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Production of Myxopyronin a Antibiotic from Different Soil Environments in Egypt

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Article

Extraction of *Myxopyronin A* Antibiotic from Contrary Soil Environments in Egypt

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Abstract: Background: Antibiotic resistance is an overwhelming serious difficulty globally. This necessitates the exploration of novel sources of antibiotics to overcome this challenge.**Aim of the study:** Bacterial *Myxopyronin A* production from various soil environments Egypt; as well as testing its antimicrobial activity in preclinical animal testing and *randomized human clinical trials phases 1/2*. **Type of the study:** Screening experimental study. **Methodology:** Different soil environments in Egypt were screened for the growth of bacterial isolates producing *Myxopyronin A* as antimicrobial agent. Purification of *Myxopyronin A* was performed via reversed phase *HPLC*. *Paper disc diffusion assay* as well as *Broth dilution technique* were exploited to assess the invitro antimicrobial and minimum inhibitory concentration(*MIC*) of the test antibiotic. Furthermore, in vivo antimicrobial spectrum, adverse drug reactions and pharmacokinetics were detected during animal models testing stages and *human randomized clinical trials phases 1/2*. **Results:** From the culture supernatant of the Myxobacterium *Myxococcus fulvus 124B02* which was the predominant soil bacterial isolate grown on *Casein yeast peptone plate*, *Myxopyronin A* was was produced. The test antibiotic blocked the growth of many *Gram +ve* bacteria with *MICs* less than *100 mcg/ ml*; whereas it inhibited the growth of few *Gram -ve* bacteria such as *Escherichia coli* at *MICs* greater than *100 mcg/ ml*. On the other hand, Eukaryotic cells such as fungal and human cells were not affected. *Prokaryotic DNA-dependant-RNA polymerase*(*RNLP*) was noticed to be inhibited via the test antibiotic suggesting its *bactericidal action*. The maximum concentration was *7-8 mcg/ ml* at maximum time *2* hours when *600 mg* dose was orally administered in *randomized human clinical trials phases 1/2; as well as T1/2* reached *2.5 hours* following *first order kinetics of elimination*. Duration of its action was nearly *12 hours* after oral administration. Rare toxicity was detected during preclinical and *randomized human clinical trials phases 1/2* in the form of *mild diarrhea* and *cholestatic jaundice* in less than *5 %* of experimental candidates. **Conclusion:** The present study was promising due to the production of the bactericidal antibiotic *Myxopyronin A* from *Myxococcus fulvus 124B02* isolated from different soil environments in Egypt.

Keywords: *Myxopyronin A*; infection; antibiotic; resistance; myxobacteria

Introduction

An antibiotic needs to show selective toxicity in order to be therapeutically beneficial.[1] It must greatly suppress the activities of bacteria in comparison to those of human cells.[2] The hunt for new sources of antibiotics is necessary because antibiotic resistance is a significant, overwhelming problem that affects the entire planet.[3] The four primary targets of antibacterial medications are nucleic acids, cell walls, ribosomes, and cell membranes.[4] Since human cells lack a cell wall and contain distinct ribosomes, nucleic acid enzymes, and sterols in their membranes, these medications have no effect on human cells.[5] Bactericidal medications are well known to kill bacteria.[6] Bacteriostatic medications, on the other hand, stop germs from growing.[7] Bacteriostatic medications rely on the patient's phagocytes to eradicate the pathogen.[8]

Bacteriocidal medications should be administered to a patient if their neutrophil count is low.[9] Myxopyronins are a category of alpha-pyrone antibiotics.[10] A family of heterocyclic chemical compounds is known as pyrones or pyranones.[11] One oxygen atom and a ketone functional group are present in an unsaturated six-membered ring that they possess.[12] 2-pyrone and 4-pyrone are the names of the two isomers.[13] Since Myxopyronins do not cross-resistance with any other

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medicine, they might help address the increasing issue of drug resistance in TB.[14] Methicillin-resistant *Staphylococcus aureus*(*MRSA*) may also benefit from their therapy.[15] The current study's objective was to evaluate the synthesis of *Myxopyronin A*, a new antibiotic, in several Egyptian soil environments. In phase *1/2* randomized human clinical studies, it was also tested for antibacterial activity.

Patients and Methods

Ethical Statement

All relevant institutional, national, and/or worldwide guidelines for the care and use of humans and animals were prioritized in the current survey. The Ethical Committee for Human and Animal Handling at Cairo University(*ECAHCU*), at the Faculty of Pharmacy, Cairo University, Egypt, approved all study procedures involving humans and animals by the recommendations of the Weatherall Report(approval number *T-11-3-2023*). The number of humans and animals included in the study, as well as their suffering, was minimized at all costs. The randomized human clinical trials phase *1/2* registration number was *NCT00000714/ 2023*.

Type of the Study

Screening experimental study.

Place and Date of the Study

This study was finished at Faculty of Pharmacy, Cairo university, Egypt between *March 2023* and *January 2024*.

Source of Animal Models

They were obtained and legalized from the pharmacology and toxicology department of the faculty of pharmacy, Cairo university, Egypt.

Inclusion Criteria for Animal Models

Adult male obese rabbit animal models weighing about *2 kg*; can be inoculated with different bacterial infections. *100* rabbits were acclimatized for one week before the experiment. At a humidity(*50 % ± 5*), light-dark cycle(*12/ 12 h*), and a controlled temperature(*25±2 ^{0}C*). The rabbits were fed with fresh grass.

Exclusion Criteria for Animal Models

Young and female rabbits; Non-obese rabbits weighing less than *2 kg*.

Collection of *100* soil samples:

The samples were randomly selected grassland soils taken from various soil settings in Egypt at a depth of *30 cm*. Before being processed, samples were kept at *4 ℃* in sterile containers. Each soil sample was weighed at one gram, and each *250 ml* Erlenmeyer flask had *99 ml* of sterile distilled water. The flasks were shaken at *400 rpm* using a gyrator shaker for five minutes. Following dilutions from 10^{-1} to 10^{-6} in sterile distilled water, the soil suspensions were plated on selective *Casein yeast peptone agar*(*CYP*) medium(*bought from Sigma-Aldrich, USA*). *50 cc* of *nutrient broth liquid* at *PH 7* was added to *250 ml Erlenmeyer flasks* to create *the inoculum* for the bacterial isolate under investigation.

The medium was autoclaved and then infected with a loopful of culture from a nutritional agar slant that had been left overnight. The inoculum was the inoculated flasks, which were shaken for a whole day at *150 rpm*.

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Instruments

Table 1. List of instruments.

Instrument	Model and manufacturer
Autoclaves	*Tomy, japan*
Aerobic incubator	*Sanyo, Japan*
Digital balance	*Mettler Toledo, Switzerland*
Oven	*Binder, Germany*
Deep freezer -70 ^{0}C	*Artiko*
Refrigerator 5	*whirlpool*
PH meter electrode	*Mettler-toledo,UK*
Deep freezer -20 ^{0}C	*whirlpool*
Gyrator shaker	*Corning gyrator shaker, Japan*
190-1100nm Ultraviolet visible spectrophotometer	*UV1600PC, China*
Light(optical) microscope	*Amscope 120X-1200X, China*

Material

All chemical and biochemical substances were purchased from Algomhuria pharmaceutical company and Alnasr chemical company, Egypt. All chemical reagents used were of analytical grade.

Isolation of Myxococcus fulvus 124B02 Producing Myxopyronin Antibiotics

Dilution plating was used to directly obtain the selective isolation of *Myxococcus fulvus 124B02* species from several soil samples. The method involved wet heat treating soils and air drying them, along with suppressing competing microbes by using antibiotics like 10 mcg/ml Vancomycin and/or 10 mcg/ml Chloramphenicol. By adding 2 mcg/ml of Terbinafine HCl to the plating medium, fungi were eradicated. *Myxococcus fulvus 124B02* colony swarming was managed using Casein Yeast Peptone (CYP) plates incubated for five days at 30°C and pH 7.2. The contents of the CYP plate were 0.4% tryptically digested casein peptone, 0.3% CaCl2.2H2O, 0.1% MgSO4.7H2O, and PH 7.2. 16 S rRNA sequencing was used to identify the powerful bacterial isolate that produces Myxopyronin. With the use of 16S rRNA sequencing and other biochemical testing, the predominant bacterial isolate with strong antibacterial activity was found. Using bead pounding in a buffered solution including phenol, chloroform, and Isoamyl alcohol, nucleic acid was recovered from a swab. Next, using PCR, the variable region of the 16S rRNA gene was amplified from the resultant nucleic acid. Using a DNA purification kit [PurreLinkTM Genomic DNA Mini Kit, Catalogue number: K182002, obtained from Invitrogen, USA], the genomic DNA was isolated from 120-hour-cultured cells in accordance with the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using forward [*5-AGAGTTTGATCCTGGCTCAG-3-*] and reverse [*5-GGTTACCTTGTTACGACTT-3-*] primers [PCR SuperMix kit was acquired from Invitrogen,USA]. Following that, PCR amplicons from hundreds or even thousands of samples were pooled and sequenced in a single run. In order to calculate the relative bacterial abundances, the resultant sequences were compared to a reference database. amplifying specific DNA segments was made possible by the potent technique known as polymerase chain reaction, or PCR. Using a single-stranded DNA template, the PlatinumTM Taq DNA polymerase enzyme (catalogue number 10966018; acquired from Invitrogen, USA) guided the synthesis of DNA from deoxynucleotide substrates throughout the PCR process. The first step of the PCR cycle was denaturation. At 94° C, the DNA template was heated. As a result, single-stranded DNA was created when the weak hydrogen connections holding DNA strands together in a helix were broken.That second PCR cycle was called annealing. The mixture was allowed to cool to a temperature of 50–70°C. As a result, the primers were able to attach (anneal) to the template DNA's complementary sequence. In the PCR cycle, extension was the last phase. After that, the reaction was raised to 72°C, which is the ideal temperature for DNA polymerase to function. The number of copies of the template expanded exponentially as the cycles were repeated, producing an increasing number of duplicates. Applied Biosystems, USA, sold a genetic analyzer 3130XL, which was used to sequence

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the amplified PCR result. Using the Blastn programme on the NCBI website, a DNA sequence homology search analysis of the main bacterial isolate was accomplished.

Detection of Fruiting Bodies of Myxobacteria

A stereomicroscope (dissecting microscope) MSC-ST45T (bought from Infetik, China) was used to examine fruiting bodies. Crushed fruiting bodies were used to create wet mounts. Phase contrast microscopy was used to determine the refaractility, shape, and size of Myxospores. In contrast, the plates were subjected to UV light with a wavelength of 360 nm in order to measure the fluorescence of the fruiting bodies.[16]

Identification Myxopyronin A producing bacterial isolates:

Gram stain It divided bacteria into two groups according to the composition of their cell walls. On a microscope slide, the bacteria were treated with a crystal violet solution and then iodine, causing the cells to become purple. Gram-positive bacteria retained the stain when coloured cells were treated with a solvent like acetone or alcohol, whereas gram-negative organisms lost the stain and became colourless. When the gram-negative, transparent bacteria were mixed with the counter-stain safranin, they became pink.Spore shape was identified by the application of spore staining technique. The slide was cleaned with a Kim-wipe and alcohol to remove any fingerprints. Two circles were drawn with a Sharpie on the bottom of the slide. Using an inoculation loop, two small droplets of water were added to each circle. Using an aseptic technique, a very little number of germs were removed from the culture tube. Microorganisms were present in the droplet of water on the slide. Air dried the slide fully. The slide was heat-fixed by passing it through the flame three or four times with the smear side up. The slide took a long time to cool entirely. The stains were covered up using a piece of paper towel that was positioned inside the slide's border. Above the slide was a beaker filled with heated water. The paper towel was drenched in a malachite green liquid and the slide was left to steam for three to five minutes. The stained paper towel was taken out and disposed of. Water was used to carefully wipe the slide in order to remove any loose paper towel particles.Safranin was used as the counter-stain for a minute. The slide's bottom was dried before it was placed on the microscope's stage and examined using the oil immersion lens.18Spore site: the location of the spores was determined using the Gramme stain technique.[19]Cell shape: The cell shape was evaluated by the Gramme stain test.[20]Blood haemolysis: the ability of antibiotics to haemolyze blood was assessed on blood agar medium.[21] Using the motility test, it was possible to distinguish between moving and stationary bacteria. The medium was punctured with a sterile needle up to 1 centimetre from the tube's bottom in order to identify a motile colony and assess its isolation. Without a doubt, the needle was held in place both throughout its insertion and withdrawal from the medium. It needed to be incubated for eighteen hours at 35°C, or until there was observable growth.[22] Nitrate reduction test: After adding 0.5 ml of nitrate broth to a sterile test tube, it was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. It was then allowed to defrost to room temperature. A substantial inoculum of fresh bacterial culture was added to the tube, and it was then incubated for two hours at 35°C. Two drops were added of each reagent (A and B) and well mixed. It was noted that the colour became crimson after two minutes. If no red colour appeared, a tiny quantity of zinc dust was added, and after five minutes, the colour was checked to see if it had formed.[23]Methyl red test: Prior to adding the methyl red pH indicator, an infected tube of MR broth was utilised. When an organism employed the mixed acid fermentation pathway and generated stable acidic end products, the acids in the medium overcame the buffers, creating an acidic environment.[24] Catalase test: To determine if a particular bacterial strain could make catalase, a small inoculum was added to a 3% hydrogen peroxide solution. The quick development of oxygen bubbles was seen.[25]In the Oxidase test, a small piece of filter paper was treated with 1% Kovács oxidase reagent and left to air dry. Using a sterile loop, a well-isolated colony was removed from a recently cultured bacterial plate (it took 18 to 24 hours), and it was then rubbed onto prepared filter paper. Changes in colour were observed.[26]Citrate Utilization: After autoclaving at 15 pounds for 15 minutes, five millilitres of Simmon Koser's citrate medium were taken.

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The test tube holding the melted citrate medium was tilted to provide a distinct slant and butt. The designated microbe samples were injected on the inclined medium using labelled tubes and sterile wire. The tubes were incubated at 37°C for a whole day. The colour change of the medium was observed.[27]Starch hydrolysis was introduced into the bacterial plates and left for 48 hours at 37°C. Following incubation, the plates' surface was smeared with an iodine solution using a dropper for 30 seconds. Overabundance of iodine was then released. We examined the region around the bacterial growth line.Agar medium was made using 1% Tween 80 hydrolysis and Tween 80 hydrolysis.[28] Using an inoculating loop, the provided microbe was introduced to the Tween 80 agar plates, resulting in a single centre streak. The plates were incubated at 37 °C for a whole day. The plates were covered with a HgCl2 solution. The plates were inspected after a little period of time. Test result was positive; Tween 80 hydrolysis was evident in the clear halo-zone that surrounded the injected area.[29]On nutritional agar media, growth was seen to be possible at 45°C when the temperature was between 10 and 45°C.Five drops of the Kovács reagent were immediately added to the test tube holding the microbe for inoculation in the Indol test.[30] An indol test that was positive was indicated by the reagent layer turning pink to red (a cherry-red ring) a few seconds after the reagent was added to the medium.[31] Its ability to grow on nutrient agar while responding to 5% and 7% NaCl was investigated using the tolerance salinity test.[32]Voges-Proskauer(VP) test: Alpha-naphthol and potassium hydroxide were combined with Voges-Proskauer broth, a glucose-phosphate broth brimming with microorganisms, for the test. A cherry red hue represented a favourable conclusion, whereas a yellow-brown tint represented an unfavourable outcome.[33] Casein hydrolysis test: A single line streak of the test culture was formed in the centre of the skim milk agar plate under aseptic circumstances. The plate was then incubated at 37°C in an incubator for 24 to 48 hours to test the test antibiotic's ability to hydrolyze casein.[34]

Effect of Different Carbon Sources

Using a glucose fermentation test and glucose purple broth, the fermentation responses of glucose were examined. The purple soup was prepared with peptone and the PH indicator bromcresol purple. 1% of a glucose solution was added. As an inoculant, isolated colonies from a pure culture of microorganisms cultured for 24 hours were added to the glucose purple broth. A control tube containing purple broth base was employed in parallel with the inoculation of the glucose-based medium. For three days, the infected medium was incubated aerobically at 35–37 °C. The liquid started to become yellow, indicating that the experiment had succeeded. The absence of development of yellow colour showed a weak reaction to carbohydrate fermentation.[35]

In a sterile tube containing phenol red fructose broth, the inoculum of a pure culture was aseptically transferred for the fructose fermentation test. The infected tube was incubated at 35–37 °C for 18–24 hours. An acidic PH change, shown by a colour shift from red to yellow, was a positive reaction.[36]For the maltose fermentation test, phenol red maltose broth was aseptically added to a sterile tube along with a pure culture inoculum. The infected tube was incubated at 35–37 °C for 18–24 hours. An acidic pH change, shown by a colour shift from red to yellow, was a positive reaction.[37] The inoculum of a pure culture was aseptically transferred to a sterile tube filled with phenol red sucrose broth for the sucrose fermentation test.

The infected tube was incubated at 35–37 °C for a whole day. An acidic pH change, shown by a colour shift from red to yellow, was a positive reaction.[38]

Purification of *Myxopyronin A* Antibiotic

The reversed phase-HPLC chromatography method was used to accomplish this. The rate of aeration was 0.142 V/min. A 500 rpm stirring rate was used. After 18 hours, PO2 had dropped to around 20% of saturation from over 90% at that point. After 40 hours, the fermentation was halted in a gyrator shaker by centrifugation at 500 rpm. Following the collection of the supernatants, the antimicrobial sensitivity of the samples was assessed using the agar paper diffusion disc technique and the broth dilution technique to determine MICs. Using 2/ 10 volume ethyl acetate, the test

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antibiotic was removed from the 2 litres of culture broth. After that, the ethyl acetate was extracted at 40°C at a lower pressure.

The residue was then dissolved in 398 millilitres of methanol-water (90:10) and subjected to reversed phase HPLC chromatography. The phase that moved was methanol. The eluent had a flow rate of 300 ml per minute and contained 70 parts methanol, 16 parts water, and 4 parts acetic acid. By using the refractive index, the antibiotic components were found. Utilizing paper discs and Staphylococcus aureus as an indicator organism, the agar diffusion test was used to evaluate the biological antibiotic activity present in the major peak, which had a retention duration of five minutes. On the other hand, NaHCO3 was used to neutralize the primary peak. The extraction of Myxopyronin A was done using 10% V/V ethylene chloride. Approximately 85% of the antibiotic material that remained pure after the solvent evaporated. It was observed that Myxopyronin A had a retention time of 11 minutes. The pure Myxopyronin A's molecular formula was found using a mass spectrometer (Quadrupole mass spectrometer, Advion, USA).[39]

Procedure of *Broth Dilution Assay* for Determination of *MICs* of *Myxopyronin A*

Several microtiter plates were loaded with a particular broth during testing in accordance with the requirements of the target bacterium.The bacteria to be examined and various antibiotic concentrations (ranging from 1 to 200 mcg/ml) were then put to the plate. After that, the plate was incubated for sixteen to twenty hours at thirty-seven degrees Celsius in a non-CO2 incubator. The plate was taken out after the specified amount of time and examined for bacterial growth. Bacterial growth was evident as the soup turned murky. Minimum Inhibitory Concentration (MIC), or the lowest concentration of antibiotics that inhibited bacterial proliferation, was used to report the broth microdilution technique results.[40]

***Agar Diffusion Assay* with *Paper Discs* Procedure for the Determination of *Myxopyronin A* Antimicrobial Activity**

The test microorganism-seeded agar medium allowed the test antibiotic extract to spread from its reservoir, which is why the disc diffusion method (DDM) was categorized as an agar diffusion technique (ADM). A filter paper disc was often used as the reservoir, and it was set on top of an agar surface. An inhibitory zone formed around the filter paper disc upon incubation when the chemicals in the studied extracts were microbiologically active. The test extract's antibacterial effectiveness was accurately characterized by the diameter of the inhibition zone.[41] Table 2 lists the methods used to extract the test microbes: broth, selection or enrichment growing medium.

Table 2. It demonstrates different isolation media for different pathogenic m.os. utilized in the *Broth microdilution* test and agar diffusion assay using paper discs.

Pathogenic m.o	No of strains	Isolation media
Bacillus subtilis	5	Mannitol egg yolk polymixin agar(MEYP)
Bacillus cereus	7	Polymixin egg yolk mannitol bromothymol blue agar(PEMBA)
Staphylococcus aureus	6	Salt mannitol agar(SMA)
Pneumococci	13	Todd Hewitt broth with yeast extract
E. coli	17	Sorbitol- Macconkey agar
Pseudomonas aeruginosa	10	Pseudomonas isolation agar(PSA)
Candida albicans	1	Potato dextrose agar(PDA)
Saccharomyces cerevisiae	5	Sabourad dextrose agar(SDA)
Salmonella typhimurium	4	Bismuth sulfite agar(BSA)
Haemophilus influenza	3	Enriched chocolate agar
Gonococci	4	Thayer martin medium
Meningococci	6	Mueller Hinton agar

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Serratia Marcescens	4	Caprylate thallous agar medium
Mucor hiemalis	1	Potato dextrose broth
Shigella dysenteriae	8	Hekteon enteric agar
Micrococcus luteus	1	Tryptic soy agar
Proteus mirabilis	1	Blood agar

Estimation of Myxopyronin A Effect on Bacterial RNA Synthesis

In order to calculate the concentration of RNA extracted using Rneasy Kits (bought from QIAGEN, USA), the absorbance at 260 nm in a spectrophotometer was measured. 40 μg of RNA per millilitre are represented by an absorbance of 1 unit at 260 nm (A260 = 1 = 40 μg/ml).[42]

Estimation of Myxopyronin A Effect on Bacterial Protein Synthesis

By comparing the obtained absorbance at 205 nm to a standard curve, the protein content was determined. Total protein in crude lysates and purified or partly purified protein might be measured using the (205) technique. The UV spectrophotometer was calibrated to read 205 nm, giving it fifteen minutes to reach equilibrium. With a solution of the buffer and all ingredients present—aside from the protein—the absorbance measurement was reset to zero. After adding the protein solution to the 1 mL cuvette, the absorbance was measured. Sample dilution and readings were carried out in duplicate.Throughout the test method, the matching cuvettes for the samples and controls were used.

Since the protein's extinction coefficient was known, the following formula was used. To get the protein concentration, take the absorbance = extinction coefficient × path length (1 cm).[43]

Estimation of Pharmacodynamic and Pharmacokinetic Effects of Myxopyronin A during Experimental Animal Testing in Preclinical Clinical Trials

The current investigation assessed the pharmacokinetics and pharmacodynamics of Myxopyronin A in male rabbit animal models that weighed around 2 kg following dose. Moreover, LC-MS/MS was used to ascertain the concentrations of compounds in target compartments, including lung, kidney, and thigh tissue. The pharmacodynamic profile of Myxopyronin A was evaluated using the common neutropenic thigh and lung infection models, taking into account the pharmacokinetic findings. At the conclusion of the trial, 0.01 ml/g IP was used as the euthanasia technique. Every rabbit animal model received ketamine injections to produce anaesthesia, which resulted in a 27–30 minute loss of consciousness before the animals were beheaded.[44]

Estimation of Pharmacodynamic and Pharmacokinetic Effects of Myxopyronin A in Randomized Human Clinical Trials Phases 1/2

In order to demonstrate the test antibiotic's bioavailability, pharmacokinetics, and pharmacodynamics, 150 human volunteers participated in the study. With a one-week washout interval, the study was set up as a randomized, single-dose, two-treatment, two-period crossover experiment. At 0 (baseline), 10, 20, and 40 minutes, as well as 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 hours after the treatment, blood samples were taken. The four medications' plasma concentrations were determined using a tandem mass spectrometry-rapid chromatography technique. Noncompartmental approaches were utilised to compute the pharmacokinetic parameters. If the predefined range of 80% to 125% contained the 90% CIs of the log-transformed test/reference ratios AUC(0-24), AUC(0-∞), and Cmax, then Bioequivalence was established.

Subject reports and clinical data were used to evaluate tolerability. Pharmacodynamic effects were assessed by using the broth dilution technique and the agar diffusion assay to determine MICs. Phases 1 and 2 of randomized human clinical trials used spectrophotometric estimation of infectious bacterial cell numbers.[45]

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Estimation of of Phototoxicity, Mutagenicity and Carcinogenicity of the Test Antibiotic

The 3T3 neutral red uptake phototoxicity approach was used to determine the phototoxicity.[46] On the other hand, the Ames test was used to determine the test antibiotic's mutagenicity and carcinogenicity.[47]

The Determination of Toxokinetics and Toxodynamic Effects

For this, the up-and-down approach for detecting Myxopyronin A's acute toxicity was applied.[48]

The Determination of Maximum Bactericidal Activity of *Myxopyronin A*

A concentrated culture of a designated microbe was incubated for a full night and subsequently diluted to a concentration of 1 x 10^5 to 1 x 10^6 cfu/ml in growth-supporting broth (usually Mueller Hinton Broth). The antimicrobial test material was diluted to a stock level around 100 times the predicted minimum inhibitory concentration. In test tubes, more 1:1 dilutions were created. Equal amounts of the designated microbe were added to each dilution of the test antibiotic. For each test microorganism, a positive and negative control tube was inserted to show sufficient microbial growth during the incubation time and medium sterility, respectively. To create a baseline concentration of the used microorganism, an aliquot of the positive control was plated. The tubes were then incubated for the proper amount of time and at the right temperature. The bacterium was growing when the turbidity increased, and the minimum inhibitory concentration (MIC) was the point at which no visible growth was seen. The dilution that represented the MIC and at least two of the more concentrated test product dilutions were plated, and the viable CFU/ml was counted to estimate the MBC. When compared to the MIC dilution, the MBC was the lowest concentration that showed a predetermined reduction (such as 99.9%) in CFU/ml.[49]

Determination of Plasma Protein Binding Capacity of *Myxopyronin A*

Using an ultrafiltration technology, 30 patients who had received an infectious pneumococci vaccination at the same time were administered test antibiotic medications, and the protein binding (PB) extent and changeability of the medications were determined. The microbiological laboratory at Cairo University's pharmacy faculty regularly received the clinical samples that were utilised. Similarly plumbed were plasma proteins. The nonspecific binding was measured in a protein-free media. Thirty patient plasma samples were collected, of which plasma proteins were considered for twenty-four patients.

Determination of Liver, Kidney and Heart Function Tests

Functional tests were conducted throughout stages 1/2 of randomized human clinical trials to evaluate the health of the liver, kidney, and heart. On the other hand, graded dosages of Myxopyronin A were administered to all experimental individuals, resulting in urine and stool analyses as well as the measurement of blood complete counts.

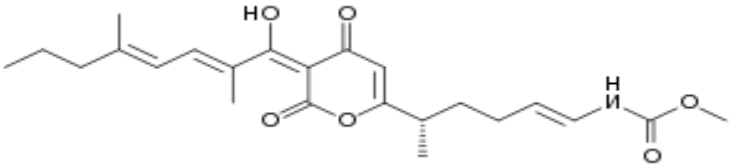

Figure 1. It demonstrates the structure of *Myxopyronin A* extracted from bacterial isolates *Myxococcus fulvus strain Mx f50* collected from different soil environments in Egypt . Molecular formula of the purified test antibiotic was noticed to be $C_{23}H_{31}NO_6$ determined through mass spectrometer.

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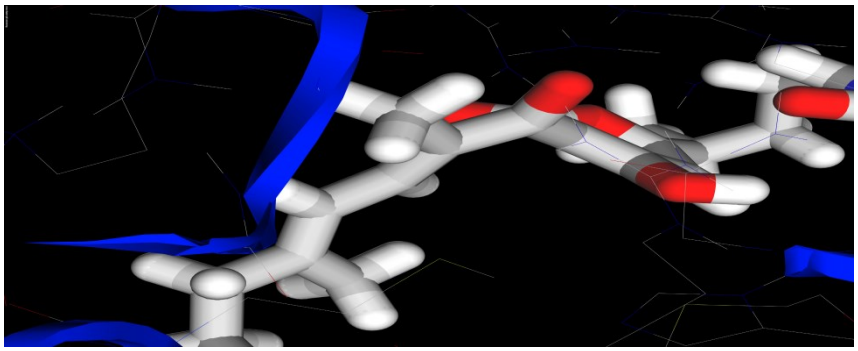

Figure 2. It represents docking of Myxopyronin A ligand on Bacterial *RNA* polymerase. Myxopyronin showed high affinity and inhibitory effect towards the switch region.

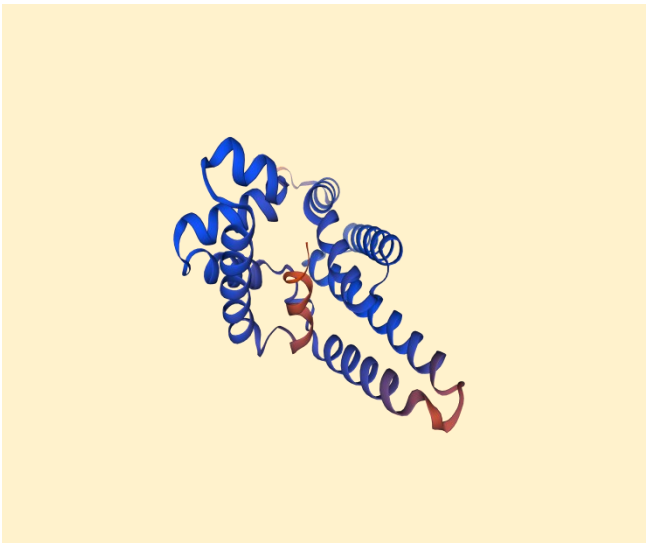

Figure 3. It demonstrates *3D* structure of bacterial prokaryotic *RNA polymerase* comprising the switch binding site to which *Myxopyronin A Ligand* strongly bound inhibiting bacterial *RNA polymerase* activity selectively leading to the inhibition of *mRNA* transcription and subsequently the mortality of the microbe. The secondary structure of RNA polymerase enzyme consisted of spiral alpha and beta sheets. Its molecular mass was approximately *198 amino-acids*.

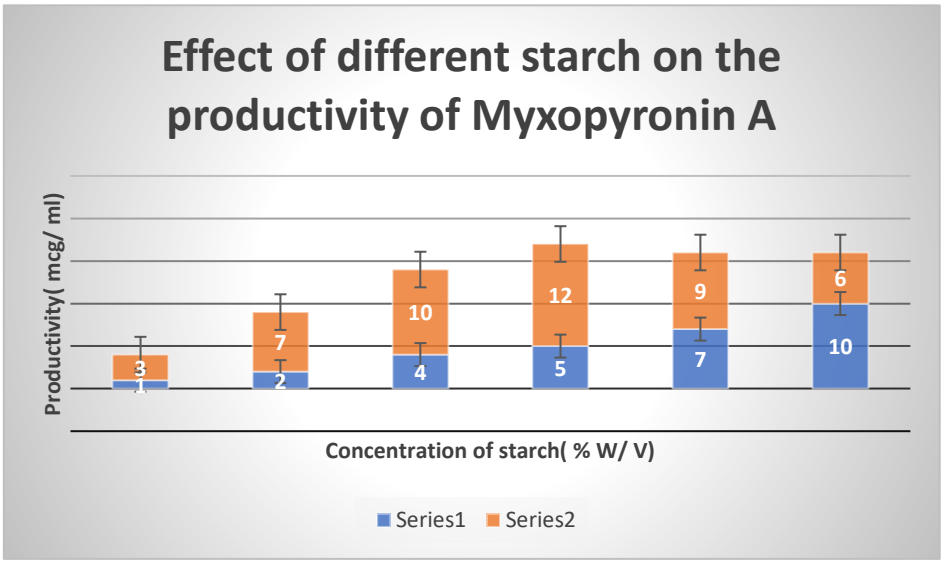

Figure 4. It shows the impact of various concentrations of *Soluble starch* on the production of *Myxopyronin A*.

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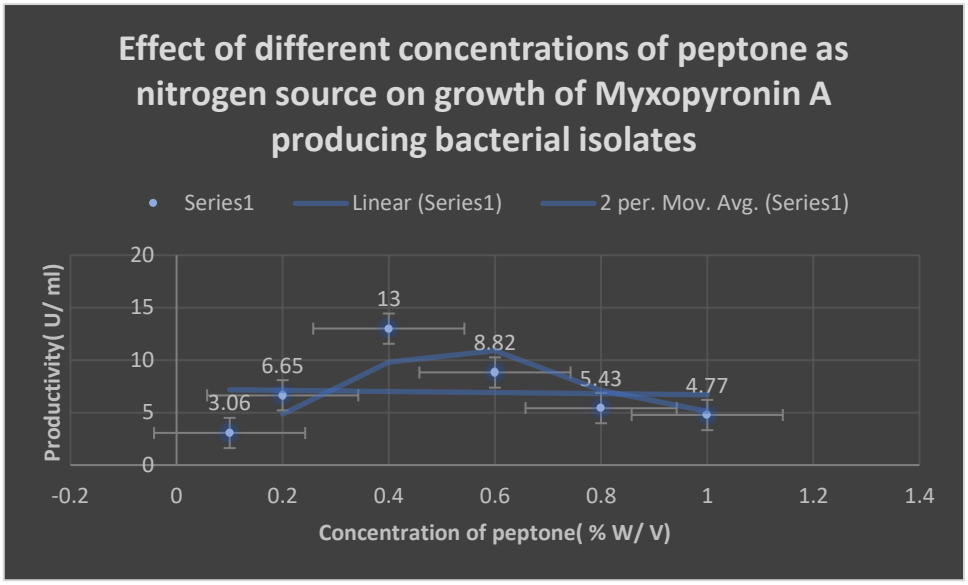

Figure 5. It shows the effects of different Peptone concentrations as nitrogen growth factor on the productivity of *Myxopyronin A*.

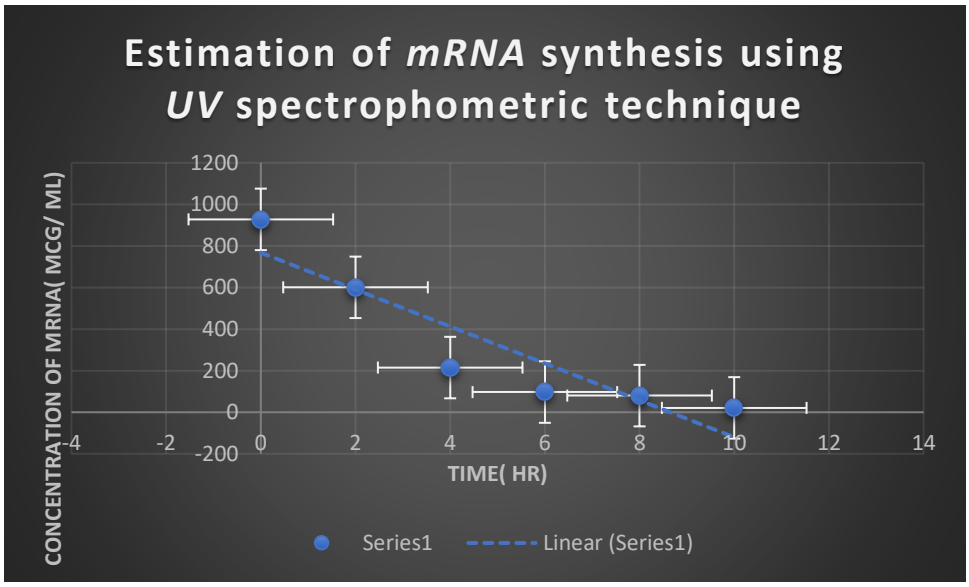

Figure 6. It refers to the estimation of effect of *Myxopyronin A* on microbial *mRNA* productivity. *mRNA* synthesis was detected to be diminished proportionately up on employment of exploding doses of *Myxopyronin A* antibiotic.

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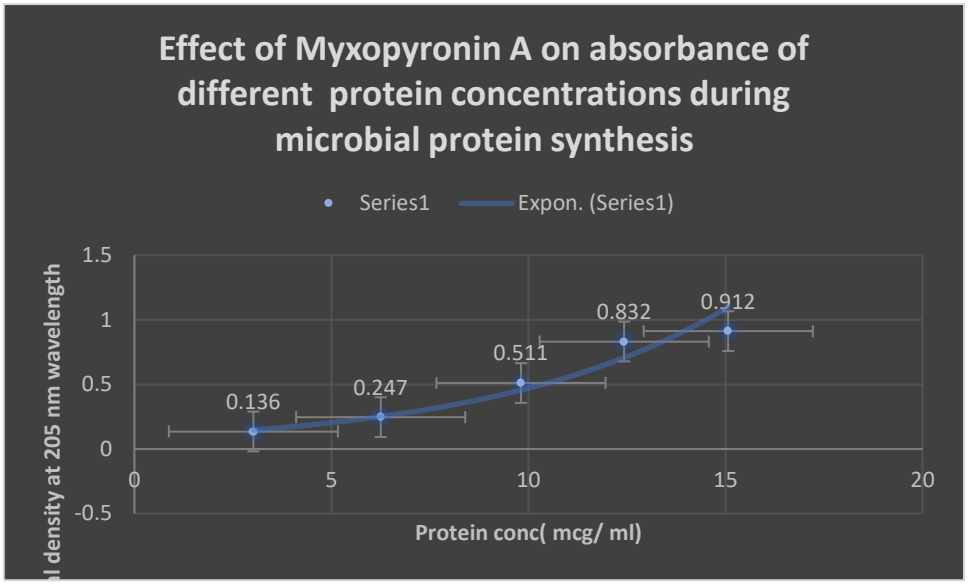

Figure 7. It demonstrates the influence of *Myxopyronin A* on protein synthesis using *UV* spectrophotometer absorption at *205 nm*. Protein synthesis was noticed to be decreased dramatically up on utilization of increasing doses of *Myxopyronin A* antibiotic.

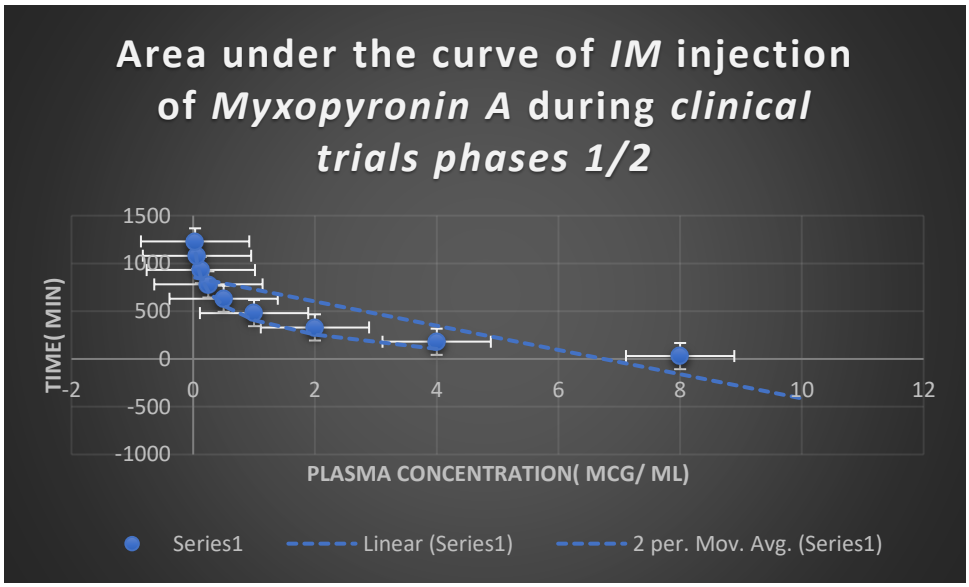

Figure 8. It shows *AUC* of *Myxopyronin A* following *IM administration in clinical trials stages 1/2*. Efficacious dose ranged from 5-*6 mg/ kg* of body weight. Onset of action was observed following closely 15 *minutes*. It followed first order of elimination kinetics.

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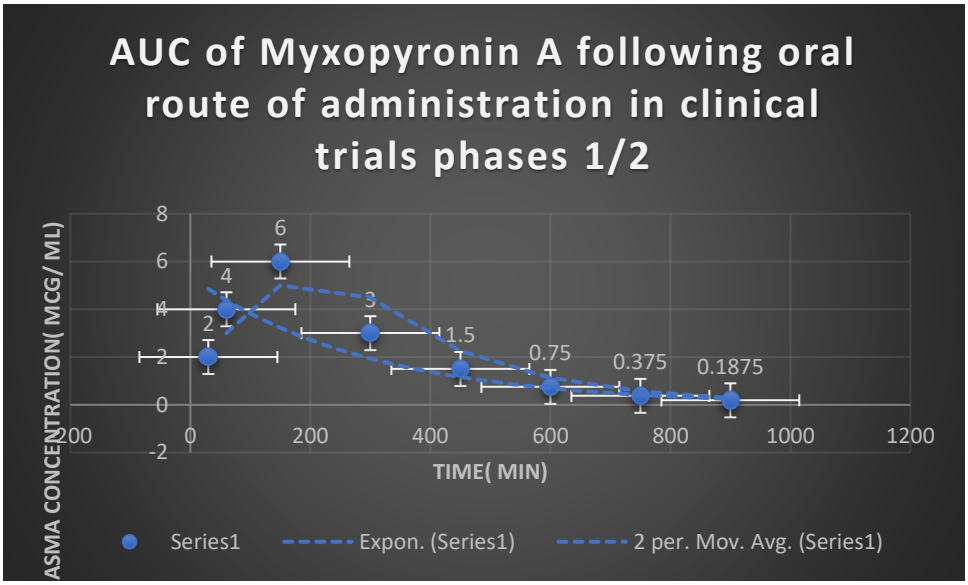

Figure 9. Area under the curve(*AUC*) following oral administration of *Myxopyronin A* during *clinical trials phases 1/2*. Efficacious dose ranged from *7-8 mg/ kg* of body weight. Onset of action was observed following nearly *30 minutes*. It followed first order of elimination kinetics.

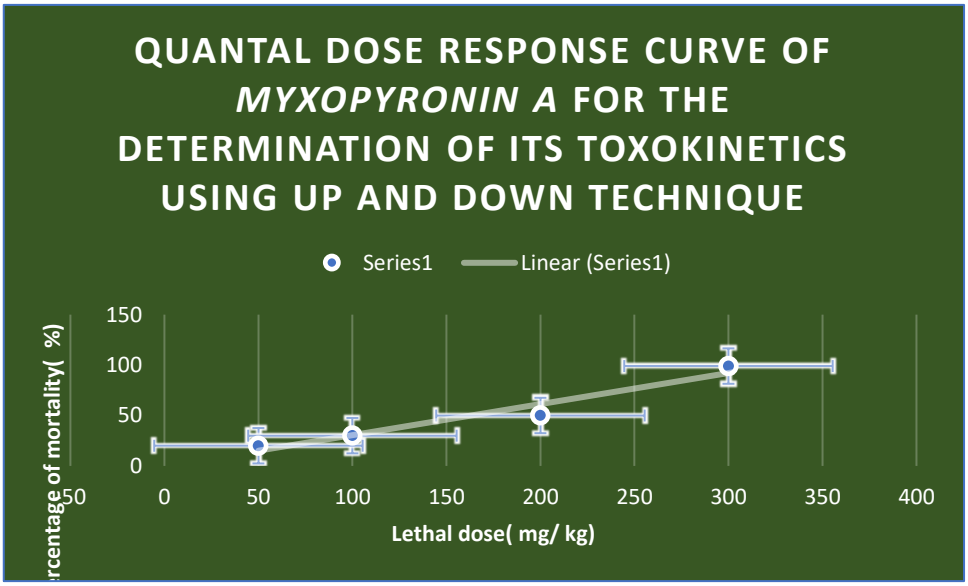

Figure 10. Quantal dose response curve for the determination of toxokinetics of *Myxopyronin A*. LD_{50} % was found to be *200 mg / kg;* while *LD99* % was nearly *300 mg/ kg*.

Statistical Analysis

All cultures were conducted in triplets. Their presentation was by means and standard deviation. One way analysis of variance(*p value≤ 0.05*) was used as means for performing statistical analysis and also, statistical analysis was based on excel-spreadsheet-software. The *F statistical analysis test* was utilized during the present study.

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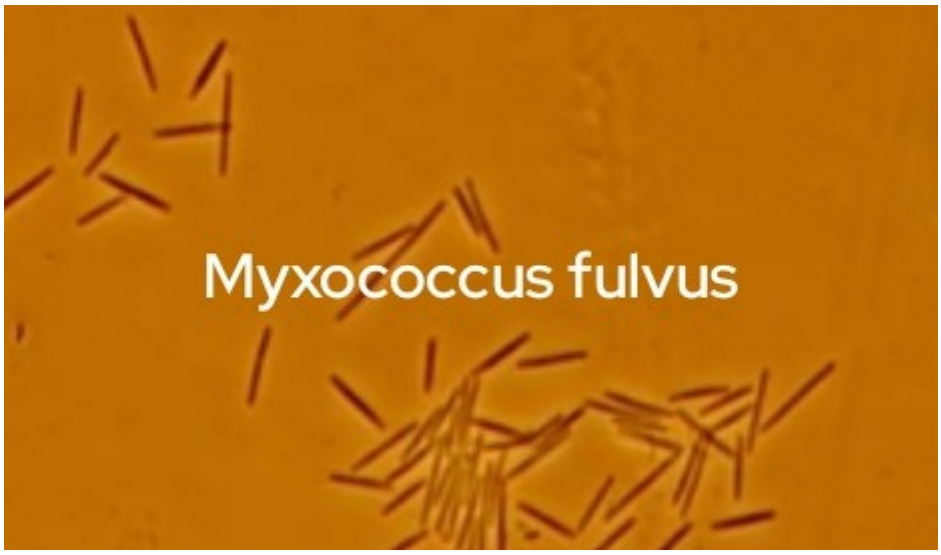

Figure 11. It demonstrates the major Gram negative bacterial isolates producing *Myxopyronin A* antibiotic using Stereomicroscope.

Figure 12. It shows *Myxococcus fulvus colonies* on *CYP* isolation plates secreting *Myxopyronin A* antibiotic.

Results

From the culture supernatant of the Myxobacterium *Myxococcus fulvus 124B02* which was the predominant soil bacterial isolate(*19* isolates, Table 3) grown on *Casein yeast peptone*(*CYP*) plate, *Myxopyronin A* was was produced. The test antibiotic blocked the growth of many *Gram +ve* bacteria with *MICs* less than *100 mcg/ ml*; whereas it inhibited the growth of few *Gram -ve* bacteria such as *Escherichia coli* at *MICs* greater than *100 mcg/ ml*. On the other hand, Eukaryotic cells such as fungal and human cells were not affected. *Prokaryotic DNA-dependant-RNA polymerase*(*RNLP*) was noticed to be inhibited via the test antibiotic suggesting its *bactericidal action*. *Cmax* was *10 mcg/ ml* at *Tmax 2* hours when *600 mg* dose was orally administered in *randomized human clinical trials phases 1/2; as well as T1/2* reached *2.5 hours* following *first order kinetics of elimination*. Duration of its action was nearly *12 hours after* oral administration. Rare toxicity was detected during preclinical and *randomized human clinical trials phases 1/2* in the form of *mild diarrhea* and *cholestatic jaundice* in less than *5 %* of experimental candidates. *Myxopyronin A* was the predominating compound after reverse phase *HPLC* technique was utilized for the refinement and the purification of of the test antibiotics (Table

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4). The phototoxicity was determined via *3T3* neutral red uptake phototoxicity technique which showed no phototoxicity. On the other hand, mutagenicity and carcinogenicity of the test antibiotic were assessed using Ames test which demonstrated no carcinogenicity and genotoxicity at all.

Table 3. It shows the distribution of *Myxopyronin A* producing bacterial isolates.

No of *+ve* bacterial isolates producing *Myxopyronin A*	**No of *-ve* bacterial isolates producing *Myxopyronin A***
19	31

Table 4. It demonstrates the degree of purity of test antibiotics following the purification via *reversed phase HPLC technique*.

Test antibiotic	**Degree of purity(%)**
Myxopyronin A	*80*
Myxopyronin B	*20*

Table 5. It demonstrates *16 S rRNA* detection of *Myxopyronin A* producing isolates using *BLASTn* software.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident
Myxococcus fulvus 124B02, complete genome	*Myxococcus fulvus 124B02*	597	1167	99%	6.00E-166	98.81
Myxococcus sp. MH1 DNA, complete genome	*Myxococcus sp. MH1*	525	1051	99%	3.00E-144	94.94
Myxococcus sp. SDU36 chromosome, complete genome	*Myxococcus sp. SDU36*	436	870	99%	1.00E-117	90.15
Myxococcus hansupus strain mixupus chromosome, complete genome	*Myxococcus hansupus*	126	126	65%	3.00E-24	77.23
Cystobacter fuscus strain DSM 52655 chromosome, complete genome	*Cystobacter fuscus*	124	124	54%	1.00E-23	78.92
Cystobacter fuscus strain Cbf 8 chromosome, complete genome	*Cystobacter fuscus*	124	124	54%	1.00E-23	78.92

Table 6. It shows the estimation of zones of inhibition and minimum inhibitory concentrations of *Myxopyronin A via* Agar diffusion assay using paper discs.

Test organism	***MIC(µg/ ml)***	**Diameter of inhibition zone(*mm*)**
Bacillus subtilis	5	12
Staphylococcus aureus	7	18
Streptococcus pneumonae	13	9
Escherichia coli	108	11
Pseudomonas aeruginosa	120	0
Candida albicans	105	0
Sacchromyces cerevisiae	100	0
Salmonella typhimurium	101	17
Bacillus cereus	16	10
Micrococcus luteus	21	13
Serratia Marcescens	105	9
Mucor hiemalis	0	19

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Shigella dysentery	113	7
Proteus mirabilis	139	6

1 The initial density of each organism during Agar diffusion assay for the determination of minimum inhibitory concentrations and zones of inhibition of gtowth was nearly 10^5/ ml.

Table 7. It demonstrates *MICs* of *Myxopyronin A* on different microorganisms using broth microdilution technique.

Pathogenic m.o	***MIC(µg/ ml)***
Bacillus subtilis	8
Bacillus cereus	7
Staphylococcus aureus	13
Pneumococci	15
E.coli	110
Pseudomonas aeruginosa	142
Candida albicans	0
Sacchromyces cerevisiae	0
Salmonella typhimurium	103
Haemophilus influenza	0
Gonococci	127
meningococci	134
Serratia Marcescens	105
Mucor hiemalis	0
Shigella dysenteriae	113
Micrococcus luteus	0
Proteus mirabilis	0

Table 8. It demonstrates Minimum bactericidal concentrations(*MBCs*) of *Myxopyronin A* on different microorganisms using broth microdilution technique.

Pathogenic m.o	***MBC(µg/ ml)***
Bacillus subtilis	24
Bacillus cereus	21
Staphylococcus aureus	39
Pneumococci	46
E.coli	331
Pseudomonas aeruginosa	412
Candida albicans	0
Sacchromyces cerevisiae	0
Salmonella typhimurium	307
Haemophilus influenza	0
Gonococci	370
meningococci	390
Serratia Marcescens	320
Mucor hiemalis	0
Shigella dysenteriae	303
Micrococcus luteus	0
Proteus mirabilis	0

Protein synthesis and *mRNA* synthesis were decreased significantly with increasing the dose *Myxopyronin A*, as demonstrated in Tables *10* and *9* respectively. Docking studies using *MCULE* and *SWISS DOCK* softwares demonstrated that the mechanism of action of the test antibiotic was probably due to the inhibition of *RNA Polymerase* through binding with its switch region. High ΔG of the test antibiotic was observed to be approximately *17 J/ mole* as determined via *SWISS* model software. On the other hand, low *Kd* of the test antibiotic towards the switch region was found to be approximately *250 nM* using *SWISS MODEL* software. The biochemical profile and the morphology

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of the potent bacterial isolates producing the test antibiotic in the present study was summarized in Table 11. The morphology and biochemical reactions indicated that the predominant bacterial isolates secreting the extracellular test antibiotic were *Myxococcus fulvus.* A total of *150* human subjects(mean *SD* age, *27.3*[*9.8*] years were enrolled and completed the study. For the test antibiotic *90% CIs* for the long transformed ratios of *Cmax*, *AUC*(*0-24*) and *AUC*(*0-∞*) were *85.1*to *96.4*, *87.2* to *97.3* and *91.7* to *98.4* respectively. The mean *PB* was observed for *Myxopyronin A* which approximated *70%*. The major protein binding for *Myxopyronin A* and rifampicin was detected to be albumin. The unbound fraction was found to be responsible for the therapeutic activity.

Table 9. It shows the estimation of *mRNA* quantity via *UV* spectrophotometer at *260 nm* after addition of *Myxopyronin A*.

***mRNA* concentration(*ng/ ml*)**	***Absorbance(optical density)* at *260 nm***
810	0.973
705	0.601
341	0.397
43	0.213

Table 10. It shows the effect of *Myxopyronin A* on the microbial protein synthesis using *UV* spectrophotometer at *205 nm*.

Bacterial protein concentration(mc*g*/ *ml*)	**Time(hr)**
70.3	1
41.06	3
18.62	5
3.01	10
0.79	12

Table 11. The resolution of biochemical reactions.

Test	**Result**
Gram stain	-
Cell shape	Elongated bacilli with tapered ends
Spore shape	Ellipsoidal
Spore site	Central
Motility	+ via gliding
Catalase	+
Oxidase	-
Blood haemolysis	-
Indol	-
Methyl red	-
Nitrate reduction test	+
Vogues proscauer	-
Citrate utilization	-
Starch hydrolysis	+
Casein hydrolysis	+
Growth at 45 °C	Bacterial isolates did not grow at 45 °C; but were grown at 10-37 °C
Tween 80	+
Tolerance salinity	
5% NaCl	-
7% NaCl	-
Saccharide fermentation	
Glucose	-
Fructose	-
Maltose	-
Sucrose	-

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Discuss

The in vitro and in vivo antimicrobial activity of *Myxopyronin A*, a novel antibiotic was evaluated in the present study. It demonstrated excellent bactericidal activity against a broad spectrum of *G +ve bacteria* with *MICs* did not exceed *20 mcg/ ml*. On the other hand It showed few bactericidal activities against *G -ve bacteria*.with minimal inhibitory concentrations were greater than *100 mcg/ ml*. Its mechanism of action was realized during the investigation of *RNA synthesis* to be via the inhibition of prokaryotic *DNA-dependant-RNA polymerase*; whereas no inhibitory impact was observed for Eukaryotic one. Docking studies through *SWISS DOCK* software confirmed this as well. The antibiotic activities *Myxopyronin A* and *B* were isolated from the culture supernatant of *29* bacterial isolates of *Myxobacterium Myxococcus fulvus 124B02* detected molecularly using *16 S rRNA* technique (Table 3).

The antibiotic activity did not inhibit the growth or kill eukaryotic cells such as human and fungal cells reflecting selectivity towards the inhibition of the growth of prokaryotic bacterial cells. This selectivity effect minimized the adverse effects noticed during the present study. Docking studies via *SWISS DOCK* software revealed that desmethylation of either *Myxopyronin A* or *B* enhanced its biological activity. Purification was performed through reversed phase *HPLC*. *Myxopyronin A* was the main refined antibiotic. Its purity degree reached approximately *80 %*; while, the remaining purified antibiotic was detected to be *Myxopyronin B*. The antibacterial activity was assessed via the determination of *MICs* of the test antibiotics using the agar diffusion technique utilizing paper discs *5 mm* in diameter and the broth dilution assay. The initial density of each test microorganism was about 10^5*/ ml* of the culture suspension. The *MICs* of test antibiotic against *G +ve bacteria* ranged from *5* to *20 mcg/ ml;* Whereas *MICs* reached above *100 mcg/ ml* against some selected *G -ve bacteria.* On the other hand no effect was detected against the growth of fungi and yeasts. (Irschik H et al., 1983) stated that *Myxovalargin A* was a novel peptide antibiotic isolated from the culture supernatant of the *myxobacterium Myxococcus fulvus strain Mx f65*. It was active against *Gram-positive bacteria*(*MIC 0.3* approximately *5 micrograms/ ml*), at higher concentrations also against Gram-negative ones(*MIC 6 approximately 100 micrograms/ ml)*, and not at all against yeasts and molds. Its mechanism of action involved the inhibition of the bacterial protein synthesis.[50] According to(Glaus F et al., 2018) *Ripostatin*, a novel antibiotic, isolated from the culture supernatant of *Myxobacterium*, *Sorangium cellulosum strain So ce377*. On the other hand it interfered of the bacterial *RNA* synthesis.[51] On the other hand, *Myxopyronin A* was found to be structurally related to *α*-pyrone antibiotics from *myxobacteria*. Its ability to inhibit *RNA polymerase* was through interaction with the switch region of *RNA polymerase*; while *Rifampicin* inhibited the same enzyme through different region.[52] *Myxopyronin A* showed no phototoxicity and mutagenicity in rabbit animal models during *the preclinical trials stage*, in the present study. Rare adverse effects including mild diarrhea and cholestatic jaundice were reported in less than *5 %* of the experimental subjects received the test antibiotics during *randomized human clinical trials phases 1/2*. The biological half life of *Myxopyronin A* reached approximately *2.5 hours*. *0.4 % peptone* and *5 % soluble starch* were detected to be the optimal nitrogen and carbon growth factors for bacterial isolates producing the test antibiotics, respectively (Figures 4 and 5). High free delta energy(*ΔG*) of the test antibiotic was observed to be approximately *17 J/ mole* as determined via *SWISS MODEL* software reflecting high catalytic activity of the test antibiotic towards the switch region. On the other hand, low dissociation constant(*Kd*) of the test antibiotic towards the switch region was found to be approximately *250 nM* using *SWISS MODEL* software indicating high affinity and binding capacity. Bioavailability studies were performed using *HPLC* during randomized human clinical trials phases 1/2 revealed that *Myxopyronin A* reached nearly *70% oral bioavailability*, *82% IM bioavailability* and *100% IV bioavailability*. Metabolic studies using *HPLC* revealed that the test antibiotic showed no in vivo induction of hepatic metabolizing C*ytochrome P450* enzymatic system; while rifampicin induced *CYP3A4* hepatic metabolizing enzyme potently. Up and down procedure intended for the evaluation of acute toxicity profile of the test antibiotic showed that LD_{50}% was about *200 mg/ kg* body weight; while LD_{99}% reached *300 mg/ kg*. On the other hand, therapeutic margin of the test antibiotic ranged from *7 mcg/ ml* to *100 mcg/ ml*. *Myxopyronin A* producing bacterial isolates were gram negative, spore

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forming *obligate aerobes* and *chemoorganotrophic*. They were *elongated rods* with *tapered ends*. No *flagella* were present; but the cells moved via *gliding*. They fermented *Tween 80*, *starch* and *casein*. On the other hand they were positive for *catalase* while negative for *oxidase* tests. They reduced *nitrates* and were able to grow at *10-37 ℃*. A total of *150* human subjects(mean *SD* age, *27.3*[*9.8*] years were enrolled and completed the study. For the test antibiotic 90% CIs for the long transformed ratios of *Cmax*, *AUC*(*0-24*) and *AUC*(*0-∞*) were *85.1*to *96.4*, *87.2* to *97.3* and *91.7* to *98.4* respectively. The point estimates for *Cmax* in the present study were outside the limit for bio-equivalence for rifampicin standard drug. The mean protein binding(*PB*) was observed for *Myxopyronin A* which approximated *70%* while that of rifampicin reached *88%*.[53] It was noticed that plasma protein binding was proportionally increased with increasing the doses of the test antibiotic. The plasma protein binding participated in extending the *Myxopyronin A* duration of action. The major protein binding for *Myxopyronin A* and *Rifampicin* was noticed to be albumin. The unbound fraction was detected to be responsible for the therapeutic activity.

The assessment of *Myxopyronin A's* impact on microbial *mRNA* production is shown in Figure 6. An increase in the dosage of the antibiotic *Myxopyronin A* was shown to cause a commensurate decrease in mRNA production. The antibiotic *Myxopyronin A* is secreted by Myxococcus fulvus colonies on *CYP isolation plates*, as seen in Figure 12. The quantal dosage response curve for *Myxopyronin A's* toxokinetics is shown in Figure 10. It was discovered that *LD50%* was *200 mg/kg* and *LD 99%* was over 300 mg/kg. The main isolates of Gram-negative bacteria that produce the antibiotic *Myxopyronin A* are shown in Figure utilizing a stereomicroscope. The *AUC* of *Myxopyronin A* after intramuscular(*IM*) injection in stages *1/2* of clinical trials is displayed in Figure 8. The range of effective doses was *5–6 mg/kg* of body weight.

The beginning of the action was noted after a close *15 minutes*. It adhered to the kinetics of first order elimination. Figure 7 uses the *UV* spectrophotometer absorption at 205 nm to illustrate how *Myxopyronin A* affects protein production. As *Myxopyronin A* antibiotic dosages were increased, a significant reduction in protein synthesis was observed. The area under the curve(*AUC*) after oral *Myxopyronin A* treatment throughout phases *1/2* of clinical trials is displayed in Figure 9. The range of effective doses was *7-8 mg/kg* of body weight. The action started after over thirty minutes. It adhered to the kinetics of first order elimination.

Conclusion

Antibiotic resistance is a global challenge that the current study shows promise in solving. According to the findings of the current study, *Myxopyronin A*, which was isolated from the bacterial isolates *Myxococcus fulvus 124B02* that were collected from various soil environments in Egypt, exhibited significant antibiotic activity both in vitro and in vivo against a moderate range of pathogenic bacteria, particularly *G+ve* varieties. Future research is recommended to investigate pharmacological interactions of the synergism type between *Myxopyronin A* and different antibiotic classes.

Declarations

Author Contributions: The present study was completely achieved via the single author Dr. Mohammed Kassab, Faculty of Pharmacy, Cairo University, Egypt.

Funding: This study was funded by me.

Publication Consent: I, the undersigned, give my consent for the publication of identifiable details, which can include photographs, case history and details within the text("Material") to be published in the above journal and article.

Ethical Statement: All relevant institutional, national, and/or worldwide guidelines for the care and use of humans and animals were prioritized in the current survey. The Ethical Committee for Human and Animal Handling at Cairo University(*ECAHCU*), at the Faculty of Pharmacy, Cairo University, Egypt, approved all study procedures involving humans and animals by the recommendations of the Weatherall Report(approval number *T-11-3-2023*). The number of humans and animals included in the study, as well as their suffering, was

minimized at all costs. The randomized human clinical trials phase *1/2* registration number was *NCT00000714/ 2023*.

Data Availability Statement: These are generated by the author Dr. Mohammed Kassab, Faculty of Pharmacy, Cairo University, Cairo, Egypt. All data and materials and their sources were included inside the manuscript.

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Conflicts of Interests: There is no conflict of interest.

Abbreviations

IM: Intramuscular, IV: Intravenous SC: Subcutaneous, IP: Intraperitoneal, AUC: Area under the curve, G+ve: Gram positive, mg: Milligram, Kg: Kilogram, PB: protein binding.

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