Antimicrobial Property of Agapanthus Praecox

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ABSTRACT: Antibiotic resistance is serious global problem. Many commercial antibiotics due to over use have induced some microbes to become multi drug resistant. Polymyxins are important bactericidal agents towards gram (G-) negative bacteria. Resistance towards polymyxin includes factors like intrinsic, adaptive & mutational modes. Thereby many researchers are gravitating towards searching for novel anti-microbial agents from the natural world. In this paper, the resistance towards polymixins have been briefly stressed upon along with the evaluation of the anti-microbial role of Agapanthus praecox towards a set of bacteria. Bacteria, Klebseilla & Bacillus subtilis were grown as a lawn culture that was then exposed to varying concentrations of butanolic & aqueous extracts. After 24 hour, the butanolic extract showed higher activity as compared to the aqueous extracts. However, Colistin showed the highest activity. This result has given hope that novel anti-microbial agents can be isolated from this plant.

KEYWORDS: Ampicillin, Agapanthus Praecox, Anti-Microbial Resistance, Anti-Microbial Agents, Aqueous Extract, Butanolic Extract.

1. INTRODUCTION

Constantly rising resistance to antibiotic is a global problem, especially, major infections due to resistant bacteria & the absence of novel antibiotics towards gram negative (G-) bacteria, have revived the use of old antibiotics. In this regard, though colistin is being reintroduced, this has led to the rise of colistin-resistant bacteria. Polymyxins are a group of antibacterial cyclic oligopeptides synthesized by gram-positive (G+) bacteria; *Bacillus polymyxa* out of which polymyxin B & polymyxin E (colistin) are used clinically. They act both on both the outer & on the inner part of the cell membrane, causing loss of membrane integrity. However, resistance to polymixins have also started to being reported. In this paper, briefly the mode of polymixin resistance has been described and also the anti-microbial role of the plant, *Agapanthus praecox* has been evaluated on a set of bacteria [1], [2].

1.1 Reasons for polymyxin resistance

There are many known reasons for resistance that includes intrinsic, mutational and adaptive, among others including (i) changes in the moiety of the lipopolysaccharide (LPS), causing a reduction in the total negative charge of LPS; (ii) genetic mutations (iii) increase in drug efflux; (iv) reduction in the porin route, (v) synthesis of capsules (vi) enzymatic inhibition of the antibiotic (say colistin) [3], [4]. Due to the onsent of resistance towards polymixins the researchers are now searching for novel sources of anti-microbial agents. In table 1, the methods employed by the bacteria for polymyxin resistance has been tabulated. In figure 1, the polymixin resistance in bacteria by the usage of the 2 component system has been illustrated.

Table 1. Methods employed by bacteria in order to acquire polymyxin resistance. Polymixin like colistin is secreted by the Gram (G+) bacteria but nowadays resistance towards colistin is being noted in other bacteria [1].

Resistance mechanism		**Bacteria**	**Genes involved**
Electrostatic repulsion	L-Ara4N & PETn modification of lipid A	E.coli, Salmonella enterica	pmrAB, mcr
	Activation of LPS modifying operon by mutations in the 2 component system	Klebseilla	crrB(ccrC)
	D-Ala modification of lipoteichoic acid	Staphylococcus aureus	ciaR

	Unclear	Salmonella enterica	Outer membrane protein
Membrane fluidity/permeability	Acylation of lipid A	Vibrio cholerae	Rcp, pagP
	Putative hopanoid	Pseudomonas aeruginosa	B_mull 2133
Remodelling of surface membrane	Capsule production	Salmonella enterica	siaD, rcs
	Changes in membrane compositions	Salmonella enterica	virB, rcs
	Changes in membrane integrity	Acinetobacter	Cas9, Lol
	LPS & LOS modifications	spgM, firA	Vibrio fisceri
	Loss of LPS	Acinetobacter baumanii	iptD
Transport-Efflux	Efflux	Pseudomonas aeruginosa	Mex pumps
	Transport	Vibrio vulnificus	trkA
Other polymixin resistance factors		Thermus thermophiles, Proteus mirabilis	bacA, mucD

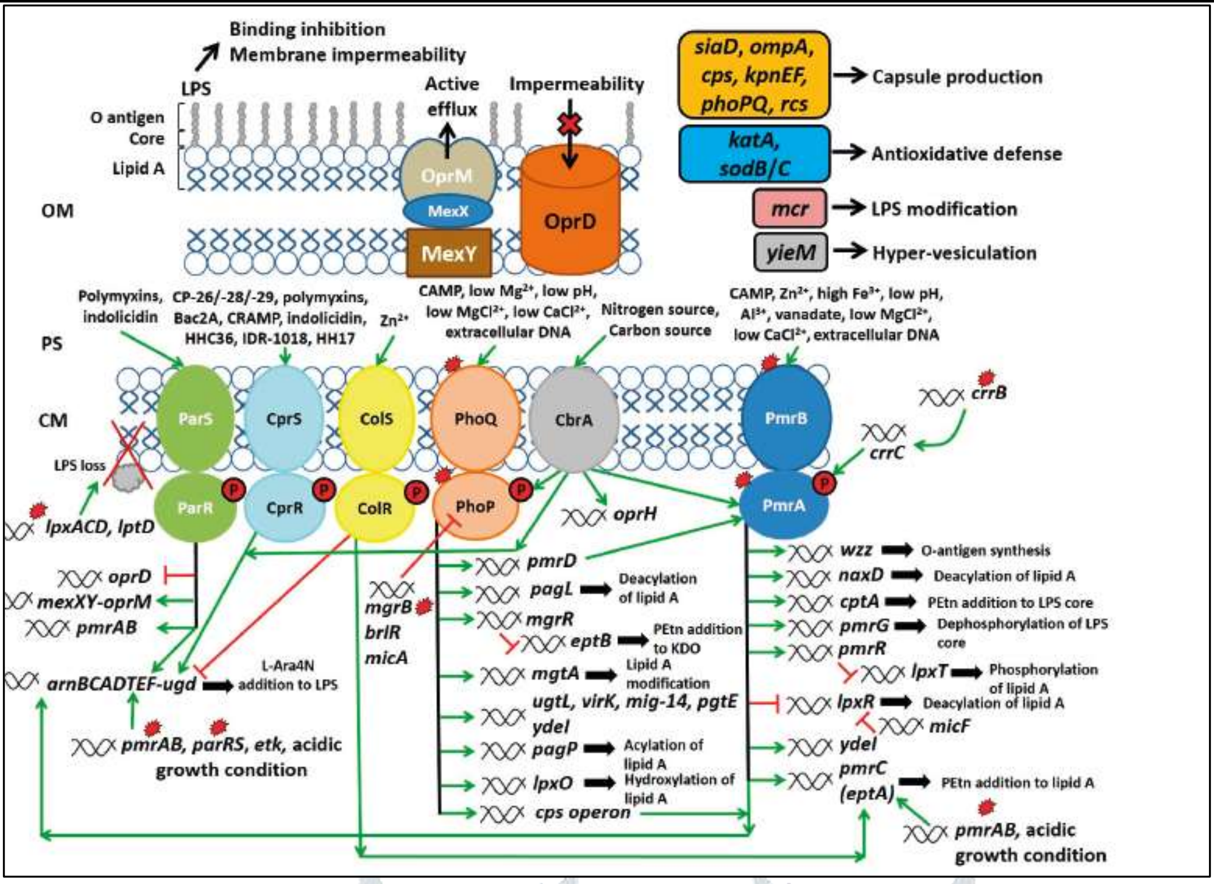

Figure1. Proposed polymyxin resistance mechanisms in bacteria.

ColR-ColS, PhoP-PhoQ, CprS-CprR, ParS-ParR, PmrA-PmrB & CbrA-CbrB are the 2-component systems that confer polymyxin resistance in bacteria. Sketch of a gram (G-) envelope that indicates inner & outer layer divided by a periplasm (The OM denotes outer membrane; the PS denotes periplasmic space; the CM denotes cytoplasmic membrane). The outer membrane's outer layer has lipopolysaccharide (LPS) that is fixed on the membrane via LPS lipid A[5]. The outer membrane's inner layer along with the whole of the inner membrane comprises only of the phospholipids & the 2 bilayers have a variety of forms of the membrane proteins Figure 1.

2. LITERATURE REVIEW

Agapanthus praecox is a medicinal plant member of the family Amaryllidaceae. However, the genus Agapanthus is difficult to group into definite species owing to its similar morphology present in its members. The leaf and the rhizome's epidermal cells are polygonal possessing curling anticlinal walls having average adaxial length is 71 micrometres & the average abaxial length is 76 micrometres. The leaves are amphistomatic having anomocytic stomata having average length of pore as 23 micrometres of the adaxial surface & 16 micrometres of the abaxial surface. The average densities of stomata on both the adaxial & abaxial surfaces were 279 $milimeter^2$ & 349 $milimeter^2$. Trichomes with secretory ducts are not found on both the surfaces. It has been reported that beryllium, oxygen, carbon, sodium, & silicon are found on both the epidermal surfaces & the rhizome while on the adaxial surface, nitrogen, chlorine & aluminium have been reported only. In Table 2, the percentages of various elements found in this plant have been discussed [6], [7].

Medicinal plants provide excellent reservoir of medicines regarding various diseases. Easy availability, with lower side effects has been associated with traditional medicine. Unfortunately, species misidentification is a major issue. The replacement of medicinal plants with nearly identical species evokes the issue of contamination in herbal based products that could be risky to the end users. The near similar morphological features within genus Agapanthus has led to replacement of *A. praecox* with other nearly similar species resulting in contamination of the medicinal plant products. The many medicinal roles of Agapanthus praecox make its proper identification a necessity.

Micromorphological features studies are important tools for plant nomenclature. Trichomes are epidermal limbs which vary from plants to plants; the morphological variation and spread have come up as useful taxonomic tools for plant species identification. Stomata which are features of the epidermis are found in mostly different regions of the plants. Differences in stomata features are taxonomically important features which have been identified as species particular and are utilized as important yardsticks in plant taxonomy.

Epidermal Leaf Cells: They have polygonal shape having curling anticlinal walls on both the abaxial and adaxial regions. They were larger on the abaxial region as compared to the adaxial surface. Stomata are found on these regions but trichomes along with secretory ducts are not found. The average adaxial cell epidermal length is 79 micrometres and the average adaxial cell breadth is 20 micrometres while the average abaxial length is 84 micrometres and the average width is 18 micrometres.

Stomata: Both the Adaxial and abaxial regions have features of anomocytic stomata that are more prominent on the abaxial rather than on the adaxial region. The stomata donot have subsidiary cells and are amphistomatically found on both regions. They are haphazardly found on both regions. The stomata are completely opened on both the regions.

Agapanthus praecox is popularly called as agapanthus or lily of the Nile comprises of 3 subspecies namely subspecies: praecox, subspecies: orientalis, & subspecies: Minimus. In the traditional medicines of South Africa, this plant is used for fertility & pregnancy & for treating heart related diseases, chest illness etc. The plant's root's decoction has antidiarrhoeal roles in goats & sheep. It has been reported to have anti-inflammatory, anticancer, antimicrobial roles etc. [8]–[10]. Based on these reports of its traditional usages, the anti-microbial role of this plant was further investigated.

3. METHODOLOGY

Experiment design: Firstly, roots of the plant *Agapanthus praecox* would be collected & their extracts would be prepared & bacterial strains would be treated with them for evaluating its anti-microbial roles.

3.1 Collection and preparation of plant material:

The plant *Agapanthus praecox* was collected from the local nursery & a voucher specimen was submitted to the institute. The roots were removed, dried, pulverized into powder & their respective butanolic & aqueous extracts were prepared. These extracts were then suspended in DMSO at room temperature for further use.

3.2 Antibacterial efficacy:

The antibacterial roles of the extracts were evaluated against *Klebsiella* & *Bacillus subtilis* by using the agar well diffusion assay.

3.3 Method:

Firstly, Luria agar media was prepared & poured onto the plates. Once the media in the plates got solidified, specific pure bacterial lawn culture was established, following which wells were punched into it via a 1mililiter sterile tip. Nearly 50 microliters of each solvent extract (0, 1, 2.5, 5, 7.5 & 10 µg/ml) was separately poured into each well. Blank well having DMSO (0, 1, 2.5, 5, 7.5 & 10 µl) was noted as negative (-ve) control & wells having Colistin (0, 1, 2.5, 5, 7.5 & 10 µg/ml) as positive (+ve) control. Following incubation for 24 hours & at 37°Centrigrade, inhibition was observed by the measurement of the zone of inhibition's diameter. The experiments were repeated thrice.

4. RESULTS AND DISCUSSION

The anti-bacterial role of the root extracts of the plant *Agapanthus praecox* was evaluated on 2 bacterial species. The root extract of the butanolic extract (10 µg/ml) was found to be highly active against *Klebsiella* & *Bacillus subtilis* as compared to the aqueous extract (10 µg/ml) towards the same bacterial species. The DMSO had minimal efficacy towards all the 2 bacterial species studied. The known antibiotic, Colistin (10 µg/ml) however showed excellent activity against all 2 bacterial species (Figure 6) (Table 3). The results indicate that this plant can be researched further for its anti-bacterial role.

Table3. Anti-bacterial role of the roots of *Agapanthus praecox* towards the bacterial species by the agar well assay. The root extract of the butanol (10 µg/ml) was found to be highly active against *Klebsiella* & *Bacillus subtilis* as compared to the aqueous extract (10 µg/ml) towards the same bacterial species.

Bacterial species	Zone of inhibition (mm)			
	DMSO	Aqueous extract	Butanolic extract	Colistin
Klebsiella	4	7	12	20
Bacillus subtilis	5	8	14	22

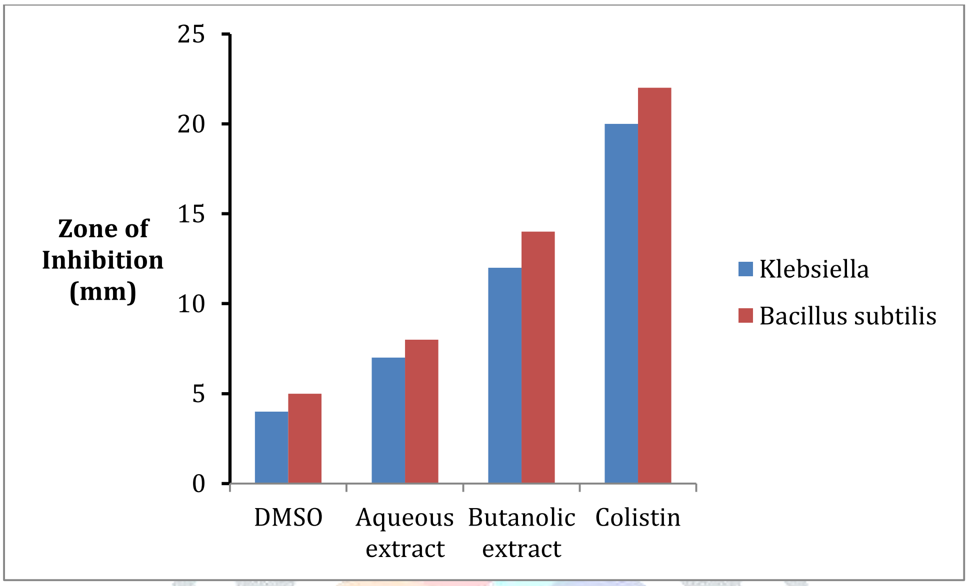

Figure6. Graphical representation of the zone of inhibition induced in both *Klebsiella* & *Bacillus subtilis*.

The positive (+) control, Colistin induced highest zone of inhibition for both the bacterial species, whereas among the extracts, the Butanolic extract showed higher inhibition as compared to the Aqueous extract while the negative (-) control showed the least inhibition.

5. CONCLUSION

Antibiotic resistance is serious global problem. Many commercial antibiotics due to over use have induced some microbes to become multi drug resistant. Polymyxins are important bactericidal agents towards gram (G-) negative bacteria. Resistance towards polymyxin includes factors like intrinsic, adaptive & mutational modes. Thereby many researchers are gravitating towards searching for novel anti-microbial agents from the natural world. In this paper, the resistance towards polymixins have been briefly stressed upon along with the evaluation of the anti-microbial role of *Agapanthus praecox* towards a set of bacteria. Firstly, roots of *Agapanthus praecox* were collected, washed, dried and Butanolic extracts & aqueous extracts were prepared. The extracts were suspended in Dimethyl sulfoxide (DMSO) and stored at room temperature. Next, bacterial species like, *Klebsiella* & *Bacillus subtilis* were grown as a lawn culture who were then exposed to varying concentrations of butanolic & aqueous extracts. After 24 hour, the butanolic extract showed higher activity as compared to the aqueous extracts. However, Colistin showed the highest activity. This result has given hope that novel anti-microbial agents can be isolated from this plant.

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