# Bio Synthesis of Silver Nanoparticles from Leaf Extract of Cassia Auriculata Flower Extract and its Antioxidant, Antimicrobial, Anticancer Activities

Dr.P.Gowsalya, M.Sc., M.Phil., Ph.D., PGDCA[1], Ms.B.Gokulpriya M.Sc., DBMI[2]

[1] *Associate Professor and Head, Biochemistry, Navarasam Arts and Science College for Women, Tamilnadu, India*

[2] *PG Scholar, Biochemistry, Navarasam Arts and Science College for Women, Tamilnadu, India*

## ABSTRACT

*Cassia auriculata Linn commonly known as Tanners Senna, is also known as Avaram tree. Senna auriculata is a distributed thorough hot deciduous forest of India. Cassia auriculata is an evergreen shrub that grows in many parts of India and in other parts of plants. The genus cassia comprises over 500 species, is a large group of flowering plants in the family Fabaceae. It includes almost 751 genera and about 19,000 different species, which are widely, distributed in the dry forests of different regions mainly Africa, America and tropical rainforests (Burnham & Johnson, 2004; Christenhusz & Byng, 2016). The flower, buds, leaves, stem, root and unripe fruit are used for treatment especially in Ayurvedic medicine. Cassia plants exhibit pharmacological activities at large scales such as antimicrobial, anticancer, anti inflammatory, antioxidant, hypoglycemic, hyperglycemic, anti mutagenic, and antiviral.*

**Keyword:** - *Cassia auriculata, Fabaceae, Tanners senna, and Avaram etc….*

## 1. INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use.Medicines derived from plants are widely famous due to their safety, easy availability and low cost (Iwu et al., 1999). Herbal medicines may include whole parts or mostly prepared from leaves, root and seed.

Tanner`s Cassia, Tanner`s Senna, awal, Tarval (Hindhi), Tarwad (Marathi), Manojyna, Charmaranga (Sanscrit), Mature Tea Tree(English), Tangedu, Pitapuspua, Avartaki, Pitkala, Taravada, Merakatangeedu (Telugu), Arusal. The flower, buds, leaves, stem, root, and unripe fruit are used for treatment especially in Ayurvedic medicine.

## 2. LITERATURE REVIEW

The review of literature of the study on " Bio Synthesis Of Silver Nanoparticles From Leaf Extract Of Cassia Auriculata Flower Extract And Its Antioxidant, Antimicrobial, Anticancer Activities" are under discussing the following.

### 2.1 CASSIA AURICULATA

Cassia auriculata is a lowland trophical evergreen tree, belonging to the fabaceae (Caesalpiniaceae) comprising approximately 500 genera and19,000 species, and is widely grown distributed throughout hot deciduous forests of India.

**Fig- 1: Cassia auriculata**

Avaram Senna (Cassia auriculata) is a common tree in Asia, which has been widely used in Indian traditional medicine. The tree grows wildly and foun abundantly near the river beds. Avaram Senna flower, which is called as Tanner`s Cassia is bright yellow in color. The dried flowers and buds have many therapeutic benefits. In Ayurveda, Avaram Senna flower is used for treating diabetes. The wonder flower gives longevity to life. Avaram Senna flower is widely used as a home remedy for skin disorders and body odor. It is also used for the treatment of pain, fever, urinary tract disorders, rheumatism, conjunctivitis, ulcers and liver diseases. Consumption of Avaram Senna flowers in the form of tea lowers blood sugar level and flowers have properties that prevent urinary tract infection, especially in women. It acts as a detoxifying agent and excretes harmful toxins from the body. Regulates menstrual cycle and prevent excessive menstrual flow. Dried Avaram Senna flower is good for external application and helps treat many skin disorders (Shirley Johanna., 2018).

**2.2 GREEN SYNTHESIS**

Rapid industrialization and urbanization has led to the release of large amount of hazardous, poisonous and unwanted, chemicals, gases and substances causing only a great of damage to the environment but also making life miserable on this beautiful planet earth. So there is a need to explore and learn more about the ways and means that are present in nature which could lead to the advancement in the synthesis process of NP`S. Biological molecules are more suitable and less hazardous for nanotechnology applications, because of their exclusive properties. They assemble themselves in highly controlled manner for the synthesis of reliable and eco-friendly metal NP`S. Synthesis of metal NP`S is an important are to research due to their potential applications. Novel applications of NP`S and NP`S in the various fields are emerging rapidly.

Most of the chemical methods used for the synthesis of NP`S are too expensive and they also involve the use of toxic, hazardous chemicals that are responsible for various biological risks. This enhances the growing need to develop environment friendly processes through green synthesis and other biological approaches. "Green synthesis" of NP`S makes use of environment friendly and non-toxic reagents. Green nanotechnology is the utilization of various plant resources for the biosynthesis of metallic NP`S. The present review gives an overview of plant mediated synthesis of NP`S, possible biomolecules of the plants involved in it and various applications of NP`S in different fields.

A survey of green synthesis of thiazines and oxazines revealed the moiety have attracted a great deal of interest of medicinal chemist, biochemist, Pharmacologist and rendered as lead molecule for designing potential bioactive agents. This review accompanying supplementary green synthetic information & its references would extend great deal of help to researches in determining the best and most productive, economical, suggestive and clinically important compound of thiazine and oxazine derivatives which will be expected to show potent pharmacological activities. This has led to the discovery of a wide variety of compounds that re high interest from the point of view antimicrobial, antioxidant, anti diabetic, anticancer, antidepressant effects among others.

**2.3 PHYTOCHEMICAL ANALYSIS**

Pytochemicals (From the Greek word phyto) are chemicals produced by plants through primary or secondary metabolism. Biologically active and naturally occurring chemical compounds found in plants(Hasler CM, Blumberg JB.,1999). Versatile pharmacological effects of medicinal plants are basically dependent on their phytochemical constituents. Secondary plant metabolities are numerous chemical compounds produced by the plant cell through metabolic pathway derived from the primary metabolic pathways (Jones ME et al.,1953).

Modern chemistry has described the role of primary plant metabolities in basic life functions such as cell division and growth, respiration, storage and reproduction (Seigler DS et al.,1995). Phytochemistry takes into account the structural compositions of these metabolities, the biosynthetic pathways, functions, mechanism of action in the living systems. Accumulates in different parts of the plants, as in the roots, stems, leaves, flowers, fruits or seeds (Costa MA et al., 1999).

**2.3.1 Sterols**

They are considered bioactive, due to their recognized activity as antioxidants, anticarcinogenic, cardiovascular protectors and antiviral capacity. Without sterols does not exist cellular protection and organization (Deamer, D., 2017).

**2.3.2 Terpenes**

They are important plant metabolities. They include substances like floral fragrances, which serve as insect attractants, pine oil, growth inhibitors, two plant hormones, gibberelic acid and abscisic acid, and some which are insecticidal (Kaiser 1993).

**2.3.3 Saponins**

Saponins are widespread among plants, having been reported from more than 500 plants from at least 90 different families. These substances have been isolated from all parts of plants: leaves, stems, roots, bulbs, flowers and fruits, although they tend to be concentrated in the roots of many species (Assa Y, Shany S, et al.,).

**2.3.4 Alkaloids**

The term alkaloid is used as a name for plant- derived compounds, containing one or more nitrogen atoms, usually in a heterocyclic ring and which have a marked effect on animals, including humans. They are widely distributed in plants. It have the property that have work in nervous system of human body and used for antispasmodic and bacterial effects (Babjide et al.,), and (Okour et al., 2004).

**2.3.5 Carbohydrates**

Although carbohydrates are primary metabolites, they are incorporated in plenty of secondary metabolites through glycosidation linkages. Polymers of simple sugars and uronic acids produce mucilages and gums (Asif HM., et al., M 2011). 2.3.6 Tannin

Tannins are sequestered in vacuoles within the plant cell, which protects the other cell components. They occur normally in the roots, woods, bark, leaves and fruits of many plants particularly in the bark of oak (Quercus) species and in sumac (Rhus) and myrobalan (Terminalia chebula). They also occur in galls.

**2.3.7 Flavonoids**

Its chemical structure contains a cyclic carbon ring instead of being merely straight or branched chains. They also occur in galls. Flavonoids are commonly recommended because of their antioxidant activity. Is a potent antioxidant with anti allergic and anti-inflammatory properties. Flavonoids have a wide a range of pharmacological activities (Lin et al., 2014).

**2.3.8 Lactones**

Sesquiterpene lactones (SLs) are a clas of sesquiterpenoids that contain a lactone ring. They are most often found in plants of the family Asteraceae(asters). Other plant families with SLs are umbelliferae(celery, Parsley, carrots) and Magnoliiaceae(Ghantous, Akram et al., 2010).

**2.3.9 Amino acids**

Amino acids though considered a product of secondary metabolite. Contrary to the observation that sterols are secondary metabolities that are indispensable part of many structural frame work of a cell (Yeoman MM et al).

**2.3.10 Resins**

Plant resins are secondary metabolites of complex mixtures, which include volatile and nonvolatile terpenoid and or phenolic compounds. They often exist in combination with essential oils (oleoresins), gums (gum resin), sugars(as glycosides), and benzoic and cinnamic acid(Andrew G. Mtewa et al., 2021).

**2.3.11 Strach**

Starch is the plant storage polyglucan that accumulates in plastids. It is composed of two polymers amylase and amylopectin with different structures and plays several roles in helping to determine plant yield. In leaves, it acts a buffer for night time carbon starvation (James R. et al., 2019).

**2.4 ANTIOXIDANT ACTIVITY**

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. They are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. In nature there are wide variety of naturally occurring antioxidants which are different in their composition, physical and chemical properties, mechanism of site of action (Prior RI et al., 1998. Antioxidant significantly delay or prevents oxidation of oxidizable substrates when present at lower concentration than the susbtrate (Halliwell B., 2007).

**2.4.1 DPPH Radical Scavenging Assay**

DPPH it a familiar abbreviation for the organic compound 2,2-diphenyl-1-picrylhydrazyl. DPPH is a dark colored crystalline powder Composed of stable free-radical molecules. It possess 2 major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most particularly it is a general antioxidant assay, and another is a standard of the position and intensity of electron paramagnetic resonance signals (Om P. Sharma and Tej K. bhat., 2009)

**2.5 ANTIMICROBIAL ACTIVITY**

Medicinal plants constitute a rich source of antimicrobial agents, as they protect itself from different various pathogens via the production of different chemicals including tannins, terpenes, and alakaloids (Obeidat et al., 2012).

2.5.1 Gram positive Bacteria

2.5.1.1 Staphylococcus aureus

Staphylococcus aureus is a gram positive bacterium which is one of the major cause of human infections of the skin, soft tissues, bones and joints as well as normal heart valves (Karlowsky et al., 2003). MRSA (Methicilin Resistant Staphylococcus aureus) it is a multi resistant strain that has been documented worldwide showing rising resistance to different classes of antimicrobials (Ness, 2010).

2.5.2 Gram negative Bacteria

2.5.2.1 Escherichia coli

Also known as E. coli is a gram negative bacteria, facultative anaerobic, rod shaped coliform bacterium of the genus E. coli that is generally found in the lower intestine of warm-blooded organisms (Wells,J.C., 2000). One of the most persistent causes of many common bacterial infections, cholescystitis, urinary tract infection and other clinical infections.

**2.6 ANTICANER ACTIVITY**

Cancer is the leading cause of mortality worldwide. At national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer(within 5 years of diagnosis) in 2012 worldwide (GLOBOCAN 2012). This rapid increasing is due to both aging and growing population, along with carcinogens, infections, genetics mutations, hormones, immune conditions and adoption of dietary risk factors such as smoking, unhealthy diet.

Thus research has developed into investigating the potential properties and uses of terrestrial plants extracts for the preparation of potential nanomaterials based drugs for diseases including cancer (Sivaraj R, et al., 2014). Many plant species are already being used to treat or prevent development of cancer. Researchers have identified species of plants that have demonstrated anticancer properties with a lot of focus on those that have been used in herbal medicine. Traditionally, the in vitro determinations of toxic effects of unknown compounds have been

performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity in developing countries (Freiburghaus F et al., 1996).

Cassia auriculata derived compounds to a variety of anticancer effects including cytotoxity, induction of apoptosis, necrosis, and inhibition of proliferation on a variety of cancer cell lines, including breast, prostate, colorectal, lung leukemia, renal, pancreatic, hepatic, oral, melanoma, cervical and ovarian cancers.

**2.6.1 MTT Assay**

The MTT assay is a colorimetric assay for assessing cell metabolic activity (Stockert Jc., 2018) NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the umber o viable cells present.

## 3. AIM AND OBJECTIVE OF THE STUDY

### Aim

The current study is focused on " Biosynthesis of Silver Nanoparticles, Evaluate antioxidant, antimicrobial, anticancer activity of the flower extract of Cassia auriculata.

### Objective

- To collect the leaves of Cassia auriculata
- To synthesis Silver Nanoparticles
- To prepare the aqueous alcoholic extract of flower of Cassia auriculata using Harborne method
- To perform the qualitative analysis of phytochemical screening alcoholic extract.
- To determine the free radical scavenging of plant extract by DPPH (2,2- Diphenyl- 1- picrylhydrazyl).
- To study the antimicrobial activity of alcoholic extract of Cassia auriculata against the following bacterias.
  Staphylococcus aureus
  Escherichia coli
- To evaluate the anticancer activity by MTT Assay.

## 4. MATERIALS AND METHODS

The material and method of the present study on "Biosynthesis of Silver Nanoparticles from flower extract of Cassia auriculata and its Antioxidant, Antimicrobial, Anticancer activities are discussed under the following**.**

**4.1 SYNTHESIS OF AgNO3 NP'S**

**4.1.1 MATERIALS**

**4.1.2 Collection of samples**

**4.2 METHODS**

**4.2.1 PREPARATRION OF CASSIA AURICULATA LEAF EXTRACT**

All the glass wares are washed with normal water, distilled water and acetone. 10g of cassia auriculata leaf was taken in a 500ml of beaker of adding 100ml distilled water. The process was boiled at 75º C for 50 minutes. The solution is changed to yellow color the extract was filtered & stored at room temperature.

### 4.2.2 SYNTHESIS OF LEAF CAPPED AgNO3 NANOPARTICLES

To synthesized AgNO3 NP`S 10g of silver nitrate was taken into separated beaker with 100 ml of distilled water. Again the separated beaker with 100 ml of distilled water then added 10ml of leaf extract was stirred at 30 minutes. The 100ml leaf extract was mixed with AgNO3 solution and this mixture was stirrer for 30 minutes by using magnetic stirrer. After the 30minutes of progressing, the color of solution was changed from yellow to dark green is obtained. The synthesized sample was aging for 24 hours. Thus the settled precipitate was kept in microwave oven at 350 watts for 25minutes and the sample was kept into muffle furnace in 250 C for 4 hours. The dried sample was grained in a mortar and then fine leaf capped AgNO3 NP`S where obtained.

### 4.2.3 MICROWAVE IRRADIATION METHOD

Microwave radiation is an alternative to conventional heating as a method to introduce energy into reactions. Microwave heating exploits the ability of some compounds (liquids or solids) to transform electromagnetic energy into heat. The use of microwaves as a mode of heating in situ has many attractions in Chemistry because, in contrast to conventional heating, its magnitude depends on the dielectric properties of the molecules. As a guide, compounds with high dielectric constants tend to absorb microwave radiation while less polar substances and highly ordered crystalline materials are poor absorbers. In this way absorption of the radiation and heating may be performed selectively. The use of microwave irradiation has led to the introduction of new concepts in Chemistry because the absorption and transmission of the energy is completely different from the conventional mode of heating. In addition, the shape and size of the sample in question can have an influence and these factors have an effect on the scale-up of some reactions.

### 4.2.4 CHARACTERIZATION TECHNIQUES

#### 4.2.4.1 SEM (SCANNING ELECTRON MICROSCOPE)

SEM stands for scanning electron microscope. Electron microscopes use electrons imaging, in a similar way that light microscope use visible light. SEM use a specific set of scan the beam in a raster-like pattern and use the electrons that are reflected or knocked off the near-surface region of a sample to form an image. Since the wavelength of electrons is much smaller than the wavelength of light, the resolution of SEMs is superior to that a light microscope.

#### 4.2.4.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectroscopy is an analytical methodology used in industry and academic laboratories to understand the structure of individual molecules and the composition of molecular mixtures. FTIR spectroscopy uses modulated, mid-infrared energy to interrogate a sample. T he infrared light is absorbed at specific frequencies related to the vibrational bond energies of the functional groups present in the molecule. A characteristic pattern of bands is formed, which is the vibrational spectrum of the molecule. The position and intensity of these spectral bands provide a fingerprint of molecular structure, making FTIR spectroscopy a highly adaptable and useful technique. FTIR spectroscopy is a great advance over the traditional dispersive infrared approach for a number of reasons including that the entire FTIR spectrum is collected in a fraction of a second and co-adding spectra, signal to noise is improved.

#### 4.2.4.3 X – RAY DIFFRACTION (XRD)

X-ray power diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimension. The analyzed material is finely ground, homogenized and average bulk composition is determined.

### 4.3 PHYTOCHEMICAL SCREENING (CK Kokate1994)

A systematic and complete study of crude drugs includes a complete investigation of both primary and secondary metabolites derived from plant metabolism. Different qualitative test were performed for establishing profiles of extract for their nature of chemical composition. The extract obtained was subjected to following chemical tests for identification of various phytoconstituents as per the methods given by Harborne.

### 4.4 ANTIOXIDANT ACTIVITY

#### 4.4.1 DPPH scavenging method

DPPH (1, 1diphenyl-2-picryl hydrazyl radical) is a stable violet colored radical which converts to yellow color on reduction. The decrease in the optical density was measured spectrophotometrically at 517nm according to the procedure described by ( Alam et al., 2013).In this assay, 1.5ml of a serial concentrations of various plant extracts in methanol was added to 1.5ml of a freshly prepared DPPH solution (DPPH was dissolved in methanol and absorbance was adjusted to 0.1±0.05).The tubes were kept in dark for 30 min followed by measuring the absorbance against blank sample at 517 nm. Ascorbic acid, vitamin E and BHT were used as standards and all experiments were carried out in triplicate. The DPPH scavenging activity of the extracts was calculated and SC50 (Concentration of sample required to scavenge 50 % of DPPH radicals) value was determined from this equation:

DPPH scavenging activity (SA) %= [(Acontrol – Asample)/Acontrol] ×100

Where Asample is the absorbance of a sample solution, and Acontrol is the absorbance of the control solution (containing all of the reagents except the test sample).

## 4.5 ANTIMICROBIAL ACTIVITY

[A] Preparation of Mueller-Hinton agar

(1) Beef infusion : 300 g
(2) Acid hydrolysate of casein : 17.5 g
(3) Starch : 1.5 g
(4) Agar : 17 g
(5) Distilled water : 1 Lit.

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121ºC for fifteen minutes. The sterilized medium (20 ml) was poured in sterilized Petri dishes under aseptic condition, allowing them to solidify on a plane table.

[B] Preparation of samples and standards

The test sample and Std controls (Chloramphenicol, Ciprofloxacin) were dissolved in dimethyl formamide (DMF).All the samples and standards was taken at concentration of 100 µg/ml for testing antibacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm. The tabulated results represent the actual readings control and samples.

[C] Test cultures

Following common standard strains were used for screening of antibacterial activities:
Escherichia coli [Gram negative]
Staphylococcus aureus [Gram positive]

[D] Inoculum's preparation

The inoculum was standardized at 1* 106 CFU/ml comparing with turbidity standard (0.5 MacFarland tube)

[E] Swabs preparation

A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

[F] Experimental procedure

The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.

The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60oC after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed.

Ditch the bore in plate. Add compounds solution in bore.

The plates were placed in an incubator at 37oC within 30 minutes of preparation for bacteria

After 48 hrs incubation for bacteria, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

## 4.6 ANTICANCER ACTIVITY

Objective

The purpose of this SOP is to provide clear and concise instructions on performing cytotoxicity assay by MTT method.

Materials & Methods

MTT Powder (the solution is filtered through a 0.2 µ m filter and stored at 2–8 °C for frequent use or frozen for extended periods)
DMSO
CO2 incubator
Tecan Plate reader

Preparation of test solutions
For cytotoxicity studies, serial two fold dilutions from 100µm to 0 µm were prepared which is then used for treatment.

Cell lines and culture medium
All the cell lines were procured from ATCC, stock cells wasculturedin DMEM/ F12 supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO2 at 37oC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells /well of Jurkatwas seeded in a 96 well plate and incubated for 24 hrs at 37oC, 5 % CO2 incubator.

Source of reagents: F12, DMEM, FBS, PenStrep, Trypsin procured from Invitrogen.

Procedure
The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37oC for 24hrs in 5% CO2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37o C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line.

**Calculating Inhibition:**

**% Inhibition =** 100 – (OD of sample/OD

### 4.6.1 MTT ASSAY

The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxcity caused by the test material.

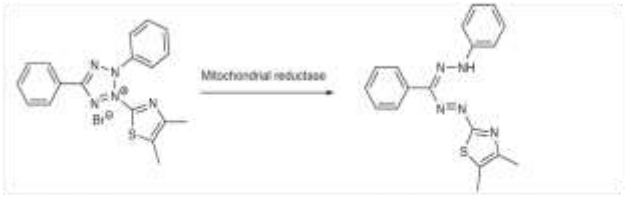


**Fig-5: MTT, Formazan**

## 5. RESULTS AND DISCUSSION

### 5.1 PHASE I

#### 5.1.1 SEM ANALYSIS

The morphological structure of the prepared nano composite was revealed SEM. The synthesized micrograph of GCS NP`S where crystalline in nature. The synthesized capped silver oxide NP`S (GCS) predicts non-spherical shape structure. The particle size of the AgNO3 was 60 to 108 nm in diameter

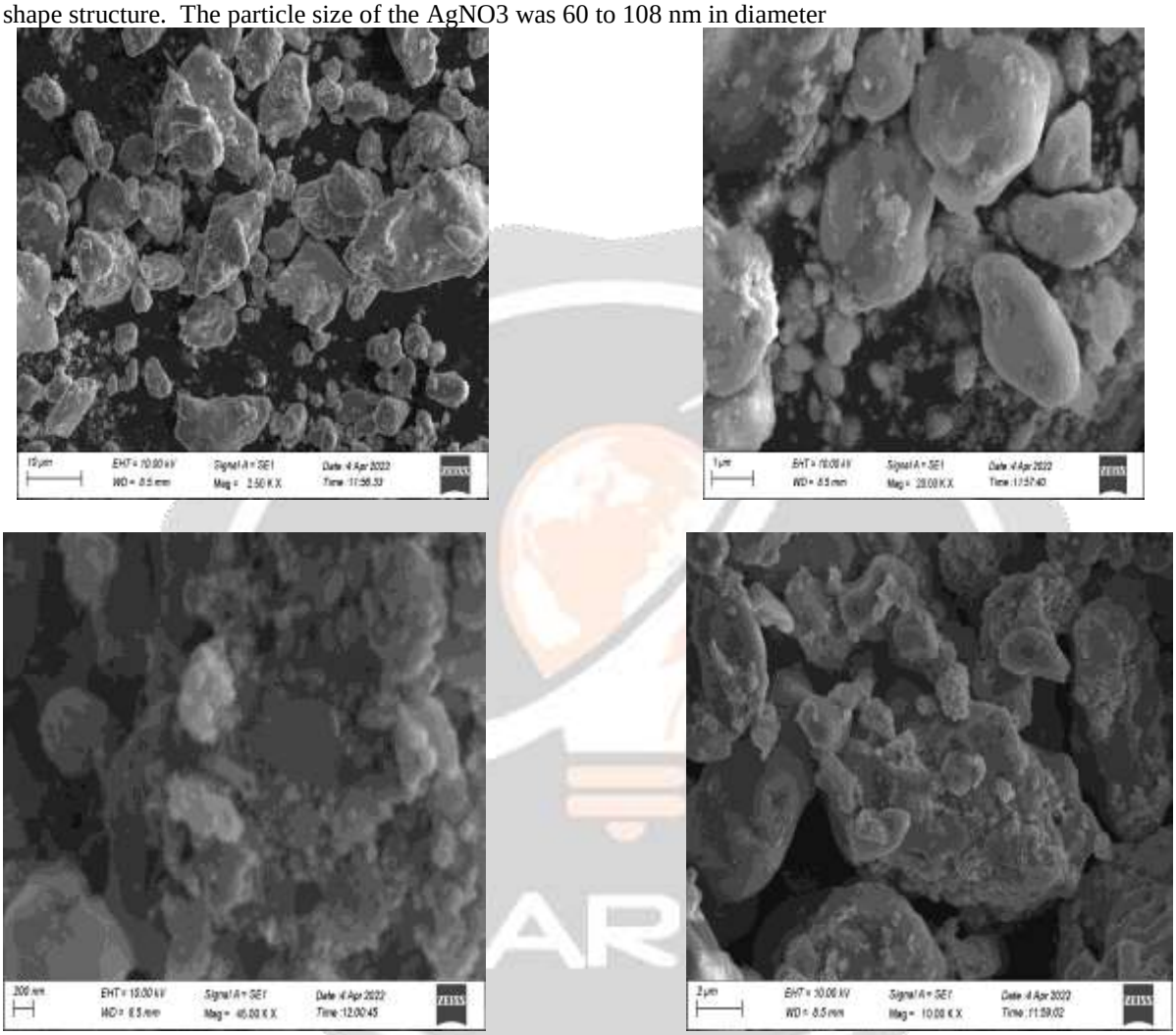

**Fig-6: SEM Analysis of Silver Nitrate**

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#### 5.1.2 FTIR ANALYSIS

FTIR Spectroscopy is used to determine the nature of functional group present in the Ag NP`S. The chemical structure of Ag NP`S was observed in the range of 4000cm-1 to 400cm-1. The FTIR spectrum of AgNO3 NP`S shows the stretching and vibrations 2357.18cm-1 (OH stretching), 1722.24cm-1(O-H stretching), 1553.75cm-1(N-H plane in bending), 1378.82-1(SO asymmetric stretching), 824.40cm-1 (S-O stretching), 477.13cm-1 (S-S stretching), 417.22cm-1(out of plane bending.

| S.NO | WAVE NUMBER $cm^{-1}$ | TYPE OF VIBRATION | INTENSITY |
|---|---|---|---|
| 1 | 2357.18 | O-H Stretching | Strong |
| 2 | 1712.24 | C=O Stretching | Strong |
| 3 | 1553.75 | N-H plane in bending | Strong |
| 4 | 1378.82 | $SO_2$ asymmetric | Strong |
| 5 | 824.40 | S-O Stretching | Strong |

| | | | |
|---|---|---|---|
| 6 | 477.13 | S-S Stretching | Strong |
| 7 | 417.22 | Out of plane Bending | Strong |
| 8 | 2924.01 | O-H Stretching | Strong |

**Table: 1 FTIR data of Cassia auticulata leaf extract**

### 5.1.3 XRD ANALYSIS

XRD Pattern of AgNo3 NP's is shown in fig. The spectra of XRD indicates that the synthesized Ag NP`S using Cassia auriculata leaf extract which confirms crystalline nature .XRD peaks located at 2θ values 32.7313, 38.0466, 44.2424, 46.1151, 53.7282, 64.3859, 74.0137, 77.3988 corresponding to hkl values(111), (200), (200), (220), (220), (220), (331), (311) and standard diffraction peaks shows the Cubic structure (JCPDS cord No: 65-2871, 31-1238,04-0783, 76-1489, 12-079). The remaining minor peaks are crystalline organic molecules of Ag NP`S, the Debye-Scherrer formula was used

$$D=K\lambda/\beta \cos \theta$$

Where,

β is full width half maxima of the peak in XRD pattern

θ is peak obtained angle

λ is X-Ray wavelength

K is the constant of 0.9

According to debye-scherrer equation the average crystalline size of the particles are calculated as

| 2 Theta (deg) | d Spacing (deg) | FWHM (deg) | h k l values | Crystallite size D(nm) | Dislocation Density ρ | Strain η | Micro Strain S | Average crystallite size D(nm) |
|---|---|---|---|---|---|---|---|---|
| 32.7313 | 2.81268 | 0.1171 | 111 | 33.4066 | 8.9605 | 8.3555 | 2.3643 | |
| 38.0466 | 2.36518 | 0.1171 | 200 | 36.4700 | 7.5284 | 9.3953 | 0.1558 | |
| 44.2424 | 2.04726 | 0.1338 | 200 | 70.9901 | 1.9842 | 4.8267 | 9.6177 | |
| 46.1151 | 1.96841 | 0.2342 | 220 | 63.4037 | 2.4875 | 5.4042 | 0.0167 | 43.44 |
| 53.7282 | 1.66615 | 0.0836 | 220 | 44.3644 | 5.0807 | 7.7173 | 1.1101 | |
| 64.3859 | 1.44703 | 0.1004 | 220 | 10.5378 | 9.0051 | 9.3953 | 1.3504 | |
| 74.0137 | 1.26570 | 0.1020 | 331 | 24.5450 | 1.1664 | 1.3979 | 0.8334 | |
| 77.3988 | 1.22243 | 0.1224 | 311 | 66.8657 | 2.2366 | 5.1244 | 0.3233 | |

**Table 2: X-ray diffraction data of Cassia auriculata leaf extract**

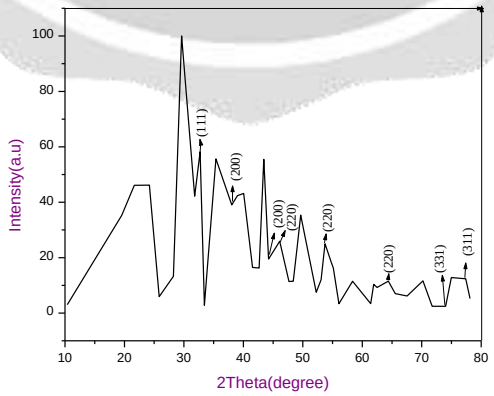



**Fig-8 X- ray diffraction spectra of *Cassia auriculata* leaf extract**

## 5.2 PHASE II

### 5.2.1 Phytochemical Analysis

The results of phytochemical analysis of Cassia auriculata was shown in the Table 1.

Table:3 Qualitative Phytochemical Analysis

| S.No | Plant constituents | Results |
|---|---|---|
| 1 | Test for Alkaloids | - |
| 2 | Test for Glycosides | + |
| 3 | Test for carbohydrates | + |
| 4 | Test for phytosterols | + |
| 5 | Test for Steroids | - |
| 6 | Test for Flavanoids | + |
| 7 | Test for Saponins | + |
| 8 | Test for Polyphenols | + |
| 9 | Test for tannins | + |
| 10 | Test for proteins and amino acids | + |
| 11 | Test for Terpenoids | - |
| 12 | Test for Triterpenoids | - |
| 12 | Test for Fixed oils and fats | - |

'+' Present,
'++' Moderately present,
'+++' Highly present
'-' Absence

## 5.3 ANTIOXIDANT ACTIVITY

### 5.3.1 Determination of Radical Scavenging Activity By DPPH Method

DPPH scavenging method DPPH (1, 1diphenyl-2-picryl hydrazyl radical) is a stable free radical having a maximum absorbance at 517 nm in methanol and its color changed from purple to yellow after accepting an electron or proton radical from antioxidant molecules (antioxidant sample) to become a stable diamagnetic molecule(Singh et al., 2016). The result showed that free radical scavenger of sample found to be (SC50= 21.31±0.38µg/ml.

**Table: 4 The Antioxidant activity of *Cassia auriculata* using aqueous alcoholic extract**

| S.NO | SAMPLES | DPPH scavenging activity $SC_{50}$ (µg/ml) |
|---|---|---|
| 1 | Test Sample | 21.31±0.38 |
| 2 | Ascorbic acid | 14.52±0.31 |
| 3 | Vitamin E | 26.22±0.35 |
| 4 | BHT | 19.54±0.17 |

## 5.4ANTI MICROBIAL ACTIVITY

The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60oC after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed. Ditch the bore in plate. Add compounds solution in bore. The plates were placed in an incubator at 37oC within 30 minutes of preparation for bacteria. After 48 hrs incubation for bacteria, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extract against Gram positive, Gram negative bacteria. The extracts exhibited antimicrobial activities against tested microorganisms.

**Table:5 Antimicrobial activity of Cassia auriculata using aqueous alcoholic extract.**

| **Samples** | **Zone of inhibition* (mm)** | |
|---|---|---|
| | **Gram positive** ***S. aureus*** | **Gram negative** ***E. coli*** |
| **Test sample** | **19** | **21** |
| **Ciprofloxacin(std)** | **23** | **28** |

*= average zone of inhibition in mm,
Activity index = Inhibition zone of the sample / Inhibition zone of the standard

The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant Cassia auriculata showed good antimicrobial activity against several organisms like Staphylococcus aureus and E.coli as supported by previous studies.

The result of the antimicrobial activity is similar with the work done by Anonymous, 1996.

## 5.5 ANTICANCER ACTIVITY

**Table: 6 MTT absorption value GCS**

| **HeLa** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GCS** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| **A** | **0.026** | **1.245** | **1.124** | **0.996** | **0.945** | **0.861** | **0.785** | **0.745** | **0.684** | **0.624** | **0.512** | **0.462** |
| **B** | **0.037** | **1.284** | **1.186** | **0.984** | **0.912** | **0.845** | **0.745** | **0.623** | **0.620** | **0.561** | **0.464** | **0.412** |
| **C** | **0.024** | **1.263** | **1.163** | **1.102** | **0.984** | **0.864** | **0.723** | **0.715** | **0.602** | **0.534** | **0.523** | **0.463** |
| | | **1.264** | **1.158** | **1.027** | **0.947** | **0.857** | **0.751** | **0.694** | **0.635333** | **0.573** | **0.500** | **0.446** |
| | | **0** | **8** | **19** | **25** | **32** | **41** | **45** | **50** | **55** | **60** | **65** |

**IC50 = 70**

### 5.5.1 MTT Assay

The result of MTT Assay that the plant extract was decreased percent viability of all the cells but to different extent. Plant extract was found to induce more cytotoxicity towards cell lines. These results revealed morphological changes and shrinkage of cells leading to cell death induced by the extracts in the liver cancer cell lines. The IC50 values of plant extracts of against the cell line.

The result of the anticancer activity is similar with the work done by Crouch S.P.M. et al., 1993.

### 5.5.2 Statistical evaluation

### $IC_{50}$ Value

The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC50 of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

IC50 values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable).

5.5.3 Nonlinear regression

In statistics, nonlinear regression is a form of regression analysis in which observational data are modeled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations.

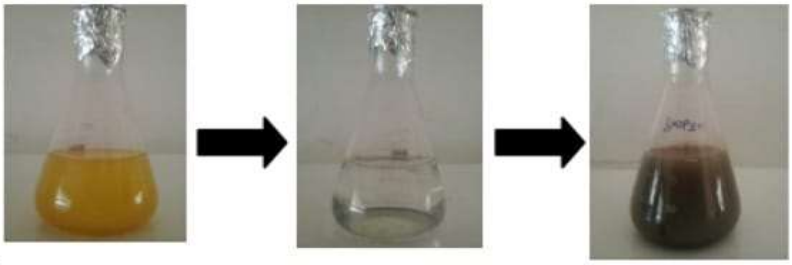
**Table: 7 MTT absorption value**

**Cisplatin (STD)**

| **HeLa** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cisplatin** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| A | 0.05 | 1.124 | 0.752 | 0.645 | 0.697 | 0.645 | 0.443 | 0.315 | 0.352 | 0.163 | 0.102 | 0.063 |
| B | 0.046 | 1.345 | 0.731 | 0.624 | 0.497 | 0.341 | 0.364 | 0.378 | 0.302 | 0.294 | 0.164 | 0.102 |
| | 0.064 | 1.024 | 0.915 | 0.702 | 0.512 | 0.512 | 0.402 | 0.315 | 0.246 | 0.164 | 0.102 | 0.074 |
| | | 1.164 | 0.799 | 0.657 | 0.569 | 0.499 | 0.403 | 0.336 | 0.3 | 0.207 | 0.123 | 0.080 |
| | | 0 | 31 | 44 | 51 | 57 | 65 | 71 | 74 | 82 | 89 | 93 |

## 6.0 PLATES

**PLATE 1: SYNTHESIS OF $AgNO_3$ NP'S**



**leaf extract + $AgNO_3$ = $AgNO_3$ NP's**

**Fig-15: Synthesis of $AgNO_3$ NP'S**

### 6.1 ANTIOXIDANT ACTIVITY DPPH ASSAY

**Fig-16: DPPH Assay**

**PLATE: 2 ANTIMICROBIAL ACTIVITIES**

**Fig-17: Microbial plates**

**PLATE3: ANTICANCER ACTIVITY**

**HELAcells (Control)-GCS**

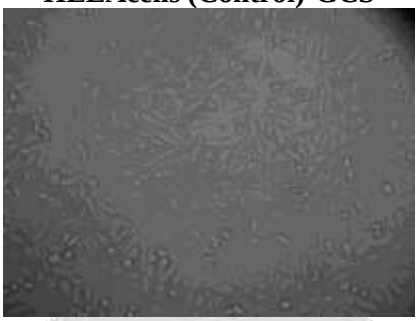


**Fig-18: HELAcells(control)-GCS**

**HELAcells (Treated)–GCS**

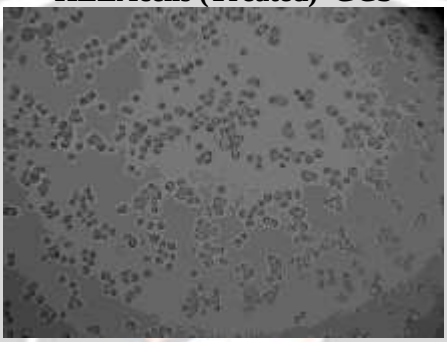


**Fig-19: HELAcells (Treated)-GCS**

**HELA cells (Control)–Cisplatin (STD)**

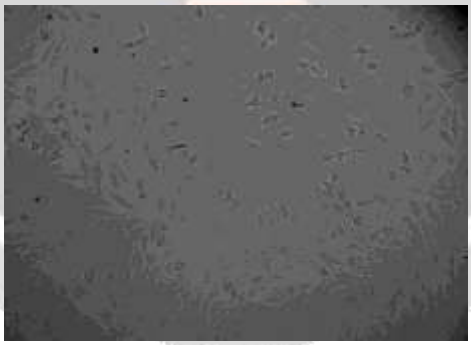


**Fig-20: HELA cells (Control)-Cisplatin(STD)**

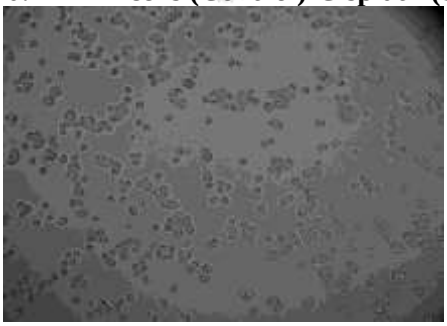


**Fig-21: HELA cells (Treated)-Cisplatin (STD)**

## 7. SUMMARY AND CONCLUSION

The present investigation was carried out in the aqueous alcoholic extract of Cassia auriculata in terms of phytochemical, antioxidant, antimicrobial and anticancer activities. Pyhtochemicals are non nutritive plant chemicals that have protective or disease preventing properties. Most pyhtochemical have antioxidant activity against oxidative damage and reduce the risk of developing the certain types of cancer. The result obtains in the study indicates that the leaves of Cassia auriculata have the potential to act as the source of useful drugs because of pyhtoconstituents. Alkaloids, Flavonoids, Carbohydrates, Lactons, Saponins, Tannins, Sterols and resins were present in the leaf extract.

Determination of natural antioxidant compounds in the plant extracts will help to develop the new medicine constituents for antioxidant therapy. In this study of plant extracts was in the investigated with DPPH method. From the result it was observed that Cassia auriculata has good antioxidant activity. So it can be used for preventing or minimizing lipid oxidation in pharmaceutical products.

This study shows that the aqueous alcoholic extract of flower of Cassia auriculata have the antimicrobial activity against Staphylococcus, E.coli and can be used as a source for developing broad spectrum antimicrobials. Natural products found in medicinal plants have great promise for the treatment of cancer. According to anticancer study of Cassia auriculata shows a effective property. The natural anticancer components present in this plant extracts used for therapeutic purpose.

All the above observations of different activity of flower extract of Cassia auriculata shows strong antioxidant activity, strong antimicrobial and good anticancer activity. These could find the potential application in today's urban lifestyle which increases our exposure to various harmful oxidants. The study can be further extended to analyse the effect of flower of Cassia auriculata in healthcare industry.

## 6. REFERENCES

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