SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

Project Report of 2022: SVP-2207

"*In vitro* morphogenetic studies of some selected medicinal plants and their involvement in green synthesis of nanoparticles"

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Sri Venkateswara College
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"*In vitro* morphogenetic studies of some selected medicinal plants and their involvement in green synthesis of nanoparticles"

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SRIVIPRA PROJECT 2022

Title: *In vitro* morphogenetic studies of some selected medicinal plants and their involvement in green synthesis of nanoparticles.

List of students under the SRIVIPRA Project

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2	Vanija Raj Sinha	B.Sc.(P) Life Sciences Sem-V	
3	Gauravya Mohan	BSc.(H) Biological Sciences Sem-III (Volunteer)	

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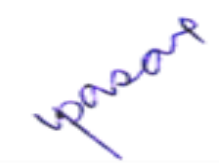
Dr. Neeti Mehla



Dr. Upasana Sharma

Certificate

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2207 titled- "In vitro morphogenetic studies of some selected medicinal plants and their involvement in green synthesis of nanoparticles." The participants have carried out the research project work under our guidance and supervision from 21st June 2022 to 7th October 2022. The work carried out is original and conducted in an online and offline mode.

Signature of Mentors:

Dr. Neeti Mehla

Dr. Upasana Sharma

Acknowledgement

It takes hard work and cooperation of many individuals for any project to become reality. We would like to take this moment to express our immense gratitude to each and every person who has extended their kind support and help during the course of this project.
First and foremost, we want to offer this endeavor to the Almighty God for the good health, strength and wisdom he bestowed upon us for the completion of this project. We express sincere gratitude to our college for rolling out the Sri Venkateswara Internship Program for Research in Academics (SRI VIPRA), helping us to gain some hands-on experience of research in the lab during our graduation years. It is a genuine pleasure to express our deep sense of gratitude to our esteemed Principal ma'am, Professor C. Sheela Reddy for her constant support and for providing us endless opportunities to always move forward.

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Sri Venkateswara College

University of Delhi

SRIVIPRA-2022

(Sri Venkateswara College Internship Program in Research and Academics)

This is to certify that this project on "***In vitro* morphogenetic studies of some selected medicinal plants and their involvement in green synthesis of nanoparticles"** (SVP-2207) was registered under SRIVIPRA and completed under the mentorship of Dr. Neeti Mehla and Dr. Upasana Sharma from 21st June to 7th October 2022.

Sharda Pasricha and S. Krishnakumar

Coordinators

Prof. C Sheela Reddy

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CONTENTS

15	Effect – *Tinospora cordifolia* Nanoparticles	15

List of Tables

List of Figures

Tinospora cordifolia

Introduction

Tinospora cordifolia is a climbing deciduous shrub which is commonly known as Giloy. It is found in tropical areas such as India, China, Bangladesh, Myanmar & Sri Lanka. It is an angiosperm belonging to the Menispermaceae family. *T.cordifolia* can be used to extract a myriad of substances such as alkaloids, steroids, glycosides, aliphatic compounds, diterpenoid lactones, polysaccharides etc.(Spandana *et al.*, 2013) It is known as a rasayana (chemical) since it enhances vitality and longevity. It is enormously used in Ayurveda for a plethora of purposes related with inflammation, allergic reactions, glucose metabolism and neurology (Sinha and Sharma, 2015).

Fig. 1: *Tinospora cordifolia*

Classification

Kingdom: Plantae
Order: Ranunculales
Family: Menispermaceae
Genus: *Tinospora*
Species: *cordifolia*

Tinospora

The *Tinospora* genus consists of about 15 species. Some medically paramount species include *T. cordifolia, T. malabarica, T. tementosa, T. crispa, T. uliginosa etc.*

Common Names

Latin : *Tinospora cordifolia* (Willd.) Hook.f. & Thomson
English : Tinospora Gulancha / Indian tinospora
Sanskrit : Guduchi, Madhuparni, Amrita,Chinnaruha, Vatsadaani, Tantrika Kundalini and Chakralakshanika
Hindi : Giloya, Guduchi (Hindi)
Bengali : Gulancha
Telugu : Tippaatigo (Telugu)
Tamil : Shindilakodi
Marathi : Shindilakodi
Gujarati : Galo
Kannada : Amrita balli
(Sharma *et al.*, 2010)

Morphology and Anatomy

Stem of this plant is succulent, long and filiform. It is a climber. Aerial roots arise from the branches of the plant. The bark is creamy and greyish in colour and deeply left spirally. Leaves of this plant are simple, alternate, estipulate, circular, pulvinate, heart-shaped and with twisted aestivation. The petiole is elongated (15 cm approx.). The membranous lamina is ovoidal shaped, 10-20 cm long, 7 nerved and deeply cordate at the base. The greenish-yellow unisexual flowers appears when plant is defoliated. Inflorescence is of racemes type. Male flowers are clustered while female flowers are found in single specific positions. Calyx is in 2 series of 3 sepals each (6 sepals in total). Corolla is free and membranous with 6 petals. The plant flowers during late spring and early summer (March to June). The succulent fruits are reddish-orange coloured. They are in aggregates of 1-3 smooth ovoidal drupelets. Fruits develop during winter months. *T.cordifolia* belongs to the menispermaceae (moonseed) family since it has curved seeds.(Mittal *et al.*, 2014).

Medicinal Aspects of *Tinospora cordifolia*

The pharmaceutical significance of this plant is due to its leaves, barks and roots. These parts of the plant contain bioactive molecules (viz. alkaloids, glycosides, lactones, saponins, tannins, steroids, polysaccharides and aliphatic compounds) with important medical applications. The plant is known to possess immunomodulatory,

immunostimulatory, anti-neoplastic, anti-oxidant, anti-hyperglycemic, anti-hyperlipidemic, anti-tuberculosis, hepatoprotective, anti-osteoporotic, anti-angiogenic, anti-malarial and anti-cancer activities. (Sinha and Sharma, 2015).

The aqueous extract of *T.cordifolia* can be used to lower the serum cholesterol and it can stabilize HDL cholesterol level to the basic value. The anti-hyperglycemic properties of this plant are essential in the treatment of diabetes mellitus. *T.cordifolia* is the best remedy for children suffering from upper respiratory tract infections.(Spandana *et al.*, 2013)

Anti-diabetic properties

Stem extracts of *T.cordifolia* can be used to cure diabetes by regulating the level of blood glucose. It has been reported to act as anti-diabetic drug since it promotes insulin secretion by inhibition of gluconeogenesis and glycogenolysis. These properties are attributed to the presence of alkaloids (Magnoflorine, Palmetine, Jatrorrhizine), tannins, cardiac glycosides, flavonoids, saponins, steroids etc. (Mittal *et al.*, 2014).The crude extract of stem in ethyl acetate, dichloromethane, chloroform and hexane restricts enzymes-salivary amylase and glucosidase. This results in the increase of post-prandial glucose level and exhibits potential activity against Diabetes mellitus. The root extract of this plant also exhibits anti-diabetic properties which causes a decline in the level of glycosylated hemoglobin, hydroperoxidase and vitamin E. (Mittal *et al.*, 2014)

Tissue culture studies (Micropropagation) in *T.cordifolia*

The medicinal uses of *T.cordifolia* have led to its commercial exploitation. Wild populations of the plant are declining due to overexploitation. Conventional vegetative propagation is inadequate for large scale cultivation due to poor seed setting and germination. (Mangal *et al.*, 2012)

Micropropagation protocols can be used as an alternative method for the propagation of *T.cordifolia*. This also allows for conservation of the species. Culture of shoot meristems, especially through amplified axillary branching, allows for swift propagation of plants with genetic uniformity of the progeny. (Mangal *et al.*, 2012)

Plant cells exhibit the property of totipotency. The totipotency of plant cells allows for plantlets to be grown through micropropagation techniques. Tissue culture is carried out by growing of tissues or cells in an artificial medium. This technique is also called micropropagation. This property allows for a plant to be fully regenerated from cells acquired from any living part (shoot, leaf, nodes etc.) of the plant. Table.1 displays some of the studies pertaining to micropropagation in *Tinospora cordifolia*.

Table 1: Micropropagation studies in *Tinospora cordifolia*

S.no.	Explant	Growth Medium	Morphogenic Response	Reference
1	Nodal segments	MS, WPM 2.32µM KIN	Clonal propagation=> Axillary shoot proliferation=> Shoot elongation	Raghu *et al.*, 2006
2	Shoot tips, leaves and nodal segments	MS 1.5 mg/L KIN	Callus induction, organogenesis => Shoot proliferation	Singh *et al.*, 2009
	Stem, leaf and nodal segments	MS 1.0 mg/L BAP, 2.5 mg/L NA	Callus formation=> Root proliferation	
3	Nodal and inter nodal segments	Half MS 0.4mg/L NAA	Organogenesis => root proliferation	Khanapurkar *et al.*, 2012
4	Nodal Segments	MS 8.0µM Kinetin	Callus formation=> Shoot proliferation	Bhalerao *et al.*, 2013
5	Nodal Segments	MS 2.0mg/L BAP, 4mg/L KIN, 0.2mg/L TDZ	Organogenesis=> Shoot proliferation	Sultana *et al.*, 2013
6	Axillary bud and cotyledonary node	MS 3.0 mg/L KIN	Embryogenesis=> shoot proliferation	Handique PJ, 2014
	Nodal segment	MS, WPM 2.32 µm KIN	Clonal propagation => shoot proliferation	
7	Nodal segment	MS medium 4.36 µM KIN	Organogenesis=> shoot proliferation	Sivakumar *et al.*, 2014
8	Stem	MS	Clonal	Sinha and

		1.5mg/L KIN, BA	propagation=> Shoot proliferation	Sharma *et al.*, 2015
9	Nodal and apical shoot tip segments	MS 2.0mg/L BAP, 0.2mg/L IAA	Organogenesis=> Shoot proliferation	Tupe and Pundhure, 2015
10	Shoot tip	MS 5.0 mg/L BAP	Organogenesis=> Shoot proliferation	Chatterjee and Ghosh, 2016
11	Cotyledons	MS 2.0mg/L IAA	Callus formation, Organogenesis=> Shoot proliferation	Mridula *et al.*, 2017
	Cotyledons	Half MS 0.5mg/L IBA, 0.5mg/L NAA	Callus formation, Organogenesis=> Root proliferation	
12	Nodal Segments	MS 4.44µM BA, 2.45µM 2-Ip	Organogenesis=> Shoot proliferation	Mittal *et al.*, 2017
13	Nodal Segments	MS 1.0mg/L BAP, 0.5mg/L 2-Ip	Organogenesis=> Shoot proliferation	Mittal and Sharma, 2017
14	Nodal Segments	MS 0.5mg/L BAP, 0.5mg/L KIN, 0.1mg/L IAA	Organogenesis=> Axillary Shoots=> Shoot proliferation	Panwar *et al.*, 2018
15	Young and mature shoot tip	MS 2.0 mg/L BA, 1.0 mg/L KIN	Organogenesis=> Shoot proliferation (shoot bud break, shoot development)	Mridula *et al.*, 2019
	Young and mature shoot tip	Half MS 0.5 mg/L IAA	Organogenesis=> Root proliferation	
16	Nodal segments	MS 2 µM BA	Organogenesis => Shoot proliferation	Pillai *et al.*, 2019
17	Shoot buds	MS 8.87 µM BAP	Organogenesis=> Axillary shoot proliferation=> Shoot proliferation	Sahu *et al.*, 2020

18	Nodal segments	MS 8.0µM/L KIN, 2.0µM/L BA	Organogenesis=> Shoot proliferation	Shankar *et al.*, 2020
19	Nodal segments	MS 2 mg/L BAP, 0.5 mg/L NAA	Organogenesis=> Shoot proliferation	Sudan *et al.*, 2020
20	Nodal Segments	MS 2.0 mg/L BAP, 0.2mg/L NAA	Organogenesis=> Shoot proliferation	Singh *et al.*, 2021
21	Nodal Segments	MS 2.0mg/L BA, 1.0mg/L KIN	Organogenesis=> Shoot proliferation	Patel and Pandya, 2022

Plumbago zeylanica

Introduction

Plumbago zeylanica is a perennial shrub. The roots of this plant are used in China and other Asian countries due to their medicinal properties. (Wei *et al.*, 2006) It is an angiosperm belonging to the family Plumbaginaceae. (Selvakumar *et al.*, 2001) The roots of the plant contain a secondary metabolite—an alkaloid called plumbagin. This compound has antimalarial, antibiotic as well as antifertility properties. (Mallikadevi *et al.*, 2008) The overexploitation of this plant has caused a steep decline in its population numbers in the wild. (Sivanesan *et al.*, 2009)

Fig 2: *Plumbago zeylanica* (Source: https://indiabiodiversity.org)

Classification

Kingdom: Plantae
Order: Caryophyllales
Family: Plumbaginaceae
Genus: Plumbago
Species: *zeylanica*

The genus is represented by about 10 species. (Selvakumar *et al.*, 2001) It includes species such as *P. indica*, *P. auriculata* and *P. zeylanica*. They are also known as leadworts.

This species has important medicinal properties. The roots are used in several herbal preparations for diseases such as diarrhea, piles and dyspepsia. (Selvakumar *et al.*, 2001)

Morphology

It is a much-branched perennial shrub. It has semi-woody stems. The leaves are simply alternate, ovate and oblong-lanceolate. The flowers are borne in spikes. The rachis is pubescent or glandular. Flowers are white, bisexual, pentamerous, pedicellate and have a sweet scent. The roots are cylindrical and irregularly bent with transverse shallow fissures. The fruit is oblong and the capsules are enclosed by persistent viscid calyx. (Yuvaraj *et al.*, 2011, Pant *et al.*, 2012)

Therapeutic Usage of *P. zeylanica*

The roots are the main source of an alkaloid—plumbagin, a natural naphthoquinone.It has a myriad of pharmacological properties such as antimalarial, cardiotonic, anticancer, antifertility, antibiotic and antineoplastic.The root stimulates the secretion of body fluids such as sweat, urine and bile. It also has a stimulatory effect on the nervous system. (Mallikadevi *et al.* 2008)
Traditionally, it has been used to treat skin diseases such as scabies, dermatitis and acne. It is also used for other diseases such as piles, ulcers, leprosy and ringworm. (Pant *et al.*, 2012)
Some new isolated constituents are Beta – Sitosteryl- 3 Beta – glucopyranoside – 6-O-palmitate (yield 0.009%), Plumbagin (0.004%), lupeol acetate (0.008%), lupenone (0.044 %, trilenolein (0.001%), Beta – sitosterol (A.T. Nguyen *et al.*, 2004)

Tissue culture studies (Micropropagation) in *P. zeylanica*

Due to erratic germination rates, propagation through seeds is difficult. (Mallikadevi *et al.*, 2008) Hence, alternative propagation methods need to be used to maintain a sustainable population. Tissue culture is well-suited for its propagation since it eliminates the need for seeds for propagation. Moreover, artificial conditions and choice of media ensure better survival of seedlings which is a problem in traditional propagation methods. Table.2 displays some of the studies pertaining to micropropagation in *Plumbago zeylanica.*

Table 2: Micropropagation studies in *Plumbago zeylanica*

S.No.	Explant Used	Growth Medium	Morphogenic Response	References
1	Nodes	MS 1 mg/L IBA	root number (24.1 ± 0.73) after incubation of cultures at 25 ± 2 °C with 16/8 h photoperiod.	Roy *et al.*, 2019
2	Shoot tip	MS 3.0 mg/L BAP	8 shoots/explant with an 85% of response was seen after 12 hours of incubation	Raja *et al.*, 2018
3	Nodes	MS 6.66 µM BAP, 4.44 µM KIN	maximum number of shoots (47.3±0.06) in time period of 6 weeks	Krishna *et al.*, 2018
4	Nodes	MS 1.5 mg/L BAP, mg/L IAA	Highest number of shoot buds induction obtained was (6.27 ± 0.31) incubated in regular cycle of 14 hours light and 10 hours dark	Majumder *et al.*, 2016
5	Nodes	MS 2.0 mg/L BAP, 0.2 mg/L NAA	10 to 12 shoots explant-1 after 3 weeks of incubation	Chatterjee *et al.*, 2015
6	Nodes	MS 13.3µM BAP, 135.74µM AdS	Maximum number of shoots obtained was (15.8±1.81) after 15 days of incubation	Chandravanshi *et al.*, 2014
7	Nodes	MS 1mg/L BAP, 1mg/L NAA	maximum shoot length was recorded at (5.38±0.99 cm.)	Dohare *et al.*, 2012

8	Nodes	MS 2.0 mg/L BAP, 1.5 mg/L IAA, 1.0 mg/L IBA	(19.56±0.04) mean number of shoots per explants were obtained within 12 to 15 days of inoculation	Satyajit *et al.*, 2012
9	Nodes	MS 1mg/L BAP	Mean number of shoots obtained were 20.2 ± 0.32 after 30 days of inoculation	Lubaina *et al.*, 2011
10	Nodes	MS 1.5 mg/L BAP 0.75 mg/L IBA 0.75 mg/L AdS 10% CM	shoot proliferation of 41.77 shoots per explants was obtained	Jain *et al.*, 2011
11	Leaf and stem	MS 1.0mg/L IBA, 0.5mg/L NAA	Number of roots/explant obtained were 19.2 (leaf), 14.0(stem) .	Sivanesan *et al.*, 2009
12	Leaf	MS 3.5mg/L BAP, 0.3 mg/L NAA	No. of shoots obtained was 17.00n +.3.00 after 6 weeks of culture	Mallikadevi *et al.*, 2008
13	Leaf callus culture	MS 0.75 mg/L BAP, 1.0 mg/L IAA, 1.0 mg/L NAA	Maximum shoot regeneration (16.3+.0.51) in 5 weeks	I. Sivanesan, 2007
14	Hypocotyl segments	MS 2.0 mg/L BA, 0.75 mg/L NAA, 50 mg/L Adenine, 10% (v/v) coconut milk under subdued light at 25±2°C	30 shoots per hypocotyl segment in 3 weeks of direct embryogenesis	Wei *et al.*, 2006
15	Nodes	MS 27.2 µM AdS, 2.46 µM IBA	8 plantlets obtained from one twig in 5 months (eight responsive nodes per explant shoot)	Selvakumar *et al.*, 2001

Green Synthesis of Silver Nanoparticles (with extract of *Tinospora cordifolia*)

Introduction

Nanoparticles

A particle of matter with a diameter of one to one hundred nanometers (nm) is commonly referred to as a nanoparticle or ultrafine particle (U.S. Environmental Protection Agency, Vert *et al.*, 2012). In contrast to colloidal particles, which typically range in size from 1 to 1000 nm and are more prone to brownian motion, they typically do not sediment. Nanoparticles are significantly smaller than the visible light spectrum (400–700 nm), making it impossible to observe them with standard optical microscopes. Instead, they must be viewed with electron microscopes or laser microscopes. For the same reason, nanoparticle suspensions in transparent media may be transparent, (Chae *et al.*, 2003) in contrast to suspensions of bigger particles, which often scatter some or all incident visible light. Nanoparticle separation from liquids necessitates unique nanofiltration techniques since nanoparticles readily pass-through ordinary filters, such as everyday ceramic candles. (Simonis *et al.*,2011) Numerous nanoparticles can be found in nature and are the subject of research in many fields of science, including chemistry, physics, geology, and biology. (Cai *et al.*, 2016, Chen *et al.*, 2013).

Silver nanoparticles

Silver nanoparticles (AgNPs) have attracted a lot of attention recently due to their numerous applications. As compared to other metal nanoparticles, they are relatively easy to synthesize, non-toxic, stable, and eco-friendly. To further enhance these benefits, researchers are now concentrating on the green synthesis of these nanoparticles. In general synthesis of AgNPs, silver ions are reduced to neutral atoms with a powerful reducing agent. Biological substances such as microorganism and plant extracts have been proven to be efficient sources of natural reducing agents for the biochemical synthesis of silver nanoparticles. It has been discovered that phytochemicals (including polyphenols, alkaloids, and others) are appropriate reducing agents in the creation of metal nanoparticles (Spandana *et al.*, 2013).

Synthesis of silver nanoparticles: Conventional and green methods

To create nanoparticles, one can use one of three basic techniques. Physical, chemical, and biological processes are among them (El-Nour *et al.*, 2010 , Irvani *et al.*, 2014). The most popular but least advantageous approaches are chemical ones. Their primary flaw is that they are not environmentally friendly methods of synthesis. Although physical approaches appear to be more environmentally friendly, biological approaches appear to adhere to the green chemistry principles almost entirely. (Ijaz *et al.*, 2020, Aisida *et al.*, 2021)

There are many chemical processes available for creating silver nanoparticles. They employ water or organic solvents to create silver nanoparticles. A metal precursor, reducing agents, and a stabilizing agent are the three reactant components required in chemical synthesis techniques for the creation of nanoparticles. The simplest of the several chemical processes suggested includes reducing silver nitrate in an aqueous solution while a reducing and stabilizing agent are present. There are several reducing substances used, such as hydrogen gas, citrate, ascorbate, and borohydride. Surfactants, ligands, or polymers with certain functional groups, like polyvinylpyrrolidone and polyethylene glycol, are utilized as stabilizing agents. (Gudikandula *et al*., 2016).

Silver nanoparticle creation frequently uses the polyol process. In this procedure, ethylene glycol, which serves as both a solvent and a stabilizing agent, helps to decrease the use of silver nitrate. For instance, inorganic reducing agents like sodium citrate and sodium borohydride are used in chemical synthesis processes. When compared to borohydride, a powerful reducing agent, sodium citrate produces larger-sized nanoparticles most of the time. Oleyl amine-liquid paraffin combination is another common chemical reagent used to create spherical nanoparticles. (Zewde *et al.*, 2016) Chemical synthesis methods' adaptability, affordability, and capacity to create specified nanoparticles with certain sizes, dimensions, and structures are their main advantages. However, in terms of greenness, chemical techniques come in last. The toxicity of the employed solvents has a long-lasting effect. On the surface of the synthesized silver nanoparticles, chemical residue from the solvent is frequently still present. These nanoparticles are especially dangerous if utilized for drug delivery. (Ovais *et al*., 2018).

The biological approaches (Aisida *et al.*, 2019) are a suitable and environmentally friendly substitute for chemical and physical methods of synthesis. Living organisms like plants, algae, microbes and fungi, and even animals can be used in the green synthesis of nanoparticles. The main advantages of biological synthesis are the safety of the procedure and the quality of the produced nanoparticles. We can be certain that the product produced will be free of any contamination because this approach uses only benign substances to enable the synthesis of silver nanoparticles. This has a negligible effect on health. In addition, and perhaps even more so than physicochemical methods, biological methods provide a high production of well-defined, uniformly sized nanoparticles. (Mittal *et al*., 2013) These features of biological synthesis support several of the twelve core tenets of green chemistry, making the process of biosynthesis considered to be environmentally friendly. Moreover, because the capping and stabilizing agents used are all biomolecules, the microbial activity of nanoparticles synthesized using biological techniques is improved. This increases the silver nanoparticles' propensity to assault microorganisms.

Method of green synthesis - An environmentally responsible technique to create nanoparticles is by using the biological method, which is provided as an alternative to chemical and physical methods. Additionally, this procedure doesn't involve pricy, hazardous, or dangerous substances. The biological technique, which has been utilized frequently in recent years, allows for the synthesis of metallic nanoparticles with a wide

range of sizes, shapes, compositions, and physicochemical characteristics. Utilizing biological agents like bacteria, actinobacteria, yeasts, molds, algae, and plants, as well as their byproducts, synthesis can be completed in a single step. Proteins, enzymes, phenolic compounds, amines, alkaloids, pigments, and other molecules found in plants and microorganisms are examples of molecules that carry out reduction-based nanoparticle synthesis. (Shah M *et al*.,2015)

Traditional chemical and physical procedures present a risk of toxicity to the environment and the cell when using reducing agents to reduce metal ions and stabilizing agents to prevent unwanted agglomeration of the generated nanoparticles. Additionally, the shape, size, and surface chemistry of the generated nanoparticles are thought to be hazardous. These substances are already present in the biological organisms used in the green synthesis approach, which produces biocompatible nanoparticles.

Bacteria are obviously targets in the manufacture of nanoparticles due to their quick development, low cost of culture, and ease of control and manipulation of the growing environment. In addition, it is well known that several bacteria species have unique defenses against the toxicity of metals or heavy metals. For these reasons, bacteria are favored since they can produce nanoparticles both in- and ex-situ. Metal ions can be reduced and precipitated for the creation of nanoparticles via metabolic pathways and reducing agents found in bacteria, such as proteins, enzymes, etc. (Korbekandi *et al*.,2009; Gao *et al*.,2014).

Tinospora cordifolia plant extract has been proved to be an effective reducing agent to synthesize silver nanoparticles. A greener process can be used for AgNPs synthesis, potentially eliminating the negative effects that are often accompanied with chemical agents to make the nanoparticles more environment-friendly. Additionally, the synthesis of AgNPs in the plant extract strengthens the medicinal qualities of *Tinospora cordifolia* and improves its therapeutic effectiveness. The nanoparticles can be assayed through a variety of techniques such as TEM, SEM and XRD to assess their properties. The current study focuses on the review of environment-friendly synthesis of silver nanoparticles through the use of various *Tinospora cordifolia* extracts and their relative bio efficacy.

Table 3: Bioefficacy studies of silver nanoparticles synthesized with *T. cordifolia* extract

S.no.	Part Used	Particle Size	Shape	Activity	IC50	Cell Line	References
1.	Stem	4-20 nm	Spherical	Antimicrobial	1.1µg/mL		Anuj SA et al., 2013
2.	Stem	9±36 nm and 12.49 nm	Spherical	Antibacterial	200 µg/mL		SIngh *et al.*, 2014

S.no.	Part Used	Particle Size	Shape	Activity	IC50	Cell Line	References
3.	Leaf	30 nm	Spherical	Antibacterial, Antioxidant	10 µg/mL		Selvam *et al.,* 2017
4.	Leaf	25-50 nm	Spherical	Anticancer	100 µg/mL	cell line A549	Jitendra Mittal *et al.,*2020
5.	Stem	0.4 nm	Spherical	Anticancer	200 µg/mL	HepG2 cancer cell line	Sakthi Priya M *et al.,* 2020
6.	Stem	182.9 ± 3.8 nm	Spherical	Antioxidant	250 µg /mL		Jeimmy González-Masís *et al.,* 2020
7.	Stem	20-30 nm	Spherical	Antibacterial	200 µg/mL		Prajwala *et al.,* 2021
8.	Stem	100-200 nm	Spherical	Antioxidant	28.62 ± 0.63 µg/mL		Abhijeet Puri *et al.,* 2022
9.	Leaf	25 nm	Spherical	Antialgal	5-10 µg/mL		Bijula *et al.,* 2022
10.	Stem	100-200 nm	Spherical	Anticancer	31.29 ± 0.22 µg/mL	MCF-7 Cell line	Abhijeet Puri *et al.* 2022

Techniques Learnt During the Project

Methodology

Murashige and Skoog (MS) medium is the most widely used media among the all the different media available like B5, N6, White and Nitsch media. It was originally prepared for the induction of organogenesis and regeneration in plant tissue cultures and has since been used in various plant tissue culture studies for several plant species with different types of culture systems.

Table 4: Components of Murashige and Skoog (MS) medium.

Components	Murashige and Skoog (MS) (mg/l)
Macronutrients	
$MgSO_4.7H_2O$	370
KH_2PO_4	170
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
Micronutrients	
H_3BO_3	6.2
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.83
$FeSO_4.7H_2O$	27.8
$Na_2EDTA.2H_2O$	37.3
Organic supplements vitamins	

Thiamine HCL	0.5
Pyridoxine (HCL)	0.5
Nicotinic acid	0.5
Myoinositol	100
Others	
Glycine	2

z

Media Preparation

4.4 g/l of MS medium was dissolved in 1000ml of distilled water and further fortified with 3% of sucrose and 0.8% of Agar. The pH of the media was calibrated to 5.7 by adding suitable amounts 1N HCl / 1N NaOH before the gelling of agar was completed. After the adjusting of pH the media was autoclaved at 121°C at 15 lb pressure for 20 minutes. Once the sterilization of the medium was concluded the medium was poured into conical flask and test tubes (Slant culture at a 45-degree angle) under the laminar air flow cabinet and made airtight using cotton plugs in order to prevent from contamination. The medium was then allowed to set.

Fig 3: MS media kept in laminar air flow ready for inoculation.

Explant Culture

Standardization of the explant culture technique was done using *Brassica* seeds. The seeds were initially surface sterilized using 95% of ethanol for three times followed by the treatment with 0.1 % of HgCl2 for 2- 3 minutes. The explants were then rinsed with autoclaved distilled water for 3-4 times in order to remove any residual traces of HgCl2. After sterilization the explants were inoculated on the MS medium and kept under controlled environmental conditions of culturing. Similar process was repeated for the nodal explants obtained from the plants of *Tinospora cordifolia* growing in Botanical Garden of Sri Venkateswara College. The explants were thoroughly washed with Teepol (Detergent) for 2-3 times and then the above sterilization process was repeated before inoculating them on to the medium.

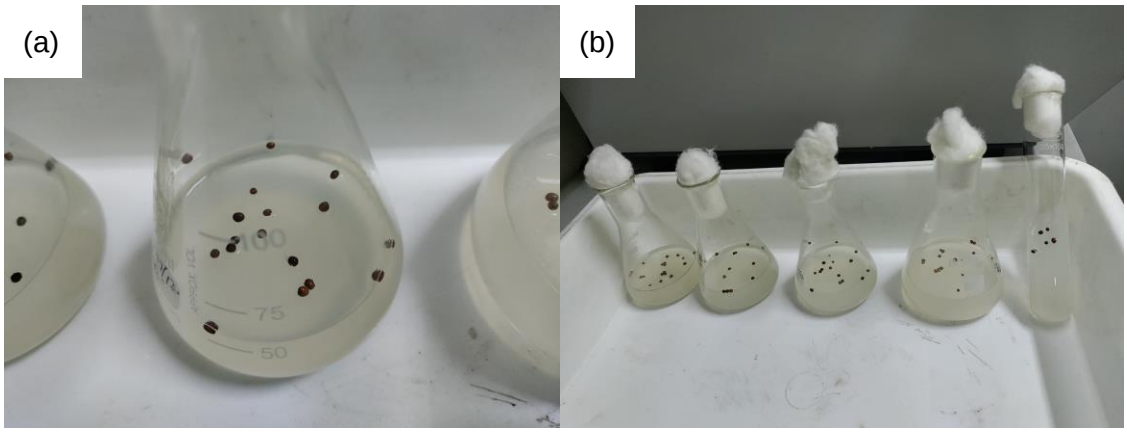

Fig 4: Inoculated media. (a) Flask culture close-up with *Brassica* seeds. (b) Four flask cultures and one tube slant culture of *Brassica* seeds, with cotton plugs.

Morphogenic Response

After 1 week of culture, seed germination along with rooting was seen in the cultures for *Brassica* seeds. Elongated seedlings were monitored over the subsequent week and average shoot length of 8-9 cm observed.

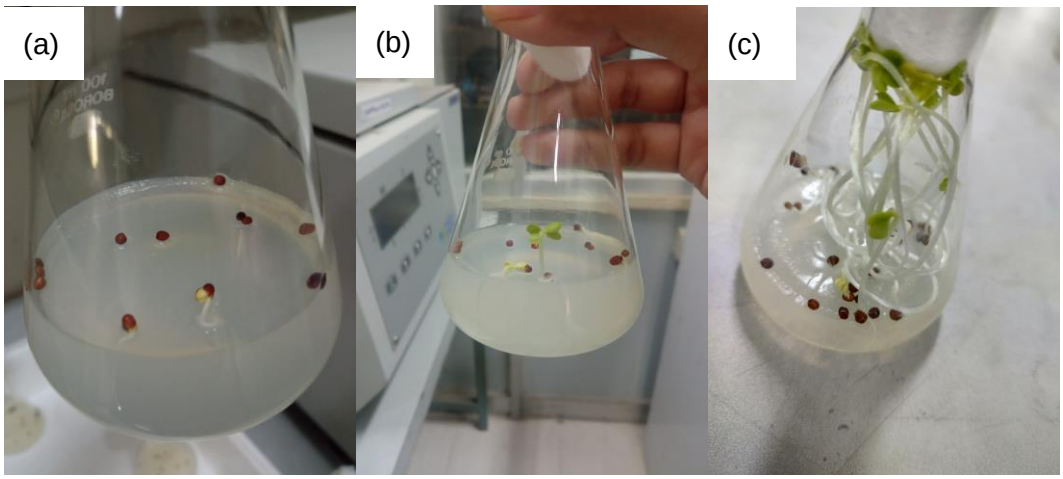

Fig 5: Successive germination steps of *Brassica* seeds in flask cultures. (a) Emergence of Radical. (b)germinated seedling. (c) elongated seedlings.

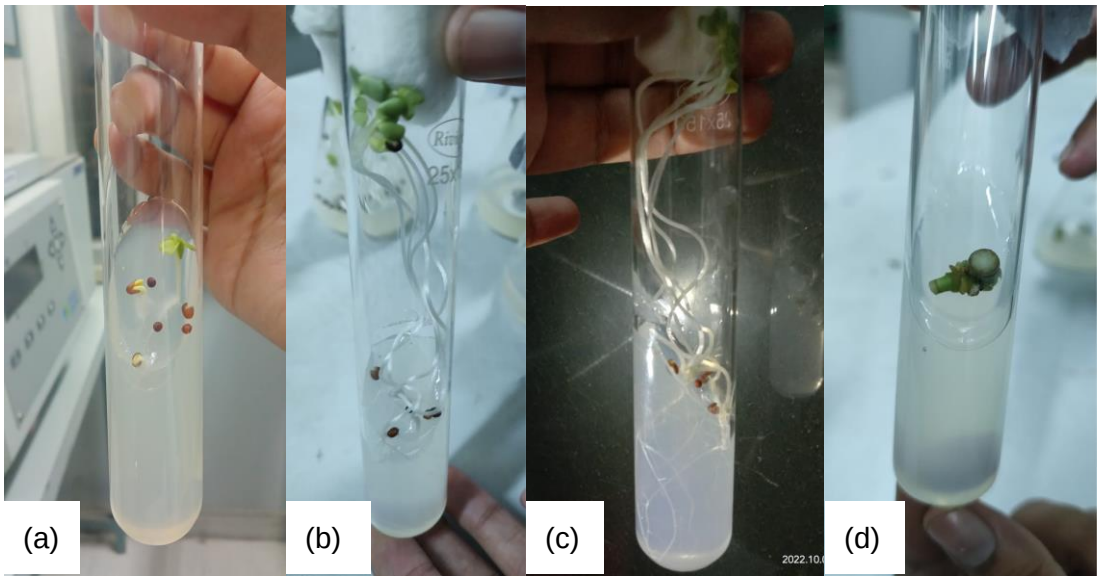

Fig 6: *In vitro* cultures. (a) Initial germination of *Brassica* seeds. (b) germinated seedlings of *Brassica*. (c) Root growth seen in *Brassica* plantlets. (d) Nodal explants of *Tinospora cordifolia*

Abbreviations

1. MS – Murashige and Skoog medium
2. WPM – Woody Plant Medium
3. KIN – Kinetin
4. BAP – 6-Benzylaminopurine
5. NA – Noradrenaline
6. NAA – 1-Napthalenacetic acid
7. TDZ – Thidiazuron
8. BA – Benzyl Adenine
9. IAA – Indole-3-acetic acid
10. IBA - Indole-3-butyric acid
12. 2-Ip -6-(γ,γ-Dimethylallyamino)purine
13. AdS – Adenine sulphate
14. CM – Coconut Milk
15. HDL – High-density lipoprotein
16. AgNPs – Silver nanoparticles
17. TEM – Transmission electron microscopy
18. SEM – Scanning electron microscope
19. XRD – X-ray diffraction analysis
20. Au – Gold
21. Ag – Silver
22. Pt – Platinum

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