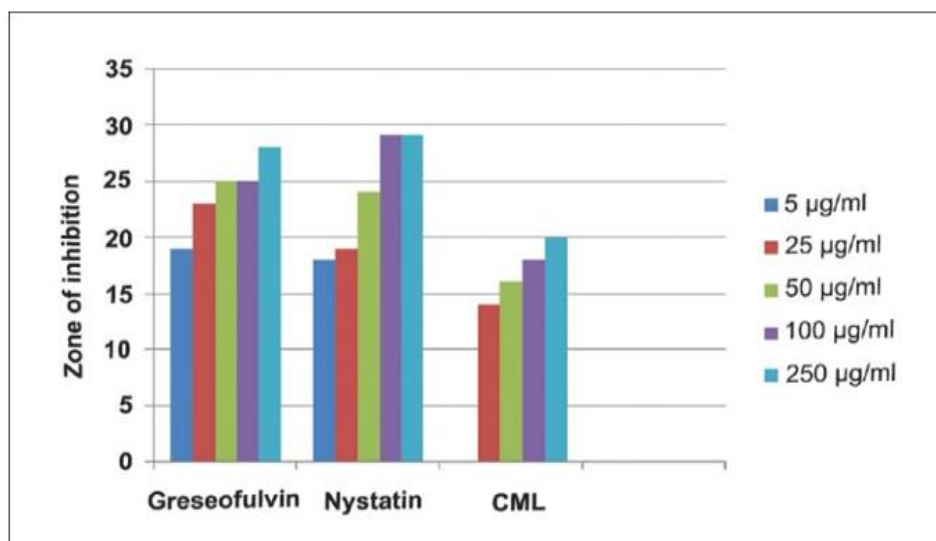


Fig-4: Antifungal activity against *A. niger*.

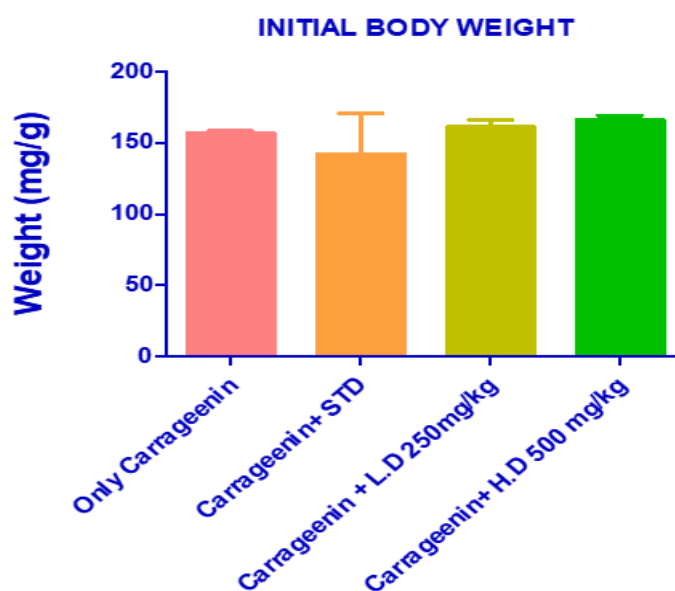


Fig-5: Effect of *Cassia auriculata* on carrageenan -induced paw edema in rats (body weight)

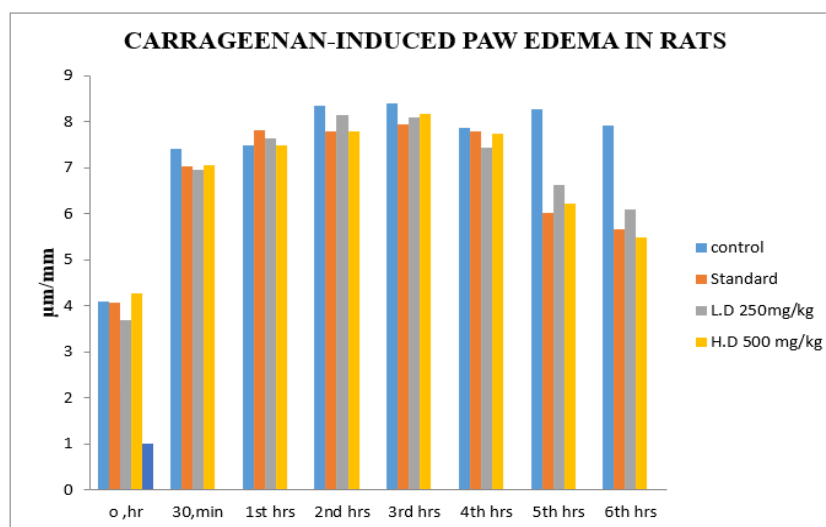
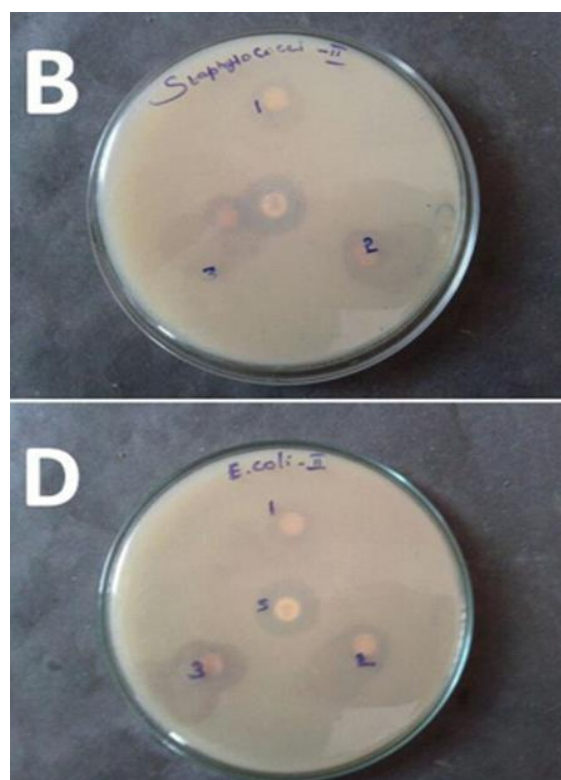


Fig-6: Carrageenan-induced paw edema in rats



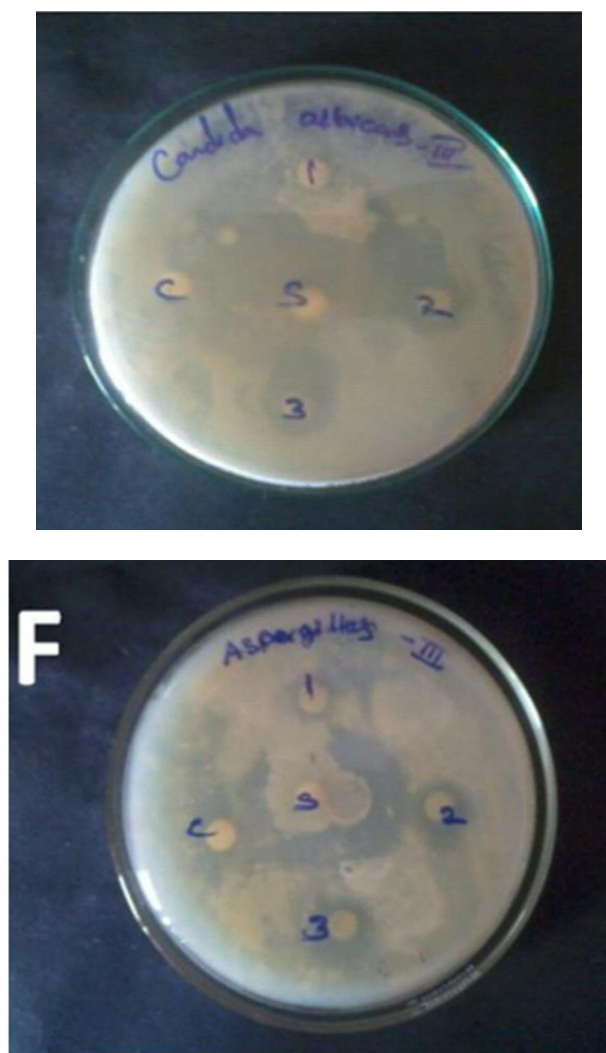


Fig: Inhibition of bacterial and fungal growth by ethanolic extract of *Cassi. auriculata* by disc diffusion method (*E. coli*, *S. coccus*, *A. niger* *C. albicans*)



Group -I only Carrageenan Group-II Carrageenan + STD



Group-III Carrageenan+ L.D.250 mg/kg Group -IV Carrageenan+H.D.500mg/kg
Fig -7: Effect of *Cassia auriculata* on Carrageenan-induced paw edema in rats