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Formulation and Characterization of Anti-Bacterial Activity of Silver Nanoparticles Using Root and Leaf of *Wedelia trilobata*

Sai Prasanna K*[1], Jyothi Reddy G[1], Kiran M[1], Appa Rao C[2]

[1]Department of Pharmacology, Sri Venkateswara University of Pharmaceutical Sciences, Tirupati-517501, Andhra Pradesh, India
[2]Department of Biochemistry, Sri Venkateswara University of Pharmaceutical Sciences, Tirupati-517501, Andhra Pradesh, India

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ABSTRACT

In the present study one such species, *Wedelia trilobata*, has be chosen to review the *In vitro* antibacterial activity of the root extract by using solvent ethanol for the selected plant *Wedelia triolobata*. The deduction of nanoparticles used to be unalterable by UV-Visible spectroscopy, FTIR, particle size, and zeta potential. The anti-bacterial activity of *Wedelia trilobata* mediated synthesis silver nanoparticles was tested by disc diffusion assay against standard organisms like *Escherichia coli* and *staphylococcus aureus* bacteria. The formation of silver nanoparticles by *Wedelia trilobata* was initially demonstrated by color changes confirmed by UV-Visible root exhibited a specific absorbance peak around 450 nm and leaf exhibited a specific absorbance peak around 418nm respectively. The Zeta potential of silver nanoparticles was found to be root 0.1 and leaf 0.4, so it indicates the dispersion and stability. The antibacterial activity against Gram-positive *Staphylococcus aureus* was increased, which was indicated by an increase in the inhibition zone diameter from 3Mm for normal (9.5Mm), standard (12.3Mm), control (11.3Mm), extraction for AgNPs *Wedelia trilobata* (13.8). 5Mm for normal (7.3Mm), standard (19.4Mm), control (10.5Mm), extraction for AgNPs *Wedelia trilobata* (19.8). The antibacterial activity against Gram-negative *E.coli* was increased, which was indicated by an increase in the inhibition zone diameter from 3Mm for normal (7.8Mm), standard (8.5Mm), control (10.2Mm), extraction for AgNPs *Wedelia trilobata* (14.3). 5Mm for normal (13Mm), standard (19.6Mm), control (15.3Mm) extraction for AgNPs *Wedelia trilobata* (19.8). The present investigation revealed that the ethanolic Root and aqueous leaf extracts the chosen plant have potential to suppress the expansion of infective bacterial strains.

*Corresponding Author

Name: Sai Prasanna K
Phone: +91 9063531316
Email: kandipallisaiprasanna24@gmail.com

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INTRODUCTION

Medicinal plants can attain render an informant for producing novel drug compounds. Plants becoming in bottom the development medicine or they are used phytomedicine the treatment of disease. Many plants go through proven to with success aid booming diseases leading to deal auditing for their therapeutic components [1]. Today, the search for natural compounds rich in antimicrobial is escalating due to their medicinal importance in controlling many related chronic disorders. The speedy emer-

gence of medication resistant strains of pathogen to up to date antimicrobial agents has carried an urgent intensive search for antibiotics delight in medicinal plants [2]. Several medicinal plants were screened widely for their anti-microbial activity of silver nanoparticles using root and leaf of *Wedelia trilobata* has been historically used for amenorrhea they contain the diterpene and luteolin. Kaurenoic acid has antibacterial, larvicidal and tripanocidal activity; It's also a multipotent stimulant of uterine contractions [3].

MATERIALS

The fresh plant of *Wedelia trilobata* was procured in Tirumala, Tirupati. Andhra Pradesh, India. Ethanol, petroleum ether was purchased from Aristo Pharmaceuticals, Mumbai, India. Amikacin was purchased from Ranbaxy Pharmaceuticals, Mohali, India.

METHODOLOGY

Extraction of *Wedelia trilibata* Root

Wedelia trilobata root was collected and washed then shade dried coarsely powdered. The 50gm of root powder was defecting with petroleum ether and filtered. Then 50gm of powder was macerated with 500ml of ethanol for 3 days with osculation string [4]. It was filtered and marc was re-extracted with 500ml of ethanol both the extract was combined and the concentration for rotary evaporator obtained extract was collected and stored [Figure 1]

Synthesis of Silver Nanoparticles on *Wedelia trilibata* Root and Leafs

Ethanolic extract of *Wedelia trilobata* root 10 ml was added to the 90 ml of 1mM AgNO3 a ratio of 9:1. This solution was kept at ambient room temperature and stirred continuously for 10 min using a magnetic stirrer. After 24 hours changed green color upto dark brown colour that signifies the formation of silver nanoparticles. The silver nanoparticles obtained were stored for further analysis [5].

Characterization of Silver nanoparticles

UV-Vis spectroscopy

The UV-Visible spectra were recorded after 18hrs from the initiation of the reaction in which the absorption maxima was scanned from 300 to 700 nm [6]. The sample was analyzed by its maximum absorbance v/s wavelength to confirm the formation of AgNPs.

Fourier Transform Infra-Red spectroscopy

FTIR trend analysis used to be carried out to spot the functional groups in *Wedelia trilobata* which have been liable for the shelter of the silver nitrate and the stabilisation of AgNPs [7]. FTIR analysis was carried out for plant extract in the wavelength range 4000-500 cm-1.

Particle size

The desiccated powders of *Wedelia trilobata* silver nanoparticles dispersed in water to obtain the scattering intensity of *Wedelia trilobata* AgNPs. The particle size was determined by the Horiba zeta size analyzer [8].

Zeta potential

Decides the surface strength of AgNPs and it is unexpendable the depiction firmness of the stability of NPs [9].

Phytochemical Screening

Test for flavonoids: Alkaline reagent′s, Shinoda test

Test for alkaloids: Dragendroffs test, Mayers test, Wagner's test.

Test for carbohydrates: Benedict's test, Molischs test, Fehling's test

Test for proteins: Biuret test, Xanthoproteic test, Millon's test

Test for glycosides: Liebermann's test, Salkowskis test, Killer Killian test

Test for phenols: Ferric chloride test, Test for tannins, Gelatin solution

Test for free amino acids: Ninhydrin test, Test for quinines, HCL test

Test for the steroids: Liebermann Burchard test

Test for saponins: Foam test, Froth test [10]

Experimental Design

Determination of Antibacterial activity (MIC and ZOI)

Disc diffusion method

The 10^8 colonies forming unit per ml bacterial inoculums in a nutrient broth which is prepared by picking 3-5 isolated colonies from the plate. The streak wipe on the surface of nutrient agar [11] goes away for 5-10 mins that one way dry the agar surface that allows for the bacterium to establish for themselves and on the media. Invert the plate and incubate them at 37^{o}C for 18-24 hours as shown in Table 1.

RESULTS AND DISCUSSION

Phytochemical screening [Table 2] of *Wedelia trilobata* ethanol: Flavonoids, carbohydrates, glycosides, phenols, tannins, steroids, saponins.

Table 1: Treatment schedule for antibacterial activity

Groups	Treatment				A dose of Ethanol extract of *Wedelia trilobata* AgNPs
	Media	Inoculums	Antibiotic	Standard Dose	
Standard	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*.	Amikacin,	10mg/kg	-
Control	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*	-	-	-
Test 1 (low dose)	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*	-	-	EWT 10 mg/kg
Test 2 (high dose)	Nutrient agar media	*Staphylococcus aureus, Escherichia coli*	-	-	EWT 20 mg/kg

Table 2: Phytochemical screening report

Sl. No	Phytochemical	Inference
1.	Flavonoids	
	a)Alkaline reagent	+
	b)Shinoda test	+
2.	Alkaloids	
	a)Wagner's test	-
	b)Mayers test	
3.	Carbohydrates	
	a)Benedicts test	+
	b)Molischs test	
	c)Fehling's test	
4.	Proteins	
	a)Biuret test	-
	b)xanthoproteic test	
	c)Millon's test	
5.	Glycosides	
	a)Liebermann's test	-
	b)Salkowskis test	+
	c)Keller Killian's test	
6.	Phenols	
	a)Ferric chloride test	+
7.	Tannins	
	a)Gelatin test	+
	b)Braymer's test	
9.	Steroids	
	a)Liebermann Burchard test	+
10.	Saponins foam test	
	a) Foam test	+

(+ indicate present, -indicate absent)

Table 3: FTIR on *Wedelia trilobata* root extract of silver nanoparticles

S.No	Type of Vibration	Wave Number		Possible Serious For Shift Alteration
		Plant Extract	Nano Silver	
1	N-H stretching vibration of secondary amines	3328	3329	Chelation of N-H groups with silver
2	O-H stretching vibration of carboxylic acids	2129	2116	Interaction of silver with OH group of flavonoids
3	Stracting vibration of C=O aldehydes or ketones	1637	1637	Chelation of C=O groups with silver
4	C-H group of phenyl ring substitution or alkynes	599	598	Interaction with phenyl ring or alkynes with silver

Table 4: Zone of Inhibition of *Wedelia trilobata* root plant

Plant	AgNPs(mM)	Zone of inhibition			
		normal	Standard	*S.aureus* (control)	Extraction (AgNPs)
Wedelia trilobata root plant	3mM	9.5	12.3	11.3	13.8
	5mM	7.3	19.4	10.5	19.8
		normal	Standard	*E.coli* (control)	Extraction (AgNPs)
	3mM	7.8	8.5	10.2	14.3
	5mM	13	19.6	15.3	19.8

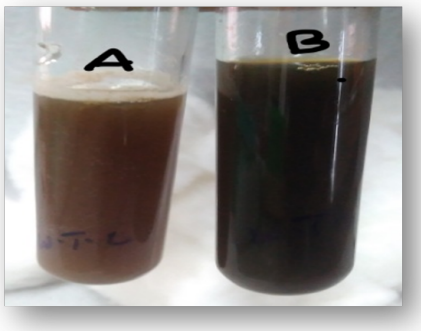

Figure 1: a. Control (*Wedelia trilobata* leaf extract) b.standard (AgNPs of *Wedelia trilobata*)

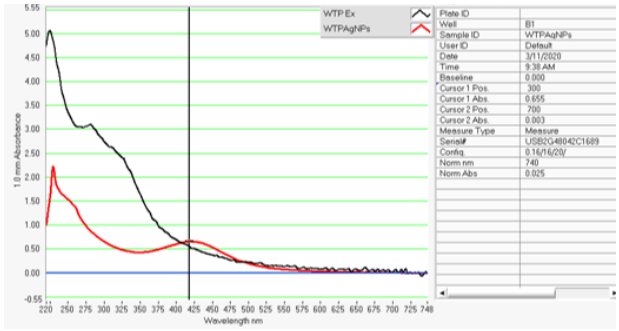

Figure 3: UV-Visible Spectroscopy of *Wedelia trilobata* Leaf Extract and AgNPs

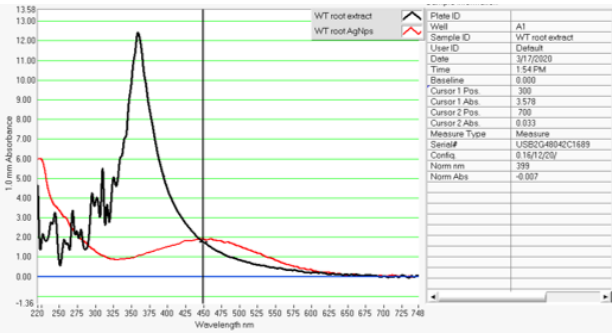

Figure 2: UV-Visible Spectroscopy of *Wedelia trilobata* Root Extract and AgNPs

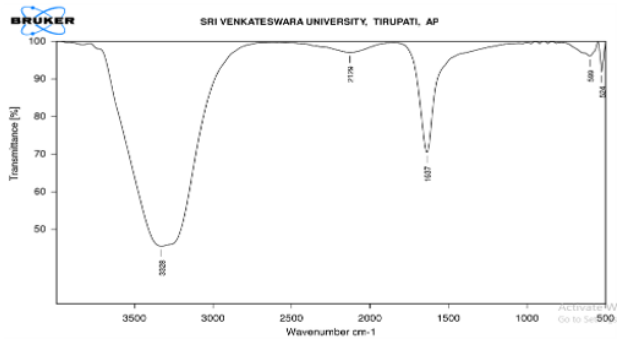

Figure 4: FTIR of *Wedelia trilobata* Root Extract

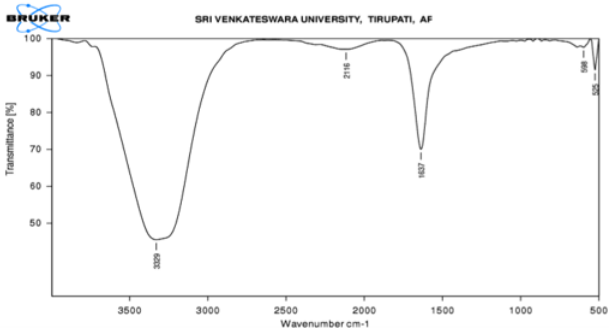

Figure 5: FTIR of *Wedelia trilobata* Silver Nano Particles

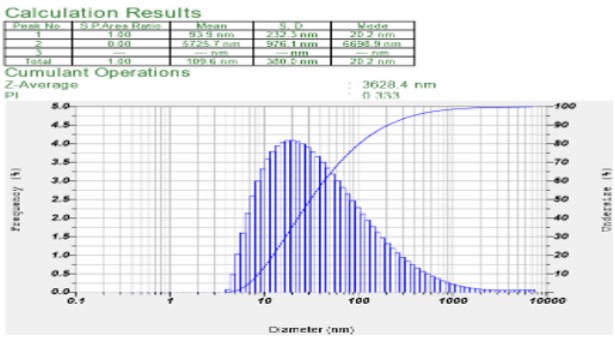

Figure 6: Particle Size of *Wedelia trilobata* Root Extract of AgNPs

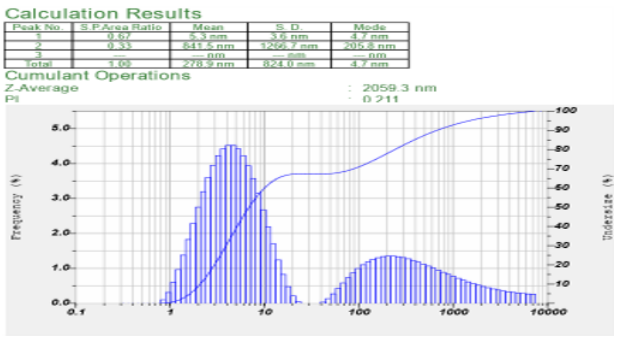

Figure 7: Particle Size of *Wedelia trilobata* Leaf Extract AgNPs

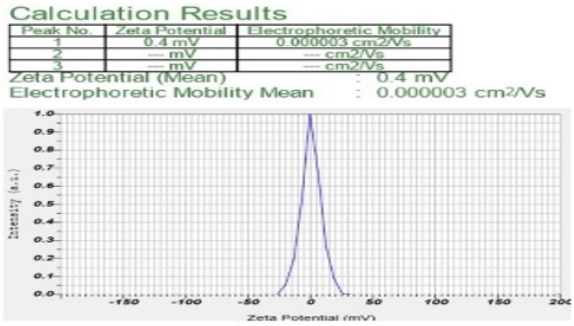

Figure 8: Zeta Potential of *Wedelia trilobata* Root AgNPs

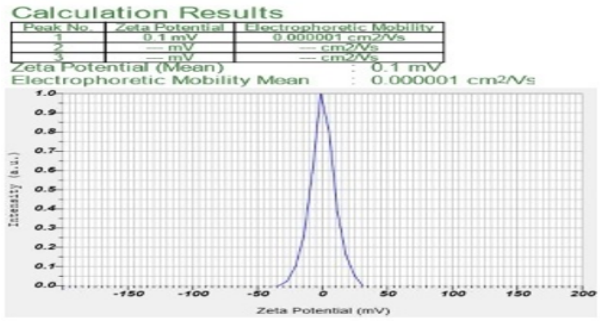

Figure 9: Zeta Potential of *Wedelia trilobata* Root AgNPs

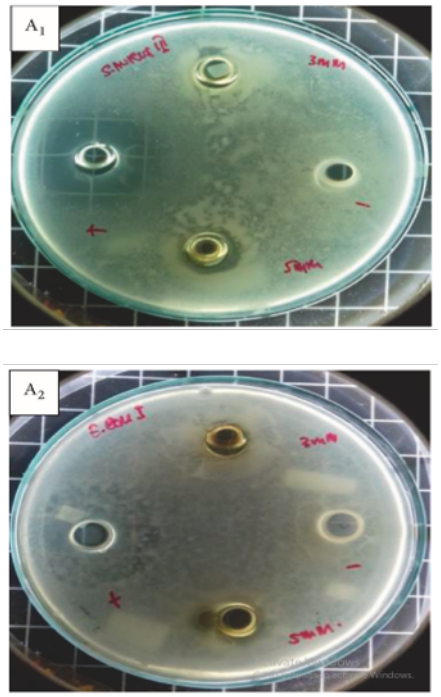

Figure 10: Antibacterial Activity of Ag Nanoparticles sequenced Using Ethanolic Root Extract Extracts of *Wedelia trilobata* Against Gram-Positive *S.Aureus* And Gram-Negative *E. coli* Bacteria

Characterization of Silver nanoparticles

UV–Visible Spectroscopy

Silver nanoparticles of *Wedelia trilobata* root exhibited a specific absorbance peak around 450nm shown in Figure 2

The *Wedelia trilobata* leaf exhibited a specific absorbance peak around 418nm shows in Figure 3.

FTIR Analysis of Green Synthesized Silver nanoparticles

The FTIR measurement was dispensed to spot the potential phytochemicals in the *Wedelia trilobata* root squeeze. FTIR spectroscopy of dried ethanolic root extract of the plant and synthesized silver

nanoparticles are shown in Figures 4 and 5 functional groups were shown in Table 3.

Determination of particle size

The *wedelia trilobata* root and leaf the synthesized AgNPs shown by the strength as well as laser diffraction unconcealed that particles acquired will be a polydisperse mixture with the size starting from 10 to 30nm. The average diameter of the particles was found to be root 3628.4nm and leaf 2059.3nm shows in Figures 6 and 7.

Determination of zeta potential

The *wedelia trilobata* root and leaf sequenced AgNPs is decided in water as dispersant. The zeta potential found to root and leaf 0.1-0.4 as shown in Figures 8 and 9.

Antimicrobial activity

Antibacterial activity show by the inhibition zone that used to be characterized by a visible zone in between the wells along with a sure distance. The standard drug amikacin 250mg solution in addition to operates the as control of your test way out by comparison the length of the inhibition zone formed. The antibacterial activity opposed to Gram-positive *S. aureus* was increased that used to be designated by a rise that in holdup zone diameter Figure 10 & Table 4.

CONCLUSION

The phytochemical constituents present in ethanolic extract like alkaloids, glycosides, steroidal, saponin, amino acids, tannins, proteins, carbohydrates, phenols. The present study conformed anti-bacterial activity of green silver nanoparticle prepared anti-microbial root extract of *Wedelia trilobata*. Water-soluble flavonoids present in the root extract might have acted that as a reductant within the formation of AgNPs. The AgNPs were spherical with average size ranges between root 3628.4nm and leaf 2059.3nm. Silver nanoparticles synthesized using ethanolic root extract of *wedelia trilobata* against gram-positive s.aureus zone of inhibition 3mM (9.5Mm), standard(12.3Mm), control(11.3Mm), 5mM normal (7.3Mm), standard (19.4Mm), control (10.5Mm). Intense preclinical and clinical studies to evaluate the efficacy of this plant product results could be beneficial for future study.

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Conflict of interest

The authors attest that they have no conflict of interest in this study.

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