GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *CORDIA SEBESTENA* AND ITS EFFECT ON INFECTIOUS MICROORGANISMS

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ABSTRACT

The present study was carried out to investigate the antimicrobial activity of silver nanoparticle from *Cordia sebestena*. Plant extract shown that the great antibacterial activity against the E.COLI. The plant extract showed the high antioxidant activity and the pharmacognosy activity on the pathogenic microbes.

INTRODUCTION

Nanobiotechnology is rapidly growing as an interdisciplinary eco-friendly research area and used in broad research section such as biology, chemistry, physics, biomedicine and material engineering **(M.Daniel *et al*.,2004].** It deals with various shapes and size of particles in the range of 1 to 100 nm From last two decades, top down and bottom-up approaches are used to produce metal nanoparticles with different morphologies, compositions and structures. It is known for its antimicrobial, anti-inflammatory activities other than that it is used in electrical batteries, an optical receptor in solar batteries, bio-labeling and in cancer treatment.

Nontoxic and new methods in the field of nano research have been developed that involves microorganism and plants for the synthesis of nanomaterials **(Mittal AK *et al*., 2013)** nanoparticle can be fabricated by using different physicochemical methods their synthesis by a biological method such as using microorganisms, single cell plant, plant tissues, fruits or plant extract is a more attractive option as they are nontoxic and therefore environmentally safe especially for their application in food, pharmacy or in medicine. Although several routes are developed for biosynthesis of metal nanoparticles from the salt of the corresponding metal, the most advantageous option is to use plant extract as they are less expensive and very easy, simple, rapid and less energy extensive process as synthesis can be carried out at room temperature within few seconds to few minutes and easy to scale up also Biogenic synthesis is advantageous not only that it is environmentally friendly but also it is free of contamination and has a well defined size, shape, and morphology than another physicochemical synthesis method. Plant extract acts both as reducing and stabilizing agent in the synthesis of nanoparticles. The activity of the plant extract varies with the varying source as it has different composition and concentration of the particular organic component in the reducing extract. In view of the number of different chemicals involved the bioreduction process is relatively complex.

Nanoparticles are gaining reputation as multifaceted materials exhibiting novel or advanced characteristics compared to larger particles . Smaller sized nanoparticles display higher surface-to-volume ratio; a feature vital to catalytic reactivity, thermal conductivity, antimicrobial activity, chemical steadiness, and non-linear optical performance . Such characteristics have nanoparticles currently playing significant roles in medical diagnostics, drug delivery systems, anti-sense and gene therapy applications, and tissue engineering . With nanoparticles integrated in consumers' health and industrial products, it is necessary to develop techniques that implement a "green" path for the synthesis of nanoparticles . In order to provide a more environmentally sound synthesis of nanoparticles, various biological routes are considered including the use of plant extracts enzymes , bacteria , fungi, and algae**.(M.Gilaki *et al*., 2010).**

MEDICINAL USES OF CORDIA SEBESTENA

Bronchitis catarrh Cough Emollient Fever Flu Intestine Pectoral Pertussis Stomach. Medicinal plants are known to play vital roles in the management of various disease especially in traditional setting where access to western medicine is either remarkably low or highly expensive. In fact, the use of medicinal plants as therapeutic agents for both humans and animal pre dates recorded history**.(O.Atoni *et al*.,2009).** Various

pharmaceutical and nutraceuticals have been developed from ethno-medicinal plants, and many are widely recorded n pharmacopoeias for use in global health-care system**(JG.Graham *et al* 2000).** It has been stated that of the 877 novel medicines developed between 1981 and 2002, natural products constituted 6%, derivatives constituted 27%, and synthetic developed on the model of natural products constituted 16%(**D.Newman *et al* 2002)** this goes to show the importance of nature as a repository for developing novel leads for medicines. It has also been reported that at least 80% of the world's population still uses traditional medicines in primary health care such as the 40,000 – 70,000 medicinal plants that constitute about 20% of all higher-plant species (**R.verpoorte *et al*., 2000).**

Cordia sebestena, also called Geiger tree, is a species of a flowering plant belonging to the family Boraginaceae and native to the American tropics where it grows up to 30 m high, bearing green or white scenty fruits of about 7.5 cm. The plant is grown as an ornamental tree in Nigeria where its medicinal importance is unclear.

The chemical compositions of the petroleum ether and ethyl acetate extracts of the flowers have been reported.**(CB.Adeosun *et al*.,2012)** The dyeing potential of the flower have also been evaluated.**(M.Kumaresan *et al*.,2012)** Bioassay guided fractionation of the ethyl acetate extract of the fruit of the plant have led to the isolation of sebestinoids A-D, which exhibit a moderate inhibition of aspartic protease.**(J.Dai *et al*.,2010)** However, the chemical composition and free radical scavenging activity of essential oils of *C. sebestena* stem bark have not been studied to date. Hence the present investigation was undertaken to evaluate the green synthesis of silver nanoparticles using *Cordia sebestena* and its effect on infectious microorganisms.

MATERIALS AND METHODOLOGY

COLLECTION OF SAMPLE

The various microbial specimens was obtained from various clinics of Chennai. The organisms were isolated from the clinical samples by performing the following tests.

COLLECTION OF PLANT

The plant *Cordia sebestena* was collected from Chennai, TamilNadu, India. Collected plants were carefully examined and identified with the help of regional flora .

PREPARATION OF POWDER

The leaves of plants were collected and dried under shade. These dried materials were powdered by using mortor and pestle and store in an airtight container. These powdered material were used for further process.

PREPARATION OF EXTRACT

20 g of dried plant powder of *Cordia sebestena* mixed with 180ml of distilled water for the extraction .It was heated upto 60ºc for 45 minutes.The extract was filtered with Whattman No:1 filter paper and clear filtrate was concentrated in room temperature by evaporation.This total extract was used for finding the Antimicrobial activity.

PHYTOCHEMICAL ANALYSIS OF CORDIA SEBESTENA EXTRACT

GLYCOSIDES, ALKALOIDS, TANNIS, FLAVONOIDS, STEROIDS, RESIN, SAPONIN were carried out.

SYNTHESIS OF SILVER NANOPARTICLE

200 ml of Cordia sebestena extract mixed in 300 µl of silver nitrate. It was incubated for 24 hours in dark room. The mixer was filtered with Wattman no:1filter paper. The colour change from yellow to brown color was observed, after the 24 hours of incubation. The solution was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was collected. Add petroleum ether solution to drain off the excess water from the collected pellet. The dried silver nanoparticle pellet used the Antimicrobial activity.

CHARACTERISATION OF THE SYNTHESIZED SILVER NANOPARTICLE

UV-VIS SPECTROPHOTOMETER ANALYSIS, XRD MEASUREMENTS,SEM ANALYSIS FOR THE SILVER NANOPARTICLES, FTIR ANALYSIS OF SILVER NANOPARTICLES

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM *CORDIA SEBESTENA* BY SPOT OVELAY MATHOD

The muller hinton agar medium was prepared and solidified.

The collected bacterial specimens *E.coli, Streptococcus, Bacillus subtilis*

Pseudomonas aeruginosa were swapped on the muller hinton agar separately.

A drop of silver nanoparticles from *Cordia sebestena* was placed at centre of plate.
The plates were incubated for 24 hours at 37ºC.

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLE FROM *CORDIA SEBESTENA* USING WELL DIFFUSION METHOD

The nutrient agar medium was prepared. Allow to solidifying in 4 petri plates at 15 minutes. The organisms used for the Antibacterial activity are *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus.* Each culture was swabbed in the petriplate.Using well borer wells were made in the petriplate. 20µl, 40µl, 60µl, 80µl different concentration added the sample in well from petriplate.The centre wells 20µl of positive sample was added.The plates were incubated at 37ºc for 24 hours.The zone of inhibition were calculated

MINIMAL INHIBITORY CONCENTRATION (MIC) OF SILVER NANOPARTICLES FROM *CORDIA SEBESTENA* USING WELL DIFFUSION METHOD:

Nutrient agar medium was prepared Allow to solidifying in 4 petri plates at 15 minutes.The organisms used for the Antibacterial activity are *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus* .Each culture was swabbed in the petriplate.Using well borer wells were made in the petriplate 20µl ($AgNO_3$), 40µl (AgNPs), 60µl (plant extract), 80µl (water) different concentration added the sample in well from petriplate. The plates were incubated at 37ºc for 24 hours.The zone of inhibition were calculated.

RESULT AND DISCUSSION

Medicinal plants are known to play vital roles in the management of various disease especially in traditional setting where access to western medicine is either remarkably lower high expensive. In fact, the use of medicinal plants as therapeutic agents for both humans and animals predates recorded history. Various pharmaceuticals and nutraceuticals have been developed from ethano medicinal plants and many are widely recorded in pharmacopoeial for use in global health care system. The herbal products usually symbolize safety in contrast to the synthetic drugs that are considered harmful to human beings and their manufacturing pollute the environment. Although herbs had been priced for their medicinal and aromatic qualities since time immemorial, the synthetic products of the modern age have surpassed these for a while. *Cordia dichotoma* is an important plant as its leaves extract has been used in wound healing **(Kuppasta.I.J et al., 2006),** anthelmintic activity and other pharmaceutical purposes. However, its antibacterial activity has not been mentioned any where in literature. *Cordia sebestena* an understudied medical plant is used intraditional medicine for the treatment of gastrointestinal disorders.In the present investigation was to made the silver nanoparticles of *cordia sebestena* as the nanomedicine to cure microorganisms on the leather. The results revealed that the methanolic extract has shown more degree of antimicrobial activity than other extract when compared to the standard drug (ampicillin). It shows antimicrobial activity whe compared to standard drug. It is due to presence of chemical constituents like carbohydrates, phenolic compounds, tannis, triterpenoids ,saponins, terpenoids, protein and amino acid which was confirmed by phytochemical studies**.(Rewat suman et al.,2013)**

Antibacterial Activity of Silver Nanoparticle of *Cordia sebestena* well Diffusion Technique

S. NO	Bacteria	ZONE OF INHIBITION (mm)			
		Silver nanoparticles			
		20µl	40µl	60µl	80µl
1	*Bacillus subtilis*	21mm	20mm	20mm	23mm
2	*Pseudomonas aeruginosa*	20mm	20mm	21mm	21mm
3	*E.coli*	15mm	15mm	16mm	17mm
4	*Streptococcus*	21mm	19mm	20mm	21mm

Minimal inhibitory concentration Activity of Silver Nanoparticle of *Cordia sebestena* well Diffusion Technique

S. NO	Bacteria	ZONE OF INHIBITION (mm)			
		Silver nanoparticles			
		20µl ($AgNO_3$)	40µl (AgNPs)	60µl (plant extract)	80µl (aqeous)
1	*Bacillus subtilis*	23mm	22mm	22mm	24mm
2	*Pseudomonas aeruginosa*	20mm	21mm	21mm	23mm
3	*E.coli*	20mm	21mm	22mm	25mm
4	*Streptococcus*	23mm	20mm	21mm	24mm

CONCLUSION

Preliminary phytochemical study of Cordia was carried out for Flavonoids, Saponins and tannins. Cordia showed positive result for Flavonoids and Saponins but negative for Tannins**.** Prepared plant extract was tested for antibacterial activity by disc diffusion and well diffusion method against pathogenic procured *E. coli*. Plant extract showed maximum zone of inhibition 12 mm, 10 mm, 8 mm, 6 mm and 4 mm respectively at 12.5mg/disc, 6.25mg/disc, 3.12mg/disc, 1.56 mg/disc and 0.78mg/ disc concentration in disc diffusion method, while 10 mm, 8 mm, 6 mm and 4 mm at same concentration in well diffusion method. Earlier reports have suggested that flavonoids and triterpenoids are responsible for antibacterial activity. However, pharmacognosy investigation of present study revealed flavonoids active constituents in *Cordia* **.**

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