1. INTRODUCTION

Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions in which, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines the metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the activation energy for a reaction, that dramatically increase the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts in that they are highly specific for their substrates. Enzymes are known to catalyze about 4,000 biochemical reactions. A few RNA molecules called RIB-Ribozymes also catalyze reactions, with an important example being some parts of the ribosome. Synthetic molecules called artificial enzymes also display enzyme-like catalysis.

Enzymes as drugs have two important features that distinguish them from all other types of drugs. First, enzymes often bind and act on their targets with great affinity and specificity. Second, enzymes are catalytic and convert multiple target molecules to the desired products. These two features make enzymes potent drugs that can accomplish therapeutic biochemistry in the body that small molecules cannot. These characteristics have resulted in the development of many enzyme drugs for a wide range of disorders.
Enzyme therapy has long been part of the methods of treatment of traditional medicine. Meanwhile, enzymes have even become one of the most innovative and expanding drug groups, and systemic therapy especially the proteolytic enzymes are extensively used in natural medicines. There is hardly any regulatory system in the human body which does not depend on enzymes. Enzymes control coagulation and fibrinolysis, inflammation and complement activity, phagocytosis, wound healing and tissue regeneration as well as the specific and nonspecific defense systems. In medical fields, enzymes are used in: (1) analysis, (2) pharmaceuticals and, (3) therapy.

Enzymes are used for diagnostic purposes. For example, in the past it took a long time to determine the blood sugar level. Today, one can use a special tape by wetting it with blood or urine to know the blood sugar level within 60 seconds. The knowledge about the enzyme reactions is needed to use them judiciously in diagnostic purposes. In the pharmaceutical area, many of the drugs used today are in reality enzymatic inhibitors, such as cytostatics, antibiotics, steroids, etc. resulting in different adverse side effects. They are used to correct metabolic disorders including genetic defects.

Therapeutic enzymes (digestive and metabolic) can be used medically either isolately or adjunctly with other therapies for treatment of various diseases like cancer, cystic fibrosis, dermal ulcers, inflammation, digestive disorders etc. Enzymes as direct pharmaceutical products find numerous applications. Some important therapeutic enzymes along with their uses include: L-asparaginase (antitumour); urokinase (blood clots); collagenase (skin ulcers); uricase (gout); L-glutaminase (antitumour); L-arginase (antitumour); L-tyrosinase (antitumour); Glucosidase (antitumour); streptokinase (anticoagulant); urokinase (anticoagulant); hyaluronidase (heart attack); ribonuclease (antiviral); trypsin (inflammation); lysozyme (antibiotic); rhodanase (cyanide poisoning); β-lactamase (penicillin allergy); serratiopeptidase (anti-inflammatory); lipase (digest lipids); laccase (detoxifier); dornase α
(cystic fibrosis); rasburicase (hyperuricemia); sacrosidase (congenital sucraseisomaltase deficiency (CSID), peptidase (celiac disease) etc., Some important enzymes used for some lysosomal diseases include: Aglucerase, Imiglucerase, Taliglucerase alfa, Velaglucerase-A (Gaucher’s disease); Pegademase bovine (severe combined immunodeficiency disease SCID); α-Galactosidase A, Agalsidase beta (Fabry’s disease) and Idursulfase (Hunter syndrome). At present, Enzyme Replacement Therapy (ERT) has been approved for six lysosomal storage diseases, and clinical trials are ongoing with recombinant human enzymes in several others (Desnick and Schuchman, 2012).

Urate oxidase or uricase (urate: oxygen oxidoreductase, EC 1.7.3.3), is the enzyme that catalyzes specifically the oxidation of uric acid to allantoin and plays an important part in nitrogen metabolism (Wakamiya et al., 1994). Higher primates (apes and humans) lack functional uricase and excrete uric acid as the end product of purine degradation (Friedman et al., 1985). In some individuals, uric acid precipitate, leading to gout symptoms.

This enzyme is widely present in most of the vertebrates (Schiavon et al., 2000) and first observed in bovine kidney. Various natural sources such as bacteria (Mansour et al., 1996), fungi (Farley and Santosa, 2002) and eukaryotic cells (Montalbini et al., 1997) were found to be uricase producers.

Uricase was originally isolated from mammals and recently from microbial sources such as viz., fungi, yeast and bacteria etc are preferred due to various reasons such as (i). Shorter generation time, (ii). Versatility to use cheaper sunstrates, (iii). Technically easier for genetic manipulation, (iv). Smaller sized and hence easily grown in fermentors under controlled conditions, (v). Seasonal variations can be controlled, (vi). Lack of ethical issues, (vii). Avalilability of technical expertise and techniques in-place etc., Uricase is an enzyme in the purine degradation pathway that catalyzes the oxidative breakdown of uric acid to allantoin. Uric acid, the primary end-product of purine metabolism is present in biological
fluids, including blood and urine. Various medical conditions increase the amount of uric acid in biological fluids. Such conditions can lead to chronic renal diseases, some organic acidemias and Lesch–Nyhan syndrome.

Many attempts have been made to fabricate uric acid sensors using uricase as a biocatalyst (Yutaka et al., 1992). The uricase molecule catalyzes the in vivo oxidation of uric acid in the presence of oxygen, which oxidizes uric acid to allantoin and CO$_2$, leaving hydrogen peroxide as the reduction product of O$_2$. Most of these enzymes either have high thermo stability or active over a wide pH range. Only one uricase, from Bacillus sp. TB-90, was observed to have both high activity and thermo stability over a wide range of pH values (pH 6–9). Hence there is a need to find more novel sources with desirable properties.

The biological reason for the loss of urate oxidase activity in humans and certain primates is unknown. According to one view, this loss has had a distinctly beneficial effect. It has been shown that uric acid is a powerful antioxidant and a scavenger of free radicals; therefore, a high serum uric acid level caused by the loss of urate oxidase activity may have contributed to a decreased cancer rate and a lengthened hominoid life span (Friedman et al., 1985). However uricase is needed when hyperuricemic condition is developed in human body. Elitek T$^M$ is the available intravenous dosage form of uricase manufactured by Sanofi Aventis and Elitek International Corporation.

Hyperuricemia is not only a direct cause of gout and related diseases but also an independent risk factor for certain kidney and cardiovascular diseases (Hosoya et al., 2011). Therefore, it is essential to reduce the level of uric acid in blood and tissues to prevent and treat many uric acid-related diseases. Because the human body cannot synthesize urate oxidase by itself, reducing the level of uric acid requires long-term or even lifelong treatment.
However, currently various uric acid-lowering drugs cause different degrees of damage to the human body and thus are not suitable for a long-term treatment.

Gout is a painful disorder, characterized by uricemia, recurrent attacks of acute arthritis, deposition of sodium urate in and around joints, and in many cases, formation of uric acid calculi (Laemmli et al., 1970). Gout treatment generally includes allopurinol, a potent competitive inhibitor of xanthine dehydrogenase, the enzyme that catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid (Massey et al., 1970). In the case of gout associated with renal complications, direct injection of urate oxidase allows a much more rapid resorption of urate nephrolithiases and it is done to prevent or treat hyperuricemia disorders that may occur during chemotherapy. Pegloticase (commercial uricase) is a recombinant uricase that can be used by man to low the levels of uric acid by catalyzing the oxidation of uric acid to allantoin that was eliminated by kidneys. Thus, uricase is a promising enzyme with high specificity towards uric acid and usually needed in large quantities for medical uses including analysis of human serum or urine for uric acid and using as a protein drug to reduce toxic urate accumulation.

Approximately two million people suffer with gout, with between 75-90% being middle-aged men. Women usually get gout only after menopause, possibly due to the drop in estrogen. There are numerous risk factors that are associated with gout, such as alcohol consumption, purine rich diet, sex (men more affected), genetics, other health problems like high blood pressure, hypothyroidism, psoriasis, hemolytic anemia, or even some cancers, Kelley-Seegmiller Syndrome or Lesch-Nyhan Syndrome, Lead exposure, medication like cyclosporine, furosemide, hydrochlorothiazide, metolazone, levodopa, aspirin, obesity and renal insufficiency. There are four stages that the disease gout can pass through (i.e.) 1. Asymptomatic hyperuricemia, 2. Acute gout, 3. Interval or intercritical gout and 4. Chronic tophaceous gout. Allopurinol, Febuxostat, Pegloticase and Probenecid are the drugs of
choice. Apart from these four drugs, no other drug is available to hyperuricemia associated diseases. This situation warrants further research on this important enzyme. Uricase has advantages for gout treatment compared with allopurinol. Uricase is also useful for enzymatic determination of urate in clinical analysis by coupling with 4-aminoantipyrine peroxidase system (Klose et al., 1978). Hyperuricemia and tumor lysis syndrome are serious complications that can occur during chemotherapy for hematologic malignancies. Rasburicase (Fasturtec/Elitek) is a market name given to the Aspergillus flavus urate oxidase expressed in Saccharomyces cerevisiae which has been approved for clinical use. Rasburicase, is a safe and effective alternative to allopurinol for lowering uric acid levels, is made up of a single polypeptide chain with 301 amino acids.

Several forms of uricase from microorganisms are currently used as diagnostic reagents to detect uric acid. Uricase is used in medicine and clinical biochemistry as a diagnostic reagent for measurement of uric acid in blood and other biological fluids (Adamek et al., 1989).

Although several microbial sources of uricase have been proposed for this clinical indication, only one has actually been used commercially under the trade mark of uricozyme and isolated and purified from Aspergillus flavus. In this back drop, the present study aimed at searching for potential uricase producing fungal sources.

Mangroves are inhabited by large number of fungal communities called manglicolous fungi. They include mostly marine and small group of terrestrial fungi – categorized into (i) Soprophytic (ii) Parasitic (iii) Symbiotic fungi. Fungi from mangrove environment play an important and ecological role in decomposition of organic matter by production of variety of extracellular degradative enzymes. Such enzymes can be used for biotechnological applications, and in pharamaceutical industries also be used as therapeutic agents.
Inspite of immense ecological role and biotechnological potentials mangrove fungi are not extensively studied. The present study aims to screen and isolate mangrove fungi producing uricase enzyme as a source of novel drug.

Despite a better understanding of the importance of mangroves they continued to be destroyed at an alarming rate (Ong 1995). Therefore it is imperative to record and quantify the abundance of marine fungi from mangrove ecosystem and to culture them to ensure their conservation for future biochemical genetic and molecular studies (Jones and Mitchekk – 1996).