Emerging Concepts in Chemical and Biological Sciences

Edited by

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Editorial

Major developments in chemistry, molecular cell biology and genetics have enabled a deeper understanding of numerous life processes around us. However, much remains to be explored and revealed. The purpose of this book is to provide a more complete theory of emerging concepts in chemical and biological sciences.

Cancer is still a terrible and overpowering disease in present society that has only treatment but not cure. L-asparaginase has been a basis of cancer treatment due to its effective application in the cure of lympho-proliferative disorders and lymphomas along with acute lymphoblastic leukemia. It has been detected that pegylation and immobilization of the enzyme can make it more effective anti-neoplastic agent. As more and more advances are made, more comprehensive understanding of the pharmacokinetic and pharmacodynamic properties of asparaginase will bring better efficiency as well as the maximum utilization of the enzyme as an antineoplastic agent.

Another natural compound carbonyl methylglyoxal plays a significant role in cancer therapy. It is commonly called Retine and are highly effective in controlling cell division. The anti-cancer strategies of methylglyoxal can help in the replacement of the anti-cancer drugs which produce many adverse effects and can aid in the advancements the effectiveness of Allopathic system of medicine and cancer therapy.

Cell migration is a main process involved in many biological mechanisms like embryological development, tissue formation, immune defense or inflammation, and cancer progression. The physical, chemical, and molecular factors affecting cell motility must be studied to understand migratory cells behavior. In vitro assays are crucial approaches to infer in vivo conditions. Thus, studies are conducted to explore new methods and the development novel approaches for measuring tumor cell migration to understand the mechanism of cancer metastasis and for the development of novel anticancer therapies.

Medicinal plants are valuable sources of medicinal products. In India, the use of medicinal plants in health care practices is relatively high. They are a crucial part of the ecosystem for the human health benefits, livelihood and knowledge. Nevertheless, in spite of their increasing economic significance, medicinal plants are not being utilized sustainably. As result they are disappearing at a high speed. Therefore, global movements, developments and prospects for the strategies and procedures regarding the conservation and sustainable use of medicinal plant resources is necessary for the conservation and sustainable use of medicinal plants.

Ventilago madraspatana, presently studied for the phytochemical and functional properties by the biomedical researchers. Physcion and Emodin is extracted from this

traditional medicinal plant, have shown to have anti-inflammatory as well as anticancer potential necessary for designing novel therapeutic agents. Thus, as we step into the new era there is a need to assimilate the traditional herbal medical information with the cutting-edge scientific advances for healthcare advancement and disease prevention.

The exploration for simple and effective descriptors of biological ecosystem mechanism is a major challenge of monitoring aquatic ecosystem health. The understanding of macro-invertebrates with respect to alteration in environmental quality make them an essential part of any biomonitoring program. The researcher uses many methods to collect benthic macroinvertebrates from aquatic habitats and these macroinvertebrate data are then used to measure the extent of environmental damage often caused by pollutants. So, it can be said that benthic macroinvertebrates are significant biomonitoring instrument that can be utilized as a complimentary method to chemical analysis.

The Sundarbans forest is the largest mangrove forest ecosystem in the world. It is home to much of India's immense biodiversity. Due to overexploitation, rapid population growth, unsustainable usage, are the main causes of biodiversity loss in the Sunderban area. So, we need to review the status and threats of biodiversity in the Sunderbans areas of India to evaluate the current state of biodiversity here. This will help the resource users to assign benefits justifiably and sustainably in the long run.

The aquatic ecosystem is thoroughly dependent on water quality and biological diversity. Physicochemical factors of water play a crucial role in the biology and physiology of fish. Lakes and reservoirs house the single largest inland fishery resources. Fishes are the significant indicator of aquatic ecosystem and constitute a notable position in socioeconomic structure. Fish is very rich source of protein along with vitamins and other minerals. Therefore, we need to stop all kinds of human activities that are leading to the damages of the aquatic ecosystem. We need to adopt of scientific fishery management process in order to save the river for us and for the forthcoming generations.

Long horned beetles are the richest species which are important for forestry, pollination or as bioindicator. This group of insects is yet to obtain serious consideration in India especially in the North East India, one of the two hotspots of the country, an area known for its floral diversity. Based on this the study shows the role of this beetle in impending management of controlling timber damage.

The C-type lectins are the major and most varied of the lectin families present in animals. It is vital to understand the importance of C-type lectins in the immune system owing to their ubiquity and varied range of functions in animal cells. With the progress of more algorithms to predict sequence and structural characteristics on C-type lectins, more and more likely cellular functions of lectins may be discovered. With all the

assembled results, upcoming work will comprise probabilistic methods for accepting or rejecting prediction results.

Isolation and characterization of beneficial bacteria with probiotic property is needed. Lactic acid bacteria (LAB) are one of the most important groups of probiotic bacteria, normally used in fermented dairy products. These bacteria are very beneficial as these microorganisms can improve lactose digestion, stimulate the immune system, and avert and treat diarrhea. Bacteria are the main group of biosurfactant producing microorganisms. Biosurfactants have a wide range of potential applications in the medical field, antiviral agents and they also have the possibility for use as chief immunomodulatory molecules, in vaccines and gene therapy. Thus, studies based on biosurfactant producing food grade LAB are very significant for obtaining stable, high quantity of biosurfactants.

Protein-DNA interaction is one of the utmost important activities in cell which controls the life of a cell by regulating gene expression. Reviewing the regulation of transcription of the gal operon determined a plethora of mechanism that operate in prokaryotic gene regulatory processes. Gal operon is one of the most studied catabolite-sensitive operons of Escherichia coli. More studies are required in this area to determine role of the gal operon of E. coli in cellular metabolism.

The control of microbial growth in foods from 'farm to fork' is crucial to safeguard consumer health and welfare and prevent foods from spoilage. Food preservatives are used to guarantee safety and avoid quality loss derived from microbial, physical–chemical, or enzymatic reactions. There are different kinds of antimicrobial and antioxidant agents, each one with diverse modes of action. But studies show that it is better to take food having less harmful preservative.

In the present scenario suicide is a major public health concern. Suicidal behavior is the cause of death and disability globally. Fortunately, current progresses in suicide theory and research is searching for useful developmental information for its prevention. Suicide prevention initiatives are required, and studies must consider the varied range of cultural and socioeconomic backgrounds of the different population worldwide.

The optical imaging methods accessible for clinical skin imaging are basically inadequate in their depth possibility to a few hundred microns and face complications in their functional and molecular imaging abilities. The visible light camera was incapable to capture image in vivo along with nano or less than 1mico level image. So, a definite technique that was appropriate to the sample surface being imaged must be developed. In general, an amalgamation of different methods, with careful explanation of the information is the best possibility in such cases

Every day, Earth's limited resources are being exhausted for energy, for material goods, for transportation, for housing, and for drugs. As we progress scientifically and

technically, we must consider sustainable use of Medicinal agents for the continuous availability of drugs for future universal health care. The incorporation of novel ideas and other technology sound policies is necessary to meet the global needs for medicinal agents to assure the preservation and progression of public health in the future.

The editors would like to thank all the authors for their contribution in such varied topics that will help to create awareness in various aspect of life. Editors also thankful to Principal, Surendranath College, Kolkata, India, Management of Lincoln University College, Malaysia and Lincoln Research & Publishing Limited, Australia for giving us the necessary permission to publish this book. This book provides a valuable window on information assurance and covers the necessary areas that humanity is facing in the present situation. As is evident from the articles the challenges in information assurance is both difficult and interesting. Researchers have presented their work with eagerness and dedication to develop new procedures of investigation and provide new answers to cope with the ever-changing threats.

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L-Asparaginase: Challenges and Development of Next Generation ASNase Therapeutic Molecule

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Abstract

L-asparaginase has been a cornerstone in the history of cancer treatment because of its effective application in the cure of lymphoproliferative disorders and lymphomas, in addition to acute lymphoblastic leukemia. In recent days, the utilization of L- asparaginase, owing to its anti-neoplastic properties has expanded its horizon as an anti-cancer drug, while it is being tested for effectivity against other types of malignancy as well. L-asparaginase exhibits extensive properties as an anti-neoplastic agent and provides a potential treatment regime in combination with other similar agents. On clinical grounds, the action of the enzyme is ascribed to the depletion of L-asparaginase in the serum, making it difficult for the malignant cells to synthesize it under scarce concentration of L-asparagine Synthetase. Even though it has emerged as a potent drug against cancer over the decades, prolonged use of it has paved way to hypersensitivity, thrombosis and other side effects observed mostly in adults rather than in infants. This problem too has been addressed with the pegylation and immobilization of the enzyme, rendering it a more viable anti-neoplastic agent.

Keywords: *L-asparaginase, Acute Lymphoblastic Leukemia, Antineoplastic agent, Pegylation*

Introduction

L-asparagine amidohydrolase (e.c. 3.5.1.1) is a member of the amidase class of enzymes that shows potency in hydrolyzing the amide bond present in L-asparagine to give L-aspartic acid and ammonia as end products (Kumar & Verma, 2012). In addition, the enzyme also shows enzymatic activities in breaking glutamine to glutamic acid and ammonia (L-glutaminase side activity). It has been noted for its effective activity against the proliferation of lymphomas and acute lymphoblastic leukemia in particular (Hann *et al.*, 2000). In combination with chemotherapeutic protocols, L-asparaginase has successfully portrayed positive results in the treatment of pediatric acute lymphoblastic leukemia, acute myeloblastic leukemia and other tumor malignancies in human (Hann *et al.*, 2000). Though the extensive use of it as a drug has shown prominent effectiveness, one of the major issues of concern raised by its continual incorporation in an ailing patient is the occurrence of hypersensitivity pertaining to the clinical dosage (Rizzari *et al.*, 2006). Addressing the

various shortcomings of the use of the enzyme as an anticancer drug, several solutions have also been proposed to negate its harmful effects on the body.

Role of L-Asparagine metabolism in normal cell and Malignant Cell Proliferation

L-Asparagine, a non-essential amino acid is synthesized by the enzymatic activities of L-Asparagine Synthetase upon its action on aspartic acid and glutamine. However, cancer cells are capable of very slow synthesis of the enzyme pertaining to the absence of L-Asparagine Synthetase. Therefore, they are dependent on the asparaginase available in the circulating pool. Consequently, low concentration of the synthetase enzyme in the pool starves the malignant cells, paving way to their eventual death. On the contrary, malignant cells require a huge amount of L-Asparagine for growth because of its activity as an amino acid exchange factor.

On the other hand, L-asparaginase also impedes protein synthesis by hydrolyzing L-Asparagine along with the depletion of the L-Asparagine present in the serum. Normal cells can cope up with this phenomenon due to an abundance of L-Asparagine Synthetase, but cancer cells fail invariably in doing so. Since cancer cells lack the Asparagine Synthetase, they depend on the utilization of serum and cerebro spinal fluid (CSF) asparagine. Asparaginase works by hydrolyzing the serum as well as CSF Asparagine (Lubkowski *et al.*, 1996). This phenomenon causes the inhibition of DNA, RNA and protein biosynthesis in ALL, AML and other asparagine dependent malignant cells leading to cell cycle arrest in G0/G1phase and eventually apoptosis (Gong & Basilico, 1990).

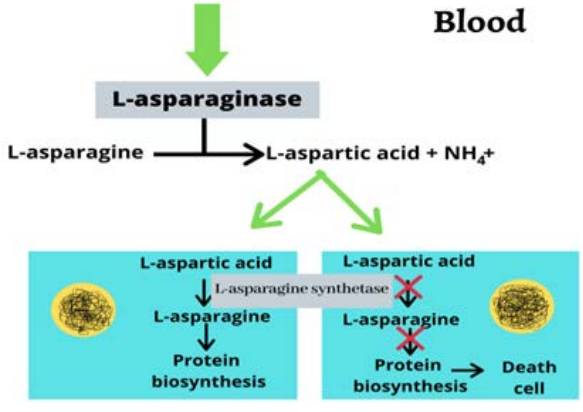

Figure 1: Role of L-Asparagine metabolism in normal cell and Malignant Cell Proliferation

Chemical Features:

Native Enzyme:

As the attainment of the enzyme from the serum of the guinea pig in large quantities was not a viable option, the focus was shifted towards microbes. In 1964, Mashburn and

Wriston, and a few years later in 1969 Campbell and Mashburn outlined the extraction and consequent purification of the enzyme from bacterial sources such as *E.coli* and successfully illustrated its tumoricidal effects (Mashburn & Wriston, 1964). An array of microbes such as *Erwinia carotovora*, *Pseudomonas 7A, Corynebacterium glutamicum, Vibrio succinogenes*, *Bacillus sp*, and *Aspergillus terreus* were found to be very productive producers of the enzyme, though the properties consistently varied from organism to organism. Inspite of the availability of several sources for the production of L-asparaginase, the bacterial strains of *E.coli* and *Erwinia caratovora* are clinically accepted and currently used till date for the treatment of lymphoblastic leukemia.The purified form of the *E. coli* L-asparaginase enzyme sums up to 133–141 kDa (Jackson & Handschumacher, 1970). All asparaginases are found to be constituent of four different subunits that have an active site unique to a particular sub unit. The molecular weight of each sub unit accounts for 22 kDa (Whelan & Wriston, 1969). The experimental works conducted by Koerholz *et al.*, (2009) reveals that the L-asparaginase isolated from an *E.coli* bacterial strain has subunits weighing about 32kDa. Molecular weight of Erwinia L-asparaginase, on the other hand, has been reported as 138 kDa. The specific activity of the enzyme in purified form lies in a range of 300 and 400 umol of the substrate/min/mg of protein while the isoelectric point lies between pH of range 4.6 to 5.5 in case of *E. coli* enzyme. The isoelectric point of Erwinia enzyme amounts to 8.7(Howard & Carpenter, 1972). The Michaelis-Menten constant for L-asparaginase reaches an approximation value of 1×10−5 mol/l (Jackson & Handschumacher, 1970).

Toxicity

L-asparaginase demonstrates side activity as L-glutaminase which causes the hydrolysis of L-glutamine to give L-glutamic acid and ammonia. The extensively used forms of L-asparaginase extracted from *E.coli* and *Erwinia chrysanthemi* have a L-glutaminase activity ranging between 2% to 10% of the primary L-Asparaginase activity (Narta, Kanwar & Azmi, 2007). It possesses a disparate virulentprofile, that diversifies through a range of acute hypersensitivity, hyperglycemia, pancreatitis and hepatocellular dysfunction (Liu *et al.*, 2012). The toxicity profile of asparaginase can be categorized as that concerning hypersensitivity in reaction toward a foreign protein and as the protein synthesis inhibition causing detrimental effects.

Hypersensitivity

Asparaginases when derived from *E. coli* or *Erwinia chrysanthemi* induce responses pertaining to the immune system in patients since they are foreign protein. These immune responses range from transient erythema to acute life-threatening anaphylaxis. Anti-asparaginase antibodies are responsible for triggering such reactions (Tong *et al.*, 2013; Wang *et al.*, 2003). Asparaginase-inactivating antibodies are found to be developed even if they do not give rise to clinical symptoms. This phenomenon has been observed in one-third of patients and it causes a decrease in the concentration of

asparagine. It causes an increase in the extracellular asparagines and increases drug resistance (Tong *et al.*, 2013). Inspite of the recorded shortcomings of the protocol, the administration of pegylated form of asparaginase has comparatively shown less resistance (Avramis *et al.*, 2002).

Thrombotic Complications:

Thrombotic complication is a commonly occurring clinical condition. Patients receiving the L-asparaginase treatment cause the inhibition of the L-asparaginase dependent hepatic synthesis of hemostatic protein, of which anti-thrombin protein is one. Thrombosis can occur with the immediate induction of asparaginase treatment or the post-remission treatment. It appears to account for a higher percentage in adult populations rather than in infants (Elliott *et al.*, 2004). Upper central venous thrombosis is the most common followed by venous thrombosis and arterial thrombosis (Avramis *et al.*, 2002).

Modern Approach

The L-glutaminase side activity of L-asparaginase accounts for the side effects observed in patients treated with the therapeutic drug. Asparaginase obtained from *E.coli* and *Erwinia chrysanthemi* also shows shorter serum half life, low trypsin tolerance, hemolysis, and formation of antibodies (Piątkowska-Jakubas *et al.*, 2008). In order to eliminate the negative effects, biobetter or biosuperior synthesis of asparaginase has been the key to side effect free chemo-therapeutic treatment. Various techniques have been adopted to synthesize biobetter Asparaginases involving site-directed mutagenesis, molecular dynamics, nanoparticle conjugation, PEGylation, bioconjugation, and PASylation. Other similar alternatives include the use of encapsulation and immobilization.

Research work conducted by Derst and his colleagues (Derst *et al.*, 2000) revealed the role of Asn 248 present in native Eca II in hydrogen bonding, which in turn influences substrate binding. Though this reduced the Asparaginase activity by about 12%, it also caused a decrease in glutaminase activity. On the other hand, Offman *et al*., (2011) put to application the technique of site-directed mutagenesis to create double mutants N24A/Y250L, which demonstrated almost negligible glutamine activity, with 72% of its activity retained. Another effective approach included the glycosylation of the enzyme which showed improved pharmacokinetics, effector function, solubility, serum half-life and binding receptor Nadeem *et al*., (2018).

In 2017, Husain *et al.,* (2016) addressed the issue using L-asparaginase procured from *Enterobacter cloacae*. The extracted enzyme lacked glutaminase activity which gave the form advantage over the occurring side effects. The purified enzyme was found to have a molecular weight of 106 kDa. With the application of SDS PAGE the physicochemical properties of the improved asparaginase gave an optimum activity at a pH ranging between 7-8 and temperature lying between 35-40ºC. The remarkable *in vitro* serum

half-life (T1/2~39h) and trypsin half-life (T1/2~32h) are also advantageous to its efficacy against cancerous cells. Husaain *et al.*, (2016) also examined the effectivity of the enzyme against HE-60, MOLT-4, MDA-MB-231 and T47D cell lines to procure positive results in each case. In addition, the mitochondrial pathway leading to apoptosis gets activated too. The improved features of the *Enterobacter cloacae* extracted L-Asparaginase was tested against normal cell lines FR-2 and human erythrocytes to which the enzyme failed to show non-toxic and non-hemolytic action.

Since the L-Asparaginase extracted from bacteria caused the appearance of immunogenic side effects, researchers tried to develop L-Asparaginase of human origin. While the human ASPG exhibits viable asparaginase activity due to its N-terminal region (Emadi, Zokaee & Sausville, 2014), according to experimental data and reports, the serine/threonine-protein kinase enzyme encoded by theSGK1 gene happens to be the new molecular partner of ASPG (Yu *et al.*, 2012). ASPG partnered with serine threonine kinase protein exhibits lysophospholipase activity in regulating the conversion of lysophosphatidylinositol to glycerophosphoinositol, where the later is an essential intracellular messenger obtained from the MAPK/EPK pathway. This phenomenon in turn controls the proliferation of malignant cells (Yu *et al.*, 2012). The experimentally obtained results contribute to establishing ASPG as a therapeutic agent causing remarkable inhibition of uncontrolled cell growth. The pharmacokinetic study of the compound suggest allosteric mode of regulation as prompted by the study of its kinetic graph. Experimental evaluations were also conducted to find out that the presence of $MgCl_2$ play an important role in increasing the activity of the Asparaginase residue in ASPG, though it failed to show any effects on its Km value. Later, Rigouin *et al.*, (2017) tried to humanize guinea pig L-Asparaginase (gp ASNase 1) with low Kmby generating chimers with the human L-Asparaginase (hASNase 1) using DNA shuffling. This paved way to the identification of clones by transforming BWΔ.The end result culminated in identifying two clones 63-hc and 65nhc which exhibited a 100-140 fold increase in catalytic efficiency when compared with hASNase 1, while retaining the anti neoplastic character of the clones.

Another team of researchers (Qian *et al.*, 1997) executed the improved efficacy of the L-asparaginase combined with lower antigenicity by modifying the enzyme with N,O-carbpoxymethyl chitosan.

The modifications led to an identical Km value but also exhibited enhanced stability against the proteolytic capacity of protease enzyme. Upon the analysis of experimental data, it can be concluded that the reduction of antigenicity bears a direct proportional relation with the molecular weight of the modifier used. In addition to the affirmative results, the modified enzymes also exhibited an increased half-life which amounted to 33 times longer than the native form of the enzyme.

Conclusion

The conclusion that can be drawn from the aforementioned information pertaining to

existing reports and experimental evaluation is that L-asparaginase is an indispensable part of the age old war against cancer. The pioneer observation of L-asparaginase as an anti-cancer agent has opened new horizons in our approach towards cancer treatment. Owing to its myelosuppressive nature and reduced amount of side effects in the PEGylated form, the enzyme has shown high effectivity and efficiency in the prevention of the proliferation of malignant cells. Improved versions of Asparaginases lacking glutaminase activity had a promising cure of patients without triggering immune responses, which consequently has reported the survival of the majority of patients undergoing the treatment. With the advancement of technology, detailed studies of the pharmacokinetic and pharmacodynamic properties of asparaginase preparation have been possible, which endows us with the hope of better efficacy as well as themaximum utilization of the enzyme as an antineoplastic agent.

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Exploitation of Medicinal Plants

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Abstract

India has more than three thousand years of medicinal heritage based on medicinal plants which are largely used as folk medicine or preparation of recent pharmaceuticals. The study of medicinal plants has assumed great importance in India and abroad. These medicinal plants are endangered due to anthropogenic activities like urbanization, overexploitation and various environmental changes. The booming of traditional medicine has resulted in an increased demand on medicinal plant products. Excessive commercial demand from a rapidly expanding pharmaceutical industry, for which no collection regulations exist, affects medicinal plants of various taxa. Asansol and Raniganj coalfield region in West Bengal is an important mineral resource area with a rich vegetation of medicinal plants. But the important medicinal plants which are used in traditional medicine are disappearing due to the impact of environmental changes, prolonged mining, industrial and urban developments. These resources are depleting at an alarming rate and they will soon be extinct and endangered. It is very important that medicinal plants should be conserved as exploitation of medicinal plants can affect the balance of our ecosystem. The knowledge in this field would enable introduction of timely cultivation in necessary cases and maintain the required balance between proper sustainable use and overexploitation of these limited natural resources. Medicinal plants are suitable raw materials for production of new synthetic drugs to treat different health problems. The pharmacologists can use this important natural resource sustainably for the preparation of herbal and modern drugs to cure diseases which can be a boon for mankind.

Keywords: *Medicinal plants, Asansol, Raniganj, Exploitation, Sustainable*

Introduction

Indian subcontinent is being inhabited by different tribal people in forest dominated villages of tribal community and represents one of the greatest emporia of ethnobotanical wealth. India is one of the richest countries in the world in terms of biodiversity as it has 15 agro-climatic zones. These medicinal plants have been used over the millennia for human welfare in between man and his environment continues even today as a large proportion of people in developing countries still live in rural areas. Ethnopharmacological information is an important tool in drug discovery. India's

biological diversity is very rich but unfortunately its wealth is being eroded due to various reasons. This diversity needs to be preserved and the immediate task will be to devise and enforce time bound plans for saving the endangered plant species as well as habitats of the biological resources. Nowadays, forest resources have been exhausted due to overgrazing, overexploitation, encroachments, unsustainable practices like shifting of cultivation and various anthropogenic developmental activities. These led to the loss of the wild habitat and resulted in the extinction of certain flora especially medicinal plants. Ethnomedicinal plants have been used as sources of modern drugs, either by providing pure compounds, starting materials for partial synthesis of useful compounds or models for synthesis of new drugs (Kamali, 2009).

Status of Medicinal Plants in India

India harbors a wide range of medicinal and aromatic plants. Out of the 17000-18000 species of flowering plants, more than 7000 are estimated to have medicinal usage in folk and documented systems of medicine like Ayurveda, Unani, Siddha & Homoeopathy (AYUSH System of Medicine). Out of 18,000 flowering plant species known from India, more than 400 species are used as medicinal plants of which 300 species yield gum and dyes and about 100 species yield essential oils and are used as raw materials in drug industry (National Medicinal Plant Board, Govt. of India, 2019). Plants are directly used as medicines by a majority of cultures around the world like Indian medicine and Chinese medicine, etc. The Indian systems of medicine have identified 1,500 medicinal plants of which 500 species are mostly used in preparation of drugs. More than 150 plant species have been categorized as endangered (WHO, 2011). India is a hub of wild collected plant medicine industry in Asia but key species have declined due to over collection and opportunistic marketing of medicinal plants to supply domestic and foreign medicinal markets. The current interest surge in herbal medicines all over the world has led to the unregulated cropping of the India's bioresources which pose stress and threat of extinction on the medicinal plant species. Researchers from TRAFFIC and IUCN, examined the trade in seven medicinal plants species with very different life histories, uses and trade patterns, to give a broad overview of Asia's medicinal plant trade and India emerged as a major destination for trade in all. Many studies have confirmed that pharmaceutical companies are responsible for inefficient and opportunistic marketing of medicinal plants (Roberson, 2008).

Commercial value of medicinal plants

The commercial evaluation of plant based drug is different from that of the source raw material of ethnomedicinal plants, for both depend much upon the demand and the supply potential. For medicinal plants, the source material, the land use value, cost of collection, cultivation, costs of preparation, packaging and transport are the criteria. According to Moran & Pearce (1994) economic value of medicinal plants and plant

based drugs mostly depend upon the following criteria a) The actual market value of the medicinal plants being treated b) The market value of the drugs of which the plant are the source material and c) The value of the drugs in terms of their life saving potential. Most of the traders are not much aware of the trade restrictions and conservation laws. During 2000, TRAFFIC – India conducted an all-India survey on trade in medicinal plant parts which also covered the two local markets of West Bengal i.e. Kolkata and Siliguri and observed that the markets dealing with medicinal plant parts in India is a highly disorganized and less studied sector. The demand, supply and price structures are highly unstable. There are much confusion and controversy regarding the trade names and the scientific names of the items in trade. Moreover there exist practices of adulteration and there is no definite system of quality control. There are items banned to be exported without cultivation certificate. In majority of the cases, the traders are not mixing inferior quality product with that of superior quality. Instead they are asking for cheaper rate against quality sacrifice and the buyers are going for less wanted compromise (Brown, 1994). In 2000, the medicinal plant part trading community of Kolkata formed an association in the name 'West Bengal Herb & Crude Drug Dealers' Association, to look after the interest of the community and medicinal plant trade as a whole. Cultivation of medicinal plants is being attempted with great effort in South India and also reportedly in Western India and North Western India. But in the Eastern region the attempts so far made are scanty and casual. The wild stock of medicinal plants are being depleted at a very fast rate due to over exploitation or premature extraction Medicinal plant diversity has its economic value and it has local, regional, national and international implications like patent right, Intellectual property right etc. It also has an alternative value and an intrinsic value which is academic and scientific (Pearce, 2001).

Overexploitation of Medicinal Plants in Asansol Raniganj coalfield area, West Bengal

Herbal medicine keeps working as the most popular medicine in solving health problems in the Asansol Raniganj coalfield region and people have strong trust in the efficacy of herbs. The reasons for the frequent use of traditional medicine being (i) the strong association of people with local flora and their belief on traditional knowledge regarding plants as medicine, (ii) easy availability of local medicinal plants, (iii) relatively poor access to synthetic drugs and their high cost and (iv) lower economic profile of the people. Mining of coal has affected the cultivation of existing vegetation especially medicinal plants, destroyed genetic soil profile, degraded air quality and altered current land uses. Although both mining and agriculture are primary activities of the people in this region but mining is preferred as it yields quicker returns. However, mining has destroyed the agricultural potential of the study area. The effect of mining on groundwater level, silting of water bodies are of great concern. It causes land degradation that affects the development of the plants. Due to underground mining, the top soil in tensile zones loses its vegetation supporting capability. Coal mining is associated with the degradation of

natural resources and destruction of habitat which causes invasive species to occupy the area which poses a threat to the ecosystem. Huge quantities of waste material are produced due to mining and if they are not disposed with care it affects the environment of the region (Ghose, 2004). There are several reports of overexploitation of medicinal plants from different parts of India like Himachal Pradesh, Rajasthan, Karnataka and Uttarakhand, etc. The State Medicinal Plant Board, West Bengal (2003) has released a list of 32 mostly consumed medicinal plants used as raw materials by Drug Manufacturing units in West Bengal and a list of 29 very prioritized and prohibited species.

Major causes for the loss of medicinal plants

The major causes for the loss of medicinal plants in India especially in Asansol Raniganj coalfield area are anthropogenic. However, some causes for the loss of bio-diversity which have been identified are– i) Habitat loss and fragmentation, ii) Introduced Species, iii) Overexploitation of medicinal plants, iv) Pollution, v) Global climate change, vi) Expansion of Industry and agriculture. Overexploitation and indiscriminate use of wild resources in commercial demand nowadays play a great role in the decline of the medicinal plants and has also become a threat for the survival of other associated species, thus acting as the major factors disturbing the entire ecosystem. Premature-exploitation of medicinal plants is another factor which is additionally responsible for rapid destruction of wild stock.

Conclusion and Recommendations

India plays an important role in exporting medicinal plants as raw material to various other countries. It is important to assess the level and extent of exploitation of medicinal plant from the wild stock and to put in place necessary checks. The survey in Asansol Raniganj coalfield area provides a veritable source of information for medicinal plant researchers and help in developing strategies for future conservation. The role of sustainable development, management, control of environmental changes is needed for the study area to preserve our indispensible natural resource of medicinal plants. Some recommendations are as follows - i) Collection of medicinal plants from wild should be banned ii) To employ latest techniques to improve the production of medicinal plants so that it can compete in the international market iii) To develop public awareness on the importance and needs for conservation of medicinal plants as it is our duty to preserve the herbal wealth for welfare of mankind iv) To understand the floral diversities in relation to local human needs and knowledge with special emphasis on indigenous species. The cultivation and preservation of medicinal plants protect biological diversity. The knowledge in this field would enable introduction of timely cultivation in necessary cases and maintain the required balance between proper sustainable use and exploitation of limited natural resources. Therefore, the interrelationship between flora, fauna and human should be urgently addressed in order to maintain a healthy eco-system.

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Methylglyoxal: A Short Review

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Abstract

Methylglyoxal a simple carbonyl compound containing a reactive aldehyde and a ketonic group which stops the growth of cancer cells without poisoning normal cells. It is also called as Retine. These are very small molecules that are highly potent in controlling cell division. This compound inhibits the enzymes required for cancer cell and infected cell to grow by respiration and does not harm normal cells. Methylglyoxal (MG), a dicarbonyl compound that is produced as a side product during glycolysis, is highly reactive and induces the formation of advanced glycation end-products that are implicated in several pathologies including cancer. As cancer cells require large amount of energy to multiply which was provided by ATP. Methylglyoxal inactivates the enzyme Glyceraldehyde-3-phosphate Dehydrogenase (GA3PD) needed for the ATP production in cancer cells and there by starves the cell to death and normal cells remain unaffected. As it is a carbonyl group, it inhibits the mitochondrial respiration followed by Glycolysis and Kreb's cycle which play a major role in the production of ATP and supplies the energy to infected cell up to demand. It also plays a role in binding of oxygen at cellular level and preventing the proteins to desaturate and inhibits the production of free radicals. Hence suitable energy and oxygen are unavailable to cancerous cell to grow, leading to death of the cell. It desaturates the proteins of malignant cell at cellular level by means of its ketoaldehyde group with an amino acid of a protein causing the death of cell.

Keywords: *Methylglyoxal, Anticancer drug, Mitochondrial respiration, Glycolysis.*

Introduction

Cancer, being a dreadful and uncontrollable disease in present society has only treatment but not cure. Cancer is due to the destructive desaturation of protein and DNA leads to mutation and uncontrolled division of cell (Gyorgyi, 1977; Sundra, 2013; Sharma, 2014; Vinayak, 2014). As several methods and techniques are developed to treat cancer has a wide range of adverse effects leading to reduce duration of patient life time. This is because the cancer therapy may be chemotherapy or radiotherapy is not only damaging the cancerous cell but also the normal cells due to its wide range of side effects. So, research is going on to treat only the tumour but not the other cells. As it is impossible that, if a foreign compound enter in to the body, it is not compatible with the

body environment and body irritates leading to side effects. So it became difficult for the scientists to work on the molecules to target the particular cell. Recently several methods are developed which target only the malignant cell by inhibiting the enzymes and catalysts that help for its growth of a cell. The rational of the study may provide ideas to improve the efficacy of Allopathic system of Medicine and cancer therapy.

Glyoxal and Methylglyoxal commonly called as Retine is a natural compound plays a significant role in cancer therapy. In 1937, Albert Szent Gyorgi, a Nobel laureate, for the discovery of vitamin-C worked on regulation of cancer and identified that Methylglyoxal inhibited the uncontrolled growth of the cell which was published in Science magazine in the year 1963. According to him, methylglyoxal is the primary electron acceptor before oxygen, as it is believed to be a universal acceptor (Gyorgyi, 1977). Methylglyoxal was produced by the nature during the evolution of life on earth. When life originated, the oxygen was not present in free form for the proteins as it is in bound form as water vapour, carbonates etc. Nature achieved it by taking a molecule of water and crowding all the oxygen at one end of molecules, the hydrogens at other end forming Methylglyoxal causing proteins to desaturate. The brown colour of the liver is due to the slight desaturation of proteins by methylglyoxal (Fodor, 1978). After availability of oxygen these desaturated proteins are used in the origin of life on earth. At cellular level, defective desaturation increase cell division. Retine is normally produced by body, when it is, it prevent growth of cancer. But the body can lose its ability to produce this substance leading to mutation. Putting the Retine back in to the body can stops the growth. His laboratory isolated and manufactured Retine in the year 1967 (Gyorgyi, 1977). Not only Dr. Albert but also scientists like Dr. Egyud and William. F. Koch made a remarkable approach on Methylglyoxal has anti-tumour activity.

Structure: Methylglyoxal is a carbonyl group with three carbon atoms. It contains an aldehyde group at the first carbon, a ketonic group on the second carbon and hydrogens at the end. These keto-aldehyde group interacts with that of amine group of protein and making it desaturate.

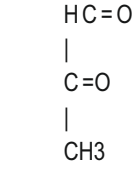

Figure 1: Structure of Methylglyoxal

Chemical Properties:

- **Formation of Schiff base:** Methylglyoxal and methylamine are mixed together in aqueous solution. Appearance of yellow color due to the formation of Schiff base. When solvents like acetone or methanol is added purple color appears (Gyorgyi, 1977).

- **Reaction with proteins:**

i) Solution of Lysine is treated with Methylglyoxal which turns into yellow color (Gyorgyi, 1977).

Table 1: Physical properties of Methylglyoxal	Yellowish hygroscopic liquid
Colour	
Odour	Pungent
Solubility	Soluble in water, alcohol, ether and benzene
Molecular weight	72.06266 g/mol
Molecular formula	C3H4O2
IUPAC name	2-Oxopropanal
Synonyms	Acetyl form aldehyde, Pyruvaldehyde
Density	1.0455

ii) Casein is treated with aqueous Methylglyoxal, Its granules become suspended in the solution and turns yellow. If methanol is added turns to brown. The brown colour of the liver is due to the slight desaturation of proteins by methylglyoxal (Fodor, 1978).

- **Absorption spectrum:** Mixture of 0.0256M solution of methylglyoxal and methylamine 0.077M and 0.0077M ascorbic acid was added, a very strong absorption appears at a wavelength of 400 nm and disappears slowly. A second peak appeared at 500 nm.

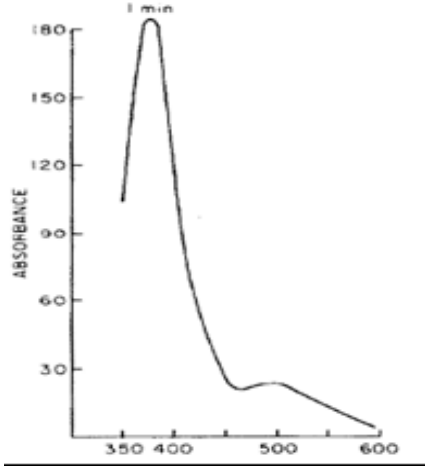

Figure 2: Absorption spectrum of Methylglyoxal

Fate of Methylglyoxal in the body: Methylglyoxal is produced in the body from glucose as a precursor. This is a parallel cycle to Glycolysis and Kreb's cycle. Methylglyoxal is

synthesized from three carbon containing molecule called Dihydroxy Acetone Phosphate (DHAP) in the presence of enzyme Methylglyoxal synthase. The produced Methylglyoxal inhibits Glyceraldehyde-3-phosphate dehydrogenase enzyme which is important rate limiting factor for the production of ATP. It also reduces the intermediates of Glycolysis and reduces the generation of ATP in normal body functioning situations. The production of Methylglyoxal in the body is restricted due to the continuous oxidation of glucose in to pyruvate during Glycolysis and Kreb's cycle. It is catalyzed by Glyoxalase system which is a two-step reaction using Glutathione as a coenzyme in which Lactic acid is produced (Gyorgyi, 1977).

Mechanism of action: Various theories are postulated on the action of Retine, out of which two of them are illustrated.

1. Inhibition of Mitochondrial respiration and Glycolysis: Methylglyoxal acts primarily by triggering a cells oxidative mechanisms to regenerate the impaired aerobic oxygen respiration that causes the cell to develop in to a cancerous. This reduces the formation of free radicals mutation and chain reactions at the DNA level. It is the fact that, cancer cell needs high amount of energy to multiply abnormally which was provided by ATP (Biswas, 1997). It is believed that Methylglyoxal inactivate the enzyme Glyceraldehyde-3-phosphate Dehydrogenase needed for ATP production in cancerous cells by inhibiting glycolysis here by starves them to death without affecting the normal cells. Recent studies have indicated that mitochondrial complex-1 and the glycolytic enzyme G-3-P Dehydrogenase may be critically altered specifically in malignant cells. Retine selectively inhibition of mitochondrial respiration and glycolysis in human leukaemic cells at lower concentrations (Biswas, 1997). Methylglyoxal strongly inhibits mitochondrial respiration in leukaemic leucocytes, whereas, at a much higher concentration, Methylglyoxal fails to inhibit mitochondrial respiration in normal leucocytes (Misra, 1996). Methylglyoxal strongly inhibits ADP-stimulated a-oxoglutarate and malate plus NAD+-dependent respiration, whereas, at a higher concentration, Methylglyoxal fails to inhibit succinate and a-glycerophosphate-dependent respiration.

2. Charge transfer and Electron permittivity: This theory was postulated by Albert szentgyorgi in his article "Living state and cancer" published in the science journal. Electrons can be taken out of molecules by other molecules by means of a charge transfer. Oxygen is an universal biological acceptor that can accept electrons from proteins and make it desaturate. The higher the desaturation of proteins higher the cell division (Gyorgyi, 1977). Oxygen also important for mitochondrial respiration for the regulation and production of ATP to meet the energy demand of cells to become cancerous. The charge transfer to proteins of mitochondria like SMAC [Small Mitochondria derived Activator of Caspases] binds to IAP [Inhibitor of Apoptosis pathway] and deactivates them, preventing the arresting of cell to degrade.

Methylglyoxal a carbonyl compound binds with a bond to oxygen transferring the acceptor power of oxygen to ketoaldehyde group forming Ascorbic acid. Albert szentGyorgi got Nobel Prize for the discovery of Vitamin C. The charge transfer takes place intramolecularly in between methyl-glyoxal and oxygen in which no net charge develops and formation of free radicals reduces preventing desaturation of protein leading to apoptosis. This charge transfer is referred as "Doping" (Gyorgyi, 1977). This reaction is catalysed by ascorbic acid (Fodor, 1978). So Vitamin C complex is used along with methylglyoxal. This reduces the ROS [Reactive Oxygen Species] and free radicals in the body preventing a tumor to develop.

Methylglyoxal in cancer treatment:

This method was developed by Dr. William. F. Koch in the year 1980. It is composed of homeopathic sized remedies of Methylglyoxal quinine molecules, one of their main functions is their ability to repair damaged respiratory enzymes in cells which is fundamental to the development of cancer. This work was promoted by Dr. Albert szentGyorgi, a Nobel laureate for discovering vitamin C. TMT uses a very slight amount of Methylglyoxal that states a self replicating process causing the cell and passes on to restore the Methylglyoxal in another cell causing a cascade of cellular response and stopping uncontrolled replication. If cancer cells does not replicate, it will die. Koch's TMT consists of five different formulations of homeopathic remedies of Glyoxal and Methylglyoxal in cells. The five formulations are Parabenzochinon, Rhodizonsaure, carbonyl gruppen [SSR], carbonyl gruppen, carbonyl gruppen (SSRI). These formulations produce methylglyoxal in the body and also reduces the formation of free radical formation.

Conclusion

It was found that leukaemic leukoytes have a high rate of aerobic glycolysis compared with other normal cells, although there is some variation with the type of leukaemic cells which derive most of their energy from glycolysis from many research works, it was observed that Methylglyoxal inhibits both mitochondrial respiration and glycolysis in leukaemic leucocytes, whereas it has no effect on the similar functions of normal leucocytes. Excessive ATP formation in cells also may lead to malignancy. Methylglyoxal inhibits electron flow through complex I of the mitochondrial respiratory chain and inactivates GA3PD in malignant cells, which suggests that in malignant cells are critically altered. It not only inhibits mitochondrial respiration but also reduces desaturation of proteins, reduced production of free radicals. By these anticancer strategies of methylglyoxal, it may replace the anti cancer drugs which produce many adverse effects and improves the efficacy of cancer therapy.

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Benthic Macroinvertebrates as Fine Descriptors of Pollution: A Review

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Abstract

This review discussed the occurrence, composition and distribution of macrobenthic invertebrates around a sewage outfall region in a stretch of river Hugli. Analysis of the benthic community helped in identifying the pollution tolearant organism and supported the use of bioindicator organisms in biomonitoring programme of the rivers. The importance of oligochaetes of the tubificidae family as bioindicator was also established.

Keywords: *Macroinvertebrates, Bioindicator, Tubuficidae*

Introduction

Benthic organisms are an important constituent of aquatic ecosystem. The benthic macroinvertebrates can be identified as those that are attached to the bottom and are retained by a sieve or mesh with a pore size of 0.2 to 0.5mm. These organisms can serve as a tool for biological monitoring. As they are sedentary with comparatively longer life cycles, they can provide valuable information by their presence/ absence/abundance/species composition, about the surrounding habitat even when the effects of chemical changes in water (if any) retreats. Their importance as bioassessment tools is recognized world wide (Mason, 1981; Hellawell,1986; Metcalfe,1989; Finogenova,1996; Markert, *et.al.*, 2003; Li, *et. al*., 2010).

Benthic macroinvertebrates as bio indicators

A bioindicator should reflect some characteristics such as – wide distribution, low mobility, well known ecological characteristics, can be used for laboratory experiments, high sensitivity to environmental changes and should be easily recognized by taxonomic characteristics. They should contain information about the quality of the environment (Markert *et. al*., 2003). Investigators such as Gaufin & Tarzwell (1952,1956), Brinkhurst (1966), Wilhm & Dorris (1966) have relied mostly on benthic organisms. Benefits of biomonitoring are manifold as described below:

- Source of Pollutants/toxicants/anthropogenic fallouts/agricultural run offs containing harmful agrochemicals, pesticides, etc. can be noticed.
- Identification of pollutants/ toxicants
- Seasonal variations of the pollutants

- Number of macroinvertebrate taxa at the site
- Species richness, variations,
- Vulnerability of key species
- Identification of species sensitive to pollutants and tolerant species

The most frequently used organisms for biomonitoring are benthic macroinvertebrates, periphytons and fishes. However, here oligochaetes as bioindicator organisms are discussed in relation to a case study in river Hugli. Oligochaetes are used in biodiversity studies, pollution surveys and environmental assessment widely.

Oligochates as bioindicator organism – A case study

Along Hugli river, three stations were selected and recorded with the help GPS device. A sewage outfall region (near Bagbazar area, Kolkata) was numbered as station 2(22.6050oN 88.3648oE).Station 1 and station 3 were respectively upstream and downstream of station 2.

Physico-chemical parameters such as Dissolved oxygen, Biological oxygen demand were measured in premonsoon, monsoon and postmonsoon seasons following APHA, 2005. Macrobenthic organisms were also surveyed along with the physicochemical parameters.

Figure 1: Sewage outfall region

The results obtained revealed that the mean value of dissolved oxygen varied around 6.0mg/l at stations 1 and 3 but station 2 reflected a poor concentration of oxygen throughout the year, never exceeding 3mg/l. The mean values of BOD recorded at station 1 and station 3was not so high and ranged from 12-15mg/l. But station 2 with poor concentration of oxygen showed ahigh value of BOD of 51-55mg/l.

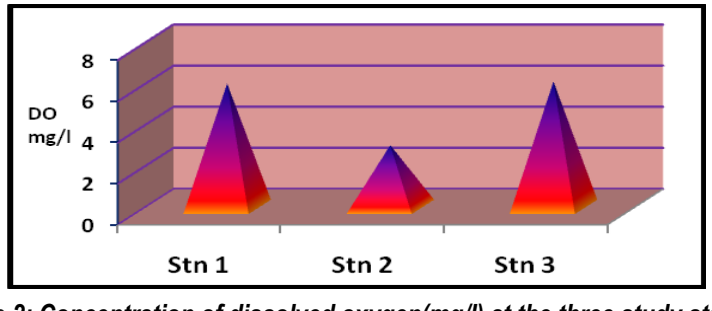

Figure 2: Concentration of dissolved oxygen(mg/l) at the three study stations

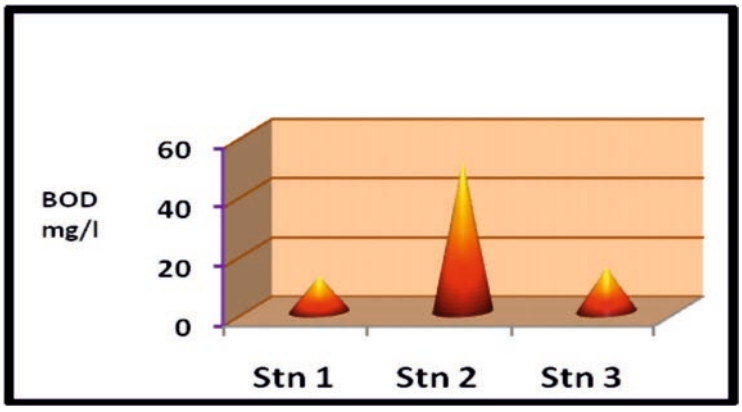

Figure 3: Sewage outfall region: Value of Biological oxygen demand(mg/l) at the three study stations

Faunal analysis revealed the presence of mainly four groups of organisms, viz., Gastropoda, Bivalvia, Polychaeta and Oligochaeta. Stations 1 and 3 showed higher percentage of Gastropoda followed by Polychaeta and Bivalvia. Oligochaetes were very sparce at these two stations.

But the fauna recorded at station 2 which is also a sewage outfall region, was mainly represented by oligochaeta-tubificid worms identified as *Limnodrilous hoffmeisteri* which contituted 99% of the total fauna indicating its dominance over the other groups.The other groups were seldom recorded from this station. So station 2 with such poor concentration of oxygen, high BOD values and high densities of these tubificid worms throughout the year indicates its polluted nature due to the inflow of sewage water and other anthropogenic activities. Also the pollution tolerant nature of *L. hoffmeisteri* was ascertained.

The use of the above mentioned tubificid worm as pollution indicator species is widely accepted by various authors (Brinkhurst,1965; Aston,1973; Metcalfe, 1989; Finogenova,1996; Yap *et.al*., 2006; Rodriguez & Reynoldson, 2011; Chowdhary & Sharma, 2013).

Conclusion

From the preceding review, it is evident that benthic macroinvertebrates are important biomonitoring tool and can be used as complimentary to chemical analysis. Oligochaetes are used in various pollution surveys and in environmental assessment studies. Also the importance of *Limnodrilus hoffmeisteri* of family Tubificidae of class Oligochaeta, in depicting the environmental condition is clear. These worms are found to indicate heavy sewage pollution in different lakes and rivers of the world.

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Understanding Suicide Attempt and Suicide Ideation in Patients: The Potential of Biomarkers for Rapid Detection

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Abstract

Suicide is currently one of the greatest health threats worldwide claiming nearly 1 million death every year across different age groups. Numerous studies have shown the significant role of abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis, noradrenergic, and serotonergic structures which are linked to patients with suicidal attempt and ideation. The involvement of genetic factors such as gene expression and DNA methylation plays a pivotal role in suicidal behaviour as shown in some of the family studies, twin and adoption studies, candidate gene analyses and genome-wide affiliation studies (GWAS). Vast literature evidence has revealed the change in suicide attempts is directly linked to rising stress, traumatic events, addiction and/or illnesses in people with changes in neurobiological and genetic factors. Additionally, suicide completers are most serious and vulnerable phenotype in society as it is very challenging to facilitate them as cohorts due to change in their behaviour, degree of lethality/vulnerability and suicidal intent. Molecular biological and biomedical tools are being used to identify genetic biomarkers that can link directly with suicidal behaviour across age groups. It has been proposed that investigating. DNA methylation rate and its stage with genes intrinsically linked to suicides along with detection of single nucleotide polymorphism approaches can potentially be game changers to identify the most vulnerable people in our society. This mini-review provides a glimpse on some of these challenging components that can be used to identify and stop suicides across age ranges globally.

Keywords: *Suicide, Molecular diagnostics, Epigenetics, Biomarker*

Introduction

Every year nearly 1 million death occurs worldwide due to suicides making it a serious health issue in teenagers and adults (Patel *et al.*, 2012). This accounts to approximately 1.4% of all deaths worldwide (Brådvik, 2018). For example, in USA, 5079 deaths in 15-

24 age group (male and female combined), 6569 deaths in 25-34 age group and 15473 deaths in 35-54 age group have been reported under suicide category (WHO Mortality Database). However, such critical information is largely missing from countries in South Asia. 2009 data suggested suicide as major cause of death crossing the motor vehicle accidents in United States alone (Rockett, 2012). On average 10-12 individual out of 100,000 committed suicides based on the records of last six decades. It is challenging to ascertain the reasons behind rise in suicide rates globally because of unpredictable complexities (Figure A1) including at the age group levels or gender, irrespective of major advances in modern mental health diagnostics (Cox *et al.*, 1987; Brådvik, 2018). Suicide phenotypes are unique in terms of its identity and nearly 90% cases reported had found psychiatric disease to be the major cause (Kellam *et al.*, 1994; Rice *et al.* 2008). However, the occurrence of same is not always dependent on depression but also on other factors such as sexual abuse, substance-based disorder, mental setbacks, personality, stress and trauma in daily life (Kellam *et al.*, 1991; Cao *et al.* 2010; Langfelder & Horvath, 2008; Guintivano *et al.* 2013).

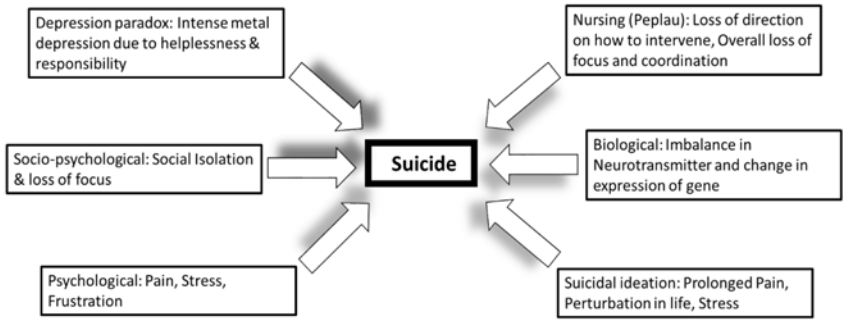

Figure A1: Factors influencing suicidal minds and ideation of suicidal thoughts

The impairment of gene expression in hypothalamic pituitary – adrenal axis (HPA), serotonergic systems and noradrenergic systems had been associated with observed abnormalities in suicide victims (Coryell & Schlesser, 2001, Oquendo *et al.*, 2014). Genetic association studies on suicidal behaviour encompassing family-based association studies and genome-wide association studies have focused on understanding link of genes such as serotonin receptors and tryptophan (Bondy *et al.*, 2006; Mirkovi *et al.*, 2016). The evidence suggests prolonged stress, neurobiological impairments with illness along with genetic risk and traumatic events are directly involved in suicidal ideation. Suicidal ideation poses a serious challenge in the age group of 12-30 years globally (Scott *et al.*, 2012). To date limited number of studies has been undertaken to understand genetic factors and diagnostic biomarkers that can be linked to suicidal ideation. This could be due to obscurity in behaviour of a person with suicidal ideation and/or attempts and difficulty in collecting samples for undertaking

robust analysis and subsequent interpretations. Suicide completers largely go unnoticed in our society and thus it is challenging to understand their motives unless intervention such as verbal counselling is undertaken.

A) Aberrant telomeres length:

Recently an interesting study has shown aberrant telomere length and mitochondrial copy number among suicide completers (Otsuka *et al.*, 2017). The repetitive nucleotide sequence of chromosome is known as telomeres and studies have shown shortening of length is directly involved in genomic instability and resulting apoptosis (Zhang *et al.*, 1999). Therefore, length of telomere can be used in the field of stress biomarker discovery. In a study, postmortem samples from 528 suicide completers in comparison to 560 normal individual considered as control were analysed by quantitative PCR reaction and the results suggested significant shorter length of telomeres among suicide completers (Otsuka *et al.*, 2017). It is noteworthy to add with course of age telomere shortening is reported in somatic cell like leucocytes (Armanios & Blackburn, 2012). The biological age of cells is therefore reflected by telomeres (Mather *et al.*, 2011). Other studies have also incorporated mental illness and other stress related disorder with telomere shortening (Lindqvist *et al.*, 2015). For example, studies including anxiety disorder (Hoen *et al.*, 2013), Post-Traumatic Stress Related ailment or PSTD (O'Donovan *et al.*, 2011), major depressive disorder (MDD) (Simon *et al.*, 2006; Verhoeven *et al.*, 2014), Schizophrenia (Kao *et al.*, 2008) and Bipolar disorder (Rizzo *et al.*, 2013) were found to be linked to telomerase length shortening. In another study the role of shortening of TL was found in patients diagnosed with MDD, PTSD and schizophrenia based on meta-analysis (Darrow *et al*., 2016). However, there are also few exceptions reported with no significant link between TL shortening and stress related disorders (Needham *et al.*, 2015; Nieratschker *et al.*, 2013). The microscopic study of hippocampus cells and oligodendrocytes from brains of post-mortem samples of suicide victims also depicted short telomeres length (Szebeni *et al.*, 2014; Mamdani *et al.*, 2015).

B) Mitochondrial DNA (mtDNA) copy number

Studies have shown correlations between neuropsychiatric condition and mitochondrial dysfunctions. Various important functions including cell proliferation, tissue differentiation, calcium signaling, cellular bioenergetics, apoptosis are driven by mitochondria (Johannsen & Ravussin, 2009). Cellular aging process and typical age related disorders are often associated with changes in mitochondrial functions (Lagouge & Larsson, 2013). Mitochondrial DNA copy number (cn) serve as index of mitochondrial dysfunction; play crucial role in biogenesis and can be assessed directly by measuring it per molecule of cell (Sahin & DePinho, 2012). Limited number of studies have been conducted to ascertain the relation of stress related disorders among psychiatric cases with mitochondrial DNA copy numbers (Giulivi *et al.*, 2010; Kim *et al.*,

2013; Chang *et al.*, 2014; Li *et al.*, 2015; Bersani *et al.*, 2016). Recently, a study on post-mortem samples of suicide completers in comparison to normal individuals was analysed through quantitative PCR and the result confirmed increased copy number of mitochondrial DNA among the former (Otsuka *et al.*, 2017). In another study, it was observed that copy number of mtDNA was higher in case of patients with childhood adversity and related neurological disorders (Cai *et al.*, 2015; Tyrka *et al.*, 2016). Studies on mitochondrial dysfunction was also correlated with aberrant telomere shortening in case of P53 mediated repression of peroxisome PGC-1α and PGC-1β (Sahin *et al.*, 2011; Sahin & DePinho, 2012; Tyrka *et al.*, 2015). Therefore cellular aging and neurological conditions are strongly related to mitochondrial dysfunction and shortening of telomere length.

C) DNA methylation studies in suicide completers

Individuals with early life trauma happens to influence stress later in their life due to its influence in several biological systems (Agorastos *et al.*, 2019). There impairments lead to various disorders such as alcohol and drug abuses, psychiatric disorders, mood disorder, disturbances in cognitive abilities and enhanced impulsivity which ultimately results in suicidal attempts (Kellam *et al.*, 1991). Molecular pathways involving the hypothalamic pituitary adrenal axis and its sensitivity is influenced by epigenetic alteration such as DNA methylation. Experiments using rats have shown changes in mental behaviour due DNA methylation in NR3C1 gene coding glucocorticoid receptor (Le-Niculescu *et al.*, 2013). The similar alteration was also documented among suicide completers who had early life traumatic experiences (Rayssiguier *et al.*, 2010). The studies conducted showed reduced level of glucocorticoid receptor (GR) leads to improper management of stress among individuals due to impaired epigenetic event in their early life traumatic events (Juruena *et al.*, 2015). Therefore, most of the cases of stress and suicide related behaviour have shown molecular variation such as change in DNA methylation and gene expression in NR3C1. In the stress hormone level often act as agonists to glucocorticoid receptors and therefore these can act as suitable biomarker for assessing stress pathway in person with suicide ideation as well as on suicide completers. The epidemiological and molecular pathways associated with suicide victims are often linked to glucocorticoid (Reznikov *et al.*, 2008; Nock *et al*., 2009; Bougea 2013; Shonkoff & Garner, 2012; Bali *et al.*, 2013; Turecki, 2012). Similarly, the level of cortisol also serves as a marker for stress response as the expression of glucocorticoid receptor found to vary with elevation or reduction in response to early life stressors (Juruena *et al.*, 2015). A new theory of dual risk hypothesis on environmental factors affecting gene expression was proposed to identify how biological state might negatively influence stress response in human (Coryell & Schlesser, 2001; Ridder *et al*., 2005; Smalheiser *et al.*, 2012; Morlando *et al.*, 2008; Choi 2010).

Another notable biomarker in studying the molecular genetics of suicide behaviour is the Brain-derived neurotrophic factor or BDNF. It is a member of neurotrophin family and act as growth factor involved in synaptic plasticity (Bibel & Barde, 2000) also plays crucial role in development, neurogenesis and survival of neural network (Russo-Neustadt, 2003). Changes in BDNF regulation adversely affect central neuronal circuit with abnormalities in stress response and resulting in reduced plasticity (Dwivedi, 2012). Interestingly, lower mRNA expression and higher-level methylation in promoter/exon IV associated with BDNF genes was observed in brain tissues of suicide completers when compared to control (Keller *et al.*, 2010). Additionally increased DNA methylation and resulting downregulation of expression was noticed in genes coding NTRK2 (Ernst *et al.*, 2009), γ-aminobutyric acid (GABA) A receptor α1 subunit (Poulter *et al.*, 2008) and spermine oxidase (SMOX) (Fiori & Turecki, 2010). Another candidate of genes including like ErbB3 (Mahar *et al.*, 2017) and kappa opioid receptor (Lutz *et al.*, 2018) was also reported to be associated with victims of suicidal ideation.

D) Genome-wide DNA methylation studies

Deep sequencing methods enable us to investigate altered methylation pattern in whole genome sample of suicidal versus normal individuals at more precise scale. Recent study on sequencing of 366 promoters has revealed differential methylation with upregulation of genes associated with cognitive processes (Labonte *et al.*, 2013). On contrary, astrocyte marker of glial cells showed opposite methylation pattern with lower level of expression in male suicide victims (Nagy *et al.*, 2015). In another study, CpG sites of interest showed hypomethylation patterns in the genes PSORS1C3 when compared with suicide completers and non-psychiatric sudden death victims (Murphy *et al.*, 2017). Therefore, larger number of cohorts showed differential expression in suicide victims including male and female case studies. The patterns can also vary with anatomical position of studied group. For example, patients with major depressive disorder provide evidence of greater methylation in ventral prefrontal cortex region of the brain (Haghighi *et al.*, 2014). Genome wide study on individuals died committing suicide by hanging when compared to control group exhibited statistically significant difference in methylation level in hipocampus and prefrontal cortical region (Kouter *et al.*, 2019). The same study reported genes such as, ZNF714 and NRIP3 also showed altered expression. Studies conducted on patients of bipolar disorder with suicidal behaviour also highlighted involvement of three candidate genes from their blood methylation profile (Jeremian *et al.*, 2017). Brodmann area 10 region of the brain essentially showed significant overlap of expression profile since this region is involved in development and function of brain (Aberg *et al.*, 2018). Therefore next generation based whole genome profiling and thorough computational biology approaches are proving to be essential tools for analysing hidden part of genomes that are linked to suicidal ideation.

E) Convergent functional genomic

Health risk associated with suicide causes irreversible damage to individual suffering from depression or any other disorder and irrespective of victim's socio-cultural background (Nock *et al.*, 2008; Berngruber *et al.*, 2013). It is very difficult to predict the mid of individual with suicidal ideation especially for patients with various mental disorders. Convergent functional genomics had been an indispensable tool for predicting person with suicide risk without asking the individual or undertaking any counselling session. Patients with fear for hospitalization and difficult to judge by counselling can now be alternatively diagnosed by convergent functional genomics approach to identify biomarkers relevant to suicidality. For example, previous studies on psychosis patients showed evidence of blood biomarker traceable to the disease symptom (Kurian *et al.*, 2011). Similarly blood gene expressions can also indicate mood disorders (Le-Niculescu *et al.*, 2009). A fairly reproducible protocol was developed with convergent genomic approach in case of suicide patients with postmortem sample of prefrontal cortex and was reported to be associated with genes involved in bipolar disorder and schizophrenia (Kim *et al.*, 2007). Interestingly, spermidine/spermine N1–acetyltransferase 1 coding SAT1, tensin homolog coding PTEN, myristylated alanine-rich protein kinase C substrate (MARCKS), and mitogen-activated protein kinase kinase kinase 3 (MAP3K3) genes showed significant upregulation in suicide patients (Le-Niculescu *et al.*, 2013). The convergent genomics approach has also helped to identify pathways and mechanism of neuropsychiatric disorder associated with chronic alcohol addiction (Rodd *et al.*, 2007) and anxiety (Le-Niculescu *et al.*, 2011). The predictive ability of convergent genetic modelling has been tested in independent cohorts of patients with bipolar disorder (Niculescu *et al.*, 2000; Ogden *et al.*, 2004; Patel *et al.*, 2010; Le-Niculescu *et al.*, 2009).

2. Conclusions and Future Perspective

In this short communication we discussed the epigenetic basis of suicidal behaviour and ideation (Figure A2).

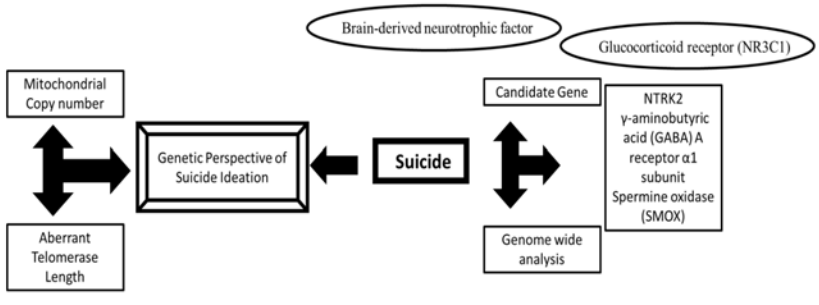

Figure A2: Molecular approach of pre-diagnosing victims with suicidal ideation by biomarkers

It is noteworthy to mention environmental factors greatly influence biological and

physiological stress among individuals. The suicidal ideation among them thus can become more challenging to understand and interpret by general counselling sessions. This limitation can easily be counter diagnosed by study of genetic biomarkers which can reflect actual status of stress among victims. The complexity of suicidal behaviour and the role of environmental factors on the ideation process areinterlinked to epigenetic mechanism as detailed in the earlier sections. Changes in DNA methylation rates and certain patterns are observed in animal model system as well as human samples with suicidal thoughts and commits. Postmortem samples analysed for gene expression studies exhibited changes in expression profile of glucocorticoid receptors, BDNF coding genes and few other candidates by whole genome next generation sequencing methods. Understanding the underlying molecular pathways triggering suicidal ideation is therefore a frontier topic of research in modern diagnosis and can form the basis to develop new strategies to defend mortality caused as a result of suicides. New insights of integrating case history, counselling report, genetic blueprints along with neuroimaging data can enable towards developing new tools for quicker identification of patients with chronic depression and suicidal thoughts. In today's stressful world irrespective of age, sex or location genetically predisposed individual might show maladaptive behavioural traits sometime under cover and impossible to detect by common methods. Moreover the approaches discussed in the review could also help us to develop potential drug targets for regulation of epigenetic changes and therefore would be an indispensable tool for finding new avenues of therapeutics. Future studies on post translational modifications, analysis of non-coding RNA such as miRNA along with role of gut microbiome would highlight more details of molecular basis of signalling and background of suicidal thoughts.

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Structural Studies on C-type Lectins Using Molecule Modeling Techniques

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Abstract

Letins, the sugar-binding proteins, are gaining importance because of their participation in immune systems, apoptosis, pathological conditions, host-pathogen interactions and cell-cell communications. The C-type lectins represent a large family of Ca^{+2}-dependent lectins that play important roles in recognition events in a variety of biological processes and share primary structural homology in their carbohydrate-recognition domains. Ca^{2+} ion mediates binding of carbohydrate ligands to the C-type CRD and stabilizes the local conformation of the protein and makes coordination bonds to the acidic residues of the protein and the hydroxyl groups of the sugar ring. Amino acid residues that coordinate the bound Ca^{2+} also make hydrogen bonds to the sugar hydroxyl groups. Calcium plays major roles in maintenance of lectin structure and directly involved in the sugar binding. However, little is known about whether calcium could affect an overall conformation of C-type lectins that determines lectin activity or whether calcium is required locally as an essential component within carbohydrate binding sites. In the present study, I want to predict the 3-D structures of some C-type lectins from the newly sequenced hypothetical proteins to examine their structural details.

Keywords: *Molecular modeling, Lectin, Calcium ion, Carbohydrate Recognition Domain, Structure.*

Introduction

Computer aided molecular modeling

The knowledge of the 3-D structures of proteins is very important in understanding their functions in living systems and designing new ones for biological and medical purposes. While the amino acid sequences of proteins are being worked out in an explosive rate, the experimental determination of 3-D structures by x-ray crystallography and NMR spectroscopy is rather slow, costly and needs highly skilled man powers. Therefore, a need exists for theoretical and computational techniques for the prediction of protein structures from their sequences.

The discovery that proteins occur in homologous families provided the original impetus for attempting to model the three-dimensional structure of one member of the family from the known experimental structure of another. This methodology has since been used many times in a wide variety of systems to model new protein structures for the purpose of studying protein functional properties, performing biochemical or mutagenesis experiments, designing new ligands or inhibitors of known biological functions or antibody antigen interactions. It is important to identify parts of the structure which are preserved in a class of proteins as well as those likely to deviate significantly from the usual family structural rubric.

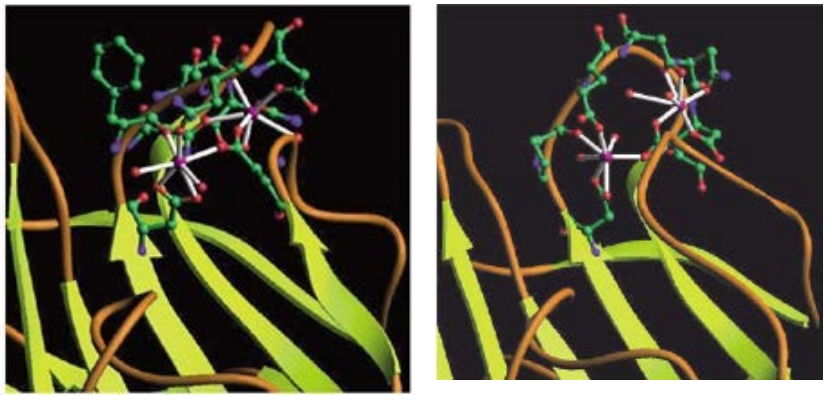
Methods

The initial structures of the C-type lectins were obtained by knowledge-based homology modeling using our in-house software package of ANALYN and MODELYN (Mandal, 1998). The modeled structures were refined using the InsightII (2005) of Accelrys equipped with DISCOVER as the energy minimization and molecular dynamics module. CLUSTALW (Thompson *et al.*, 1994) was run through the Internet for multiple alignment of the amino acid sequences. The electrostatic potential surfaces of the proteins were determined by MOLMOL (Koradi *et al.*, 1996). PROCHECK (Laskowski *et al.*, 1993) was used for checking the structural parameters. Both MOLMOL and PROCHECK were run on FUEL in the UNIX operating system. The free energies of binding of the complexes were calculated using the DOCKING module of InsightII. Protein BLAST (Altschul *et al.*, 1997) was used through the Internet for finding homologous sequences.

Results and Discussion

Calcium binding environment

Both the calcium ions in the x-ray structure of p58/ERGIC-53, a calcium-dependent mannose-selective animal lectin, are hepta co-ordinated (Velloso *et al.*, 2003). Five co-ordination positions are satisfied from the residues of proteins and two from water molecules. Equivalent AAs involved in calcium co-ordination were identified on the modeled structures. Calcium co-ordination in p58/ERGIC-53 and one of the modeled proteins from Homo sapiens which have 46% AA identity with the starting scaffold are shown in Figure.1. For most of the modeled proteins I found that five equivalent atoms of the protein are involved in ligand co-ordination of calcium ions. In addition, two water molecules could be placed in the similar positions as those of the x-ray structure.



(A) (B)

Figure 1: Residues of protein involved in co-ordination with calcium ions are depicted in balls-and-sticks representation and calcium ions in pink spheres. (A) p58/ ERGIC-53 (B) a hypothetical protein of Homo sapiens (Patra et al., 2006).

In periplasmic GGBP of Salmonella typhimurium the calcium ion is surrounded by seven ligands (Vyas *et al*., 1987 & 1991) in two calcium-binding loops (CBL-1 and CBL-2) (Figure 2). The amino acids (DLNKDGKIQ) forms the major calcium binding loop (CBL-1), and this sequence is found to be highly conserved in the three target sequences selected for modeling.

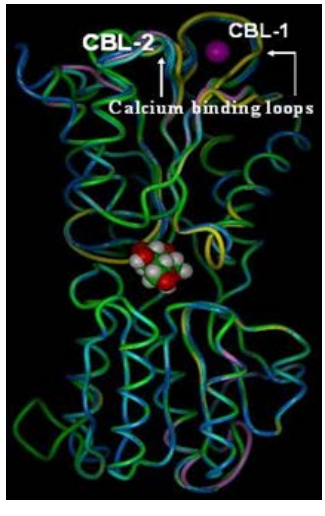


Figure 2: Ribbon representation of the superposed structures of the modeled proteins with the crystal structure of 3GBP. The ligand molecule is shown in space filling representation and is colored by atoms (Patra et al., 2006).

The loop CBL-2 only participates in calcium binding via Glu-205 side chain, whereas the side chains of Asp-134, Asn-136, Asp-138 and Gln-142 and main chain of Lys-140 of loop CBL-1 are involved in calcium-binding (Figure: 3). We have identified all the conserved equivalent residues and calcium-coordinated atoms in the modeled structures. Similar seven ligand calcium-coordination was also observed in case of a fucose binding lectin (Mitchell *et al.*, 2002).

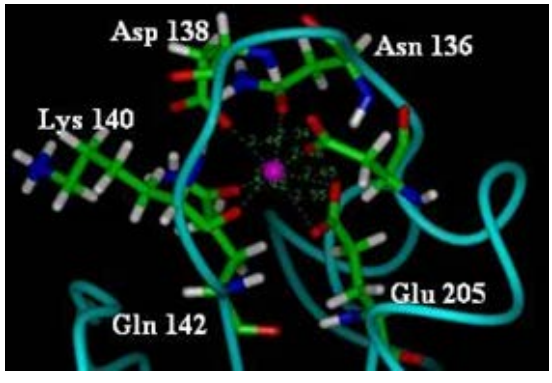


Figure 3: Mode of calcium binding in 3GBP. The calcium ion is shown in pink. The residues of protein involved in binding are colored by atom and shown in sticks. The distances of calcium ion from their coordinating atoms are also shown. The calcium ion has a classical seven-ligand coordination (Patra et al., 2006).

Conclusion

We have modeled the structures of some proteins from various species based on the experimental structure of p58/ERGIC-53(PDB ID: 1R1Z) and the structures of four bacterial proteins taking the crystal structures of a periplasmic glucose/galactose binding receptor protein from Salmonella typhimurium (PDB ID: 3GBP/1GCA) as templates. I have identified the acidic residues involved in the coordination with calcium ion in modeled protein structures and found they are conserved.

Abbreviations

3D, three dimensional; AA, amino acid; CBLs, calcium-binding loops; CRD, carbohydrate-recognition domain; GGBP, glucose/galactosebinding protein.

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Status, Threats and Impacts of Biodiversity Groups in Sundarbans Ecosystem: A Review

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Abstract

Discharge of untreated industrial effluents and domestic waste water are impacting aquatic habitats and biodiversity groups in Sundarbans ecosystem. Natural events in the form of storms and sea level rise have increased salinity levels, effecting mangrove growth and leads to the loss of Sundari *(Heritiera formes)* and nipa palm, which are species with low salinity tolerance. Indiscriminate seed collection results in the loss of variety of aquatic species. Thousands of untrained workers who collected shrimp try from sea, channels and rivers cause significant losses to the fry of other species. Various study revealed that there are a marked increase in the area of saline banks, which have increased from 38.93 square km to 74.79 square km (w.e.f. 2011) and many species have lost due to transformation in saline zone. This paper will represent a review of the on the status and threats of biodiversity groups in the Indian Sunderbans was published by the WWF (2017) to provide a critical evaluation of the current state of biodiversity in this area.

Ke*ywords:* *Salinity, Biodiversity Groups, Ecosystem*

Introduction

The Indian Sundarbans Delta (ISD) is part of the delta of the Ganga-Brahmaputra-Meghna (GBM) basin in Asia. The Sundarbans, shared between India and Bangladesh is home to one of the largest mangrove forest in the world. The ISD spread over about 9630 between 21˚40'04"N and 22˚09'21"N latitude, and 88˚01'56"E and 89˚06'01"E longitude. This part of the GBM delta as we see it today came to be formed between 2500 and 5000 years ago by the silt carried by the river Ganges (Allison *et al*., 2003) as well as its tributaries like Mayurakshi, Damodar, Ajay, and Kansai rivers. It is part of the tide dominated lower deltaic plain.

The Indian Sundarbans Delta is bounded by the Ichamati-Raimangal River in the east, by the Hugli River in the west, by the Bay of Bengal in the south, and the Dampier1 Hodges line drawn in 1829-1830 in the north. A little over half of this area has human settlements on 54 deltaic islands the remaining portion is under mangrove vegetation. Soils of ISD are principally Alfisols (older alluvial soil) and Ardisols (coastal saline soil).

The landscape is characterised by a web of tidal water systems. The average tidal amplitude is between 3.5-5 metres, with the highest amplitudes in July-August and the lowest in December-January. Of the 8 rivers that dominate the landscape only the Hugli and Ichamati-Raimangal carry freshwater flow of some significance. Being the moribund part of the lower delta plain of the GBM system, the ISD is experiencing both declining freshwater supplies and net erosion, as has been recorded since 1969 (Hazra *et al*, 2002; Hazra, 2010).

The climate of the region is tropical with high relative humidity between 70-88 percent. The mean maximum temperature is 34°C during June and the mean minimum temperature is 11°C during January. Although the region experiences occasional rains through most of the year barring January and February (Chaudhuri & Choudhury, 1994), the monsoon period, which occurs between June and October accounts for about 80 percent of the annual precipitation. The ISD is prone to extreme storm events which are frequent during the pre-monsoon period, and from September through November. Historical records indicate a high frequency of extreme weather events, such as severe storms or cyclones.

A pronounced ecological change is evolving in this delta due to huge discharges of untreated domestic and industrial effluents carried by tributary rivers as well as the disposal of contaminated mud from harbour dredging and resulting from the rapid emergence of the Haldia Port Complex, a major oil disembarkment terminal in eastern India. The Sundarbans delta has become susceptible to chemical pollutants such as heavy metals, organochlorine pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons which may have changed the estuary's geochemistry and affected the local coastal environment (Mahadevia, Vikas; Climate Change, 2012). Due to a diversity of inputs such as agricultural runoffs, wastewater and sewage discharges, and agricultural wastes, maximum concentrations of organochlorine pesticide residues were recorded at sites located along the main stream of the Hugli (Ganges) estuary. Among the hexachlorocyclohexane isomers (HCHs) and dichlorodiphenyltrichloroethane DDTs, β-HCH and DDE predominate. From an eco-toxicological point of view, the impacts of DDT and HCH are much pronounced.

Sundarbans Bioiversity Groups

In addition to this, the ecosystem provides habitat for a wide range of terrestrial and aquatic species, including large numbers of migrant and resident bird species, fish, and invertebrate assemblages as well as important endangered and highly threatened species. The Sundarbans ecosystem also contains numerous species of microbes, algae, and lichens (table 1). It also serves as a breeding ground for two of the four most primitive Horse Shoe Crabs which travel across the Asia Pacific region. In some groups—for example, mollusks—the Sundarbans has a very high number of species, genera, and families compared to similar ecosystems. The existence of such a wide

array of globally threatened animals significantly increases the value of the Sundarbans in the context of the global commons. Reclamation over time has led to a number of local extinctions as well as reduction in habitat for a number of species. Five species are known to be extirpated from the Indian Sundarbans: the water buffalo (*Bubalus bubalis*); the swamp deer (*Cervus duvaucelli*); the Javan rhinoceros (*Rhinoceros sondaicus*); the gharial (*Gavialis gangeticus*); and the chitra turtle (*Chitra indica*) (Chaudhuri & Choudhury 1994, Das & Nandi 1999). The faunal composition is undergoing changes, with more species being included in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) as a result of habitat degradation and ecological changes. A brief assessment of the biodiversity in the Indian Sundarbans is given in Table 1. The matrix takes into account the ecosystem services provided by biodiversity groups are discussed here. It also lists the role that each of the biodiversity groups play in the dynamics of the Sundarbans ecosystem and the threats to each of these biodiversity groups.

Table 1: Status, Threats, and Impacts of different biodiversity groups on Sundarbans eeosystem:

Sl. No.	Biodiversity Group	No of Species	Importance	Threats	Impacts of Sundarbans Ecosystem if absent
1.	Lichens	167	Pioneers in habitat colonization Fix nitrogen and fertilize forest soil Sequester carbon; 30 percent o body weight Accumulate toxic chemicals or radioactive nucleotides Medicinal usage, from secondary metabolites Primary producer Home for invertebrates an d provide benefits to insects-camouflage and mimicry	Decline in vegetation cover Developmental activities Fuelwood collection Impact of climate change	Impact on ecosystem energetics
2	Mangroves	105	Promote wide array of ecosystem services including: Act as buffer against natural calamities Mangrove swamps support wide variety of aquatic, benthic, and terrestrial organisms. Mangrove detritus acts as substrate for microbial activity and nutrient generation, thus a nutrient and carbon sink. Medicinal importance Promote ecotourism	Devoid of any high-elevation zone for the species to reestablish due to sea level rise Habitat degradation due to industrial pollution. Timber poaching and fuel wood collection	Land mass vulnerable to tropical cyclones Absence of substrate for microbial activity, thus impact on nutrient dynamics of estuarine ecosystem
3.	Non-mangroves	145	Coastal stability through increasing planctonic productivity	Anthropogenic disturbances along beaches	Successional stages of plant communities would be impacted Habitats for a umber o faunal resources would be impacted

4.	Mollusca	17	Role in formation of organic detritus in estuaries Source of bird food Aesthetic, commercial, gastronomic, biomedical importance	Habitat and shoreline change Indiscriminate exploration and collection of undersized specimens Commercialization of marine shell Industrial pollution	Impact on energy flow in food chain
5.	Protozoa	67	Initiate decomposition process.	Change in sea surface temperature.	Impact on energy flow in food chain.
6.	Polychaetes	57	Diet of fish (demersal) and invertebrates. Act as an indicator of status of benthic community.	Anthropogenic and climate change impact in shore habitat.	Impact on energy flow in food chain. Impact on detritus food chain
7.	Crustacea	329	Recycling of minerals and organic matter Maintain balance of productivity of oceans Degradation of plant matter to detritus particles Aquaculture and fisheries are very much dependent upon them	Destruction of habitat Change in salinity and erosion Pollution from inland waters (oil pollution) Shrinking of tiger prawn population	Impact on detritus food chain Impact on livelihood
8.	Xiphosurans	2	Play a vital role in the ecology of estuarine and coastal communities Carapaces frequently serve as substrate for encrusting invertebrates and algae Biomedical research and traditional usage	Change in shoreline and formation of undulating terrain Red crabs destroy their nests and breeding grounds	No information.
9	Insects	497	Ecology of forest ecosystems Role in nutrient cycle, nutrient availability in soils, and biogeochemical cycles Role in carbon cycle during decomposition process Pollination	Climatic variability (trends in precipitation, soil temperature, moisture, and organic carbon, thus affecting trophic cascade of detrital web) Impact of pesticides on nontarget species	Significant impact on tree growth, from survivorship curve, reproductive output, and forest ecology. Impact on pollinator- dependent host plants.
10	Mites	121	Decomposer and helps in nutrient cycling	Impact on population of mites due to changing trends in precipitation, soil temperature, moisture, and organic carbon, thus affecting trophic cascade of detrital web	Impact of detritus food chain

11	Spiders	114	Regulate insect populations Ecological indicators of overall biodiversity in many terrestrial communities	Change in composition and properties of mangrove flora Extremely sensitive to small changes in habitat structure , including habitat complexity, litter depth, and microclimate characteristics	Impact on pest population and detrital food chain
12	Fish	364	Major source of livelihood for local community	Pollution from inland water (oil pollution) Usage of destructive fishing gear, such as mosquito nets Indiscriminate seed collection	Socioeconomic, and reduced protein source
13	Herpetofauna	Amphibia: 11 Reptiles: 71	Indicators of microhabitats in ecosystems Determine relative health of ecosystem Role in energy flow	Increase in salinity Industrial pollution	Impact on energy flow in food chain
14	Aves	234	Nutrient transport to or from ecosystem Pollination	Habitat disturbance, land use change Sea level rise Unplanned tree plantation at mud flats Climate variability	Impact on ecosystem energetic Impact on mangrove species that are dependent on bird pollinators
15	Mammals	47	Serve as primary, secondary, and tertiary consumers Crucial member of local food web Recycle nutrients, agents of pollination and germination, seed dispersal, modification of vegetation structure and nutrition pathways Disperse seeds and mycorrhizae	Urbanization Change in crop pattern Breaches in embankments along riverbanks due to flood	Impact of large predators and prey base

Protection and Conservation of Sundarbans

The Sundarbans came under forest management in 1875. Vide Act VII of 1878, parts of the forests were constituted as 'Reserved' or 'Protected' forests, designed to protect the Sundarbans forests against the forces of the land market and reclamation pressures. The state preserved these mangrove forests primarily as a means of ensuring a continuing supply of timber and other forest products. The Sundarbans forests remained a production unit run as a state monopoly until 1980. Since then the Sundarbans' stature as a significant biogeographic region has only increased, culminating in 1987 when the Sundarbans National Park was included in the list of World Heritage Sites. However, the watercourses outside the National Park and sanctuaries remain open to fishing and are accessible as a commons where excluding potential beneficiaries from obtaining benefits

is impossible. This has given rise to a situation where absolute degradation of the commons is a possibility in the near future, about which marine biologists and conservationists remain concerned. Given the socioeconomic state of the eco-region and lay of the land, riparian Sundarbans as of now does not lend itself to institutional arrangements that help resource users to allocate benefits equitably and sustainably over long periods. A new sanctuary spread over 556 2 km has been constituted (taking the count to four) in the western part of the Sundarbans forests where existing licensed fishers continue to exercise their rights—essentially turning the common pool resource into common property—which may help resource users (with the exclusion of some) to allocate benefits equitably and sustainably over long periods.

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Effect of Seasonal Drought on Ichthyofauna of a Rainfed River

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Abstract

Rivers of India are the fulcrum on which livelihood of millions of people are balanced. River Damodar is rainfed and one of the important tributary of River Ganga. Every summer this river has to experience severe drought condition and may loose most of the natural aquatic fauna as well as flora. The study aims to find the fish diversity status at the time of severe seasonal drought and rejuvenation and repopulation status of fish fauna of this river after. Fish faunal diversity, distribution and abundance during summer drought was studied for four years (2016-2019) in three season in the Damodar river system. The result indicates that the cumulative mean value of the fish catch from six study sites in three seasons, shows a larger value during postmonsoon season and the least during premonsoon. While studying the catch data of each study sites the dry summer showed the lowest catch and diversity. The study indicated that there are distinct changes in diversity and abundance of fish fauna among seasons. The summer drought destroys fish faunal diversity of the river but the rejuvenation power of the river replaces, thus diversity is conserved naturally.

Keywords: *Drought, Rejuvenation, Refuge, Assemblage, Migration*

Introduction

River Damodar is a major east flowing river and River water is extensively used for irrigation, navigation, hydroelectric power generation, water supply for domestic, industrial, fish culture, mining, waste disposal, and various other anthropogenic activities. In Damodar valley drought is worse during the premonsoon period, but in monsoon the river swells up with full burst so, seasonal flooding occurs during the post monsoon. The Damodar river valley is barely one degree south of the tropic of cancer, with narrow and high river bed, the valley is parallel but opposite to the direction of rainstorm (Ghosh, 2011). For this reason, this area is suffering from severe dried condition, specially the areas which are in the upper valley and those sites where river water is dammed. The River is rainfed and the headwater regions of Damodar river, the Tori, Ramgarh, Bhandaridaha are completely dried up during summer months. This seasonal drying starts from late postmonsoon and extends upto early monsoon, acute

summer drought is recorded during every premonsoon season. Severe drought ultimately shrinks the wetted area of the river bed and isolation of marginal habitat occurs. During the last phase of drought, cease to flow occurs, river bed almost dries up leaving series of stagnant pools in the riverbed. The river biota gradually becomes confined in those stagnant pools. As a result, aquatic biota has to face these extreme challenges almost every year, specially the fish. The biota need to survive there for they have to rejuvenate the river by repopulating its ecosystem as before. Fishes adapt various methods to cope up with the severe drought condition in Damodar river valley, by taking shelter in shallow pool refuges inside the river bed , by migration to the suitable upstream or downstream refuges. The Fig. 1 shows graphical representation of the summarized mean catch data variation in four study years. The study reveals that, the post monsoon catch is highest. The catch data revealed that, it causes about 33% reductions from fish catch from post monsoon collection, 16% reduction from monsoon fish collection . The cumulative mean of four years catch data of six study sites indicates that all the catches are more in post monsoon season whereas premonsoon catch is the lowest in almost all study site catch. The mean of fish catch data reveals that, the catch data of year 2016 at Tori shows more or less same fish catch at premonsoon and postmonsoon season. Fig 2. shows the site wise fish catch data in three seasons. Ramgarh shows a more or less same catch data during premonsoon and post monsoon season. And at Panchet the catch data of monsoon and post monsoon season is more or less same. As maximum effect of drought was observed at Tori and Santhoshpur the fish catch was very low during premonsoon season. The catch variation among season at Ramgarh varies at low margin. Throughout the year most of the fishes are found in the large pool near Ramgarh bridge.

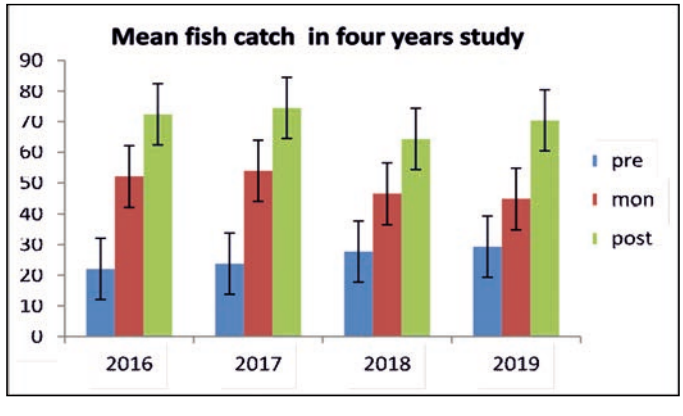


Figure 1: Graphical representation of mean catch of fish in four years at the Drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean± SEM of Four consecutive years.

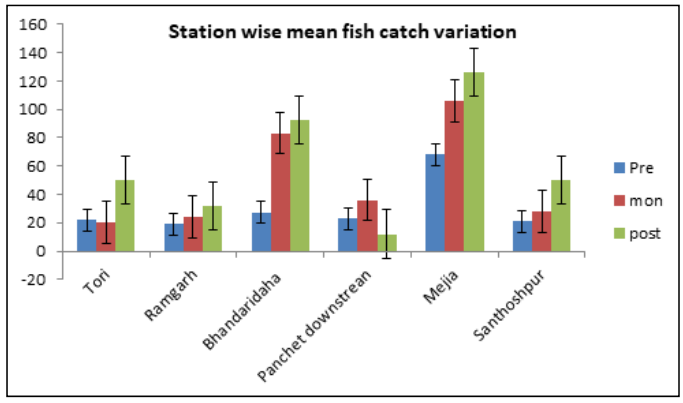


Figure 2: Graphical representation of mean catch of fish at mentioned sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

Some fishes (*Glossogobius giuris giuris*), exhibited higher density during drought, other like *Mastacembelus* armatus almost disappeared during low flow but *Notopterus notopterus* remained unaffected. The study reveals that, the overall occurrence of fishes in different stretch varies seasonally. The statistical analysis indicates that, occurrence of some fishes varies significantly between drought season and post monsoon season.

During mid premonsoon, dry condition becomes severe, Damodar river floodplain dries up, and river water become confined to its low-flow channel or deep and shallow pools. These discreet pools represent different lentic environment temporarily, because longitudinal fragmentation restricts normal natural nutrient , biota and organic matter flow towards downstream flow. Lake (2003, 2005) while working on Australian rivers indicated the process of perturbation restoration power of lotic system, the present study finds similar condition while studying the fish faunal status of Damodar River during drought. Drought is unusual as a natural hazard in that it is a disturbance of deficiency rather than excess (Bond *et al.*, 2008). It is obvious that anthropogenic modification of rivers may act synergistically to make drought worse for fish. This study intended to determine whether the severe summer drought is effecting the fish population of Damodar river system and destroying the natural breeding grounds. The study also aims to bring out the significant difference in fish catch during summer drought and post monsoon season to analyze the extent of damage.

Materials and Methods

The present study was conducted for consecutive four years (2016, 2017, 2018 and 2019) during pre monsoon (March to June), monsoon (July to October), and post monsoon (November to February) periods throughout the upper valley, middle valley

and lower valley of the Damodar river. The sites were selected which are affected by yearly drought most. Tori, Ramgarh, Bhandaridaha, Downstream to Panchet dam, Mejia, and Santhoshpur,

Collections of fish samples

All the study sites were sampled by engaging fishermen . The stagnant pools inside the river bed were thoroughly sampled.

The preserved specimens were identified to the lowest practical taxonomic level following standard methods (Day, 1957-78; Talwar & Jhingran,1991; Jayram,1999). The oozing females and juveniles were identified live and released immediately.

Description of Damodar river System

This river originates from the hills of Latehar at Chotonagpur district Chandwa in Jharkhand and drains into a fan shaped catchment area of about 25,820 sq km. The river has a total length of 540 km, out of which 380 km is in Jharkhand and the remaining 160 km is in West Bengal. The Damodar river joins the river Hooghly at Attannogate.. The main tributaries of the river are the Barakar and the Konar.

Results

The result indicates that the cumulative mean value of the fish catch from six study sites shows a larger value during postmonsoon season and lowest is in premonsoon season. Comparing the mean of the catch data of premonsoon and monsoon, catch mean is much more than the summer catch data mean. May be due to the rejuvenation property of the river the population has been re-established.

The statistical analysis of fish catch data during premonsoon, monsoon and post monsoon season reveals that during 2016 only 15.49% of total fish catch was recorded in the premonsoon season while it increased to 35.56% in the monsoon and in the post monsoon season the fish catch was maximum 49.388%. In the year 2017 the result percentage of premonsoon has increased 0.6% and monsoon catch was same as before postmonsoon catch decreased by 0.6%., The percentage of premonsoon during 2018 has increased by 5.63%, monsoon catch is same, and the post monsoon catch has increases by 0.6%. There is a 3% decreasement in fish catch during monsoon and 2.3% increasement during postmonsoon season observed during 2019, the monsoon catch mean was same as 2018. The study finds the study site Mejia as most productive in all three seasons. The graphical representation of the year wise mean catch data is represented in the Fig.1. and the Fig. 2. represents the stations wise study results of fish catch data during premonsoon, monsoon and post monsoon season.

The catch data of fishes in summer drought and post monsoon season has been compared and some of the fishes show significant difference between summer drought and post monsoon season (Table I). Statistical analysis of the fish catch data results

presented in Table-I indicates, that there are significant differences between catch data of some fishes in pre monsoon and post monsoon season.

Type of Fish	Significant level	Value of *T*-test of difference between Premonsoon and postmonsoon means
1. *Notopterus notopterus* (Pallas)	36.03	1.441
2. *Gudusia chapra* (Hamilton Buchanan)	19.11	0.792
3. Gonialosa manmina (Hamilton Buchanan)	-44.38**	-2.046
4. *Cirrhinus mrigala* (HamiltonBuchanan)	-21.95	-1.515
5. *Cirrhinus reba* (Hamilton Buchanan)	-58.33***	-2.806
6. *Labeo bata* (Hamilton Buchanan)	-51.75***	-4.043
7. *Labeo boga* (Hamilton Buchanan)	-54.67***	-3.679
8. *Labeo dero*(Sykes)	-39.63**	-2.214
9. *Labeo calbasu* (Hamilton Buchanan)	-54.00	-1.391
10. *Osteobrama cotio cotio* (Hamillon Buchanan)	-18.83	-1.605
11. *Puntius phutunio* (Hamillon Buchanan)	-49.05*	-1.736
12. *Puntius sarana sarana* (Hamillon Buchanan)	-104.50**	-2.026
13 . *Puntius sophore* (Hamilton Buchanan)	-36.00**	-2.045
14 . *Puntius ticto* (Hamilton Buchanan)	-51.58**	-2.374
15. *Chela cachius* (Hamilton Buchanan)	-43.90*	-2.576
16. *Chagunius chagunio* (Hamillon Buchanan)	-5.50	-0.275
17. *Chela labuca* (Hamilton Buchanan)	-3.86	-0.257
18. *Satmostoma bacaila* (Hamillon Buchanan)	10.75	0.736
19. *Satmostoma phulo* (Hamillon Buchanan)	-6.00	-0.380
20. *Amblypharyngodon mola* (Hamillon Buchanan)	-9.60	-0.403
21. *Aspidoparia morar* (Hamilton Buchanan)	12.69	0.649
22. *Barilius bendilisis Bendilisis* (Hamilton uchanan)	3.17	0.173
23. *Danio aequipnnatus* (McClelland)	-5.58	-0.477
22. *Barilius bendilisis Bendilisis* (Hamilton uchanan)	3.17	0.173
23. *Danio aequipnnatus* (McClelland)	-5.58	-0.477
24. *Esomus danricus* (Hamilton Buchanan)	-72.92**	-2.155
25. *Crossocheilus latius latius* (Hamilton Buchanan)	-1.25	-0.334
26. *Garra lamta* (Hamilton Buchanan)	-1.25	-0.202
27. *Lepidocephalicthys guntea* (Hamilton Buchanan)	-6.45	-0.851
28. *Aorichthys aor* (Hamilton Buchanan)	-16.38**	-2.197

29. *Mystus tengara* (Sykes)	**10.19**	**0.028**
30. *Ompok pabo* (Day)	**6.50**	**1.508**
31. *Wallago attu* (Hamilton Buchanan)	**-32.25****	**-2.378**
32. *Ailia coila* (Hamilton Buchanan)	**-18.06***	**-1.826**
33. *Glyptothorax telchitta* (Ham Buch)	**-34.42**	**-0.779**
34. *Gagata cenia* (Hamillon Buchanan)	**-8.00**	**-0.892**
35. *Nangra itchkeea* (Hamilton Buchanan)	**-53.50*****	**-2.573**
36. *Xenentodon cancila* (Hamillon Buchanan)	**-7.88**	**-1.112**
37. *Monopterus cuchia* (Hamillon Buchanan)	**-33.25****	**-2.413**
38. *Chanda nama* (Hamilton Buchanan)	**-4.75**	**-0.850**
39. *Parambasis thomasii* (Hamillon Buchanan)	**-7.65**	**-1.183**
40. *Rhinomugil corsula* (Hamilton Buchanan)	**-53.40*****	**-3.943**
41. *Sicamugil cascasia* (Hamilton Buchanan)	**21.75**	**0.745**
42. *Glossogobius giuris* (Bloch Schneider)	**-4.38**	**-0.373**
43. *Anabas testudineus* (Bloch)	**24.38*****	**2.715**
44. *Colisa fasciatus* (Bloch)	**4.13**	**0.367**
45. *Channa amphibious* (Bloch)	**2.00**	**0.466**
46. *Macrognathus armatus* (Hamilton Buchanan)	**22.68****	**2.138**

According to Table I the catch data of fishes in the River system pre monsoon and post monsoon season shows significant difference. The fishes whose abundance differs are *Puntius phutunio* (Ham Buch); *Aila coila* (Ham Buch); *Chela cachius* (Ham Buch), shows significant difference in catch among dry and post monsoon season at 10% level in the river system. The fish *Gonialosa manmina* (Hamilton Buchanan), *Labeo dero* (Hamilton Buchanan), *Puntius sarana sarana* (Hamilton Buchanan), *Puntius sophore* (Hamilton Buchanan), *Puntius ticto* (Ham Buch) *Aoricthys aor* (Hamilton Buchanan), *Wallago attu* (Hamilton Buchanan), *Monopterus cuchia* (Bleeker), *Macrognathus armatuss* (Hamilton Buchanan), catch data varies between pre monsoon and post monsoon season at 5% level of significance. Whereas Cirrhinus reba (Hamilton Buchanan), *Labeo boga* (Hamilton Buchanan), *Labeo bata* (Hamilton Buchanan), *Nangra itchkeea* (Hamilton Buchanan), *Rhinomugil corsula* (Ham Buch); *Anabas testudineus* (Bloch) species catch data shows significant difference in catch among premonsoon and postmonsoon season at 1% level in the river system. It can be summarized that, the test value reveals, according to Table I. two fish species catch data varies at 10% level of significance , ten fish species catch data varies at 5% level of significance, seven fish species catch data varies at 1% level of significance in the entire river system including the reservoir. As a result a change in composition of fish fauna occurs season to season and diversity also changes. Recovery from drought is driven by migration of many species from refuge and by high recruitment of some other species immediately after the drought brakes. Table II. shows Diversity of

Ichthyofauna in Damodar River system during Summer drought and post monsoon season along with mean of four years catch data. The Fig. 9 A,B,C, represents the mean catch data in premonsoon and post monsoon season in individual fishes.

Fig.9A

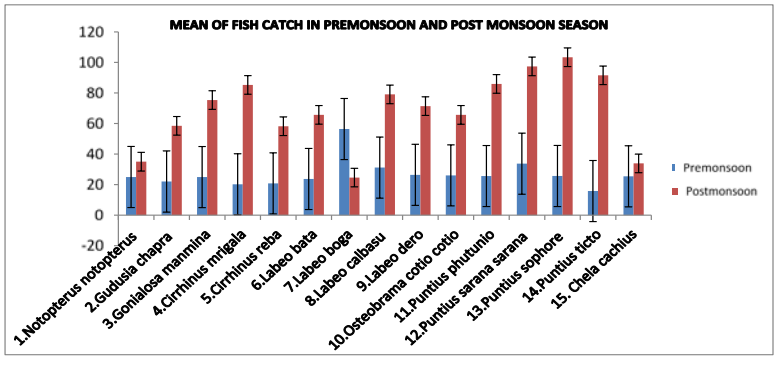


Fig.9B

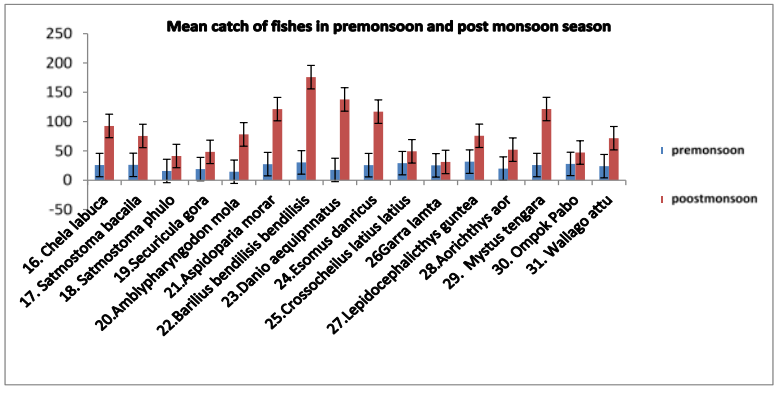


Fig.9B

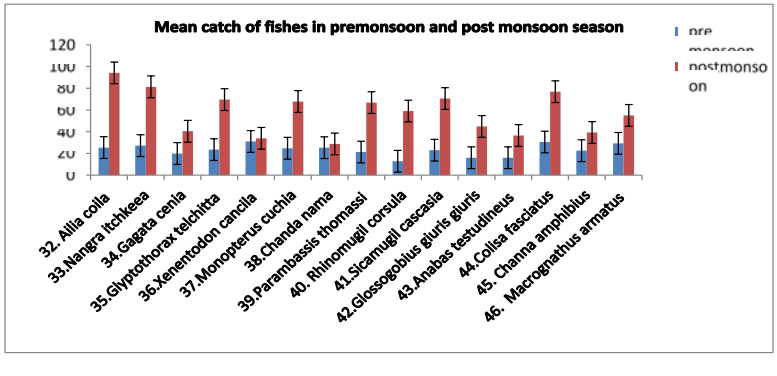


Figure 9 (ABC): Diversity of Ichthyofauna in Damodar River system During Summer drought and post monsoon season along with mean of four years catch data

Distinct hydrographic conditions of different stations in Four study years (2016, 2017, 2018, 2019) are shown in (Fig. 3-8). Maximum water temperature was recorded 48°C at Tori during premonsoon season, whereas minimum water temperature was found 14°C at Bhandaridaha and Panchet downstream during postmonsoon season Fig. 6. Transparency is one of the hydrological impact factors playing role in species distribution. Transparency was recorded at highest values (48.20 cm) during postmonsoon at Mejia where minimum value observed at Tori in the monsoon (2.14 cm) Fig. 8. Water pH values varies between 8.19 (Ramgarh) to 4.50 (Bhandaridaha). pH value during premonsoon season in all study sites are low and varies between 6.82 to 4.50 Fig. 3. Water alkalinity values varies between 53.72 ppm (Santhoshpur) to 16.8ppm (Panchet downstream) during premonsoon season only Fig. 4. Dissolved Oxygen ranges from 9.25 mg/l (recorded during postmonsoon season at Panchet downstream) to 4.17 mg/l (recorded during Monsoon season at Assansole) Fig. 5. Carbondioxide ranges from 83 mg/l (recorded during postmonsoon season at Mejia and 10mg/l (recorded during post Monsoon season at Tori) Fig. 7.

During prolonged dry season which may be due to late occurrence of rain or, less precipitation, river flow reduces to a series of diminishing pools, this condition leads to loss of biota. The study reveals that in case of this river seasonal drought imparts similar effect on river biota so as supra seasonal drought does.

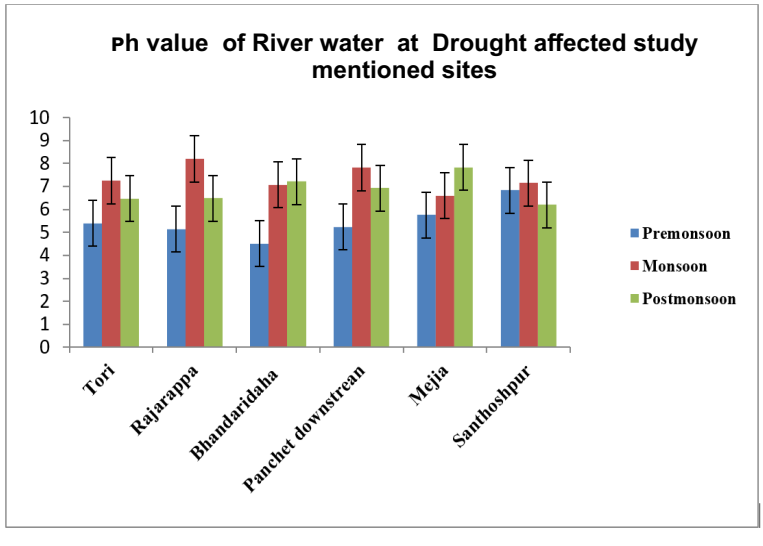


Figure 3: Graphical representation of mean pH value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

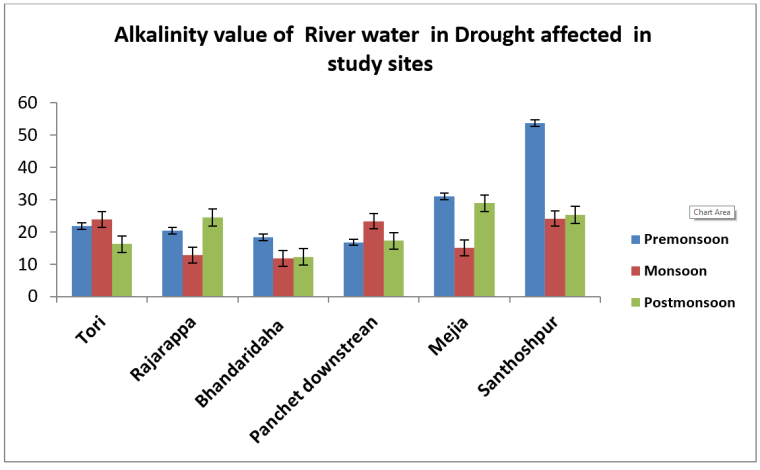


Figure 4: Graphical representation of mean Alkalinity value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

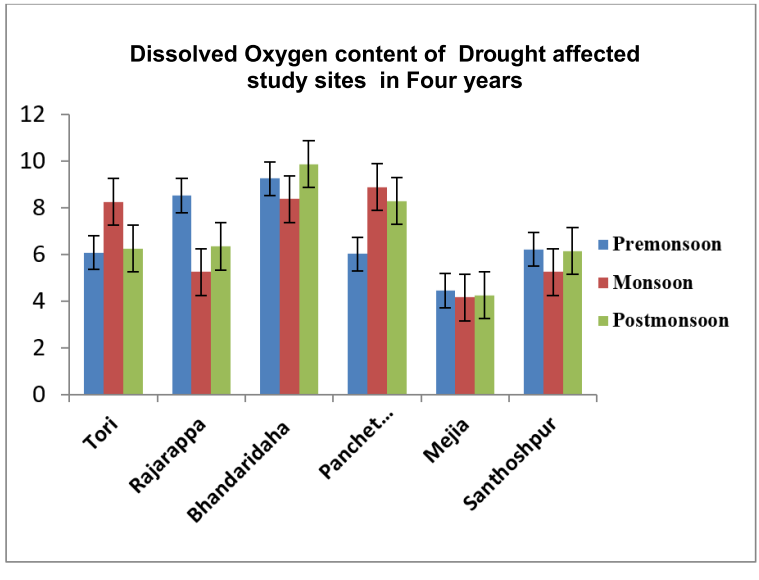


Figure 5: Graphical representation of mean Dissolved Oxygen value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season . Data represented as mean ± SEM of Four consecutive years.

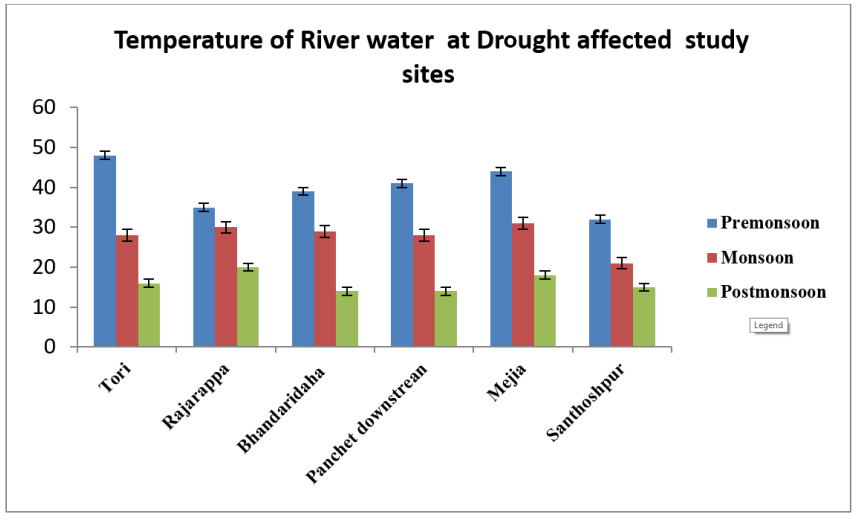


Figure 6: Graphical representation of mean Temperature value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

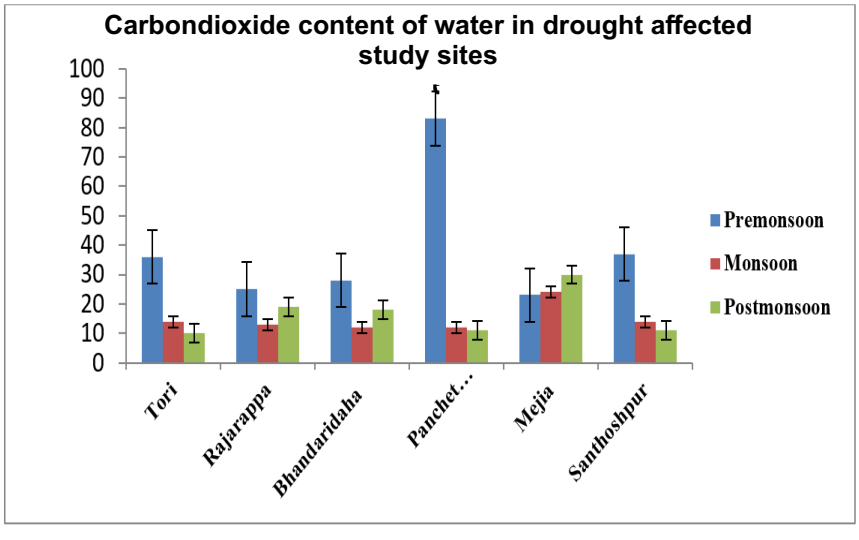


Figure 7: Graphical representation of mean Carbondioxide content value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

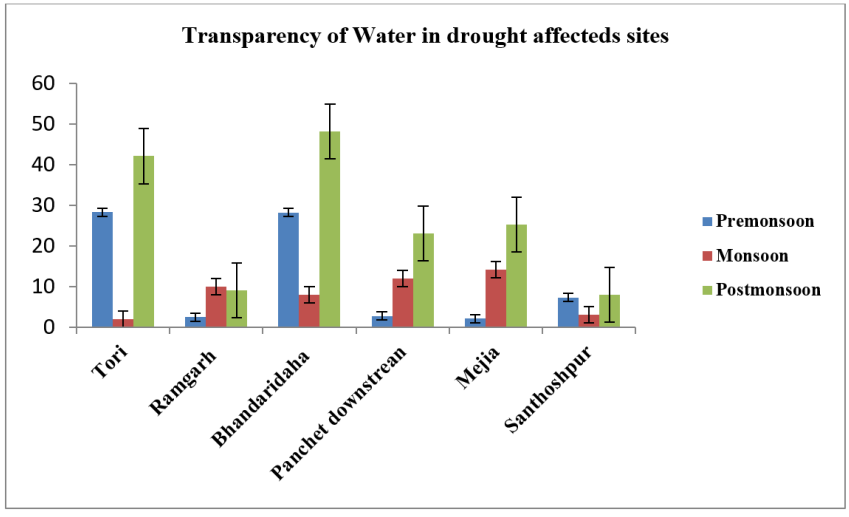


Figure 8: Graphical representation of mean Transparency value of River water at the mentioned drought affected study sites at premonsoon, monsoon and postmonsoon season. Data represented as mean ± SEM of Four consecutive years.

Discussion

The areas which are most affected by drought and are intensively investigated to know the fish faunal diversity status as well as abundance. The study reveals that, at Tori, Ramgarh in upper valley, Bhandaridaha and Panchet downstream in the middle valley and Mejia and Santhoshpur in the lower valley drought is severe and changes the fish faunal assemblage as a whole. In those area during the late summer as drought progresses, shallow pools inside the river bed decrease in size, where decomposition creates a low pH ranging from 4.5 at Bhandaridaha and 6.8 at Santhoshpur which is a stressful condition for fish to survive (Bond *et al.*,2008) The water temperature rises to 48°C to 32°C, and Dissolved oxygen ranging from 4.45-9.25mg/l definitely stresses aquatic fauna. The stagnant pool at Panchet downstream showed similar condition, there free Carbondioxide was 83mg/l and pH 5.24, water temperature 41°C. At Mejia discreet pools are also not very frequently observed in the driedup riverbed. Patches and shallow flow with obnoxious smell found where pH value5.75 was recorded.,CO2 23mg/l , Transparency 2.23cm, and Dissolved oxygen was found 4.45mg/l. Panchet downstream riverbed site was covered with bushes and natural vegetation, river flow was very narrow and almost dried up. Small minnows were found to swim freely in that long stretch of refills but dissolved oxygen in water was 9.25mg/l which is not suitable to the fishes to survive long.

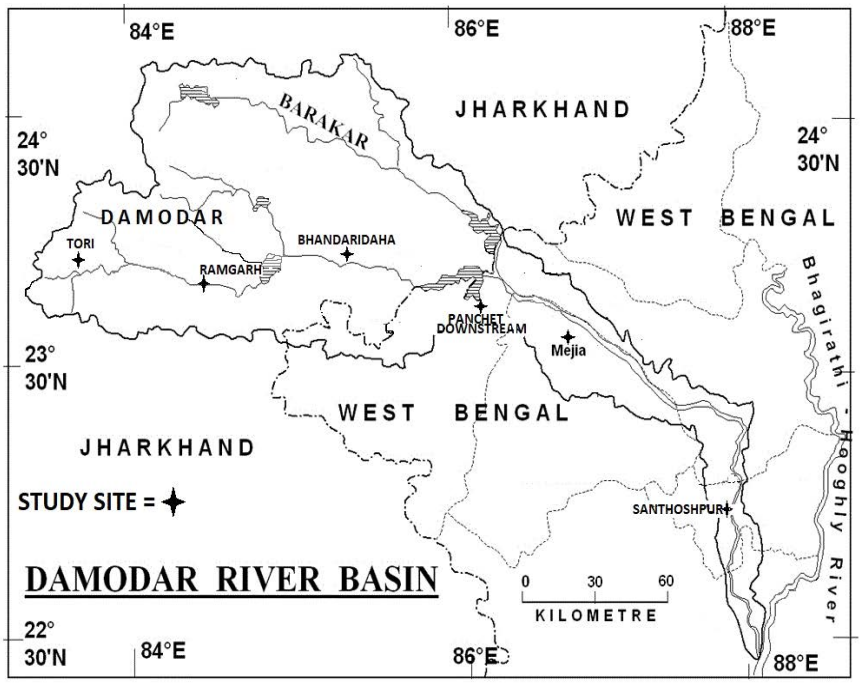
Drought results in reduction of lateral connectivity thus results in habitat loss, struggle for

existence in those stagnant pools which may be the cause for destruction of diversity and assemblage of fishes. The fish populations have to cope up to survive in the lentic environment instead of healthy flow of the river. The severely affected fish population, in the first phase fishes adapt various methods to cope up with the adversity, either by migrating in shallow pool refuges or to suitable natural flow of the river which may be far from the location. During late summer, water flow almost ceases and only very shallow surface flow through gravel bars continues to move towards downstream between pools. The condition becomes more serious when oxygen level becomes critical or when these pools became completely dry the fishes within them died. The shallow flow of river may not allow the large fishes to migrate to suitable refuges. Water flowing through gravels and sand lanes in Damodar riverbed helps fingerlings of some fishes move to deeper water pools. The study finds the pools at Tori, Ramgarh and Bhandaridaha, Panchet downstream show high inter and intraspecific interaction there, water transparency showed an alarming result, 2.58cm only at Ramgarh, similar result is observed in Panchet downstream, Mejia and Santhoshpur. The fingerlings of *Notopterus notopterus, Amblypharyngodon mola. and Mystus vittatus* were observed at Tori, Ramgarh and Bhandaridahas' discreet pools. At Tori, Ramgarh and Bhandaridaha small fishes, like *Danio acquipinnatus, Puntius ticto, Rasbora daniconius, Glossogobius giuris giuris, Nemacheilus denisoni and Macrognathus armatus* were found to migrate into pools and others were trapped in small pools within riffles.

Most of the fish fingerlings are found to exist in the late monsoon after summer drought in the refill pools of Bhandaridaha also and below the Ramgarh bridge stagnant pool. Another large stagnant pool exist at Mejia where oozing female fishes of *Amblypharyngodon mola, Ailia coila, Osteobrama cotio* were observed . This particular study site may act synergistically the conserved breeding ground and considered as good hope for future reimbursement. At Santhoshpur the fingerlings of *Aoticthys aor, and Xenentodon cancila, Cirrhinus reba were* found in good number. The fishes which are observed to survive in restricted habitat increased in number by the post drought and early monsoon are *Amblypharyngodon mola, Rinomugil corsula, Osteobrama cotio. and Sicamugil cascasia* found in the Tori, Ramgarh, Bhandaridaha and Mejia study sited. The migration through shallow flow from critically affected drought area to the more suitable habitat will definitely increases the aggregation of fish spawns by number and diversity in partial stagnant water, where inter and intra specific struggle may decrease the number of fish count but, provide a better option to the fishes to complete their lifecycle .The *Chagunius chagunio* species shows a sharp decline during summer drought but the study finds a good number of fingerlings at Bhandaridaha during the drought season only. The fingerlings were swimming freely in the waterlogged pool in the riverbank. All these fingerlings will rapidly invade the area by the resumption of normal flow. The damage impacts reached a critical point, resulting in species removal like Nandus nandus and Chitala chitala during a prolonged drought in

due to broad-scale habitat loss and drying of refuges. However, 19 fish species have already been lost from the region, revealed from early study (Sarkar and Banerjee, 2000, 2002, 2012). A strong natural disturbance like excessive drought and flood can disturb the riverine ecosystem (Pandit *et al*.,1994)but river can regenerate biotic community similar to the previous one within a short period if provided the system is able to take refuge in interstitial zone or being adapted to combat adversity(Jefrey *et al*., 2003). In spite of all adversity, the river has the capability to recover its biotic community easily unless the changes are either very strong or too frequent (Matthews *et al*., 1998). During the study a very special short distance migration of the Glossogobius giuris, and Sicamugil cascasia species from river to the adjacent crop field on the bank was recorded during the outbreak of monsoon. The small pocket pools at Ramgarh, Bhandardaha, Mejia and Sanhoshpur refuge for many small fishes during the drought, these small overcrowded refuges may join the mainstream with all the fishes, if rain is not late or water is released from the reservoirs to save the biodiversity. The study revealed that, diversity of ichthyofauna of the Damodar river system has not been affected much till today, after experiencing severe drought or construction of dams across this river. But, abundance and distribution range of many fish species have considerably hampered.

Map of Damodar River showing Study sites.



Recovery from stress condition adopted by fish species

The oozing females and juveniles of all total twenty eight fish species that were collected from Tori and Ramgarh, Bhandaridaha, Mejia, Santhoshpur were found during early monsoon and postmonsoon seasons throughout the study period.

Conclusion

Endless drought condition prevails during every summer season in Damodar river valley. The natural flow of the river water goes down to few centimeters in some places of upper valley region of Damodar river.

The most important and effective outcome of the recent study is that the upstream region at Ramgarh, and Panchet were found to be very good breeding ground of fishes since oozing females and juveniles of almost all the adult fishes collected from these stations were found in the early monsoon and postmonsoon seasons throughout the study period. Natural pool like areas as there in Ramgarh should be preserved to save natural breeding grounds. Proper care should be taken and preservation of breeding ground should be done at the upstream region of the river. There may be several other breeding grounds which should be searched and subsequently preserved. Construction of fish ladder and fish pass at the inflow and out flow region of the reservoir should be made to maintain regular fish migration. Proper conservation is needed to conserve the areas where mainstream and tributaries meet, to conserve native fish faunal composition. Proper care should be taken for all govt. recognized culture ponds near the river bed as in Durgapur to rear culturable food fishes of the river for fish seed generation. Dredging silts from reservoirs at regular interval is utmost necessary. In the Damodar river basin, it is essential to preserve the upstream diverse areas that are not yet strongly affected by development. In these reaches, protection efforts should be directed toward discouraging physical modifications. In the already modified intermediate sections, the challenge is to recreate the diversity of the natural conditions.

The river has the capability to recover its biotic community easily unless the changes are either strong or too frequent. Fishes are the integral part of the riverine ecosystem, we have to stop all those anthropogenic activities that are causing immense damages to the riverine and aquatic forms of life. It is late but not too late and it is the crucial time to take an oath to save the river for us and for the coming generations.

Acknowledgement

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Authors' Contribution: The authors contribution specifically includes Collection of fishes from study sites, identification of each specimens, in three seasons. The catch data was statistically analysed and the interpretation was drawn by the author. The author has prepared the manuscript .

Ethics Approval and Consent to Participate: Ethics approval is not applicable.

Conflict of Interest: The author declared that this research work has no conflict

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Isolation and Screening of Some Food Grade Lactic Acid Bacteria for Biosurfactant Production

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Abstract

Lactic acid bacteria (LAB) are considered as one of the most important types of friendly bacteria found in the digestive tract and possess positive roles in maintaining good health and immune system in humans. LAB are extensively studied and used in a variety of industrial and food fermentations. They are widely used for humans and animals as adjuvants, probiotic formulation, and dietary supplements and in other food fermentation applications. They have also been reported to produce important metabolites, among which biosurfactants in particular have shown antimicrobial activity against several pathogens in the intestinal tract and female urogenital tract partly through interfering with biofilm formation and adhesion to the epithelial cells surfaces. This property helps their applications in food industry, medical field and also in bioremediation of environment. In this present study total six biosurfactant producing food grade LAB were isolated and screened for obtaining stable, high amount of biosurfactants.

Keywords: *Lactic acid bacteria, Lactobacillus, Probiotic, Biosurfactant, Biofilm*

Introduction

Surfactants are widely used in agricultural, food, drug and cosmetic industry due to their ability to reduce the surface tension at air-water and water-oil interface. However they are mostly chemical in nature, persist long time in ecosystem and for this reason they are considered as environmentally hazardous and toxic component. So nowadays emphasis is given on the searching processes for newer environment friendly source of surfactants. According to Fakruddin, (2012), microorganisms may be one of such alternative resource for production of different types of biosurfactants. The surface active hydrophobic and hydrophilic biomolecules produced by different micro-organisms which may remain either attached to cell surfaces or excreted extracellularly are termed as Biosurfactant Desai & Banat, (1997). Properties like broad substrate availability, tolerance to high range of pH, temperature and ionic strength, biodegradability, low toxicity, emulsifying and demulsifying ability and antimicrobial activity make microbial surfactant comparable to chemically synthesized surfactant Vijayakumar & Saravanan, (2015). Among different types of micro-organisms bacteria are the most important

producers of biosurfactant (Priya *et al*., 2011). Recently, biosurfactants from various probiotic bacteria including Lactic acid bacteria (LAB) are gaining importance as most of the representatives of this group of friendly bacteria possess 'generally recognized as safe' or GRAS status by United States food and drug administration (FDA) for human consumption (Rodriguez *et al*., 2002). Biosurfactants from LAB have several advantages over synthetic surfactants such as: higher biodegradability, lower toxicity, good compatibility with eukaryotic organisms and effectiveness at a wide range of temperatures, pH values and salinities (Rodrigues *et al*., 2006). Because of their natural existence in various raw and fermented foods, soil, mucosal surfaces such as the vagina and the gastrointestinal (GI) tract of human and other animals, they can be widely used in medical field, food industry as well as for bioremediation purposes. According to Rodrigues *et al*., (2006), the biosurfactants from probiotic bacteria can be used as antibacterial, antifungal, antiviral agents as well as major immunomodulatory molecules, antiadhesive agents and in vaccines and gene therapy. Due to their anti-adhesive activity biosurfactants by probiotic bacteria have the potential for use against the biofilm producers including several human pathogenic microorganisms like *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans* etc. both *in vivo and in vitro* condition. They are found to reduce the infection for various diseases in the urogenital, respiratory and gastrointestinal tracts. They also lower down the adhesion of pathogenic micro-organisms to glass, silicone rubber, surgical implants and voice prostheses (Van Hoogmoed *et al*., 2004; Velraeds *et al*., 1996). Previous application of biosurfactants on the outer surfaces of catheters and other medical insertional devices can delay the onset of pathogenic biofilm growth which reduces the use of immunosuppressive drugs. (Busscher *et al*., 1997). This property is also applicable during fruits, vegetables, meat and fish preservation processes in food industry. During 2011, Thavasi *et al.* reported that biosurfactant from *L. delbrueckii* can be used for bioremediation purposes. Even in the crude form of extracts, the lactic acid bacteria derived biosurfactants certainly find suitability for environmental applications.

In this context, the aim of the present study is to screening out some biosurfactant producing food grade LAB with wide antimicrobial activity against some biofilm forming pathogenic bacteria.

Materials and Methods

Collection of samples

Several vacuum packed refrigerated food products (meat and fish) were collected from local market of Haldia, East Midnapur, West Bengal for isolation of Lactic acid bacteria.

Sample enrichment and isolation of Lactic acid bacteria

Samples were enriched overnight in 0.1% sodium chloride solution. Then serial dilution

of samples was carried out and 100µL diluled sample was spread over sterile Lactobacillus MRS agar media (De Mann *et al.*, 1960). Then the plates were incubated at 30°C for 48 hours.

Identification of isolates by morphological and biochemical studies

Partial identification of isolates was performed based on the conventional methods including Gram staining, IMVIC test, carbohydrate utilization test and catalase test (Tamang *et. al.*, 2005). Motility test was performed growing the isolates within MRS stab culture (Table A1).

Table A1: Result of morphological and biochemical assays of different isolates

Tests	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Morphology	r	r	r	r	r	r
Gram staining	+	+	+	+	+	+
Methyl Red	+	-	-	+	-	-
Voges Proskauer	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-
Indole test	-	-	-	-	-	-
Motility test	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+

+ : positive reaction; - : negative reaction; r : rod shaped

Antimicrobial Activity

Antibacterial activity of the LAB isolates was tested by inoculating the colonies and cell free culture aliquot of isolates directly on the lawn of target organisms (Ko Hyun & Ahn, 2000). The inhibitory spectra of the isolates were evaluated against five numbers of biofilm forming human pathogenic bacteria (Table A2) of which three Gram positive and two Gram negative bacteria. Pathogenic strains were procured from MTCC, Chandigarh and maintained on Trypticase Soy Broth (TSB, Himedia) media.

Table A2: Antibacterial spectra of different isolates

Indicator strains	S1	S2	S3	S4	S5	S6
Lactococcus lactis MTCC 3038	+	+	-	+	+	-
Staphylococcus aureus MTCC 96	-	+	+	+	-	-
Escherichia coli DH1 MTCC 1667	-	-	+/-	-	+	+
Bacillus subtilis MTCC 121	-	+/-	-	+	-	-
Pseudomonas aeruginosa MTCC 741	-	-	-	+	+/-	-

+ : diameter of zone of inhibition >8mm, +/- : <8mm, - : absent

Biosurfactant Extraction

All the LAB isolates were tested for biosurfactant production by cultivating them overnight in MRS (HiMedia) broth. They were inoculated into the flask containing 100 ml of MRS broth and incubated for 24 hrs. Two methods were followed for the extraction of biosurfactant from isolates to obtain both the extracellular form and the cell-bound form (Tillawala & Shah, 2016). After 24 hrs of growth, the cells were harvested by centrifugation at 8000g for 10 min at 4˚C. The cells were washed twice in distilled water and resuspended in 100 ml of phosphate buffer saline containing 10 mM potassium dihydrogen phosphate and 150 mM sodium chloride and the supernatant was kept aside for further use. The cells were then incubated at room temperature for 2 hrs with gentle stirring for biosurfactant production and sonicated for cell rupture. Centrifugation was performed at 8000g for 10 mins at 10˚C. The supernatant was then filtered through sterile 0.22 mm pore size filter (Millipore) and was used for biosurfactant assay and biofilm inhibition assay. To obtain extracellular form of biosurfactant, the supernatant which was previously separated was acidified with 6N HCl to attain a pH of 2. It was incubated overnight at 4°C. Chloroform and methanol was added in the ratio 3:1 (v/v) to extract the biosurfactant. The solvent was evaporated and the crude biosurfactant was obtained. Collected biosurfactant was dissolved in 10% DMSO.

Screening assay for biosurfactant production (Tillawala & Shah, 2016)

Drop collapse test: To screen the isolates for biosurfactant production, the ability to collapse a droplet was tested. In this method, the interfacial tension between the drop containing the surfactant and the parafilm surface is reduced which results in the spread of the drop. Twenty-five microlitre of extracted biosufactant was pipetted as a droplet on the parafilm. Distilled water and tween 80 were used as negative and positive controls respectively. The flattening of droplet and spreading of the droplet on the parafilm surface was observed. Methylene blue was added to the water for staining purpose. The

droplets were allowed to dry and diameter of dried droplet was recorded.

Oil spreading test: To compare the surface activity among various isolates, oil spreading test was performed. Briefly, 20 ml of distilled water was added to a petriplates followed by the addition of 20 µl of vegetable oil to the surface of water. Twenty microlitre of supernatant from each LAB obtained above was placed onto the centre of oil membrane and checked for the formation of zone of clearance.

Emulsification index: Both the cell bound form and extracellular form of biosurfactant was used to check the emulsification of vegetable oil. 1 ml of culture broth from both the sources was mixed with 1 ml of vegetable oil and vortex-shaken for 2 minutes and the emulsion mixture was allowed to stand for 20 minutes. It was then checked for presence of foam layer. After 24 hours, the foam layer was again checked for stability. A negative control was maintained only with the buffer solution and vegetable oil and Tween-80 was used as the positive control.

Results and Discussions

Isolation of Lactic acid bacteria

From the collected food samples six numbers of lactic acid bacterial colonies (S1, S2, S3, S4, S5 and S6) were selected for further experiments based on their small, whitish colonies (Figure B1). The purity of the suspected producer colonies was checked by streak plate method and the isolates were maintained in MRS slants at 4°C.

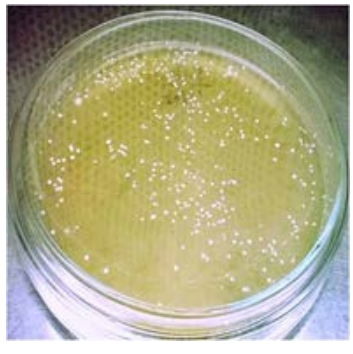


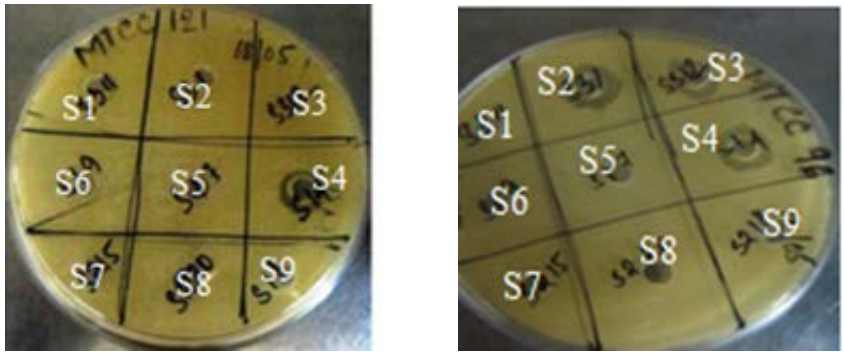
Figure B1: Small, whitish colonies of LAB isolate

Identification of isolates by morphological and biochemical studies

Table A1 shows the result of identification tests. After Gram staining under light microscope all the isolates appeared as rod shaped in structure. Single, paired and chain form of rod were observed. All the isolates were Gram positive, Catalase negative and nonmotile. All the isolates showed acid production in all the carbohydrates checked. S1 and S4 appeared as positive in methyl red test, all were negative in VP test.

Antimicrobial activity

Among the isolates S3, S4, S5 and S6 showed activity against both the Gram positive and Gram-negative pathogenic bacteria after 24 hours of incubation. Figure B2 showed presence of zone of inhibition against Staphylococcus aureus MTCC 96 and Bacillus subtilis MTCC 121.



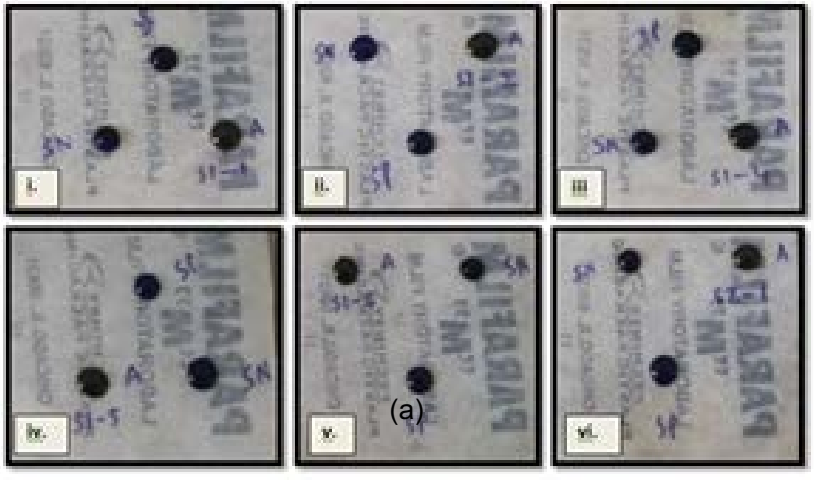
(a) (b)

Figure B2a and B2b: Antimicrobial activity of isolates against MTCC 121 and MTCC 96

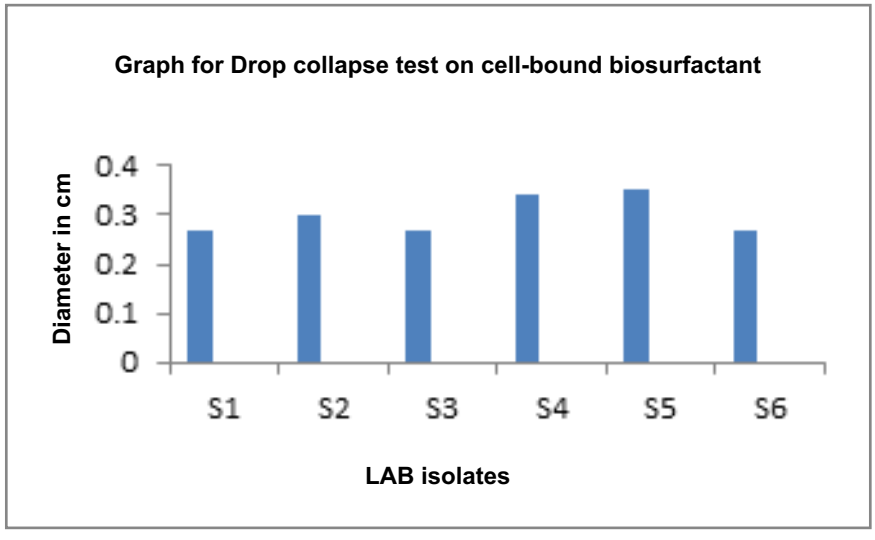
Screening assay for biosurfactant production

1. Drop collapse test –

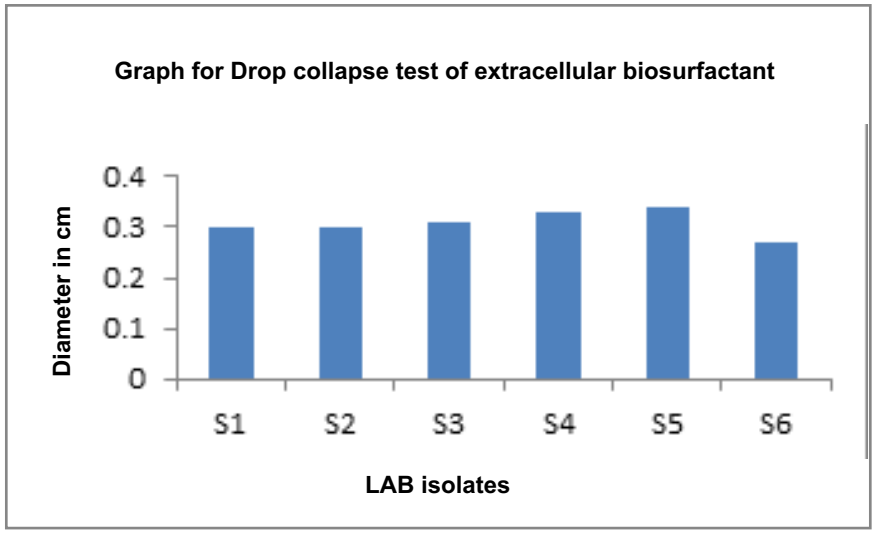
Both cell-bound and extracellular form of the samples from six isolates showed collapsed droplet formation. Figure B3 shows the diameters formed by the respective samples.



(a)



(b)



(c)

Figure B3: Drop collapse test for six isolates (a); Graph showing Drop collapse test for cell-bound form (b) and extracellular form (c).

2. Oil spreading test –

Zone of clearance was observed for two samples i.e S4 and S5. Therefore, result was considered positive for these two samples (Figure B4).

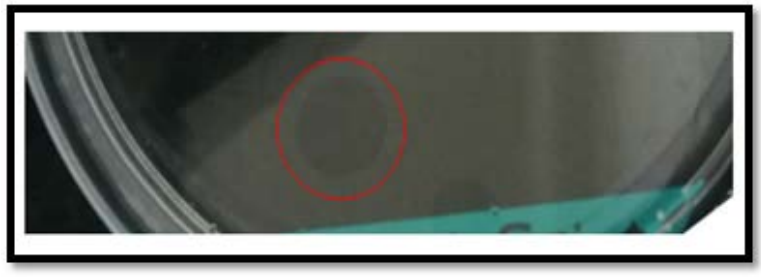
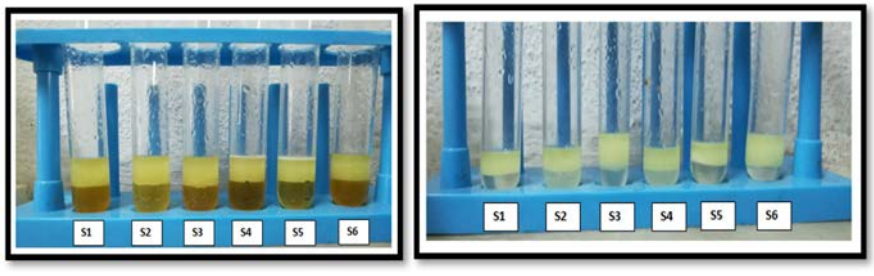


Figure B4: Zone of clearance formed by biosurfactant from S4 on vegetable oil.

3. Emulsification index –

Figure B5 showed the result of Emulsification index test which was performed on all the six samples, both from the extracellular and cell-bound form of biosurfactants. Foam layer was observed only cell-bound form of S4 and S5. Therefore, the result was considered positive for these two samples.



(a) (b)

Figure B5: Emulsification of extracellular form (a) and cellbound form (b) of biosurfactant with vegetable oil.

Conclusion

In the current study, we have extracted biosurfactants from different isolates of lactic acid bacteria and screened them for their biosurfactant producing ability among which two samples (S4 and S5) yielded positive results, six different isolates of lactic acid bacteria were Gram stained and observed. All the isolates were found to be Gram positive and their

morphology was found to be rod shaped. All the isolates were catalase negative and nonmotile. Two different protocols were followed for biosurfactant extraction, one for cell-bound form of biosurfactants and other for extracellular form of biosurfactant. For screening of the biosurfactants, several tests were carried out and the results were observed. For drop collapse test it was found that all of the isolates produced collapsed droplets and for oil spreading test a zone of clearance was observed for samples S4 and S5. Emulsification activity was observed in two of the samples which are sample S4 and S5 only for extracellular form. Among all the isolates S4 and S5 produce stable biosurfactant after 24 hours. They also show antimicrobial activity against some biofilm forming pathogens. So further experiments can be proceeding with these two isolates.

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Comparative Study of Sequence Specific Protein-DNA Interaction by *in vivo* Gene Expression in Gal Operon

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Abstract

Protein-DNA interaction is one of the most essential activities in cell which controls the life of a cell by regulating gene expression. Transcription forms the basis of gene expression which involves the protein-DNA interaction. Gene expression occurs through positive or negative regulation of molecular interaction. In the classical model of negative regulation of gene expression, a repressor protein binds to its cognate operator DNA, at or near the promoter region and inhibits transcription initiation. In positive regulation, an activator activates the promoter by binding near the transcription start site. In *E. coli*, expression of Gal operon is under both positive and negative control. The former is initiated by cyclic adenosine 3':5'-monophosphate (cAMP) and its receptor protein (CRP complex), which binds near the transcription start site and activates gene expression. The negative regulation occurs by simultaneous binding of the repressor protein (GalR) dimer to the operators regions (Oe and Oi). Binding of GalR dimer to wild type Oe and Oi inhibits the gene expression with high affinity. Mutation in any of the operators' sequence destroys the repression mechanism. To examine the sequence specificity for binding of repressor protein to the operators, DNA sequence of one operator is replaced by another. Then result comes with decreased value of repression. This experiment has been performed by *in vivo* gene expression study of different plasmids having a construct of gal promoters and different combinations of gal operators in the regulatory region. To understand the functional importance of sequence variants in transcription regulation *in vivo* expression study was done by monitoring the expression of green fluorescent protein (GFP), which acts as a downstream reporter gene, cloned under the control of the Gal promoter.

Keywords: *Gal Operon, Gene expression, Transcription, Positive and negative regulation of gene expression, Activator, Repressor protein, Operators, Reporter protein*

Introduction

Protein-DNA interaction which plays an essential role in transcription is crucial for gene expression in cell. This transcription process may be positively or negatively regulated by the interaction of different proteins and its interacting DNA partner (Semsey *et al.*, 2004). In classical negative regulation of gene expression, a repressor protein binds to cognate

operator element in DNA at or near the promoter and inhibits transcription initiation. In positive regulation, an activator activates the promoter for gene expression. Gal operon is one of the best-studied catabolite-sensitive operons of *Escherichia coli*. It contains four structural genes, *galK*, *galT*, *galE* and *galM*, which specify the enzymes galactokinase, galactose transferase, galactose epimerase, and mutarotase respectively. The gal operon has a promoter region containing two overlapping promoters P1 and P2 maintaining the distance of 5 bp, two operators, *galOE* (Oe) and *galOI* (Oi), and a cAMP•CRP active site. The structural gene for the repressor protein, *galR*, is located far from the structural genes for the galactose enzymes (Figure 1) (Adhya *et al.*, 1966, 1979; Aiba *et al.*, 1981; Buttin, 1963; Michaelis *et al.*, 1967; Musso *et al.*, 1977; Shapiro *et al.*, 1969; Kalckar *et al.*, 1959; Saedler *et al.*, 1968).

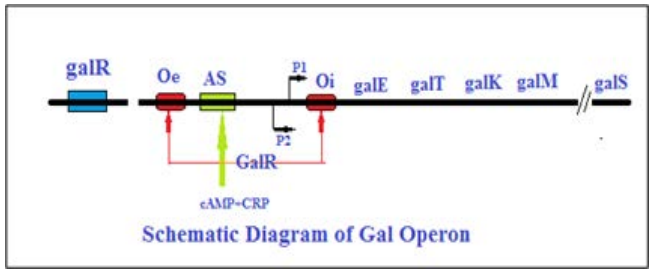


Figure 1: Schematic diagram of Gal operon

β-galactosidase hydrolyzes lactose to form β-D-galactose. Mutarotase converts the β-D-galactose into α-D-galactose. Then galactokinase, galactose transferase, galactose epimerase act in a sequence of steps to yield the overall reaction Galactose + ATP → glucose-1-phosphate + ADP.

Repression in gene expression occurs due to binding of repressor protein, GalR to both of the operators Oe and Oi in dimeric form. Both operators participate in repression in a constitutive manner, such that mutation of either operator destroys the switching off mechanism (Irani *et al.*, 1983). One dimer binds to Oe and another to Oi. The histone-like protein, HU which actually acts as a co-factor, binds to the gal promoter region and causes DNA super coiling by formation of a DNA loop. DNA super coiling makes the promoter inactive for transcription initiation by deforming it (Roy *et al.*, 2005; Aki *et al.*, 1997). HU binds at +6.5 position of the DNA and helps for co-operative binding with Oe and Oi operators. Then interaction between Oe- and Oi-bound GalR molecules occurs, followed by formation of a DNA loop and GalR tetramer (Figure 2) (Aki *et al.*, 1997). HU and DNA super coiling acts simultaneously to stabilize the GalR tetramer (Semsey *et al.*, 2002). Repression of the gal operon is overcome by addition of inducers such as D-galactose or D-fucose. The gal repressor has an N-terminal domain with a helix-turn-helix motif that binds to Oe or Oi half-site and a C-terminal domain that binds the inducers (Parks *et al.*, 1971).

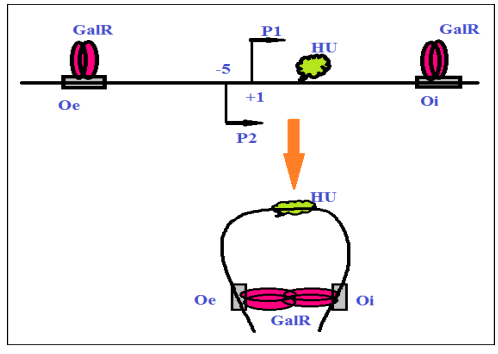


Figure 2: Repression mechanism of Gal Operon due to binding of GalR dimer to Operators

In this study it has been tried to understand how both the operator sequences are important for repression of expression of the genes present in the gal operon. Here all the experiments were done *in vivo*.

Objectives

Study of repression mechanism in Gal operon was done by *in vivo* gene expression. Following are the works which have been done in this study:

1. Designing of regulatory regions having various combinations of operator sequences and other genes. Here three combinations were formed. (Table1)
2. Cloning of the regulatory region in working plasmid vector pSA11.
3. Transformation of plasmid containing the above genes (according to Table1) in *E.coli* XL1-blue (XL1B) strain.
4. Expression of the plasmids in bacterial strain for fluorescence study in presence and absence of inducers.
5. Measurement of fluorescence intensity using fluorometer.

Table 1: Nomenclature and sequence of the Regulatory region of the reporter gene constructs used in this study

Sl. No.	Types of Plasmids	Regulatory region of gene construct	Sequence
1	**OeOi (wild type)**	Operator Oe & Oi, promoter P1 & P2 and active site AS	5'GGATCC TGTAAACGATTCCACTAATTTATTCCATGTCACACTTTTCGCAT CTTGTTATGCTATGGTTATTTCATACCATAAGCCTAATGGAGCGA ATTATGA GAGTTCTGGTTACCGGTGGTAGCGGTTACATTCTAGA –3'
2	**OeOe**	Operator Oe & Oe, promoter P1 & P2 and active site AS	5'GGATCCGTGTAAACGATTCCACTAATTTATTCCATGTCACACTTTTCGCAT CTTGTTATGCTATGGTTATTTCATACCATAAGCCTAATGGAGCGA ATTATGA GAGTTCTGGTTACCGGTGTAAACGATTCCACTCTAGA –3'
3	**OiOi**	Operator Oi & Oi, promoter P1 & P2 and active site AS	5'GGATCCGTGGTAGCGGTTACATTAATTTATTCCATGTCACACTTTTCGCAT CTTGTTATGCTATGGTTATTTCATACCATAAGCCTAATGGAGCGA ATTATGA GAGTTCTGGTTACCGGTGGTAGCGGTTACAT TCTAGA–3'

Materials and Methods:

Materials used:

Agarose, Ampicillin, ethidium bromide, D-Galactose and D-Fucose were purchased from Sigma Chemical Company (St. Louis, MO, USA). Luria Broth (LB) and agar powder were purchased from Hi-Media (India). Anhydrous glycerols, $CaCl_2$, Tris base, boric acid, EDTA (disodium salt) were purchased from J.T. Baker.

Bacterial strain and plasmids:

E.coli strain XL1-blue was maintained in our lab as glycerol stock at –80 ºC.

Methodology:

Expression of plasmids having the regulatory region followed by fluorescence measurement:

Table 1 contains the gene sequences of the regulatory region of the gal operon constructed in this study. *E.coli* XL1B strain was used as the host. The above genes were cloned in pSA11 vector at BamHI/ XbaI cloning site. In all cases the gene responsible for green fluorescence protein (GFP) was under the control of the cloned promoter regions.

The plasmids containing the above genes were transformed in XL1B strain and selected on a plate containing 2.5% Luria broth, 1.5 % agar and 100 µg/ml of ampicillin and incubated at 37° C for overnight. A single colony was picked and inoculated in 50 ml of LB medium and then allowed to grow for overnight at 37°C in presence of 100 µg/ml of ampicillin.

Then the concentration of cells were normalized at $O.D_{600}$=2.

At that point, 20 mM D-galactose was added to the culture medium and it was allowed to incubate with gentle shaking at 180 rpm up to 6 hrs at 37° C.

The medium containing cells was collected before addition of D-galactose and also at various time points after addition of D-galactose to examine the time dependent derepression of gene expression in presence of inducer. After addition of galactose, aliquots were collected after 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr and finally at 6 hr. 4 ml medium with cells was collected at every time point before and after the addition of D-galactose. The growth of cells in the collected aliquot was stopped by keeping the medium on ice after collecting it. The above process was also repeated in case of D-fucose and there 20 mM D-fucose was added instead of D-galactose. The fluorescence of the cells was measured using Quantamaster 6 (PTI) fluorometer. The excitation wavelength was 488 nm. The emission was scanned from 500 nm to 600 nm. The maximum of the recorded spectra was at 515 nm.

Results

***In vivo* Expression study**

To study the regulation of gene expression of gal promoters containing different combinations of gal operators, three plasmids (pSA11 vector was used) were constructed, each having a different combination of operators in the regulatory region (Table1). The

downstream reporter gene was the green fluorescent protein (GFP), cloned under the control of the Gal promoter in between BamHI/ XbaI cloning site. These constructed plasmids (one with the wild-type OeOi configuration and other two in which the wild-type configuration was replaced with OeOe and OiOi) were allowed to grow in LB media and then the fluorescence was measured. Gal repressor protein binds to operator regions as dimer and form a loop to inhibit transcription; this repression can be overcome using the inducer like D-galactose or D-fucose. Thus, the expressions of the constructed plasmids were done in absence and presence of D-galactose and D-fucose. A time dependent expression study was also done in presence of inducer.

Figure 3A and 3B shows the relative steady-state expression levels of three plasmids in absence of any inducer, D-galactose or D-fucose. Both the mutant plasmids OeOe and OiOi have significantly higher fluorescence values than wild type sequence OeOi. This result indicates that both the operator sequences are simultaneously needed for the complete repression.

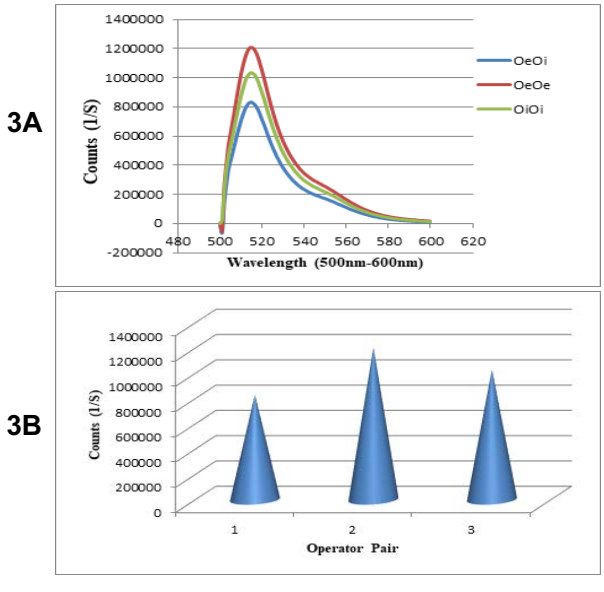


Figure 3: GFP expression of three plasmids in absence of Inducer

3A: Relative fluorescence intensity of three plasmids along the wavelength range 500nm - 600nm

3B: Relative fluorescence intensity of three plasmids at the maxima. Along X- axis, 1: denotes for OeOi (wild type), 2: denotes for OeOe, 3: denotes for OiOi

To examine the derepression of transcription, 20 mM D-galactose and 20 mM D-fucose were added separately to the culture media of XL1B cell containing the constructed plasmid

in it and then it was allowed to grow for six hours. The experiment in presence of D-Galactose and D-Fucose were done as two different sets. At different time interval 4 ml media with cell was collected and fluorescence was measured. With time the GFP expression increases in presence of both D-galactose (Figure 4A, 4B and 4C) and D-fucose (Figure 5A, 5B and 5C) indicating the derepression in presence of inducer.

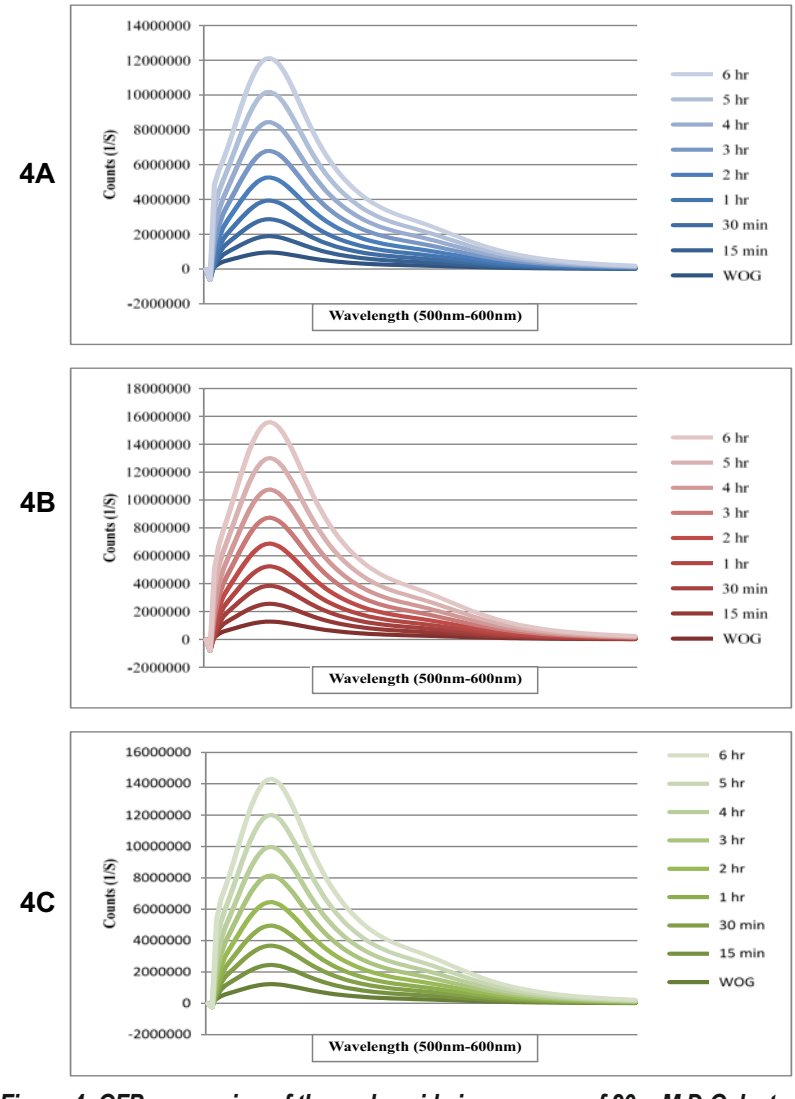
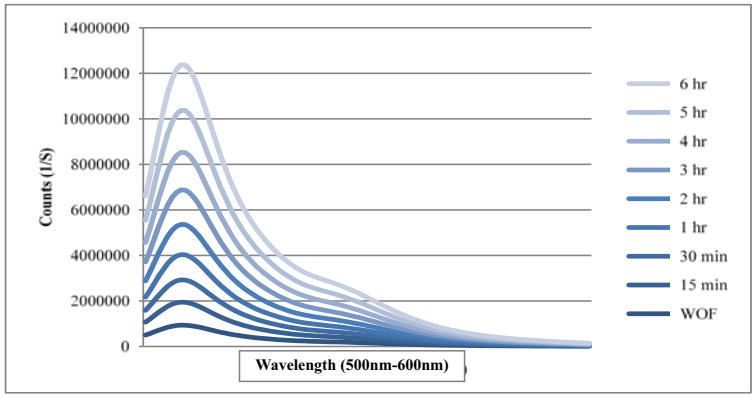


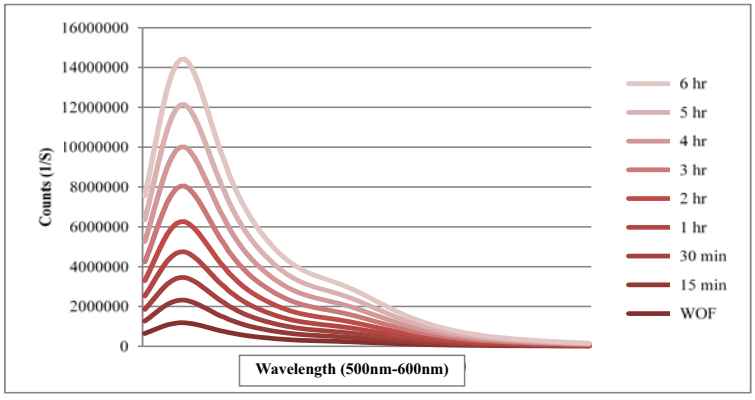
Figure 4: GFP expression of three plasmids in presence of 20 mM D-Galactose with different time:

4A: For OeOi, 4B: For OeOe and 4C: For OiOi

5A



5B



5C

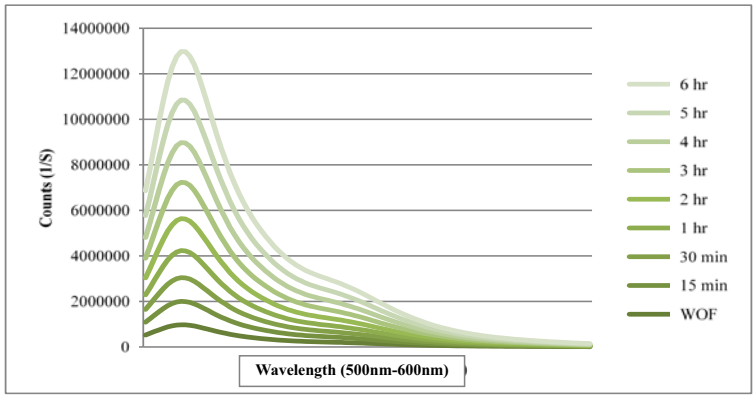


Figure 5: GFP expression of three plasmids in presence of 20 mM D-Fucose with different time:

5A: For OeOi, 5B: For OeOe and 5C: For OiOi

Discussion

The above results clearly emphasize the actual sequence information is essential for the functional outcome in protein-DNA interaction. Figure 3 shows the relative expression of fluorescence of GFP in absence of any inducer. Both OeOe and OiOi plasmids have significantly higher fluorescence value than that of wild type OeOi plasmid that clearly signifies the derepression due to substitution of one of the natural variants with other sequence. Upon addition of the inducers (D-Galactose and D-Fucose) the expression levels of three plasmids also increases with time (Figure 4-5). That phenomenon indicates the derepression of gene expression due to presence of inducer. These results clearly underline that in addition to the affinity, the actual sequence information is also important for the functional outcome.

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Evaluation of Anticancer Activity of Physcion and Emodin Isolated from *Ventilago madraspatana*

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Abstract

Two hydroxyanthraquinonoids, viz., physcion and emodin were isolated from the stem bark of *Ventilago madraspatana* Gaertn. by column chromatography followed by preparative thin layer chromatography. The quantitative estimation of these compounds in the chloroform extract of the stem bark of *V. madraspatana* was standardised by LC-UV technique. The *in vitro* antiproliferative activity of these quinones was assessed against Ehrlich ascites carcinoma (EAC), a transplantable murine tumour model, two human cancer cell lines, viz. human malignant skin melanoma (A375), and epidermoid laryngeal carcinoma (Hep2) and also in normal peripheral blood mononuclear cells (PBMC) by MTT reduction assay. In all these experimental tumour cells, emodin exhibited more cytotoxicity than physcion. Further, both compounds showed a comparatively lower toxicity in normal human lymphocytes. Generation of intracellular reactive oxygen species (ROS) by these quinones in EAC cells was measured fluorimetrically, where emodin was found to be greater ROS generator than physcion in cancer cells which was also corroborated with their respective IC_{50} values.

Keywords: *Emodin, Physcion, Antiproliferative activity, Reactive oxygen species*

Introduction

Plants are known to provide unique chemical templates and synthons for design of novel pharmaceutical agents (Newman & Cragg, 2016). Among the various classes of phytochemicals characterised so far, quinonoids belong to a special category of compounds ubiquitously found in all respiring plant and animal cells (O'Brien,1991). They play crucial roles in the cellular metabolism, mainly involve in the photosynthesis and electron transfer reactions in their hosts. Moreover, some of them show pronounced cytotoxic and allergenic actions, serving their hosts as weapons for defense against invading pathogens (Bolton & Dunlap, 2017). This observation gave rise to the fruitful utilization of quinonoid natural products for development of many important therapeutic agents, e.g. the adriyamycin group of anticancer drugs, the anthracycline antibiotics (Lu *et al.*, 2013; Mc Gowan *et al.* 2017) and the atovaquone series of naphthoquinonoids antiparasitic agents (Mustafa & Agrawal, 2008). Thus, it would be worthwhile to make

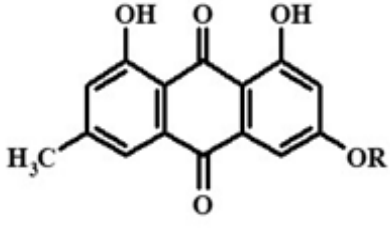
anattempt to identify and isolate new bioactive quinonoid templates from the indigenous natural resources.

One of such large woody plant *Ventilago madraspatana* Gaertn. (Family Rhamnaceae), commonly known as Red creeper (Raktavalli in Sanskrit) is distributed throughout the forests of low elevations in India and Sothern parts of Asia. This herb is well- documented in Ayurveda and other indigenous systems of medicines and traditionally it is used for the control of various ailments such as fever, dyspepsia, leprosy, colic disorder, pruritis, skin diseases etc (Chopra *et al.*, 1999). Phytochemical studies on this plant show the presence of a wide variety of quinonoids, triterpenoids, iridoid glycosides and flavonoids (Chatterjee & Pakrashi, 1997). Pharmacological studies on different parts of this plant have exhibited anticancer, antioxidant, antimicrobial, anti- inflammatory, immunosuppressant, insect repellent activities (Dalu & Dhulipala, 2015; Periyasamy & Kaliyaperumal, 2016; Vidya *et al.*, 2019). It has also been reported that the hydroxy anthraquinonoid moiety is the key 'pharmacophore' responsible for exhibiting anticancer property of this plant (Ghosh *et al*., 2010). So, it is our endeavour to explore similar other quinonoid compounds from this plant and also to investigate their various biological properties.

A search for novel quinonoid compound in this plant led to the identification of physcion (**1**) and emodin (**2**), two hydroxy anthraquinonoids, as active antiproliferative agents. Isolation and characterisation of physcion and emodin from the stem bark of *V. madraspatana* Gaertn. were achieved and *in vitro* antiproliferative activity of these compounds was evaluated against murine and human cancer cells. Cytotoxicity of **1** and **2** was also assessed against human peripheral blood mononuclear cells (PBMC). Further, capacity of these quinonoids to generate ROS in tumour cells was measured.

Isolation and characterisation of Physcion and Emodin from the stem bark of *V. madraspatana*

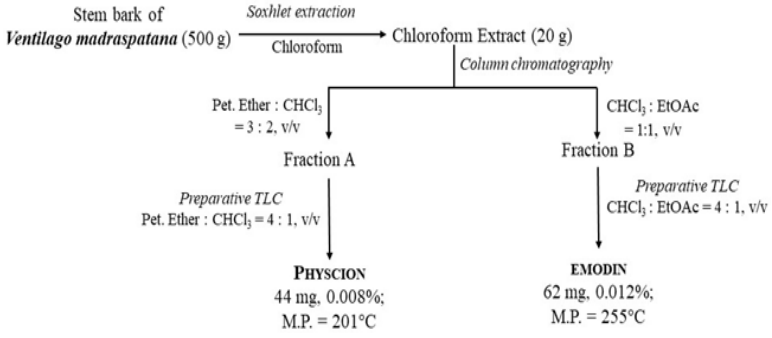
Physcion (**1**) and emodin (**2**) (Figure 1) were isolated from the chloroform extract of the stem bark of *V. madraspatana* according to the procedure (Scheme1) developed by



Physcion (1) : R = CH_3
Emodin (2) : R = H

Figure 1: Structures of physcion and emodin

modification of earlier report Kesava Rao *et al.* (1983). These two compounds were purified by crystallization, followed by characterisation through routine spectroscopic analysis (Appendix 1).



Scheme 1.Isolation of physcion and emodin from V. madraspatana

Quantitative estimation of physcion and emodin by LC-UV method

The quantitative analysis of physcion and emodin in the chloroform extract of the stem bark of *V. madraspatana* was done by using LC-UV technique. LC analysis was performed under isocratic condition (methanol: 0.5% acetic acid in water = 80:20, v/v), at a flow rate of 1.0 mL/min at ambient temperature, followed by UV detection at 255 nm (Figure 2).

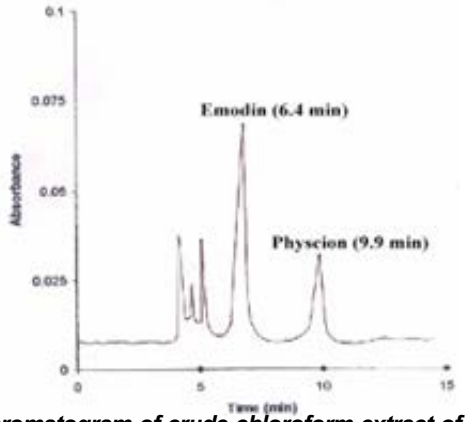


Figure 2: LC-UV chromatogram of crude chloroform extract of V. madaraspatana

A distinct separation and simultaneous determination of emodin and physcion was achieved within 10 minutes and these compounds could be quantified in the concentration range of 30 - 50 ng/mL under the specified chromatographic conditions.

In vitro antiproliferative activity of physcion and emodin against cancer cell lines Cytotoxicity study

The cytotoxicity of physcion (**1**) and emodin (**2**) in various cancer cell lines and PBMC was evaluated *in vitro* by MTT reduction assay and the IC_{50} of each compound was determined (Mosmann, 1983). Doxorubicin, a clinically used quinonoid anticancer agent was taken as positive control in this study (Table 1).

Compound	IC_{50} (μM)			
	Murine tumour	Human cancer cell lines		Normal human cells
	Ehrlich Ascites Carcinoma **(EAC)**	Malignant skin melanoma **(A375)**	Laryngeal carcinoma **(Hep2)**	Peripheral blood mononuclear cells (**PBMC**)
Physcion (**1**)	8.4 ± 1.5	> 100	>100	>100
Emodin (**2**)	5.2 ± 1.2	54.8 ± 1.5	31.2 ± 1.1	>100
Doxorubicin	>10.0	0.007 ± 0.001	0.42 ± 0.04	15.51 ± 1.74

From Table 1, it was shown that, in almost all types of tumours, emodin (**2**) was more active than its 3-methoxy derivative physcion (**1**), exhibiting 1.5- to 3- folds enhanced cytotoxicity. Again, these compounds when tested in the two human cancer cell lines, were found to be more sensitive towards A375 than Hep2 cells. Furthermore, none of the tested compounds were found to be cytotoxic against normal PBMC up to 100 μM concentration.

ROS generation in cancer cells *in vitro*

Generation of reactive oxygen species (ROS) by physcion (**1**) and emodin (**2**) in murine Ehrlich ascites carcinoma cells (EAC) was measured fluorimetrically using DCFH-DA (Gao *et al*., 2004). It was found that, substantial generation of ROS was recorded by both the compounds **1** and **2** at 0.1 μM concentration (Figure 3). In a parallel experiment

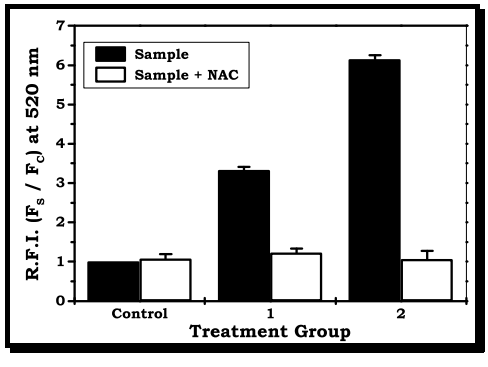


Figure 3: ROS generation, in vitro, in EAC cells treated with physcion (1) and emodin (2)

with addition of N-Acetyl-L cysteine (NAC), a specific scavenger for H_2O_2, showed dramatic reduction in fluorescence intensity, almost to the level of the 'untreated' control. This observation would confirm the formation of H_2O_2 as the predominant ROS generated by **1** and **2**. It was interesting to find that the order of ROS generation in terms of relative fluorescence intensity (R.F.I.) by **1** and **2** could be commensurate more or less with their respective *in vitro* cytotoxicity (IC_{50}) values in EAC cells.

Conclusion

Physcion and emodin isolated from the stem bark of *Ventilago madraspatana* exhibited cytotoxicity against certain tumour cells in corroboration of some earlier reports on these compounds, which are also found in other medicinal plants. The findings from the present work would encourage more extensive study towards the isolation and characterisation of other bioactive quinonoid compounds from indigenous plants of potential therapeutic significance.

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Appendix A

Physico - chemical data of physcion (1,8-dihydroxy-3-methoxy-6-methyl-9,10-anthraquinone)

TLC R_f 0.53 (petroleum ether: chloroform = 20: 80, v/v). UV/Vis (EtOH): λ_{max} (log ε) 224 nm (4.46), 256 nm (4.18), 264 nm (4.19), 286 nm (4.17), 436 nm (3.99). IR (KBr): ν_{max} (cm^{-1}) 2918, 1629, 1566, 1479, 1450, 1367, 1296, 1163, 1035. ^{1}H-NMR ($CDCl_3$): δ 2.45 (3H, s, CH_3-6), 3.94 (3H, s, 3-OCH_3), 6.69 (1H, d, J= 2.6 Hz, H-2), 7.09 (1H, bs, H-7), 7.38 (1H-d, J=2.6 Hz, H-4), 7.64 (1H, bs, H-5), 12.12 (1H, s, peri-1-OH), 12.32 (1H, s, peri-8-OH). ^{13}C NMR ($CDCl_3$): δ 22.2 (6-CH_3), 56.1 (3-OCH_3), 106.8 (C-7), 108.2 (C-5), 113.7 (C-8a and C-9a), 121.3 (C-4), 124.5 (C-2), 133.3 (C-10a), 135.3 (C-4a),148.5 (C-3), 162.5 (C-8), 165.2 (C-1), 166.6 (C-6), 182.0 (C-10), and 190.8 (C-9). EI-MS (70 ev): m/z 284(M+), 256, 241.

Physico - chemical data of emodin (1,3,8-Trihydroxy-6-methyl-9,10-anthraquinone)

TLC R_f 0.67 (chloroform: ethyl acetate = 90: 10; v/v). UV/Vis (EtOH): λ_{max} (log ε) 222 nm (4.47), 253 nm (4.38), 266 nm (4.29), 289 nm (4.36), 438 nm (4.16). IR (KBr): ν_{max} (cm^{-1}) 3377, 2925, 1629, 1481, 1456, 1336, 1211, 1103, 1033. ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s, 6-CH_3), 6.67 (1H, d, J= 2.3 Hz, H-2), 7.09 (1H, bs, H-7), 7.29 (1H, d, J=2.3Hz, H-4), 7.63 (1H, d, J= 0.9Hz, H-5), 9.88 (1H, s, 3-OH) ; 12.12 (1H, s, peri-1-OH) ; 12.29 (1H, s, peri-8-OH). ^{13}C NMR ($CDCl_3$): δ 20.9 (6-CH_3), 107.6 (C-7), 108.7 (C-5), 109.0 (C-9a),113.1 (C-8a), 120.3 (C-4), 123.6 (C-2), 132.7 (C-10a), 134.8 (C-4a), 147.5 (C-3), 161.6 (C-1), 164.7 (C-8), 165.6 (C-6), 181.9 (C-10), and 189.8 (C-9). EI-MS (70 ev): m/z 270(M+), 242, 217, 214,213,189.

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Longhorned Beetles *(Coleoptera: Cerambycidae)* of Dooars, West Bengal

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Abstract

The beetles *(Coleoptera)* comprise one of the largest and most diverse groups of insects. The Cerambycidae (longhrned beetles), characterized by long antenna, is one of the most species rich family within Coleoptera. The larvae of cerambycids usually feed on the tissues of woody plants and can be important pests, damaging and even killing trees in managed and natural landscapes. Some are beneficial insects through their role as pollinators on some plant species and may be valuable bio-indicators of woodland biodiversity, important for silvicultural management. The study area Dooars is a flat terrain region at the foot of the Himalaya (District- Jalpaiguri, West Bengal) with large patches of forest and tea gardens. Besides a large number of tea gardens Dooars has reserve forests namely, Buxa Tiger Reserve, Jaldapara Wildlife Sanctuary, Gorumara National Park and Chapramari Wildlife Sanctuary. Survey of entomofauna was conducted in different ranges of the 4 above mentioned reserve forests. The diversity spectrum of these 4 reserve forests of Dooars have yielded in the recognition of 55 species over 3 subfamilies. Out of these 2 species are new to the India, 18 from the state while 34 from Dooars. One species is reported as endemic to India. On the basis of species richness the decreasing order of reserve forests are BTR> JWLS> GNP> CWLS. The forests are dominated by the members of the subfamily Lamiinae. Premonsoon appeared to be the most preferred season. Distribution analysis further reveals that the fauna is largely Oriental (100%), followed by Palearctic (53%), Ethiopian (11%), Australian (5%), Nearctic (5%) and Neotropical (2%). The longicorn beetle fauna at subfamily, genus and species level in Dooars represents 43%, 10% and 4% of the Indian fauna respectively.

Keywords: *Biodiversity, Cerambycidae, Dooars, Reserve Forests, Fauna*

Introduction

Cerambycids are quite widespread, occurring in many diverse habitats around the world, from tropical rainforest to high altitude pine forests to scrubland and can be ecologically important by recycling dead plants (Solomon, 1995). The larvae of cerambycids develop in plant tissues, usually feed on the tissues of woody plants, but different species may require hosts that are healthy, moribund, dead, or even in various stages of

decomposition. In study area Dooars, none of the earlier workers ever attached importance to the fauna of those reserve forests though an abode of floral diversity and hence the present work has been done.

Study Area

The Dooars, a green strip of land lying along foot of the Himalaya is the gateway to the North-Eastern parts of India and stretches from the river Teesta on the west to the river Sankos on the east. Major part of Dooars is in the District Jalpaiguri and rest in Coachbehar district. It includes 181 tea gardens with a total area of 1187.06 sq. km., alpine landscape, transparent rivers and 4 reserve forests (spread over 1731.03 sq. Km.) (Anonymous, 1987). It represents the biogeographic provinces of Central Himalayas (2C) and lower gangetic plains (7B) situated in between latitudes 26˚ 16' and 27˚ 00' North and longitudes 88˚ 04' and 89˚ 58' East. Survey was confined to the reserve forests only expecting more of diversity as compared to the tea gardens. Those 4 forests of the area are-

Buxa Tiger Reserve (BTR, 23˚30' - 23˚55' N, 89˚25' - 89˚55' E) is located in the North-eastern corner of Jalpaiguri district, West Bengal with an area of 760 km2. It covers 385 km2 of core zone and 375 km2 of buffer zone. It is mainly Moist Tropical Forest and subdivided into 8 subtypes: Sal Forest, Moist Mixed / Dry Mixed Forest, Wet Mixed Forest, Semi-evergreen Forest, Evergreen Forest, Hill Forest, Savannah Forest and Riverine Forest. The reserve includes 14 forest ranges. Jaldapara Wildlife Sanctuary (JWLS, 25˚58' - 27˚45' N, 89˚08' - 89˚55' E) is one of the major sanctuaries of West Bengal with an area of 216.51 km2. It includes 7 ranges. The basic vegetation structure of this region is mixed deciduous with other types like: Riverine Forest, Sal Forest, Wet Mixed Forest, Semi-evergreen Forest, Evergreen Forest, Riverine Grassland, Savannah Grassland, Open low lying herbland, hydrophytic vegetation etc. (Ghosh and Das, 2007). Gorumara National Park (GNP, 26˚42' - 26˚7' N, 88˚48' - 88˚8' E) with an area of 79.99 km2 in Dooars. The vegetation of the park can be classified into 4 main types: Moist Deciduous and Dry Deciduous Forest, Semi-evergreen Forest, Riverine Forest and Savannah Forest. Chapramari sanctuary (CWLS) covers an area of 9.49 km2, bounded by river Neora on one hand and on the other by Bamni and Murti rivers. It consist of Dry Mixed Forest with a small patch of pure and mature Sal (Anonymous, 1996).

Material and methods

Insect samples are collected during 2001 to 2014 in the aforesaid forests by the author and his team with hand picking, sweep net, bush/foliage/herb beating. Many of the samples are collected by operating UV light trap during 18-22 hrs of the day. Further processing of the insect material is done following the recommended practices (Alfred & Ramakrishna, 2004) and studied under Stereozoom bionocular microscopes, model Zeiss SV11 and OlympusSZX7, each with necessary photographic attachment.

Reported species are in the deposition of Department of Agricultural Biotechnology, Ramakrishna Mission Vivekananda University.

Identification of the collected samples

Identification of the collected samples are done following various literatures. Samples are studied using Stereozoom binocular microscope. Primary identification of cerambycid samples are done following Gahan, 1906; Rondon & Breuning, 1970 and Cherepanov, 1990. Other literatures and websites are also consulted. Determined status of the taxa are also compared with the type specimen deposited in the collection of Zoological Survey of India.

Results

Present work represents diversity spectrum of referred bettles sampled during the period 2001 - 2014. Collection is made throughout the year except the period of June 15 to September 15 when the forests remain closed for rejuvenation.

Morphological variations are the basic parameter, by which the status of the taxa is established, for which the family Cerambycidae provides innumerable features. The cerambycid taxa as realised during the study is presented with a diagnosis, dichotomous keys and descriptions supplemented by necessary illustrations and photographs. Phenotypic variations in cerambycids are remarkable. Males and females can differ in length of their antenna or also in their coloration. Adaptive phenotypic plasticity in individuals are able to express two or more discrete phenotypes in response to differences in the internal or external environment experienced by the developing organism (Roux *et al.*, 2009).

The list of Indian Cerambycids includes 1500 species under 381 genera (Bisby, 2014). Prior to present study, West Bengal was representing 178 species belonging to 80 genera (fig: 1).

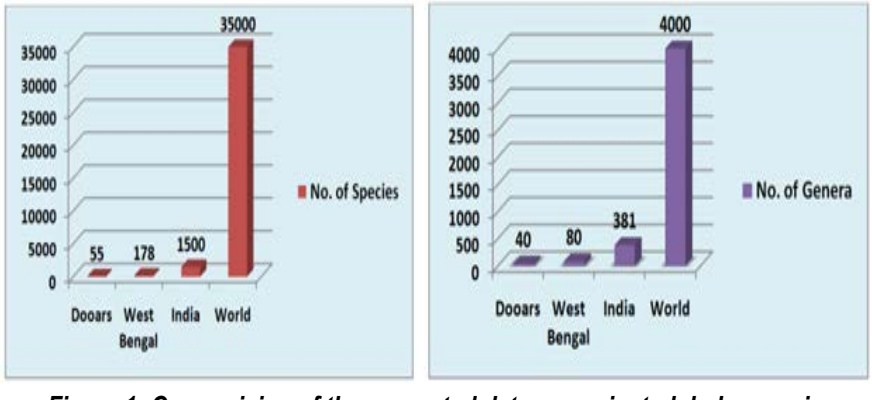

Figure 1: Comparision of the generated data as against global scenario

The diversity spectrum of these 4 reserve forests have yielded in the recognition of 55 species under 40 genera (fig: 1). The longicorn beetle fauna at genus and species level in Dooars represents 10% and 4% of the Indian fauna respectively (fig: 1). Out of these 2 species are reported as new to the subcontinent (fig: 2).

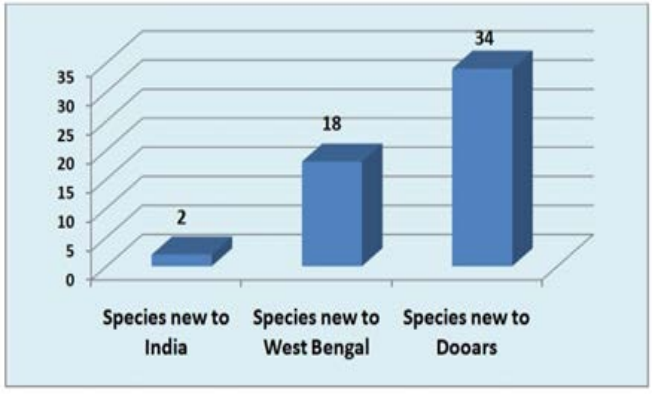

Figure 2: New findings

Subfamily: Cerambycinae- *Derolus mauritanicus* (Buquet)

Subfamily: Lamiinae- *Pterolophia (Hylobrutus) lateralis Gahan*

While 18 species are recorded first time from West Bengal.

Subfamily: Prioninae- *Dorysthenes (Paraphrus) granulosus (Thomson), Dorysthenes (Lophosternus) buqueti (Guerin-Meneville), Nepiodes costipennis (White)* ***Subfamily: Cerambycinae-*** *Chlorophorus annularis (Fabricius), Pachydissus parvicollis Gahan, Stromatium longicorne (Newman), Zoodes compressus (Fabricius)*

Subfamily: Lamiinae- *Aristobia approximator (Thomson), Astathes (Tetraophthalmus) gibbicollis Thomson, Astathes (Tetraophthalmus) violaceipennis (Thomson), Coptops aedificator (Fabricius), Glenea (Stiroglenea) cantor (Fabricius), Olenecamptus dominus Thomson, Ostedes (Trichostedes) assamana Breuning, Pharsalia (Cycos) subgemmata (Thomson), Stibara (s. str.) tricolor Fabricius, Tetraglenes hirticornis (Fabricius), Thysia wallichii wallichii (Hope)*

Species found to be endemic to India is *Pachydissus parvicollis Gahan*.

Most of the species recorded, are the members of the subfamily Lamiinae (29), followed by Cerambycinae (19), Prioninae (7) (fig. 3).

On the basis of species richness the decreasing order of the reserve forests are BTR>JWLS>GNP>CWLS (fig. 4). Analysis of floral pattern explains the reason of such concentration. Buxa Tiger Reserve predominated by all forest types with 7 subtypes

provides wide variety of host plants for these borers, while grassland is a major part of Jaldapara Wildlife Sanctuary and Gorumara National Park. Chapramari Wildlife Sanctuary includes mainly the Sal trees. All these forests are dominated by the members of the subfamily Lamiinae (fig: 3).

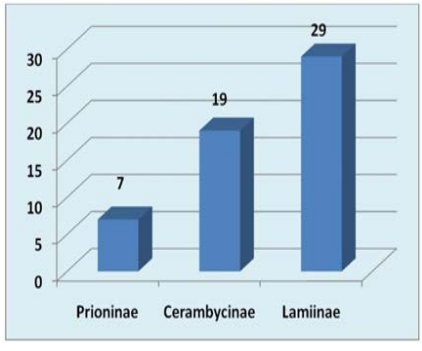

Figure 3: Total number of species under subfamilies

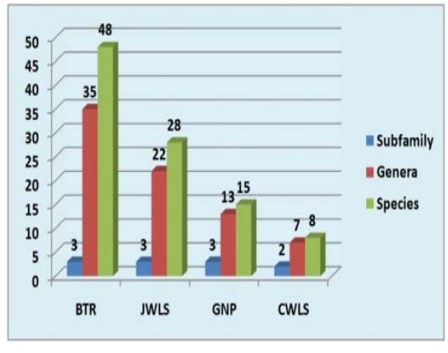

Figure 4: Comparative account of taxa trapped

Their seasonal distribution is mostly during Premonsoon (50%), followed by Postmonsoon (35%) and Monsoon (15%) (fig. 5). Distribution analysis reveals the fauna is largely Oriental (100%), followed by Palearctic (53%), Ethiopian (11%), Australian (5%), Nearctic (5%) and Neotropical (2%) elements (fig. 6). The find of exotic elements reveals Indochinese and Indomalayan connection with immigration and colonization of host plant species especially the Malayan floristic elements from bordering countries of northeast India.

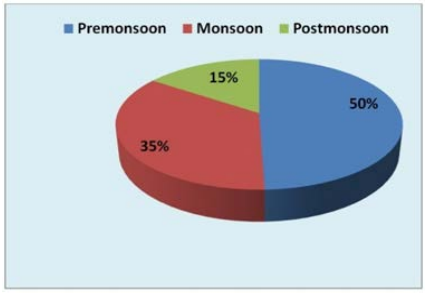

Figure 5: Seasonal distribution (%) of Cerambycidae taxa trapped

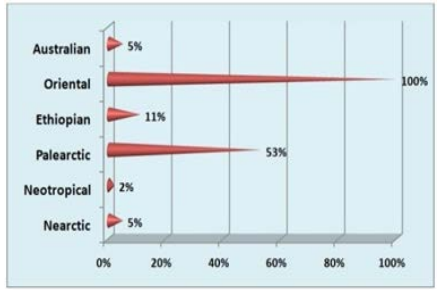

Figure 6: Zoogeographical distribution (%) of Cerambycidae taxa

Conclusion and Significance

Many *Cerambycid* species are important pests of forests, plantations, street trees and

shrubs. They cause an annual loss of millions of dollars by their destruction, both to living trees as well as to felled trees. It is difficult to make an assessment of the revenue loss caused by forest insect pests in a vast and diverse country like India. One earliest estimate of annual loss due to insect damages puts down the figure as Rupees 53.70 millions (10% of the total revenue of the forests which was Rupees 537 millions in 1959-60) (Chatterjee & Sen-sarma, 1968). However, some are beneficial insects through their role as pollinators on some plant species (Ponpinij *et al*., 2011) and may be valuable bio-indicators of woodland biodiversity, important for silvicultural management (Mantia *et al*.,2010). Inspite bing a large producer of commercial timbers, Cerambycids of Dooars as well as from india have never been studied in a systemic manner and hence the present work has been done for future management of controlling timber damage.

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An Overview on Food Preservatives

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Abstract

People around the world are very much busy in their tight and hectic schedule. So dependency on packed and processed food has been increased. Food additives are widely used by the food industry to increase the product shelf life and/or attribute as well as enhance certain characteristics of particular foods like taste, freshness, and colour of the foods. Addition of additives has been increased by the food industry. So our bodies are getting contaminated with a large number of synthetic industrial chemicals, many of which are known to be toxic and carcinogenic while others remain untested for their health effects. In this article some of the adverse effects of food preservatives have been discussed. These chemical compounds, however, can have many undesirable side effects in human body.

Keywords: *Preservatives, Chemicals, Antioxidants, Antimicrobial, Side effect*

Introduction

Demand for stable, safe and durable foods has been increased due to very much busy tight and hectic schedule of modern life style. Food preservatives are substances that can prevent or delay changes in the food. Food preservation (Food preservation, 2020) is one of the oldest practices of human being. Preservatives may be natural or artificial. Food preservation by artificial way can be done by nuclear radiation (Anon, 1991), vacuum packing and hypobaric packing. Chemical preservation (Chemical preservation of food, 2020) is one of the most effective ways of preservation. In the preservation process the growth of bacteria either stopped or delayed; again the reaction of food with oxygen or heat is suppressed by this process and the quality of the food product is well maintained for a long time.

Preservation Process

Factors that change the food properties are microbes and oxidation. Microbes like bacteria and fungi invade food and feed off its nutrients. Some of these can cause diseases. Others turn foods into smelly and greasy items. Whereas, oxidation brings a chemical change in the food's molecules caused by enzymes or free radicals, like apples (Jean, 1994) and potatoes became brown when they kept in open air after cutting. Preservatives help to maintain the food quality by inhibiting the oxidation process or by ceasing the activity of microbes. One of the ways microbes can be controlled by making

the food more acidic. People have been preserving food using bacteria that produce lactic acid for a long time for e.g. yogurt. Sodium Chloride is probably the oldest preservative that human using. It acts as antimicrobial agent (Jay, 1995) by sucking moisture out of cells, and thus growth of the microbes prohibited. Antioxidant activities are exhibited by inhibiting free radicals by chemical compounds like BHT (butylated hydroxyanisole) and tocopherol. Antioxidants (Carocho, 2018) are present in wood smoke, which is used to preserve foods. Smoking and salting together was an effective way of preservation of meat before refrigeration. Antimicrobial nitrates and nitrites are used to inhibit *Clostridium botulinum* in foods containing raw meat. Sulphur dioxide and sulphites are extensively added to control the growth of microorganisms in dry fruits, juices and wines. Sulphites can acts as both antimicrobials and antioxidants. A significant association between chelation and antibacterial properties exists. Many of the chelators found in food occur naturally like oxalic acids, succinic acids, lactic acids, citric acids, glycine, leucine and various macromolecules (peptides, proteins). Commonly used chelators in foods are citrates, pyrophosphates and EDTA (ethylenediamine-tetraacetic acid). Some antibiotics also used for preservations such as nisin, natamycin.

The Commission of the European Union assigns E-followed by numbers as codes for chemicals for food additives (Food additives, 2020). This numbering is followed by the food industry all over the world. List of some Preservatives given in Table 1.

Table 1: Name of some preservatives

Number	**Name of Preservative**
E 200	Sorbic acid
E 202	Potassium sorbate
E 203	Calcium sorbate
E 210	Benzoic acid
E 211	Sodium benzoate
E 212	Potassium benzoate
E 213	Calcium benzoate
E 214	Ethyl p-hydroxybenzoate
E 215	Sodium ethyl p-hydroxybenzoate
E 216	Propyl p-hydroxybenzoate
E 217	Sodium propyl p-hydroxybenzoate
E 218	Methyl p-hydroxybenzoate

E 219	Sodium methyl p-hydroxybenzoate
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium hydrogen sulphite
E 223	Sodium metabisulphite
E 224	Potassium metabisulphite
E 226	Calcium sulphite
E 227	Calcium hydrogen sulphite
E 228	Potassium hydrogen sulphite
E 230	Biphenyl, diphenyl
E 231	Orthophenyl phenol
E 232	Sodium orthophenyl phenol
E 233	Thiabendazole
E 234	Nisin
E 235	Natamycin
E 239	Hexamethylene tetramine
E 242	Dimethyl dicarbonate
E 249	Potassium nitrite
E 250	Sodium nitrite
E 251	Sodium nitrate
E 252	Potassium nitrate
E 281	Sodium propionate
E 282	Calcium propionate
E 283	Potassium propionate
E 284	Boric acid
E 285	Sodium tetraborate (borax)
E 1105	Lysozyme

Some Effects

Chemicals used for preservation has some harmful effects. Most common effects are itching, flushing, abdominal pain, nausea, vomiting, diarrhoea, asthma, cough, rhinitis, anaphylaxis etc. Some of the commonly used preservatives with their use and harmful effect are listed (Voss, 2002) in Table 2.

Table 2: Uses and side effects of Preservatives

Examples	Uses	Effects
Sulphites	fruits	headaches, palpitations, allergies, and even cancer
Nitrates and Nitrites	meat products	stomach cancer
Benzoates	Fruit products, acidic foods, margarine	hyper activity, allergies, asthma and skin rashes
Sorbates/sorbic acid	Dairy products, fruit products, syrups, sweets, beverages, fermented products	possibility of allergies
Potassium Bromates	breads	cancer
Butylated Hydroxyanisole(BHA)	Bakery products, cereals, fats and oils	high blood cholesterol
Nisin	Cheese product	resistance to microorganism developed
Disodium ethylenediaminetetraacetic acid (EDTA)	Dressings, margarine, canned vegetables	abdominal cramps, nausea, vomiting, diarrhoea, headache, low blood pressure,

Conclusion

Preservatives increase the shelf life and maintain the quality of food for longer time. Though some of these chemicals preservatives have harmful effects and people are shifting to natural preservatives which are non-toxic in nature also with a wide range of health benefits. Food Safety and Standards Authority of India (FSSAI) has given the standard or approved ingredients for the food safety and standard. Already the use of

potassium bromate and cyclamates in any food category has been banned. It is better to take food having less harmful preservative.

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In vitro Study of Cell Migration by Matrigel Invasion Assay

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Abstract

Migration or dissemination of cancer cells from the site of primary tumor to distant body parts is the major cause of cancer deaths. A crucial step in tumor metastasis is the invasion of basement membranes by tumor cells. Basement membranes are thin continuous sheets of extracellular matrix that underlies epithelia and endothelia and form a barrier to macromolecules and cells. A breakthrough method for studying both cell migration and invasiveness *in vitro* is the matrigel invasion assay or transwell migration test. It follows the principle of the Boyden Chamber chemotaxis assay where migration of cancer cells across a reconstituted membrane 'Matrigel' quantifies metastatic cell invasive potential. Significant amounts of matrix degrading enzymes mainly metalloproteinases, serine proteases, cathepsins and others are expressed by metastatic cells. Of them, collagenase-IV as well as serine proteases appear to play key roles for basement membrane invasion. The results obtained using this assay reveal that there is a strong correlation between the ability of tumor cells to invade *in vitro* and their invasive behavior *in vivo*, thus validating the use of this technique as a measure of invasive potential. Balance between proteolytic enzymes and their inhibitors determine the degree of invasion through the matrigel membrane and so the matrigel 'chemoinvasion' assay has been a useful tool for the screening of anti-invasive agents. Thus the study of existing methods and the development of newer and novel strategies for quantifying tumor cell migration and their invasiveness are the key factors for understanding the basis of cancer metastasis and developing novel anticancer therapies.

Keywords: *Tumor, Invasion, Matrigel, Cell Migration*

Introduction

Development of malignant cancers involves the growth of tumors accompanied with increased requirements for both oxygen and nutrients. Neovasculature formation or angiogenesis not only helps the tumor to grow in size, provides oxygen and nutrient supply, but also helps in dissemination of neoplastic cells. One of the most crucial event in cancer metastasis is the invasion of basement membranes. The dynamic process of tumor cell invasion requires these cells to activate specific proteases so as to initiate a local and limited degradation of matrix components (Kleinman & Martin, 2005). In the early stages of angiogenesis, the degradation of the basement membrane is required for

the endothelial cells to migrate towards the tumor and has mechanisms similar to those mentioned for metastatic cells. One of the best *in vitro* methods used till date to assess the invasiveness of cells is the matrigel invasion assay.

Matrigel

In the past, isolated basement membranes, particularly from the amnion, were used to study the invasiveness of cells (Hendrix *et al.*, 1989). This was later replaced by 'matrigel' which is a reconstituted membrane. Commercially prepared matrigel is available in aliquots of 10-12 mg/l. However, Boyden chambers are preferred nowadays for the assay.

The Assay

The use of filtered chambers to quantify cellular invasive activity dates back to 1978 (Hart & Fidler, 1978). Since then, modifications have been included like the introduction of Boyden chambers and transwell chambers. Polycarbonate filters (8 or 12µm pore), depending on the cell size, were coated with matrigel diluted with water. The coating of matrigel required to block invasion of endothelial cells is generally lower than that used for metastatic cells since they are less aggressive than most of the tumor cells (Albini *et al.*, 1995).

The cells to be tested are suspended in culture medium ad placed in the upper compartment of the Boyden chamber. Medium along with chemoattractant is placed in the lower chamber and then incubated at 37 ºC in 5% CO2. A large range of chemoattractants can be used in the chemoinvasion assay. Tumor cell conditioned medium mimicking a highly angiogenic environment is generally used for endothelial cells. Invasion can be assayed after the desired time period. At the end of the incubation time, the cells remaining on the upper surface of the filter are removed by wiping them with a cotton swab. The cells on the under surface of the filter are quantitated after staining with toluidine blue, hematoxylin/eosin or others (Fig1)(Roy *et al.*, 2008). An invasion index can be calculated and indicate the specific contribution of matrix degradation using the following formula (Albini, 1998).

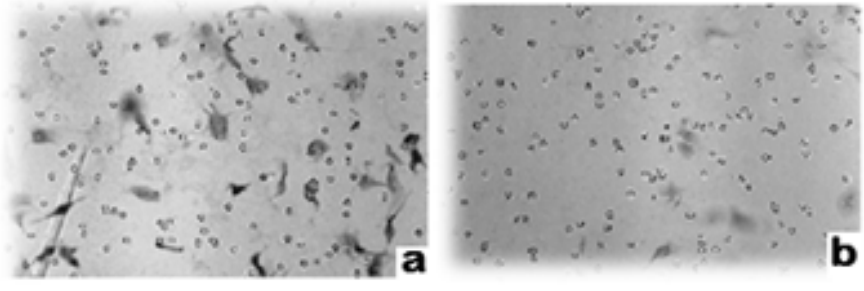

Figure 1: Invasion of matrigel membranes by B16F10 cells after 24 hr incubation. Migrated cells show blue coloration due to Giemsa staining; a=control; b= cells treated with 5µg cisplatin, 1

Invasion index= (Invaded cells/migrated cells) ×100

Assays are generally performed in triplicate and repeated twice or thrice.

Discussion

Most of the deaths due to cancer are caused due to tumor metastasis. Multiple classes of enzymes such as matrix metalloproteinases, serine proteases, cathepsins etc. are upregulated during tumor metastasis. The assay is an important tool for measuring cell migration and invasion *in vitro* in the presence or absence of test drugs. Likewise, preliminary screening for agents which may have invasion-inhibiting or reducing effects can also be done by the transwell migration assay. Moreover, it correlates between *in vitro* behavior of cells on matrigel and their *in vivo* metastatic potential. By facilitating learning of the intimate mechanisms of cell movement, invasion and differentiation, it helps in analyzing cell lines and their clones. A whole array of potential pharmacological modulators can be validated using experiments based on the invasion assays. Altogether, it can be said that the inception of the matrigel era has deeply influenced our conception of tumor invasion; the turf is now set for strategically dealing with tumors.

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Imaging Beyond Optical Light Camera Technique: A Short Review

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Abstract

We, each and every one are different from each other and direct prove of it is the passport photo i.e. image of a face. Similarly every material is different to each other, since materials have no face but have surface so image of surface is a direct proves. But for material surface study, micro and nano scale image or single molecule as well as atomic level image is the necessity condition. So image is a very good tool to differentiate material to material, face to face, organ to organ etc. Here I will discuss about imaging with some various technique in macro, micro,nano and atomic level which are using to characterize material and to detect disease without visible camera.

Keywords: *X-Ray, MRI, CT Scan, SEM, TEM etc.*

Introduction

Photography and motion picture are arts. Here the image-forming device is camera, and Photographic film electronic image sensor is the sensing medium. The respective recording medium can be the layer itself, or a digital electronic or magnetic remembrance. The camera is a dark room or chamber from which, as far as possible, all light is excluded except the light that forms the image. But by this visible light camera we would unable to take image *in vivo* as well as nano or less than 1mico level image. So we need different technique to do such.

1. X-ray technique

An X-ray machine is essentially a camera. Instead of visible light , however , it uses X-ray to expose the film. X-ray are like light in that they are electromagnetic waves, but they are more active so they can penetrate many resources to varying degrees. When the X-rays hit the layer, they representation it just as light would. Since bone, fat muscle, tumors and other masses all absorb X-rays at different levels, the reflection on the film lets you see different (distinct) structures within the body because of the different levels of disclosure on the film (Figure 1) Beyond UV (still shorter wavelength or higher frequency) are X-rays and the even more dominant gamma rays. Their high energy allows them to penetrate through solid material. They can cause serious scratch to the macromolecules of living.

Understanding the Image As X-rays from the source pass all the way through the body, they lose their energy. The loss of energy, called attenuation, depends on some tissue distinctiveness. As a simple clarification we may say that some tissues are “translucent” to X-rays, some are “transparent” (partially transparent) and some are “dense” to X-rays. A totally dense material will absorb all the X-rays, allowing none to pass throughout. A “transparent” tissue between the source and the film implies that more X-rays hit the film, affecting more silver halide, significant to a black image, an “dense” tissue will block a lot of X-rays, less or no silver is exaggerated and the image is white. Intermediate degrees of clearness give rise to shades of gray in the image. keep in mind that an X-ray image on film is seen as a negative film!

The actual darkness in the image also depends on the initial energy and the 'quantity' of the X-rays as they appear from the source. This is analogous to the reflectivity of the subject and the amount of available light in common photography. The 'quantity' of X-rays is related to the electron flow from the cathode (measured in milliamperes), and the energy is correlated to the anode voltage (kilovolts) – the greater the anode voltage, the faster the electrons and the more energetic the X-rays when the electrons are stopped.

The most important (but not exclusive) factor is the existence of 'heavy' elements in the tissues. The word 'heavy' refers to the atomic mass (as in the periodic table of elements), which does not necessarily match up with the density or specific gravity. mainly body tissues are carbon, hydrogen, oxygen and nitrogen based. The atomic masses of these fundamentals are 12, 1, 16 and 14 respectively. The ordinary heavier elements are calcium (40) and iron (56). Bone has a enormous concentration of calcium. Muscle tissue has a reasonable degree of calcium abundance and blood, of iron. Remember, this does not make all bone or blood dense to X-rays! The thickness of the tissue and the relative loads of heavy elements also matters. Thus, a solid mass of muscle or blood may be more opaque than a thin coat of bone. Remember also that an X-ray image for studying 'soft' tissues uses a smaller amount energetic X-rays or shorter exposure than one taken for studying bone. These scientific details need not worry us. What we do need to understand is the difference generated by different tissues.

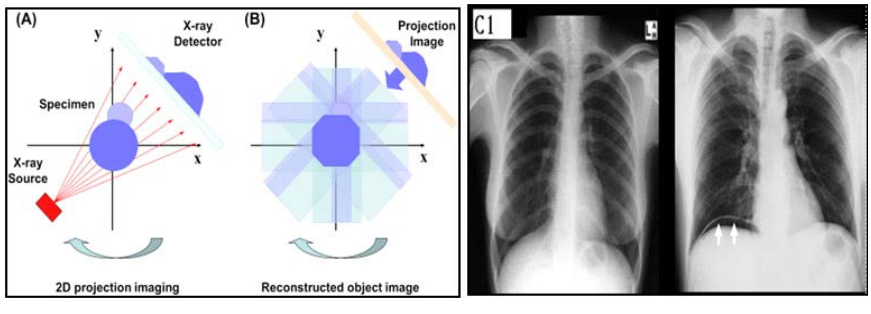

Figure 1: X-ray Technique

2. CT Scan technique

In computerised tomography (CT) the X-ray source rotates around a plane of the body, taking sequential pictures with a detector (instead of a film) which is synthesized by a computer. The resulting picture formed by the computer is like a section of the body and can be recorded on a film (Figure 2). CT pictures are consequently like X-ray images. A CT image can be taken as a plain image or with the foreword of a contrast medium. Like conventional X-ray images, bone appears white, air black and soft tissues contain intermediate densities depending on their composition and thickness. However, the contrast and resolution is better than in conventional tomography.

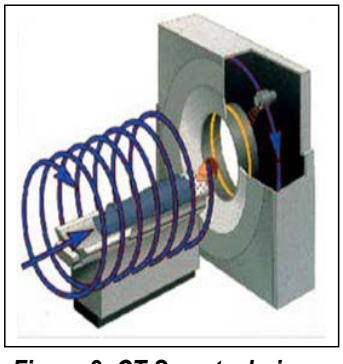

Figure 2: CT Scan technique.

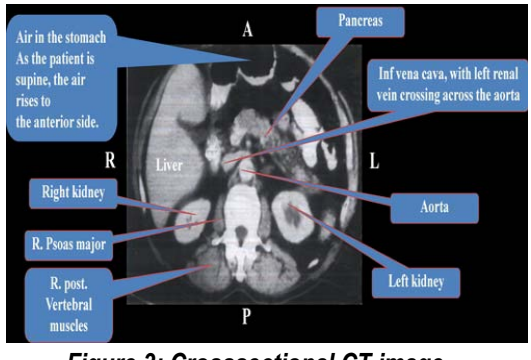

Figure 3: Crosssectional CT image (taken from reference)

Compare this crosssectional CT image (Figure 3) with abdominal organs as you have seen them! Remember, a CT image is seen as if one is screening a slice from below.

3. MRI Technique

Magnetic resonance imaging (MRI) uses the property of protons aligning themselves in a magnetic field and their feedback to radio frequency waves. The protons 'resonate' to the radio frequency and relapse to ordinary ('decay') when the radiation is stopped. Efficiently it is the imaging of protons. The most usually imaged proton is a hydrogen nucleus. So far it is believed that this method does not injure body tissues as X-rays do. MRI images are even more realistic than CT images. MRI explain your science studies have shown you that your body is made up of living cells…Which are made up of molecules and molecules are prepared up of atoms. In the 1940's physicists exposed that the nuclei of some atoms have a property called “SPIN”… Like a wobbling spinning top. This causes the nucleus perform like a tiny magnet. After many years of investigation physicists found they could affect the tiny nuclear magnets of hydrogen atoms using very strong magnets and radio waves. When this happens energy is

released as a tiny beat of radio waves !!! This little pulse of radio waves that can be detected and analysed. The timing, and the energy of these signals, reveals information about the Hydrogen atoms and what types of molecules they are emotionally involved to. The grey or white manifestation of fat is an indicator that this is an MR image. It evident as a deep layer in the abdominal wall. It is also easily recognisable around the kidneys (perirenal fat) and in the greater momentum in front and the characterization of soft tissue structures is sharper.

4. Ultrasonography technique

Ultrasound or ultrasonography is a medical imaging skill that uses high frequency sound waves and their echoes. The ultrasound apparatus transmits high-frequency (1 to 12 megahertz) sound pulses into the body using a probe. The sound waves pass through into the body and strike a boundary between tissues (like, between fluid and soft tissue, soft tissue and bone). Some of the sound waves reflect back to the probe, while some take a trip on further until they reach another boundary and then reflect back to the probe. The reflected waves are recorded by the probe and relayed to the machine. he machine calculates the distance from the probe to the tissue or organ (boundaries) using the velocity of sound in tissue (1540 m/s) and the time of the each echo's return (Generaly on the order of millionths of a second). The machine displays the distances and intensities of the echoes on the monitor, forming a two dimensional image. The tricks are relatively inexpensive and portable, especially when compared with modalities such as magnetic resonance imaging (MRI) and computed tomography (CT).

Electron Microscopy technique

Electron Microscope are two types and they are Transmission (TEM) and Scanning (SEM)

5. TEM:

Transmission electron microscopy is a microscopy method whereby a beam of electrons is transmitted through an ultra thin sample, interacting with the specimen as it passes through. An image is created from the interaction of the electrons transmitted though the specimen. The image is magnified and focused onto an imaging tool, such as a fluorescent screen, on a layer of photographic film. Also we can speak TEM works like a slide projector. A projector shines a beam of light that is transmits through the slide. The patterns decorated on the slide only alow certain parts of the light beam to pass through . Thus the transmitted beam replicates the patterns on the slide , forming an blown up image of the slide when falling on the screen.

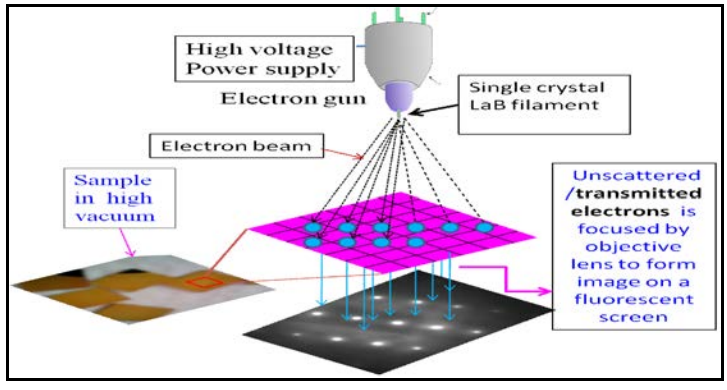

Figure 4: This figure shows that our Golden Colour Hydrogenated Diamond Like Carbon sample Characterized by TEM to the actual thickness and plan to plan distance.

6. SEM

Scanning electron microscopy is used for inspecting topographies of specimens at very high magnifications using a part of equipment called the scanning electron microscope. SEM magnifications can go to more than 300,000 X but most semiconductor manufacturing applications involve magnifications of less than 3,000 X only. SEM inspection is often used in the analysis of die/package cracks and fracture surfaces, bond failures, and physical defects on the die or package plane.

During SEM examination, a beam of electrons is focused on a spot volume of the specimen, resulting in the transfer of energy to the spot. Figure 5. is the schematic diagram SEM imaging technique that how electrons is emitted from the surface of our Hydrogenated Diamond Like Carbon to the detector for the formation of SEM image.

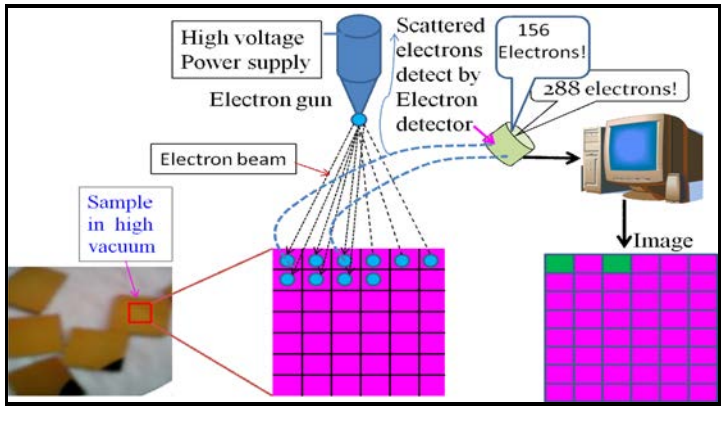

Figure 5: is the schematic diagram SEM imaging technique that how electrons is emitted from the surface of our Hydrogenated Diamond Like Carbon to the detector for the formation of SEM image.

These bombarding electrons, also referred to as main electrons, dislodge electrons from the specimen itself. The dislodged electrons, also known as secondary electrons, are attracted and composed by a positively biased grid or detector, and then translated into a signal.

To produce the SEM image, the electron beam is swept across the region being inspected, producing many such signals. These signals are then enlarged, analyzed, and translated into images of the topography being inspected. Finally, the reflection is shown on a Cathode ray tube (CRT).

Optical Microscopy is typically what one thinks of when studying microscopy. It is prevalent and widely available. Its resolution is restricted by the diffraction of light, which is limited to about 1/2 a wavelength of the light being diffracted through the optical lensing element of the microscope. While this is several hundred nanometers, in practice it is challenging to get good optical resolution down to the 1 μm range.

The next most widely available tricks would be that of Scanning Electron Microscopy. Here we are limited by the diffraction of electron waves. Since these are in the Angstrom range, there are instruments with resolution of a little nanometers quite widely deployed. Current information have described a new instrument able to study atoms (a few Ångstroms in size) but this is not available for use yet. Each instrument achieves high resolution by focusing the electron beam as strongly as possible. The minimum spot size achievable by focusing the electron beam defines the resolution of a particular tool.

Scanning Auger Microscopy (SAM) is closely related to SEM, except that the scattering in the sample leads to a "smearing" of the incident beam, and broadens the useful spot size. Its resolution general lags behind that for SEM. Probe Microscope techniques like STM and AFM have resolution that is restricted by the sharpness of the probe tip. This can be in the range of a few nanometers, but the differing contrast mechanisms mean that STM can easily attain atomic resolution while atomic resolution for AFM is difficult to achieve.

7. Atomic Force Microscopy

HOW DOES THE AFM WORK? AFM provides a 3D profile of the surface on a nanoscale, by measuring forces between a sharp probe (<10 nm) and surface at very short distance (0.2-10 nm probe-sample separation). The probe is supported on a flexible cantilever. The AFM tip "gently" touches the surface and records the small force between the probe and the surface. How are Forces Measured ? The probe is placed on the end of a cantilever (which one can think of as a spring) (Figure 6). The amount of force between the probe and sis dependant on the spring constant (stiffness of the cantilever and the distance between the probe and the sample surface. This force can be described using Hooke's Law:

F=-K. X

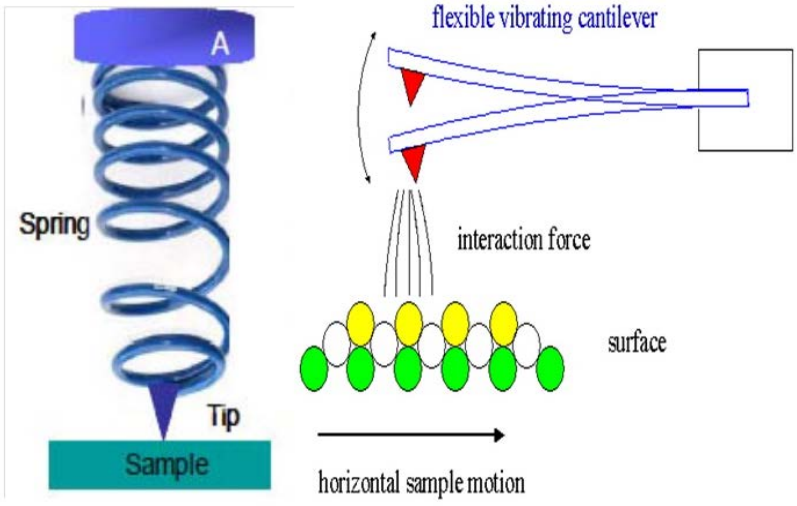

Figure 6: Schematic diagram of spring and deflection of cantilever

F= Force, k = spring constant, x = can tilever deflection

If the Spring constant of the cantilever (typically ~ 0.1-1 N/m) is less than surface, bends and the deflection is monitored.

This typically results in forces ranging from n N (10^{-9}) to µ N (10^{-6}) in the open air

AFM provides a 3D profile of the surface on a nanoscale, by measuring forces between a sharp probe (<10 nm) and surface at very short distance (0.2-10 nm probe-sample separation). The probe is supported on a flexible cantilever. The AFM tip "gently" touches the surface and records the small force between the probe and the surfaces. The AFM and the STM have vertical resolutions in the sub-atomic range of tens of picometers. The best instruments can resolve down to a few hundred femtometers.

Conclusion

The most appropriate technique depends on the sample surface being imaged and the required information to be obtained. All technique were able to characterize the samples, although for SEM image metal coating is required to increase contrast and obtain accurate dimensional measurements; this introduced an error of up to 14 nm.

SEM is least appropriate for small nanoparticles, out of the three techniques discussed in this in literature.

AFM is rather independent of nanoparticle materials, delivering high contrast and signal-to-noise ratio on all samples. On the other hand, AFM is very sensitive to cleanliness of

the sample being imaged. TEM offers the largest throughput, and is most attractive for rapid characterization of a large number of nanoparticles. The smallest nanoparticle imaged in this job was 15 nm in diameter, although there are a whole class of nanoparticles smaller than this. For illustration, quantum dots are typically nanocrystals which have a diameter below 6 nm. Both AFM and TEM are able of adequately characterizing these nanoparticles.Which technique a researcher chooses often depends on the accessibility and familiarity with the methods. In general, there are more SEM installed than AFM universal; additionally, TEM costs about double the price of SEM, and twenty times that of AFM. The higher cost and high maintenance of SEM mean that entrance to them can be difficult. In terms of availability and cost, AFM is the greatest option. It is recommended that researchers conclude the type of information they desire to obtain and the appropriateness of techniques to particular samples, with particular consideration given to nanoparticle size. In general, a combination of methods, with careful clarification of the data is the best option.

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The Importance of Sustainable Development of Natural Products in Global Health Care

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Abstract

Three fourth of the world's population still depends on medicinal plants and other tools of traditional system of medicines for their primary health care. Climate change and global environmental impacts attributable to the population growth have negative impacts on human health. Natural products keep us healthy but there is relatively little discussion as yet, about the long term effects of the current, non-sustainable harvesting methods for medicinal plants and other sources of natural products from the wild, which are depleting day by day without concurrent initiatives to commercialize their cultivation. However, maintaining and enhancing the availability of quality natural products on a sustainable basis is an important public health care concept. To achieve these goals for future health care, and restore the health of mother Earth, a great transformation is necessary. This book chapter entails fundamental significance and prospect of sustainable development of natural products and various difficulties in the path of natural product research and their potential resolutions.

Keywords: *Natural products, Drug, Global, Biodiversity, Ecology*

Introduction

"And the earth brought forth grass, and herb yielding seed after his kind, and the tree yielding fruit after his kind, whose seed was in itself, and after his kind: and God saw that it was good."

-Genesis 1:12

The use of herbs as medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. Two hundred and fifty years ago there were no synthetic medicines. The 250,000-300,000 species of higher plants were the main source of drugs for the world population.

"We're Depleting Natural Resources Twice as Fast as Nature Can Recover."

The global population has now exceeded 7.5 billion, and forests and other resources around the world are being irreversibly depleted for energy, food, shelter, material goods, and medicines to accommodate population needs. Climate change is predicted to negatively impact on human health across the globe.

"……the ability of ecosystems to sustain future generations can no longer be taken for granted...". (United Nations Millennium Ecosystem Assessment, April, 2005)

Drugs are primarily derived from two sources, synthetic and natural, or in some cases, as semi-synthetic compounds, a mixture of the two. For the developed nations , efforts have been initiated to make drug production in more sustainable manner with milder reagents, shorter reaction times, and more efficient processing, thereby using less energy, and reactions which are more atom efficient, and generate fewer by-products.(GREEN CHEMISTRY)

"Green chemistry is sustainable chemistry."

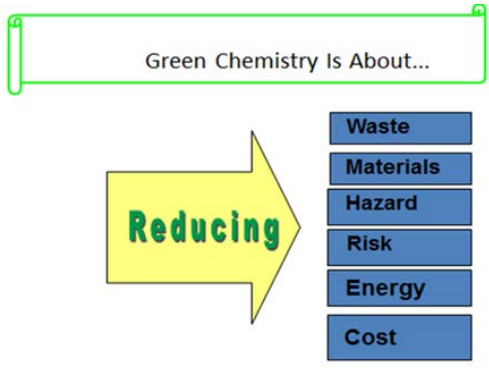


Figure 1 : An overview of Green Chemistry

75% of the world's population still depends on medicinal plants and other tools of traditional system of medicines for their primary health care. Natural product medicines have come from various source materials like terrestrial plants, terrestrial microorganisms, marine organism, terrestrial vertebrates and in vertebrates more over they are renewable resources.

"Natural Product… keeps us healthy."

There is relatively little discussion as yet, about the long term effects of the current, non-sustainable harvesting methods for medicinal plants from the wild, which are depleting these critical resources without concurrent initiatives to commercialize their cultivation.

"All medicinal natural products should be regarded as a sustainable commodity, irrespective of their source."

However, maintaining and enhancing the availability of quality medicinal agents on a sustainable basis is an unappreciated public health care concept. To accomplish these goals for future health care, and restore the health of the Earth, a profound paradigm shift is necessary:

"Saving our planet, lifting people out of poverty, advancing economic growth... these are one and the same fight. We must connect the dots between climate change, water scarcity, energy shortages, global health, food security and women's empowerment. Solutions to one problem must be solutions for all."

- Bani K Moon

Global health care

"It is the health that is real wealth and not pieces of gold or silver"

- Mahatma Gandhi

Global health is the health of populations in the global context; it has been defined as "the area of study, research and practice that places a priority on improving health and achieving equity in health for all people worldwide". It is about worldwide health improvement (including mental health), reduction of disparities, and protection against global threats that disregard national borders.

"When it comes to global health, there is no 'them' only 'us'."

- Global health Council

Globally, the rate of deaths from non-communicable causes, such as heart disease, stroke, and injuries, is growing. At the same time, the number of deaths from infectious diseases, such as malaria, tuberculosis, and vaccine-preventable diseases, is decreasing.

Some of the major diseases currently affecting countries around the globe include HIV/AIDS, malaria, Zika, and tuberculosis, COVID-19. Climate change is also an international problem which can affect people's health.

The concept of sustainable development aims to maintain economic advancement and progress while protecting the long-term value of the environment; it "provides a framework for the integration of environment policies and development strategies" (United Nations General Assembly, 1987)

Concerns with respect to future energy resources, and for continuing access to quality water and food have received widespread discussion in both the scientific and popular arenas, and are starting to develop political interest at the policy level.

On the other hand, much less attention has been given to the burgeoning global need for synthetic and natural medicinal agents; as a topic of concern it barely makes it to the level of discussion of scientific societies.

A Sustainable Civilization is one where the needs of the present can be met without compromising the ability of future generations to meet their own needs.

"Human beings are at the center of concerns for sustainable development. They are entitled to a healthy and productive life in harmony with nature"

Ultimate Inequality

Access to quality health care is an enormous public health global issue at the scientific, clinical, economic, political and policy levels. It is one aspect of the "ultimate inequality" that exists between and within every country in the world; it is the difference in access to health care between the rich and the poor. There is a very different mix of health care practices and a corresponding diversity of natural and synthetic drugs being used in different countries by various populations. Populations in the urban areas are more likely to follow allopathic medical practices, while those in rural areas, who have no access to allopathic medicine, rely on local healers and local medicinal plants.

"The essence of global health equity is the idea that something so precious as health might be viewed as a right."

-Paul Farmer

The quality of the health care that is associated with the different origins of medicinal agents is likewise extremely different, and is reflected in the regulations applied to traditional medicines and synthetic drugs.

Herbal Medicines

"Nature itself is the best physician"

- Hipocrates

There is increasing use of herbal medicines, extracts, oils or dietary supplements in today's world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings. These herbal remedies being available not only in drug stores, but now also in food stores and supermarkets. The herbal plants with several medicinal properties are used to treat a variety of disease conditions.

According to the World Health Organization (WHO) Guidelines, herbal medicines are considered to be: Plant-derived materials or products with therapeutic or other human health benefits which contain either raw or processed ingredients from one or more plants. In some traditions materials of inorganic or animal origin may also be present.

Herbal remedies considered as "dietary supplements" by the U.S. FDA. Traditionally, dietary supplements referred to vitamins, minerals, other essential nutrients. Dietary Supplement Health Education Act of 1994 expanded the category to include other products such as herbs, other botanicals, amino acids, and metabolites. Dietary supplements are not required to undergo the same type of testing or approval that are

required for prescription drugs or over-the-counter drugs. FDA requires extensive testing and clinical studies of drugs to determine their safety, proper dosages, effectiveness, possible side effects and interactions with other substances. Dietary supplements are not subject to these categories.

Herbal drugs are plants or plant parts that have been converted into phyto pharmaceuticals by simple processes involving harvesting, drying, and storage. Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting. Standardization of herbal medicines is the set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon local standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control processes.

Several problems not applicable to synthetic drugs often influence the quality of herbal drugs. For instance:

- Herbal drugs are usually mixtures of many constituents.
- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- Chemo-varieties and chemo cultivars exist.
- The source and quality of the raw material are variable

Herbal remedies and medicines are widely available in our country without a prescription. While the general population may believe that anything “natural” is safe, the fact is that herbal remedies, like manufactured pharmaceuticals, can be toxic and have significant side effects.

To focus on Herbal materia medica we have to note that, a large and increasing number of patients in all over the world use medicinal herbs or seek the advice of their physician or Traditional Healers and Hakim’s yet patients and physicians often lack accurate information about the safety and efficacy of herbal remedies.

Herbal medicinal products have become popular because of perceived safety and economy and inability of allopathy to cure everything.

But in reality, there may be fewer differences between natural medicines and pharmaceuticals. While the use of herbal medicines continues to grow, recent reports of adverse therapeutic drug interactions, toxic side-effects and altered laboratory tests underscore the need for users to inform their healthcare providers of such use to ensure

their safety. The adverse reactions have tempered the enthusiasm of consumers for these natural cures resulting in decline of sales of herbal medicines. The actual limitation is that less number of national level institutes providing knowledge of herbal therapeutics due to lack of relevant research evidences is expected to hinder the market growth.

Sustainable drugs and Global health care

By definition, sustainability aims to promote healthy, viable, and equitable communities. In the same way the healthcare industry promotes healthy behaviors such as eating right and exercising; it should encourage environmental stewardship since the health of our environment affects public health.

Sustainable Medicine is based on the premise that twenty-first century Western medicine-driven by vested interests-is failing to address the root causes of disease.

Medicinal plants, both endemic and widespread, their resources and knowledge about their usage must be preserved since these plants could be renewable source for new drugs.

"Plants love us. They help us reclaim our health and our whole selves. Plants are healers"

-Robin Rose Bennette

On a daily basis, we often forget to acknowledge with gratefulness the vast contributions that plants, their extracts, and the various products derived from them, make to human health and well-being: i) as food stuffs, ii) as flavoring agents and spices, iii) as perfumes and cosmetics, and iv) as pharmaceutical and biological agents. These categories are not mutually exclusive, for it is apparent that there is significant overlap between foods, spices, cosmetics, and biological (medicinal) agents.

Actual scenario

Natural products have good values in treating many diseases including infectious diseases, hypertension, etc. That they can save lives of many, particularly in the developing countries, is undisputable.

"Nature does not hurry, yet everything accomplished"

- Lao Tzo

The major challenges of any pharmaceutical scientist are serious problems with the overall quality, safety and efficacy of natural products. Preservation and dosage measurement are serious problems in developing countries. The label claim and other information provided for the use of a herbal preparation may be far from what is in the `bottle'. Therefore Standardization of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, standardization, and *In-vitro, In-vivo* parameters.

"When we have to do with an art whose end is saving of human life, any neglect to make ourselves through masters of it becomes a crime."

– Samuel Hahnemann

In India, indigenous herbal remedies such as Ayurveda, Siddha and Unani and other Indian traditional system of medicines have since ancient times used medicinal plants in treatment of various diseases.

"Ayurveda is the science of life"

Indian traditional medicines or medicinal plants are also considered as a vital source of new drugs. Mainstreaming of such medicine is important for the people. Several steps have been taken in India to promote such medicine and to integrate them into clinical practice. Evidence based incorporation of Indian traditional medicine in clinical practice will help to provide quality health care to all.

"From Yoga to Ayurveda, Indians are proud of our heritage, we must never forget the innovative spirit that our ancestors were blessed with"

- Narendra Modi

India is already a major exporter of medicinal plants. It is estimated that annually Rs, 86 crores worth of raw materials and drugs from medicinal plants are exported from India.

"*To improve global health, it's not enough just to have a really good new product and to obtain marketing approval. You still need to market the product and bring it to patients, follow up, create the infrastructure, and so on - the whole pipeline, the network. That's something that companies are extremely good at: organizing a whole pipeline in a cost-effective way."*

- Thomas Pogge

Another important issue is we often treat diseases with preparations originating from very distant countries. Even nowadays, we are facing a paradox with the same problem present for centuries: Outside parties frequently manipulate and interfere with local policy makers in order to gain access to local communities' environmental resources. In addition, mainstream science and more developed society exploit environmental knowledge for locating and extracting natural resources, and making use of medicinal plants for commercial purposes. Developing communities or countries rarely benefit economically.

"*Lack of accountability weakens the environmental and health rights of citizens; it damages peace- building and reconciliation initiatives; impedes the implementation of global health policies; leads to the loss of ecosystems and biodiversity; and weakens democracy, justice, human rights, and international security."*

-Widad Akrawi

At a time when we are facing global economic crisis, which most severely affects developing countries, assistance in raising their own capacities, including development

of renewable natural products, would strengthen the economy of these countries, and economically unburden the rest of the world.

"We are more capable of turning around our global health crisis than we think."

-Kris Carr

The Great Need

"Humankind is not sufficiently aware that natural products drug discovery is important for new generations as a tool for their health care".

-Cordell & Colvard 2012

For approximately 85% of the world's population, plant materials are a primary source of health care (Fabricant & Farnsworth, 2001). This fact is not sufficiently accepted by pharmaceutical companies that are producing synthetic drugs for decades as solutions for incurable diseases. Knowledge of plants and their medicinal properties that were transmitted from generation to generation is in danger of disappearing. Developed countries in alliance with their large pharmaceutical companies, constantly in the struggle for new markets, do not permit the development of local pharmaceutical companies in developing countries.

"The Indians' botanical knowledge is disappearing even faster than the plants themselves."

- Richard Evans Schultes

Medicinal plants, both endemic and widespread, their resources and knowledge about their usage must be preserved since these plants could be renewable source for new drugs.It is known that chemicals and chemical reagents are typically non-renewable, and their use depletes our future resources.

"Consequently, all drug discovery programs, synthetic or natural, must be the concept of sustainability."

-Cordell, 2011.

The Fundamental significance of sustainable development of natural products

" The economic imperative at the heart of innovation is fundamental to the process of societal transformation that the sustainable development goals aim to achieve."

- Francies Gurrey (WIPO Director General)

We know that for the major lethal diseases, there are no truly effective drug treatments. In addition, drug resistance to existing chemotherapeutic regimens for fungal and bacterial infections, AIDS, cancer, and malaria is increasing. Because of the challenges for health care in the future, this is the call for decision-makers, governments, international agencies, and pharmaceutical companies to commit to the sustainable development of natural products as medicinal agents, particularly in developing countries.

Biotechnology companies may play crucial role in the sustainable development of natural product drugs. Trans location of the genes from slow-growing to fast-growing, large biomass plants or other organisms will enable the large-scale production of medicinal agents. Genetic engineering will play an important role in saving medicinal *plants, which are rare or endangered.*

Key Prospects

"Look deep into nature, and then you will understand everything better"

- Albert Einstein

Plant extracts and products contribute in four major areas to human health and well-being: (Cordell , 2004).

I. Foodstuffs,
ii. Flavoring agents and spices,
iii. Perfumes and cosmetics
iv. Pharmaceutical and biological agents

Their exploitation in sustainable manner would provide significant commercial perspective to:

- Global pharmaceutical companies
- Small to medium size biotechnology companies,
- Botanical supplement companies,
- Food industry

"If we surrendered to earth's intelligence, we could rise up rooted like trees."

-Rainer Maria Rilke

Development of natural products in a sustainable manner may interface different scientific fields.

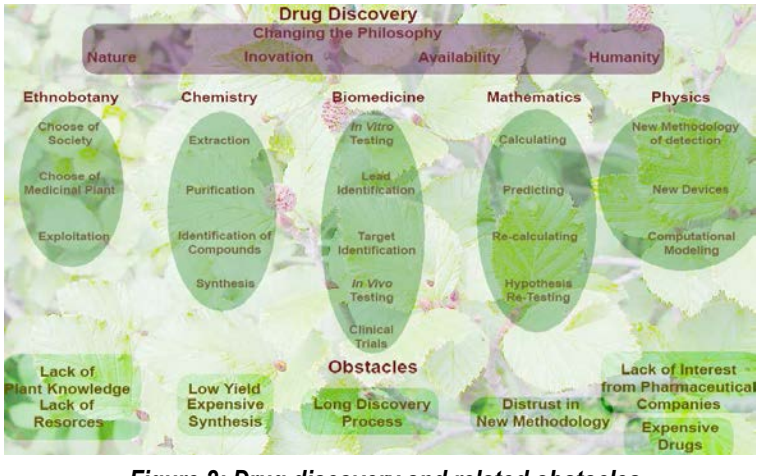


Figure 2: Drug discovery and related obstacles

To achieve sustainable development goals, there is a strong need for changing the philosophy and establishing new connections among nature, invention, availability and humanity .

"Adopt the pace of nature: her secret is patience."

- Ralph Waldo Emerson

Natural products and drug discovery

Plants have been utilized as medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poulitices, powders and other herbal formulations. The specific plants to be used and the method of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals . In more recent history,the use of plants as medicines has evolved the isolation of active compounds,beginning with the isolation of morphine from opium in the early 19thcentury.

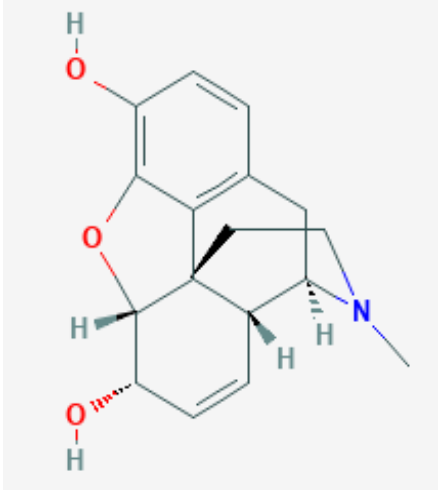
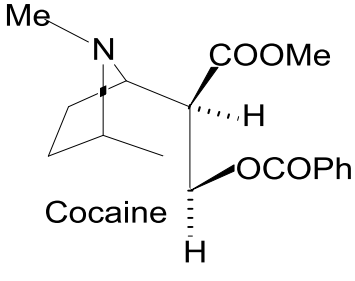
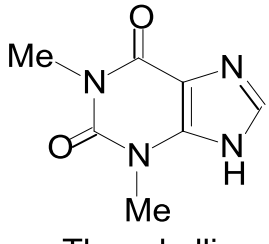
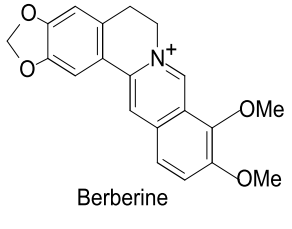


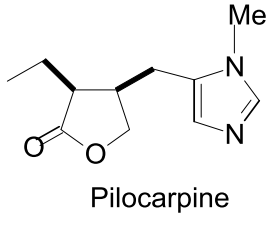
Figure 3 : Morphine

Some Other Early Developed Active Molecules From Plant Resources









Drug discovery from natural sources is a chance for the reunion of natural and human sciences to improve the health care systems all over the world.

"I believe in GOD, Only I spell it NATURE"

- Frank Lloyd Wright

Changes in the perception of natural treasures and necessity to help developing countries to build their own facilities and engage own scientific potential will ensure sustainable development of natural product drugs.

"Let your food be your Medicines"

- Hippocrates

Interdisciplinary research will enable the overcoming of obstacles that are present for many decades in the process of natural products drug discovery.

" The doctor of future will no longer treat the human frame with drugs but rather will cure and prevent disease with nutrition"

- Thomas Edison

Threats to biodiversity

Biodiversity is under serious threat as a result of human activities. The main dangers worldwide are population growth and resource consumption, climate change and global warming, habitat conversion and urbanisation, invasive alien species, over-exploitation of natural resources and environmental degradation

The imbalance between humans and other species on our planet is a threat to the survival of the humankind.

" We should preserve every scrap of biodiversity as priceless while we learn to use it and come to understand what it means to humanity."

- E. O. Wilson

Therefore, plantecology should be considered in the sustainable development of natural products.

Accordion to the World Wildlife Fund (data from 2004), due to the human consumption, 20% of medicinal plants in the world are in threat of disappearing.

"If the bee disappeared off the face of the earth, man would only have four years left to live."

- Albert Einstein

The ethical rules should be considered during the exploitation of medicinal plants.Humankind should not satisfy its own needs at the expanse of other Earth species. In less developed countries, plants are the primary source of health care. When large pharmaceutical companies confiscate medicinal plants in order to make

new drugs, these drugs will not be available to the local people because of the costs. This is also an ethical issue.

"The greatest threat to our planet is the belief that some one else will save it"

- Robert Swan

"Traditionally used plants are far less expensive than the new drugs isolated from them and eventually synthesized."

Pan *et al*., 2013

Difficulties and Potential Resolution in the path of natural product research

" It is important to get results from experiment but the most important is the process in getting that result"

- Dr. Nick Ahmad Nizam

Over the past decades, pharmaceutical companies have shown an increased interest in exploring new compounds from plants. However, the lack of a rational approach in many aspects has limited their success.

Pharmaceutical companies often give up from capital investment in the development of natural products because collection of plants in some countries may be a time-consuming process and require extensive (re) negotiations related to access. Their approach includes bringing as much chemical diversity as possible to the biological screening interface but with no consideration given to the origin of the plant derived materials. They do not care about chemo-diversity, functional diversity of the constituents, or ethno-medical association of the plant.

"Another reason for quitting is concern about plant extracts, which frequently yield a known, rather than a novel, active constituent."

-Cordell, 2004

Certainly, safety issues must be considered before accessing the plants:

- Plant authentication,
- Cytotoxic, mutagenic and therapeutic perspective,
- Free of potentially toxic insecticides,
- pesticides and heavy metals,
- Free of fungal and insect infestation, and
- Free of radiation contamination

Developed countries are not willing to invest, in the people or the places in developing countries because of bureaucracy, cost, and time. Consequently, biorich developing countries will not be able to access their biome and enhance their scientific (taxonomic, chemical, and biological) capacity. Local pharmaceutical development will be inhibited.

Therefore, developing countries will continue to relay on externally acquired (imported) pharmaceutical and medicinal agents.

Ethno-medical information, biological evaluation of plant extracts and their constituents, the chemistry of natural sources, and the clinical evaluation of plant extracts are still not accessible globally.

"A failure is not a loss.It' s a gain. You learn. You change . You grow".

- Michael Barata

The useful databases that are available like Indian plant anticancer compounds database (InPACdb), The herb information Knowledge base (THINKherb), Traditional Chinese medicines integrated database (TCMID) and Traditional Chinese medicine information database (TCHM-ID)etc. (Pan *et al.*, 2013).

There is a strong need for indexing eco and ethno-information of plants, chemistry and biology of their products in such way that information can be analyzed and accessed globally in real time

There is another problem regarding access of active principles. They are often extracted and analyzed only at a single moment in time, ignoring daily metabolic flux, seasonal variation in enzyme activities, and the biosynthetic genes which are present, but not fully functional. Development of methodology able to characterize the majority of constituents in an extract without isolation may help in the process of extracts validation and standardization (Cordell, 2004).

While there are important ecological concerns regarding genetically modified crops, they present economically effective way to bring preventative health care to humankind (Cordell, 2011).

"Natural species are the library from which genetic engineers can work. Genetic engineers don't make new genes, they rearrange existing ones."

- Thomas E. Lovejoy

There are numerous fungi, bacteria, and, in some cases, algae, symbiotically associated with the plant, which are capable of independent biosynthetic production. Investigation of this potential will enhance the production of active agents in a sustainable manner (Cordell, 2004).

Conclusion

"We need to rethink our health care systems to offer every body equitable access to quality care"

- HRH Princess Diana Mired

We require programs to assist developing countries to potentate their facilities, and scientists in order to evaluate natural product-based medicinal agents in a sustainable manner. This would significantly improve global health care systems.

"What is growth for if not to help ordinary people thrive?"

- Winnie Byanyima

International efforts and investment in new medicinal agents will require the creation of numerous new alliances. These alliances must work on both local and global level and involve individuals who can set aside their ego for the greater good. Therefore, the alliances should be composed of international agencies, government agencies, pharmaceutical companies, academic institutions, non-government organizations, scientific societies, and private foundations (Cordell, 2004).

"Unity is strength ……...When there is team work and collaboration, wonderful things can be achieved."

- Mattie J.T. Stepnek

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