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Anti-Bacterial Role of Asparagus Racemosus: A Review

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ABSTRACT
The antimicrobial peptides (AMPs) are integral portion of the human's innate immune system showing broad-spectrum antimicrobial roles. AMPs are used for antimicrobial therapy due to the increase in the cases of resistance towards conventional antibiotics. Unfortunately, resistance towards AMPs and their derivatives are also showing an increase. In this paper, the flowers of the vegetable Asparagus racemosus were evaluated for its anti-microbial activity on two species of the genus lactobacillus, Lactobacillus acetotolerans & Lactobacillus acidifarinae. Firstly, various extracts of Aqueous, methanol, ethanol, butanol were made. The AMP, Cecropine A was chosen as positive control & the Dimethyl sulfoxide (DMSO) was chosen as the negative control. Then the paper discs were soaked in these extracts and were kept on the lawn culture. After 24 hours, the ethanolic extract showed the highest inhibition whereas DMSO showed the least activity. These results open up possibility that this common vegetable can be utilized for the commercial production of anti-microbial agents.

Keywords
Antimicrobial peptides, Asparagus racemosus, Antimicrobial resistance, Cecropine A, extracts, paper disks, zone of inhibition

1. INTRODUCTION
Among the various facets of the human immune system is the presence of the Antimicrobial peptides (AMPs) [1-4]. However, there are many reports that describe the modes by which the microbe can lead to resistance towards AMPs, the rate of which depends upon a complex role-play of various different factors that includes the mutation rate of supply, resistant mutant's fitness at various AMP amount, and the selective pressure's strength. Many reports are there that proves that AMP-resistant microbes displays cross broad resistance towards AMPs with divergent structures and mechanism of action. Thereby, regular clinical treatment of AMPs regarding bacterial infections can give rise to resistant pathogens that are capable of escaping the human's innate immune system. The consequences of clinical levels of exhibition regarding the link between AMP resistances with microbial pathogenesis are yet to be understood[5-7].

The generation of anti-microbial resistance occurs in microbes due to various factors like efflux of antibiotics, modification of targets, among others that have been illustrated in figure 1. Interestingly many microbial genes have been located that have been implemented in antimicrobial resistance[8-11]. For example, Staphylococcus aureas has hemB gene conferring resistance towards anti-microbial proteins (AMPs) like LL-37, lactoferricin B and this gene acts by preventing the uptake of such AMPs, Klebseilla pneumoniae has the mcr-1 gene that confers resistance towards polymixin B, Listeria monocytogenes has the mptACD gene conferring resistance towards Leucocin A. Such a pattern of resistance towards AMPs has been tabulated in table 1. In table 2, various anti-microbial peptides (AMPs) in various stages of clinical development have been tabulated. Some like OP-145 which is equivalent to LL-37 in humans are in Phase I/II. These clinical trials provide a hope that new AMPs are in the pipeline. However, a major factor that needs to be considered is that most of the clinical trials end in failure. In this scenario, plants used traditionally for their anti-microbial roles are being searched for isolation of novel anti-microbial compounds[12-15]. Among such plants which are the most popular are such that being daily consumed as vegetables. In this regard, this paper has investigated, the anti-microbial properties of the vegetable Asparagus racemosus. In table 3, list of AMPs for clinical development has been listed [16].

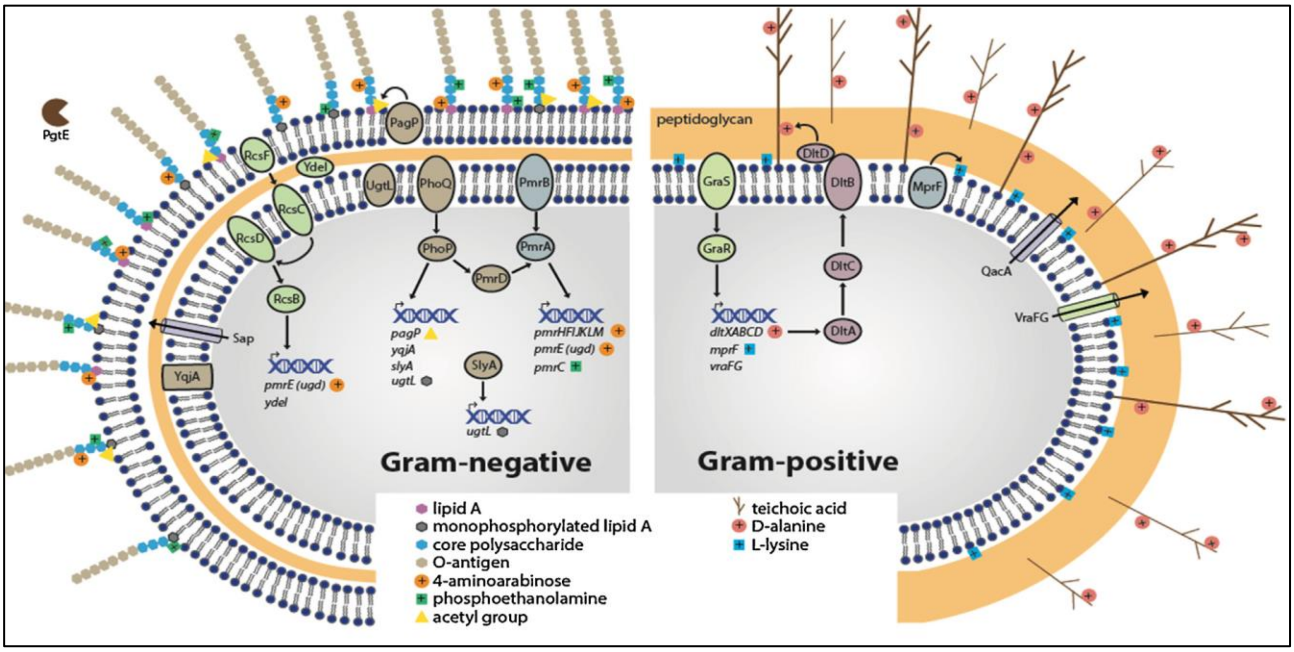

Figure 1: Synopsis of the inherent antimicrobial peptide resistance mode in Gram negative & Gram- positive bacteria. The major routes that results in temporary high-grade AMP resistance in bacteria are modifications in membrane, increase in efflux & proteolytic deterioration Figure courtesy [17]

Table 1: Tabulation of known routes of obtained resistance to peptides of antimicrobial origin. Knowing which genes are responsible for imparting resistance would help in prevention of such resistance [18]

Bacteria	**AMP resistance**	**Isolation method**	**Proposed mechanism**	**Genes causing AMP resistance**
Staphylococcus aureas	LL-37, lactoferricin B	Clinical samples	Inhibition of AMP uptake	hemB
Klebsiella pneumoniae	Polymixin B	Clinical samples	Encodes PEtN transferase	mcr-1
Listeria monocytogenes	Leucocin A	Plating directly with Leucocin A	Unkown	mptACD
Acinetobacter baumannii	Colistin A	Plating directly with Colistin	Decreased AMP binding	IpxA
Salmonella typhimurium	Polymixin	DES mutagenesis	Reduces anionic charge	phoQ
	Polymixin B	Plating directly with Colistin	Reduces anionic charge	pmrA
	PR-39	Plating directly with PR-39	Inactivation inhibits AMP uptake	sbmA
	Protamine	Plating directly with protamine	Inhibited AMP uptake	hemA

Table 2: Antimicrobial peptides (AMPs) used for clinical development. As AMPs have been used for anti-microbial activity, hence they have the interest of the pharmaceutical industry[19]

Peptide	Status	AMP host	Administration	Company	Indication
OP-145	Phase I/II	LL-37 (humans)	Ear drops	OctoPlus Inc	Ear infection
HLF1-11 (Lactoferrin)	Not specified	Lactoferrin 1-11 (human)	Intravenous	AM-Pharma B.V.	Transplantation
Pexiganan	Phase III	Magainin	Topical cream	Dipexium pharmaceuticals	Diabetic foot
Iseganan	Phase III	Protegrin-1	Mouth wash	IntraBiotics Pharmaceuticals	Ventilator infection
Omiganan	Phase III	Indolicidin	Topical cream	Mallinckrodt	Catheter infections
Lytixar	Phase II	Synthetic	Topical cream	Lytix Biopharma AS	Skin infections
C16G2	Phase II	Synthetic	Mouth wash	C3 Jian	Prevents tooth infections

2. LITERATURE REVIEW

D I Andersson in his study discloses about 100 distinct human AMPs are known to show broad-spectrum antibacterial action. Because of the increasing incidence of resistance to traditional antibiotics there is an interest in creating AMPs as an alternative antibacterial treatment. Several cationic peptides that are derivatives of AMPs from the human innate immune system are presently under clinical development. There are currently ongoing therapeutic research aiming at regulating the expression of AMPs to enhance the human innate immune response. In this review we address the possible difficulties connected with various treatment methods. There is extensive experimental evidence outlining ways by which bacteria may acquire resistance to AMPs plants [20].

B Bechinger in another study discloses about more than 40 antimicrobial peptides and proteins (AMPs) that are expressed in the mouth cavity. These AMPs have been grouped into 6 functional categories, 1 of which, cationic AMPs, has garnered considerable study in recent years for their promise as possible antibiotics. The aim of this review is to explain current advancements in our knowledge of the various modes of action of cationic AMPs and the bacterial resistance to these peptides. The newly discovered peptide GL13K is presented as an example to demonstrate several of the mentioned topics [21].

Hwang-Soo Joo in one of his study diclosis about the antimicrobial peptides (AMPs) that are a crucial component of the host's innate immune system, targeting invasive and colonizing microorganisms. For effective survival and colonization of the host, bacteria have a variety of mechanisms to interfere with AMP function, and AMP resistance is closely linked with the pathogenicity potential of bacterial infections. In particular, since AMPs are regarded as possible new antibacterial medicines, it is essential to study bacterial AMP resistance mechanisms. This study provides a comparative overview of Gram-positive and Gram-negative bacterial methods of resistance to different AMPs, such as repulsion or sequestration by bacterial surface structures, change of membrane charge or fluidity, breakdown and elimination by efflux pumps. This paper is part of the topic issue 'Evolutionary ecology of arthropod antimicrobial peptides [22].

3. DISCUSSION

Asparagus racemosus grows in the tropical areas of India. This plant is used in traditional medical systems like, Ayurveda, Unani etc. Many bioactive compounds like Saponins, flavonoids etc. have been held responsible for many medical roles. This plant has been traditionally used regarding treatment of some diseases. Its parts are useful for regarding treatment of piles, gonorrhoea, etc. along with increasing lactation. Animal studies have shown that its extract has antioxidant roles, along with immune system regulation.

The ayurvedic composition of this plant 'Satavari mandur' has been reported to provide protection towards pyloric ligation-influenced gastric ulcers with a comparative efficacy to the drug ranitidine. As per another report, the oral feeding of the root methanolic extract induced protection against acute gastric sores induced by acetic acid & ligation of pylorus. Moreover indomethacin-influenced sores in rats were reversed upon feeding with methanolic extract. The biological activity of this plant is due to the presence of some phytochemicals like Shatavarin I, Shatavarin IV, Shatavarin V, etc. Moreover the chemical structures of some of these phytomolecules have also been elucidated which would further help in determining the mechanism of the biological activity of such compounds (Table 3, Figure 2). Based upon these reports, this paper has attempted to evaluate the anti-bacterial property of the flowers of this plant[22,23].

3.1. Experimental design

Firstly, flowers of Asparagus racemosus were collected and dried following which various extracts of various solvents were prepared & their anti-microbial roles were evaluated [17].

3.2. Antimicrobial role

Antimicrobial role of the flowers of Asparagus racemosus was observed with the extracts of methanol, ethanol, butanol & water with Cecropine A [an antimicrobial peptide (AMP)] as positive control & DMSO as negative control. Two lactobacillus species like Lactobacillus acetotolerans & Lactobacillus acidifarinae were selected for this purpose [18].

3.3. Extract preparation

The flowers were first collected, dried and extracted in a Soxhlet extractor by use of various solvents like methanol,

ethanol, butanol & water. The extracts were then suspended in DMSO at 4°C for future use [19].

3.4. Bacterial culture

Pure culture of Lactobacillus acetotolerans & Lactobacillus acidifarinae were cultured in Mueller-Hinton agar (MH) for 24 hours & were subsequently sub-cultured to form a pure culture [20].

3.5. Evaluation of the anti-bacterial role

Firstly, a bacterial lawn culture was established. Then discs of 6mm were laced with the reconstituted extracts in DMSO & then placed onto the lawn culture and observed for the formation of zone of inhibitions measured in millimetres. Cecropine A was used a + control with DMSO as - control.

The antimicrobial role of various extracts of Asparagus racemosus was observed on 2 bacterial species (Lactobacillus acetotolerans & Lactobacillus acidifarinae). An antibiotic zone reader was employed to measure the zone of inhibition. The ethanolic extract showed maximum inhibition in both the bacterial species which was even higher than the + control, whereas both the aqueous extract & DMSO showed the least activity (table 6) (Figure 5).

The antimicrobial role of various extracts of Asparagus racemosus was observed on 2 bacterial species (Lactobacillus acetotolerans & Lactobacillus acidifarinae). An antibiotic zone reader was employed to measure the zone of inhibition. In both Lactobacillus acetotolerans and Lactobacillus acidifarinae, the ethanolic extract showed the highest zone of inhibition, followed by the butanolic extract which was followed by the methanolic extract. The anti-microbial peptide used as positive control here is showed lesser activity as compared to the alcoholic extracts. However, both DMSO and the aqueous extract showed minimal activity. The main inference that can be gained from this experiment is that the ethanolic extract had the necessary phytomolecules that showed the highest anti-microbial activity [22].

Table 4: Zone of inhibition (millimetres) where the ethanolic extract showed highest inhibition & the aqueous extract & DMSO showed the least activity. Cecropine A as + control showed moderate activity[16]

Bacterial species		**Zone of inhibition (mm)**				
	Aqueous extract	**DMSO**	Cecropine A	**Methanolic extract**	**Ethanolic extract**	**Butanolic extract**
Lactobacillus acetotolerans	2	3	7	9	16	14
Lactobacillus acidifarinae	3	4	8	11	17	15

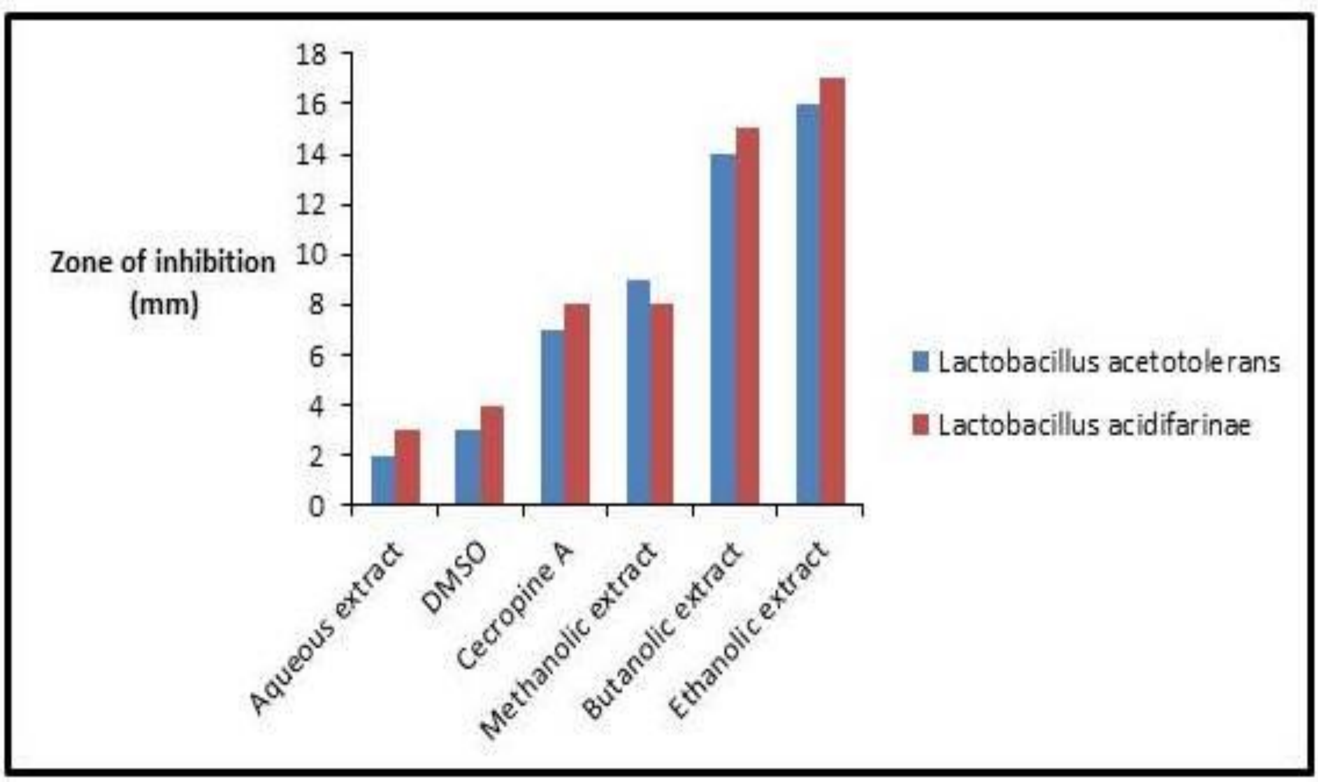

Figure 3: Graphical representation of the zone of inhibition of various extracts. Cecropine A as + control showed moderate activity whereas the ethanolic extract among the extracts showed highest activity[19]

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4. CONCLUSION

The human's innate immune system possesses antimicrobial peptides (AMPs) that show broad-spectrum antimicrobial roles. AMPs are nowadays used for antimicrobial therapy as resistance towards conventional antibiotics is increasing. Unfortunately, even resistance towards AMPs and their derivatives are also increasing. In this paper, the flowers of the vegetable Asparagus racemosus were evaluated for its anti-microbial activity on 2 species of the genus lactobacillus, Lactobacillus acetotolerans & Lactobacillus acidifarinae. Firstly, various extracts of flowers of this plant of the following solvents like Aqueous, methanol, ethanol, butanol were soaked on paper discs. The AMP, Cecropine A was chosen as positive control & the Dimethyl sulfoxide (DMSO) was chosen as the negative control. Both Lactobacillus acetotolerans & Lactobacillus acidifarinae were separately cultured as lawn culture on which the paper discs were placed. After 24 hours, the ethanolic extract showed the highest inhibition followed by the butanolic and the methanolic extracts whereas DMSO and the aqueous extract showed the least activity. Cecropine A showed moderate activity implying that the ethanolic extract may have compounds which have higher activity than Cecropine A. These results open up possibility that this common vegetable can be utilized for the commercial production of anti-microbial agents.

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