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# EVALUATION OF ANALGESIC ACTIVITIES OF *BACILLUS CEREUS* AND *BACILLUS PUMILUS* METABOLITES

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## ABSTRACT

Soil samples from Malnad regions of Karnataka were screened for bacteria with antibiotic production potential. The two isolates, which showed significant antibacterial activity were identified as *Bacillus cereus* and *Bacillus pumilus*. The metabolites of the two bacteria were subjected to various solvent extractions based on polarity and the extracts were tested for analgesic activity by tail warm water-immersion method and Eddy's Hot-plate method using Swiss albino mice. The methanol extract of *B. cereus* and ethyl acetate extract of *B. pumilus* showed significant analgesic activity with a reaction time of 33.63 ± 0.61 seconds and 34.63 ± 1.24 seconds at a dose of 50 mg/Kg body weight. The obtained result promises that both the bacteria can be exploited for bioactive molecules with therapeutic potential.

**Key words**: *B. cereus*, *B. pumilus*, Analgesic

## INTRODUCTION

Natural products still remain the most important source for discovery of new and potential drug molecules. Large number of bioactive compounds from plants and animals have been discovered and isolated as pharmaceutical agents from both terrestrial and marine sources. The compounds derived from plants referred as secondary metabolites form the ingredients for bioactive compounds like analgesics, heart drugs, laxatives, anticancer agents, contraceptives, ulcer treatments and diuretics[1]. These secondary metabolites have complex and unique structures and their production is often enhanced by both biotic and abiotic stresses.

Microbes are also known to produce secondary metabolites under normal or stressed conditions. The discoveries of number of antibiotics since 1930's are the best examples. These antibiotics have been useful in our battle against bacteria and fungi for over 70 years. About 1,00,000 secondary metabolites of molecular weight less than 2500 have

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been characterized, mainly produced by microbes and plants; some 50,000 are from microorganisms[2-4]. Plenty of antibiotics are already in commercial use as successful antimicrobial compounds. Some antibiotics are also found to be potentially useful in medicine for activities other than their antibiotic action. They are used as antitumor agents, immunosuppressive agents, hypocholesterolemic agents, enzyme inhibitors, antimigraine agents and antiparasitic agents[5]. New bioactive products from microbes with diverse pharmacological activities continue to be discovered at an amazing pace; 200-300 per year in the late 1970's, increasing to thousands per year at present[6-8].

Though microbial metabolite with various pharmacological activities is an emerging area with new molecules, the available literature has very little information regarding analgesic activity. Therefore, the present study was to examine the analgesic effects of *B. cereus* and *B. pumilus* metabolites using standard methods.

## EXPERIMENTAL

### Materials and methods

### Solvent extraction and preparation of samples

The two bacteria *B. cereus* and *B. pumilus* were grown separately in large quantity in nutrient broth medium and incubated for three days at 35°C. The broth was centrifuged to separate the cells at 10,000 rpm for 20 minutes. The clear supernatant containing the metabolites was collected. The metabolites of both organisms were subjected to successive solvent extraction with petroleum ether, ethyl acetate and methanol (1 : 1) in a separating funnel. All the three solvent extracts were dried in separate plates. The *B. cereus* (BC) petroleum ether extract was labeled as sample BC-1, ethyl acetate extract as BC-2 and methanol extract as BC-3. Similarly, the *B. pumilus* (BP) extracts were labeled as BP-1, BP-2 and BP-3, respectively.

### Selection and preparation of experimental animals

Healthy adult albino mice weighing 25-30 grams were used. Each animal, at the commencement of its dosing were between 8 to12 weeks old. The experimental protocols were approved by institutional animal ethical committee (Ref. No. NCP/IAEC/CL/02/ 12/2010-11) prior to the experiments. Procurement of animals and all the experiments were carried in the Department of Pharmacology, National College of Pharmacy, Shimoga, Karnataka. The temperature in the experimental animal room was maintained around 25°C (± 3°C). The relative humidity was maintained between 50-60 percent. They were kept in normal lighting of 12 hours light and 12 hours dark. For feeding, conventional laboratory

diet with an unlimited supply of drinking water was provided. The animals were marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance was administered in a single dose by intraperitoneal route.

### Study of $LD_{50}$

The $LD_{50}$ studies for all the test samples were carried out as per OECD guidelines 423.

### Evaluation of analgesic activity by tail immersion method

Young Swiss albino mice weighing 25-30 grams were used. The distal 3 cm part of the tail was marked. Prior to analgesic experiments, the animals were screened for their sensitivity to heat by immersing the last part of the tail gently in hot water maintained at 55$^{o}$C. The animals withdrawing their tail within 5 seconds were selected for the study.

The selected animals were divided into eight groups of six animals each. The groups 1, 2 and 3 received the sample extracts of *Bacillus cereus* BC-1, BC-2 and BC-3, respectively. Similarly, groups 4, 5 and 6 received the sample extracts of *Bacillus pumilus* BP-1, BP-2 and BP-3, respectively. All the samples were prepared in sterile water at a dose of 50 mg/Kg body weight. The group seven received the control drug Pentazocine at 10 mg/Kg body weight. The control group received plain water of 1 mL each (group eight). After administration of the drug and the test samples, the tail withdrawal reflex time was noted. The basal reaction time was measured after 1 hour, 2 hours and 3 hours considering the post-drug reaction time.

### Evaluation of analgesic activity by Eddy's hot plate method

Swiss albino mice of either sex weighing about 25-30 grams were selected. They were divided into 8 groups of 6 animals each. The groups 1, 2, and 3 received the sample extracts of *Bacillus cereus* BC-1, BC-2 and BC-3, respectively. Similarly group 4, 5 and 6 received the sample extracts of *Bacillus pumilus* BP-1, BP-2 and BP-3, respectively. The group seven received the control drug pentazocine at 10 mg/Kg body weight. The control group received plain water of 1 mL each (group eight). After administration of the drug and samples, the time of basal reaction to pain stimulus of the mice placed on the hot plate heated at 55$^{o}$C were recorded after 1 hour, 2 hours and 3 hours. The increase in reaction time against control group was compared.

### Statistical analysis

The mean value ± SEM were calculated for each parameter. The results were

analyzed statistically by ANOVA were followed by Dunnet's test. The minimum level of significance was fixed at $p \leq 0.001$

## RESULTS AND DISCUSSION

### L $D_{50}$

All the test samples (BC-1, BC-2, BC-3, BP-1, BP-2 and BP-3) exhibited $LD_{50}$ at 500 mg/Kg body weight. Hence, the dose level was fixed at 50 mg/Kg weight (the $^{1}/_{10}^{th}$ of the $LD_{50}$ dose is equal to therapeutic dose).

### Tail warm water immersion method

#### *Bacillus cereus*

The significant analgesic activity was observed for the sample BC-3 with a reaction time of 30.0 ± 0.61, when compared to control with 3.62 ± 0.35 seconds. This reaction time was better than standard, which showed maximum reaction time of 15.43 ± 0.41 seconds. This result was observed after three hours of administration of control, standard and test sample. The sample BC-3 also exhibited increasing analgesic activity with increase in time. The reaction time was 20.58 ± 0.73 after one hour, 25.33 ± 0.39 seconds after two hours and 30.0 ± 0.61 seconds after three hours of administration. The test sample BC-1 also showed significant increasing activity with a reaction time of 22.03 ± 0.98 and 24.88 ± 0.62 seconds after one hour and two hours of administration, respectively. However, the activity decreased to 16.58 ± 0.77 seconds by the third hour. The sample BC-2 showed no activity.

#### *Bacillus pumilus*

The significant analgesic activity was observed for the sample BP-2 with a reaction time of 32.25 ± 1.05, when compared to control with 3.55 ± 0.49 seconds. This response time was better than standard, which showed a reaction time of 15.0 ± 0.45 seconds. In addition, the sample BC-2 had increasing activity up to two hours with a reaction time of 15.13 ± 0.55 seconds after one hour, 32.25 ± 1.05 seconds after two hours followed by decreasing activity by the third hour with 21.25 ± 0.51 seconds.

The sample BP-3 showed no activity after one hour, slightly increased activity after two hrs with 10.75 ± 0.55 reaction time and no activity by the third hour with 3.9 ± 0.21 seconds. The sample BC-1 did not exhibit any activity.

The data obtained for all the samples, standard and control are shown in Table 1 and Histogram (Fig. 1a and 1b).

**Table 1: Analgesic activity of *Bacillus cereus* and *Bacillus pumilus* extracts by tail immersion method**

| Treatment groups | Dose mg/Kg body wt. | Reaction time in seconds | | |
|---|---|---|---|---|
| | | After 1 hr | After 2 hrs | After 3 hrs |
| **Control (water)** | - | 3.0 ± 0.35 | 3.55 ± 0.49 | 3.62 ± 0.35 |
| **Standard (Pentazocine)** | 10 | 12.63 ± 1.028** | 15 ± 0.45** | 15.43 ± 0.41** |
| **BC-1** | 50 | 22.03 ± 0.98*** | 24.88 ± 0.62*** | 16.58 ± 0.77** |
| **BC-2** | 50 | 3.82 ± 0.41 | 3.60 ± 0.79 | 24.38 ± 0.87*** |
| **BC-3** | 50 | 20.58 ± 0.73*** | 25.33 ± 0.39*** | 30.0 ± 0.61*** |
| **BP-1** | 50 | 4.32 ± 0.21 | 3.72 ± 0.17 | 3.65 ± 0.08 |
| **BP-2** | 50 | 15.13 ± 0.55** | 32.25 ± 1.05*** | 21.5 ± 0.45*** |
| **BP-3** | 50 | 3.97 ± 0.27 | 10.75 ± 0.55** | 3.9 ± 0.21 |

Values are mean ± SEM, n = 6, *p < 0.001 significant, p < 0.01 moderately significant
(In comparison to control sample)

**BC-*Bacillus cereus***; BC-1 Petroleum ether extract; BC-2 Ethyl acetate extract; BC-3 Methanol extract

**BP-*Bacillus pumilus***; BP-1 Petroleum ether extract; BP-2 Ethyl acetate extract; BP-3 Methanol extract

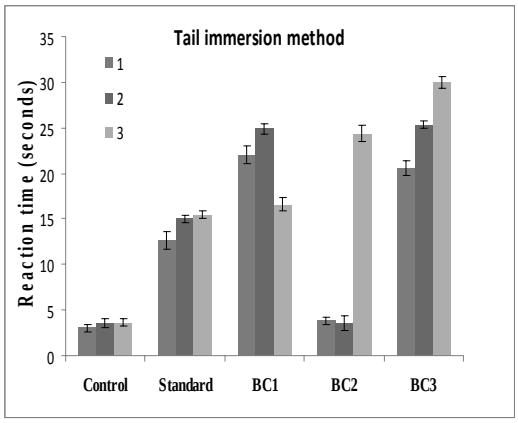


**Fig. 1a: The histogram showing the basal reaction time for *B. cereus* samples after 1 hr, 2 hrs and 3 hrs in comparison with control and standard by tail immersion method**

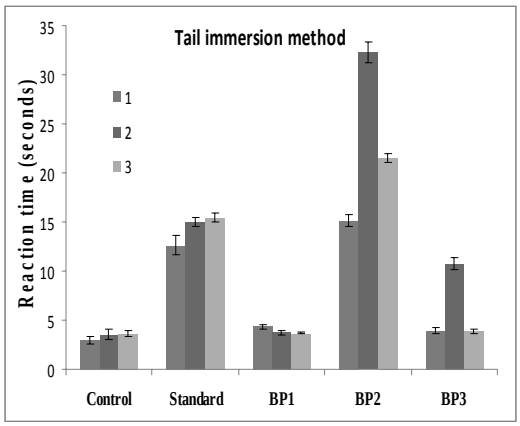


**Fig. 1b: The histogram showing the basal reaction time for *B. pumilus* samples after 1 hr, 2 hrs and 3 hrs in comparison with control and standard by tail immersion method**

## Eddy's hot-plate method

### *Bacillus cereus*

The significant analgesic activity was observed for the sample BC-3 with a reaction time of 33.63 ± 0.68, when compared to control with 3.55 ± 0.49 seconds. This reaction time was found to be better than standard, which showed maximum reaction time of 17.88 ± 0.59 seconds. This response was observed after three hours of administration of control, standard and sample. The sample BC-3 also exhibited increasing analgesic activity with a response time of 23.13 ± 0.96 seconds after one hour, 30.18 ± 0.82 seconds after two hours and 33.63 ± 0.68 seconds after three hours of administration.

The sample BC-1 also showed increasing activity with time. It showed a response time of 22.30 ± 0.78 and 26.50 ± 1.13 seconds after one hour and two hours of administration, respectively. However, the activity decreased to 16.38 ± 0.62 seconds by the third hour. The sample BC-2 showed no activity up to two hours. By the third hour, there was significant activity with a reaction time of 21.13 ± 0.51 seconds.

### *Bacillus pumilus*

The significant analgesic activity was observed for the sample BP-2 with a reaction time of 34.63 ± 1.24, when compared to control with 3.63 ± 0.35 seconds. The reaction time was better than standard response of 15.40 ± 0.57 seconds. This reaction was observed after two hours followed by decreasing activity by the third hour with 21.13 ± 0.51 seconds reaction time.

The sample BP-3 showed no activity after one hour, good activity after two hours with 12.25 ± 0.91 seconds and decreased activity by the third hour with 4.87 ± 0.67 seconds. The sample BP-1 did not exhibit any activity.

The data obtained for all the samples, standard and control are shown in Table 2 and Histogram (Fig. 2a and 2b).

In the present work, the metabolic extracts of two bacteria *B. cereus* and *B. pumilus* were tested for their analgesic activity. The two organisms were originally isolated from soil samples for their antibiotic production potential. The results obtained showed the methanol and petroleum ether extracts of *B. cereus* and ethyl acetate extract of *B. pumilus* exhibited significant analgesic activity by both tail-immersion and Eddy's hot-plate method.

**Table 2: Analgesic activity of *Bacillus cereus* and *Bacillus pumilus* extracts by Eddy's hot plate method**

| Treatment groups | Dose mg/Kg body wt | Reaction time in seconds | | |
|---|---|---|---|---|
| | | After 1 hr | After 2 hrs | After 3 hrs |
| **Control (water)** | - | 3.08 ± 0.67 | 3.63 ± 0.35 | 3.55 ± 0.49 |
| **Standard (Pentazocine)** | 10 | 11.50 ± 0.53** | 15.4 ± 0.57** | 17.88 ± 0.59** |
| **BC-1** | 50 | 22.30 ± 0.78*** | 26.50 ± 1.14*** | 16.38 ± 0.62** |
| **BC -2** | 50 | 5.17 ± 1.15 | 4.92 ± 1.03 | 21.13 ± 0.42*** |
| **BC-3** | 50 | 23.13 ± 0.96*** | 30.18 ± 0.82*** | 33.63 ± 0.68*** |
| **BP-1** | 50 | 4.00 ± 0.29 | 5.22 ± 0.24 | 5.25 ± 0.45 |
| **BP-2** | 50 | 21.65 ± 1.55*** | 34.63 ± 1.24*** | 21.13 ± 0.51 |
| **BP-3** | 50 | 4.92 ± 0.55 | 12.25 ± 0.91 | 4.87 ± 0.67 |

Values are mean ± SEM, n = 6, *p < 0.001 significant, p < 0.01 moderately significant
(In comparison to control sample)

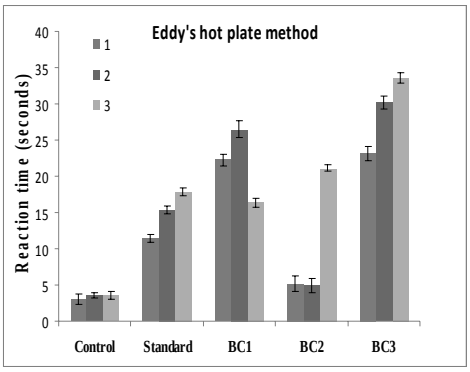


**Fig. 2a: The histogram showing the basal reaction time for *B. pumilus* samples after 1 hr, 2 hrs and 3 hrs in comparison with control and standard by Eddy's hot plate method**

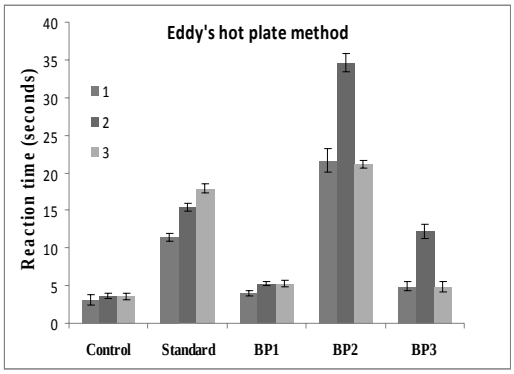


**Fig. 2b: The histogram showing the basal reaction time for *B. pumilus* samples after 1 hr, 2 hrs and 3 hrs in comparison with control and standard by Eddy's hot plate method**

The plant and animal based metabolites are known to have bioactive compounds with analgesic activity and are in use since centuries. But microbial based analgesic compounds are seldom reported. There are several reports on bioactive metabolites produced

by microorganisms with pharmacologically active substances by both bacteria and fungi. Most of these studies are related to antimicrobial, anticancer, anti-inflammatory, antioxidant, immunomodulatory, enzyme inhibitors and antiparasitic activities. The reported sources of these microorganisms are from diverse environments like marine, endophytic, epiphytic or terrestrial environments[9-12]. But less information is available regarding screening and evaluation of microorganisms for analgesic activity from these sources.

Most of the microbial metabolites reported on analgesics are crude extracts obtained from various solvents extraction. Karunakaran et al.[13] have reported analgesic activity for ethyl acetate extract of the fungus *Aspergillus oryzae* at 100 mg/Kg body weight on albino rats. The ethyl acetate extract of four bacterial strains isolated from sea weeds have been reported with antinociceptive activity by Ramaswamy and Kumar[14] Jebasingh and Murugan[15], have reported two marine bacteria *Bacillus megaterium* associated with cone snail, and *pseudomonas aeruginosa* isolated from tubeworm with analgesic activity. The chloroform extracts of the culture supernatants of these two bacteria were tested at a dose of 100 mg and 200 mg/kg body weight on albino rats.

Apart from crude metabolites, the intermediary compounds produced during metabolism has been reported. The morphine alkaloid Hydromorphone, which is an intermediary metabolite produced during degradation of morphine by a bacterial strain *pseudomonas putida* M10, has been reported with analgesic activity[16]. "Scutigeral", a secondary metabolite isolated from fruiting bodies of *Albatrellus ovinus*, has been reported with an affinity to the brain dopamine D1 receptors, stimulates rat dorsal root ganglion neurons and may act as an orally active pain killer[17].

In the present work, the successive extracts of metabolites by petroleum ether, ethyl acetate and methanol of *B. cereus* and *B. pumilus* were tested for analgesic effects. Analgesic activity on these two bacteria has not been reported in the past. Hence, as per the available literature and to the best of our knowledge, this may be the first report on analgesic activity on *B. cereus* and *B. pumilus* metabolites.

## CONCLUSION

The present study indicated potential analgesic effects for both *B. cereus* and *B. pumilus* metabolites. The study reveals that when compared, the *B. pumilus* extract showed better activity than *B. cereus*. Among the three solvent extracts, the ethyl acetate extract of *B. pumilus* with a reaction time of 34.63 ± 1.24 seconds was found to be better than petroleum ether and methanol extracts. Similarly, the methanol extract of *B. cereus* was found better than the other two extracts with a reaction time of 33.63 ± 0.68 seconds. The methanol

extract of *B. cereus* and ethyl acetate extract of *B. pumilus* can be subjected to further isolation of compounds in pure form. Further identification and purification of the active components and exploration of the chemical structures of the same can lead to potentially useful compounds of biomedical importance.

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