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ANTIBACTERIAL ACTIVITY OF *BEAUVERIABASSIANA* MYCELIA EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Beauveriabassiana was a well know fungi used in IPM (Integrated Pest management) for control of agricultural pests. Earlier studies on Beauveriabassiana(Bb) reported exogenous metabolites processing medicinal activities. Present study focused on effect of ten Bb strains mycelia extracts (hexane, ethyl acetate, methanol and water) against seven human bacterial pathogens. Antibacterial activity was performed by disc inhibition assay and it was observed that ethyl acetate extract of ten Bb strains possesses antibacterial activity. Further work was carried out for quantification of Minimum inhibition activity (MIC) against seven tested human pathogenic bacteria, 8 strains showed MIC at 25µg/ml and remaining 2 strains at 50µg /ml.Standard antibiotic chloramphenicol inhibited 5 pathogens in total 7 pathogens. Chloramphenicol did not inhibited B.subtilis and K.pneumonia.

Key Words: *Beauveriabassiana, Mycelia extract, Anti-Bacterial.*

INTRODUCTION

Fungi are one of the most important microbial kingdoms for having various medicinally important activities [1]. Antibacterial compounds isolation and usage in medicine was started from a fungi *Penicillium*sps andit was still continuing as there wasa greatdemand for new antibacterial compound due to development of drug resistance with present antibacterial compounds [2]. Antibacterial research occupied a major portion of medicinal research which tremendously exploiting all the possible ways to overcome drug resistance. Drug resistance of pathogens rising alarm for developing alternate antibacterial compounds [3]. Microorganisms are good source for this as microorganisms are easily cultured and handled.In isolating and discovering metabolites, known microorganisms not having any toxicity toward humans are very useful [4]. In this contest there are some fungi that are using commercially for various activities can helps in screening and discovery of antibacterial compounds [5]. Microorganisms using in agriculture are generally nontoxic and their toxicity was highly regulated by respective government departments [6]. So agricultural important fungi were chosen to isolate antibacterial compounds as their application was regulated for their toxic effects. *Beauveriabassiana* was one of the mostly used fungi in agriculture that was discovered by AgostinioBassi in 1835 and using in IPM for control of various pests on crops [7]. Before to use of *Beauveria*sps as bio pesticide, it was used in Chinese and Korean medicine for Infantile convulsions, epilepsy, sore throat, wounds using *Beauveria* infected silkworm larvae [8].Entomopathogenic fungi are studied for their mechanism against pests that leads to isolation of many metabolites and compounds which are known for their pesticide activities [9]. Additional screening of isolated metabolites from *Beauveria*sps for various medicinal activities provided information that metabolites from *Beauveria*spshave antioxidant, anticancer, anti-viral, anti-ulcer, anti-inflammatory etc [10]. Having huge potentiality of entomo pathogenic fungi and mainly *Beauveria*sps in both and agriculture and medicinal use, metabolites responsible for medicinal activities are highly focused for screening and isolation of target compounds [11]. Till date majority of the compounds screened from *Beauveria* are secreted externally into broth or into hemolymph of pest [12]. There are very few reports on metabolites that are extracted from the homogenized mycelia. Present study was focused on the endogenous metabolite(s) activity against human pathogenic bacteria.

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METHOD AND MATERIALS

Isolation and culturing of *Beauveriabassiana:*

Ten *Beauveriabassiana* (Bb) isolates are used in the study in which two isolates of *Beauveriabassiana* (Bb1 & Bb2) are isolated from local field infected *Helicoverpaarmigera* and remaining isolates (ARSEF 1166, ARSEF 3286, ARSEF 1512, ARSEF 1314, ARSEF 1149, ARSEF 1788, ARSEF 3120, ARSEF 326) were obtained from Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF). *Beauveriabassiana* isolates were cultured on modified Sabouraud's dextrose broth (SDB) media added with 1% yeast extract and incubated in shaking incubator at temperature of 25ºC for 10 d. After incubation, mycelium was separated using whatman No.1 fitter paper under aseptic conditions and rinsed with sterilized double distilled water. The mycelium was dried in hot air oven at 45 ºC for 48 h and packed in sterilized poly propylene bags in aseptic conditions and weighed.

Solvent extraction of*Beauveriabassiana*mycelia:

Dried mycelium was homogenized using motor and pestle in sterile conditions and 10g of mycelium was extracted with 100ml of solvent (hexane, ethyl acetate, methanol) for 24h by using Soxhlet extraction apparatus and water extract was prepared by boiling mycelia powder in sterilized distilled water at 80 ºC using shaking water bath for 3h. The crude extracts were concentrated at 40ºC using a rotary evaporator apparatus. The crude extracts were preserved in deep freezer at -20ºC until further use [13].

Antibacterial activity:

Mycelium extracts of *Beauveriabassiana* were screened against a total of seven bacterial strains by disc diffusion technique. Bacteria cultures were inoculated in nutrient broth and incubated in shaking incubator at 37 ºC for 24 hrs. 100 µl of bacterial culture was spread on sterilized nutrient agar plates under aseptic conditions.

100 µL of each extract was loaded on sterile Whatman No.1 filter disc and placed on bacteria plate and incubated at 37 ºC for 24 h and zone formation around disc was measured.Antibacterial activity was tested against Gram positive bacteria (*Bacillus subtilis* (MTCC2394), *Staphylococcus aureus* (MTCC3160)) and Gram negative bacteria (*Escherichia coli* (MTCC448), *Klebsiella pneumonia* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Enterobacter aerogenes* (MTCC 111) and *Serratiamarcescens*(MTCC8708). The experiment was repeated in triplicates [14].

Minimum inhibitory Concentration (MIC):

10ml of sterilized nutrient broth was added with the Bb extracts separately such that final concentration of the extract in broth was 10,25,50,75,100 mg/ml and standard antibiotic Chloramphenicol of same concentrationsas mycelia extract was used as antibacterial control. Positive control was prepared without extracts in nutrient broth and added with a loop full of 24 h old pathogenic bacteria culture and incubated at 37° C for 24 h. OD was observed at 600nm and concentration at which no bacterial growth was observed was determined as MIC for the bacteria [15].

RESULTS AND DISCUSSION

Antibacterial activity of mycelia extracted with four solvents (hexane, eethylacetate, methanol and water), mycelia extracted in ethyl acetate has potential in inhibiting pathogenic bacteria followed by methanol extracted mycelia. Hexane and water mycelia extracts did not have any antibacterial activity against tested pathogens and standard antibiotic chloramphenicol also inhibited 5 pathogens (*S.aureus*, *E. coli*, *P. aeruginosa*, *Serratiamarcescens*, *Enterobacter aerogenes*) but there was no inhibition effect on *B.subtilis* ,*K.pneumonia*. MIC for the ten Bb mycelia extracts, it was observed 8 Bb strains (BB1, BB2, ARSEF 1166, ARSEF 3286, ARSEF 1512, ARSEF 1149, ARSEF 3120, ARSEF 1788) inhibited all the bacteria at 25µg/ml and remaining 2 Bb strains (ARSEF 1314, ARSEF 326) inhibited bacteria at 50µg/ml where as standard antibiotic chloramphenicol inhibited *S. aureus*, *E. coli*, *Serratiamarcescens*, *E. aerogenes* at 25µg/ml and *P. aeruginosa* at 50µg/ml and there was no inhibition on *B. subtilis* and *K. pneumonia*. *Beauveriasps* contains many metabolites and bioactive studies demonstrated that some of the metabolites belong to class cyclodepsipeptide (like iso-isariin D) isolated from marine fungi *Beauveria feline* EN-135 strain [16]. *Beauveria*sps was most studied entomopathogenic fungi for isolation of bioactive compounds which have medicinally potential activity [17]. One of the most studied bioactivity was antimicrobial activity as pathogenic microorganisms acquiring resistance to synthetic antibiotics there was always in need for antibiotic compounds.Most of the antibacterial compounds from *Beauveria*sps are extracted from cell free culture filtrate. To my knowledge reports on mycelia extracts of *Beauveria*sps with potential antibiotic activity are very few or not studied well. Further research should be continued to isolate potential antibacterial compound from mycelia extract of *Beauveriabassiana*.

Table No. 1: Antibacterial activity of extracts (water, methanol, ethyl acetate and hexane) of 10 Bb isolates.

Strain	Extract	*B.subtilis*	*S.aureus*	*E. coli*	*K.pneumonia*	*P.aeruginosa*	*S.marcescens*	*E. aerogenes*
BB1	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
BB2	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 1166	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 3286	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 1512	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 1314	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 1149	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 3120	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 1788	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
ARSEF 326	*Aqueous*	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+
	Ethylacetate	++	++	++	++	++	++	++
	Hexane	-	-	-	-	-	-	-
Chloramphenicol (Std)		-	+	+	-	+	+	+

The results are mean ± S.E. of three replicates; + indicates zone of inhibition less than of 10mm in diameter; ++ indicates zone of inhibition more than 10 mm in diameter; - Indicates no zone of inhibition.

Table No. 2: MIC of extracts (water, methanol, ethyl acetate and hexane) of 10 Beauveria bassiana isolates.

Strain	Extract	*B.subtilis*	*S.aureus*	*E. coli*	*K.pneumonia*	*P.aeruginosa*	*S. marcescens*	*E. aerogenes*
BB1	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	75	100	75	75	-	100	100
	Water	-	-	-	-	-	-	-
BB2	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	100	-	-	75	100	100	100
	Water	-	-	-	-	-	-	-
ARSEF 1166	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	100	75	75	100	-	-	75
	Water	-	-	-	-	-	-	-
ARSEF 3286	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	-	75	100	75	75	100	100
	Water	-	-	-	-	-	-	-
ARSEF 1512	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	50	-	-	100	75	-	100
	Water	-	-	-	-	-	-	-
ARSEF 1314	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	50	50	50	50	50	50	50
	Methanol	75	100	100	-	-	75	100
	Water	-	-	-	-	-	-	-
ARSEF 1149	*Hexane*	100	100	100	100	100	100	100
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	100	-	75	100	100	75	-
	Water	-	-	-	-	-	-	-
ARSEF 3120	*Hexane*	100	100	100	100	100	100	100
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	100	75	75	100	-	-	100
	Water	-	-	-	-	-	-	-
ARSEF 1788	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	25	25	25	25	25	25	25
	Methanol	75	100	-	-	100	75	75
	Water	-	-	-	-	-	-	-
ARSEF 326	*Hexane*	-	-	-	-	-	-	-
	Ethylacetate	50	50	50	50	50	50	50
	Methanol	-	100	75	75	-	-	100
	Water	-	-	-	-	-	-	-
Cholarmphenicol		-	25	25	-	50	25	25

Concentrations mentioned in above table are in µg/ml; The results are mean ± S.E. of three replicates; - Indicates no zone of inhibition.

CONCLUSION

Based on the experimental results obtained entomopathogenic fungi contains metabolites endogenous which are having potential bioactivity against pathogenic bacteria. Further research should be done for isolation of compounds from the mycelia of Bb with potential activity which might have a valuable drug activity against drug resistance pathogenic bacteria.

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