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Assessment of antibacterial, cytobiochemical and genotoxic activities in search for biotherapeutic applications from vegetative parts of *Cassia fistula* L.

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Abstract

The medicinal plant *Cassia fistula* L., sometimes referred to as Amaltas or Golden Shower, is widely distributed in India. In this study examination of the phytochemical makeup of *Cassia fistula* L., vegetative parts, viz., leaf and pod for its antibacterial efficacy against a variety of bacteria that were frequently linked to infection in addition to genotoxic cum mitodepressive potential of the pod against *Cassia fistula* L., root tip cells were taken up. The extracts were subjected to phytochemical analysis and antibacterial activity with the well diffusion method. The extracts from *Cassia fistula* L. contained a range of phytochemicals, including as phenolics, terpenoids, flavonoids, and alkaloids. The methanol extract exhibited the strongest antibacterial action. Additionally, out of these five different extracts of *Cassia fistula* L., dried pod aqueous and methanolic extract exhibited maximum inhibitionof normal mitotic index (MI) and maximum production of genotoxicity index (GenI) in growing root tip cells of *Cassia fistula* L., and there were statistically significant differential responses in terms of electrolyte leakage (the potency of electrolyte being: Aqueous extract>Methanolic extract>Ethanolic extract>acetone extact>Petroleum Ether extract in comparison to control sets) coupled with attenuation cum inhibition of root dehydrogenase activity (mitochondrial activity) in root tip cells of *Cassia fistula* L., (Aqueous and methanolic extract of *Cassia fistula* L., pod powder produced maximum inhibition of dehydrogenase acitivity and subsequent mitochondrial poisoning in comparison to Ethanolic, Chloroform and Petroleum Ether Extracts in the same doses) indicating probable cytotoxic cum metabolic inhibitory activities as at higher doses, which may be utilized further for drugs for greater phytoremidiation. *Cassia fistula* L., may be utilized to create fresh antibacterial medications and as a natural supply of antibacterial chemicals.

Keywords: *Cassia fistula* L., leaf and fruit, phytochemicals, dehydrgogenase activity, cytotoxicity, membrane leakage, antibacterial activity, genotoxicity and *Allium sativum* L.

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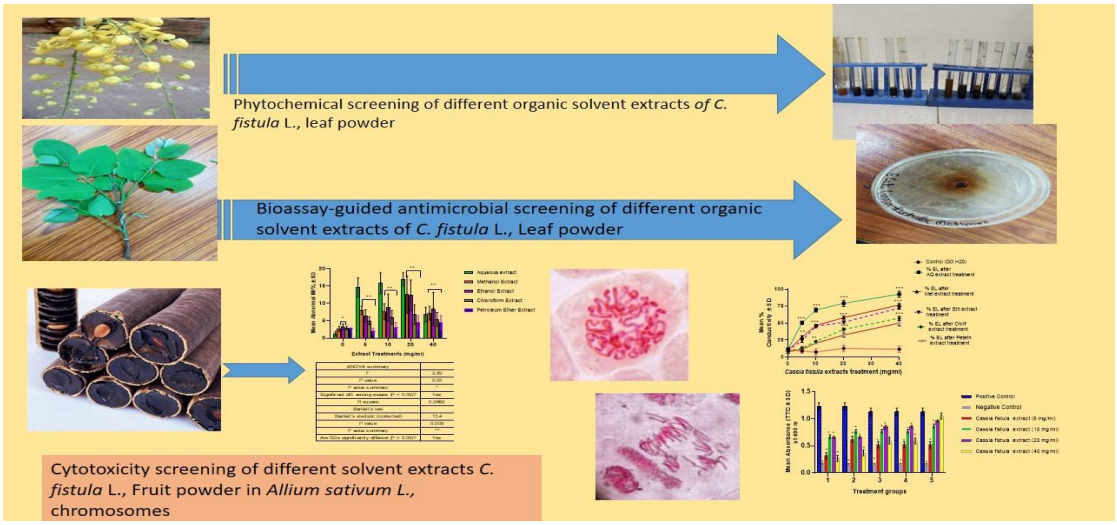


1. Introduction

People have been using plants for medicinal purposes for many years. India's traditional medical systems, such as Ayurveda, Siddha, and others, have long used plants as remedies for a wide range of illnesses. Since ancient times, Ayurvedic treatments have been used in Indian traditional medicine to manage chronic illnesses, often saving lives. When it comes to side effects and eventual patient dependence, traditional medications always have the upper hand over conventional ones. Consequently, by easing a patient's health problems and suffering, traditional medicines can significantly enhance that patient's quality of life [1]. Due to the efficacy, accessibility, and inherited usage of traditional medicines, the World Health Organization has recognized Ayurvedic traditional medicinal systems in disease control programs [2]. Numerous chemicals with therapeutic benefits are being extracted from various herbs and utilized to treat long-term illnesses [3]. In recent past Deresa and Diriba (2023) [4] stated that they had been also employed as pesticides. Studies show that between 70 and 80 percent of people in underdeveloped nations use herbal remedies to treat their illnesses, and that 25 percent of commercial medications are made from medicinal plants [5].

Cassia fistula L., also referred to as Golden Shower or Amaltas, is one such herb that has been used for generations to treat a range of Amaltas [6]. It originated in India and Sri Lanka and has since spread to Mexico, China, Mauritius, East Africa, South Africa, and the West Indies, among other nations [1]. It is commonly found in the Bhilai region of the Indian state of Chhattisgarh and has mostly been used in folklore and tribal medicine for decades due to its widespread availability and affordability for altruistic applications in different ailments among masses [7]. Decoctions of different parts of *Cassia fistula* L., plant have been enjoying applications through decades owing to its pharmacological and therapeutic uses [8]. The deciduous *Cassia fistula* L., tree, which is a member of the Fabaceae family, can reach a height of 20 metres. The plant is distinguished by its lengthy pods that hold a large number of seeds and its yellow blossoms. Traditional medicine has made use of *Cassia fistula's* leaves, bark, flowers, and fruits. Numerous pharmacological characteristics of the plant, including as antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer effects, have been documented. Herbal Medicinal Use of *Cassia fistula* L., includes to treat a fever, handling of Jaundice, to allow the wound to heal, to eliminate pimples and acne and whooping cough remedies [9].

Worldwide, bacterial infections remain a serious public health risk. Antibiotic-resistant bacteria have emerged as a result of the overuse and abuse of antibiotics, which are frequently used to treat bacterial diseases. New antibacterial agents are therefore desperately needed. Plants possess a variety of bioactive chemicals that make them a promising source for novel medications. Since all of the plant's parts have been successfully utilized to treat infectious disorders, *Cassia fistula* L., (Leguminosae) has the potential to be an alternative therapy for those conditions [10]. Anthraquinone, oxyanthraquinone, tannin, volatile oils, and rhein have all been found to be present in *Cassia fistula* L., flowers and leaves. These chemicals are known to have antibacterial activity [6]. Diarrhea has been treated using the

whole plant. Fever, skin conditions, and stomach ache have all been treated using the flowers, fruits, and seeds. Additionally, it has been noted to have hepatoprotective and anti-inflammatory properties [10]. According to other studies, *Cassia fistula* L., extract can treat wounds [11], The cytotoxic impact of *Cassia fistula* L., flower extract on the breast cancer cell line MCF-7 was revealed in a study on the formation of AgNPs [12]. The extract's pharmacological and cytotoxic effects using different solvent extractions in vivo, however, have not been thoroughly investigated to determine their exact potency of action.

There are still worries about the safety of herbal medications, even though their market demand is expanding. According to Bardoloi and Soren (2022) [13] all herbal extracts have been shown to be a storehouse of various proportions of allelochemicals that may not be a safer option in different seasons due to their varying phyopharmacological activities that might cause genotoxic responses. Because traditional herbal treatments are natural and have been used for many years, there is a widespread perception that they are safe and harmless. Herbal goods must be standardized for tight quality control and to guarantee the safety of their active ingredients [14].A toxicological study is crucial, per OECD standards ([15, 16], to demonstrate the efficacy and safety of a novel medication. Finding negative effects and figuring out the exposure levels at which they happen are the main goals of every herbal medicine's toxicological evaluation [17]. The kind and degree of any adverse effect, as well as the exposure level at which the impact is noticed, are two crucial variables that are taken into account when assessing the safety of any form of herbal medicine [18].

The widespread perception that herbal remedies are safer and more effective than their allopathic equivalents has led to an increase in the usage of these items. Toxicological research on herbal medicines should be carried out, nevertheless, as this presumption is unfounded and dangerous [19]. According to studies, certain herbs that are commonly used in traditional medicine may be genotoxic [13]. Any combination of hazardous materials can result in a variety of chromosomal abnormalities, such as exchanges, sister chromatid unions, gaps, chromatid breaks, isochromatid breaks, and chromosomal fragments; in some cases, even a change in DNA [20]. According to Virolainen et al. (2023) [21], the effects of such DNA impairment may include altered heritable features, higher morbidity/mortality, the development of diseases, and/or a tendency to them.

Although *Cassia fistula* L., is a widespread in different parts of India, but its toxicological profiling in correlation to its strong exhibition of antibacterial action against a range of bacteria, its phytochemical makeup and cytotoxic actions using eukaryotic model genetic systems in Plants like *Lathyrus sativum* L., , *Allium Cepa* L., and *Allium sativum* L., are almost undocumented. Additionally how the alleolochemical actions of different decoctions in cytological systems of higher plantshas not been explored yet. In this study the authors have tried to fully elucidate the antibacterial activity of different extracts of *Cassia fistula* L., fruits in four different solvents (polar and nonpolar in addition to aqueous solvent extractions) and subsequent bioassay guided screening of antibacterial and cytogentoxic activity using *Allium sativum* L., ana-telophase aberrations systems in addition to its membrane damaging assays and root mitochondrial activity (dehydrogenase activity) to elucidate the mechanism of toxic actions (if any) *in vivo*.

Taxonomical Identification:

1. Kingdom – Plantae
2. Order – Fabales
3. Family – Fabaceae
4. Subfamily – Caesalpinioideae
5. Genus – *Cassia*
6. Species – *C. fistula* L.,

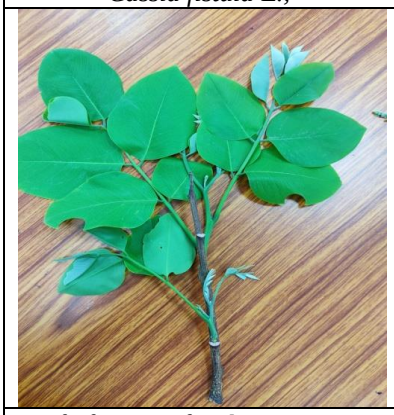
Common Name: Golden shower tree, Amaltash, Golden tree, Golden rain tree

Regional Name:

1. Assamese – Sonalu, Sonaru
2. Bengali – Bandaralathi, Sonali
3. English – Indian Laburnum, Pudding-pipe tree, Purging fistula, The Indian laburnum
4. Gujarati – Garmalo
5. Hindi – Amaltash, Amaltas, Swarnpushpi,
6. Irula – Kakkai
7. Kannada – Kakke
8. Konkani – Balo
9. Urdu – Amaltas

Image showing the plant of *Cassia fistula* L.,

The flower of *Cassia fistula* L.,



Leaf of *Cassia fistula* L.,

The Fruit of *Cassia fistula* L.,

Figure 1. *Cassia fistula* L.

2. Materials and Methods

2.1. Collection and preparation of plant material

In April, plant material belonging to *Cassia fistula* L., *was* gathered from various locations within the Bhilai district of Chhattisgarh state, India. After being divided, the leaves were cleaned and allowed to air dry in the shade for a week. Using a mechanical grinder, the dried plant material (leaf and fruit) was ground into a fine powder and kept for later use in an airtight container [22].

2.2. Extraction and phytochemical analysis of leaf and fruit of *Cassia fistula* L.

Using the Soxhlet extraction method (200 ml), the powdered plant material (20 g each of leaf and fruit) was extracted using a variety of solvents, including ethanol, petroleum, methanol, and distilled water. After filtering, the extracts were concentrated at 40°C in a hot air oven, weighed and kept in containers with labels as dried extracts.Standard procedures were used for the extracts' phytochemical examination. Salkowski's reagent was used to identify terpenoids, Mayer's reagent was used to identify alkaloids, the ferric chloride solution was used to identify flavonoids, and the phenolics were identified using the ferric chloride solution [23].

2.3. Preliminary Phytochemical screening of leaf and fruit of *Cassia fistula* L., extracts

2.3.1. Test for the presence of Carbohydrate

To 2ml of the extract, 2-3- drops of 1% α-naphthol and then 2ml of Sulphuric acid were added along the side of the test tube. The appearance of a violet ring at the junction indicates the presence of carbohydrates in the sample [23].

2.3.2. Test for the presence of Protein

To 3ml of sample extract 5ml of Million's reagent was added. It was then heated. Conversion to brick red color indicates the presence of protein [24].

Test for the presence of Phenol:

To 3ml of sample extract 2ml of 5% aqueous ferric chloride solution was added. The formation of a deep blue or black color indicated the presence of phenol in the extract used [25].

2.3.3. Test for the presence of Secondary metabolites

a) Test for the presence of Sterol:

To 1 ml of sample extract, 3 ml of Chloroform and 1 ml of Sulphuric Acid were added. The presence of sterol is confirmed if the lower layer appears red in color [26].

b) Test for the presence of Alkaloids:

A few drops of Mayer's reagent were added to 1 ml of the extract by the side of the tube. A creamy white precipitate indicates the presence of alkaloids [26].

c) Test for the presence of flavonoids:

To 1 ml of the extract, 2.5 ml of dilute ammonia was added followed by a few drops of concentrated sulphuric acid. The yellow color indicates the presence of flavonoids [26].

d) Test for the presence of Cardiac glycosides:

To 0.5 ml of the extract 2 ml of glacial acetic acid was added along with 1-2 drops of 1% aqueous ferric chloride. Then 1ml of sulphuric acid was added to this solution. A brown ring was observed at the interface, indicating cardiac glycosides' presence [27].

e) Test for the presence of Anthraquinones:

To 0.5 ml of the extract 2 ml of Sulphuric acid was added. The solution was then heated. 2.5 ml of Chloroform was added and the solution was shaken and mixed well. Furthermore, 1 ml of

dilute ammonia was added. The change in color indicates the presence of anthraquinones [28].

f) Test for the presence of Terpenoids:

2 ml of chloroform was added to 0.5 ml of the extract, and 3 ml of concentrated sulphuric acid was added. The appearance of reddish-brown color at the interface indicates the presence of terpenoids [24].

g) Test for the presence of Tannins:

To 5 ml of extract, a few drops of 1% aqueous ferric chloride were added. The appearance of brownish green or blue-black color indicates the presence of tannins [24].

h) Test for the presence of Saponins:

To 0.5 ml of extract, 5 ml of distilled water was added and shaken vigorously. Appearing of froth indicated the presence of Saponins [20].

2.4. Antibacterial activity assay

2.4.1. Media Preparation

The process of creating nutrient agar medium (NAM) involved combining 5.0g of peptone, 3.0g of beef extract, 5.0g of NaCl, and 15.0g of agar with 1L of distilled water. The mixture was then autoclaved at 15lb pressure for 15 minutes. Using a gel borer, wells were created in the sterilised media by pouring it into Petri dishes [29].

2.4.2. Antibacterial Activity of *Cassia fistula* L, extracts

Tested against a panel of microorganisms frequently linked to infections, such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Lactobacillus sp,* the antibacterial activity of *Cassia fistula* L, extracts was conducted. The bactericidal activity of the extracts was assessed using the borer technique. In summary, bacterial cultures were cultured for 24 hours at 37°C in nutritional broth. The bacterial culture was distributed onto Nutrient agar plates using a sterile cotton swab, and 20 µL of various plant extracts (20 g/200 mL) were then impregnated into the well diffusion (4 mm). Following a 24-hour incubation period at 37°C, the diameter of the zone of inhibition surrounding each disc was measured on the plates [30].

2.5. *In vivo* cytotoxicity assay of *Cassia fistula* L, extracts using root tip cells *Allium sativum* L., ana-teolophase assay

Fresh healthy garlic (multiclove; *Allium sativum* L.,) of 4-7 ±1.5 g was chosen and procured from the local market. The cloves of the *Allium sativum* L., were gently separated by hand tearing and the old roots and scales were carefully removed. The denuded cloves were placed over sand pots moistened with water sprinkling were placed in an isolated space at 25 ± 1ºC for the root induction. The freshly emerged roots of about 0.5-1 cm length were treated by placing the rooted cloves on test tubes containing 5mg, 10 mg, 20 mg, and 40 mg/ ml respectively from the stalk solutions obtained from different solvent mediated *Cassia fistula* L., extracts (i.e., methanol, ethanol, acetone, petroleum ether and aqueous) for 24h. Distilled water was used as a negative control. Cytological investigations were carried out following the protocol of [31]. Evaluation of genotoxicity was determined as per the following formulas in meristematic cells of *A. sativum* L. Genotoxicity in meristematic cells was evaluated according to de Souza et al. (2022) [32]. Approximately, 2,500 meristematic cells were analyzed per treatment. Cytotoxicity was evaluated based on the mitotic index (MI) according to the formula:

$$MI = \frac{\text{Number of cells in division}}{\text{Total number of cells observed}} \times 100$$

Genotoxicity was analyzed based on the number of cells carrying chromosomal and nuclear aberrations, such as nuclear buds, anaphasic and telophasic bridges, and chromosomal losses and breaks. The genotoxicity index (GenI) was calculated using the formula:

$$\text{Gen I} = \frac{\text{(No of cells showing chromosomal abnormality + nuclear buds+ nuclear breakage)}}{\text{(Total no of cells counted)}} \times 100$$

$$\text{Mitotic Inhibition} = \frac{\text{(MI in Control-Abnormal Mitotic index after treatment)}}{\text{(MI in Control)}} \times 100$$

The frequency of micronuclei (FMN) was determined by assessing the number of cells containing MN:

FMN= (No of cells showing MN) ÷ (Total no of cells counted) x 100.

The presence of MN can be used as a biomarker for genotoxicity and chromosomal instability-related events. When this damage is not repaired, it has the capacity to produce mutagenic effects.

a) Detection of Morphological Characters for cell death, computation the Percentage of Dying Cells: We chose nucleus migration from centre to margin of cell wall, condensation, vacuolation of cytoplasm, nuclear fragmentations as characteristic hallmarks of dying cells. Nucleus margination is displacement of nucleus in a cell wall margin [20].

b) Percentage of Dying Cells= (No of cells dying or dead cells) ÷ (Total no of cells counted) x 100.

c) Observation and manual scoring of cell dimensions of Giant cells with vacuolated cytoplasm: to detect the extent of protoplasmic shrinkage in *A. sativum* L., extracttreated root cells were transferred to an incubation solution (containing 0.1 M sodium phosphate buffer pH 7.2 containing 0.3 M sucrose with 6µM neutral red) for 30 minutes. The cells roots were then hydrolyzed in 1N HCl and counterstained with 2% acetoorcein and safranine solution. The root tips were then squashed in 45% acetic acid and Photographs were taken from all the morphogenic regions of the root tip (in x 100 magnification) and cell dimensions like shrinkage areas, legth, breadth and cytoplasmic areas were observed and scored [33].

2.6. Determination of Membrane Permeability/ Electrolyte Leakage after *Cassia fistula* L, extracts pre-treatment on growing roots of *A. sativum* L.

Ions that were leaking into deionized water from tissue were used to measure membrane permeability or electrolyte leakage (EL). Test tubes containing 10 mL of deionized water and segments of fresh root samples (extract pretreated and controlled sets of 100 mg root tissues of *Allium sativum* L. in each tube) were used. The tubes were immersed in water that was 32 °C-heated for 6 hours. Following incubation, the bathing solution's electrical conductivity (EC1) was measured using an electrical conductivity metre (Systronics M-308, Kolkata, India). After that, the samples were autoclaved for 30 minutes at 121°C to totally destroy the tissues and liberate all electrolytes. The final electrical conductivity (EC2) of the samples was then calculated after they had been cooled to 25 ° C. The formula EL%=EC1/EC2X100 was used to convert the EL into a percentage [33].

2.7. Evaluation of root metabolic/mitochondrial activity after *Cassia fistula* L, extracts pre-treatment on growing roots of *A. sativum* L.

The best method for determining a cell's viability is TTC (2, 3, 5-Triphenyl tetrazolium chloride) staining. *Allium sativum* L., germinating roots were subjected to 24 hours of treatment with various concentrations of different solvent mediated *Cassia fistula* L., extracts (i.e., methanol, ethanol, peteroleum ether and aqueous) for 24h. The same procedure was followed while using pure water as the positive control and 0.2% hydrogen peroxide (H_2O_2) as the negative control. In 0.5% (w/v) TTC stain for five hours in the dark, all the roots were submerged. After that, distilled water was used to cleanse the roots. Using a spectrophotometer and 95% ethanol as a blank, absorbance was measured at 490 nm. The test O.D.s had been converted into percentages representing the following rise or fall in metabolic activity, and the positive control (O.D. of 0.2% hydrogen peroxide treated sets) was taken to represent 100% metabolic/respiratory activity (dehydrogenase) activity, out of root mitochondrial activity [33].

2.8. Statistical analysis

The data obtained from the studies were represented as Mean ± SEM. The data were analyzed by one-way analysis of variance (ANOVA), 'P' value less than 0.05 was considered as statistically significant. Graphpad Instat version 9 and Microsoft Excel 2010 were used for statistical analysis and production of tables.

3. Results

3.1. Phytochemical analysis

The phytochemical analysis of *Cassia fistula* L., extracts revealed the presence of a variety of compounds including Carbohydrates, Protein, and Phenol. The Methanol extract showed the highest zone of inhibition for all tested bacteria.Secondary metabolites in the phytochemical analysis of *Cassia fistula* L., extracts revealed the presence of a variety of compounds including Sterol, Alkaloids, flavonoids, glycosides, Terpenoids, saponins

Table 1. Different solvents extract in the preliminary test.

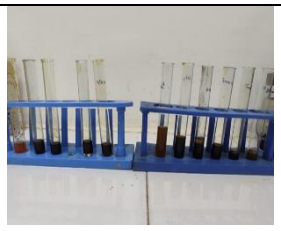
Type of solvent	Color and Consistency of *Cassia fistula* L., extracts
Aqueous	Light orange and sticky
Methanol	Dark green and sticky
Ethanol	Dark green and sticky
Chloroform	Radish and sticky
Petroleum either	Dark green and sticky

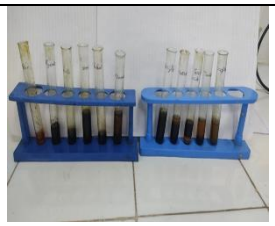
Table 2. Preliminary & Secondary metabolites screening *Cassia fistula* L., extracts

Test	Aqueous Extract		Methanolic Extract		Ethanol Extract		Chloroform Extract		Petroleum Extract	
	Leaf	fruit	Leaf	fruit	Leaf	fruit	Leaf	fruit	Leaf	fruit
Carbohydrate	+	+	-	+	-	+	-	-	-	+
Protein+aminoacids	+	+	+	+	+	+	+	+	+	-
Phenolics	+	+	+	+	+	+	+	+	+	+
Sterol	+	-	+	+	+	_	+	+	+	-
Alkaloids:	+	+	-	+	+	+	-	+	+	+
flavonoids	+	+	-	+	+	+	+	+	+	-
glycosides	+	+	-	+	-	_	-	+	+	-
Anthraquinones	+	-	+	+	+	_	+	-	+	-
Terpenoids	-	-	+	+	+	+	+	+	-	+
Tannins	+	+	+	+	+	+	-	+	+	+
Saponins	+	+	+	+	-	+	+	-	-	-


Aqueous extract


Methanol extract


Ethanol extract


Chloroform extract


Petroleum either extract

Figure 2. Showing the results of different secondary metabolites presnt in different *Cassia fistula* L., in extracts, + denotes the presence of metabolites, and – denotes the absence of metabolites.

3.2.Detection of Antibacterial activity of diffent extracts of *Cassia fistula* L.

To detect the antibacterial activity, against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Lactobacillus sp* and a lawn of these bacteria in NAM plate were made using extract from different solvents. In we found the maximum zone in the Methanol extract, then any other extract.

			
Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Lactobacillus* sp

Figure 3a. Photomicrographs showing Antibacterial activity of Methanol extract of *Cassia fistula* L.

			
Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Lactobacillus* sp

Figure 3b. Photomicrographs showing Antibacterial activity of Aqueous Extract of *Cassia fistula* L.

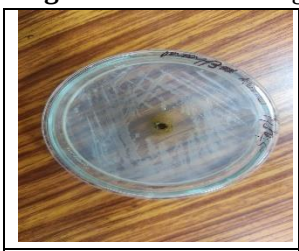
			
Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Lactobacillus* sp

Figure 3c. Photomicrographs showing Antibacterial activity of Ethanolic Extract of *Cassia fistula* L.

Antibacterial effects of different extracts on *Staphylococcus aureus* with respective zone of inhibitions (in cm)			
			
Treatments	Aqueous Extract	Methanol extract	Ethanol Extract
Treatment dose	20g/200ml	20g/200ml	20g/200ml
ZOI observed (in cms)	0.7cm	1.2cm	1.0cm
Antibacterial effects of different extracts on *E.coli* with respective zone of inhibitions (in cm)			
			

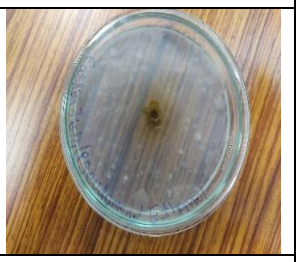
Treatments	Aqueous Extract	Methanol extract	Ethanol Extract
Treatment dose	20g/200ml	20g/200ml	20g/200ml
ZOI observed (in cms)	0.3cm	1.4cm	0.3cm
Antibacterial effects of different extracts on *Pseudomonas sp* with respective zone of inhibitions (in cm)			
			
Treatments	Aqueous Extract	Methanol extract	Ethanol Extract
Treatment dose	20g/200ml	20g/200ml	20g/200ml
ZOI observed (in cms)	0.5cm	0.9cm	0.6cm
Antibacterial effects of different extracts on *Lactobacillus sp* with respective zone of inhibitions (in cm)			
			
Treatments	Aqueous Extract	Methanol extract	Ethanol Extract
Treatment dose	20g/200ml	20g/200ml	20g/200ml
ZOI observed (in cms)	0.8cm	1.1cm	0.4cm

Figure 3d. Comparative antibacterial activity of Aqueous, Methanolic and Ethanolic extracts in different bactieral strains.

Table 3. Showing the zone of inhibition of diffent extracts of *Cassia fistula* L., *on diffent microorganisms*

Tested microorganisms	Aqueous extract	Methanolic extract	Ethanolic extract	Chloroform extract	Petroleum Either extract	Zone of inhibition highest in
Staphylococcus aureus	0.7cm	1.2cm	1.0cm	-	-	Methanol extract
Escherichia coli	0.3cm	1.4cm	0.3cm	-	-	Methanol extract
Pseudomonas aeruginosa	0.5cm	0.9cm	0.6cm	-	-	Methanol extract
Lactobacillus sp	0.8cm	1.1cm	0.4cm	-	-	Methanol extract

From the experimental results it could be found that the methanolic extract of *Cassia fistula* L., was found to be the most potent in expressing antimicrobial activity in *E. coli* cells with the maximum inhibition zone (1.4 cm in diameter) followed by *Staphylococcus aureus* (1.2cm in diameter), followed by *Lactobacillus sp* (1.1cm in diameter) with least effects on *Pseudomonas aeruginosa* (0.9 cm in diameter).

3.3.In vivo Cytogenotoxic activities of different extracts of fruit of *Cassia fistula* L., in *Allium sativum* L., root tip cells

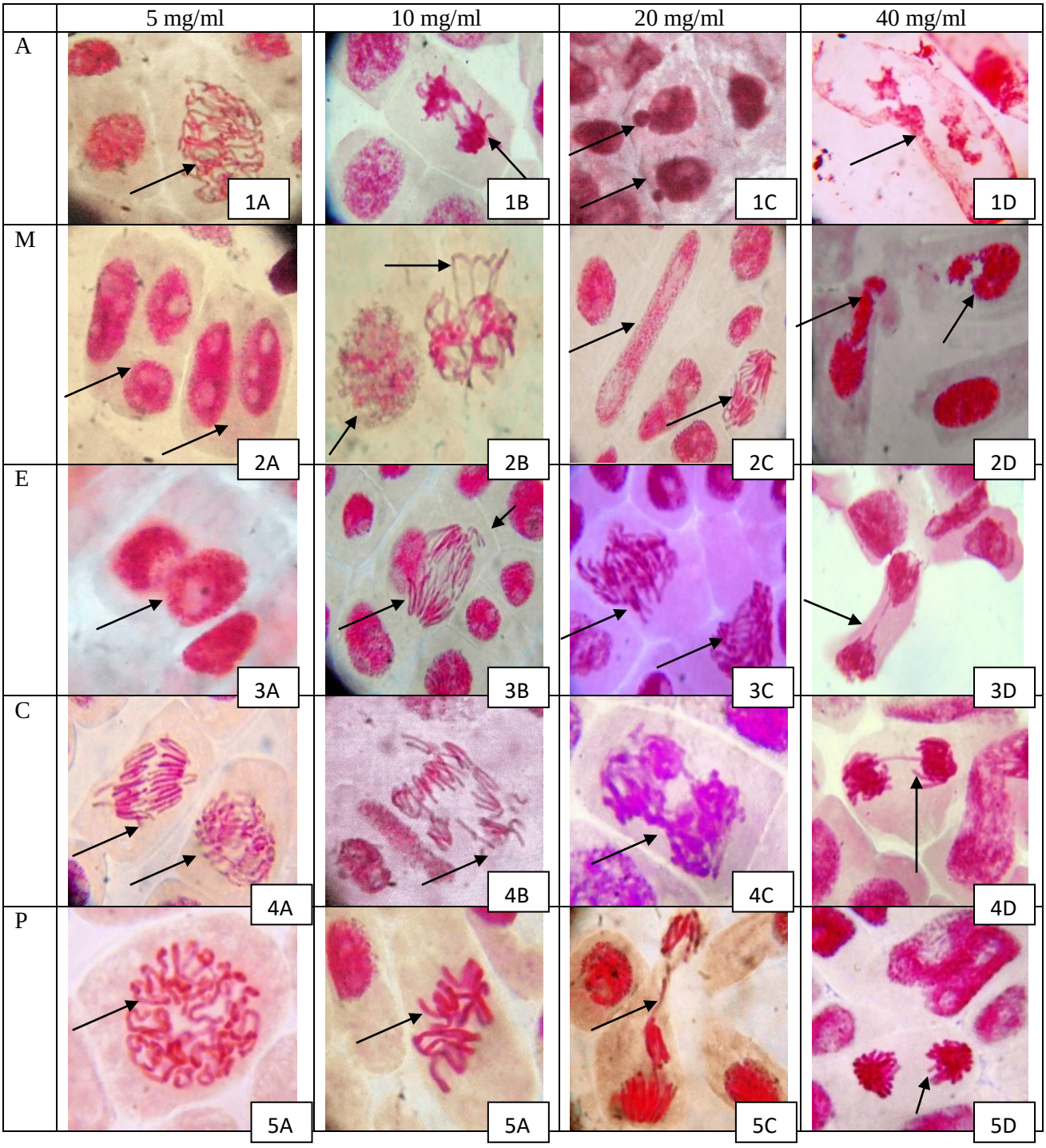
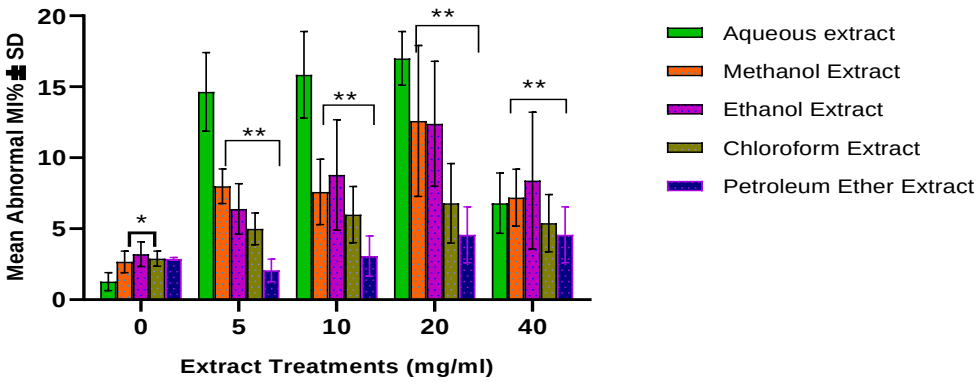


Figure 4. Photomicrographs showing meristematic root tip cells of *Allium sativum* L., representing chromosomal alterations after 24 hrs of treatment with various extracts and increasing concentrations of *Cassia fistula* L., dried pod powder.

Photomicroplates (A-D) showing induction of chromosomal abnormalities after varying concentrations of different extract pretreatments on germinating root tips of Allium sativum L., step by step induction of cellular death from early stages of cellular toxicity (from 5, 10, 20 and 40 mg/ml doses). 1A= Pulverized chromatin at prophase, 1B= Stellate anaphase bridge with stickyness, 1C= Nucleuar budding with micronuclei formation at interphase, 1D= ghosht cells with karyolysis leading to apoptosis; 2A= Tri and Binucleolate condition showing lesions, 2B: Nuclear erosion plus multiple metaphasic ring formation, 2C= Ghost cell formations with strap-like nucleus with erosion., 2D= Karyorrhexis with nuclear breakage; 3A= Trinucleate state with nuclear lesions, 3B= Anaphasic stickiness with early separation, 3C=

Distrurbed ball metaphase, 3D= Displace anaphase showing chromosome bridge; 4A= tropokinetic anaphase with vagarant chromosomes and bridge formation, 4B= Distrubed multipolar anaphase in hyperploid cell, 4C= double telophasic bridge showing stellate anaphase in hyperpolid cell, 4D= stellate teolophase showing bridge; 5A= Early Ball metaphase, 5B= Partial c-metaphase, 5C= Stricky anaphasic bridge with hyperploidy, 5D= stellate anaphase. A= Cassia fistula L., aquatic fruit extract; M= Cassia fistula L., methanolic fruit extract, E= Cassia fistula L., Ethanolic fruit extract, C= Cassia fistula L., Chloroform fruit extract, P= Cassia fistula L., Petroleum fruit extract.

Chromosome aberrations (CA) were seen during the majority of the mitotic cycle. The rates of chromosome abnormalities increased in a concentration-dependent manner with varying extracts and increasing concentrations of *Cassia fistula* L., dry pod powder. Chromosome aberrations consisting of fragments, sticky, and disturbed chromosomes were the most frequently detected at high concentrations (20 and 40 mg/mL). In addition, c-mitosis, bridge, and binucleate to trinucleated cells were detected as chromosomal aberrations. However, the Aqueous, methanolic and ethanolic extracts of dried pod powder from *Cassia fistula* L., at 40 mg/ml pretreated roots showed signs of apoptosis and the production of ghost cells. Figure 4 Plate: 1D, 2C, 2D. The induction of nuclear budding was typically detected in the control and methanolic, ethanolic, acetone, and ethyl acetate extract treatment sets, and was significantly different from the 20 mg/ml concentration of *Cassia fistula* L., aqueous extract. The generation of micronuclei was much greater at 20 mg/ml than it was at the other doses. Thus varying cytotoxicological effects of *Cassia fistula* L., pod extract on *A. sativum* L., meristem cells are also visible in all the microphotographs in Figure 4.



ANOVA summary	
F	2.89
P value	0.05
P value summary	*
Significant diff. among means (P < 0.05)?	Yes
R square	0.3662
Bartlett's test	
Bartlett's statistic (corrected)	13.4
P value	0.009
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

Figure 5. Bargraphs showing comparative cum differential abnormal MI% induction in *Allium sativum* L., root tip cells after pretreatment with different extracts for 24 hrs.
The Bars with Asterisks (, **) within each panel are significantly different at P < 0.05 according to one way ANOVA (aqueous extract vs respective treatment groups) followed by Tukeys's multiple comparison tests within treatment groups within 95% CI of diff.*

Compared to the mitotic index of 10.7 in the control, exposure to *Cassia fistula* L., pod extract suppressed the mitotic index in a concentration- and time-dependent manner. Acute toxicity was seen at 40 mg/ml when *Cassia fistula* L., pod aqueous extract was treated for 24 hours, resulting in the lowest Mitotic Index (MI) value of 3.2. When *Cassia fistula* L., pod aqueous extract was compared to control and various organic solvent extracts (methanolic, ethanolic, acetone, and ethyl

acetate extract treatment sets), the mitotic index for the extract dropped significantly ($p < 0.01$) at 10, 20, and 40 mg/ml.These cytogenetic findings point to the genotoxic and cytotoxic effects of the *Cassia fistula* L., pod extract on *Allium sativum* L., meristem cells. The microscopic analysis discovered significant apoptosis, also referred to as necrosis, in animal tissues.

3.4. Determination of *in vivo* Membrane Permeability/ Electrolyte Leakage after pretreatment with different extracts of *Cassia fistula* L., fruit powder (5 to 40 mg/ml; 24 hrs) in growing roots of *Allium sativum* L.

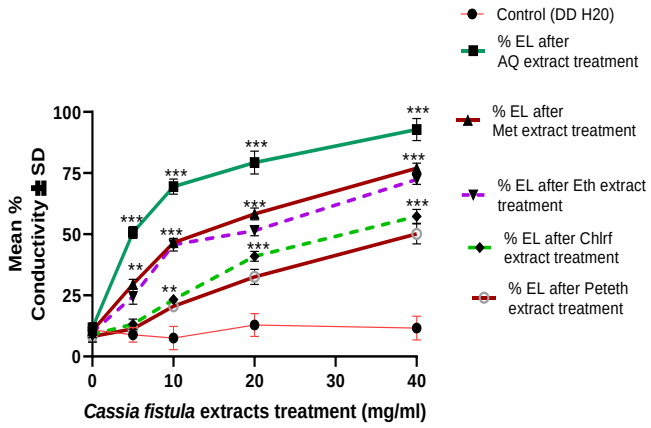


Figure 6. Line diagram showing the values are expressed as mean % electrolyte leakage±SD (n=6), after pretreatment with different extracts of *Cassia fistula* L., in etiolated *Allium sativum* L., roots. *ANOVA is followed by post-hoc Dunnet's test (vs control). Asterisks denote the level of significance; ***$p \leq 0.001$.*

In untreated (distilled water treatment) roots of *Allium sativum* L., the root electrolyte leakage was minimal which was below 20% (Figure 6). After incubation at different extracts of *Cassia fistula* L., fruit powder (5 to 40 mg/ml; 24 hrs) the etiolated roots could show a differential response in terms of electrolyte leakage/membrane permeability. There was a concentration dependent disruption of membrane leakage in a significant manner in etiolated roots of *Allium sativum* L., in comparison to control (Figure 6) showing membrane hydrolyzing/disruptive properties of different extracts of *Cassia fistula* L., pod powder. The maximum electrolyte leakage could be observed after 24 hrs pretreatment with aqueous extract of *Cassia fistula* L., pod powder where even at 5 mg/ml pretreatment produced nearly 50% electrolyte leakage from *Allium sativum* L., roots in comparison to control sets. At 20 mg/ml pretreatment there was production of 75% electrolyte leakage from *Allium sativum* L., roots in comparison to control sets and at 40 mg/ml pretreatment there was production of 95% electrolyte leakage from *Allium sativum* L., roots in comparison to control sets. This was followed by pretreatment with Methanolic extract of *Cassia fistula* L., pod powder where at highest dose, i.e., 40 mg/ml nearly 76% electrolyte leakage could be accounted, followed by Ethanolic extract pretreated sets where at 40 mg/ml doses, nearly 71.25% electrolyte leakage could be counted. The least effect was accounted in roots pretreated with chloroform and peteroleum ether extracts of *Cassia fistula* L., pod powder, where at the highest dose (40 mg/ml) could produce 57.24 and 52.14% electrolyte leakage in *Allium sativum* L., roots. So the potency of electrolyte leakage could be represented by Aqueous extract>Methanolic extract > Ethanolic extract > acetone extact > Petroleum Ether extract in comparison to control sets.

3.5. Evaluation of root *in vivo* metabolic/mitochondrial activity of different extracts of *Cassia fistula* L., dried pod powder (5 to 40 mg/ml; 24 hrs) pretreatment on etiolated roots of *Allium sativum* L.,

The effect different concentrations of Aqueous Extract of *Cassia fistula* L., pod powder on root metabolic activity (dehydrogenase) activity on growing roots of *Allium sativum* L., exhibited differential activity. TTC staining was employed in the present study as an indicator to evaluate the effect of plant extract on mitochondrial metabolism. In TTC staining 2, 3, 5-triphenyl tetrazolium chloride is reduced to red formazan by mitochondrial enzymes. The result showed a dose dependent decrease in mitochondrial activity as visualised by decrease in staining and absorbance in comparison to positive control (*$p < 0.001$) (Graph 3). Negative control remained unstained with minimum absorbance indicating least mitochondrial activity and Positive control showed maximum activity. In the case of treatments, it was found that at 10 and 20 mg/ml in comparison to 5mg/ml and 40 mg/ml pretreatments, there was an increase in root metabolic activity (dehydrogenase activation) reaching more than 50% activity but much lesser in comparison to positive control (0.2% H_2O_2 treatment).But at the highest concentration i.e. at 40 mg/ml pretreatments there was a significant loss of root metabolic activity probably due to cellular poisoning and disruption of

dehydrogenase activity in comparison to both negative and positive control sets. From this result, it may be deciphered that Aqueous Extract of *Cassia fistula* L., pod powder at higher treatment (40 mg/ml) probably had been acting as mitochondrial poison disrupting root metabolic activity vis a vis ETS chain in root mitochondria out of ROS accumulation.

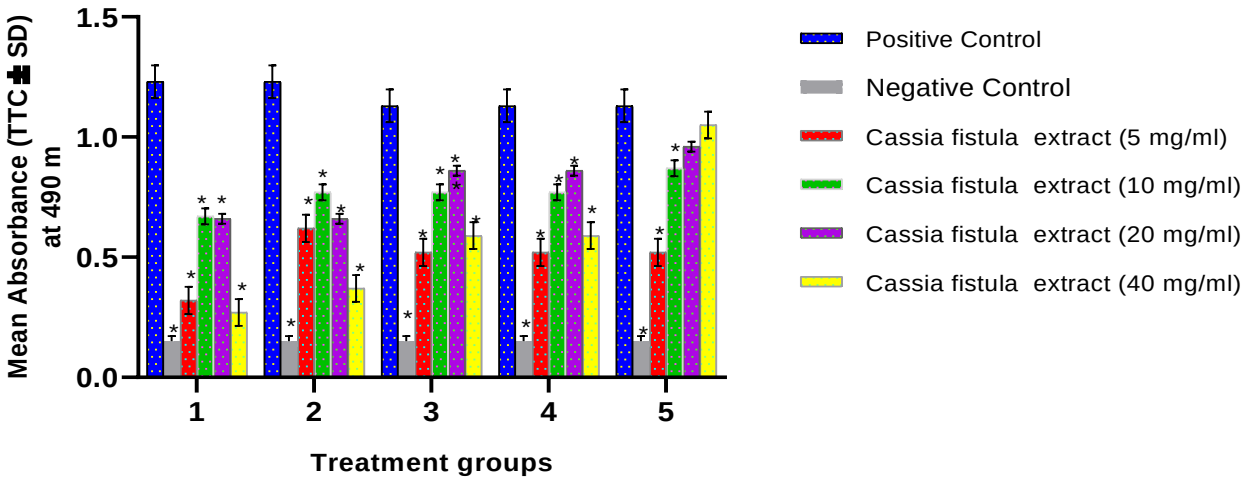


Figure 7. Determination of root metabolic activity (dehydrogenase activity by TTC staining, i.e., root mitochondrial activity) after pretreatment with different extracts of *Cassia fistula* L., pod powder (24 hrs) in growing roots of *Allium sativum* L.,

(*NC) Negative control - Distilled water; (PC) Positive control – 0.2% hydrogen peroxide showing the mean O.D. of formazan produced in root tissues of Allium sativum L., after 24 hours of growth due to root dehydrogenase activity. P verses negative control (*=<0.001) following ANOVA and Dunnet's multiple comparison test with each dose with positiver control in each treatment groups. (1= Cassia fistula L., Aqueous extract; 2= Cassia fistula L., Methanolic Extract; 3= Cassia fistula L., Ethanolic Extract; 4= Cassia fistula L., Chloroform Extract; and 5= Cassia fistula L., Petroleum Ether Extract)*

The impact of varying amounts of powdered *Cassia fistula* L., pod methanolic extract on the root metabolic activity (dehydrogenase) of developing *Allium sativum* L., roots produced interesting results. In the current investigation, TTC staining was used as a biomarker to assess how plant extract affected mitochondrial metabolism. Mitochondrial enzymes convert 2, 3, and 5-triphenyl tetrazolium chloride to red formazan in TTC. The staining and absorbance decreased in comparison to the positive control (*p<0.001), indicating a dose-dependent decrease in mitochondrial activity (Graph 3). The positive control displayed the highest level of mitochondrial activity, while the negative control stayed unstained and had the lowest absorbance. When comparing the treatments to the 40 mg/ml pretreatment, it was discovered that at 5, 10, and 20 mg/ml, the root metabolic activity (dehydrogenase activation) increased to above 50% activity, but it was less than the positive control (0.2% H_2O_2 treatment). However, compared to both negative and positive control sets, there was a notable decrease in root metabolic activity at the highest dose, or 40 mg/ml pretreatments, most likely as a result of cellular poisoning and disruption of dehydrogenase activity. From this result, it may be deciphered that Methanolic Extract of *Cassia fistula* L., fruit powder at higher treatment (40 mg/ml) probably had been acting as mitochondrial poison disrupting root metabolic activity vis a vis ETS chain in root mitochondria out of ROS accumulation.

The impact of varying doses of powdered *Cassia fistula* L., pod ethanol extract on the activity of the enzyme dehydrogenase in the developing roots of *Allium sativum* L., produced varying responses. In the current investigation, TTC staining was used as a biomarker to assess how plant extract affected mitochondrial metabolism. Mitochondrial enzymes convert 2, 3, and 5 - triphenyl tetrazolium chloride to red formazan in TTC staining. The staining and absorbance

increased in comparison to the negative control (*p<0.001) (Graph 3) but were less than the positive control set, indicating a dose-dependent increase in mitochondrial activity. The positive control displayed the highest level of mitochondrial activity, while the negative control stayed unstained and had the lowest absorbance. When comparing the treatments to the 40 mg/ml pretreatment, it was discovered that at 5, 10, and 20 mg/ml, the root metabolic activity (dehydrogenase activation) increased to above 50% activity, but it was less than the positive control (0.2% H_2O_2 treatment). Root metabolic activity (dehydrogenase activity) increased significantly even at the highest dose, 40 mg/ml pretreatments, when compared to both negative and 5 mg/ml pretreatment sets. From this result, it may be deciphered that Ethanolic Extract of *Cassia fistula* L., pod powder at higher treatment (40 mg/ml) could not found to be active in disrupting root metabolic activity via mitochondiral ETS chain as compared to Aqueous and Methanolic extract pretreatment in the same dose regime.

The toxic effects of varying doses of powdered *Cassia fistula* L., pod chloroform extract on the root metabolic activity (dehydrogenase) of developing *Allium sativum* L., roots produced varying outcomes. In the current investigation, TTC staining was used as a biomarker to assess how plant extract affected mitochondrial metabolism. Mitochondrial enzymes convert 5-triphenyl tetrazolium chloride to red formazan in TTC. The staining and absorbance increased in comparison to the negative control (*p<0.001) (Graph 3) but were less than the positive control set, indicating a dose-dependent increase in mitochondrial activity. The positive control displayed the highest level of mitochondrial activity, while the negative control stayed unstained and had the lowest absorbance. In the case of treatments, it was discovered that pretreatment at 5, 10, 20, and 40 mg/ml increased root metabolic activity (dehydrogenase activation) to above 50% activity, but less than the positive control (0.2% H_2O_2 treatment). In contrast to negative control sets, there was a notable increase in root metabolic activity (dehydrogenase activity) even at the maximum dose, i.e., 40 mg/ml pretreatments. This finding indicates that, when compared to aqueous and methanolic extract pretreatment in the same dose ranges, chloroform extract of *Cassia fistula* L., fruit powder at all pretreatment doses significantly improved the root metabolic activity. Therefore it may be said that at higher dose (40 mg/ml pretreatment) Chloroform Extract of *Cassia fistula* L., pod powder had not been a mitochondrial posion as like Aqueous and Methanolic extract pretreatments.

The influence of different doses of *Cassia fistula* L., pod powder Petroleum Ether Extract on root metabolic activity (dehydrogenase) activity in developing *Allium sativum* L., roots resulted in attenuated mitochondrial toxicity. In TTC staining mitochondrial enzymes convert 2, 3, and 5-triphenyl tetrazolium chloride into red formazan. The results showed a dose-dependent increase in mitochondrial activity, as indicated by an increase in staining and absorbance, as compared to the negative control (*p<0.001) (Graph 3). Formazan formation was marginally lower than the positive control set at the maximum dose of pretreatment, although it was nearly equal to the positive control. The positive control displayed the highest level of mitochondrial activity, while the negative control stayed unstained and had the lowest absorbance. It was discovered that after 5, 10, and 20 mg/ml pretreatment, root metabolic activity (dehydrogenase activation) increased to about 50% activity, but less than that of the positive control (0.2% H_2O_2 treatment). In contrast to negative control sets, there was a notable increase in root metabolic activity (dehydrogenase activity) even at the maximum dose, i.e., 40 mg/ml pretreatments. From this result, it may be deciphered that Petroleum Ether Extract of *Cassia fistula* L., pod powder at all pretreatment doses significantly increased the root metabolic activity in comparison to Aqueous and Methanolic extract pretreatment in the same dose regimes. Therefore it may be said that at higher dose (40 mg/ml pretreatment) Petroleum Ether Extract of *Cassia fistula* L., pod powder had not been a mitochondrial posion as like Aqueous and Methanolic extract pretreatments.

From these comparative results it was found that Aqueous and methanolic extract of *Cassia fistula* L., pod powder produced maximum inhibition of dehydrogenase acitivity and subsequent mitochondrial poisoning in comparison to Ethanolic, Chloroform and Petroleum Ether Extracts in the same doses.

4. Discussion

Medicinal herbs have been used for treating a variety of illnesses since ancient times. *Cassia fistula* L., *i*s one such plant that has been used in traditional medicine to treat a range of ailments.

In this study, we examined the phytochemical composition and antibacterial capabilities of *Cassia fistula* L., extracts. Phytochemical analysis revealed a wide range of compounds, including alkaloids, phenolics, terpenoids, and flavonoids. These compounds have a number of known pharmacological properties, including antibacterial activity. Depending on their polarity, different phyto-constituents are soluble to varying degrees in various solvents. Water is primarily utilized as a solvent in a conventional environment to create these mixtures [34]. According to Chatepa et al. (2024) [35], methanol has been described as an effective solvent due to its ability to dissolve phytochemicals such as alkaloids, flavonoids, tannins, and saponins. Secondary metabolites of plants, phytochemical elements like alkaloids, flavonoids, tannins, phenols, saponins, and several other fragrant chemicals act as a defense against numerous microbes, insects, and other herbivores. Plants are known to generate flavonoids, which are hydroxylated phenolic substances, in response to microbial infection [36]. By inhibiting the synthesis of bacterial cell walls and enzymes, phenolics, alkaloids, flavonoids, and terpenoids have been demonstrated to have antibacterial effect. As a result, we discovered potent antibacterial properties in the *Cassia fistula* L., methanol extract. Strong antioxidant, antibacterial, and antifungal properties have been reported for plant phenolic compounds [37, 38]. There were notable phenolic presences in both the aqueous and methanolic extracts of the *C. fistula* leaf powder, which are consistent with the previously published research.

Numerous researches have revealed that the flavonoids derived from plant extracts have anti-inflammatory, antibacterial, and antioxidant qualities. According to Takó et al. (2020) [39], phenolic contents were emphasized as extremely potent antibacterial agents that directly affect microbial systems by neutralizing them and harming fungal hyphae. According to a different study, plant extracts high in flavonoids from several plants have antibacterial properties [40]. Plant extracts inhibit the growth of microbial pathogens by a variety of methods, including as damage to the cytoplasmic membrane, loss of energy activity, and disruption of cell membrane function [41]. Researchers [42, 43] have found that phenolic components, which may interact with free radicals through hydrogen or electron exchange, are closely linked to plant extracts' antioxidant ability. Accordingly reports revealed that to the flavonoids may have a direct impact on the antibacterial and antioxidative actions [40].

Given that tannins are known to have strong antibacterial properties, the presence of tannins in all extracts may account for their strong bioactivities [44]. Antimicrobial activity of the saponins has already been demonstrated [45]. According to some research, bioactive saponins' antibacterial qualities could be connected to how they interact with cell membranes [46]. Protein synthesis is hampered by tannins' binding to proline-rich proteins [47]. According to experiments, saponins can alter the morphology of microbial membranes and potentially compromise their integrity [48]. According to some research, hydrophobic saponins have a greater antibacterial effect because they can attach to microbial cell membranes more readily [46]. The capacity of saponin to induce protein and specific enzyme leaks from the cell accounts for its antimicrobial properties [49].

Citations from the literature indicate that the various plant parts of *Cassia fistula* L., are an important source of secondary metabolites that have been shown to have antibacterial action and can be used to treat a variety of ailments. Proanthocyanidins are widely distributed throughout *Cassia fistula* L., leaves, flowers, and pods. About 7.8% of *Cassia fistula* L., leaves contain phenolic chemicals [50]. The leaves and fruits of *Cassia fistula* L., include sennosides B, tannins, oxyanthraquinones, oxalic acids, and anthraquinones [51]. Leaf cuticular wax has been found to include a number of other secondary metabolites, including hextriacontanoic, triacontanoic, nonacosanoic, and heptacosanoic acids [52]. The roots of *Cassia fistula* L.**,** include rhamnetin 3-O-gentiobioside [53], β-sitosterol, stigmasterol, β-sitosterol-3-O-βglucopyanoside, lupeol, betulinic acid, and fistucacidin [54]. Polyphenolic substances such as alkaloids, flavonols, proanthocyanidin, xanthones, and anthraquinones are widely distributed in stem bark [50]. The flower extract contains a variety of essential oils, including 2-hexadecane and (E) nerolidol [52, 55]. Linoleic acid, oleic acid, stearic acid, and palmitic acid are the main fatty acids present in *Cassia fistula* L., seeds; myristic acid and caprylic acid are present in smaller amounts [52] . The aril of *Cassia fistula* L., seeds contains a variety of substances, including triterpene, lupeol, emodin, physcion, citreorosein, rhein, ziganein, coumarins, scopoletin, chromones, isovanillic acid, and vanillic acid [56]. Aqueous, methanolic, and chloroform

extracts from *Cassia fistula* L., leaves were examined for their ability to screen for antibiotic activity against *P. mirabilis, K. pneumoniae, E. coli, S. aureus*, and *B. cereus*. The entire set of examined organisms is susceptible to strong antibacterial activity of the methanol extract from leaves, according to the results. The chloroform extract efficiently suppressed *B. cereus, S. aureus, E. coli, K. pneumoniae, P. aeruginosa*, and *P. mirabilis* [57]. Finally, according to Khadim et al. (2016) [58], the aqueous extracts showed a moderate level of effectiveness against *P. mirabilis*, *K. pneumoniae*, *B. cereus*, and *S. aureus*. As of late, *Proteus vulgaris*, *Bacillus mycoides*, *E. coli*, *Mycobacterium smegmatis*, *B. subtilis*, *Klebsiella aerogenes*, and *P. aerogenes* have all been shown to be susceptible to the moderate antibacterial activity of *Cassia fistula* L., [59]. According to Valsaraj et al. (1997), *S. aureus* was inhibited at a concentration of 6.25 mg/mL, whilst *E. coli* and *Bacillus subtilis* were seen to be inhibited at a concentration of 12.5 mg/mL. Interstingly, in this experimental investigation it was found that the methanolic extract (10 mg/ml) of *Cassia fistula* L., brought forth superior antimicrobial activity in *E. coli* cells with the maximum inhibition zone (1.4 cm in diameter) followed by *Staphylococcus aureus* (1.2cm in diameter), followed by *Lactobacillus sp* (1.1cm in diameter) with least effects on *Pseudomonas aeruginosa* (0.9 cm in diameter) which is in harmony with previous reports.

The antibacterial activity of *Cassia fistula* L., fruit was observed using the agar dilution streak method. Only *E. coli* had shown a moderate amount of inhibition, while *B. subtilis* and *Staphylococcus epidermidis* did not and then it was argumented that extracts of this plant might be lacking broad antimicrobial activity but rather having effective against only some specific strains. Later, the ethanolic and methanolic extracts of *Cassia fistula* L., were reported to cause greater sensitivity in gram-negative bacteria than in gram-positive bacteria [29] too. According to Seyyednejad et al. (2014) [29], the extract may also have an impact on the protein synthesis pathways or outer membrane of Gram-negative bacteria, which may result in a suppression of bacterial cell density. Our findings are in line (table 3, fig. 1d) with the earlier findings, which indicated that the methanolic extract was the most effective in expressing antibacterial activity in *E. Coli* cells. Parts of the plant and secondary metabolites, like phenolic compounds, are responsible for most of *C. fistula's* antibacterial qualities [60]. According to reports [60], *Cassia fistula* L., is a rich source of several secondary metabolites, including steroids, anthraquinone, glycosides, saponins, triterpenoids, and flavonoids, which are also in charge of preventing the growth of certain bacterial strains. It was observed that the *Cassia* species showed significant activity against bacteria that are Gram-positive. According to Rizvi et al. (2009) [61], it was claimed that some substances, such flavonoids and polysaccharides, are primarily responsible for inhibiting the growth of bacteria.

Many tests using various techniques and approaches were created in order to maximize the effectiveness of the toxicity investigation. Studies showing the relationship between mutagenicity and carcinogenicity as well as the relationship between genotoxicity and both parameters served as the foundation for the development of these methods [62]. Since then, established procedures have been used to evaluate the potential for genotoxicity posed by the use of pharmaceuticals, food additives, pesticides, industrial and environmental chemicals, and natural products like medicinal plants and their oils vis-a-vis different solvent-mediated extracts for future drug developments [63, 64, 13, 20, 65, 66] and their biosafety evaluations. Every plant has the potential to be genotoxic fully in certain mechanistic cycles or partially, *in vivo* or *in vitro*, in addition to the solvent systems used to prepare the extract and the model organism used both where both can produce a translatable bifunctional diagnostic modality. One important component of ethnicity that needs to be thoroughly investigated is the innate knowledge of folkloric herbal medicine [67]. It has been shown that several common plants contain substances that can damage DNA [20, 64] in several model organisms.To cross-verify the safety profile of each extract, there are particular steps and processes known as "genotoxic assays." Numerous endpoints, including single- and double-strand breaks, point mutations, deletions, chromosomal aberrations, development of micronuclei, DNA damage and cell cycle interactions, nucleolar frequency and morphometric changes, are present in genotoxic experiments [69, 66]. The comet assay (CA), the micronucleus test (MN), the Ames test, the Salmonella/microsome assay [70], the Bacterial reverse mutation assay (BRM), the *Allium cepa* test (ACT), and the chromosomal aberration assay (ChA) are among the various tests [71], that have been found to be employed while

investigating these plants. In Addition to *Allium cepa* test, *Allium sativum* L., is another well-liked *in vivo* model, in which the root tip of the plant is examined for chromosomal aberration during the mitotic phase following treatments with different xenobiotics [72, 20]. The outcome of this present study is in conformity with other reports where genotoxicity assessment were done with different solvent extracts from *Euphoria hirta* [73], *Jatropha gossypiifolia* [74], *Curcuma caesia* [75], *Cymbopogon khasianus* [76], *Chaptalia* sp [77], *Cascabela thevetia* L., [20], *Cannabis sativa* L., [78], *Vitis vinifera* L., [65], and *Punica granatum* L., [66].

The *Allium sativum* L., root tip ana-teolophase experiment has been recognized as a respectable genetic model for assessing cytotoxic and genotoxic effects, including chromosome abnormalities, micronuclei, mitotic index, and mutational frequency during mitosis, in higher plants like *Allium cepa* L., The benefits of using *Allium sativum* L., root tips cells for evaluating the genotoxic effects of plant extracts were confirmed by the study's [79, 20]. In order to assess chromosomal abnormalities and mutational frequency, a variety of chromosome aberrations were taken into consideration in the four phases of mitosis (prophase, metaphase, anaphase, and telophase). Analysis of chromosome aberrations made it possible to assess the genotoxic effects as well as the clastogenic and molecular actions of the agents [80]. Significant information was provided by chromosomal abnormalities, which might be regarded as an effective test to look into the genotoxic potential of the examined treatments [71]. The disruption of the correct chromosomal arrangement at each stage or phase of cell division and obstruction in the phases of cell division may be the cause of the increase in chromosomal abnormality.After being exposed to varying concentrations of the extracts and fraction, *Allium sativum* L., cells' aberrant chromosome conditions may have resulted from the plant extract's genotoxic chemicals (polyphenols, tannins, phenolics, saponins, alkaloids, terpenoids, etc.). Chromosome abnormalities may result from suppression of spindle formation, disruption of DNA synthesis, or blocking of their production [81, 82]. These chromosome abnormalities may result from blocking prophase, stopping one or more mitotic phases, or slowing down the pace at which cells go through mitosis [69]. Chromosome stickiness, bridges, c-mitosis, and vagrant chromosomes were the chromosome abnormalities seen in the current investigation at all treatment doses. These abnormalities may potentially have been caused by the extract's impact on spindle formation, which disrupted cell division. Stickiness and disruption chromosomal aberrations were the most common ones seen. Cell division disruptions were caused by these abnormalities, which were brought on by the extract's impact on spindle formation. Chromosomal fragments resulting from chromosome breaks that show the clastogenic effect; on the other hand, aneuploidy risk is increased by bridges and c-metaphases [83]. According to previous reports chromosome bridges provide evidence of the clastogenic impacts of chromosome breakage, vagrant chromosomes, and c-metaphases, which also raised the chance of aneuploidy [82].The observed stickiness in prophase and metaphase cells, as well as the bridges in anaphase and telophase cells, may be the consequence of incorrect chromosomal fiber folding, which leads to subchromatid bridges connecting the chromatids. Bridges between chromosomes were seen in both telophase and anaphase in this experimental set up (plate 4c and 4d: fig 2). According to Yuet Ping et al. (2012) [73], chromatids or subchromatids broke and fused to generate the bridges observed in the cells whereas a proportionately low frequency of c-mitosis that could be linked to the spindle apparatus's inability to properly assemble and function [84]. Binuclear cells that were produced with significant frequency are indicative of the ability of one ingredient of *Cassia fistula* L., extract to interfere with spindle and cell wall formation. At 20 and 40 mg/ml of *Cassia fistula* L., aqueous extract, the proportion of micronuclei cells was clearly higher than the control ($p < 0.01$). Micronuclei are defined as segments of chromosomes or entire chromosomes that are lagging at anaphase and consist of cytoplasm encircled by a membrane [85]. Chromosomes with sticky ends suggested an extremely harmful and permanent impact that might perhaps result in cell death [86, 82, 87]. It is likely that chromatids or subchromatids broke and fused to generate the bridges observed in the cells [88, 89]. Uneven chromosomal distribution resulting from nondisjunction of chromatids in anaphase may be the cause of stray chromosomes that were not grouped to a particular stage of mitotic division [90]. The observation of apoptotic and ghost cells in the NA concentrations may be attributed to the detrimental impact of the plant extract's allelochemicals on the nuclear components of

Allium sativum L., roots. This may have something to do with the extract's and fraction's ability to restrict growth, as seen in this investigation. Several plant secondary compounds have a well-established history of obstructing microtubules, vesicle transport, and cell division cycle [91]. The extract may damage or alter nucleic acid structure, preventing cell division and elongation, which could have an impact on the plant's ability to grow.

The microscopic investigation discovered a large-scale cell death known as pyrosis, which is the irreversible condensation of chromatin in the nucleus of a dying or necrotic cell. The nucleus's fragmentation occurs next (Karryorrhexis). The chromatin condenses into numerous pieces or a solid mass lacking structure, while the nucleus shrinks in size (Karyolysis) (plate 1D, 2D, Fig: 2). Previous studies [88, 20, 65, 66] produced results that were comparable. At the non-proliferative G0/G1 phase, the dividing cells were blocked, and the expression of the cell cycle protein clearly decreased in the proliferative S phase cells.Due to the creation of strap nuclei inside the large ghost cells, these modifications may increase the rate of apoptosis [20]. In the meantime, morphology—which showed the cell nucleus condensing and pyknosis with vivid staining—proved the occurrence of rising cell apoptosis [92, 20, 33]. When taken as a whole, these findings provide compelling evidence for the carcinogenic and cytotoxic properties of the watery extract of *Cassia fistula* L. The mitotic index, which calculates the percentage of cells in the mitotic phase of the cell cycle, is used as a biomarker for cell proliferation [66, 93]. Therefore, it is possible to interpret the decline in the mitotic index of *Allium sativum* L., meristematic cells as cellular death. This study used the mitotic index to assess the genotoxicity of treating *Allium sativum* L., with *Cassia fistula* L., extract. Following treatment of *Allium sativum* L., root tips' meristem cells with *Cassia fistula* L., extracts, the mitotic index reduced as treatment concentration and duration increased. When comparing the mitotic index at high concentrations (5, 10, 20, and 40 mg/ml) of *Cassia fistula* L., to the control, there were significant changes ($p < 0.01$) (Fig 2. Graph: 1). The mitotic index's depression effect implies that *Cassia fistula* L., extract may have had some effect on stopping cells from entering prophase, stopping one or more mitotic phases, or slowing down the pace at which cells undergo mitosis [94]. This type of induction causes a molecular alteration in the genetic material, which may indicate a DNA lesion or interfere with the cell cycle, stopping the phases of cell division, as stated by Clay et al. (2021) [95]. One or several metabolite components of *Cassia fistula* L., extracts may be connected or intercalated with proteins (histones) or DNA nucleotides in interphase to cause a shift in mitotic phases [96], showing the overall mitodepressive action altogether. The immense possibility of *Cassia fistula* L., aqueous extract to bring forth any instant epigenetic modifications [97] out of abiotic stress, thus disturbing cellular homeostasis though ROS outburst vis-à-vis generation of heat shock protein pools [98] and thereby modifying the histone groups in *Allium sativum* L., through deployment of structural alterations in finer chromatin organization must not be also completely ruled out.

Another pertinent plant-based physiological marker for determining the degree of water stress in plants is electrolyte leakage. The stability and integrity of the cell membrane under stress are measured indirectly [99]. A less stable cell membrane results from increased electrolyte leakage. The cell membrane is an essential barrier, and when it breaks down, the cell may die [100]. Under stressful circumstances, oxidative breakdown and ROS buildup degrade the lipid bilayer, resulting in electrolyte leakage [101]. Cell membrane integrity and permeability can be changed by allelochemicals found in plant aqueous extracts, which can cause electrolyte leakage [102]. Cell membrane damage resulting from different biotic and abiotic stresses is commonly measured using this technique [31,33]. Certain investigations have linked the phytotoxic compounds found in plant extracts to a notable rise in the electrolyte leakage of plant cells. The electrolyte leakage and dose dependency of the aqueous and methanolic extract of *Cassia fistula* L., pod powder showed a strong inverse correlation in the current study (Fig. 3; $p < 0.01$), indicating that the allelochemicals effectively inhibited cellular viability by causing damage to the permeability of the cell membrane to electrolytes, which in turn led to cell death. It is well known that allelochemicals, including flavonoids, block the mitochondrial membrane's electron transport chain [103, 104]. The author noted that phenolic substances, including benzoic and cinnamic acids, can modify the integrity and permeability of cell membranes by causing structural alterations in the proteins that make up the membrane [105]. The notable electrolyte outflow in the quantities

examined suggests that the application of *Cassia fistula* L., aqueous and methanolic extracts to *Allium sativum* L., roots may have changed the permeability of the cell membrane.

Numerous stresses can cause damage to the cell membrane, and one of the primary variables influencing cell injury is the lack of membrane integrity response. In fact, Kaur et al. (2010)[106] found that modifications to membrane permeability have an impact on every major physiological and biochemical mechanism associated with membrane function. Tanatson et al. (2013) [107] demonstrated that the loss of membrane integrity caused by *Cymbopogon citratus* essential oils results in an increase in electrolyte leakage from the roots of barnyard grass (*Echinochloa crus-galli*), increasing its permeability. According to studies by Kaur et al. (2010)[106], electrolyte leakage causes certain terpenes to suppress plant development which shows prominent hindered effects on cell division and differentiation, gene expression and signal transduction, and truncated cell membrane permeability [108]. . In fact, a rice cultivar's electrolyte leakage has increased due to alleolopathic effects elvolic acid, a terpenoid, [109] in root cells. It has been reported that a number of additional steroidal chemicals have increased the rate at which numerous weeds and crops of various tissues leak electrolytes [110]. Strong electrolyte leakage and elevated lipid peroxidation [111] were indicators of the plasma membrane destruction in lettuce germinated seeds and roots caused by allelochemicals found in the aqueous extracts of diploid and mixoploid fenugreek [112]. Furthermore, the pigment concentration and mitochondrial respiration have been reduced in seeds, roots, and leaves by these allelochemicals. When aqueous extracts are present, the growth of lettuce seedlings is reduced due to all of these disruptions.

Thus, some examples of secondary metabolites that are important in reducing oxidative stress are total phenols, total flavonoids, and total alkaloids, which are present as a concoction in organic extracts. This harmful effect may result from the loss of membrane integrity brought on by increased electrolyte leakage [113]. A decrease in membrane permeability could be caused by malondialdehyde (MDA) and other products of polyunsaturated fatty acid peroxidation in the biomembranes [114]. It has recently been shown that measurements of electrolyte leakage may be associated with a number of physiological and biochemical markers that regulate how plants respond to their surroundings [115]. It has been documented in the literature that many allelochemical stressors initially target cell membranes. According to Wang et al. (2009) [116], the leachate of *Jatropha curcas* leaves increased the electrolyte leakage in marigold roots, hence inducing an allelopathic stress. More recent research indicates that a disruption in membrane integrity is the cause of the increased electrolyte leakage from *Echinochloa crus-galli* roots under *Cymbopogon citratus* essential oil stress [117]. According to Ladhari et al. (2014)[113], lettuce's cytological, physiological, and biochemical processes were impacted by the allelochemical stress of aqueous (15 g/L) and methanol (6 g/L) extracts of the leaves of *Capparis spinosa* L. and siliquae of *Cleome arabica* L. The findings demonstrated that aqueous extracts mostly had a cytotoxic impact on root tip cells, causing morphological changes and necrotic events. This was connected with a significant decrease in the mitotic index. Indeed, the presence of allelochemicals in these extracts caused oxidative damage to lettuce, which was characterized by lipid peroxidation and a rise in malondialdehyde content. A substantial electrolyte leakage indicated the rupture in the membrane permeability in reaction to this. So from the present finding it may be concluded that the aqueous and methanolic extract of *C. fistula* L., pod powder definitely brought about *Allium sativum* L., root cell membrane damage and which ultimately resulted in profuse electrolyte leakage as a combined effects of phenols, total flavonoids, and total alkaloids (Table: 2; Figure). An essential process during germination is cellular respiration, which gives the embryo a supply of ATP so it can restart its metabolic processes. The inhibition of germination in the presence of plant extracts may be due to the development of roots or reduced respiration in seeds. Scientific literatures [118, 119] reported on the impact of allelochemicals or plant extracts on breathing. The reduction in dehydrogenase activity may indicate cellular harm from exposure to allelochemicals found in fenugreek extracts. One measure of mitochondrial respiration is the formazan level. A common method for measuring mitochondrial dehydrogenase activity is to assess the decrease in TTC (2,3,5-triphenyl tetrazolium chloride). According to Rashid et al. (2010) [119], the formazan level in the roots of radish and lettuce was lowered by the aqueous and methanol extracts from *Pueraria Montana* (Fabaceae). The extracted phenolic acids from *Saccharum*

officinarum (Poaceae) leachate, [118], decreased the formazan rate in lettuce roots. These authors link the suppression of root growth to a decline in ATP synthesis caused by a drop in dehydrogenase activity. This present study's findings demonstrated that pretreatment with an aqueous and methanolic extract of *C fistula* L., pod powder reduced mitochondrial respiration in the roots of *Allium sativum* L., more than pretreatment with an ethanolic, chloroform, and petroleum ether extract (Fig: 4). Previous research has established that some allelochemicals may impact plant growth by altering the respiration mechanism. This decrease may be attributed to decreased oxygen intake, suppression of mitochondrial ATP generation, and eventually disruption of plant oxidative phosphorylation [120]. Furthermore, the primary impact of active substances on respiratory metabolism has been shown to be the blockage of the electron transfer chain and the lowering of the mitochondrial membrane potential [121]. Research has shown that a number of flavonoids exhibit the following characteristics: they exhibit an uncoupling activity [122], inhibit oxidative phosphorylation [123], inhibit electron flow at the level of complexes I and III [124], inhibit phosphate uptake [125], and inhibit the activity of exogenous NADH-dehydrogenase [126]. Furthermore, it has been shown that phenolic acids block a variety of processes involved in mitochondrial metabolism, including phosphate and Ca^{2+} transport, electron flow, and L-malate oxidation [127]. Salicylic acid functions as an uncoupler at low concentrations (< 1.0 mM) hence could stimulate the ADP-limited electron transport. However, at greater concentrations, it prevents the ubiquinone pools and dehydrogenases from losing electrons [128]. Monoterpenes accelerate respiration and decrease respiratory regulation, as established by Abrahim et al. (2000, 2003a) [129, 130], indicating that they function as uncouplers. On the other hand, when ROS production is elevated, they have the potential to build up and cause harm to the cells. Peroxidation of membrane lipids, oxidative damage to proteins and DNA, and the opening of the mitochondrial permeability pore—which is linked to apoptotic cell death—are some examples of this damage [131, 132, 133] . However, several factors have been demonstrated to assist in this process, including membrane potential reduction [134], ATP depletion associated with Ca^{2+} influx [135], ROS-induced membrane permeability transition (MPT), and elevated intramitochondrial Ca^{2+} levels [136]. Similar signaling pathways have been connected to both apoptotic and necrotic cell death. Since several allelochemicals have been demonstrated to interact, allelochemicals may act either directly or indirectly by increasing oxidative stress and ultimately inducing necrosis or apoptosis affecting mitochondria. Based on certain facts, maize plant roots experience oxidative stress due to α-pinene. It incites KCN-insensitive respiration in intact tissues; in isolated mitochondria, it decouples electron transport from ATP synthesis, lowers membrane potential, and blocks alternative oxidase routes [137]. It has been demonstrated that each of these actions results in the formation of ROS, and several medicines that cause apoptosis also share these properties [134].The increased levels of lipoxygenase activity observed in unharmed roots further corroborate this theory. Membrane damage from the peroxidative attack may liberate polyunsaturated fatty acids and other substrates for lipoxygenases [138, 114]. Lipoxygenase activity has been shown to be associated with necrosis, apoptosis, and cellular proliferation [139]. In conclusion, we hypothesize that allelochemicals present in *Cassia fistula* L., pods and leaves are equipotentially able to damage mitochondria, leading to oxidative stress, necrosis, or apoptosis in cells, which is detrimental to microorganisms or plants.

5. Conclusion

According to published research, *Cassia fistula* L., ethanol, methanol, and aqueous extracts include a variety of groups of phytochemicals with antimicrobial activity, including rhein, saponin, triterpenoids, glycosides, anthraquinone, steroids, flavonoids, and β-sitosterol. The extract of *Cassia fistula* L., possesses the ability to inhibit the growth of *E. coli* due to its distinct chemical makeup or the synergistic effects of its ingredients. This work suggests that *Cassia fistula* L., may be used to identify phytocompounds of pharmacological value that may be used to treat various bacterial illnesses. The preparations of *Cassia fistula* L., organic extract contain a variety of phytochemicals, including phenolics, terpenoids, alkaloids, and flavonoids. The varied extracts showed the strongest antibiotic effect when tested bacteria were exposed to methanol. These findings suggest that *Cassia fistula* L., has a strong marketable potential to be explored and applied as a natural resource of antibacterial compounds as well as a could open up a potential avenue for the development of novel antibacterial drugs. Further investigation is needed to isolate

and identify the active compounds responsible for the antibacterial effect that has been described. The data obtained strongly imply that *Cassia fistula* L., leaves and pods are clastogenic, mutagenic at high doses, and maybe anti-carcinogenic at low doses. More research on this plant's potential as an anti-cancer agent and the identification of safe and effective dosages are necessary before it can be used safely in traditional medicine.

The high rate of fragmented cells in the root tip cell bioassay of *Cassia fistula* L., is indicative of its mutagenic and carcinogenic properties, which are mediated via ROS generation-induced DNA oxidation and electrolyte leakage in root cell membranes. Oncogene activation is seen in the induction of more ghost cells and less frequent apoptosis. The propensity for defective cells to proliferate is demonstrated by the development of various clastogenic chromosomal defects in vitro and the appearance of hyperpolid ghost cells with strap-like nuclei. As an assay plant, *Cassia fistula* L., demonstrated greater sensitivity as a genotoxin. It is advised that extracts from *Cassia fistula* L., be used infrequently.The fruit of *Cassia fistula* L., (Amaltas) is also used to treat leukoderma, pruritus hematemesis, diabetes, and other conditions[140, 141]. The potential for dose- and time-dependent genotoxic effects is still unscreened for. Strong cytotoxins known as antitumor plant extracts are typically employed in nuclear medicine to eradicate damaged cells and prevent the growth of decayed ones in the wake of cancer. On the other hand, healthy cells may sustain double stranded DNA damage and mutation from the same dosage. Fruit extract from *Cassia fistula* L., (Amaltas) is cytotoxic and can halt mitosis.Consequently, it is necessary to avoid using alternative therapeutic plant parts that have the potential to be genotoxic. Numerous researches have demonstrated cytotoxic properties for the fruit extract of *Cassia fistula* L., (Amaltas), including anti-fungal, antibacterial, laxative, anti-inflammatory, and anti-tumor properties. It has been observed that consumption of fruit extract from *Cassia fistula* L., (Amaltas) causes liver damage in albino rats[141]. According to Yang et al. (2019)[143], pyrrolizidine, a well-known alkaloid of *Cassia fistula* L., and its N-oxide derivatives are cytotoxic. In the ileum of mice and guinea pigs, anthraquinone, glycosides, and Rhein component of *Cassia fistula* L., have been shown to be cyto-genotoxic [144]. It has been determined that evaluating the hazardous qualities of *Cassia fistula* L., is crucial for protecting the public's health in order to prevent DNA damage that could cause consumers to become cancerous [145]. As a result, this indirect strategy using a plant assay was tried to forecast potential dose-dependent genetic harm to humans. The current research will help with *Cassia fistula* L., dosage formulation and appropriate administration, which may lower the occurrence of cancer.

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