Biological activity of some plant extracts in Diyala Governorate

Mohanad W. Mahdi Alzubaidy [1a]

Biology Department, College of Education for Pure Science, University of Diyala
Email: spatialresearch@coehuman.uodiyala.edu.iq
a) mohanad@uodiyala.edu.iq as Corresponding Author

Mohammed Yousif Hajim [1]

[1] Spatial Research Unit, University of Diyala, Iraq
a) mohanad@uodiyala.edu.iq as Corresponding Author

ABSTRACT

The goal of this work was to determine the antimicrobial activity of wild plants found in Diyala (*Nerium oleander, Peganum harmala, and Alhagi mauroram*), whose active components (alkaloids and glycosides) were extracted using a hot alcoholic extract. In this work, several solutions were made and the antibacterial activity of *Escherichia coli* was tested using the Minimum Inhibitor Concentration (MIC) technique at various concentrations. PCR was used to test the extracts' activity against the *ddpC* gene. The study samples revealed five mutations: three substitution mutations and two silent mutations, indicating that plant extracts have good efficacy due to the presence of effective compounds capable of inhibiting microbial growth as well as a clear effect on DNA by controlling enzymes, transcription, and translation processes in DNA bacteria. Due to the urgent and ongoing need to detect new antimicrobials with diverse chemical structures and valuable mechanisms of action, it is possible to make good use of the species of wild plants found throughout Diyala city. This is because there is an increase in the incidence of new and frequent infectious diseases as a result of antibiotic resistance.

Keywords: Antimicrobial, *Escherichia coli*, plant Extracts, Diyala Governorate

Introduction

"Everything of plant origin that is utilized medicinally is a medicinal plant," according to the scientist Dragendroff. We can deduce from this definition that it encompasses all plants without exception [1]. It is undeniable that medicinal plants play a significant role in the pharmaceutical industry and other sectors, as well as the critical need for them in this field due to the active chemicals they contain, such as alkaloids, glycosides, and volatile oil [2]. Pharmacology is related to many sciences such as Botany phytochemistry [3]. Therefore, obtaining inhibitory compounds of therapeutic importance in its pure form is still a concern for chemists, biologists and pharmacologists [4]. Scientific research has focused on increasing the quantity of these compounds by exploiting plants spread in the wild or that are medicinally effective in terms of content. Diyala Governorate is famous for the diversity of many different types of these oleander plants, *Nerium oleander*. It is found in subtropical and temperate regions as well. This family is characterized by economic importance. The plants of this family are a source of medicinal drugs, alkaloids, vegetable milk, rubber and ornamental plants [5]. Another plant spread in the province is *Peganum harmala*. The *Peganum harmala* plant is one of the most

widely used medicinal plants in the field of folk medicine, and it is a herbal plant.

It is used as a medicinal herb in many countries in Africa, Europe, Central, East and North Asia, especially in the Arabian Gulf region, such as Saudi Arabia and the surrounding countries. *Peganum harmala* has been used for a long time against diabetes and rheumatism and as a treatment for headaches, epilepsy, sciatica, jaundice, forgetfulness and all kinds of pain. The Greeks also used the powder of *Peganum harmala* seed to treat fevers and worms, [6]

The *Alhagi mauroram* plant is also spread. It is a perennial, many-branched shrubs, with abundant and strong thorns, solid leaves, small, with a length of between 0.5_2 cm which are simple. The flowers are in clusters scattered from 3 to 8 in one cluster. *Alhagi mauroram* is used as a depressant in case of fever, aids digestion, tonic, laxative, diuretic, and bronchitis [7]. *Escherichia coli* bacteria cause food poisoning cases as well as wound infections [8] and can be considered the first pathogen that causes urinary tract infections [9]. This bacterium belongs to the family *Enterobacteriaceae*, a Gram-negative bacilli with dimensions (5-6 x 1-5.1) microns, motile by flagella, and lactose fermenter.

Therefore, its colonies appear dry and pink, as well as give a positive test for indole [10]. The colon bacteria produce two types of enterotoxins, one of which is stable at high temperatures and the other is unstable at high temperatures that cause summer diarrhea in infants [11].

Methods of processing:

Plant sample collection:

Three types of widespread wild plants were collected: *Nerium oleander*, *Alhagi mauroram*, and *Harmal* from the regions of Diyala city, during March and April. The samples were washed with water for several times to remove dust. Infected and weak leaves were also removed after being classified by the herbarium (College of Science - University of Baghdad) and based on the book "Flora of Iraq".

It was dried in the shade at room with temperature (25-30) C until it became completely dry, then it was ground by an electric grinder to obtain samples in the form of powder. They were kept in plastic containers marked with the name of the plant, the plant part, and the date of the collection preserved in the herbarium.

Preparation of raw plant extracts:

The method of hot alcoholic extract was employed based upon what was mentioned in [12], as the weight of (30) gm of dry leaf powder separately was placed in a saxolith apparatus containing (250) ml of methanol at a concentration of (98%). The extract was left for (7) hours at a temperature of (60) Celsius, after which it was filtered with a Whatman type filter paper No. (1). The filter was subjected to evaporation by means of the Rotary Evaporator until a thick liquid was obtained. The residue was evaporated at room temperature to obtain a dry powder, which represents the outcome of the crude extract.

Specific chemical detections of some active ingredients:

Detection of alkaloids:

I followed the method [13] to detect alkaloids. 10g of dried leaf powder was boiled in (50) ml of distilled water acidified with (4%) of HCl. The solution was filtered after cooling and the filtrate was tested for an hour beaker with each of the following reagents:

1. Dragendroff`s Reagent: There was an orange precipitate indicates the positive result for the presence of alkaloids.
2. Mayer`s Reagent: There was a white precipitate indicates the positive result for the presence of alkaloids.

Detection of glycosides:

The method mentioned [13] was adopted to conduct this detection. Equal parts of extracts of plant leaves were mixed with Banadact`s Reagent. There was a red precipitate indicates the positive result of the presence of glycosides. Fehling reagent was also used by mixing it with the extract in equal quantities and leaving the reaction in a boiling water bath for (10) minutes. The positive result was that there is a red precipitate.

As for the confirmatory detection, the method mentioned in [14] was adopted. (5) ml of each extract was taken and several drops of Kedde`s Reagent were added, with the formation of a violet color indicating the presence of glycosides.

Measuring the absorption spectrometry of the crude extract alkaloids:

The absorption spectrum of the alkaloids of the crude extract of the study plants was measured using a spectrophotometer at specific wavelength (250-470) nanometers.

Estimating the percentage of alkaloids:

The method mentioned by [15] was adopted to separate the alkaloids from the leaves of the studied plant species, where the percentage was estimated based on the dry weight of the plant sample.

Determining the percentage of glycosides:

The method [16] was followed to extract and separate the glycosides from the leaves of the studied plants.

Isolating and diagnosing *Escherichia coli*

E. coli was isolated from stool samples by culturing experimental soy broth (TSB) at 37 °C in MacConkey agar, Eosin methylene blue agar (EMB) Biochemical identification assays were performed by the VITEK@2 system.

Bio-Efficacy test

The efficacy of plant extracts of three studied plants against *E.coli* was verified. (MIC) assay was conducted for all the total extracts on bacteria. The method presented in [17] was followed with some modifications: A series of gradual dilutions of plant extracts were prepared using Nutrient Broth medium with concentrations ranging (500, 400, 300, 200, 100, 50, 25, 12.5) mg/ml. The above tubes were inoculated with (100) microliters of bacterial culture aged (24) hours containing 1.5 x 106 cells/ml. The tubes were incubated at (37) Celsius for (24) hours. The results were compared with the control represented by culture medium inoculated with bacteria only, and culture medium with plant extract only, respectively. The value of the (MIC) was determined as the lowest concentration of the plant extract that prevents a clear turbidity that can be observed with the naked eye in the culture medium.

Extracting DNA from the bacterial genome:

DNA was extracted from *E. coli* bacteria using a Promega DNA extraction kit (USA). DNA fragments were detected by using agarose gel electrophoresis at a concentration (1.5%). The DNA concentration was also checked using a nanodrop and kept in a refrigerator at -20 °C [14,15]. Bacterial DNA was amplified by **Forward** primer: CGGGCCGAGTTTGTTTAACG and **Reverse** primer: GCGACCATTTGCTACCATCG which were designed by the researcher and supplied by Macrogen/Korea Corporation and the resulting size was 307 bp.

PCR mixture conditions were prepared and reactions were performed using a reaction mixture consisting of 12.5 µl of Go Tag Green Master Mix/Promega, 2 µl of each primer, 5.5 µl of DW and 5 µl of DNA, with a final volume of 25 µl. The amplification conditions for the final thermal cycling program were as follows: initial denaturation was 1 cycle of 95°C at 2 min, 35 cycles each including denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extensions at 72 °C for 30 seconds, then extend the final cycle at 72 °C for 4 minutes. The size of the PCR product is 307 bp.

Statistical Analytics:

The arithmetic means were compared using the analysis of statistical variance method to compare the results of determining the (MIC) of plant extracts for the study samples. Bacterial DNA primers were designed by primer3 Plus program and the mutation type was determined by using Geneious Software Eleventh Edition. [18]

Results and discussion

The results of the preliminary chemical detections showed in the active compounds of the crude extracts of the leaves of plants shown in Table (1), as the leaves of all three species contained alkaloids and glycosides. Alkaloids and glycosides are effective metabolic compounds that characterize wild plants [19]. It was mentioned that alkaloids are stored inside plant vacuoles, where they play an important role in plants as they are considered a reservoir for the manufacture of proteins or

defense materials against insects or detoxifying compounds. In addition, they are regulators of growth and other processes [20].

Table (1) results of the chemical detections of the active ingredients.

Active components	Leaves			Positive result	Reagent
	Nerium oleander	*Peganum harmal*	*Alhagi maurorum*		
Alkaloids	+ +	+ +	+ +	orange precipitate white precipitate	Drajendroff Mayer
Glycosides	+ + +	+ + +	+ + +	red precipitate red precipitate Purple	Banadact Fehling

Preliminary findings to investigate the presence of active compounds in the plant residues of the three crops indicated the presence of alkaloids, glycosides, which could be attributed to the inhibitory effect on bacterial germination and growth. The reduction that was observed through the results may be attributed to the presence of the active compounds in the residues of the plants used in the study, which may justify the difference in the effect of the three plants. Studies have indicated the possibility of converting it to simpler or more complex compounds after liberating it into the bacteria wall. The plant extracts contain many compounds, including organic acids, aldehydes and aromatic acids, as well as some toxic gases [21].

Diagnosis and efficacy:

Bacterial isolates were diagnosed based on phenotypic properties when grown on differential and facultative media. It was cultured on Maconkey agar medium containing crystal violet pigment and bile salts, which inhibits the growth of members of the Gram-positive family of enteric bacteria while allowing Gram-negative bacillus to grow, and the result was the appearance of all colonies in pink color, *E.coli* From other bacterial species belonging to the intestinal family [22].

Table (2): Biochemical tests for *E.coli* isolates

Biochemical test	Catalase	Oxidase	Lactose fermention	Indole	Methyl-red	Ureas	Simmon citrate	Voges - proskaur
E.coli	+	-	+	+	+	-	-	-

(+) : positive : (-) negative

It was reported [23] that the compound Salicylic acid (one of the phenolic acids found in wild plants) has an inhibitory action in the uninhibited concentrations of P. aeruginosa for bacteria adhesion to Arabidopsis roots and reduces biofilm formation and inhibits the formation of pyocyanin, Protease, and Elastase. Salicylic acid compound affects the representation of 331 genes and inhibits the transcription of foreign proteins without affecting the housekeeping genes, so it is an inhibitor of virulence factors

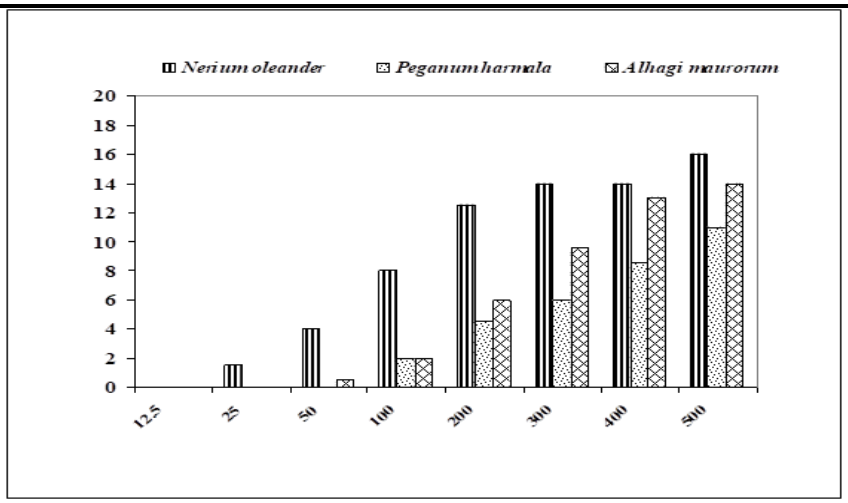

Figure (1) The inhibition diameters of the plant extract of plant species

Analysis of the *ddpC* gene in *E.coli* . isolates

DNA extraction of the study isolates was performed using PCR technology (Molecular Methods) in a one-way pattern with specific primers. This method was used to identify the effect of the plant extract, depending on the results of the current study and through the evidence obtained from Figure 1. The extract of oleander was used because it gave the highest inhibition values and ranged between 2-16 mm on the biofilm gene in this study for *E.coli*. . The PCR products appeared as a DNA band of up to 307 base pairs. This was confirmed by gel electrophoresis in 1.5% agarose stained with ethidium bromide. It was photographed under UV light as shown in Figure (2)

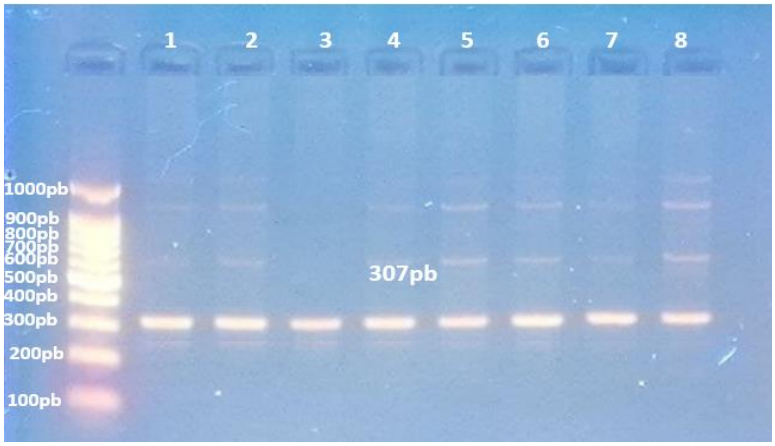

Figure2: PCR product of the *ddpC* gene (307bp). electrophoresis on 1.5% agarose at 70 volts. For 1:00hours, DNA Ladder (100bp-2000bp)

Evaluating the effect of plant extract on the *ddpC* gene of *E. coli*

The nitrogenous base sequences of *E.coli* isolates were determined after reading the DNA sequences, and after deleting defects in either, analyzed and matched with (NCBI) online at www.ncbi.nlm.nih.gov. Meanwhile, data analyzes using Geneious Software showed. The reference sequences are very similar to the 98% *ddpC* gene sequence. By comparing the observed DNA sequences of these samples with their stored reference sequence (Gen Bank: CP087110.1)

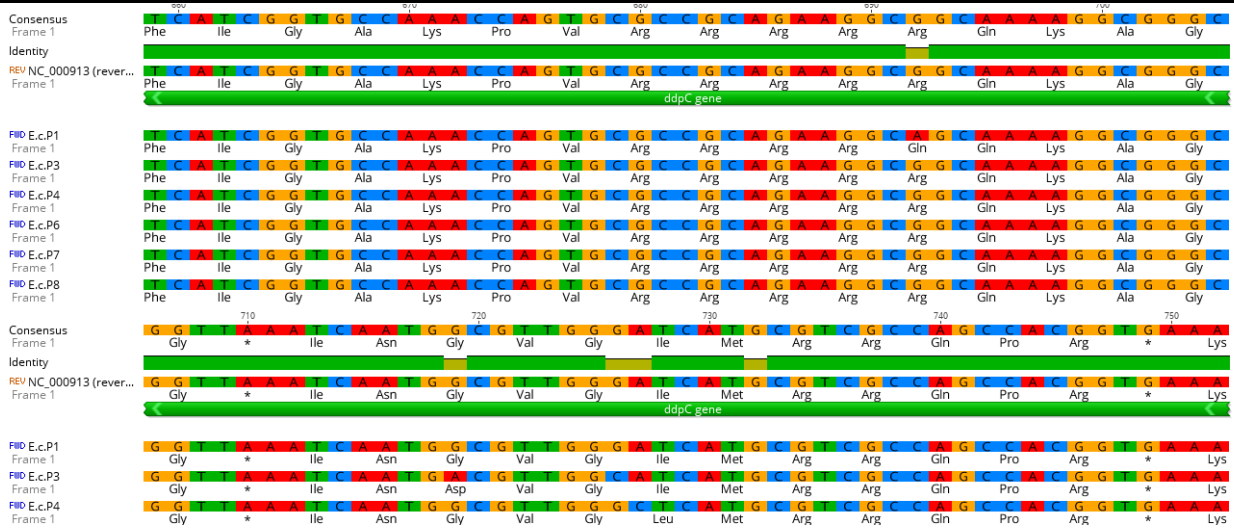

Figure 3: DNA sequences in six *E.coli* isolates compared with the reference sequences of isolates *E.coli* Gen Bank: CP087110.1. by Geneious Software

By analyzing the results of sequencing the *ddpC* gene according to (Fig. 3), the data showed 5 mutations: the first leads to the change of amino acid Arg to amino acid Gln through a change of A<G, as well as the occurrence of a second mutation of substitution type leading to a change of amino acid Gly to amino acid Asp through A<G.

The third mutation was of the silent type and was repeated in two isolates, as there was a change in the nitrogenous base through the change of C < G as well as the change of C < A. This shows the effect of alkaloids and clacosides, which may have interfered with the metabolic chain reactions of proteins forming the cell membrane of bacteria, leading to mutations. This explains the killing and growth inhibition of bacteria [24].

Alkaloid compounds also have the ability to inhibit the activity of topoisomerase enzymes, which play an important role in the processes of DNA replication and replication [25]. This inhibition is due to the intrusion of alkaloids and its binding with the main groove of the DNA or the cleavage of the complex TopoIsomerase-DNA are among the mechanisms that can be used by the toxins and inhibitors of the Isomerase Topo DNA as an inhibitor of mixtures. [26]

Conclusion:

It is highly possible to benefit greatly and make good use of the species of wild plants spread in Diyala city. Because there is an increase in new and frequent infectious diseases due to the increase in resistance to the antibiotics used continuously, there is an urgent and continuous need to detect new antimicrobials with diverse chemical compositions and valuable mechanisms of action.

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