1. INTRODUCTION

L-asparaginase (EC 3.5.1.1) is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid. L-asparaginases are naturally occurring enzymes expressed and produced by microorganisms. Colaspase from *Escherichia coli* in some context is also known as L-asparaginase (Rossi, 2011). Different types of asparaginases can be used for different industrial and pharmaceutical purposes. The most common use of asparaginase is as a processing aid in the manufacture of food. L-asparaginase have been used as food processing aid in order to reduce the formation of acrylamide, which is a suspected carcinogen in starchy food products such as snacks and biscuits and is marketed under the brand names Acrylaway and PreventASe (Kornbrust *et al.*, 2010). The Food and Drug Administration (FDA) has approved L-asparaginase for the effective treatment of acute lymphoblastic leukemia and lymphosarcoma. L-asparaginase is marketed as a drug under the brand name Elspar for the treatment of acute lymphoblastic leukemia (ALL) and is also used in some mast cell tumor treatment protocols (Appel *et al.*, 2007) Unlike most of other chemotherapeutic agents, it can be given as an intramuscular, subcutaneous, or intravenous injection without fear of tissue irritation.

Today, L-asparaginase used for therapeutic purposes is available in three preparations: two unmodified or native forms, both purified from bacterial sources and one form modified from one of the native preparations. The native preparations are derived from *E. coli* (marketed commercially by Merck & Co. as Elspar), or *Erwinia chrysanthemi* (*Erwinia*), (available as Erwinia L-asparaginase from Ogden BioServices Pharmaceutical Repository in the United States) for patients allergic to the *E. coli* product, The *Erwinia* product is commercially available in Canada and Europe as Erwinase, marketed by Porton. Both native preparations are approved for use in the therapy of patients in the front line and at relapse. A third preparation, PEG - L-asparaginase (nonproprietary name pegasparaginase), is a chemically modified form of the enzyme in which native *E. coli* L-asparaginase has been covalently conjugated to monomethoxy polyethylene glycol (PEG). Pegasparaginase (available commercially from Rhone - Poulenc Rorer as Oncaspar) is approved by the Food and Drug Administration for use in combination chemotherapy for the treatment of patients with acute lymphoblastic
leukemia (ALL) who are hypersensitive to native (unmodified) forms of *E. coli* L-asparaginase.

Although L-asparaginase is produced by various microorganisms (Verma *et al.*, 2007), L-asparaginase from *Er. chrysanthemi* and *Escherichia coli* are currently in clinical use (Kozak and Jurga, 2002). Unfortunately, a therapeutic response by patients rarely occurs without some evidence of toxicity (Narta *et al.*, 2007). The side effects are due to allergic responses and the use of L-asparaginases from different organisms can alleviate this problem. In addition, contamination with glutaminase is one of the causes of toxicity (Gallagher *et al.*, 1989; Manna *et al.*, 1995). Therefore, there is a need to find novel sources of the enzyme that are free of glutaminase.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and lies third as a cause of death from cancer (World Health Organization, 2013). Once rare in Western countries, HCC now is the most rapidly growing cause of cancer deaths in the USA and UK (El-Serag and Rudolph, 2007 and Cancer Research UK, 2013).

Hepatocellular carcinoma is one of the most common cancers with an incidence of 4 to 15 per 100,000 in Western countries, compared with 120 per 100,000 in Asia and Africa (Mishra *et al.*, 2009). Most cases of HCC are secondary to either a viral hepatitis infection (Hepatitis B or C) or cirrhosis (alcoholism being the most common cause of hepatic cirrhosis) (Kumar *et al.*, 2003). Although advanced research in the clinical study of HCC has been made, the prognosis remain poor, HCC is one of the leading causes of worldwide cancer mortality, with an estimated number of 1 million annual deaths and 5 - year survival rate of less than 5 per cent (Ahmad and Rabinovitz, 2007; Blum and Spangenberg, 2007).

Notably, men are about three to five times more likely to develop HCC than women (Wands, 2007). Hormonal factors are underlining the hepatocarcinogenesis. Sex disparity characteristic in HCC could be attributed by both sex hormone pathways, with distinct role in each sex (Liu *et al.*, 2009). The increased activity of estrogens in female patients might be attributed to protect them form carcinogenesis process (Liu *et al.*, 2009; Naugler *et al.*, 2007). Therefore, up - regulation of the androgen pathways in male patients is considered to accelerate liver carcinogenesis (Liu *et al.*, 2009). The prognosis for patients with HCC is poor; only 20 per cent are eligible for curative surgery upon
diagnosis at the initial stage, with limited therapeutic options for the others. The inability to make a timely diagnosis and the limited efficacy of palliative treatments for HCC contribute to the poor outcome of therapeutic cure of the disease.

The population most at risk for HCC are those with cirrhosis; the highest risk, estimated at 3 to 8 per cent per year, is associated with cirrhosis due to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection (Yuen et al., 2009; Yang et al., 2010; Lok et al., 2009). Liver diseases associated with intermediate risk include hereditary haemochromatosis (HH) (Elmberg et al., 2003), an inherited condition causing iron overload and iron deposition in the liver and other organs, non-alcoholic fatty liver disease (Stickel et al., 2010), alcohol-related liver disease (El-Serag et al., 2000) and primary biliary cirrhosis (Cavazza et al., 2009), while those with autoimmune liver disease probably have a lower risk (Wong et al., 2011).

Actinobacteria represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria, including five subclasses and 14 suborders (Stackebrandt, 2000). Marine actinomycetes have been traditionally a rich source for biologically active metabolites. Although heavily studied over the past three decades, actinomycetes continue to prove themselves as reliable sources of novel bioactive compounds. Among the well-characterized pharmaceutically relevant microorganisms, actinomycetes remain major sources of novel, therapeutically relevant natural products (Jensen, 2000). Majority of these compounds demonstrate production of one or more bioactivities, many of which have been developed into drugs for treatment of wide range of diseases in human, veterinary and agriculture sectors (Berman, 1997). Isolated compounds from marine actinomycetes has a broad spectrum of biological activities such as antibiotic, antifungal, toxic, cytotoxic, neurotoxic, antimitotic, antiviral and antineoplastic activities (Newman and Cragg, 2007).

Recently, research on L-asparaginase producing microorganisms has been focused mostly on biotechnological applications involving the preparation of glutaminase free L-asparaginase and new applications related to drug delivery against hepatocellular cancer (Tardito et al., 2011). Marine actinomycetes are a prolific source of secondary metabolites and the vast majority of these compounds are derived from the single genus Streptomyces (Das et al., 2006). Streptomyces species are distributed widely in marine
and terrestrial habitats (Pathom-aree et al., 2006) and are of commercial interest due to their unique capacity to produce novel metabolites. While most of the studies on marine Streptomyces have focused on antibiotic production, only few reports have dwelt on their enzymatic potential.

Various methods have been proposed for isolation of potential L-asparaginase producing microorganisms through enrichment techniques, like supplementing with different carbon and nitrogen source. Generally, extracellular microbial products, like enzyme producers are screened by plate assay and potential strains can be evaluated based on the zone of hydrolysis around the colony. A rapid plate assay technique was often suggested and attempted, for easy isolation of L-asparaginase producing actinomycetes (Magda et al., 2013). Bacteria are mostly preferred group because of their fast growing, easy handling and efficient enzyme producing nature as well as to meet wide variety of industrial needs.

Culture optimization is a major factor in L-asparaginase process. Optimal environmental conditions are greatly influencing the L-asparaginase enzyme activity and growth of L-asparaginase producing bacteria. Several research studies about the production of L-asparaginases have been reported from different source of microorganisms that either produces this enzyme constitutently or through induction by specific compounds. The ranges of optimal conditions are quietly varied for L-asparaginase producing bacteria because these are species specific nature. Therefore, optimization of culture parameters is very essential in L-asparaginase production management. Many reports have been carried out for optimization of L-asparaginase enzyme production such as incubation period, pH, temperature, agitation rate, inoculum level, composition of fermentation media, substrate concentration, (Gurunathan et al., 2012; Hymavathi et al., 2009). Although many researchers have reported on optimization of carbon and nitrogen sources for L-asparaginase production, very less research work is found on the influence of incubation conditions (Kumar et al., 2010).

Several methods are available currently for producing L-asparaginase commercially by using the modern biotechnological approaches such as Recombinant - DNA technology, gene cloning into novel producers etc. Presently, L-asparaginase is produced throughout the world by submerged fermentation. This
methodology has many disadvantages such as the low concentration of product formation and consequent handling (purification of product from source), reduction and disposal of large volumes of water during the downstream processing etc. Therefore, the submerged fermentation methodology is a cost intensive, highly problematic and poorly understood unit operation (Naeel et al., 2012 ). An alternative solution to molecular cloning and genetic engineering are the promising key tools which has ability to produce recombinant L-asparaginase. Large quantity of L-asparaginase mRNA was measured by RQ - PCR in some of the earlier studies (Irino et al., 2004). The L-asparaginase mRNA level was paralleled to the L-asparaginase enzyme activity and the protein levels obtained could be measured through these methods.

Purification and analytical separation of L-asparaginase have been carried out by using different chromatographic methods, example ion exchange chromatography with DEAE cellulose and recently Sephadex G - 200 chromatography and also by different electrophoretic methods. Many of the above separation methods are either uneconomical or too time consuming to be used routinely for large scale purification. Because protein purification is typically tedious and labor - intensive, an efficient, simple and economical “Quantitative preparative native continuous polyacrylamide gel electrophoresis” has been commonly used in protein analysis due to its high resolution, but the relative higher expenses and denaturing conditions of preparative gel electrophoresis considerably limit its practical application as a method for protein purification (Hassan et al., 2011).

Few of the purified preparations have shown to possess antitumour activity. Like bacteria, actinomycetes are also a good source for the production of L-asparaginase. The oncolytic enzyme, L-asparaginase also finds wider application in the treatment of hepatocellular carcinoma (Ghobrial et al., 2005). Proteomics analysis on oncolytic studies could easily characterize the qualitative alterations and quantitative protein expression level changes in response to varying conditions during apoptosis (Yim et al., 2006). However, the traditional methods are complex and time consuming, exact protein spots are often unattainable and contamination is often faced in the whole process (Hengartner, 2000). Moreover, apoptosis is a dynamic process where different conditions should be detected over time (Denicourt and Dowdy, 2004). A simple, easy and rapid method for proteomic analysis would greatly benefit this field.
With this background, the present study focusses on all the above discussed criteria and thus aims at attempting to identify a suitable alternate microbial source for production of L-asparaginase. The present research work also aims at characterization and molecular modelling process of the enzyme and to evaluate the effect of L-asparaginase on treatment of Hepatocellular Carcinoma.