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Antibiotic Resistance of "*Pseudomonas aeruginosa*" and the Effect of *Euphorbia trigona rubra* Leaves Crude Extract

Isaac John Umaru

Department of Biochemistry, Federal University, Wukari, Nigeria

Email address:
umaruisaac@gmail.com

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Abstract: *Introduction:* Antibiotic resistance is a global problem that threat the public health. New patterns of antimicrobial resistance emerge daily which get through the international boundaries and easily spread. The aim of this study is to evaluate the activity of *Euphorbia trigona rubra* leaves crude extract on this drug resistance bacteria's. *Materials and methods:* This study was carried out in natural product laboratory in collaboration with Microbiology Laboratory. Antimicrobial sensitivity patterns of the bacteria were done by using Kirby Bauer Disc Diffusion method. Antibiotics which were tested in congruent with the crude extract include Augmentin (10 µg), Amoxicillin (10 µg), Ampicillin (10 µg), Cefixime (30 µg), Ticarcillin (30 µg), Ceftazidime (30 µg), Azithromycin (15 µg), Gentamycin (10 mcg), Ciprofloxacin (10 µg), Trimethoprim-Sulfamethaxazole (10 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), and Rifampicin (30 µg). *Results: Euphorbia trigona rubra* leaves crude extract has shown a significant activity of 21.33±0.55 mm when compared to the drug (4.63±0.71 mm, 7.33±0.55 mm, 4.00±0.06 mm, 4.58±0.21 mm, 7.11±1.00 mm, 5.97±0.87 mm, 4.63±0.71 mm, 7.33±0.55 mm, 5.00±0.06 mm, 5.58±0.21 mm, 5.97±0.87 mm, 4.63±0.71 mm, and 9.13±0.55 mm) at 500 µg/mL, respectively that the bacterium was resistance to, this shows that the crude extract from the leaves of this cactus plant has an effective activity for resistance bacteria and should be considered as an agent in the pharmaceutical industries.

Keywords: Antibiotic, *Pseudomonas aeruginosa*, *Euphorbia trigona rubra*, Leaves

1. Introduction

Resistance pathogens, only entry of any microorganism inside the body can not initiate any disease among any individual. Establishment of a disease depends on many factors, among them individual resistance is very important and All the living entities of our planet are struggling continuously for their existence. Starting from the minute viruses, the fungus, bacteria, protozoa, parasites, plants and animals of various differentiated species are struggling for their existence and multiplication. In the way of such struggle, as a part of evolution, microorganisms developed their system to secrete some antibacterial chemicals. Some of these chemicals are identified by the scientists and afterwards used as antibiotics. Due to uncontrolled use of these chemicals to kill other microorganisms, the susceptible organisms get ample opportunity to alter their susceptible systems and to develop some new system to bypass the detrimental effect of those chemicals. It is called as microbial resistance to antibiotics [1-3].

Development of resistance among microorganisms against antimicrobial agents and spread of mainly plasmid based genetic materials related with such resistance to many other new species of microorganisms continuously with accelerated speed is becoming a threat for antimicrobial chemotherapy. The spread is mainly due to indiscriminate, unnecessary use and residual effect of antibacterial substances [4]. Transport of such resistance power is performed by transport of related genetic materials from one microorganism to another. Resistance in bacterial population spread from person to person by bacteria, from bacterium to bacterium by plasmids, from plasmid to plasmid or chromosome by transposons [5, 6].

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, P. aeruginosa is a multidrug resistant pathogen recognized for its ubiquity, it's

intrinsically advanced antibiotic resistance mechanisms and various sepsis syndromes [7].

The organism is considered opportunistic in so far as serious infection often occurs during existing diseases or conditions, most notably cystic fibrosis and traumatic burns. It generally affects the immunocompromised but can also infect the immunocompetent as in hot tub folliculitis. Treatment of P. aeruginosa infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result [8]

Theses bacterium are known for their diverse metabolism, has acquired inherently resistant to many antibiotic classes and its ability to gain resistance to all effective drugs [9]. It has been found to cause a life-threatening and complicate to treat cause of their confined susceptibility to antibiotics and their high ability to acquire resistance during therapy" [10]. Mechanisms of such resistance consist of acquisition of wide-spectrum lactamases, various antimicrobial-modifying enzymes. Multidrug efflux pumps as a result of mutational shifts, alternations in antibiotic targets and in the permeability of outer membrane and depression of ampC [11].

The bacteria is a citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal [12].

This is perhaps becoming a dangerous threat to the modern civilization in near future. Thus the aim of the study is to evaluate the Ethnomedicinal use of a plant methanol leaves crude extract of *Euphorbia trigona rubra* (Cactus) against the resistance pathogen.

This plant *Euphorbia trigona rubra* is a tender, evergreen, succulent plant occasionally known (especially in older literature) as Euphorbia hermentiana. The plant grows into a densely and compactly branched erect shrub or small tree of two metres or more. *Euphorbia trigona rubra* s widely cultivated as a pot plant [13].

It is also an attractive cultivated variant commonly called Euphorbia trigona. The stems and leaves flushed purplish-red (and some bronzish-green). The leaves are often darker red in this clone and the stems greener for a lovely bicolored look [14]. The stems can be flecked red to magenta. Some leaves may become rich magenta-red. Speciality growers may also offer crested or fasicated material. Borne along the branch ribs on the new growth from between the two thorns on each ridge is the leaves, lanceolate to drop-shaped, varying from 7 to 9 mm and deciduous, to 3-5 cm and more persistent. Usually the leaves die off in winter, but in exceptional cases (no direct sunlight) they may last for several years. The plant will produce new leaves in spring. In the frequently encountered Euphorbia trigona cv. Royal Red the leaves may become rich magenta-red [15, 13, 16, 17].

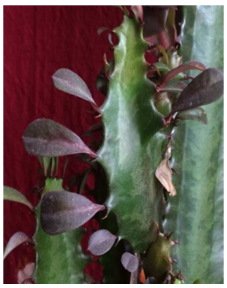

Figure 1. *Showing the plant Euphorbia trigona rubra and the leaves.*

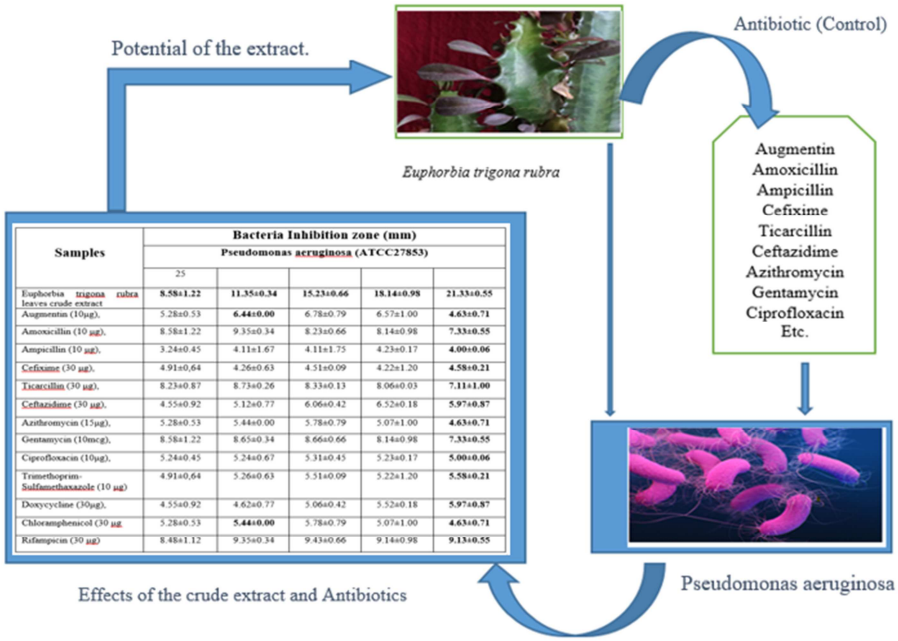

Figure 2. *Showing the graphical Abstract of Pseudomonas aeruginosa" and the Effect of Euphorbia trigona rubra Leaves Extract.*

2. Material and Methods

2.1. Chemical Reagents

Chemical reagent and standard for anti-bacterial drug Augmentin (10 µg), Amoxicillin (10 µg), Ampicillin (10 µg), Cefixime (30 µg), Ticarcillin (30 µg), Ceftazidime (30 µg), Azithromycin (15 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Trimethoprim-Sulfamethaxazole (10 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), and Rifampicin (30 µg) were purchased from Sigma-Aldrich Co, Selangor Malaysia.

2.2. Preparation of Crude Extract

The *Euphorbia trigona rubra*, leaves were air dried, ground in an electric blender, soaked in methanol for 7 days, then filtered. The residue was re-extracted with fresh methanol for 72 hrs. All the solvent were combined and evaporated in rotary evaporator (Rato-vap). The extract was stored in a sample bottle prior to use.

2.3. Micro-dilution Antibacterial Assay

The serial dilution technique described by Eloff, using 96-well micro-plates, was employed to determine the minimum inhibitory concentration (MIC) of the methanol crude extract of *Euphorbia trigona rubra* for antibacterial activity [14].

The resistance bacterial strains was employed *Pseudomonas aeruginosa* (ATCC27853) a diverse bacteria that possess an extreme tolerance to most of antibacterial drugs. They cause an array of infections to humans this was obtained from the virology laboratory Universiti Malaysia Sarawak.

3. Data Analysis

Two way ANOVA tests were used for the statistical analyzing of our results and p-values that were less than 0.05 were considered as significant.

Table 1. *Bacterial inhibition of Euphorbia trigona rubra leaves extract on Pseudomonas aeruginosa and resistance antibiotics at various concentration.*

Samples	Bacteria Inhibition zone (mm)				
	Pseudomonas aeruginosa (ATCC27853)				
	25 µg/mL	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL
Euphorbia trigona rubra leaves crude extract	8.58±1.22	11.35±0.34	15.23±0.66	18.14±0.98	21.33±0.55
Augmentin (10 µg),	5.28±0.53	6.44±0.00	6.78±0.79	6.57±1.00	4.63±0.71
Amoxicillin (10 µg),	8.58±1.22	9.35±0.34	8.23±0.66	8.14±0.98	7.33±0.55
Ampicillin (10 µg),	3.24±0.45	4.11±1.67	4.11±1.75	4.23±0.17	4.00±0.06
Cefixime (30 µg),	4.91±0,64	4.26±0.63	4.51±0.09	4.22±1.20	4.58±0.21
Ticarcillin (30 µg),	8.23±0.87	8.73±0.26	8.33±0.13	8.06±0.03	7.11±1.00
Ceftazidime (30 µg),	4.55±0.92	5.12±0.77	6.06±0.42	6.52±0.18	5.97±0.87
Azithromycin (15 µg),	5.28±0.53	5.44±0.00	5.78±0.79	5.07±1.00	4.63±0.71
Gentamycin (10 mcg),	8.58±1.22	8.65±0.34	8.66±0.66	8.14±0.98	7.33±0.55
Ciprofloxacin (10 µg),	5.24±0.45	5.24±0.67	5.31±0.45	5.23±0.17	5.00±0.06
Trimethoprim- Sulfamethaxazole (10 µg)	4.91±0,64	5.26±0.63	5.51±0.09	5.22±1.20	5.58±0.21
Doxycycline (30 µg),	4.55±0.92	4.62±0.77	5.06±0.42	5.52±0.18	5.97±0.87
Chloramphenicol (30 µg)	5.28±0.53	5.44±0.00	5.78±0.79	5.07±1.00	4.63±0.71
Rifampicin (30 µg)	8.48±1.12	9.35±0.34	9.43±0.66	9.14±0.98	9.13±0.55

The result is Mean±SD. N = 3, P-values = 0.05.

4. Result and Discussion

A total of 13 antibiotic with *Euphorbia trigona rubra* leaves crude extract were used in this study to ascertain the potential activity of the crude extract of *Euphorbia trigona* against the resistance bacteria. The antibiotic susceptibility test of p. aeruginosa towards the 13 various type of antibiotics shows slow activity when compared to the crude extract of *Euphorbia trigona rubra* especially with Augmentin (10 µg), Ampicillin (10 µg), Cefixime (30 µg), Azithromycin (15 µg), and Chloramphenicol (30 µg), of 4.63±0.71, 4.00±0.06, 4.58±0.21, 4.63±0.71 and 4.63±0.71 µg respectively when compared to the crude extract of 21.33±0.55 at the same concentration of 500 µg/mL.

The inactivity of some of this bacterial drug against p. aeruginosa was also reported in India by Javiya et al., 17.85% by Ekrem and Rokan recorded in Iraq" [18, 19] and in Egypt [20]. It was also recorded in Gujarat by Rajat et al. [21], as well as in Northeastern Nigeria by Okon et al. [22]. The variation in "P. aeruginosa" rates in various studies could related with the type of clinical specimens, and geographical locations.

In this study low rate of observed with the selected drugs which was similar to the antibiotic studied by [23-27].

This may be related to the resistance to beta-lactams as well as hyper production of "Beta lactamase" through the genes of resistance and mutational processes which was broken by the crude extract from *Euphorbia trigona rubra* which showed higher growth inhibition of the bacteria *Pseudomonas aeruginosa*.

Therefore the use of *Euphorbia trigona rubra* crude extract could be a remedy to the high resistance of the drugs and be used as an agent for other resistance bacteria's.

5. Conclusion

The dissemination of bacterial resistant strains towards the antibiotics causes losing of drug efficacy this may be as a

result of the lack of awareness, noncompliance, indiscriminate use of antimicrobial agents and unhygienic condition. Thus the use of *Euphorbia trigona rubra* will help to reduce the significant challenge for treating both of nosocomial and community-acquired infections for convenient antimicrobial agent for therapy which is necessary in minimizing the clinical outcome.

Conflict of Interest

The authors declare no conflict of interest.

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