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Screening and the Production of *Myxopyronin A* Antibiotic from different 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 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 *7-8 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. **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].

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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 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. 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*.

Instruments

Table 1. List of instruments.

Instrument	Model and manufacturer
Autoclaves	*Tomy, japan*

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Aerobic incubator	*Sanyo, Japan*
Digital balance	*Mettler Toledo, Switzerland*
Oven	*Binder, Germany*
Deep freezer -70 ^{0}C	*Artiko*
Refrigerator 5	*whirpool*
PH meter electrode	*Mettler-toledo,UK*
Deep freezer -20 ^{0}C	*whirlpool*
Gyrator shaker	*Corning gyratory 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:

The selective isolation of species of *Myxococcus fulvus 124B02* from different soil samples was directly achieved using dilution plating. The technique comprised the suppression of competing bacteria exploiting antibiotics such as *10 mcg/ ml Vancomycin* and/ or *10 mcg/ ml Chloramphenicol* combined with wet heat treatment of soils and air drying. Fungi were eliminated via supplementing the plating medium with *2 mcg/ ml Terbinafine HCl*. Swarming of *Myxococcus fulvus 124B02* colonies was controlled with *Casein Yeast Peptone*(*CYP*) plates incubated at *30℃* and *PH 7.2* for *5 days*. The composition of *CYP* plate included *0.4 % Peptone* from *Casein*, tryptically digested, *0.3 % CaCl2.2H2O*, *0.1 % MgSO4.7H2O*, *PH 7.2*. The potent bacterial isolate producing Myxopyronin was performed utilizing *16 S rRNA* sequencing technique. The predominant bacterial isolate with high antibacterial activity was identified using *16S rRNA* sequencing and other biochemical tests. Nucleic acid was extracted from a swab by bead-beating in a buffered solution containing Phenol, Chloroform and Isoamyl alcohol. Variable region of *16S rRNA* gene was then amplified from the resulting nucleic acid using *PCR*. The genomic *DNA* was extracted from *120 hours* cultured cells using a *DNA purification kit*[*PurreLink™ Genomic DNA Mini Kit with Catalog number: K182002* was purchased from Invitrogen, USA] according to the protocol provided by the manufacturer of *DNA* purification kit. *The 16S rRNA gene* was amplified by *PCR*[*PCR* SuperMix kit was purchased from Invitrogen,*USA*] using forward[*5-AGAGTTTGATCCTGGCTCAG-3-*] and reverse[*5-GGTTACCTTGTTACGACTT-3-*] *primers*. *PCR* amplicons from up to hundreds of samples were then combined and sequenced on a single run. The resulting sequences were matched to a reference database to determine relative bacterial abundances. Polymerase Chain Reaction (*PCR*) was a powerful method for amplifying particular segments of *DNA*. *PCR* used the enzyme *Platinum™ Taq DNA polymerase* with catalog number *10966018*[purchased from Invitrogen, *USA*] that directed the synthesis of *DNA* from deoxynucleotide substrates on a single-stranded *DNA template*. *DNA polymerase* added nucleotides to the 3` end of a custom-designed oligonucleotide when it was annealed to a longer template *DNA*. Thus, if a synthetic oligonucleotide was annealed to a single-stranded template that contained a region complementary to the oligonucleotide, *DNA polymerase* could use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded *DNA*. Denaturation was the initial *PCR* cycle stage The *DNA* template was heated to *94° C*. This broke down the weak hydrogen bonds that held *DNA* strands together in a helix, allowing the strands to separate creating single stranded *DNA*. Annealing was the second *PCR* cycle. The mixture was cooled to anywhere from *50-70° C*. This allowed the primers to bind (anneal) to their complementary sequence in the template *DNA*. Extension was the final step of *PCR* cycle. The reaction was ; then heated up to *72° C*, the optimal temperature for *DNA polymerase* to act. *DNA polymerase* extended the primers, adding nucleotides onto the primer in a sequential manner, using the target *DNA* as a template. *With one cycle, a single segment of double-stranded DNA template was amplified into two separate pieces of double-stranded DNA.*

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These two pieces were then available for amplification in the next cycle. As the cycles were repeated, more and more copies were generated and the number of copies of the template was increased exponentially. The amplified *PCR* product was sequenced using a genetic analyzer *3130XL*[purchased from Applied biosystems, *USA*]. *DNA* sequence homology search analysis of the predominant bacterial isolate was achieved using Blastn algorithm at *NCBI* website. Fruiting bodies were examined using a *Stereomicroscope*(*dissecting microscope*) *MSC-ST45T*(purchased from Infetik, China). Wet mounts from crushed fruiting bodies were prepared. The refaractility, shape and the size of Myxospores were determined victimizing phase contrast microscopy. On the other hand the plates were exposed to *360 nm* wavelength ultraviolet light to assess the fruiting bodies fluoresced [16].

Identification *Myxopyronin A* producing bacterial isolates:

Gram stain:

It classified bacteria into two categories based on the makeup of their cell walls. The bacterial cells became purple after being treated with a solution of crystal violet and subsequently iodine on a microscope slide. When colored cells were treated with a solvent such as alcohol or acetone, gram-positive organisms kept the stain whereas gram-negative organisms lost the stain and turned colorless. With the addition of the counter-stain safranin, the clear, gram-negative bacteria became pink [17].

Spore shape:

This was discovered using the spore staining method. To get rid of any fingerprints, the slide was wiped with alcohol and a Kim-wipe. On the bottom of the slide, a Sharpie was used to create two circles. Each circle was filled with two tiny droplets of water using an inoculation loop. A very small amount of germs was taken out of the culture tube using an aseptic method. The water droplet on the slide had microorganisms on it. The slide was thoroughly dried by air. Bypassing the slide through the flame three to four times with the smear side up, the slide was heat-fixed. It took a while for the slide to completely cool. A piece of paper towel placed inside the slide's border was used to hide the streaks. A beaker of heating water was situated over the slide. The slide was allowed to steam for three to five minutes; while the paper towel was covered with a malachite green liquid. Removed and thrown away was the discolored paper towel. To get rid of any stray paper towel bits, the slide was gently cleaned with water. The counter-stain was safranin for 1 minute. Before putting the slide on the microscope's stage and seeing it via the oil immersion lens, the slide's bottom was dried [18].

Spore site:

During the Gram stain test, the spore location was established [19].

Cell shape:

During the Gram stain test, the cell shape was assessed [20].

Blood haemolysis:

On blood agar media, the test antibiotic capacity to haemolyze the blood was tested [21].

Motility test:

It discriminated between motile bacteria and non-motile bacteria.

A sterile needle was used to penetrate the medium to within 1 cm of the tube's bottom to select a well-isolated colony and test for motility. The needle was certainly retained in the same position as it was inserted and removed from the medium. It took *18* hours of incubation at *35°C*, or until noticeable growth appeared [22].

Nitrate reduction test:

0.5 ml of nitrate broth was added in a clean test tube, was autoclaved for 15 minutes at *15 lbs pressure* and *121°C*, and was let to cool to room temperature. The tube was inoculated with a heavy inoculum of fresh bacterial culture and was incubated at *35°C* for *2 hours*. *2 drops* of reagent A and *2 drops* of reagent B were added and mixed well. The development of red color within *2 minutes* was observed for. If no red color was developed, a small amount of *zinc dust* was added and observed for the development of the red color within *5 minutes* [23].

Methyl red test:

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In the Methyl Red test, an infected tube of *MR* broth was used before adding the methyl red *PH* indicator. The buffers in the medium were overcome by the acids when an organism used the mixed acid fermentation pathway and produced stable acidic end products, resulting in an acidic environment [24].

Catalase test:

A little inoculum of a specific bacterial strain was introduced to a *3%* hydrogen peroxide solution to see if it might produce catalase. It was observed for the rapid emergence of oxygen bubbles [25].

Oxidase test:

The 1% Kovács oxidase reagent was applied to a tiny piece of filter paper, which was then allowed to air dry. A well-isolated colony was taken from a fresh (*18 to 24-hour culture*) bacterial plate using a sterile loop, and it was then rubbed onto prepared filter paper. Color alterations were noticed [26].

Citrate utilization:

Five milliliters of a Simmon Koser's citrate medium were taken after it had been autoclaved at *15* pounds for *15 minutes*. To create a clear slant and butt, the test tube containing melted citrate medium was slanted. Using sterilized wire and labeled tubes, the specified samples of microbe were injected on the media's incline. For *24 hours*, the tubes were incubated at *37°C*. The medium's color shift was watched for [27].

Starch hydrolysis:

For *48 hours* at *37°C*, the bacterium plates were injected. After incubation, a dropper was used to saturate the surface of the plates with an iodine solution for *30 seconds*. Iodine that was in excess was afterward poured out. The area surrounding the bacterial growth line was looked at [28].

Tween 80 hydrolysis:

1% Tween 80 was used to create agar media. The supplied microorganism was added to the *Tween 80 agar plates* by utilizing an inoculating loop to create a single center streak in the plate. The plates were incubated for *24* hours at *37 °C*. *HgCl2* solution was poured over the plates. After a short while, the plates were examined. Positive test result; distinct halo-zone surrounding the injected region showed *Tween 80* hydrolysis [29].

Growth at 10-45 ^{0}C:

On nutrient agar media, growth was observed to be possible at *45°C* [30].

Indol test:

The test tube containing the microorganism for inoculation received 5 drops of the Kovács reagent directly. Within seconds after introducing the reagent to the media, the reagent layer formed a pink to red colour (cherry-red ring), which was a sign of a positive indol test [31].

Tolerance salinity test:

Its capacity to develop on nutrient agar while being responsive to 5% and *7 % NaCl* was examined [32].

Voges-Proskauer(VP) test:

For the test, Voges-Proskauer broth, a glucose-phosphate broth loaded with microorganisms, was added to alpha-naphthol and potassium hydroxide. A successful outcome was indicated by a cherry red tint, whereas an unfortunate outcome was indicated by a yellow-brown color [33].

Casein hydrolysis test:

For testing the casein hydrolyzing activity of the test antibiotic, a single line streak of the given culture was made in the center of the skim milk agar plate under aseptic conditions and plate was incubated at *37°C* in an incubator for *24-48 h* [34].

Saccharide fermentation tests:

Glucose fermentation test:

The fermentation reactions of glucose were investigated using glucose purple broth. Peptone and the *PH* indicator bromcresol purple made up the purple broth. A *1%* concentration of glucose was added. Isolated colonies from a 24-hour pure culture of microorganisms were added to the glucose purple broth as an inoculant. Parallel to the inoculation of the glucose-based medium, a control tube of purple broth base was used. The inoculated medium was incubated aerobically for *3*

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days at a temperature of *35–37* ℃. The medium began to become yellow, which was a sign of a successful outcome. A poor carbohydrate fermentation response was indicated by the lack of yellow color development [35].

Fructose fermentation test:

A pure culture's inoculum was aseptically transferred to a sterile tube of phenol red fructose broth. The infected tube was incubated for *18–24 hours* at *35–37* ℃. A color shift from red to yellow, signifying an acidic PH alteration, was a sign of a favorable response [36].

Maltose fermentation test:

A pure culture inoculum was aseptically transferred to a sterile tube containing phenol red maltose broth. The infected tube was incubated for *18–24 hours* at *35–37* ℃. A color shift from red to yellow, signifying an acidic PH alteration, was a sign of a favorable response [37].

Sucrose fermentation test:

A pure culture's inoculum was aseptically transferred to a sterile tube containing phenol red sucrose broth. For *24 hours*, the infected tube was incubated at *35–37* ^{0}*C*. A colour shift from red to yellow, signifying an acidic *PH* alteration, was a sign of a favourable response [38].

Purification of *Myxopyronin A* antibiotic:

This was achieved through reversed phase chromatography technique.

The aeration rate was *0.142 V/ V. min.* The stirring rate was *500 rpm*. *PO2* was about *90 %* of saturation; but decreased to about *20 %* after *18 hours*). The fermentation was stopped after *40 hours* via centrifugation at *500 rpm* in a gyrator shaker. The supernatants were collected; then tested for antimicrobial sensitivity using broth dilution technique to detect MICs and agar paper diffusion discs technique. The test antibiotic was extracted from the *2 liters* of culture broth with *2/ 10 volume* ethyl acetate. The ethyl acetate was then removed under the reduced pressure at *40℃*. Afterwards, the residue was dissolved in 398 ml of methanol-water(*90: 10*) and chromatographed on reversed phase *HPLC*. Methanol was the mobile phase. The eluent was *70* part *methanol*: *16* part *water*: *4* part *acetic acid* with flow rate *300 ml/ min*. Detection of the antibiotic components was achieved exploiting refractive index. The main peak with retention time *5 minutes* contained the biological antibiotic activity which was determined via agar diffusion assay using paper discs and *Staphylococcus aureus* as an indicator organism. On the other hand, the main peak was subjected to neutralization via *NaHCO3*. Myxopyronin A was extracted using *10 % V/ V Ethylene chloride*. After the evaporation of the solvent, about *85 %* of the remaining antibiotic substance was pure. It was noticed that the retention rime of *Myxopyronin A* was *11 minutes*. Molecular formula of the purified *Myxopyronin A* was detected through mass spectrometer(Quadrupole mass spectrometer, Advion, USA) [39].

Procedure of *Broth dilution assay* for determination of *MICs* of *Myxopyronin A*:

During testing, multiple microtiter plates were filled with a certain broth, according to the needs of target bacteria. Varying concentrations of the antibiotics and the bacteria to be tested were then added to the plate. The plate was then placed into a non-*CO2* incubator and incubated at thirty-seven degrees Celsius for sixteen to twenty hours. Following the allotted time, the plate was removed and checked for bacterial growth. When the broth became cloudy, bacterial growth occurred. The results of the broth microdilution method were reported in Minimum Inhibitory Concentration(*MIC*), or the lowest concentration of antibiotics that stopped bacterial expansion [40].

Agar diffusion assay with *paper discs* procedure for the determination of *Myxopyronin A* antimicrobial activity:

The disk diffusion method(*DDM*) was classified as an agar diffusion method(*ADM*) because the test antibiotic extract to be tested diffused from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir was a filter paper disk, which was placed on top of an agar surface. When tested extracts compounds were microbiologically active, an inhibition zone developed around the filter paper disk after incubation. The diameter of the inhibition zone properly described the antimicrobial potency of test extract [41]. The test microbes were isolated using either selective or enrich growth media or broth(Table 2).

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.

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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)
Sacchromyces 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
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:

The concentration of *RNA* isolated with *RNeasy Kits*(purchased from QIAGEN, USA) was determined by measuring the absorbance at *260 nm* in a spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 40 µg of *RNA* per ml($A260 = 1 = 40\ \mu g/\ ml$) [42].

Estimation of *Myxopyronin A* effect on bacterial protein synthesis:

Absorbance was measured at *205 nm* to calculate the protein concentration by comparison with a standard curve. A(*205*) method could be used to quantify total protein in crude lysates and purified or partially purified protein. The *UV* spectrophotometer was set to read at *205 nm* allowing *15 min* for the instrument to equilibrate. The absorbance reading was set to zero with a solution of the buffer and all components except the protein present. The protein solution was placed in the 1 mL cuvette and the absorbance was determined. The dilution and readings of samples were performed in duplicate.The matched cuvettes for samples and controls were utilized during the test procedure. The extinction coefficient of the protein was known, the following equation was employed. $Absorbance = Extinction\ \ coefficient \times concentration\ \ of\ \ protein \times path\ \ length(\ 1\ \ cm)$ to determine the concentration of the protein [43].

Estimation of pharmacodynamic and pharmacokinetic effects of *Myxopyronin A* during experimental animal testing in preclinical clinical trials:

In the present study, the pharmacokinetics and the pharmacodynamics of *Myxopyronin A* were evaluated after dosing in male rabbit animal models weighing about 2 kg. Furthermore, compound concentrations were determined in target compartments, such as lung, kidney and thigh tissue, using

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LC-MS/ MS. Based on the pharmacokinetic results, the pharmacodynamic profile of *Myxopyronin A* was assessed victimizing the standard neutropenic thigh and lung infection models [44].

Estimation of pharmacodynamic and pharmacokinetic effects of Myxopyronin A in randomized human clinical trials phases 1/2:

This study was conducted in *150* human volunteer subjects to show the bioavailability, pharmacokinetics and the pharmacodynamics of the test antibiotic. The study was designed as randomized, single-dose, *2*-treatment, *2*-period crossover trial with a washout period of *1 week*. Blood samples were collected at *0*(*baseline*), *10*, *20*, and *40 minutes* and at *1*, *1.5*, *2*, *3*, *4*, *6*, *9*, *12*, and *24 hours* postdose. Plasma concentrations of the 4 drugs were measured by using a rapid chromatography-tandem mass spectrometry method. Pharmacokinetic parameters were calculated by using noncompartmental methods. Bioequivalence was determined if the *90 % CIs* of the log-transformed test/ reference ratios *AUC*(*0-24*), *AUC*(*0-∞*), and *Cmax* were within the predetermined range of *80%* to *125%*. Tolerability was assessed by using clinical parameters and subject reports Pharmacodynamic effects were evaluated through the determination of *MICs* via agar diffusion assay and broth dilution technique During randomized human clinical trials phases 1/2 all utilized infectious bacterial cell counts were estimated spectrophotometrically [45].

Estimation of of phototoxicity, mutagenicity and carcinogenicity of the test antibiotic:

The phototoxicity was determined via *3T3* neutral red uptake phototoxicity technique [46]. On the other hand, mutagenicity and carcinogenicity of the test antibiotic were assessed using Ames test [47].

The determination of toxokinetics and toxodynamic effects:

Up and down method for acute toxicity detection of *Myxopyronin A* was utilized for this purpose [48].

The determination of maximum bactericidal activity of *Myxopyronin A*:

A pure culture of a specified microorganism was grown overnight, then diluted in growth-supporting broth(typically *Mueller Hinton Broth*) to a concentration between *1 x 10^5* and *1 x 10^6 cfu/ ml*. A stock dilution of the antimicrobial test substance was made at approximately *100* times the expected *MIC*. Further *1:1* dilutions were made in test tubes. All dilutions of the test antibiotic were inoculated with equal volumes of the specified microorganism. A positive and negative control tube was included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period and media sterility, respectively. An aliquot of the positive control was plated and used to establish a baseline concentration of the microorganism used.The tubes were then incubated at the appropriate temperature and duration. Turbidity indicated growth of the microorganism and the *MIC* was the lowest concentration where no growth was visually observed. To determine the *MBC*, the dilution representing the *MIC* and at least two of the more concentrated test product dilutions were plated and enumerated to determine viable *CFU/ ml*. The *MBC* was the lowest concentration that demonstrated a pre-determined reduction (such as 99.9%) in *CFU/ ml* when compared to the *MIC* dilution [49].

Determination of plasma protein binding capacity of *Myxopyronin A*:

Victimizing of an ultrafiltration technique, the protein binding(PB) extent and changeability of the test antibiotic medicates were settled when given simultaneously to *30 patients* inoculated with infectious *pneumococci* inside hospitals in Egypt. Clinical samples used were routinely received by microbiological laboratory inside the faculty of Pharmacy, Cairo University, Egypt. Plasma proteins were likewise plumbed. A protein-free medium was used to determine the nonspecific binding. Plasma samples from *30* patients were enclosed, of which plasma proteins were deliberated for *24 patients*.

Determination of liver, kidney and heart function tests:

These functional tests were performed to assess the vitality of liver, kidney and heart during the randomized human clinical trials phases 1/2. On the other hand, Urine, stool analyses were achieved in addition to estimation of blood complete counts to all experimental subjects received graded doses of *Myxopyronin A*.

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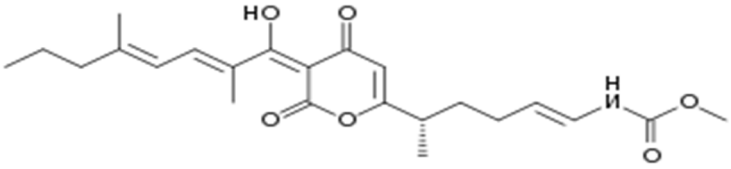

Figure 1. It demonstrates the structure of *Myxopyronin A* extracted from bacterial isolates *Myxococcus fulvus strain Mx f50* collected from different soil environments in Egypt. Molecular formula of the purified test antibiotic was noticed to be $C_{23}H_{31}NO_6$ determined through mass spectrometer.

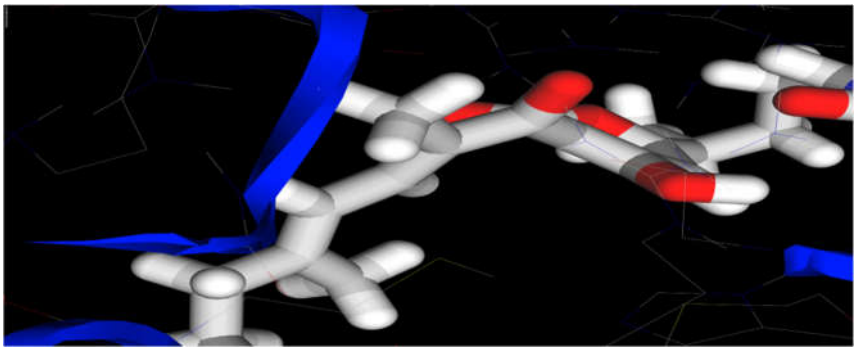

Figure 2. It represents docking of Myxopyronin A ligand on Bacterial *RNA* polymerase. Myxopyronin showed high affinity and inhibitory effect towards the switch region.

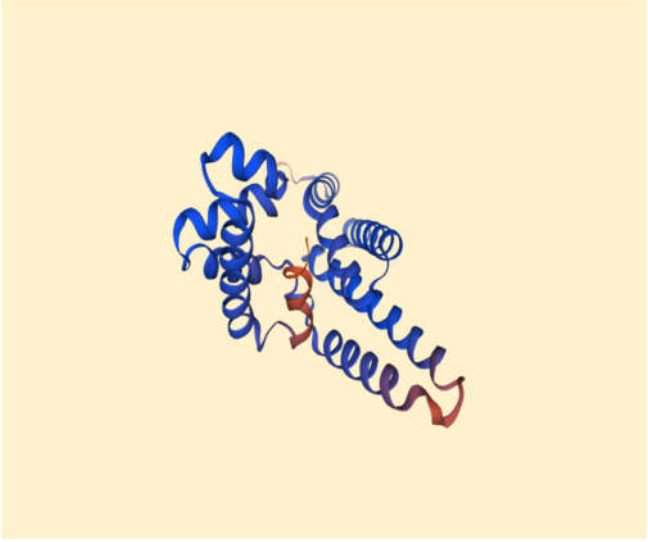

Figure 3. It demonstrates *3D* structure of bacterial prokaryotic *RNA polymerase* comprising the switch binding site to which *Myxopyronin A Ligand* strongly bound inhibiting bacterial *RNA polymerase* activity selectively leading to the inhibition of *mRNA* transcription and subsequently the mortality 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*.

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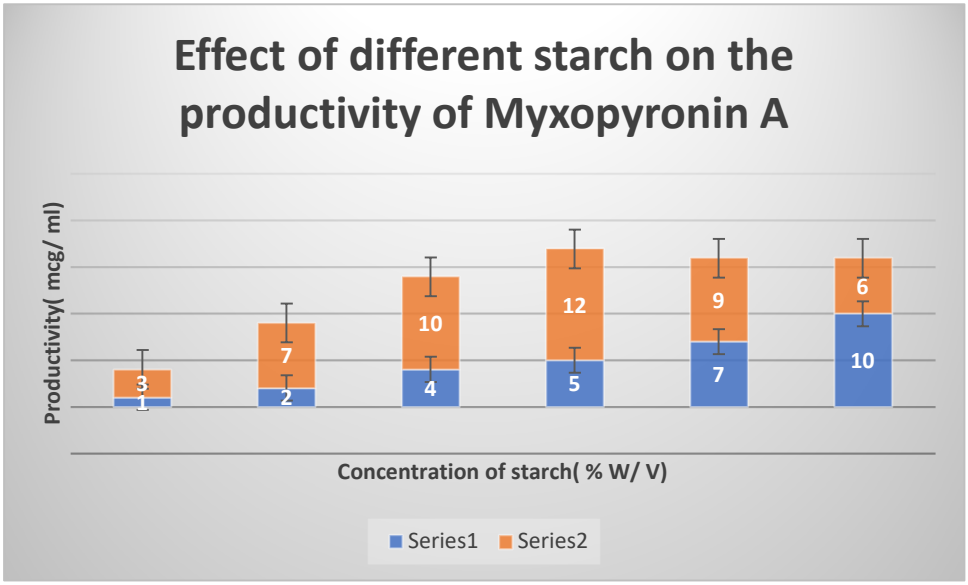

Figure 4. It shows the impact of various concentrations of *Soluble starch* on the production of *Myxopyronin A*.

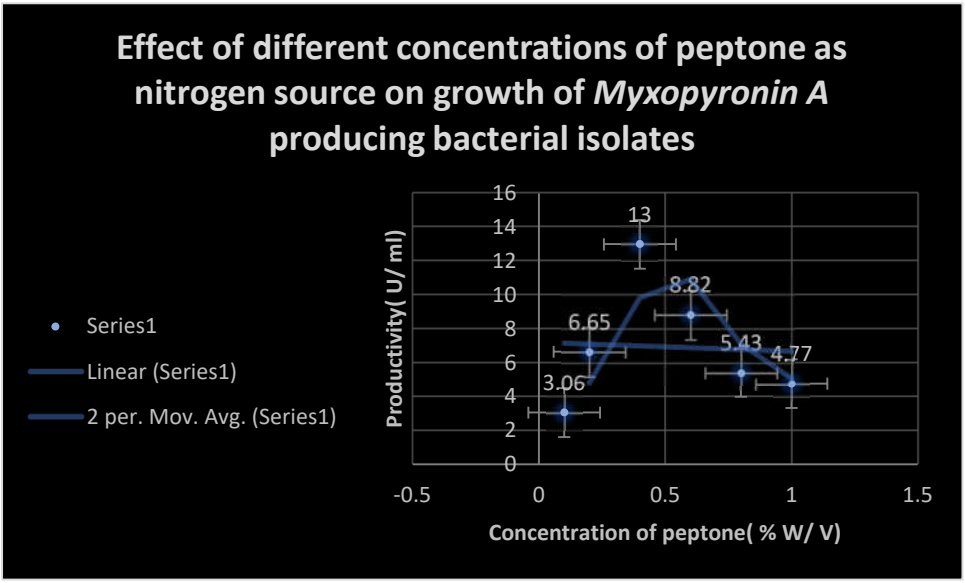

Figure 5. It shows the effects0 of different Peptone concentrations as nitrogen growth factor on the productivity of *Myxopyronin A*.

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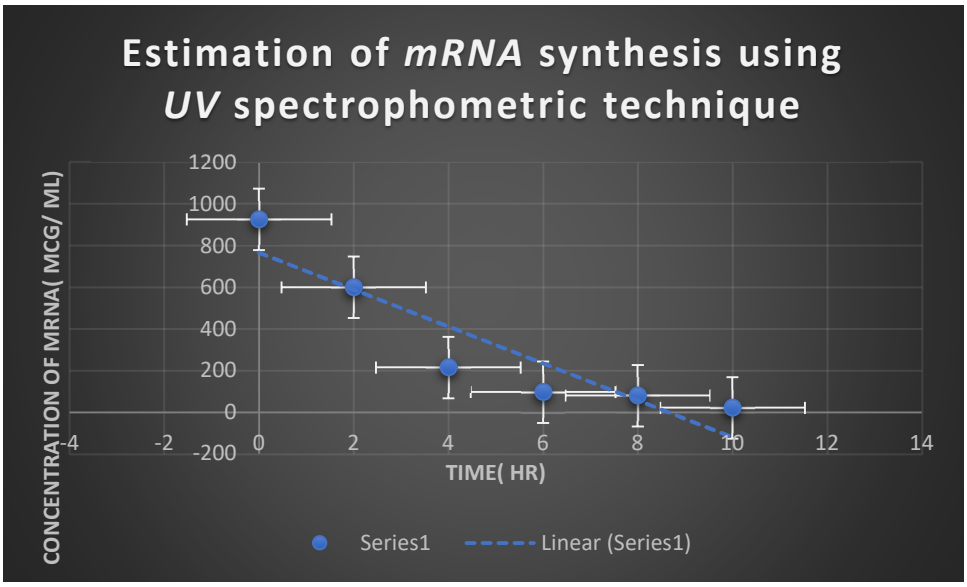

Figure 6. It refers to the estimation of effect of *Myxopyronin A* on microbial *mRNA* productivity. *mRNA* synthesis was detected to be diminished proportionately up on employment of exploding doses of *Myxopyronin A* antibiotic.

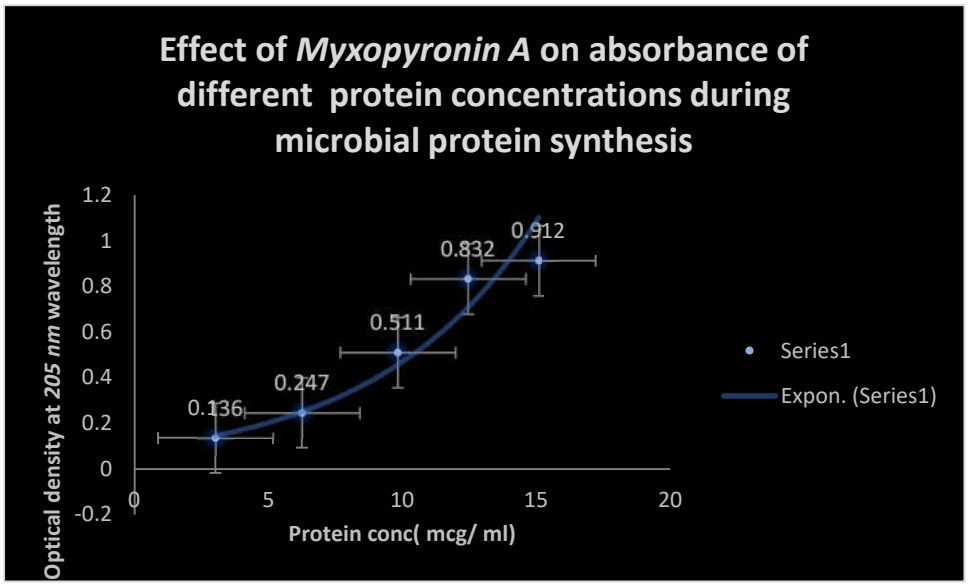

Figure 7. It demonstrates the influence of *Myxopyronin A* on protein synthesis using *UV* spectrophotometer absorption at *205 nm*. Protein synthesis was noticed to be decreased dramatically up on utilization of increasing doses of *Myxopyronin A* antibiotic.

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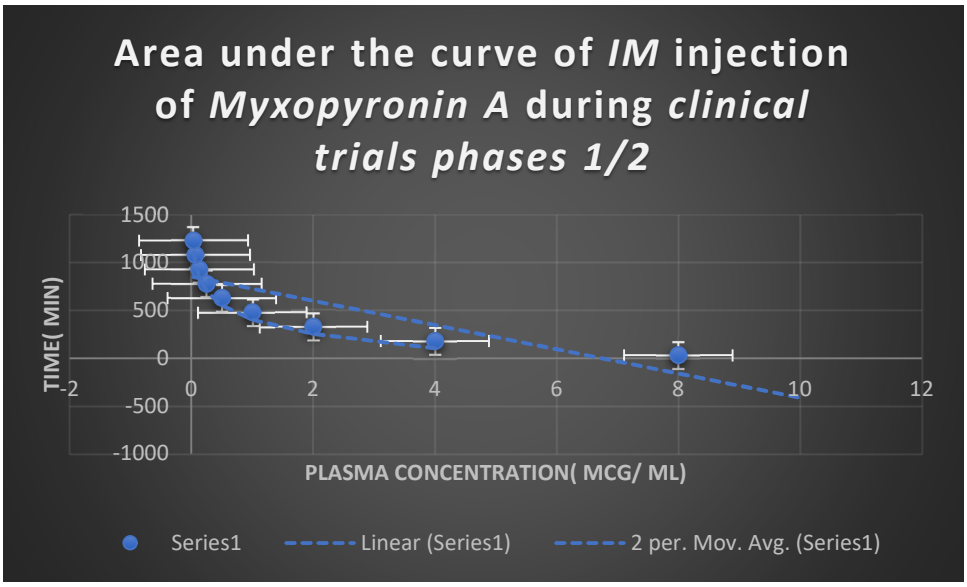

Figure 8. It shows *AUC* of *Myxopyronin A* following *IM administration in clinical trials stages 1/2*. Efficacious dose ranged from *5-6 mg/ kg* of body weight. Onset of action was observed following closely 15 *minutes*. It followed first order of elimination kinetics.

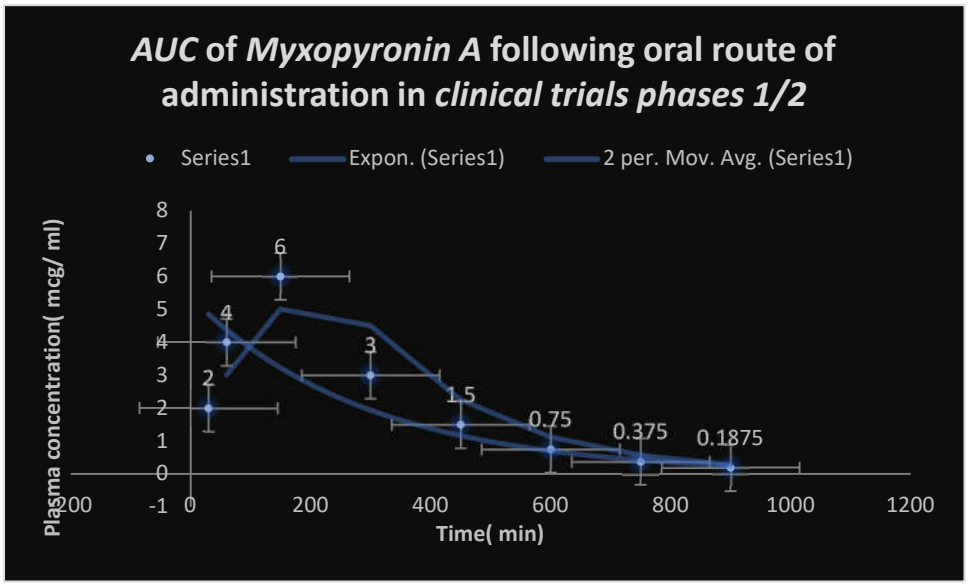

Figure 9. Area under the curve(*AUC*) following oral administration of *Myxopyronin A* during *clinical trials phases 1/2*. Efficacious dose ranged from *7-8 mg/ kg* of body weight. Onset of action was observed following nearly *30 minutes*. It followed first order of elimination kinetics.

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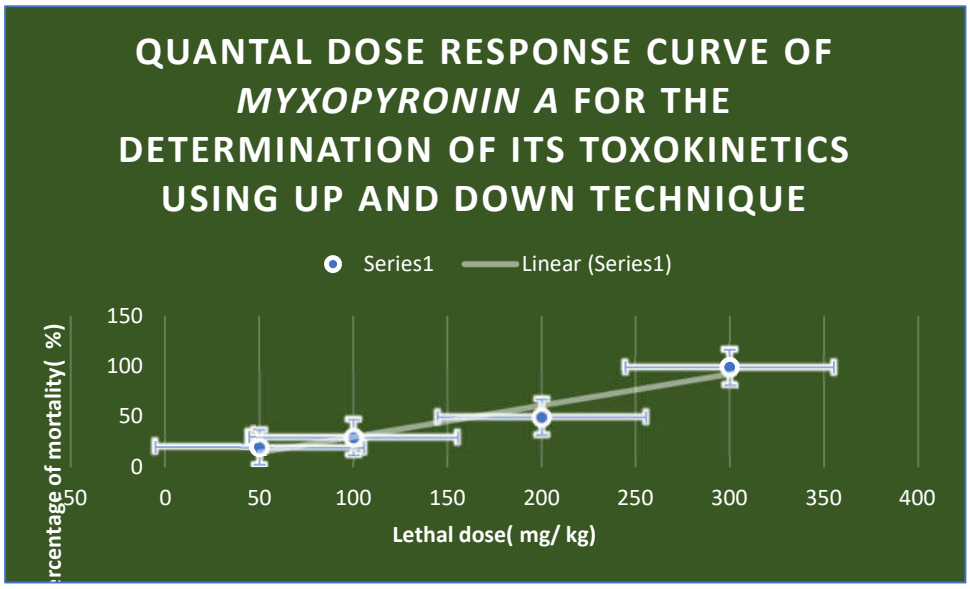

Figure 10. Quantal dose response curve for the determination of toxokinetics of *Myxopyronin A*. LD_{50} % was found to be *200 mg / kg;* while *LD99 %* was nearly *300 mg/ kg*.

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.

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

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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 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.

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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 [2]	*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
Shigella dysentery	113	7
Proteus mirabilis	139	6

[2] 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)*

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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

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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 n*m**
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

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 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 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	-

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Methyl red	-
Nitrate reduction test	+
Vogues proscauer	-
Citrate utilization	-
Starch hydrolysis	+
Casein hydrolysis	+
Growth at 45 ℃	Bacterial isolates did not grow at 45 ℃; but were grown at 10-37 ℃
Tween 80	+
Tolerance salinity	
5% NaCl	-
7% NaCl	-
Saccharide fermentation	
Glucose	-
Fructose	-
Maltose	-
Sucrose	-

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(

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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 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 *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 *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 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 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.

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.

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