



Antibacterial activity of *Azadirachta indica* and *Citrullus colocynthis* against different microorganisms

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Abstract

Multiple bacterial infections are significantly increasing with passage of time, which contributing to rate of morbidity and mortality. Frequent use of drug making bacteria resistant therefore search of new drugs with fewer side effects become an urgent necessity. The purpose of this work was to evaluate the antibacterial activity of *Azadirachta indica* and *Citrullus colocynthis* against different Microorganisms. The work was carried out in the Gomal Center of Biochemistry and Biotechnology Gomal University, D. I. Khan Khyber Pakhtunkhwa, Pakistan during the month of July 2017. The Methanolic extracts were used by agar well diffusion method and activity was measured through zone of inhibitions. According to the result methanolic extract inhibited the growth of *E. coli*, *Klebsiella pneumonia* and *S. Aurous*. Between two medicinal plants, *Citrullus colocynthis* was found more effective. Minimum Inhibitory concentrations of these both plants extracts against bacterial strains were good. Similar to agar well diffusion *Citrullus* was also found to more effective in MIC. It was concluded that methanolic extract of these plants have antibacterial activity.

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Introduction

Resistance against antimicrobial drug is emerging problem of the world in public health concerns of the 21st century. This resistance is increasing due to improper use of antibiotics against bacterial infection. Because of this prolonged exposure to the antibiotics bacteria become resistant and easy to survive and these pathogenic isolates are considered as Multi Drug Resistant isolates (MDR).

This proved to be resistant for both human and animal health during the treatment and also increase treatment duration (*Chakrapany S et al., 2014*). Therefore investigations shall be carried out to battle against the development of antimicrobial resistant. Plants play an important role in developing modern medicines as they contain active phytochemical components.

Plants were used as medicine in advanced and developing countries since long times. According to world health report 80% of world people used plants for the treatment of different disease (*WHO, 1993*).

The uses of plants as medicine have some advantages like it have less side effects and the patient have ability of good tolerance, also plants are usually acknowledged as therapeutic agents (*Vermani and Garg, 2002*). Phytochemicals are combination of two words in which "Phyto" means plant and chemical stands for chemical. They are explained as non nutrient bioactive agents which have vital role in the treatment of chronic diseases.

Plants like Vegetables, grains and fruits are the main sources of phytochemicals. Upto five thousand (5000) or more phytochemical have been studied in grains and plants. Flavonoids, terpenoids, polyphenols, tannis, minerals, vitamins, alkaloids and enzymes are types of phytochemicals having antibacterial activity (*Madhuri and Pandey., 2009*).

Azadirachta indica evergreen plant are economically more significant species in the Indian continent (*Koul*

et al., 1990), and also grow worldwide (*Verkek and Wright., 1993*).

The maximum height of this plant goes upto 30 meters. It leaves are (narrow) and live averagely 200 years. In the soil it roots goes deep to soak water and nutrients. The neem plant have the ability to grow in the hot, dry and stress condition like acidic, drought, stony and fertile.

This plant used for many reason e.g leaves ,bark, seeds and flowers are used as bug killer, medicine and also used in construction of houses (*Champagne et al.,1992*). In Pakistan such types of plants used in furnitures and for shade purpose.

Citrullus colocynthis is a famous medicinal plant from the family of Cucurbitaceae. In wild this plant seems to be a perennial herbaceous. *Citrullus colocynthis* having rough leaves sizes of five (5) to ten (10) cm long, three (3) to seven (7) lobed It stems are rough and angular, flowers are monoecious and axillary.

Fruit is pepo and nearly globular and 4 to 10 cm in diameter, shortly the size of this plant fruit near to small orange. Fruits has spongy. Pale yellow and hard rind whereas seeds are smooth and many in numbers. In born in several parts of tropical Asia. It is also originate in the desert of North Africa.

Resistance against antimicrobial drug is emerging problem of the world and outlook of these drugs in future is still uncertain. To overcome this problems serious action is needed in a different areas such as control use of antibiotics, multiple studies needed to develop new drugs with fewer side effects and to better understand genetic mechanism of resistance. Main goal is to offer effective drug to the patients.

Material and methods

Plant material collection

Two different plants used in this study *Citrullus colocynthis* (roots) and *Azadirachta indica* (leaves)

were obtained from Dera Ismail Khan and carefully taken in laboratory for further experimental analysis.

Plant washing and extraction process

According to (Momani *et al.*, 2007) protocol with some modifications. For plant extract preparation fresh part of *Citrullus colocynthis* and *Azadirachta indica* were obtained and rinsed with distilled water. Plant were dried at room temperature under shadow and then grinded to powder form. For extraction purpose 10 gm powder of plant were mixed in 100 ml of methanol in conical flask and covered with aluminum foil Mixture was left at 25°C for one day.

Powdered of both plants mixed carefully with methanol at speed of 200 round per minute (rpm) on shaker for 120 minutes, after mixing centrifugation were carried out at 4000rpm for ten minutes. Mixtures were filter through what man (No.4) filter paper and then put in 100 ml beaker.

Evaporation of methanol was carried out by placing the extracts in rotatory evaporator at 68°C. Stock was prepared by mixing the dry crude extracts in (DMSO) dimethyl sulfoxide and make final concentration of 1100 mg/ml.

Preparation of media

Conical flask of 250 ml used to prepare the nutrient media in which 100 ml of distilled water and 2.8 gm nutrient media was mixed thoroughly. Media was then sterilized by using autoclave at 121°C for 15 minutes. After sterilization media was carried to biosafety cabinet. In each autoclaved petri plates 25 ml of sterilized media was poured under biosafety cabinet to avoid contamination. Petri plates were left for solidification at 37°C.

Bacterial test

In this study *E. coli*, *K. pneumonia* and *S. aureus* were used. All the bacterial strains were obtained from Gomal Center of Biochemistry and Biotechnology culture bank. The bacteria were cultured on Nutrient agar for 24 hours at 37°C.

Agar well diffusion Method

This assay was used to check the activity of *Citrullus Colocynthis* and *Azadirachta Indica* against selected bacteria using Nutrient agar plates. Sterile glass rod was used to spread 100 µl of bacterial strains into nutrient agar plates. Sterile borer was used to make the wells of 8mm in size in bacterial inoculums agar plates and bottom of the well were. Each well were filled with 100 ul volume of plant extracts. Phosphate buffer saline used as negative control whereas Ciprofloxacin 5mcg was used as positive control. Plates were kept at 25°C for 10 minutes, so that diffusion of extract take place. After that plates were kept in incubator at 37°C for 24 hours.

Minimum Inhibitory Concentration of Azadirachta Indica and Citrullus Colocynthis Extracts

Micro broth dilution method was used to check the Minimum Inhibitory concentration of plants by using 96-well microtiter plates. Bacterial strains were inoculated in 15 ml falcon tubes containing 5ml Tryptone soya broth and incubated at 37°C for 2 hours. Two fold serial dilutions method was used by putting 100 µl of TSB in each well. In every well about 10ul of 2 hours old culture was added. Two wells were used for control in which one as for culture control and other for medium control. After that plates were incubated in the incubator at 37°C for 24 hours and Spectrophotometer was used to measure the optical density of the samples using wavelength of 630nm. At 0 hours first reading was taken and plates were left for incubation for final reading. Incubation of 24 hours, final reading was taken (Aboabaet *al.* 2006).

Results

The antibacterial activity *Citrullus colocynthis* and *Azadirachta indica* has been checked by agar well diffusion method. *Azadirachta indica* found most effect against *e.coli* (25.66 ±7.28) where its effect against other microorganisms was less then *Citrullus colocynthis*, whereas *Citrullus colocynthis* shows strong effect against all bacteria. The inhibition zone produced by both medicinal plant extracts were noted in Table 1 and 2.

Table 1. Antimicrobial activity of methanolic *Azadirachta indica* extracts against bacterial strains.

Bacterial Strains	Zone of Inhibition of <i>Azadirachta indica</i> (neem)
<i>E.coli</i>	25.66 ± 7.28
<i>S. aureus</i>	13.3 ± 5.1
<i>K. pneumonia</i>	10.6 ± 1.03

Table 2. Antimicrobial activity of methanolic *Citrullus colocynthis* extracts against bacterial strains.

Bacterial Strains	Zone of Inhibition of <i>Citrullus colocynthis</i>
<i>E.coli</i>	24 ± 1.54
<i>S. aureus</i>	19.66 ± 2.73
<i>K. pneumonia</i>	17.6 ± 2.25

Minimum Inhibitory concentration of *Citrullus colocynthis* and *Azadirachta indica* was measured by using Microdilution method against bacterial strains. Both medicinal plants found effect but same as previous experiment *Citrullus colocynthis* once again found to be more effective against all microorganism.

Minimum inhibitory concentration can be checked in tables. In (Table 3) showed the Minimum inhibitory concentration (MIC) of *Azadirachta Indica* and (Table 4) showed the *Citrullus colocynthis*.

Table 3. Minimum inhibitory concentration of *Azadirachta indica* against bacterial strains.

Bacterial Strains	Minimum inhibitory concentration mg/ml of <i>Azadirachta indica</i> (neem)
<i>E.coli</i>	3.12
<i>S. aureus</i>	12.5
<i>K. pneumonia</i>	25

Discussion

Antibiotic resistant and demand of new antimicrobial agents is major concern, to overcome this situation plant could be a useful to provide new antimicrobial agents with improved safety and efficacy (*Srivastava et al., 2000*).

The investigation for different antimicrobials from naturally available sources has received more concentration and different way have been put into recognize compounds which can perform as proper antimicrobial agent to synthetic ones. Plants produce highly numbers of phytochemicals which have healing and medicinal properties.

Table 4. Minimum inhibitory concentration of *Citrullus colocynthis* against bacterial strains.

Bacterial Strains	Minimum inhibitory concentration mg/ml of <i>Citrullus colocynthis</i>
<i>E.coli</i>	3.12
<i>S. aureus</i>	6.2
<i>K. pneumonia</i>	12.5

These phytochemicals divided into alkaloids, acid essential oil, steroids, tannins and saponins etc. These phytochemical produce less toxic and more effective

medicine which inhibit the microorganism growth (*Kelmanson et al., 2000; Ahmad and Bag, 2001*). Large numbers of therapeutic applications of

phytochemicals have been found against human pathogens. For the discovery of new antimicrobial compound different studies have been conducted and checkout the antibacterial activity of plants. Pulling out of these compounds depends upon their solubility with different solvents. Because of organic nature methanol has high ability to dissolve more active antimicrobial and organic compounds (Cowan., 1999). In current study main goals were to find the capability of of *Citrullus colocynthis* and *Azadirachta Indica* extracts to inhibit growth of different Bacterial Strains. Numbers of different techniques have used to evaluate the activity of plants extracts against microorganisms. Minimum inhibitory concentration (MIC) and Agar well diffusion are among the most usually used ones (Arora and Kaur., 2007, Gurudeeban et al., 2010 ; Pavitthra et al., 2010).

Interestingly in both experiment plants showed good antibacterial activity against *E.coli* *Pseudomonas aeruginosa* and *K. pneumoniae*. In Agar well diffusion method *Azadirachta* showed the highest activity against *E. coli* (25.66 ±7.28) where its effect against two other plants were also good.

The result is similar to the previous study of (Jagannadh and Radhika, 2006), Where they explore the *A.indica* was found very effective against on *E.coli* and *Klebsiella pneumoniae*. Whereas *Citrullus colocynthis* extrats showed strong activity against all selected microorganism. *Citrullus colocynthis* as compared to *Azadirachta indica* was more effective. *Citrullus colocynthis* was also found effective against *S. aureus* by (Keikhaie et al., 2017). Results of both plants were presented in (table 1 and table 2).

It reveal that extracts contains necessary compounds which inhibit the growth bacteria (Daud et al., 2014) after agar well diffusion method, MIC method was used to check the minimum concentration of *Citrullus colocynthis* and *Azadirachta indica* that inhibit growth of bacterial strains. Among these two methanolic plants extracts of *Azadirachta indica* showed the highest antibacterial activity against all

strains, which is also proving the result of Agar well diffusion method. Results can be checked in (table 3 and table 4).

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