# The Inhibition Effects of *Withania somnifera* leaves Extracts for Multi Drugs Resistance Bacterial Isolates

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## Abstract

The continuous resistance of pathogenic bacteria to antibiotics and failure of treatment of human diseases cause by these micrograms in addition to important of *withania somniera* plant in herbs medicine the present study carried out to assessment the antibacterial effect of three types of leaf extract : acetone , methanol and water in different concentrations to four multidrug resistance pathogenic isolates were selected two Gram positive and two Gram negative , the result showed that all types of isolated were inhibited by leaf extracts whereas the

acetone record the highest effect were the inhibition zone (millimeter ) of growth of four bacteria isolates *pseudomonas* aeruginosa ,*Klebsiella pneumonia , Staphylococcus aureus and Streptococcus pneumonia* reach 15,14,13 15 mm respectively at 100 mg / ml concentration of acetone , the current finding indicated that leaf extracts useful as a good natural alternative drug from antibiotics in treatment of numerous infectious diseases these result from both Gram positive and Gram negative bacteria .

***Key words : Withania somnifera** , leaf extracts ,antibacterial effect .*

## Introduction

Pathogenic bacteria responsible for the serious diseases to human and important causes of morbidity and mortality worldwide **,**The continuous increase and development of pathogenic bacterial resistance to antibiotic according to different mechanisms of resistance , and due to random usage of antibiotic in human treatment and veterinary medicine [(1)] , multidrug resistance (MDR) of bacteria cause reduction in the effectiveness and failure of the synthetic drugs to treatment the microbial infections either Gram negative or positive species [(2) (3)] , in addition to high cost of antimicrobial manufacture so that the scientist carried several studies and researches' to dissolve and to minimize the treatment problems to found alternative drugs from the natural source **.**

Worldwide reported the presence important compounds as constituents of medicinal plants and posses high activity against bacteria and considered natural ,low cost , available sources use as drugs in treatment of microbial diseases. [(4)]

*Withania somnifera* from Solanceae family known Ashwagandha Indian ginseng winter cherry use in Ayurvedic folk medicine has potent effect to treatment several diseases as Arthritis stress , Sedative , cough, others [(5)] · Leaves constitutes of *Withania somifera* riches with withanolides ,alkaloids , Steroidal lactones, , tannin , flavonoids, , other compounds [(6)] . Several studies carried to detect the action of Withania *somnifera* extracts against the growth of pathogenic bacteria isolated from different clinical samples , Rizwana *et al* 2012 showed in their study of antibacterial affect of acetone , Methanol , Chloroform extracts of *Withania somnifera* the high inhibition effect of the extracts on the growth of seven species of human pathogenic bacteria [(7)] .

*Withania somnifera* plant useful as drug in treatment of wounds , skin diseases , anti cancer , digestive system disorders , respiratory tracts infection [(8,9)] .Antibacterial activity of leaf extracts at concentration 6.25 - 12.25 mg/ml give high growth inhibition of *Escherichia coli , Salmonella typhi ; klebsiella pneumonia citrobacter freundii , pseudomonas aeruginosa* [(10)] **.**

**The objective of** our current study was performed to focus the light on the potential of antibacterial effect of *Withania somnifera* leaf extracts against growth of four Multi Drugs resistance bacterial isolates .

## Materials and Method

### Bacterial Isolation & Identification :

The present was carried during January 2019 to April 2019 in Baquba city for isolation and identification of bacteria from pathogenic samples ( urine , wounds swabs , nasal swab , Sputum).each samples were inoculated on to the 50 % sheep blood agar , MacConkey agar (without crystal violet ). each plate were incubated overnight under aerobic condition at 37 c the identification of bacteria performed on the base of colony characteristics , morphology of bacteria (staining ,shape and arrangement ), and biochemical tests include (IMPIVC, Oxidase test , urease test , Coagulase and DNase test for *Staphylococcus aureus* , Catalase , Hemolysin test , Triple – sugar iron agar test, **,**conformation of isolate identification performed by Vitek 2 Compact System [(11)] .

### Multi Drug Resistance (MDR) Isolates:

To detect the percentage of resistance of pathogenic bacterial isolates fourteen antibiotics were used include Gentamicin ( 10 ) , Ampicilin ( 10 ) , Tetracycline ( 30 ) , Amikacin ( 10 ), levofloxacin (10 ) , Ciprofloxacin ( 5 ) , Trimethoprim – Sulfamethoxazole (25 ) , Cefepime (30 ) , Meropenem (10) , Erythromycin (15 ) , Pencillin G (10 ) , Amoxicillin calvulanic acid (10 ) , Vancomicin ( 30 ), Norfloxacin (10 ) , using Kirby – Baure (Disc Diffusion method ), suspension of bacteria with 1.5 x10 cell / ml were prepared by transport 2 - 5 colonies of bacteria to brain heart infusion broth and compare the turbidity with 0.5 maCfarland slandered solution ,take ( 100 ul ) by sterilize pipettes and place on the surface of Muller-Hinton agar plate , and blood agar for *Streptococcus pneumonia* isolates , spread the inoculums by sterilize cotton swab ,after 15 minute distribute antibiotics disc by sterilize forceps and at equal distance between each disc ( seven discs for each plate ) , incubate for 24 hours at 37c , the diameter of inhibition zone per millimeter were recorded and compare the reading with the standard value for each bacteria species on the base of [(12)] **.** each isolates showed resistance to three or more antibiotics considered Multidrug Resistance (MDR) [(13)] .

### Plant Extracts Preparation :

Leaves of *Withania somnifera* plant were collected ,cut, and washed by tap water to removing soil and dust and exposure to direct sun light for dry , we obtained fine powder from leaves by use electric mixer grinder , store the powder in small air tight bottle at room temperature [(14)] , 50 gram of leaf powder dissolved separately in 500 ml of acetone , Methanol and water in liter conical flask size leave on a rotator shaker incubator for 24 hour . The mixture filtrate by what man No1 filter paper and double cotton of muslin cloth , distribute the filtrate in test tubes and centrifuged for 10 minutes at 3000 pmm / minutes , by rotary evaporator the filtrate concentrated in vacuum under low pressure and dried in oven at 40 C to obtain dry mixture , kept in tight sterilize glass tubes and preserved in refrigerator at 4 c until use to prepare different concentrations [( 15)] .

Four concentrations ( 25 , 50 , 75 , 100 mg / ml ) of acetone , methanol, water extracts were prepared by dissolved 1 gm of leaf powdered of each types of extract in l0 ml of acetone , methanol , distill water , then dilute to obtained the demand concentrations and sterilize by 0.22 mm Millipore filter paper [(16)] .

### *Withania somifera* leaf Extract Activity

Agar well diffusion method were used to investigate the activity of leaf of *Withania somnifera* leaf extracts at concentrations 25, 50 , 75 , 100 mg /ml in three solvents ( Acetone , Methanol , Water) against the growth of MDR isolates belongs to four pathogenic bacteria species . Colonies of isolates activate by subculture in brain heart infusion broth and incubate for 24 hours at 27 c at bacterial suspension with 5x10 cell /ml were used by compare with McFarland standard 0.5 solution , 100ul then spread by sterile cotton swabs on the surface of Muller- Hinton agar and on blood agar for *Streptococcus pneumonia* , wells with 6mm diameter were makes by sterile cork bores and transport 50uL from 25,50, 75,100mg/ml of plant extract to the wells each treatment performed in three replicates , leave the plate for 30 minute in room temperature to allow the diffusion of extract , the plates incubated for 24 hours at 37c reach the inhibition zone of each extract were recorded by millimeter and calculated the mean of the zones from three replicates [(17)] .

## Results and Discussion

The result sensitivity of isolates belongs to four species of pathogenic bacteria against fourteen antibiotics identified from clinical samples showed the presence of several isolate has multidrug resistance( resistance to more than three antibiotics . One selected multi drug resistance isolate include two species of Gram negative *Pseudomonas aeruginosa and*

*Klebsiella pneumonia , and* two species of gram positive *Staphylococcus aureus and Streptococcus pneumonia* were selected to investigate the antibacterial activity of three types of *Withania somnifera* leaf extract . our the finding revealed that multi drug resistance of each isolates , *pseudomonas aeruginosa* resistant to five antibiotics include Gentamicin , Amikacin , levofloxacin , Tetracycline , Ciprofloxacin , *Klebsiella pneumonia* resistant to four antibiotics : Amikacin , Trimethoprim – Sulfamethoxazole ,Cefepime . The results of sensitivity of Gram positive isolates showed multidrug resistance to other groups of antibiotics *Staphylococcus aureus* resistant Gentamicin , Ampicillin , Tetracycline , Amikacin and *Streptococcus pneumonia* resistant to Meropenem ,Erythromycin, Pencillin ,Ampicillin , Tetracycline , Amoxicillin antibiotics .

Table (1):- Multidrug resistance of one selective pathogenic bacteria isolates

| Bacteria species | MDR isolates | | Antibiotics |
|---|---|---|---|
| | Source of isolate | Antibiotic number | |
| Staphylococcus aureus | Nasal swab | 4 | Gentamicin , Ampicillin , Tetracycline , Amikacin |
| Psuedomonas aeroginosa | Burns infections (skin swab) | 5 | Gentamicin , Amikacin , levofloxacin , Teteracyclin , ciprofloxacin |
| Klebsiella pneumonia | Urinary tract infections ( urine) | 4 | Amikacin , Trimethoprim – Sulfamethoxazole ,cefepime |
| Streptococcus pneumonia | Pulmonary Pneumonia (sputum) | 6 | meropenem , Erythromycin, Penicillin ,Ampicllin , Tetracycline ,Amoxicillin-calvulanic acid |

The presence of different clusters gens in specific sites on plasmids or chromosomes responsible for the antibiotic resistance of bacteria and the association between those genes and other gens increase the chance of bacteria survival and adaptation may be result to the presence of multiple resistance genes in the bacteria strains [18] ., in addition of genetic processes mainly conjugation which considered one of the major mechanisms ( conjugative plasmids ) for transmission of resistance elements between bacteria strains as class 1 integron which considered as a part of antibiotic resistance Island which & into different conjugation plasmids [19(.

**Anti bacterial effect of leaf extracts :**

The result in tables ( 2 & 3 ) revealed that antibacterial activity of leaf extracts in three solvents that the acetone leaf extract of Withania somnifera were the highest inhibition effect on the four species of pathogenic bacteria in the all concentration ,the maximum inhibition zone recorded in 100 gm /ml were *Pseudomonas aeruginosa* 15 mm ; *klebsiella pneumonia* 13 mm ; the antibacterial activity on Gram positive bacteria were 13 mm for *Staphylococcus aureus* and 15 mm for *Streptococcus pneumonia* .

Methanol extract of leaf has strong inhibition activity against all multidrug resistance of pathogenic isolates , the range of their effect for different concentration about ( 8 -13 mm) , the highest effect in 100 mg/ml concentration on *(Pseudomonas aeruginosa , Klebsiella pneumonia , Staphylococcus aureus , and Streptococcus pneumonia* were the inhibition zone 12, 13 , 11, 12 mm respectively .

The third types leaf extract were used in current study water extract showed the weak antibacterial activity on bacteria the lower inhibition zone observed in all concentration and species of bacteria isolate these

use in our study the diameter of growth inhibition zone on four pathogenic isolates by well diffusion method reach at highest concentration 100 gm / ml were 4 - 5 mm .

**Table (2) : Anti bacterial effect of *Withania somnifera* leaves extract on Gram negative multidrug resistant isolates .**

| Concentration mg / ml | Extract | Pseudomonas aeruginosa | Klebsiella pneumonia |
|---|---|---|---|
| | | Inhibition zone / mm | |
| 25 | Acetone | 9 | 8 |
| | Methanol | 7 | 7 |
| | Water | 2 | 1 |
| 50 | Acetone | 9 | 11 |
| | Methanol | 8 | 7 |
| | Water | 3 | 2 |
| 75 | Acetone | 12 | 13 |
| | Methanol | 9 | 8 |
| | Water | 5 | 2 |
| 100 | Acetone | 15 | 14 |
| | Methanol | 11 | 13 |
| | Water | 4 | 4 |

**Table (3) : Anti bacterial effect of *Withania somnifera* leaves extract on Gram positive multidrug resistant isolates. .**

| Concentration mg / ml | Extract | Staphylococcus aureus | Streptococcus pneumonia |
|---|---|---|---|
| | | Inhibition zone / mm | |
| 25 | Acetone | 11 | 9 |
| | Methanol | 8 | 8 |
| | Water | 2 | 2 |
| 50 | Acetone | 11 | 9 |
| | Methanol | 9 | 8 |
| | Water | 3 | 3 |
| 75 | Acetone | 12 | 11 |
| | Methanol | 9 | 9 |
| | Water | 3 | 3 |
| 100 | Acetone | 13 | 15 |
| | Methanol | 11 | 12 |
| | Water | 5 | 4 |

The result of the study agreement with the finding of previous finding that showed acetone extract of leaf has high a potent effect against pathogenic bacteria [20][21]The high potential antibacterial of acetone and methanol leaf extract of *Withania somifera* due to leaves components well extracted in polar solvent more than non polar solvent

(22(. The result of water extract became agreement with the some studies which showed that water extracts inhibited the growth Gram negative isolate due to withanolides compounds can by well extracted in water [23] [24] obtain similar result they showed the strong ability of crude extract of *Withania sominfera* to inhibition the growth five species of clinical bacterial isolates at 100 mg / ml concentration .

The strong inhibition effects on the growth of bacteria isolates of *Withania sominfera* extracts due to the active biochemical components as withanolides (major constituents of leaves) , Amino acids , glycowithanolide , Alkaloids , flavonids and other compounds with the high effect to different diseases of extraction and purification[25] .

## Conclusion

Current investigation of antibacterial leaf extract of *Withania somnifera* showed that acetone and methanol extract exhibit strong activity against the four species of multidrug resistance pathogenic isolates , since we recommended to future studies to identify the chemical compounds to increase the drug activity and reduce the toxicity , to use the plant as antibacterial drugs for treating the disease specially for multidrug resistance isolates .

**Conflict of Interest**: non

**Source of Fundings:** self findings.

**Ethical Clearance:** non

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