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Introduction
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Section A

1.1 Antibacterial proteins

Antibacterial proteins are the natural antibiotic biomolecules that kills or slow down the growth of bacteria. However, with increased knowledge of the causative agents of various infectious diseases, led us to investigate synthetic antibiotics such as broad range of antimicrobial and antifungal substances. Bacteria have evolved abundant protection mechanisms against antimicrobial agents, which are resistant to old and current available drugs (Milton et al., 1997, Nizet, 2006). The increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics would render current antimicrobial agents insufficient to control bacterial diseases. As a result many investigators started to find alternate remedy from their sufferings (Zakir et al., 2002).

Plenty of plants and animals extracts owe their antibacterial potency to the presence of bioactive protein substances, and they have become very good resources for developing novel antibacterial drugs to replace current inefficient antibiotics. Many antibacterial proteins show a remarkable specificity against wide range of pathogenic microbes with low toxicity for eukaryotic cells; a phenomenon which has favoured their investigation and exploitation as potential new drugs (Zasloff, 1992). Besides commercial or chemotherapeutic value, antibacterial peptides provide a useful way of assessing innate immunity at the biochemical and molecular level. This finds relevance in invertebrates, where they lack acquired memory-type immunity based on clonally derived immunoglobulins or T lymphocyte subsets. Antibacterial proteins have been isolated from a wide variety of invertebrate phyla, including insects (Boman, 1995), ascidians (Azumi et al., 1990 and Lee et al., 1997), chelicerates (Nakamura et al., 1988), annelids (Milochau et al., 1997) and mollusks (Hubert & Roch, 1996), as these proteins were
involved in providing protection against foreign invading microorganism (Smith & Chisholm, 1992).

1.2 Antibacterial proteins isolated from various sources

Since many centuries antibacterial proteins were originated from different plants and animals sources, were used for the treatment of various diseases caused by pathogenic bacteria. It is obvious that human beings are susceptible to infectious diseases. The external want of human being is to remain healthy and cure from his surroundings. But in the ancient era, millions of people died from various infectious diseases like plague, cholera, diarrhea, tuberculosis etc. In epidemic form, this instigated the man to endeavor for remedy from their sufferings (Zakir et al., 2002). Plenty of plants and animals extracts owe their potency to the presence of bioactive proteins (substances). As a result, they would become very good sources for developing innovative antibacterial drugs for pharmaceutical industry. Traditional medicine was an excellent source for bioactive agents which could be applied in the preparation of medicine (Aboba et al., 2006). These medicines could be used as life-saving drugs. Increasing in failure of chemotherapeutic and antibiotic resistance activity exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plant and animal extract for their potent antimicrobial activity. There are several reports mentioned regarding the antimicrobial activity of crude extracts obtained from plant and animal species (Belboukhari and Cheriti, 2005; Charu et al., 2008). A monomeric protein with molecular mass of 35 kDa, was isolated from Murraya Koenigii L (curry leaves) has found to be a potent antibacterial activity. The protein was further designated as antioxidant protein from curry leaves (APC) (Mylarappa et al., 2009). Zheng et al., (2010) isolated novel
antibacterial protein with a molecular mass of 44 kDa from dried fruiting bodies of the wild mushroom *Clitocybe sinopica*. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis showed that the protein was composed of two subunits each with a molecular mass of 22 kDa. Chellapandi and Jebakumar (2008) purified proteins with low molecular masses ranged from 10-17 kDa which was resembled to phospholipase A2 of snake venoms. A biofilm-forming marine bacterium, D2, isolated from the surface of the tunicate *Ciona intestinalis*, was found to produce a novel, 190 kDa antibacterial protein. The protein contained at least two subunits of 60 and 80 kDa, joined together by noncovalent bonds, and was shown to be released by D2 cells into the surrounding medium during stationary phase. N-terminal sequence analysis of that revealed no close similarity of this protein to any other proteins within the Swiss Prot database (Sally et al., 1996). Roshan and Kudumula (2009) have been identified and characterized 11 kDa antimicrobial protein from Granular hemocytes of Indian mud crab, *Scylla serrata*, this protein was highly similar to scygonadin, a male-specific AMP isolated from the ejaculatory duct of *S. serrata*. Suresh et al., (2003) engrossed antibacterial properties of the sperm binding proteins and peptides of *Human Epididymis 2 (HE2)* family. Suguru et al., (1990) purified and determined potent antibacterial protein in royal jelly, the primary structure of royalisin was determined to consist of 51 residues, with three intramolecular disulphide linkages, having a calculated molecular mass of 5523 Da. Wen Shu et al., (2006) were reported novel protein 10.8 KDa exhibited no significant homology to any other antimicrobial peptides were isolated from the seminal plasma of the mud crab.
1.3 Antibacterial / Antiviral proteins isolated from silkworm sources

Silkworm *Bombyx mori* was known to have both cellular and humoral immune system which together forms a potent defense mechanism against invading bacteria (Abraham *et al.*, 1995; Boman and Hultmark, 1987; Gotz and Boman, 1985; Dunn, 1986; Kimbrell, 1991). Literature survey pertaining to antibacterial proteins revealed that the proteins from non-mulberry silkworms against *Pseudomonas aeruginosa* AC-3 causing disease flacherie (Sharma *et al.*, 2005) and induced antibacterial protein in Tasar silkworm, *Antheraea mylitta* (Jain *et al.*, 2001) were reported. Silkworm *Bombyx mori* is an economically important insect; host for different pathogenic microorganisms (Abraham *et al.*, 1995). Geographically differentiated genotypes of silkworm were known to show varying degree of tolerance to different pathogens (Chitra *et al.*, 1975), although earlier investigations have been characterized Cecropin–like antibacterial proteins (Morishima *et al.*, 1990) and lysozyme (Powning and Davidson 1973), but to what extent they involved in humoral response in silkworm remains to be understood. It was found that these worms are naturally resistant to some strains of bacteria. Therefore, there might be some proteins present or generate in the worms that could be either bactericidal / bacteriostatic in nature. Tae Won Goo *et al.* (2008) were engrossed *Bombyx mori* protein disulfide isomerase which could enhance the production of nuecin, an antibacterial protein. Masao and Morishima 1993 synthesized bactericidal protein (cecropin) and lysozyme were induced by soluble peptidoglycan fragments (SPG) from *Escherichia coli* in a culture of fat body from *Bombyx mori* larvae. Morishima *et al.*, (1992) have been observed various bacteria were tested for elicitor of anti-bacterial protein synthesis in *Bombyx mori* larvae by injecting formalin-killed cells into the hemocoel.
Morishima et al. (1990) have been isolated cecropins inducible antibacterial peptides, from the silkworm, *Bombyx mori*. Sumida et al. (1991) found antibacterial activity in the Haemolymph of silkworm, *Bombyx mori* by injecting formalin-treated *Escherichia coli* K-12 in the anterior and posterior body part of the ligated larvae. Ganesh et al. (2008) extracted midgut protein P252 from *Bombyx mori*, which was binds to *Bacillus thuringiensis* Cry1A. Further it was concluded that Chlorophyllide-Binding protein, and the resulting complex has antimicrobial activity. Attacins were antibacterial proteins synthesized by pupae of the giant silk moth, Hyalophora cecropia in response to a bacterial infection. Immune upregulation of novel antibacterial protein from silkmoths (Lepidoptera) that resemble lysozymes but lack muramidase activity (Archana et al., 2007). Few high molecular weight about 60 kDa proteins from gut juice of silkworm larvae, have been reported to precipitate nuclear polyhedrosis virus (NPV) *in vitro*. (Uchida et al., 1984) The high molecular weight substances from the gut juice of silkworm were found to inactivate nuclear polyhedrosis virus *in vitro*; anti NPV of red fluorescent protein (RFP) of 60 kDa was reported (Mukai et al., 1969). Suzuki (1936) and Aizawa (1962) investigated the inhibitory and antiviral properties of digestive juice of *Bombyx mori* larvae and reported that an unknown substance of high molecular weight in the gut juice could inactivate nuclear polyhedrosis virus *in vitro*. Hayashiya et al., (1968, 1976) have been reported the agglutination reaction of NPV and RFP, similar agglutination reaction was also found with flacherie virus (FV) of *Bombyx mori* but not between tobacco mosaic virus (TMV) and RFP.
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1.4 Antiviral/Antibacterial protein from silkworm fecal matter

Silkworm fecal matter is a natural substance and it was known to be one of the richest sources of antibacterial and antiviral substances (Hiraki et al., 1997; Da et al., 2002; Neelagund et al., 2007). The protein with molecular mass of about 530 Da purified from silkworm Bombyx mori fecal matter was reported to act as an antiviral against viruses such as human immunodeficiency virus (HIV), Sendai virus (HVJ), herpes simplex virus type-1 (HSV) (Hiraki et al., 1997). Further the dimer protein of 16 KD and 23 KD was purified and characterized an anti nuclear polyhedrosis virus (NPV) protein from silkworm fecal matter (Neelagund et al., 2007). Hirayama et al., (1993) have also reported an antiviral protein from silkworm fecal matter and reported it to be a glycoprotein. Antiviral protein (AVP) was observed to inactivate many types of cells in suspension (for instance, QMRSV cells). The protein was found to recognizes mannose on cell surface leading to aggregation. Red fluorescent proteins (SE-RFP) were reported to be 298 Da and 1100 Da from silkworm fecal matter having light-dependent activity against Bombyx mori nucleopolyhedrovirus (BmNPV). Light was observed to be essential also for the SE-RFP synthesis as it was produced only when silkworms were reared in light and also exhibited a potent broad spectrum antimicrobial activity against pathogenic bacteria and fungi was evaluated by agar dilution technique (Kalyankumar et al., 2009, 2010).

1.5 Antioxidant and Hepatoprotective Proteins

Silkworm Bombyx mori L fecal matter was investigated to having beneficial effects against some pathophysiological status. A number of reports revealed that protein molecules from various plant sources possess antioxidant and hepatoprotective protein.
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(Tsoi & Fong, 2005; Oh et al., 2006; Lee et al., 2006). Protein from Cajanus indicus Spreng protects liver and kidney against mercuric chloride-induced oxidative stress (Ayantika and Parames, 2008). Hepatoprotective effects of whey protein on D-Galactosamine-induced hepatitis and liver fibrosis in rats (Hisae et al., 2006). Sarkar et al., 2006 purified and characterized 43 kDa hepatoprotective protein from the herb Cajanus indicus L. Manna et al., (2007) isolated 43 kDa protein which was partially sequenced from the leaves of this herb Cajanus indicus L. Attenuates sodium fluoride-induced hepatic and renal disorders in vivo. Ayantika et al., (2006) absorbed protective effect of a 43 kDa protein from the leaves of the herb, Cajanus indicus L on chloroform induced hepatic disorder and the same 43 kDa protein showed anti-oxidative effect of a protein from Cajanus indicus L against acetaminophen-induced hepatonephro toxicity (Ayantika and Parames, 2007). Protein from silkworm feces was used as folk medicine in Korea and China, it was evaluated for pharmacological and phytochemical parameters to better understand their pathophysiological actions. This implied that the silkworm fecal matter has high medicinal value (Hirayama et al., 1993; Zhang et al., 2008). In particular, widespread use of silk protein from silkworm bombyx mori fecal matter as a medicine, even though there are no scientific studies carried out pertaining to antihepatotoxic and antioxidant properties.

1.6 Biochemical composition of antibacterial / antiviral proteins from silkworm Bombyx mori fecal matter

Biochemical compositions of antibacterial / antiviral proteins were analyzed to be glycoproteins / lipoproteins / simple proteins with / without chlorophyll derivatives associated with them (Spikes et al., 1983, Willem, 1997). Silkworm excrement along with
the other substances contains glycoprotein and chlorophyll derivatives. The antibacterial and antiviral activity has been reported due to the presence of chlorophyll-like substances (Drazenick et al., 1971, Hiraki et al., 1996 and 1997, Lewin et al., 1980, Hirayama et al., 1993, Neelagund and Hinchigeri, 2011).

a) Glycoprotein

Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side-chains. The carbohydrate part is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation. In proteins that have segments extending extracellularly, the extracellular segments are often glycosylated. Glycoproteins are often important integral membrane proteins, where they play a role in cell–cell interactions. Glycoproteins also occur in the cytosol, but their functions and the pathways producing these modifications in this compartment are less understood.

During the very last years, the importance of antibacterial glycoprotein has attracted considerable attention towards Beta-2 glycoproteins I (b2GPI), also known as apolipoprotein a human heparin binding plasma protein, have antibacterial activities against Gram-positive and Gram-negative bacteria (Maria et al., 2008).

b) Identification of glycoprotein

There are many methods for the identification of carbohydrate containing biomolecules. The direct method for detecting the glycoprotein on electrophoresed gel is thymol sulfuric acid method (Racuse, 1979). The method is quite sensitive and therefore, it is generally used to detect / identify glycoproteins on the PAGE gels (Venkatesh, 1998).
Lectin blotting assay method was also used for detecting the glycoproteins on the PAGE gels (Hirayama et al., 1993 and Hiraki et al., 1996).

c) Tetrapyrrole pigments

Tetrapyrroles are natural products which are essential for organic life in the biosphere. Haems are involved in oxygen and electron transport, chlorophylls and bacteriochlorophylls catalyze the conversion of solar energy to chemical energy, and Vitamin B_{12} participates in important biochemical reactions. As a consequence, tetrapyrole chemistry and biochemistry have attracted research talent for over a hundred years (Constantin et al., 1994). One of the most important sources of chlorophylls and their derivatives has been silkworm excrement, which could be supplied in large quantities in southern parts of China (Longqin and Deyu, 1989). The earlier workers isolated many antiviral proteins from sources containing chlorophyll and reported that the antiviral activity was due to the presence of chlorophyll like substance associated with a glycoprotein (Drzenick et al., 1971; Lewin et al., 1980; Hiraki et al., 1996; Hiraki et al., 1997; Neelagund and Hinchigeri, 2011). These substances have shown clear antiviral activity against HVJ, HSV and antiviral activity against selected human viruses (Hiraki et al., 1997). The digestive fluid of silkworms fed mulberry leaves contains virus-inactivating substances (Suzuki, 1936). No virus-inactivating substance has been detected in the gut fluid of silkworms fed an artificial diet devoid of mulberry components, suggesting that mulberry leaf components play an important role in the in vivo synthesis of red fluorescent protein (RFP) (Hou and Chiu, 1986). Hayashiya et al., 1969; Hayashiya, 1976; observed that light is essential in the synthesis of RFP in silkworm, Bombyx mori L, fed mulberry leaves. RFP has been detected in the digestive fluid of
anterior part of the midgut of silkworms and it was not detected in the hemolymph (Nishida et al., 1973). Their antiviral activity was reported to be dependent on light intensity and temperature. Furthermore, it was also found to possess a strong hemolytic activity under light. Hence, it was concluded that light might be necessary for the formation of the proper green pigment, and/or for causing photodynamic inactivation of the viruses (Schnipper, 1980; Spikes, 1984; Lee and Lee, 1990). Uchida et al. (1984) have been observed that the virus-inactivating protein contained in the digestive juice of the silkworm was not a glycoprotein. To understand the biochemical mechanism underlying the inactivation of NPV by RFP; it was necessary to identify the pigment portion of red fluorescent protein. Hence, the pigment moiety present in the purified RFP complex which was extracted for analysis by using spectrophotometry and spectrofluorometric methods (Neelagund and Hinchigeri, 2011).

d) Significance of tetrapyrroles

Very few studies on antibacterial / viral have been carried out with porphyrins as photosensitizers, although most viruses might be expected to be quite sensitive because they were composed of proteins, nucleic acids and in some cases lipids (Lewin et al., 1980). Enveloped viruses such as HSV and HVJ were effectively inactivated by the photodynamic effect of hemetoporphyrin derivative (HpD) (Lewin et al., 1980). Hemetoporphyrin derivative (HpD) was known to react with the lipid portion of cytoplasmic membranes of HSV and HIV (Kessel, 1977; Berenbaum et al., 1982). The antibacterial / antiviral substances from silkworm feces were reported and found to be a chlorophyll-like substance. Those substances shown clear antibacterial activities against several pathogenic bacteria (both Gram positive and Gram negative) and antiviral
activities against HVJ, HSV (Herpes Simplex Virus type-1) and nuclear polyhedrosis virus (NPV). Their mechanism of antiviral activity was reported to be dependent on light intensity and temperature. Furthermore, it was also found to possess a strong hemolytic activity under light (Hiraki et al., 1997). Antinuclear polyhedrosis activity was not observed in both individual components separated from AVP, whereas good precipitation activity was observed when protein was in intact form. Hence, it was concluded that the monovinyl pheophytin a could be the essential component for the antiviral activity of the protein (Neelagund and Hinchigeri, 2011).

1.7 Biological activity

a) Precipitation activity

b) Bioassay of antiviral activity of protein in vivo

c) Antiviral activity of protein

a) Precipitation activity

The precipitation activity was observed when purified protein was mixed with nuclear polyhedrosis virus (NPV) serial two fold dilution technique incubated and examined the precipitate under electron microscope. (Uchida et al., 1984, Hayashiya et al., 1976 and Neelagund et al., 2007).

b) Bioassay of antiviral activity of protein in vivo

The determination of antiviral activity of protein from gut juice and fecal matter was carried out in silkworms. This in vivo bioassay was performed by injecting protein along with NPV into silkworm larvae (Sethuraman et al., 1993, Uchida et al., 1984 and Neelagund et al., 2007).
c) **Antiviral activity of cells and viruses**

*Herpes simplex virus type-1* (HVJ), HVJ infection to vero cells was performed as described in Hiraki et al., 1996. Briefly, 0.2 ml of virus samples (about 50 PFU [plaque forming unit]/0.2ml) were inoculated onto Vero cells monolayers in 60 mm plates and incubated for 1 h at 37 °C. Then, the monolayers were washed twice with PBS. MEM with 10% CS containing 5% anti-HIV human serum was added to the plates. The number of plaques as HSV infection was counted at 72 h after infection.

**Section B**

1.8 **Current status of bacterial diseases**

Bacterial diseases include any type of infection caused by bacteria. Millions of bacteria normally live on the skin, in the intestines, and on the genitalia. The vast majority of bacteria do not cause disease, and many bacteria are actually helpful and even necessary for good health. These bacteria are sometimes referred to as “good bacteria” or “healthy bacteria.” Harmful bacteria that cause bacterial infections and disease are called pathogenic bacteria. Bacterial diseases occur when pathogenic bacteria get into the body and begin to reproduce and crowd out healthy bacteria, or to grow in tissues that are normally sterile. Harmful bacteria may also emit toxins that damage the body. Common pathogenic bacteria and the types of bacterial diseases they cause include:

a) *Klebsiella*

A genus of Gram-negative, nonmotile bacteria. Characteristic large mucoid colonies are due to production of large amount of capsular material. Species of *Klebsiella* are commonly found in soil and water, on plants, and in animals and humans. Harmless
strains of Klebsiella are beneficial because they fix nitrogen in soil. Pathogenic species include K. pneumonia, K. rhinoscleromatis and K. Ozaenae, also known as K. pneumonia subspecies pneumonia, rhinoscleromatis and Ozaenae.

**Occurrence and symptoms of diseases**

Klebsiella pneumonia is the second most frequently isolated colon-related bacterium in clinical laboratory. The carbohydrate-containing capsule of Klebsiella promotes virulence by protecting the encased bacteria from ingestion by leukocytes; nonencapsulated variants of Klebsiella do not cause disease. Capsular type 1 and 2 cause pneumonia; type 8, 9, 10, and 24 are commonly associated with urinary tract infections. Klebsiella accounts for large percentage of hospital-acquired infections, mostly skin infections (in immunocompromised burnt patients), bacteremia and urinary tract infections. It was most common contaminant of intravenous fluids such as glucose solutions and other medical devices (Podschun and Ullmann, 1998).

b) **Streptococcal**

Streptococcal is a bacterium often found in the throat and on the skin. People may carry groups A streptococci in throat or on the skin and have no symptoms of illness. Most Group A Streptococcus (GAS) infections are relatively mild illnesses such as “strep throat” or impetigo. Occasionally, these bacteria can cause severe and even life-threatening diseases. GAS disease may occur when bacteria get into parts of the body where bacteria usually are not found. Such as the blood, muscle, or the lungs. These infections are termed “invasive GAS disease” Two of the most severe but least common forms of invasive GAS disease are necrotizing fasciitis and streptococcal toxic shock syndrome. Necrotizing fasciitis (Occasionally described by “the media as the flesh eating
Streptococcal toxic shock syndrome (STSS) results in rapid drop in blood pressure and organs (e.g., kidney, liver, lungs) functions (Richard 2002).

d) **Staphylococcal**

Staphylococcal infections are a group of different infections that are caused by Staphylococcus bacteria. There are several types of staphylococcus bacteria, but most infections are caused by a type called Staphylococcus aureus. S. aureus is commonly found inside the nose and on the surface of the armpits and buttocks. In most cases, the bacteria do not cause any symptoms. If a person has bacteria living on their body but they do not experience any symptoms, they are said to be colonised by bacteria. In England, it was estimated that 80% of all people were colonised by S. aureus at least once in their life, and that 20-30% were persistently colonised.

**Types of Staphylococcal infections**

The different types of staphylococcal infections can be broadly classified into two groups:

- skin infections, such as boils or impetigo (skin infection that causes the skin to become crusty and itchy)
- invasive infections, such as blood poisoning or endocarditis (an infection of the heart’s lining)

Skin infections usually occur when the S. aureus bacteria invade a cut in the skin. Invasive infections can develop as a complication of a skin infection that has spread beyond the skin due to a person having a weakened immune system. Invasive infections can also occur from using medical equipment that goes inside the body, such as a feeding
tube or catheter (a tube that is used to empty the bladder). Food poisoning can occur if you eat food that was contaminated with \textit{S. aureus} bacteria. Food poisoning usually occurs as a result of eating food, usually meat, which either has not been cooked properly or has not been chilled at the right temperature (Tadayuki \textit{et al.}, 2010).

d) \textit{Bacillus subtilis}

In 1892, Ferdinand Cohn, a contemporary of Robert Koch, recognized and named the bacterium \textit{Bacillus subtilis}. The organism is Gram-positive, capable of growth in the presence of oxygen, and forms a unique type of resting cell called an endospore. The organism represented what was to become a large and diverse genus of bacteria name \textit{Bacillus}, in the family \textit{Bacillaceae}. The unifying characteristic of these bacteria is that they are Gram-positive, form endospores and grow in presence of O\textsubscript{2}. The trivial name assigned to them was \textit{aerobic sporeformers}.

There is great diversity of physiology among the aerobic sporeformers, not surprising to considering their recently discovered phylogenetic diversity. Their collective features include degradation of most all substrates derived from plant and animal sources, including cellulose, starch, pectin, proteins, agar, hydrocarbons and others; antibiotic production; nitrification; denitrification; nitrogen fixation; facultative lithotrophy; autotrophy; acidophily; alkaliophily; thermophily and parasitism. Aerial distribution of the dormant spores probably explains the occurrence of aerobic sporeformers in most habitats (Burke \textit{et al.}, 2002)

e) \textit{Pseudomonas aeruginosa}

Most of \textit{P. aeruginosa} infections occur in hospitalized patients, particularly those who are debilitated or immunocompromised. \textit{P. aeruginosa} is the 2\textsuperscript{nd} most common cause
of infections in ICUs. HIV-infected patients, particularly those in advanced stages, were at risk for community-acquired *P. aeruginosa* infections.

*Pseudomonas* infections could develop in many anatomic sites, including skin, subcutaneous tissue, bone, ears, eyes, urinary tract and heart valves. The site varies with the portal of entry and the patient's vulnerability. In hospitalized patients, the 1st sign may be overwhelming gram-negative species.

**Skin and soft-tissue infections:** In burns, the region below the eschar can become heavily infected with organisms, serving as a focus for subsequent bacteremia—an often lethal complication. Deep puncture wounds of the foot were often infected by *P. aeruginosa*. During sinuses, cellulitis, and osteomyelitis may result. Drainage from puncture wounds often has a sweet, fruity smell.

**Respiratory tract infections:** *Pseudomonas aeruginosa* is a frequent cause of ventilator associated pneumonia. In HIV-infected patients, pseudomonas most commonly causes pneumonia or sinusitis. *Pseudomonas bronchitis* is common late in the course of cystic fibrosis. These isolates have a characteristic mucoid colonial morphology. Rarely, pseudomonas causes acute bacterial endocarditis, usually on prosthetic valves in patients who have had open-heart surgery or on natural valves in drug abusers (Worlitzsch *et al.*, 2002).

*f) *Escherichia Coli*

One hundred of strains of the bacterium *Escherichia Coli* O157:H7 was an emerging cause of food borne and water borne illness. Although most strains of *E. Coli* are harmless and live in the intestines of healthy humans and animals, this strain produces
a powerful toxic and can cause severe illness. *E. coli* were first recognized as a caused if illness during an outbreak in 1982 traced to contaminated hamburgers. Since then, most infections are believed to have come from eating undercooked ground beef. *E. coli* are a type of fecal coliform bacteria commonly found in the intestines of animals and humans. *E. coli* is short for *Escherichia coli*. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage mat contain many types of diseases causing organisms (Ruiting and Reeves 2002).

g) **Salmonella typhi**

*Salmonella* a genus of rod-shaped, Gram-negative, non-spore-forming, initially, each *Salmonella* species was named according to clinical considerations, e.g., *Salmonella typhi-murium* (mouse typhoid fever), *S. cholerae-suis* (hog cholera). After it was recognized that host specificity did not exist for many species, new strains or *serovar*, short for serological variants) received species names according to the location at which the new strain was isolated. Later, molecular findings led to the hypothesis that *Salmonella* consisted of only one species, *S. enterica*, and the *serovar* were classified into six groups, two of which are medically relevant. But as this now formalized nomenclature is not in harmony with the traditional usage familiar to specialists in microbiology and infectologists (Stephen et al., 2007).

**Sources of infection**

Salmonellosis is spread to people by ingestion of *Salmonella* bacteria that contaminate food. *Salmonella* is worldwide and can contaminate almost any food type, but outbreaks of the disease involved raw eggs, raw meat (ground beef and other poorly
cooked meats), egg products, fresh vegetables, cereal, pistachio nuts, tomatoes, and contaminated water (Stephen et al., 2007).

**Section C**

1.9 **Proposed mechanism for antibacterial protein**

Most antimicrobial agents used for the treatment of bacterial infections may be categorized according to their principal mechanism of action. There are 4 major modes of action: (1) interference with cell wall synthesis, (2) inhibition of protein synthesis, (3) interference with nucleic acid synthesis, and (4) inhibition of a metabolic pathway

- **Interference with cell wall synthesis**

  Antibacterial drugs that work by inhibiting bacterial cell wall synthesis include the lactams, such as the penicillins, cephalosporins, carbapenems, and monobactams, and the glycopeptides, including vancomycin and teicoplanin. Lactam agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes required for the synthesis of the peptidoglycan layer. Vancomycin and teicoplanin also interfere with cell wall synthesis, but do so by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis (Neu, 1992 and McManus, 1997).

- **Protein synthesis inhibition**

  Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins, and oxazolidinones produce their antibacterial effects by inhibiting protein synthesis. Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells. Antibacterial agents take advantage of these differences to selectively inhibit bacterial
growth. Macrolides, aminoglycosides, and tetracyclines bind to the 30S subunit of the ribosome, whereas chloramphenicol binds to the 50S subunit (McManus, 1997).

- Interference with nucleic acid synthesis
  - Inhibit DNA synthesis: fluoroquinolones
  - Inhibit RNA synthesis: rifampin

Fluoroquinolones exert their antibacterial effects by disrupting DNA synthesis and causing lethal double-strand DNA breaks during DNA replication, whereas sulfonamides and trimethoprim (TMP) block the pathway for folic acid synthesis, which ultimately inhibits DNA synthesis (Drlica et al., 1997; Yao et al., 2003; Petri et al., 2006).

- Inhibition of metabolic pathway: sulfonamides, folic acid analogues
  The common antibacterial drug combination of TMP, a folic acid analogue, plus sulfamethoxazole (SMX) (a sulfonamide) inhibits 2 steps in the enzymatic pathway for bacterial folate synthesis (Fred and Tenover, 2006).

- Disruption of bacterial membrane structure: polymyxins, daptomycin
  Disruption of bacterial membrane structure may be a fifth, although less well characterized, mechanism of action. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents. The cyclic lipopeptide daptomycin apparently inserts its lipid tail into the bacterial cell membrane, causing membrane depolarization and eventual death of the bacterium (Storm et al., 1977; Carpenter and Chambers, 2004).
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Kinetics of bacterial inhibition

The kinetics and bacteriostatic or bactericidal nature of the antibacterial activity of bioactive protein was determined against strains by incubating the cells at a concentration of approximately $10^4 - 10^5$ cells/ml with 10 mM of the peptide in 1 X Phosphate buffer saline (PBS) at pH 6.5. Samples were withdrawn at 1, 2, 4 and 8 h post incubation with the \textit{Antimicrobial peptides / proteins (AMPs)} and plated onto Lysogeny broth (LB) agar to determine the viable colony forming units (cfu/ml). A control in which cells were incubated with equivalent amount of sterile water was also kept in parallel. For peptidoglycan (peptidoglycan) competition assay, similar experiment was carried out but bioactive protein were pre-incubated for 15 min with 32 mg of \textit{M. luteus} peptidoglycan (Sigma) and then assayed for kinetics of inhibition against strains as mentioned above (Archana \textit{et al.}, 2007).

Section D

Scope

The present investigation proves us to make well-built conformation of bioactive protein. Prose on antibacterial, analgesic, antioxidant, and hepatoprotective proteins isolated from silk worm (\textit{Bombyx mori}) fecal matter is inadequate. The study suggests that the purified bioactive protein could be productively used as an effective innate bioactive protein, which could be taken up for detailed investigation to envisage the possible mechanism of action for its bioactivity. It would also extend to achieve expression of protein in particular system to get large amount. Further, this protein could be successfully used for biopharmaceutical process.
1.11 Objectives of our work

- Isolation of antimicrobial / antiviral substances from silk worm (*Bombyx mori*) fecal matter.
- Purification of antimicrobial / antiviral substances from silkworm (*Bombyx mori*) fecal matter extracts using conventional biochemical techniques.
- Determine antimicrobial / antiviral activity of purified substances from silkworm (*Bombyx mori*) fecal matter.
- Biochemical characterization of purified antimicrobial / antiviral substances purified from silkworm (*Bombyx mori*) fecal matter.

1.12 Scope of future work

The detailed characteristic of a bioactive protein from silkworm fecal matter having major importance in facilitating the development of novel treatment for many infectious diseases. The novel bioactive protein study could be extended to investigate various pharmacological studies in clinical biochemistry. Abundance of silkworm fecal matter is available with silkworm growers economically cheaper; hence, it could be possible to isolate bioactive protein in large quantity. Biochemical characterization of novel bioactive protein, together with sequencing will provide the platform for cloning the bioactive protein gene into bacteria, leading to over expression of bioactive protein by modern biotechnology. This study could be helpful for further investigation conducting in the field of immunology, biopharmaceuticals, clinical biochemistry and proteomics etc.