Chapter - 6

Evaluation of CNS depressant and anticonvulsant activities of Bacillus cereus and Bacillus pumilus metabolites
6.1. INTRODUCTION

The modern day life is highly competitive and more demanding of mental skills. The working conditions are becoming increasingly stressful. In small quantities, stress and anxiety are good. They can motivate and help one be more productive. However, too much stress or a strong response to stress is harmful. As a result, is the surge in incidence of variety of psychiatric disorders. According to world health report (WHO 2001) approximately 450 million people suffer from a mental or behavioral disorder. This amounts to 12.3% of global burden of disease and expected to rise to 15% by 2020. Anxiety disorders or psychiatric disorders are affecting nearly 25% of the adult population at some point in their life. The prevalence of anxiety disorders is 30.5% in women and 19.2% in men. The disorders are remarkably high in young people. Children aged 7 to 11 years reported to have 15.4% prevalence rate of anxiety disorders. In spite of prevalence of disorders in so many people, only a small minority of them receives even the most basic treatment (Leon et al., 1997; Ruiz et al., 2006). Stress and anxiety can also lead to poor general health as well as specific physical or psychological illnesses like infection, heart disease, or depression (Khanum and Razack, 2010).

6.1.1. Central nervous system (CNS)

The central nervous system (CNS) mainly comprises of the brain and spinal cord. The CNS processes the information with the help of chemical messengers which can act as neurotransmitters, neuromodulators, neuroregulators, neuromediators and neurotropic factors. The compounds like nor adrenaline, adrenaline, dopamine, GamaAminoButyric Acid (GABA), glutamate, acetylcholine, 5-hydroxytryptamine (5-HT), peptides like endorphins, serotonin etc, can act as chemical messengers via specific mechanism to mediate neurotransmission or as a
neurotransmitters. The neuromodulators like prostaglandins (PGs), purines and neuropeptides interact with their recognition sites i.e. receptors and regulate the function of CNS (Seth, 2005).

6.1.2. Drugs acting on central nervous system

Drugs acting on CNS were first discovered by the primitive humans and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically because they can produce specific physiological and psychological effects. Among the natural products many plants have been reported to have activity against CNS disorders and are useful in alleviation of human suffering (Suba et al., 2002). The recent advances in science and technology have contributed to an enormous improvement in the quality of mankind. The path breaking research in psychopharmacology has lead to flow of drugs for specification. For example, Benzodiazepines (Diazepam, Alprazolam, laraepam, nitrazipam etc) are the most frequently prescribed synthetic drugs for variety of disorders particularly against anxiety, depression, epilepsy and insomnia (Abid et al., 2006).

6.1.3. Central nervous system depressants

These are a group of drugs with diverse chemical structures that induce behavioral depression. They can produce desired effects like relief from anxiety, inhibitions, induces relaxation, sleep, unconsciousness, general anesthesia and coma. The tendency of all these drugs is to inhibit the excitability of neurons. The term sedative, tranquilizers, hypnotics and anxiolytic are commonly applied to any central nervous system depressants. In smaller doses many of these drugs can produce a state of drowsiness, and when used in this manner they are referred to as sedatives.
A sedative compound decreases activity, moderates excitement and calms the recipient when used in larger doses; hypnotics may produce anesthesia, poisoning and death. These progressive dose-related effects may be indicated as follows: Sedation = Hypnosis = Anesthesia = Coma = Death. The sedatives and hypnotics are used to allay nervousness, to induce sleep and control convulsions. The hypnotics suppress cerebral activity sufficiently to blunt the patient awareness of the environment thereby establishing conditions favorable for sleep. The general action of the hypnotics and sedatives is that of the depression of the CNS (Wafford and Ebert, 2008; Clauw, 2008). Sedative-hypnotics include alcohol (ethanol), barbiturates and non-barbiturate hypnotics (Quaalude). For the past 30 years the barbiturates have been replaced by the benzodiazepines that are less addictive and have less abuse potential. But these psycho neural drugs have very serious side effects. The chronic use of benzodiazepines can cause deterioration of cognitive function, physical dependence and tolerance. Benzodiazepines can also adversely affect the respiratory, digestive and immune system of the body. Besides, it has addiction liabilities and often proves more harmful in the long run (Dhawan et al., 2003).

6.1.4. Epilepsy

Epilepsy is a common neurological disorder. It is a collective term given to a group of syndromes that involves spontaneous, intermittent, abnormal electrical activity in the brain. There is spontaneous occurrence of brief episodes associated with disturbance in consciousness and excessive EEG spikes (Rollas and Kucukguzel, 2007). The overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in
older age. It affects approximately 50 million people worldwide (Fisher et al., 2005; Poole et al., 2000).

6.1.5. Antiepileptic drugs (AEDs)

An ideal epileptic drug should suppress all seizures without causing any unwanted side effects. Unfortunately, it is observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients. The current therapeutic treatment of epilepsy with modern antiepileptic drugs is associated with side-effects, dose-related and chronic toxicity, and teratogenicity effects. Approximately 30% of the patients continue to have seizures with current AEDs therapy (Samren et al., 1997). The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate have reported several side effects, mainly neurotoxicity. Since majority of antiepileptic drugs are to be consumed life long, the administration of other drugs predisposes to the risk of drug interaction (Basavaraj et al., 2011; Venkateswarulu et al., 2012). Thus it is necessary to investigate for antiepileptic agents that are safe, efficacious and free from toxicity. The main intention of treating an epileptic is to not only to eliminate the occurrence of seizures but also to help him to have a self sustained life.

In this context, there is resurgence of interest in medicines from natural sources. It may be from plants, animals or microbial origin. The drugs obtained from the natural source will always have significantly lesser side effects than that observed with synthetic drugs and comparably with near equal efficacy (Koehn and Carter, 2005; Newman et al., 2003).
6.1.6. Drugs from natural sources

The use of the medicinal herbs for curing disease has been documented in history of all civilization. In the early sixties, the increasing needs for drugs able to control new illnesses or resistant strains of microorganisms stimulated to look for unconventional new sources of bioactive natural products. In the beginning most of the chemical studies were conducted randomly as a result of poor availability of ethnopharmacologic information. In 1970's the search for new biomedicals expanded. The look from marine organisms resulted in the isolation of more or less 10,000 metabolites, many of which endowed of pharmacodynamic properties (Faulkner, 2000). A broad spectrum of biological activities was detected such as antibiotic, antifungal, toxic, cytotoxic, neurotoxic, antimitotic, antiviral, antineoplastic and anticonvulsant activities. In more recent years, new targets have been added to the general screening. For example: AIDS, immunosuppression, anti-inflammation, alzheimer disease, ageing processes and some tropical diseases (Kelecom 1999; Faulkner, 2002).

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures, better safety and efficacy profiles (Raza et al., 2003). The untapped wealth of plant kingdom can be a novel source of newer compounds with significant therapeutic activity (Dey et al., 2012). Now, various phytochemical and pharmacological studies have been carried out on these anti-convulsant plants (Nsour et al., 2000). Variety of drugs from plant sources have been tested and are in use for psychopharmacological effects and are found to be effective in the treatment of psychiatric disorders (Evans, 1998; Bejar et al., 2000). There are several plants very effective in treating stress and anxiety. Such plants include Matricaria
chamomilla (chamomile), Hypericum perforatum (St. John’s wort), Piper methysticum (Kava kava) and Passiflora incarnate (Passion flower) (Dhawan et al., 2003).

The drugs obtained from animal life, both from terrestrial and oceanic origin have showed a wide variety of chemical compounds like terpenes, polyketides, actogenins, peptides etc with structural diversity and biomedical importance (Carte 1996; Write 1998; Donia et al., 2003). One of the major problems of these natural products coming into clinical trials is supply issue. The concentration of active compounds in these organisms are often is in minute quantities (Prokch et al., 2002). Hence, scientists have to look for alternative natural source without extinction of the respective species.

Besides plants and animals, the other alternative natural source that we can look for is microorganisms. There are many microorganisms associated with plants and animals and their metabolites have striking structural similarity of natural products suggesting that microorganisms are the real producers of these metabolites (Prokch et al., 2003).

Many classical animal models are available for preliminary pharmacological tests on CNS which provide useful information about action of compounds on psychomotor performance, motor behavior and neurotoxicity which helps in compilation of data of various models at same time and make it easy to screen drugs (Franco et al., 2005).

In the present study, the metabolic extracts of two bacteria B. cereus and B. pumilus were tested for locomotor activity in mice which is an index of wakefulness (alertness) of mental activity using photoactometer and muscle relaxant property by
rota-rod apparatus. The metabolites were also tested for antiepileptic property by maximal electric-shock method.

6.2. Review of Literature

Plenty of literatures are available on plant metabolites having CNS depressant and anticonvulsant activity. Reports on animal metabolic extracts and metabolites from flora and fauna of marine organisms having CNS depressant activity are also available. In the present search the literatures on microbial metabolites with CNS depressant and anticonvulsant activity was surveyed. Literatures are available on CNS depressant and anticonvulsant drugs which are metabolized by intestinal microorganisms resulting in biotransformation of drugs in the gut to available form or non-available forms to the body. But microbial metabolites having CNS depressant activity and anticonvulsant activity are seldom reported.

Jebasingh and Murugan (2012) isolated marine bacteria *Bacillus megaterium* associated with cone snail and *Pseudomonas aeruginosa* from tubeworm. The chloroform extract of the culture supernatants were screened for CNS depressant, anti-inflammatory, analgesic and antipyretic activities. The CNS depressant activity was performed by administration of extract at 200 mg/kg body weight in albino rats and observing the locomotor activity in photoactometer. The locomotor activity in rats was greatly reduced by the extracts of both bacteria and was dose dependent. The standard drug Diazepam (5mg/kg body weight) treated rats showed a reduction of 96% in locomotor activity whereas the chloroform extract of *B. megaterium* exhibited 92% and *P. aeruginosa* 90% reduction in locomotor activity.
Ramasamy and Kumar (2009) isolated four marine bacterial strains BR1, PC4, EM13, and EM14 from Balanus amphitrite (barnacle), Polyclinum constellatum (ascidian) and Enteromorpha compressa (seaweed) respectively. The ethyl acetate extracts of all the four strains were subjected to anti-inflammatory, analgesic and CNS depressant activities. The activity was carried on swiss mice. The locomotor activity was studied by evaluation of scores using photoactometer. Of all the four bacterial extracts the EM13 exhibited higher percentage (42%) of reduction in locomotor activity at 200 mg/kg body weight. The standard drug Diazepam treated animals exhibited 73% reduction in locomotor activity.

Kekuda et al. (2013) have reported anti-inflammatory, analgesic, antipyretic and CNS depressant activities for the crude extract obtained from Streptomyces species PO-178, isolated from Western Ghats soil of Agumbe, Karnataka, India. The CNS depressant activity was performed by spontaneous locomotor activity in digital actophotometer by administering 100 and 200 mg/kg body weight in swiss albino mice. Diazapam was used as standard drug. The percentage reduction in locomotor activity of the test samples were very less when compared with that of the standard. The extract administered at a dose of 200 mg/kg body weight showed a highest of 8% reduction when compared with standard drug showing 70% reduction.

Kamat and Kerkar (2011) isolated bacteria from salt pan water, salt and sediment samples from nine saltpans from north and South Goa. The bacterial extracts were screened for various biological activities to ascertain their biomedical importance. A total of 63 out of 1178 cultures were found to be active showing antioxidant, anti gastric ulcer, antifungal, memory enhancing activity and activity against neurological disorders, anticancer, amylase, amylase inhibitory, protease and
protease inhibitory activity. Seven cultures showed antidementia activity two cultures showed antianxiety activity and 14 cultures showed anti-depressant activity.

Choudhury et al. (2008) isolated mycotoxin MT81 from *Penicillium nigricans* and its two structural derivatives viz. acetylated MT81 (AcMT81) and benzoylated MT81 (BzMT81). The earlier studies of these compounds had showed antimicrobial activities as well as to cause hepatotoxicity and nephrotoxicity. The CNS depressant activity was performed by giving the sedative drug diazepam. The sleep induced by diazepam, chlorpromazine and pentobarbitone was prolonged following the administration of MT81, AcMT81 and BzMT81 and this effect is dose dependent.

Naik et al. (2001) have reported a compound “Pimprinine”, an extracellular alkaloid isolated from the culture filtrate of *Streptomyces* CDRIL-312. Pimprinine was subsequently purified and some physicochemical properties, antimicrobial activities and pharmacological activities of pimprinine were studied. Pimprinine showed promising anticonvulsant activity in both minimum and maximum electric seizure threshold test in mice. Its anticonvulsant activity was very much comparable to that of phenyl hydantion sodium (standard). Pimprinine also inhibited effectively tremorine-induced tremors in mice.

Mikolasch et al. (2003) isolated sixty-one strains of alkane-oxidizing bacteria and were tested for their ability to oxidize \( N-(2\text{-hexylamino}-4\text{-phenylimidazol-1-yl})\text{-acetamide} \) to imidazol-2-yl amino acids applicable for pharmaceutical purposes. After growth with \( n\text{-alkane} \), 15 strains formed different imidazol-2-yl amino acids identified by chemical structure analysis (mass and nuclear magnetic resonance spectrometry). High yields of imidazol-2-yl amino acids
were produced by the strains *Gordonia rubropertincta* SBUG 105, *Gordonia terrae* SBUG 253, *Nocardia asteroides* SBUG 175, *Rhodococcus erythropolis* SBUG 251, and *Rhodococcus erythropolis* SBUG 254. Biotransformation occurred via oxidation of the alkyl side chain and produced 1-acetylamino-4-phenylimidazol-2-yl-6-aminohexanoic acid and the butanoic acid derivative. Some substituted imidazoles were found to have antifungal activities, while various 1-substituted imidazoles have anticonvulsant properties.

### 6.3. Materials and Methods

#### 6.3.1. Test samples

The samples prepared by successive solvent extraction with petroleum ether, ethyl acetate and methanol from metabolites of *Bacillus cereus* (BC-1 to BC-3) and *Bacillus pumilus* (BP-1 to BP-3) were used as test samples.

#### 6.3.2. Selection and preparation of experimental animals

Selection and preparation of animals for experiments were done as per the procedure mentioned in chapter 5 (5.3.3.3).

#### 6.3.3. Evaluation of CNS depressant activity using photoactometer

Most of the CNS acting drugs influence the locomotor activities in man and animals. The drugs can increase or decrease spontaneous motor activity (SMA). The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamines can increase the activity. So, the locomotor activity can be an index of alertness of mental activity.

The locomotor activity can be easily measured using a photoactometer. The instrument consists of a transparent cage which is $25 \times 48 \times 18 \text{ cm}^3$ in size. It has a wire mesh at the bottom. Six lights and six photo cells are placed in the outer
Technically its principle is that a photo cell is activated when the rays of light falling on photo cells are cut off by animals crossing the beam of light. The photo cells are connected to an electronic automatic counting device which counts the number of “cut offs”.

In this experiment the normal locomotor activity of mice (control), reduction in locomotor activity of the mice after administration of standard drug (Diazepam) and test samples were measured using photoactometer.

Healthy young swiss albino mice weighing between 25-30 grams were used. The selected animals were divided into eight groups of six animals each. Before the administration of the test samples, standard and control, the basal activity score for all the animals were recorded using photoactometer. The groups 1, 2, and 3 received the sample extracts of *B. cereus* BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of *B. pumilus* BP-1, BP-2 and BP-3 respectively. All the samples were prepared in sterile water at a dose of 50mg/kg body weight. The group 7 received the standard drug Diazepam (Provizer Pharma, Mumbai) at 5 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the locomotor activity was observed. The scores were recorded for all the animals and percentage change in locomotor activity was calculated by the following formula:

\[ \text{Change in motor activity} = \frac{(A-B)}{A} \times 100 \]

Where;

A: Basal score, B: Score after treatment, (Kulkarni, 1999)
6.3.4. Evaluation of skeletal muscle relaxant activity

One of the important pharmacological actions of antianxiety agents is muscle relaxing property. The benzodiazepine classes of drugs are the best examples. They reduce the anxiety and tension by skeletal muscle relaxation along with calming effect. The loss of muscle-grip is an indication of muscle relaxation. This effect is studied by placing the animals on rotating rods. The difference in the fall off time from the rotating rod between the control and diazepam-treated animal (standard) is taken as an index of muscle relaxation. Similarly, the samples are also compared with control and standard.

Healthy young swiss albino mice weighing between 25-30 grams were used. The mice were placed on a horizontal wooden rod (diameter 3cms) that was 50 cms above the bench in order to discourage the animals from jumping off the roller. The rod was set for a rotation of 20 revolutions/minute. After the preliminary runoff naive animals, those that did not remain on the rod for 5 consecutive minutes were discarded. The selected animals were divided into eight groups of six animals each. The groups 1, 2, and 3 received the sample extracts of \textit{Bacillus cereus} BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of \textit{Bacillus pumilus} BP-1, BP-2 and BP-3 respectively. All the samples were prepared in sterile water at a dose of 50mg/kg body weight. The group 7 received the standard drug Diazepam at 5 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the mice were placed on the rotarod. The time taken for each mouse to fall off the rotarod was recorded as the endurance time. The percentage decrease in time spent on rotarod was calculated (Yasuda \textit{et al.}, 2005).
6.3.5. Evaluation of anticonvulsant activity by Maximal Electro-Shock (MES) induced convulsions

Different types of epilepsies can be induced in laboratory animals. The grand mal, petit mal or psychomotor types are examples and either rats or mice can be used. The convulsions in mice can be induced by giving high voltage current near the brain or by using suitable CNS stimulating chemicals (Chemo-convulsions e.g. Pentylenetetrazole).

In MES convulsions, electric shock is applied through the corneal electrodes (or ear electrodes) and through optic stimulation cortical excitation is produced. The MES-convulsions are divided into five phase such as tonic flexion, tonic extensor, clonic convulsions, stupor, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES-convulsions (Yadav et al., 2010).

The electro-shock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by antiepileptic but also by other centrally active drugs (Rao et al., 2002, Vogel, 2002).

Healthy young swiss albino mice weighing between 25-30 grams were used. Before the administration of the test samples, standard and control, the mice were first tested by giving current of 80 mA for 0.2 seconds using electro-convulsiometer. Those animals which showed characteristic course of convulsions were selected for experiment. The selected animals were divided into eight groups of six animals each. The groups 1, 2, and 3 received the sample extracts of *B. cereus* BC-1, BC-2 and BC-3 respectively. Similarly, groups 4, 5 and 6 received the sample extracts of
B. 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 7 received the standard drug phenytoin (Taj Pharmaceuticals Company, Raigarh, Maharashtra, India) at 25 mg/kg body weight. The control group received plain water of 1ml each (group eight). After 1 hour of administration of the standard drug and the test samples the electric shock was induced. The different phases of convulsions i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery time or death were observed. The time (seconds) spent by the animals in each phase was recorded. The percentage protection provided by the standard and test samples was calculated (Rollas et al., 2007).

6.4. Results

6.4.1. Locomotor activity

*Bacillus cereus*

Among the *B. cereus* samples, the sample BC-3 administered mice showed reduced locomotor activity with a score of 251±12.23 in 10 minutes. The scores were taken after 1 hour of administration of the drug. The score recorded before treatment was 600±2.25. This score amounts to 58.1% reduction in locomotor activity in comparison with control.

The sample BC-1 with a score of 300±4.78 (575±1.52 before treatment) and BC-2 with a score of 350±7.25 (590±4.87 before treatment) exhibited 47.82% and 40.67% reduction in locomotor activity respectively. The standard drug (Diazepam) administered mice exhibited only 75 scores (610 before treatment), thereby showing 87.05% reduction in locomotor activity.
Bacillus pumilus

Among the *B. pumilus* samples, the BP-3 administered mice showed reduced locomotor activity with a score of 250±8.89 (550±2.23 before treatment) in 10 minutes. This shows 54.54% reduction in locomotor activity in comparison with control. The samples BP-2 with a score of 310±7.23 (575±2.32 before treatment) and BP-1 with a score of 300±5.35 (550±3.25 before treatment) showed 46.08% and 45.45% reduction in activity respectively.

The activity of all the test samples were comparatively less with that of standard which exhibited 87.05% reduction in locomotor activity.

The dose administered the scores of mice before and after administration of standard and test samples, the percentage reduction in locomotor activity of BC and BP test samples are shown in table 6.1. The histogram of BC test samples showing percentage decrease in locomotor activity in comparison with standard is shown in figure 6.1. The histogram of BP test samples showing percentage decrease in locomotor activity in comparison with standard is shown in figure 6.2.
Table 6.1. CNS depressant activity of *Bacillus cereus* and *Bacillus pumilus* metabolites in mice using Photoactometer

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg body weight</th>
<th>Mean of locomotor activity scores in 10 min</th>
<th>Percentage reduction in locomotor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>-</td>
<td>555±0.96</td>
<td>560±0.36</td>
</tr>
<tr>
<td>Diazepam (Standard)</td>
<td>5mg</td>
<td>610±1.36</td>
<td>75±12.69**</td>
</tr>
<tr>
<td>BC-1</td>
<td>50mg</td>
<td>575±1.52</td>
<td>300±4.78**</td>
</tr>
<tr>
<td>BC-2</td>
<td>50 mg</td>
<td>590±4.87</td>
<td>350±7.25**</td>
</tr>
<tr>
<td>BC-3</td>
<td>50 mg</td>
<td>600±2.25</td>
<td>251±12.23**</td>
</tr>
<tr>
<td>BP-1</td>
<td>50 mg</td>
<td>550±3.25</td>
<td>300±5.35**</td>
</tr>
<tr>
<td>BP-2</td>
<td>50 mg</td>
<td>575±2.32</td>
<td>310±7.23**</td>
</tr>
<tr>
<td>BP-3</td>
<td>50 mg</td>
<td>550±2.23</td>
<td>250±8.89**</td>
</tr>
</tbody>
</table>

Values are mean ±SEM, n=6, ** p<0.001 significant (compared to control)

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
Figure 6.1. The percentage decrease in locomotor activity of *Bacillus cereus* (BC) test samples in comparison with standard.

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

Figure 6.2. The percentage decrease in locomotor activity of *Bacillus pumilus* (BP) test samples in comparison with standard.

BP – *Bacillus pumilus*, BP-1 Petroleum ether extract; BP-2 Ethyl acetate extract; BP-3 Methanol extract
6.4.2. Skeletal muscle relaxant activity

*Bacillus cereus*

Among the *B. cereus* samples, the test sample BC-3 showed significant skeletal muscle relaxant activity in mice with a fall off time of 365±16.23 seconds when compared to 775±20.22 seconds fall off time before treatment. This shows 52.90% decrease in time spent by the mice on revolving rod when compared with control. The standard drug (Diazepam) administered mice exhibited fall off time of 125±7.68 (725±2.73 before treatment), thereby showing 82.75% reduction in time spent on rotarod.

The test samples BC-1 and BC-2 with a fall off time of 420±18.20 seconds (800±7.59 before treatment) and 452±15.24 seconds (770±18.2 before treatment) exhibited 47.5% and 41.29% decrease in time when compared to control.

*Bacillus pumilus*

The BP-3 test sample exhibited skeletal muscle relaxation in mice with a fall off time of 350±2.36 seconds when compared to 755±16.25 seconds recorded before treatment. This shows 53.64% decrease in time in comparison with control. The sample BP-2 and BP-1 with a fall off time of 410±22.22 seconds (775±5.65 before treatment) and 385±19.93 seconds (720±8.2 before treatment) exhibited 47.09% and 46.52% decrease in time spent on rotarod when compared to control.

All the test samples showed less skeletal muscle relaxation in comparison to standard which exhibited 82.75% activity.

The dose administered, the fall off time of mice before and after administration of standard and test samples, the percentage reduction in time spent on the rotating rod of BC and BP test samples are shown in table 6.2. The histogram
of BC test samples showing percentage decrease in time spent on rotarod in comparison with standard is shown in figure 6.3. The histogram of BP test samples showing percentage decrease in time spent on rotarod in comparison with standard is shown in figure 6.4.

**Table 6.2. Evaluation of skeletal muscle relaxant activity of Bacillus cereus and Bacillus pumilus metabolites in mice using Rotarod**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg body weight</th>
<th>Fall off time (sec)</th>
<th>Percentage decrease in time spent on rotarod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>-</td>
<td>750±8.73</td>
<td>730±7.7</td>
</tr>
<tr>
<td>Diazepam (standard)</td>
<td>5mg</td>
<td>725±2.73</td>
<td>125±7.68**</td>
</tr>
<tr>
<td>BC-1</td>
<td>50mg</td>
<td>800±7.59</td>
<td>420±18.2**</td>
</tr>
<tr>
<td>BC-2</td>
<td>50mg</td>
<td>770±18.2</td>
<td>452±15.24**</td>
</tr>
<tr>
<td>BC-3</td>
<td>50mg</td>
<td>775±20.22</td>
<td>365±16.23**</td>
</tr>
<tr>
<td>BP-1</td>
<td>50mg</td>
<td>720±8.2</td>
<td>385±19.93**</td>
</tr>
<tr>
<td>BP-2</td>
<td>50mg</td>
<td>775±5.65</td>
<td>410±22.22**</td>
</tr>
<tr>
<td>BP-3</td>
<td>50mg</td>
<td>755±16.25</td>
<td>350±2.36**</td>
</tr>
</tbody>
</table>

Values are mean ±SEM, n=6, ** p<0.001 significant (compared 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
Figure 6.3. The percentage decrease in time spent on rotarod of *Bacillus cereus* samples in comparison with standard

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

Figure 6.4. The percentage decrease in time spent on rotarod of *Bacillus pumilus* samples in comparison with standard.

**BP** – *Bacillus pumilus*; BP-1 Petroleum ether extract; BP-2 Ethyl acetate extract; BP-3 Methanol extract
6.4.3. Anticonvulsant activity

**Bacillus cereus**

All the test samples of *B. cereus* (BC-1 to BC-3) failed to show any significant anticonvulsant activity. A maximum of 19.92% protection was shown by BC-3 sample in comparison with control. This percentage of protection is far less when compared to 90.90% protection exhibited by the standard. The test samples BC-1 and BC-2 exhibited 13.55% and 10.82% protection respectively.

**Bacillus pumilus**

The test samples of *B. pumilus* also failed to show any significant anticonvulsant activity with a maximum of 18.10% protection shown by BP-3 sample in comparison with control. The samples BP-2 and BP-1 exhibited 10.82% and 4.45% protection respectively.

The duration spent at different phases of convulsions and percentage protection obtained for all the test samples and standard are shown in table 6.3.
Table 6.3. Anticonvulsant activity of *Bacillus cereus* and *Bacillus pumilus* metabolites by Maximal Electro-Shock (MES) method

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg body weight</th>
<th>Duration in various phases (time in seconds)</th>
<th>Recovery or death</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flexion</td>
<td>Extensor</td>
<td>Clonic</td>
</tr>
<tr>
<td>Control (water)</td>
<td>---</td>
<td>2.29±0.17</td>
<td>10.99±0.21</td>
<td>5.50±0.15</td>
</tr>
<tr>
<td>Phenytoin (Standard)</td>
<td>25 mg</td>
<td>1.28±0.95</td>
<td>1.0±0.50</td>
<td>1.50±0.90</td>
</tr>
<tr>
<td>BC-1</td>
<td>50 mg</td>
<td>3.35±0.45</td>
<td>9.50±0.55</td>
<td>3.0±0.45</td>
</tr>
<tr>
<td>BC-2</td>
<td>50 mg</td>
<td>2.50±0.34</td>
<td>9.95±0.35</td>
<td>3.5±0.91</td>
</tr>
<tr>
<td>BC-3</td>
<td>50 mg</td>
<td>2.20±0.12</td>
<td>8.80±0.75</td>
<td>3.60±0.75</td>
</tr>
<tr>
<td>BP-1</td>
<td>50 mg</td>
<td>3.44±0.34</td>
<td>10.50±0.86</td>
<td>4.40±0.88</td>
</tr>
<tr>
<td>BP-2</td>
<td>50 mg</td>
<td>4.50±0.48</td>
<td>9.80±0.45</td>
<td>4.20±0.50</td>
</tr>
<tr>
<td>BP-3</td>
<td>50 mg</td>
<td>2.50±0.86</td>
<td>9.00±0.20</td>
<td>2.55±0.65</td>
</tr>
</tbody>
</table>

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

6.5. Discussion

The plant and animal based metabolites are known to have bioactive compounds and literatures are available on plant and animal originated metabolites with CNS depressant activities. They are in use to treat diseases since centuries (Yasuda *et al.*, 2005, Fernandez *et al.*, 2006). The reports on pharmacologically active metabolites of microbial origin are comparatively less. Most of the studies reported of microbial origin are on antimicrobial, anticancer, immunomodulatory, anti-inflammatory, antioxidant, enzyme inhibitors and antiparasitic activities (Demain, 1999; Kelecom, 2002; Lam 2006). But very less information is available regarding evaluation of microbial metabolites acting on CNS. Moreover, microbial metabolites with anticonvulsant activity are seldom reported.
6.5.1. Microbial metabolites having neuroactive compounds

Among the available literatures on microbial metabolites, some are neuroactive compounds and some are depressants. *Antarticum vesiculatum* and *Psychroserpens burtonensis*, the two bacteria obtained from antartic region are known to produce neuroactive compounds (Ballal *et al.*, 2007) and Komadoquinone A, a neuritogenic compound has been isolated from *streptomyces* species (Itoh *et al.*, 2003). “Lactacystin”, a low molecular weight metabolite with neurotrophic factor like activity which would be useful to treat patients suffering from neurological diseases has been reported by Barde (1989).

6.5.2. CNS depressant activity

The present studies on bacteria were performed with an aim to detect the possible CNS depressant action of *B. cereus* and *B. pumilus* metabolites. The motive behind to carryout CNS depressant activity was the signs and symptoms of CNS depression shown by the animals during determination of LD$_{50}$ studies. The LD$_{50}$ studies are mandatory and also essential for fixation of the test dose before carrying any pharmacological studies on animal models.

In the present studies the successive solvent extracts of metabolites obtained from *B. cereus* and *B. pumilus* were tested for CNS depressant activity by locomotor scores using photoactometer, skeletal muscle relaxant activity by rotarod method and anticonvulsant activity by maximal electro-shock method. The study revealed that *B. cereus* metabolites is having little better CNS depressant activity with 58.10% depression when compared to *B. pumilus* metabolites which showed 54.54% CNS depressant activity. Regarding skeletal muscle relaxation, the *B. pumilus* metabolites have shown more muscle relaxant activity with 53.64% decrease in time...
on rotarod when compared to 52.90% exhibited by \textit{B. cereus}. However, none of the test samples exhibited any significant anticonvulsant activity.

A mycotoxin obtained from \textit{Penicillium nigricans} with CNS depressant activity has been reported by Chaudhuri \textit{et al} (2008). Among bacterial metabolites, the ethyl acetate extracts of four bacterial strains obtained from marine source have exhibited CNS depressant activity (Ramasamy \textit{et al}., 2009). Kamat and Kerkar, (2011) have reported bacterial metabolites with antianxiety and antidementia activity. Jebasingh and Murugan (2012) have reported CNS depressant activity for chloroform extracts of \textit{Bacillus megaterium} associated with cone snail and \textit{pseudomonas aeroginosa} from tubeworm, both obtained from marine source.

The CNS depressant activity reported by Chaudhuri \textit{et al}., (2008) is a fungal metabolite. The bacterial metabolites having CNS depressant activity reported by Ramasamy \textit{et al}., (2009) Kamat and Kerkar (2009), Kekuda \textit{et al}., and Nail \textit{et al}., are strains of different species and none of them belongs to the genus \textit{Bacillus}. The two bacteria reported in the present studies with CNS depressant activity belongs to the genus \textit{Bacillus}, i.e. \textit{B. cereus} and \textit{B. pumilus}. Jebasingh and Murugan, have reported CNS depressant activity on bacteria from the same genus but on a different species, \textit{Bacillus megaterium}. It was marine isolate and the extracts have shown CNS depressant activity with a reduction of 92% in locomotor activity and found to be more significant than the present isolates studied.

6.5.3. Antibiotics and smooth muscle relaxation

The antibiotics like Dicloxacillin, Aminobenzylpenicillin, Chloramphenicol, and Spiramycin have reported to have smooth muscle relaxation activity (Omura 1992). The metabolites of the two bacteria tested in the present study were originally
isolated from soil samples during screening for antibacterial activity and proved to be good antibiotic producers (Kumar et al., 2013). The antibiotic fraction in the metabolite may be responsible for smooth muscle relation and depressant activity.

An aromatic amino acid obtained from *Pseudoalteromonas rubra* is known to have myorelaxant properties (Ballal et al., 2007). Some of the well known antibiotics are peptide antibiotics.

The crude extracts obtained from *Streptomyces* species has been reported to have dose dependant CNS depression. Many species among *Streptomyces* are well known for their antibiotic production (Kekuda et al., 2013).

### 6.5.4. Anticonvulsant activity

Compounds having sedative action may also exhibit anticonvulsant activities (Du et al., 2002). The sedative activity exhibited by *B. cereus* and *B. pumilus* metabolites prompted us to carryout anticonvulsant activity. Many literatures are available on bioactive compounds obtained from plant and animal sources with anticonvulsant activity (Monks et al., 2002; Venkateswarulu et al., 2012; Vinitraj, 2012). Isatin derivatives of natural origin (both plant and animal) have been reported to exhibit considerable pharmacological actions such as anticonvulsant, antianxiety and psychoactive activity. They are found in plants of the genus *Isatis, Calanthe discolor*, etc, which are also found as a component of the secretion from the paratid gland of *Bufo* frogs (Wei et al., 1982; Guo et al., 1986; Yoshikawa et al., 1998). Similar kind of isatin derivative 6-(3'-methylbuten-2'-yl) has been reported from bacteria *Streptomyces albus* (Grafe et al., 1986). “Pimprinine” an extracellular alkaloid produced by *Streptomyces* species has been reported to have anticonvulsant
activity by MES test in mice. It is also reported to inhibit effectively tremorine-induced tremors and analgesia in mice (Naik et al., 2001).

In the present studies the metabolites of *B. cereus* and *B. pumilus* tested for CNS depressant activity and skeletal muscle relaxant activity have shown promising results whereas the metabolites exhibited poor anticonvulsant activity. The CNS depressant, skeletal muscle relaxant and anticonvulsant activities for metabolites of these two bacteria have 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 these activities for these two bacteria.

6.6. Conclusion

The present study indicated the potential CNS depressant as well as muscle relaxant activity for both *B. cereus* and *B. pumilus* metabolites. The metabolites can be further tested for other activities like antianxiety, induction of relaxation, and sleep. Further purification and identification of the active compounds responsible for sedative activity and exploration of the chemical structures of the same can lead to potentially useful compounds of biomedical importance. Howeyer, none of the test samples exhibited any significant anticonvulsant activity.