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Studies on the phytochemistry, antibacterial activity and green synthesis of nanoparticles using *Cassia tora l. against amphicillin resistant bacteria*

Sathya, A* and V. Ambikapathy

Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, Tamilnadu, India

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ABSTRACT

The field of nanotechnology is one of the most active areas of research in modern materials science and technology. An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. The present study synthesis from 1 mm $AgNO_3$ solution through the leaf extract of Cassia tora L. as reducing as well as capping agent. Nanoparticles were characterized using UV –vis absorption spectroscopy's green synthesized silver nanoparticles showed the antibacterial against the isolated microorganisms. Escherichia coli, Pseudomonas sp. and Salmonella sp. were isolated from clinical specimen. Phytochemical analysis of aqueous extracts of Cassia tora L. showed the presence of anthraquniones, carbohydrates, glycosides, cardiac glycosides, amino acid, phytosterols, fixedoils and fats, phenolic compounds, tannins, flavonoids, steroids and saponins. The silver nanopaticles was characterized by using UV –visible Spectrophotometer at the range of 200-800nm. Absorption spectrum (200-800nm) at the beginning of the reaction showed three distrinct peaks , centering around 220, 280 and 430nm. The absorption spectrum showed increase in intensity over a broad spectrum in the range of 350-600nm within 10min of contact time while the remaining part of the absorption spectrum in the range of 350-600nm continued to increase with increasing incubation time till 60min. Each antibiotic resistant microorganism differs in the formation of zone of inhibition representing their sensitiveness to silver nanoparticles extracts of Cassia tora.

Key Words: *Cassia tora*, Phytochemical, Antibacterial and Silver Nanoparticles

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INTRODUCTION

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multi cellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries (Khosravi and Behzadi, 2006). Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases.

Cassia tora L. (Family: *Cesalpinaceae*), *Calendula officinalis* (Family: *Compositae*) and *Momordica charantia* (Family: *Cucurbitaceae*) are some of the very common Indian herbs having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents respectively (Nadkarni, 1982; Brown and Dattner, 1998; Grover and Yadav, 2004; Christopher, 2005) . These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis, leprosy etc (Horvath and Ferenc, 1992; Zahra *et al*., 2000; Cordova et al., 2002; Harrison and Dorothy, 2003).

The biologically inspired experimental processes for the synthesis of nanoparticles is evolving in to an important branch of nanotechnology. Metalic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable. The present study deals with cost effective and environment friendly given synthesis from 1 mm $AgNO_3$ solution through the leaf extract of *Cassia tora* L. as reducing as well as capping agent. Nanoparticles were characterized using UV –vis absorption spectroscopy's green synthesized silver nanoparticles showed the antimicrobial against the isolated microorganisms.

MATERIALS AND METHODS

Plant material and preparation of the Extract

The leaves of the plants were collected from uncultivated farmlands located at Southern parts of Tamilnadu in Thanjavur. All the plant samples were identified by the authors. The plant samples were air-dried and ground into uniform powder using a Thomas- Willey milling machine. The powder was used for extraction of bioactive compounds. The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No. 42 (125 mm).

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Microorganisms used

Escherichia coli, Pseudomonas sp. and *Salmonella sp.* were isolated from clinical specimen. Isolated bacteria were identified based on morphological and biochemical characteristics. Log phase cells were used for assays.

Green Synthesis of Nanoparticles of AgNo3

5mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Cassia tora* L. leaf extract was added into 90 ml of aqueous solution of 5 mM Silver nitrate for reduction into Ag+ ions and kept at room temperature for 4 hours. Bionanoparticles characterized by the UV visible spectrophotometer (430nm).

Analysis of Bacterial Activity

The antibacterial activity of crud *Cassia tora* L. leaf extract and green synthesized silver nanoparticles and crud extracts were tested by the agar diffusion method. For the determination of antibacterial activity, the antibiotic resistant bacteria namely, *Salmonella spp, Eshedrichia coli and Pseudomonas aeruginosa.*

RESULTS AND DISCUSSION

In the present study *Escherichia coli, Pseudomonas sp.* and *Salmonella sp.* were isolated from clinical specimen. Isolated bacteria were identified based on morphological and biochemical characteristics. The results were presented in table -1. Phytochemical analysis of aqueous showed the presence of anthraquniones, carbohydrates, glycosides, cardiac glycosides, amino acid, phytosterols, fixed oils and fats, phenolic compounds, tannins, flavonoids, steroids and saponins while alkaloids are absent in all of the tested extracts (Table - 2). Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs. (Le G A. 1989; Evans WC. 1996) Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will be different. (Kirtikar and Basu 1999)

The antibiotic sensitivity of bacteria such as *Escherichia coli, Pseudomonas spp* and *Salmonella spp* against various antibiotics was analyzed. The organisms showed resistance to antibiotics such as Ampicillin, Chloramphenicol, Streptomycin and Tetracycline (Table - 3). Aqueous extract was found to have maximum zone of inhibition against *Escherichia coli, Pseudomonas spp*, and *Salmonella spp*.

The antimicrobial activity of silver nanoparticles produced by the soil bacteria *Cassia tora* against the above mentioned antibiotic microorganisms at various concentrations 0.5mM and 1mM (Table - 3). Literature also reveals the use of aqueous paste of seeds of *Cassia tora, Momordica charantia* seed juice (prepared in water) and Calendula flower water decoctions in various skin conditions associated with bacterial infections. (Nadkarni, 1982, The silver nanopaticles was characterized by using UV –visible Spectrophotometer at the range of 200-800nm. The UV-visible spectra recorded at different time intervals showed increased absorbance with increasing time of incubation at around 420nm. Absorption spectrum (200-800nm) at the beginning of the reaction showed three distinct peaks , centering around 220, 280 and 430nm. The absorption spectrum showed increase in intensity over a broad spectrum in the range of 350-600nm within 10min of contact time while the remaining part of the absorption spectrum in the range of 350-600nm continued to increase with increasing incubation time till 60min. The absorbance at around 220 and 280nm maintained the same intensity throughout the incubation time. The nature of the absorbance spectrum representing absorbance profile of the cell filtrate of showed trend similar to *Cassia tora* cell filtrate. However the intensity of absorbance in the range of 350-600nm increased significantly resulting in gradual appearance of a peak at 420nm (Table - 4).

Table - 1Morphological and biochemical characteristics of Isolated bacteria

S. No.	Morphological and Biochemical characteristics	Results		
		Escherichia aeruginosa	*Pseudomonas Spp*	*Salmonella spp*
1.	Motility	+	+	+
2.	Gram staining	-/Rod	-/Rod	-/Coccus
3.	Indole test	+	-	-
4.	Methyl red test	+	-	+
5.	Voges Preskaur test	-	-	-
6.	Citrate test	-	+	+
7.	Triple sugar iron test	A/G	A	A
8.	Urease test	+	+	-
9.	Catalase test	+	+	-
10	Oxidase	-	+	+
11.	Carbohydrate Fermentation test (S/G/L)	+/+/+	-/-/-	+/+/-

'+' - Positive; '-' - Negative; 'A' – Acid; 'G'- Gas

Table – 2 Phytochemical analysis

S. No.	Phyto constituents	*Cassia tora* L aqueous extracts	
		Leaf	Seed
1	Alkaloids	-	+
2	Tannins	+	+
3	Saponins	+	+
4	Carbohydrate	+	+
5	Glycoside	-	+
6	Phytosterols	+	+
7	Oils and Fats	+	+
8	Phenol	+	+
9	Flavonoids	-	+
10	Protein and amino acid	+	+

'+' - Positive; '-' - Negative;

Table – 3 Antibiotic Sensitivity of isolated bacteria

S. No.	Isolated Bacteria	Zone of inhibition mm in diameter						
		Standard Antibiotics				*Cassia tora* crude extracts	*Cassia tora* silver nanoparticles	
		Ampicillin	Chloramphenicol	Streptomycin	Tetracycline		0.5 mM	1 mM
1	*Escherichia coli*	Resistant	8	10	Resistant	14	12	16
2	*Pseudomonas Spp*	Resistant	11	8	12	20	18	22
3	*Salmonella spp*	Resistant	12	Resistant	8	18	20	26

Table – 4 Characterizations of Silver Nanoparticles

S. No	Time duration (mins)	0.5 Molar of $AgNo_3$ (200-800nm)				
		Plant extract (ml)				
		1	2	3	4	5
1	30	0.3	0.1	0	0	0
2	60	0.5	0.1	0	0	0
3	90	0.8	0.4	0.5	0.5	1
4	120	0.8	0.6	1	1.1	1..5

S. No	Time duration (mins)	1 Molar of $AgNo_3$ (200-800nm)				
		Plant extract (ml)				
		1	2	3	4	5
1	30	0.5	0.1	0.5	0.6	0
2	60	0.5	0.5	0.6	0.6	0
3	90	1	0.8	0.8	0.9	1.1
4	120	1.1	1.5	1.2	0.9	1.2

CONCLUSION

Finally concluded *Cassia tora* plant extract with aqueous and synthesis of silver nanoparticles can be used as antimicrobial agent and also used as herbal medicine for curing number of disease in the form of pellets of paste. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages. Plant extract is being eco friendly and very cost effective; the presented method can be economic and effective alternative for the large scale synthesis of silver nanoparticles in nanotechnology processing industries.

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