

# GREEN SYNTHESIZED SILVER NANOPARTICLES FROM *Wrightia tinctoria* AND ITS ANTIMICROBIAL ACTIVITY AGAINST BIOFILM AND ESBL PRODUCING *Acinetobacter baumannii*

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**Abstract:** In recent years, hospitals in developed countries have faced forcefully high rates of antibiotic-resistant *Acinetobacter baumannii*. In the present study, we analyzed the evolution of Biofilm and ESBL characterization among clinical isolates of multidrug resistance *A. baumannii*. Among the 42 isolates, 38% of were an association with biofilm and ESBL. In the present study, we synthesized silver nanoparticles from two solvent extracts of *Wrightia tinctoria* (leaf and seed) were screened for their phytochemicals composition and antibacterial activity against biofilm and ESBL producing *A. baumannii*. Among them, leaf (methanol) extract showed highest phytochemicals constituents and good antibacterial activity. The GCMS result was revealed the presence of number of beneficial compounds. This present study was concluded that *W. tinctoria* leaves possessed potent antimicrobial properties against *A. baumannii*.

**Index Terms-** *A. baumannii*, AgNPs, *Wrightia tinctoria*, Biofilm, ESBL

## I. INTRODUCTION

The rise in multidrug-resistant (MDR) bacteria has emerged as a critical health complication and a big challenge in developing treatment option of infectious diseases. This phenomenon was occurred by indiscriminate and improper use of antibiotic (Fernandez J *et al* 2016). One of the major mechanisms of protection is inactivation of the antibiotics. This defense mechanism was usual and active against penicillin and chloroamphenicol antibiotics. One more defense mechanism was that bacteria produce the enzyme which was denaturing the drugs (Ibrahim 2014).

Among the various nosocomial pathogens, *Acinetobacter* an important opportunistic pathogen on the most of the countries in the world, mostly involving patients with impaired host defense. This isolates as a significant nosocomial pathogen, because its survival on low pH, dry and moist surface (Wankhede *et al.*, 2016). Among the *Acinetobacter* spp, *Acinetobacter baumannii* are highly resistant to antibiotics than *Enterobacteriaceae*; moreover, it has the propensity to obtain resistance. Because, this isolates as frequently resistance to a various group of antibiotics such as flouroquinolones aminoglycosides, ureidopenicillins and third –generation antibiotics of cephalosporins, carbapenems (Manikal *et al.*, 2000).

The beta-lactamase (MBL) producing *Acinetobacter baumannii* has become a growing therapeutic concern in worldwide. This ESBL producing isolates may co-exist with resistance to other classes of antimicrobials such as aminoglycosides, flouroquinolones, tetracyclines, chloramphenicol and cotrimoxazole. Furthermore, patients have infected with *Acinetobacter baumannii* efficacy pose a hazard for themselves or other patients within a hospital environment.

Regarding the limit of antibiotic treatment, many studies have focused on the alternative drugs for treating infectious diseases with using plants, because which are abundant in our environment, less side effect and overcome the antibiotic resistance. *Wrightia tinctoria* is a well known potential medicinal plant distributed in tropical region belongs to the family Apocynaceae. This plant is traditionally used to treat for against Psoriasis and non-specific dermatitis, dandruff, various scalp and skin disorders, anti-haemorrhagic, and hepatoprotective activity, anti inflammatory (Mahendra *et al.*, 2009 and Ravi *et al.*, 2010).

Now day's nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Silver nanoparticles with plants are commonly used for the therapeutic purpose. The antimicrobial property of silver binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication (Rajathi *et al.*, 2013).

In view of these reported medicinal values, the present work was carried out to examine the antibacterial potential of a AgNps of *Wrightia tinctoria* against clinical isolates of *Acinetobacter baumannii*.

## II. MATERIALS AND METHODS

### 2.1 Test pathogens

The clinical isolates of *Acinetobacter baumannii* were procured from Microtech, clinical laboratory, Coimbatore, India. All isolates were confirmed with cultural characteristic and biochemical test.

### 2.2 Determination of ESBL producing isolates

Double disc diffusion synergy test (DDST) ESBL production was confirmed by double disc synergy test, a phenotypic test. Synergy was determined between a disc of amoxyclav (20 µg amoxicillin and 10 µg clavulanic acid) and a 30µg disc of cefotaxime (3rd generation cephalosporin). Plates were incubated at 37°C for 24 h and the diameters of zones of inhibition were recorded. The isolates that exhibited a distinct shape/size with potentiating towards amoxyclav disc were considered potential ESBL producers (CLSI, 2013).

### 2.3 Collection of plant

The leaf and seed of *Wrightia tinctoria* was collected from Coimbatore, Tamilnadu and India and were shade dried, powdered and extracted in soxhlet apparatus successively with polar and nonpolar solvents such as methanol and chloroform. The extracts were stored at 4°C for further analysis. The Qualitative tests were carried out using solvents extract according to standard procedures for identification of major phytochemicals (Solomon *et al.*, 2013).

### 2.4 Preparation silver nanoparticles

For synthesis of silver nanoparticles, 10 ml of leaf and seed extracts of *Wrightia tinctoria* was added to 90 ml 2 mM solution of silver nitrate in 250 ml conical flask and kept at room temperature for 2 hrs. The primary detection of synthesized silver nanoparticles (AgNPs) was carried out in the reaction mixture by observing the colour change of the extracts.

### 2.5 Determination of antibacterial activity of plant extract:

This test was carried out according to the method of Jahir and Saurabh (2011). The Mueller - Hinton agar plates were inoculated with freshly prepared overnight inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10<sup>8</sup> CFU/ml of bacteria. The 6mm diameter of the well was made with borer on the agar plates. Different AgNPs concentrations of plant extract were filled in well with the help of micropipette and one well filled with plant extracts. The Ciprofloxacin 20 µg/30 µL was added in one well as a standard and added 100µl of solvent in another well, which was served as a control. Incubate the plate at 37°C for 24hrs, then observed the zone of inhibition. The MIC was carrying out with Resazurin method. Any colour change observed from purple to pink or colourless was taken as positive. The lowest concentration of plant extract at which colour change occurred was recorded as the MIC value (Satyajit *et al.*, 2007).

### 2.6 GCMS analysis

The methanol extract of *Wrightia tinctoria* was analysed by GC-MS. GC-MS was performed with Hewlett Packard HP 6890 series GC system with Hewlett Packard 5973 mass selective detector. DB-5 µm column (U.S.A) of length 30m with 0.32mm internal diameter and film thickness of 0.25 was used. Helium was used as carrier gas at a flow rate of 2.5ml/min. Temperature programme was 40°C to 290°C with a heating rate of 5°C/min. For compound identifications Wiley 275.MS Library data were used.

## III. RESULT AND DISCUSSION

In the present study, 42 MDR isolates were procured from clinical laboratory, which was carried out to phenotypic detection of ESBL producing isolates of *A. baumannii* using DDST screening method. The Number of methods were used for the detection of ESBLs in clinical isolates have been suggested, however because of the sensitivity of some *Acinetobacter* isolates to clavulanic acid the best recommended phenotypic method for detection of ESBL producing *Acinetobacter* is double disc diffusion method (Beceiro *et al.*, 2008).

Among the 42 isolates, 25 (59.5%) of were positive for ESBL character and 22 (52.3%) of were Biofilm producers. These biofilm and ESBL producing isolates were highly resistance to common antibiotics. Interestingly out of 42 isolates, 38% of were an association with biofilm and ESBL (Table.1). *A. baumannii* is caused the nosocomial infection and ability to infect healthy hosts and its bias to develop drug resistance. This phenomenon was reported worldwide and most of the isolates are resistant to a different class of antibiotics. Moreover, this biofilm formation might also enhance the receiving of novel drug resistance properties help the exchange of diffusible genetic materials (Enea *et al.*, 2017).

**Table 1. Association between Biofilm and ESBL Producing *A. baumannii***

S.No	Biofilm	ESBL (n=42)		Total %
		ESBL Positive	ESBL negative	
1.	Biofilm positive	16 (38%)	6 (14.2%)	22 (52.3)
2.	Biofilm negative	9 (21.4%)	11 (26.1%)	20 (48%)

Recently incidence of ESBL producing *A. baumannii* was increased worldwide. According to geographic variation, types and rate of  $\beta$ -lactamase production was occurred (Turner, 2005). Among the various gram negative bacterial pathogens, *A. baumannii* was a major threat to global healthcare setting especially ESBL producing isolates were not easily eradicated (Geetanjali *et al.*, 2015).

The incidence of ESBL in present study, isolates is almost higher than an earlier report from India (Geetanjali *et al.*, 2015; Neetu *et al.*, 2015; Harekrishna, 2016). The current study was contrary to the previous report (Kansal *et al.*, 2009; Kumar *et al.*, 2011). They were found that 75% of ESBL producing *A. baumannii* from clinical sources. These ESBL producing isolates were resistance to not only cephalosporins antibiotics but also many poses co-resistance to other classes of antibiotics, especially aminoglycosides and fluoroquinolon (Turner, 2005). These surveillance reports are valuable in deciding the need for new and safe antibiotics against ESBL producing isolates. According to this statement, the present study was designed to obtain the natural antibiotics from plant materials.

Plants producing therapeutic properties can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells. From the 2 decade antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. In the present study, the preliminary phytochemical screening indicated methanol leaf extracts of *W.tinctoria* showed the presence of bioactive constituents such as Flavanoids, Tannins, alkaloids, terpenoids, quinones and carbohydrates. Whereas seed extracts revealed the presence of alkaloids, Flavanoids, phenols and terpenoids the results were summarized in Table 2.

**Table 2. Qualitative Phytochemicals Evaluation of Methanol Extracts of *Wrightia tinctoria***

S.No	Constituents	leaf	Seed
1	Alkaloids	+	-
2	Saponins	-	-
3	Flavonoids	+	+
4	Tannins	+	-
5	Phenol	-	+
6	Steroids	-	-
7	Quinones	+	-
8	Terpenoids	+	+
9	Carbohydrates	+	+
10	Proteins	-	-

‘+’ Presence of compounds, ‘-’ Absence of compounds

The antibacterial activity of silver nanoparticle was tested 15 isolates of *A. baumannii*, which were selected according to the positive results of biofilm and betalactamase. All the two crude leaf and seed extracts of *Wrightia tinctoria* exhibited significant in vitro antibacterial activity against all the 15 isolates. Among the tested extracts, leaf extract exhibited more antibacterial activity when compared to the seed extract. The zone of inhibition was ranged from 10mm to 19mm for 7 isolates of *A. baumannii*. Whereas four isolates of growth were affected with moderately with zones of inhibition was ranged from 9mm to 15mm. Among the different concentration of plant extracts, the maximum zone of inhibition was observed from 10 $\mu$ g of leaf extract.

The effect of growth on bacterial strains by seed was less when compared to the leaf extract with zones of inhibition ranged from 9mm to 14mm, among the 15 isolates, 5 of were inhibited with seed extract. The above mentioned zone of inhibition was observed when using 10  $\mu$ g of seed extract. In our literature knowledge, no one report about the antibacterial activity of *Wrightia tinctoria* against ESBL and biofilm producing *A. baumannii*, this is the first study of AgNPs of leaf and seed extract of *Wrightia tinctoria* against clinical isolates of *A. baumannii*. The MIC for leaf extract was 4  $\mu$ g and 6  $\mu$ g for seed extract.

Metal nanoparticles can be synthesized by reducing metal ion using some chemicals. In biosynthesis, it is believed that extracts of natural materials act as reducing agent for generation of metal nanoparticles (Monavallil, 2010). These AgNPs in the intracellular space can bind to sulphur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes, subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death.

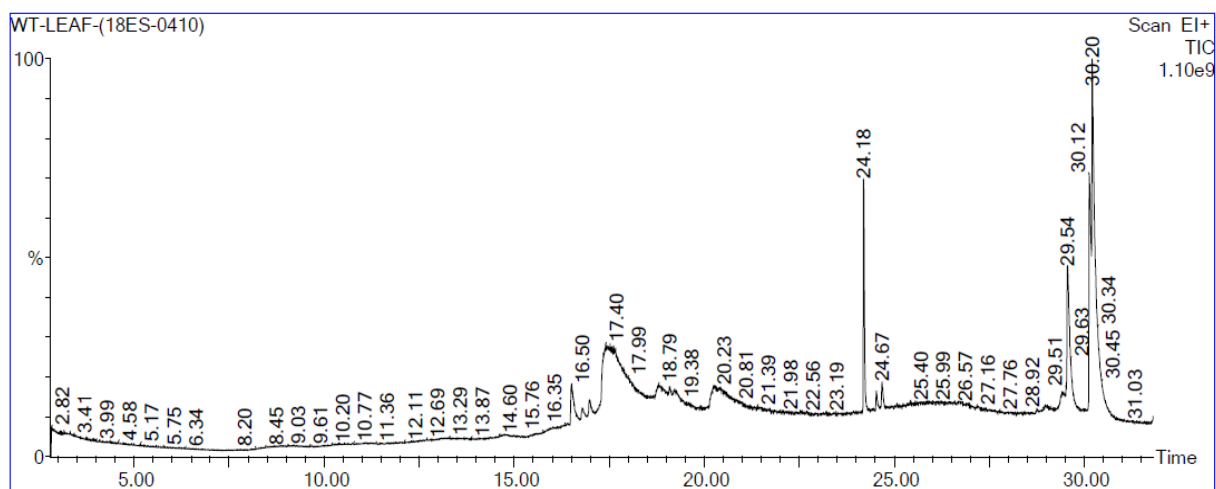
According to antimicrobial activity, methanol leaf extract showed the broad spectrum of antimicrobial activity; therefore methanol extract was carrying out the GCMS analysis. Table 3 was revealed that GC-MS analysis of *Wrightia tinctoria*. On comparison of the mass spectra of the constituents with the NIST library, the squalene, phytol, betulinand organic components such as Pentadecane and Benzylidine were characterized and identified. The major constituents were Hexadecanoic acid,

Octadecanoic acid and other minor constituents were also present. The GC-MS chromatogram shows the peak area separation of the components (Figure 1). The above mentioned isolated compounds from the methanol extract of *Wrightia tinctoria* leaf seem to possess the reported biological activity.

**Table 3. Phytoconstituents Identified in the *Wrightia tinctoria***

S.No	REV	For	Compound Name	Molecular weight	Formula	CAS
1.	884	756	Phytol	296	C <sub>20</sub> H <sub>40</sub> O	150-86-7
2.	968	926	Squalene	410	C <sub>30</sub> H <sub>50</sub>	7683-64-9
3.	822	550	Betulin	442	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	473-98-3
4.	727	471	Octadecanoic acid	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	57-11-4
5.	733	474	Dodecanoic acid	200	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	143-07-7
6.	906	906	Lupeol	426	C <sub>30</sub> H <sub>50</sub> O	545-47-1

**Fig1. GCMS Analysis of *Wrightia tinctoria***



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