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1. Introduction

Science may be defined as ordered knowledge of natural phenomena, and the rational study of the relations between the concepts in which these natural phenomena are expressed. Science, therefore, includes the following:\:

- Raising questions about nature and natural phenomena, rationally thinking about these questions for logical answers, framing hypotheses (which may be falsifiable) relevant to the possible answers, making observations, tests and experiments (often several times, at several different places and confirmed by several other people), making measurements, recording the data, presenting the results cogently and logically interpreting the results without any bias. The whole set of procedures mentioned above is often referred to as the scientific method, that has a very gradual conceptual development in history through the efforts of such great people as Aristotle, Galileo, Francis Bacon, Rene Descartes, Isaac Newton and others.

- Measurements, accurately made and very carefully documented, form the core of scientific method and these, along with the gathering and organizing of data, form the prelude to interpretation of scientific observations. These activities are routine in the everyday life of a scientist. But, in a rapid development that has suddenly engulfed science, scientists and the science that they generate, themselves have become objects of measurement by the emerging field of Scientometrics, fuelled by the rapid developments in the field of information science and technology. Therefore, it has now become mandatory for every scientist to publish his results based on which not only he but also his research findings are subjected to serious scrutiny and assessment. It is necessary for every scientist to know the methodologies in such assessments.

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2. Classical criteria for measurement of scientific achievements of a scientist

A number of criteria were employed in the past (and continued to be applied even now in many places) to measure the achievements of a scientist. The most important among these are:

   a) The number of publications that a scientist has published. The publications include the original research papers, short communications, review articles, popular science articles, books, research reports, etc.

   b) The number of patents and copy rights that a scientist holds.

   c) The quality of publications. Such publications are mandatorily to be the ones that have been assessed and peer-reviewed by experts in the relevant area of research.

   d) Peer reviewers are required to have patience, commitment, scholarship, genuine interest in the subject, unbiased attitude, etc.

   e) The recognition of a person’s scientific work by peers in his/her field and higher-ups in his/her institution/organization.

   f) The person should have won prestigious awards/prizes/honours.

   g) The person should have been elected as Fellow/Members of prestigious scientific societies at the national and international levels.

   h) The person should have been appointed to panels/special committees.

   i) The person should have been sanctioned major research projects.

   j) The person should be occupying prestigious academic/administrative positions and should have chaired/organized major seminars/conferences/symposia/workshops. He/she should also have been invited to deliver lectures and keynote addresses.

The undue dependence of making judgments of research potential and expertise of a scientist on the number of his publications has resulted in a number of undesirable side-effects. The most important among these are follows:

   a. It has encouraged scientists to publish more and has thus resulted in a ‘publish or perish’ syndrome.
b. This in turn has resulted in substandard publications.

c. The number of science journals has increased substantially within a short span of four to five decades.

d. Scientific publishing has increasingly become a profitable business enterprise. More and more corporate organizations have entered into the journal publishing business.

e. There is a growing exploitation of researchers with journals insisting on costly memberships for publication, collecting exorbitant publication charges (page charges) or compulsory paid reprint charges. These have resulted in what are called ‘reader pay models’ and author pay models’ for acquiring access to published scientific papers.

f. This has promoted plagiarism of various categories.

3. Evolution of the Discipline of Scientometrics

All the above have naturally led to the evolution of new ideas in assessing scientists based on their science publications. Thus emerged the discipline of Scientometrics, where science output is measured using tools of information science and technology which themselves are emerging at a very rapid rate. According to Weinberg, Scientometrics, with particular emphasis on citation index, dates back to 12th century CE in Hebrew literature. de Candolle made attempts to scientometrically analyze the research articles published by the prestigious Science Academies of the World.

Then came the publication of Bush entitled, “As we may think”, published in Atlantic Monthly. Often considered as the ‘internet pioneer’, Bush has contemplated an information workstation called Memex. He inspired the development of the World Wide Web (WWW) and had used phrases like “web of trials” and “association of thoughts”. These ideas and phrases greatly influenced Eugene Garfield, who is now universally acclaimed as the “Father of Scientometrics”. The epoch-making paper of Garfield (now about 83 years old)
was published in 1955 in the journal, Science: “Citation Indexes for Science: A new dimension in documentation through association of ideas”. Science 123: 108-111”. This paper sketched the conceptual foundations of Scientometrics, and the discipline was subsequently enriched by Garfield himself, Derek of Solla Price, Joshua Lederberg, Robert Merton, Belva Griffith, Blaise Cronin and others. Garfield’s ideas of citation-based searches, resource recovery and quantitative evaluation of publications revolutionized library documentation, especially subject indexing. Garfield had changed the “scholarly publication ecosystems” and he “turned lead (i.e. references, foot notes, etc.) into gold (i.e. citations)”. Unlike his predecessor Bush, he emphasized on “collective associative thinking” of many researchers and not one researcher. It must be emphasized that before Garfield, most indexing/abstracting databases were started as (and even now many remain as) discipline-oriented reference resources. As examples, we may cite Biological Abstracts, Chemical Abstracts, Psychological Abstracts, etc. Thanks to Garfield, the field of scientometrics was strongly established and there is now even a separate journal on scientometrics.

4. Citation Index

It was through the efforts of Garfield7 that the concepts of Citation Index and Journal Impact Factor (JIF) (or simply Impact Factor, IF) become very popular. Citation Index (CI) counts the number of citations of an author’s paper by other authors in their papers. The more an article is cited by others, the greater is its value. In fact, a paper is given the title “Citation Classic” if it is cited within a span of two years by more than 200 other authors. Thus Citation Index will indicate directly the merit of the paper of an author, and indirectly of the author’s scientific achievements. The major problem with Citation Index is that it is essentially a quantitative measure and will not indicate whether a paper is cited in a positive way or in a negative way. I have found that some heavily cited papers have been cited and criticized rather than appreciated.
5. Journal Impact Factor (JIF or IF)

a) Concept, definition and calculation of IF

Impact factor concept was first introduced by Garfield in 1955, although it was first used in the early 1960s to help select journals for what would evolve to become the Science Citation Index (SCI). Thomson Scientific’s Institute for Scientific Information (ISI) publishes the Journal Citation Reports (JCR) and Science Citation Index, Expanded (SCI-E) and calculates the ISI Web of Science (WoS) impact factor.

Impact factor is an indication of the standard of a scientific journal/periodical. It is calculated as the number of citations in the current year to any items (source and non-source items) published in a journal in the preceding two-year period (the numerator element in the equation to calculate IF) divided by the number of substantive articles (source items) published in the same two-year period (the denominator element in the equation to calculate IF). Items in the above statement refer to the research and review articles. For example, journal X’s year 2005 IF=citations in 2005 (in journals indexed by Thomson Scientific) to all articles published by journal X in the years 2003 and 2004, divided by the number of articles deemed to be ‘citable’ by Thomson Scientific that were published in journal X in years 2003 and 2004. Obviously, IF depends crucially on which article types Thomson deems as ‘citable’.

b) Importance and uses of IF

IF is very helpful in the evaluation of the quality of science journals. It is useful in the assessment of competitive journal’s performance. IF measures the productivity of individual researchers as well as their research departments/institutions. It also helps in estimating the efficiency of investments in scientific research. The performance of different fields of research can easily
be compared using JIF. It also helps to assess the performance of different countries of the world in scientific productivity.

c) Criticisms on IF and citation index

Several criticisms have been levelled so far on the IF and citation index. The following are the most important.\(^9,10\)

i. Since a journal’s IF is derived from citations to all the articles published in it, this number does not tell anything about the quality of work of any specific author who had published his paper in it (PLos-Public Library of Science Medicine 2006).\(^11\) In other words, IF is not a statistical representative of individual journal articles and poorly correlates with actual citations of individual articles. Campbell\(^12\), the Editor of Nature, has stated that an analyze of citations received in 2004 to papers published in it in 2002 and 2003 revealed that 89% of the journal’s IF was generated by just 25% of its papers.

ii. A journal’s IF can be substantially affected by the publication of only review articles that get more citations than original research articles/short communications. Journals that exclusively publish review articles generally have a higher IF value than journals that exclusively publish research articles only or in addition short publications.

iii. Research fields with literature that rapidly becomes obsolete are favoured by many new researchers in order to get more IF.

iv. Editors of many scientific journals are encouraged/forced to implement scientifically unethical strategies like: encouraging and forcing authors to cite only those articles published in their journals, or to substantially cut down the references relating to articles published in other journals and by decreasing the number of research articles published. In support of this allegation, The Wall Street Journal’s (5\(^{th}\) June 2006) ‘expose’ revealed how unscrupulous editors of scientific journals try to boost IF following the above-stated strategies. Nearly 80% of the journals in the Thomson Scientific database have a self-citation of nearly 20% compared to nearly 6% of the journals not in it. Also, the coverage of the Thomson Scientific journals database is incomplete (only 250 scientific disciplines and only 6,822 journals, out of 1,26,000 as of 29\(^{th}\)
January 2008 are listed; 52 of these 6,822 journals alone are from India) and dominated by American publications. Only 4% (5,900 out of 1,26,000 journals) has IF and remaining 96% are often referred to as having no IF or never formally cited. In other words, the journal inclusion criteria for Citation Index purposes in Thomson Scientific are not clear.

v. Another criticism that is related to Thomson Scientific is that it is the sole arbiter of the IF and it is a part of Thomson Corporation, a for-profit organization, responsible naturally and primarily to its shareholders and not to its stakeholders, who most care about IF.

vi. IF depends on expansion or contraction of a research field.

vii. Small research fields lack journals with high IF; this is especially true to classical fields like taxonomy, anatomy or embryology.

viii. Relation between research fields strongly determines IF.

ix. Citation rate of articles determines IF of journals, but not vice versa.

x. IF varies every year for the same journal and, therefore, every journal frantically strives to enhance its IF every year, at any cost.

xi. The cost of publishing in high IF journals has become prohibitively increased and many researchers, especially from developing countries, are unable to publish in such journals even the papers are of a very high standard or such journals start publishing substandard papers when once the prohibitive cost of publication has been met by the author.

xii. Scientists may soon argue that a cost to IF ratio is a variable parameter. As is stated by Shakespeare in Julius Caesar. “Are all they conquests, glories, triumphs, spoils shrunk to this little measure”. The usage “little measure” here implies IF.

xiii. Scientists will be tempted/forced to adapt and shift to research on popular subjects to elicit greater number of citations, rather than take the paths less trodden where important scientific discoveries/inventions may be waiting.
xiv. The joy of science seems to have disappeared for many individuals, who are now consumed by IF and citation indices. This has lead to a more calculated and purposeful (?) approach to science publication today. **Emphasis in science has now been shifted from what to publish to where to publish.**

xv. Authors’ citation behaviour is now prone to systematic bias instead of a quotidian citation behaviour. IF forces an under- or over-citation, a strategic coat-tailing, i.e. citing friends, immediate colleagues, celebrity authors, an erroneous citation/irrelevant citation, and/or authors might not have read the papers that they have cited. In other words, in a number of cases the citation is about whom one knows and not about what one knows.

**d) Suggestions for improving the Impact Factor methodology**

The above criticisms, one should understand, do not deter the importance of IF, but should be taken as ideas to improve the IF methodology. A number of suggestions, based on the above criticisms, have been made to improve the IF procedure. These include the following (see for example⁹).

i. Individual research articles should be assessed for their IF rather than a journal as a whole being assessed.

ii. IF assessing organizations like Thomson Scientific should take more responsibilities and increase transparency.

iii. User rating of scientific papers should be encouraged.

iv. IF is now calculated taking into consideration only citations of articles of the previous two years. Instead, a long-term base should be used. For instance, the importance of some scientific publications may not be immediately realised but may become prominent after a lapse of a few years. As for example, Barbara McClintock’s Noble winning paper on transposons did not have significant immediate citations, but was cited repeatedly after a few years.

v. For calculating IF, review papers and publications other than original research articles should not be taken into consideration.
vi. Some people have suggested averaging the IF of all papers of an author gained over a period of time to assess his scientific potential. This is especially followed in India by some institutions when they recruit scientists. The primary motivation for this attempt is to develop a ‘single number criterion’ to permit rapid comparison – a simple parameter to judge the productivity and impact of scientists\textsuperscript{14}. But, its significance is obscure to even its most ardent proponents.

vii. Alternate measures to IF should be used.

viii. Competition to Web of Science should be encouraged so as to make IF prepared on better bases.

6. Alternative to Journal Impact Factor and Citation Index

A number of alternative measures are being evolved so as to make assessment of scientific contribution more credible. The most important among them are the ‘h index’ and the ‘downloading index’.

(i) ‘h index’:

The ‘h index’ was introduced by Hirch in 2005\textsuperscript{15,16}. It is a single number that assesses an individual’s consistency of scientific performance (see: http://www.arxiv.org/abs/physics/0508025) and is claimed to avoid the shortcomings/pitfalls of JIF. The Hirch definition for ‘h index’ is as follows\textsuperscript{17}: “A scientist has index ‘h’ if h of his or her Np papers have at least h citations each and the others (Np-h) have < h citations each”. Here, h is the highest number of papers a scientist has that have each received at least that number of citations. For example, if a scientist has published 50 papers, 30 of which have achieved 30 or more citations, his or her h-index is 30.

This index can be applied to any aggregate of publications, permitting classification of individuals, institutions and journals. This index has been calculated by Hirch for a large number of researchers, both celebrated and less well-known scientists. On this basis Hirch recommends that an h-index of 20 after 20 years of scientific activity ‘characterizes a successful scientist’. Since
Longevity must hopefully improve a scientist’s h index, Hirch adds that “a value of 40 after 20 years…… characterizes outstanding scientists, while an h-index of 60 after 20 years or 90 after 30 years characterizes truly unique individuals”. He further adds that an h-index of ~ 12 should qualify for grant of academic tenure, h of ~ 18 is the suggested bar for a professorship (in a US institution) and h of ~ 45 appears to be a norm for election to US National Academy. It was found that the Nobel Prize winners had a median value of h=35.

The major problem with h index is posed by a paper with more than one author; whether each of the authors should be given the same whole h value of 1 or whether each author should be assigned a fractional ‘h’ credit. In case of the latter, how to decide the actual fraction to be assigned to each author, because it will be very difficult to estimate the exact relative contribution of each of the involved authors.

Braun et al proposed a h-type index for journals. This index is equal to h if the journal has published h papers each of which has at least h citations. According to these authors, this is an interesting supplement to the controversial use of JIF to rank journals because of the following two reasons: (i) It is insensitive to an accidental excess of un-cited papers and also to one or several outstandingly highly cited papers. (ii) It combines the effects of ‘quantity’ (i.e. the number of publications) and ‘quality’ (i.e. the citation rate) in a rather specific balanced way that should reduce the apparent ‘overlapping’ of some of the review journals.

Gangan Prathap has extended a Hirch-type index for institutions. He recognises two levels of h indices, h1 and h2, where h1=h, if the institution has published h papers, each of which has at least h citations, and h2=h, if the institution has h individuals, each having a h index which is at least h.
(ii) Downloading Index:

Downloading Index or Downloading Counts is an early indicator for monitoring progress of science. Here, the number of downloads of a research paper is normally taken as an early indicator of its impact, especially in digital/on-line publications. Downloading Index was introduced by Sharma\textsuperscript{21}. Download Counts refer to the number of research papers and book chapters downloaded per unit time from an institution. This is especially meant to measure the efficiency of Science and Technology Libraries\textsuperscript{22}. Downloading Index is followed by Inflibnet to rank Universities in India in the utilization of scholarly publications. Reliable data for this are available from Elsevier Publications. It will not indicate, however, whether the downloaded papers were really read and used by the person who downloaded them.

(iii) Competitions to the Science Citation Index (SCI) of Web of Science (WoS)

It was earlier indicated that for a better efficient and more transparent assessment of scientific productivity of an individual, Institution or Journal, competition of organizations involved in such an effort is a must and competition is better than monopoly. For a very long time, there were no competitors to Thomson Scientific and its Web of Science\textsuperscript{23}. Now Scopus and Google Scholar are emerging as good competitors for WoS. Scopus (http://www.scopus.com/scopus/home.url) was started in 2004 and promoted by Elsevier Company (Holland). It is a commercial database. 98% of its database are entries from science and only 2% are related to social sciences. The data available in Scopus are from 1996. Google Scholar (http://scholar.google.com) was started in November 2004, a little after Scopus was initiated. It is an open access database\textsuperscript{24}.

Mention must also be made about SCImago. The SCImago Journal and Country Rank (SJR) is operating from Spain (http://www.scimagojr.com). It is a
portal that includes journal and country scientific indicators developed from the information contained in the Scopus® database. SJR is developed by SCImago from the algorithm Google Page Rank. Its initiatives include 13,209 journals of which 157 are from India.

7. Future of Journal Impact Factor and Citation Index

It is heartening to know that we now have autonomous initiation indexes and citation parsing tools. Journal Impact Factor Concept and Citation Index Concept are going to stay as they form an inescapable activity but with greater efficiency, transparency and devoid of most, if not all, defects and discrepancies that they have now. We will soon have access to a critical mass of web-based digital objects and usage statistics with which to develop multidimensional models of scholars’ communication behaviour-publishing, posting, blogging, scanning, reading, downloading, glossing, linking, citing, recommending, acknowledging, etc. Hence, there is now a need to insist on a more scholarly approach to Scientometrics which will enable new entrants to the area to understand that rigour is an integral part of the discipline.

References:


Deuxième édition considérablement augmenté. H. Georg, Lyon meme Maison, Gené ve-Bale.


***************
Introduction:

Bioinformatics is an interdisciplinary area which may be defined as the interface between biological and computational sciences. According to Arthur Lesk, author of an introductory book on Bioinformatics, “the marriage of biology and computer science has created a new field called bioinformatics”. It is the discipline of using computers to address information problems in the life sciences and it involves the creation of electronic databases on genomes, protein sequences, protein structures etc.

In the living organisms, nucleic acids and proteins are the important informational macromolecules. Proteins carry out a variety of cellular functions such as enzymatic catalysis, transport, gene regulation, etc. These are linear chain molecules which fold into compact, three-dimensional structures in the cell to perform their specific biological functions. Proteins are made up of 20 different kinds of amino acids. The information to specify the order in which the amino acids are arranged in a given protein is specified by its corresponding gene sequence in the DNA.

Experimental Advances in Molecular Biology and Biochemistry:

Since the identification of the double helical structure for DNA, great advances have taken place in the field of Molecular Biology which has enabled us to understand how the information stored in the DNA is transcribed to Messenger RNA which, in turn, is translated into proteins with the help of the genetic code. Techniques have been developed to purify individual proteins from the cell and to elucidate their amino acid sequence. Also a number of biochemical and biophysical studies could be carried out to understand the...
structure and function of proteins. Of great importance is the technique of x-ray Crystallography which helps to elucidate the three-dimensional structures of proteins by crystallizing them. Another important technique for structure determination is the Nuclear Magnetic Resonance Spectroscopy.

With the advent of Recombinant DNA Technology in the 70s, it has become possible to clone any gene of interest and determine its nucleotide sequence. From the nucleotide sequence, one can deduce the corresponding amino acid sequence using the universal genetic code. Recombinant DNA Technology has emerged into a powerful technology not only to clone and sequence individual genes from organisms but also to sequence entire genomes of organisms, the prominent of which is the Human Genome Project which was commenced in 1990 and the draft sequence of the human genome was available in 2001.

In the subsequent years, the DNA Microarray Technique has emerged which helps to study the expression of large number genes simultaneously. Parallel to this development, the technique of 2-D gel electrophoresis, coupled with mass spectrometric techniques, has enabled to characterize the proteome (the entire set of proteins encoded by an organism).

The ability to clone and express any gene of interest from an organism in another suitable host has resulted in the production of large quantities of proteins which are purified relatively easily. This has fueled, in a large way, the three-dimensional structure determination of a number of proteins. Also the ability to engineer mutations in the coding region of a cloned gene through the technique of site-directed mutagenesis has further enhanced our understanding of protein structure, function and stability.
Data Explosion and Database Development

All the above key techniques have resulted in the explosion of sequence, structure, expression and other related data. Because of the massive nature of data it has become very essential to handle these data through computational techniques. The data are stored and organized in databases. Further, computational techniques have been developed to retrieve relevant data from the databases and to analyze them.

Major Databases

The foregoing paragraphs show that different kinds of data have been generated in the past few decades. The key molecular biological data are nucleotide sequences of individual genes and whole genome sequences, protein sequence data, protein structure data, gene expression data. The major nucleotide databases are the GenBank, EMBL Data Library, and the DNA Data Bank of Japan. Swiss-prot, Protein Information Resource (PIR) and NCBI are important protein sequence databases. Protein Data Bank (PDB) is the database which has the data on three-dimensional structures of proteins, DNA, RNA, protein-DNA, protein-protein and protein-small molecular ligand complexes. There are several secondary databases such as PFAM, BLOCKS, etc which are derived from the primary databases.

Role of Internet and World Wide Web in the Growth of Bioinformatics

The availability of internet and world-wide web has fostered the development of Bioinformatics to a large extent. It has provided quick and easy access to publicly available data in any part of the Globe. Internet enables users to submit and retrieve data and search across many databases. It has also provided a means for database developers to make available the data for users. Table 1 provides some key databases that are useful to a large number of Life Scientists.
Database and Web-Servers Catalogue – The Major Role by the Nucleic Acids Research Journal:

All over the world, a number of databases on various aspects of Molecular Biology are developed and made available for the users. The Journal, Nucleic Acids Research, from Oxford Publishers devotes its first issue every year (January) to provide a brief overview of the different databases, data content, access details and so on (http://nar.oxfordjournals.org/content/39/suppl_1). Also it devotes its July issue for web-servers available all over the world that provide access to different software and computing facilities available for scientists (http://nar.oxfordjournals.org/content/38/suppl_2).

Use of Bioinformatics Data and Tools for research

The variety of Bioinformatics data can be used for fruitful biological research to generate hypothesis and testing. Also it can help in the design of suitable experiments to answer some key questions in the understanding of biological phenomena at the molecular level. For example, plant and Animal Scientists have used morphological and anatomical characteristics to classify living organisms and to characterize their lineage and evolution. With the availability molecular sequence data from a number of organisms, Phylogenetic Tree Construction is now done using these data. Genome level comparisons enable one to find out the similarities and differences among organisms and their ancestry. Also aligning a given sequence from many organisms with the multiple sequence alignment tools provides information on conserved and variable amino acids in protein which, in turn, help to dissect their role in the structure, folding and function of the protein. The large number of protein structures available in the PDB can be fruitfully exploited to understand the principles that govern the folding and stability of proteins. The knowledge thus
gained could be used in the development of structure prediction methods, prediction of protein stability upon mutations, understand protein-DNA recognition and so on.

The Interdisciplinary Nature of Bioinformatics – Role of many Disciplines

Because of the interdisciplinary nature of Bioinformatics, scientists from various Basic Disciplines such as mathematics, physics, chemistry, computer science, statistics and life sciences can play a role in the further advancement of this area. Indeed advances in Bioinformatics Research have been made by scientists from different backgrounds.

Areas of current research

Areas of current research in Bioinformatics include genomics, proteomics, glycomics, phramocogenomics, structural bioinformatics and so on. Genomics involve DNA sequence analysis, sequence comparison, gene finding, single nucleotide polymorphism, comparative genomics, gene expression analysis, correlation of gene activity to disease or drugs etc. Proteomics involves the analysis of protein sequences, three-dimensional structures of proteins and protein-protein interactions. In Chemo Informatics, generation of libraries of chemical compounds or drug-lie molecules and predicting biological activities using in silico approaches are being pursued.

Current and future needs

Bioinformatics needs better tools to understand and interpret data as well as subsequent analysis. Data need to be integrated and presented with a consistent user interface. There is also the need for development of better algorithms to predict protein structure and protein–protein interactions. It is also essential to develop collaboration between wet-lab scientist and in-silico scientists.
Conclusion:

As an emerging area, Bioinformatics has attracted considerable attention. Careful analysis of the large amount of data is expected to provide key insight into normal cellular functioning and the onset of disease. It is expected to play a greater role in Life Science Research.

Table 1: List of major molecular biological databases

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Database</th>
<th>Comments</th>
<th>Uniform resource locator</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>National Center for Biotechnology Information (NCBI)</td>
<td>Provides access to large number of databases such as nucleotide sequences, protein sequences, structures, literature, books etc. Also provides a number of tools such as the BLAST for sequence searching and analysis</td>
<td><a href="http://www.ncbi.nlm.nih.gov">http://www.ncbi.nlm.nih.gov</a></td>
</tr>
<tr>
<td>02</td>
<td>European Bioinformatics Institutes, European Molecular Biology Laboratory (EMBL-EBI)</td>
<td>The EBI is a centre for research and services in bioinformatics. The Institute manages databases of biological data including nucleic acid, protein sequences and macromolecular structures</td>
<td><a href="http://www.ebi.ac.uk/">http://www.ebi.ac.uk/</a></td>
</tr>
<tr>
<td>03</td>
<td>Protein Databank</td>
<td>The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies</td>
<td><a href="http://www.rcsb.org/pdb/home/home.do">http://www.rcsb.org/pdb/home/home.do</a></td>
</tr>
<tr>
<td>04</td>
<td>Stanford Microarray Database</td>
<td>The Stanford Microarray Database (SMD) stores raw and normalized data from microarray experiments, and provides web interfaces for researchers to retrieve, analyze and visualize their data.</td>
<td><a href="http://smd.stanford.edu/">http://smd.stanford.edu/</a></td>
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</tbody>
</table>
Methodology is a system of ways of doing, teaching or studying something while method is the particular way of doing something or an ordered way of doing something. In other words, methodology is the way of approach and method is a technique adopted to do something.

In general, Drug Research is a very sensitive area of research the outcome of which affects human health and life. Therefore, the methodology and methods adopted in drug research should be standard and precise. Hence the methodology and methods in drug research are not different from those adopted in scientific research.

In recent years herbal drug research is gaining momentum in recent years throughout the world owing to the conviction that herbal drugs or natural drugs are safer and more effective than allopathic drugs. It is true that drugs from natural origin are more adaptive, effective, cheaper, renewable and eco-friendly. Even about 25% of the prescribed allopathic medicine at present has components or derivatives sourced from medicinal plants. In China, 80% of population uses Traditional Medicine which consists of mostly herbs. In India, 70% of rural population still depends upon Indian Traditional Medicine like Siddha, Ayurveda or Unani.

Herbal Medicine, Phytomedicine or Natural Medicine originated from the knowledge of our forefathers and pertains to the use of plants for various ailments of humans and animals; the knowledge has been passed on to the next

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generation through oral methods. Knowledge embodied in Ethnobotany (documentation of herbals used by Ethnic Communities) or ancient medical literatures like Siddha, Ayurveda, Unani or Chinese Medicine have been put to scientific scrutiny, evaluated, authenticated and the evolved standards applied for the safer use for therapeutic purpose.

The general methodologies and methods or techniques followed by researchers in the field of Herbal Drug Research are outlined.

**METHODOLOGIES:** Herbal Drug Research encompasses identification, collection, processing and analysis of individual medicinal plant or part thereof or group of plants or parts which form a drug. Researchers in the field of herbal drug development have to ensure the Quality, Efficacy and Safety of the herbal drugs.

Herbal Drug Research could be broadly classified into the following depending upon:

**The stage or level or approach:**
- FIELD RESEARCH
- DESCRIPTIVE RESEARCH
- ANALYTICAL RESEARCH
- EXPERIMENTAL RESEARCH
- COMPARATIVE RESEARCH

**The subject area of Research:**
- ETHNOBOTANY
- PHARMACOGNOSY
- PHARMACOLOGY
- PHYTOCHEMISTRY
- PHARMACEUTICS

**FIELD RESEARCH:**
In field research one could embark upon
i) documentation of traditional knowledge of Tribe or Ethnic Community about the use of plants for curing diseases or for building, for food or rituals etc. (Ethnobotany).

ii) Survey on occurrence and distribution medicinal plants in a geographical area (Floristic Survey)

iii) Survey on disease prevalence in a particular area (Health Survey)

iv) Survey on Herbal Trade

v) Survey on supply and demand on Herbal Raw Drugs.

DESCRIPTIVE RESEARCH:

External Morphology:
Description of herb or part based on external morphology (Use Standard terminology to describe parts of Plants): Organoleptic Methods (Colour, Texture, Odour, Taste and Fracture)

Internal Morphology:
Sectioning methods (hand sectioning, microtoming, staining method, photomicrograph, measurement).

Analytical Research:

Physico-chemical parameters

Ash analysis:
- Total ash
- Acid soluble ash
- Acid insoluble ash
- Sulphate ash

Extractive Values: - Extractive values in different solvents like petether, benzene, or actone, chloroform, ethanol and water.

LOD (Loss On Drying)

Liquid: Colour, Odour, Refractive Index, Iodine Value, Specific Gravity, total Lipids etc.,
CHEMICAL ANALYSIS:

- **Qualitative**: Extraction by Soxhlet or by Cold Extraction
  Separation by column or separating funnel

- **Quantitative**:
  Quantitative estimation of biological active compounds like Glycosides, Alkaloids, Steroids, Saponin, Terpenoids, etc.,

- **Identification and Characterization**: Using TLC, HPTLC, GCMS, HPLC and UV, IR, FTIR, and NMR Spectra.

**Experimental Research:**

**Pharmacological activities:**

Screening of herbal drugs for various disease using standard animal models.
Anti-inflammation activity - Carageenan-induced and cotton pellet granuloma model in rats.

- Anti-diabetic activity - Alloxan or Streptorytocin induced rat model
- Anti-Ulcer activity - Alcohol or Formalin induced model or pyloric ligated model in rats.
- Antipyretic activity - Yeast induced pyrexia in rat.
- Analgesic activity - Acetic acid induced rat model.
- Toxic activity - Mice model.

**In-vitro biological activities:**

- In-vitro antioxidant activities
- In-vitro anti ulcer activity - helicobacter pylori model
- In-vitro cytotoxic activity
- In-vitro antibacterial and antifungal activity

Case studies on individual herbal drug and disease would be discussed in detail. How selection of research problem or selection of medical plant or disease would also be dealt with.

***************
RESEARCH GUIDANCE: TRAINING SCHOLARS FOR QUALITY RESEARCH, COMMUNICATION SKILLS AND PUBLICATIONS

Prof. N. Parthasarathy*

The objective of this presentation is to emphasize the need for guiding and moulding the research scholars towards achieving the goals in their chosen research work, informing them to have clarity in thinking, listing objectives/ framing hypotheses, employing standard & recent methods, presentation of results, interpretation and discussion and preparation of the research articles for publication in high-impact journals.

The guides inform scholars to be in touch with current to old literature which provide good experience and exposure to newer developments. They further provide clarity in thinking.

- **Research communication: Key points for submission to high-impact journals**
  - **Introduction** – Introducing only the aspects dealt within the article, referring pertinent and recent literature.
  - **Lead sentences** in research papers should particularly be most recent and relevant and subsequent ones should orient the reader to the aspects dealt in the paper.
  - **Objectives** – Should be clear, achievable/achieved within the time frame.
  - **Designing research work**, careful planning, keeping in mind basic/applied nature and advancement in the field.
  - **Methodology** (Up to date, standard and most appropriate, recent methods)
  - **Effective execution** - particularly field/lab work. Should avoid hesitation in approaching people and preparedness to visit well-equipped libraries.
  - **Data analysis** with appropriate state-of the-art tools.

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- Presentation of results - in succinct and straightforward manner.
- Interpretation - Good flow, vocabulary, Command over language.
- Over a period of time - Experimental learning - one would come out with their own refined methods and will have a “style” in writing as well as oral communication.
- Research papers
- Publication in peer-reviewed National and International Journals: (Particularly under the new point system, Publications are important)
  - Select journals with impact factors.
- Experience in article preparation
- Self reviewing twice – Read as author, read as a critical reviewer, possible addition, good edition for considerable improvement.
- Submission – conforming to journal style
- Attending to revision carefully reading & responding to Reviewers’ comments
- Proof reading

Promising research avenues and preparing the research articles for publication in high-impact journals will be discussed. As the primary independent factor of the environment, the climate constitutes the major ecosystem driver. Earth’s environment has been undergoing significant changes due to increasing human population, especially during the last century. Some of the important changes that are occurring in the global environment relate to increased resource consumption; depletion of fossil fuel reserves that were generated over several hundred millions of year in the geological past; and the large scale changes in land cover and land use. The most significant changes brought about by human activities are the increase in concentration of carbon dioxide and other greenhouse gases in the Troposphere, and depletion of Stratospheric Ozone.
Tropical Forests are treasure of Biodiversity and many of the elements of biodiversity are potential Bioresources utilized by wild life as well as humans. Forests are not mere stock of wood and their functions are multi-fold. The (geologically) past vegetation yields us now coal, oil and natural gas. The existing forests provide (i) subsistence needs such as fuel wood, charcoal, building material, fodder, fruits and nuts, honey, medicine, dyes etc., (ii) various industrial uses (timber & non-timber) (iii) wildlife resources, (iv) sites for cultivation of various plantation crops such as coffee, tea, cardamom, clove, pepper etc., which emphasize the need for wiser management of forest land use, and (v) have vital ecological functions such as biodiversity, soil and water conservation (watershed and landslide protection), climate control, flood and landslide prevention, and as a source of atmospheric carbon sink.

The structural complexity of tropical forests, their high diversity and the complex web of species and environmental interactions for various ecosystem processes such as folivory, pollination, seed dispersal and nutrient cycling, need to be understood in a holistic manner to better understand ecosystem functioning for effective forest management and biodiversity conservation.

The rural people of the developing world (85%) still rely on herbal or traditional medicine for their primary health care. They are considered to be safe, cost-effective and without major side effects. By and large, these medicinal plants are obtained by commercial collection from the natural habitats. The forests and waste lands are primary source of medicinal plants over the centuries. Due to human population explosion, on one hand, the forest cover is dwindling and on the other hand, requirement of medicinal plants is increasing steeply.

This has resulted in over exploitation of medicinal plants in the forests and there is a marked decline in the availability of quality raw material used in
the manufacture of medicines and allied products. To cope up with the increasing demand for medicinal plants, the viable option is commercial cultivation of these non-agricultural species. This will not only ensure quality of raw material, efficacy of drug and safety to use but also will reduce collection pressure from the wild and will offer livelihood for local people, particularly those residing near forest reserves. The concern for Biodiversity loss increases because humans depend on nature for everything. As population systems cannot be replicated, species loss is an irreversible phenomenon. Hence, Bioresource use from ecosystems should be on a sustainable basis i.e. well within the regenerative potential of species.
Nature Worship was an ancient tradition in India and all forms of life were considered sacred. There was a general conception among the early people that the godly element was actively at work in places of natural beauty. Hence trees were sacred to the Ancient Tamils. They considered trees the abode of spirits and gods and believed that the sacredness of living beings and inanimate objects ensured their safety and persistence. Many villages set apart sanctified land to propitiate the vanadevatas, i.e. tree spirits. In certain groves, the entire vegetation was considered sacred and worshipped.

Groves persist even to the present day, and have an important role at various socio-cultural, economic, religious and political levels (Malhotra, 1998). This lecture provides an overview of the history and ecological status of the Sacred Groves in the villages of Tamil Nadu.

**History and Nature of Sacred Groves**

Historical records, legends and folk songs throw light on the Sacred Groves of Tamil Nadu. The first authentic report of the Sacred Groves is in the memoirs of Ward and Conner (1827), cited in the 1891 census of Travancore State (Census Commissioner’s Office, India, 1894). Brandis (1897), the first Inspector General of Forests in India, reported on the Sacred Groves in the hill ranges of the Salem District in the Madras Presidency.

The custom of establishing Sacred Groves originated in the remote past. Several inscriptions on stone slabs and copper plates record that rulers granted...
land to maintain temple gardens, which were called *thirunandavana*. A great variety of flowering plants were cultivated in these gardens and flowers from them were offered to the deity to perform pujas (Hindu Prayers). Even after the introduction and proliferation of Christianity and Islam, the Sacred Groves remained as cradles of ancient rural civilization not only in Tamil Nadu but also in many other States of India. Sacred Groves occur in almost every part of Tamil Nadu. Many villages have more than one. Their area ranges from a few trees to hundreds of hectares. Most of the Sacred Groves represent the natural vegetation of their geographical location.

There is little evidence about the exact number and area of Sacred Groves, since no comprehensive surveys have been carried out. A 1995 assessment documented 13,270 Sacred Groves all over India. Of these, 79 ranged in size from 0.01 to 900 ha and together embraced 10,511 ha of vegetation cover. Of this area, only 138 ha comprised totally undisturbed vegetation, and 3,188 ha had an open canopy. Most of these groves (66 of 79), covering an area of 10,251 ha, were located in the catchment areas of major rivers and rivulets; 58 (9,621 ha) were at the origin of perennial streams and 38 (6,454 ha) were on hillsides (Rao, 1996). Of the 13,270 groves, 448 were in Tamil Nadu. Another estimate, however, suggests that the number of groves in the country may be as high as 100,000 to 150,000 (Vajpeyi, 2000). Each Sacred Grove has a Residing Deity and folklore associated with it. Generally they are dedicated to one of the following:

- **Village gods and/or ancient spirits**
- **Snake gods and/or an incarnation of Vishnu, variously known in different locations as Ayyappan, Sasthana or Ayyanar, a Hindu god who unites spiritually both shaivite and vaishnavite followers**
- **Shaivite gods (located in dense forests)**
- **Vaishnavite gods (located in dense forests).**
The Village Sacred Groves are generally dedicated to Amman, the goddess of fertility and good health. The next most worshipped deity is Ayyanar, worshipped daily and also offered special prayers on full moon and new moon days.

**Beliefs, Taboos, Rituals and Folklore Associated with Sacred Groves**

The taboos, rituals and beliefs associated with the groves, supported by mystic folklore, have been the prime motivating factors for preserving the Sacred Groves in as pristine a condition as possible. Trees such as banyan, peepal, neem and tamarind are considered to be the abode of spirits. When a child is desired or born, people propitiate the spirits by tying toy cradles to the branches. Similarly, they tie on a black cloth with salt in it to ward off the evil eye. Yellow, white or sometimes red pieces of cloth and bangles are tied to the trees; people ask for material, moral and social well-being in exchange for these gifts to the spiritual realm.

In certain Sacred Groves, people fulfil their vows by tonsuring (shaving the head to make a ceremonial offer of hair to the god) or by placing granite statues of snake gods in the grove’s temples. In many places, offerings of terracotta horses of various sizes are lined up in one corner of the Sacred Grove in the hope of a good harvest.

Ritual activities are carried out in the Sacred Grove as part of annual week-long village celebrations dedicated to local deities, held in the spring or summer. In certain Sacred Groves, food is cooked using the dead wood collected from the grove. The preparations are offered to the goddess and other deities, and the food is distributed to all those who take part in the festival. Folk tales and epics are enacted at night. On the last day of the festival, animals such as fowl and goats are sacrificed to the goddess. Groves dedicated to snake gods (*Nagara kavus*) are highly respected by the believers. In most of
The *Nagara kavus*, daily pujas are performed and special prayers are offered during full moon days.

The people believe that any damage to the Sacred Grove, harm to the fauna residing in it or felling of any tree from it may invite the wrath of the local deity, causing diseases and failure of agricultural crops. Even taking a dry twig is forbidden, and any violation of the taboo, people say, will incur the wrath of the snake gods. Therefore, many people will not even take dead wood out of the Sacred Groves.

**Ecological Significance of Sacred Groves**

**Water associations**

Majority of the Sacred Groves in Tamil Nadu are associated with reservoirs, ponds, springs or streams. Many Sacred Groves are located in catchments near the origins of springs or streams. Therefore, the groves act as local-area microwatersheds which help to meet the water needs of local communities. In drier climates, reservoirs associated with the large Sacred Groves provide irrigation for agriculture. The trees prevent surface runoff and thus topsoil erosion and siltation.

**Conservation of biodiversity**

Sacred Groves protect several plant and animal species valuable for food, medicinal and other uses (Ramakrishnan, 1998). Despite increased pressures, Sacred Groves shelter many plant and animal species which might have vanished elsewhere in the surrounding environment, often including wild crop relatives and endemic and endangered species (Swamy, 1997). In general, Sacred Groves in Southern Tamil Nadu harbour many varieties of mango, jamun (*Eugenia jambolana*) and fig. The Allinagaram Grove in the Theni District was found to support four wild varieties of mango. The tree *Terminalia*
*arjuna* found in this Sacred Grove, with a girth of about 10 m, may be one of the oldest living trees. Similarly, the Kandanur Sacred Grove in Sivagangai District supports a rare rattan species (*Calamus* sp.) which might otherwise have vanished from the local landscape. Sacred Groves in Kanyakumari District support numerous rare endemic orchid species on the *Hopea parviflora* trees. The Sacred Groves in the Kanyakumari District harbour many of the rare endemic plants of the Western Ghats such as *Antiaris toxicaria*, *Diospyros malabarica*, *Diospyros ebenum*, *Feronia elephantum*, *Butea frondosa*, *Garcinia cambogia*, *Sterculia foetida*, *Gnetum ula* and *Cycas circinalis* (Sukumaran and Raj, 1999).

The Sacred Groves shelter several medicinal plants of great value not only for the primary health care of the village communities but also in the modern pharmacopoeia. The literature of the Nayaks (erstwhile rulers or kings of the state) mentioned that the Alagar Hills, venerated through the centuries because of the vast Sacred Grove there, harbour a wealth of medicinal plants. These hills became an important source of raw materials for Ayurvedic and Siddha medicines. In some Sacred Groves of Kanyakumari District, medicinal plants are raised around the temple by the priest, who generally takes care of the health and well-being of humans and cattle.

The ground flora in the Sacred Groves often include wild turmeric (*Curcuma* spp.), wild ginger (*Zingiber* spp.) and cardamom (*Elettaria cardamomum*). Water Reservoirs and Ponds close to Sacred Groves support varied flora and fauna.

Isolated Sacred Groves do not usually shelter major mammalian wildlife species. However, Sacred Groves that form part of a continuous stretch of reserved forest, as in the Alagar and Suruli Hills, harbour bison. Apart from primates and minor mammals, Sacred Groves also have numerous bird,
butterfly and bat species. However, there are as yet no detailed accounts or inventories of biodiversity in the Sacred Groves.

Management of Sacred Groves in Tamil Nadu

Most of the Nagara kavus and Sasthana kavus (Sacred Groves dedicated to the god Ayyappan) in Kanyakumari District are owned by a few families or groups of families under a trust. Traditionally these communities allot a small portion (about one-seventh) of the available landholding for the purpose of maintaining Sacred Groves. Some groves are under the custody and management of local communities or tribes. Some are owned and managed by the village communities under Hereditary Trusteeship. All management decisions are taken collectively at a gathering of the entire village during the annual rituals in the Sacred Grove. Sacred Groves, associated with large Hindu Temples are managed by local trustees of the Temple Governing Board under the Supervision of State-Run Institutions.

Threats to Ecology and to Sociocultural Traditions of Sacred Groves

Today the traditional belief systems, which were fundamental to the concept of Sacred Grove conservation, are considered mere superstitions. The rituals are now known to very few people, mostly belonging to the older generation. In a recent study, it was observed that in the larger Sacred Groves, traditional rituals are still performed in accordance with the customary beliefs, but in smaller groves, the traditional rituals are no longer performed (Swamy, 1997). The traditional values appear to be gradually disappearing with the recent advent of modernization, urbanization and people’s changing aspirations. As a result, the violation of cultural norms and taboos no longer carries heavy consequences, and the Sacred Groves are becoming degraded.

Human activities that were previously taboo, such as dead wood collection, biomass gathering, lopping of tender branches and green leaves for
goats, creation of footpaths, cattle grazing, mining of sand and clay, brick-making and collection of wild fruits, vegetables, medicinal plants, fruit-eating bats and fireflies, are affecting the ecology of the Sacred Groves.

Invasion of exotic weeds has become a serious problem in the ecology of some Sacred Groves; the domination of alien species such as *Eupatorium odoratum, Lantana camara, Prosopis juliflora* and *Hyptis suaveolens* often threatens and depletes local species in these groves.

Conflicts among the Sacred Grove Managers have also resulted in loss of biodiversity in certain Sacred Groves, when policy decisions have been made to benefit certain minority sections of the village society, against the traditions of the Sacred Grove.

**Conclusions**

Sacred Groves harbour many woody plant species as well as fauna. These groves function as genetic reservoirs of wild species. As religious beliefs and taboos weaken, the pressure on these forests increases. The temples within the groves are still used as places of worship, but the forest surrounding them has become relatively unimportant. In many places, strong taboos against biomass extraction no longer exist, while in other places, natural resources are removed from the forest under cover of darkness. The rationale behind the reverence for nature and the protective taboo seems to have been forgotten, sometimes even where religious rituals continue to be observed.

It is important that people recognize the values of these remaining patches of forest and that levels of resource extraction be kept low and regulated and this would facilitate sustainable resource use. Identifying the socio-economically important species of the Sacred Grove and raising them in buffer zones, might be a viable strategy for their conservation and sustainable use. This would not, however, address the social changes that have contributed to the
Sacred Groves’ decline. Where spiritual and ethical traditions no longer ensure the conservation of these forests, the public may need to be educated and informed about other reasons – environmental, social and economic – for conserving the forest and using it sustainably.

Bibliography


Vajpeyi, Y. 2000. Tree of Life. *Indian Express* (Sunday Magazine), September 3.
BATS ARE THE ONLY FLYING MAMMALS THAT MASTERED FLIGHT MUCH BEFORE MAN’S OWN LINEAGE BEGAN. TODAY THEY FORM THE SECOND LARGEST GROUP OF MAMMALS. ALMOST A THOUSAND SPECIES ARE DISTRIBUTED AROUND THE WORLD VARYING FROM THE TROPICAL ‘FLYING FOX’ WHICH WEIGHS ABOUT ONE KILOGRAM TO THE TINY KITTY’S HOG-NOSED BAT OF SOUTHEAST ASIA THAT WEIGHS ABOUT TWO GRAMS.

BATS BELONG TO THE ORDER, CHIROPTERA, WHICH MEANS HAND-WINGED. THEY FLY THEIR FINGERS CONNECTED TO ONE ANOTHER BY A WEB-LIKE MEMBRANE AND THEY CRAWL BY THEIR THUMBS. THE ORDER, CHIROPTERA, IS DIVIDED INTO TWO SUB-ORDERS – MICROCHIROPTERA AND MEGACHIROPTERA – AND THEY ROOST IN DIFFERENT KINDS OF HABITATS SUCH AS CAVES, CAVERNS, CREVICES, CELLARS, BRANCHES OF TREES, HOLLOWS IN TREES, CULVERTS, UNDERSIDE OF BRIDGES, TEMPLES, RUINS AND ATTICS. INTERESTINGLY, A FEW SPECIES OF BATS MODIFY LARGE PALM LEAVES BY BITING A SERIES OF HOLES ACROSS THE CENTER OF THE LEAF SO THAT THE LEAF HANGS LIKE A TENT THE BATS THEN ROOST INSIDE.

THE FEEDING HABITS OF BATS SHOW REMARKABLE DIETARY VARIATIONS. THE MEGACHIROPTERANS FEED UPON FRUITS AND NECTAR. MOST OF THE MICROCHIROPTERANS PREY MAINLY ON INSECTS. HOWEVER, THERE ARE BATS BELONGING TO THE LATTER THAT FAVOUR ADDITIONAL FOOD SUCH AS FROGS, MICE, FISH, BLOOD AND SOMETIMES EVEN SMALLER BATS.

IT CAN BE GREAT FUN TO FOLLOW THE BATS DURING NIGHTS IN THE FIELD AND THUS GAINING AN INTIMATE PICTURE OF THEIR FORAGING ACTIVITY. BY MARKING THE BATS WITH VISUAL MARKER TAGS OR CHEMILUMINESCENT TAGS, IT MIGHT BE POSSIBLE TO FOLLOW THE BATS IN THE OPEN AREA. HOWEVER, ONE HAS TO VISUALLY LOCATE THE TAGGED INDIVIDUALS WHILE USING THESE LIGHT EMITTERS. INSTEAD, IN THIS AGE OF ELECTRONIC

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gadgets, radio-tracking is seductive technique to make a systematic study on the foraging behaviour of shy or elusive animals like bats. It needs a set of expensive equipment – transmitters and receivers. By means of radio-tracking, it is possible to find out the direction and approximate distance covered by the bats and to quantify the duration spent in flight and rest through out the nights for about two weeks.

Bats fascinate us because they “see” with their ears, hang upside down to sleep by day, and can catch insects while flying even in the darkest of nights. Bats can thread their way dexterously through hill and dale and thickets and bushes in total darkness. They do it with a kind of sonar that challenges our highest technology. While flying they emit trains of ultra short pulses of patterned, high frequency, high intensity sounds through nose or mouth, and adroitly pinpoint the exact location of obstacles and of the smallest prey with the aid of the echoes coming back from them. This process is known as Echolocation.

The discovery of this phenomenon is rather recent. In 1794, the Italian scientist, Lazzaro Spallanzani demonstrated that bats can fly even when blinded. In Geneva, Ludwig Jurine added the significant discovery that bats could no longer perceive obstacles once their ears were plugged with wax. In 1920, the English Physiologist H. Hartidge hypothesized that bats emit high frequency sounds, above the range of human hearing, and use their echoes for orientation. In 1938, the American biologist, Donald R. Griffin used instruments designed by G.W. Pierce and demonstrated that bats emit ultrasonic sounds with a frequency of more than 20,000 cycles per second (inaudible to the human ear) and utilize the reflected echoes to detect obstacles and to navigate in their environment.
Echolocatory Navigation is restricted only to microchiroptera bats. The megachiroptera bats have prominent eyes, depend on vision to reach feeding areas and on the sense of smell, to locate ripe fruits. It is thus clear that the microchiroptera bats are efficient gatherers of insects such as moths, beetles, crickets, grasshoppers etc. A single bat may snatch two insects out of the air in less than five seconds and as many as 500 insects in an hour. However, some of the insects have ear-like structures sensitive to ultrasounds and are able to protect themselves from the marauding bats. When bats approach nocturid moths, which are serious agricultural pests, the latter take evasive action by making a variety of flight patterns, loops, spirals and changes in both speed and direction or they simply fold their wings and drop like pebbles. Arctiid moths have been found to emit a series of ultrasonic clicks to which are ascribed a variety of functions ranging from jamming the echolocatory system of bats, to startling them and to conveying a warning that they are distasteful.

A few species of bats switch off their echolocatory machine to circumvent insect defenses and rely on other cues. The fringe lipped bats, *Trachops cirrhosus*, use the sounds produced by their prey – frogs, while the Indian false vampire bats, *Megaderma lyra*, use the rustling noise created during the movement of their prey – frogs, mice, geckoes, spiders etc. This passively listening to the noise associated with the movement of the prey, is known as passive method of prey detection. Nevertheless, a few individuals of *M. lyra* skim at the surface of the pond in darkness for a few minutes, apparently detect a stationary frog and in a dart, the bat captures a frog with its mouth. While skimming at the water surface, the bat emits ultrasounds and thus actively uses its Echolocatory System. We call this method as active acoustic localization of prey in water. The returning echoes from the protruding targets (head of a frog) in the water surface should be easily distinguished from the echoes returning from the ripples at the surface of the water. Thus the water surface acts as an
acoustic mirror. Interestingly the bats are not able to detect a freshly killed frog if its head is submerged just below the water surface.

Being mammals, bats carry their young in the womb until they are ready to be born. The gestation period ranges six weeks to eight months. Pregnancy does not begin soon after mating. A few hibernating bats, living in temperate areas, mate before the onset of hibernation and the females store the sperm during period of hibernation (about four months). Fertilization occurs after the termination of hibernation. No other mammals store sperm for such long periods as do these species of female bats.

Each female gives birth to a single young. The newborn babies are hairless, sightless and flightless - but with claws to grip for precious life. Mothers lavish meticulous care upon babies and carry the latter with them in the abdomen while flying night after night, outmanoevering hundreds of or even thousands of insects. The babies tenaciously cling to the body of their mothers on such wild rides. Sometimes, to lighten the load, mothers leave their young at home. The youngsters wait for their mothers to return with teats full of warm milk. Remarkably each mother is able to identify her own baby in the prevailing darkness, by the baby’s sound and scent. The adults of hipposiderid bats (Hipposideros speoris) emit constant frequency (CF) sounds at 135 kHz with a frequency modulated tail (FM) with no harmonics. But in the early stages of life, especially the newborn babies, emit multi harmonic sounds with a combination of FM - CF - FM pattern. At prevalant and volant stages (about 30 days of age), most of the energy is concentrated in the second harmonic which is elevated to the frequency range of adults’ CF - FM pattern at the age about six weeks. However, the young bats wean after three months, begin to operate their own sonar system and become independent.
Although bats are known to host a variety of microbial organisms, disease transmission to man is rare. According to Merlin D. Tuttle, who has studied bats for the past 30 years in various parts of the world and examined their impact on their environment, parasites of bats are not significant to human health and even the danger of rabies ‘has been vastly exaggerated’. Rabies infection in bats is rare in Asia, Africa and Europe and unknown in Australia. The US reported a total of nine cases of human rabies from bats in more than 30 years and only single case is known in Canada.

Bats are legally protected in the Soviet Union and in all European countries. In a few areas of India, they are considered sacred. However, unawareness and ignorance of the beneficial aspects of fruit bats, coupled with misconceptions and unjust persecution as crop pests, have resulted in their categorization as Vermin in Schedule V of the Indian Wildlife Protection Act 1972 (IWPA). However, recently the IWPA shifted the Salim Ali’s fruit bat *Latidens salimalii*, Wroughton’s free-tailed bat *Otomops wroughtoni* to Schedule I, by recognizing their rarity. Tuttle founded, ‘Bat Conservation International’ (BCI) based at Austin, Texas, USA to disseminate information about bats to conservationist, health officials, educators and news media. The author of this manuscript is a member in the Scientific Advisory Committee of the BCI representing India.

The purpose of the BCI is to prevent the extinction of bats, to ensure the survival of viable bat populations and to inform the public of their value. The Zoo Outreach Organization at Coimbatore (South India) has founded a network called ‘Chiroptera Conservation and Information Network of South Asia’ (CCINSA). The purpose of this network is to link together bat field researchers and their knowledge throughout South Asia (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan and Sri Lanka) so that a clear picture can emerge, and lead to conservation action. The network encourages and promotes the
study of all bats, prioritising species assessed as Data Deficient in India for upcoming fieldwork.

The CCINSA organizes CAMP (Conservation Assessment and Management Plan) Workshops regularly to update the status of the bats of South Asia. The following Table (Molur et al. 2002) illustrates the status of the bats of South Asia, prepared during the recent CAMP Workshop held at the Madurai Kamaraj University (January 2002)

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References:

The phylum *Mollusca* includes slugs, snails, whelks, clams, mussels, oysters and squids. There are approximately 80,000 species of which about 100 species have been implicated in poisoning man, or are known to be poisonous or venomous under certain circumstances. The marine gastropods known as cone snails (Conus) coming under marine gastropods, constitute an unusually species-rich group of venomous predators. It is one of the largest single genera (>500 species) of living marine invertebrates. Species of Conus inject complex venom into their prey. Some Cone Snails have evolved what *a priori*, would seem to be exceedingly unpromising evolutionary directions for relatively slow snails that are unable to swim. A significant complement of Conus species (>50) catch fish as either their major or exclusive prey. The sting of at least one of Conus has resulted in a high frequency of human fatality.

The biological activity of a Conus venom is due to a large complement of unusually small, highly structured peptides. Each Conus species has its own distinct repertoire of venom peptides (Olivera 1997, Olivera & Cruz 2001). The great majority of Conus species are highly specialized predators and in some cases, only a single species of prey is envenomed. However, collectively, Cone Snails have a remarkably broad spectrum of prey that fall into at least four phyla (Röckel et al. 1995).

The conopeptides are highly structured mini-proteins that have evolved in ~500 species of fish, mollusk and worm hunting Cone Snails for rapid prey immobilization and defense. Their small size, relative ease of synthesis,
structural stability and target specificity make them important pharmacological probes. It is estimated that >50,000 conopeptides exist but <0.1% have been characterized pharmacologically. Of those characterized to date, a surprising number have been found to be highly selective for a diverse range of mammalian ion channels and receptors associated with pain signaling pathways, including the nicotinic acetylcholine receptors (α-conotoxins), the noradrenalin transporter (χ-conopeptides), sodium channels (μ- and μO-conotoxins), calcium channels (ω-conotoxins), the N-methyl-D-aspartate (NMDA) receptor (conantokins) and the neurotensin receptor (contulakins). Cone Snails are not the only species that produce peptides in their venom. However, those found in spiders, snakes, sea anemones and scorpions are typically of larger size.

Conotoxins can be classified into several families based on the number and pattern of disulfide bonds and biological activity. Some of the conotoxins are α-conotoxin, π-conotoxin, τ-conotoxin, ω-conotoxin, γ-conotoxin, μ-conotoxin, δ-conotoxin, κ-conotoxin, etc.

Conotoxins have been classified based on their physio-pharmacological action. Those that are seemingly associated with inhibiting the acetyl choline receptor at the post synaptic terminus has been identified as α, those which block the muscle pull a page sensitive sodium channels as μ, those that block Ca^{2+} channels in the terminals of the perivascular nerves reducing these but not affecting the contractile property of sympathetic transmission as ω, those that inhibit the shaker K^{+} as κ- Conotoxins (Shon et al., 1998), those that act by showing sodium channel inactivation as δ-conotoxins, etc.

**Overview of Conus peptides:**

The small size of conopeptides makes chemical synthesis possible in amounts sufficient to investigate the biochemistry, pharmacology, and
physiology of each gene product. Thus, a relatively large set of venom peptides from Cone Snails has been investigated to an extent that the physiological mechanisms underlying their biological activity are understood. A small subset of these, molecular interactions with their target biomolecules have been defined. The database that has accumulated is sufficient to predict, on the basis of sequence homologies alone, the probable general physiological mechanisms that underlie the biological activity of many extra Conus peptides. Thus, the extensive molecular analysis of the genes encoding venom peptides of Conus has provided a database of over 1000 genes from close to 100 different Conus species, a large enough sequence dataset to provide a window into general patterns of interspecific divergence of the gene families represented.

Recently, molecular data have provided a preliminary picture of the phylogenetic relationships among the major Conus species revealing that several distinct clades of Cone Snails, with reasonable agreement between datasets from different labs as to which species belong to a clade (Duda et al. 2001, Duda & Palumbi 1999, Espiritu et al. 2001, Monje et al. 1999).

**Conus venom Pharmacology and Toxin biochemistry:**

A significant amount of information has been collected for several gene families expressed in the venom ducts of Cone Snails, but most of the data are for a small subset of *Conus* species and were originally acquired for reasons unrelated to either the taxonomy or ecology of these snails. The lethality of *C. geographus* envenomation to humans attracted the attention of the toxicology community, and a physiological and pharmacological characterization of the venoms of a few Cone Snails was carried out. A first comprehensive study of the effects of different *Conus* venoms (Kohn et al. 1960) demonstrated that there are striking differences in potency and in particular, venoms of fish-hunting *Conus*
are much more lethal than those of the other groups when tested in vertebrates. Early work by Endean and coworkers suggested that there might be components with unusual pharmacological properties not previously observed in other venoms (Endean et al. 1974, 1976, 1977, 1979). The first biochemical attempt at characterizing the biologically active components of Cone Snail Venoms was reported by Spence et al. (1977), who purified a toxin from *C. geographus* that paralyzed muscle and inhibited muscle action potentials, without detectable effects on the electrical activity of nerves. These workers determined the amino acid composition but did not fully characterize the toxin. In retrospect, there is little doubt that they were the first to purify a peptide in a class of *Conus* venom components now known as μ-conotoxins, novel polypeptides that block sodium channels by binding the same site as tetrodotoxin (from the puffer fish, fugu) and saxitoxin (from dinoflagellates that cause “red tides”), but with much higher selectivity.

A systematic biochemical analysis of *Conus* venoms was initiated in the 1970s and originally focused on a few fish-hunting species, in particular *C. geographus*, *C. striatus* and *C. magus*. This early biochemical work, which was reviewed in 1985 (Olivera et al. 1985), showed definitively that the major biologically active components of *Conus* venoms are unusually small, highly structured peptides and that many of these potently alter the function of ion channels.

One surprising feature of Conus venoms is their complexity (Olivera et al. 1990) because each species has a large repertoire of peptides that can be expressed. The complex behavioral repertoire of different Conus species, coupled with the complexity of their environment, may be the primary factors that drive the complexity of Cone Snail Venoms.
One strategy for setting up hypotheses regarding the function of diverse venom components is to summarize the likely general utility of various venom components. Most obvious of all is the use of venom to capture prey. There is also excellent evidence that venom is used by many, if not all, species of Conus to defend themselves against potential predators. Finally, there are preliminary indications that at least certain species use their venom for competitive interactions.

The underlying general reason for accelerated evolution of Conus venom peptides is that Conus peptide genes are a molecular readout of the biotic interactions of each Conus species. Because different species have divergent biotic interactions, hypermutation resulting in the divergence of Conus venom peptides is a molecular mirror of the underlying biology and these are the genes exquisitely tuned to the interactions of an individual Conus species with the other organisms in its environment. These are the genes that must change with environmental changes, and the attendant changes in biotic interactions, occur to such an extent that new species evolve to exploit the new ecological opportunities.

**Molecular studies on the venom component.**

Woodward et al. (1990) reported the first molecular analysis of Cone Snail cDNA clones from the messenger RNA of venom ducts. This study revealed that peptides expressed in the venom duct of *C. textile*, were initially translated as prepropeptides, with a specific proteolytic cleavage required to generate the mature toxins. This study determined the sequences of several related *C. textile* toxin precursors.

Although some elements of these precursors, notably the signal sequence at the N-terminal end, were conserved, the mature peptide toxins, encoded at the C-terminus of the precursors, had diverged remarkably from each other in
amino acid sequence. Thus, this work established the striking juxtaposition of conserved and highly divergent regions in a *Conus* precursor termed as “focal hypermutation”. It is generally observed in the C-terminal, mature-toxin–encoding region whenever sequences of two *Conus* peptide precursor genes in the same family are compared. This interspecific focal hypermutation resulted in a different complement of peptides in the venom of each cone species.

The discovery that some *Conus* venom peptides have unique pharmacological properties accelerated the characterization of venom components and by 1990 (Olivera et al. 1990) it had become clear that the venom of all Cone Snails is remarkably complex and that an individual species could express 100–200 different venom peptides. Furthermore, as had been established for intraspecific divergence, the interspecific hypermutation occurs only in the C-terminal mature toxin region and not in the N-terminal section of the precursor, nor in the 30 untranslated region of the mRNA.

Thus, a consensus picture emerged that the biologically active components of *Conus* venom is mostly small peptides (referred to as conopeptides or conotoxins, terms used interchangeably in this thesis report). An enormous diversity of peptides are expressed in the venoms of living *Conus* (a conservative estimate is over ~50,000 peptides altogether), each apparently encoded by a separate gene. However, as the molecular genetic analysis continued, it also became clear that this massive peptide diversity has been generated by diversification of only a few gene superfamilies (~20–30), with members of a given gene superfamily sharing highly conserved sequence features such as the signal sequence that becomes a molecular signature for all members of that superfamily (Olivera 1997).

The conserved sequence elements in a *Conus* peptide gene superfamily have made it feasible to use PCR to uncover new conopeptides sequences that
are now easily accessed, resulting in the availability of an ever-expanding venom peptide database from many *Conus* species. The hypermutation of the C-terminal, mature toxin region as speciation occurs leads to different complements of venom peptides in different species—the growing list of available toxin sequences provides an unparalleled opportunity to analyze several gene families that have undergone accelerated evolution over a large number of congeneric species. It is arguably the largest such molecular database available at the present time for interspecific divergence within a single genus of animals. Thus, *Conus* peptides provide a window into molecular events that accompany speciation.

**Voltage-gated ion channels sensitive to conotoxins:**

Ions moving by facilitated diffusion can traverse the plasma membrane through channels created by proteins. These embedded transmembrane proteins allow the formation of a concentration gradient between the extracellular and intracellular contents. These ion channels are said to be 'gated' if they can be opened or closed. There are three types of gated ion channels, they are:

- Ligand gated.
- Mechanically gated.
- Voltage gated.

Ligand gated channels open or close in response to the binding of a small signaling molecule or "ligand". Some ion channels are gated by extracellular ligands and some by intracellular ligands. In both cases, the ligand is not the substance that is transported when the channel opens. The binding of neurotransmitter acetylcholine opens sodium channels in certain synapses.

Mechanically gated channels are beyond the scope of this piece, but stretch receptors, opening channels to create nerve impulses form one such example.
Voltage gated channels are found in neurons and muscle cells. They open or close in response to changes in the charge (measured in volts) across the plasma membrane. For example as an impulse passes down a neuron, the reduction in the voltage opens sodium channels in the adjacent portion of the membrane. This allows the influx of $\text{Na}^+$ into the neuron and thus the continuation of the nerve impulse.

**Other bioactive conopeptides:**

Several conopeptide families have been characterized that affect ion channels, but whose exact molecular target has not yet been identified. In some cases, this is because they have invertebrate targets that have not yet been cloned. For example,

\begin{itemize}
  \item $\gamma$-conotoxin PnVIIA from *C. pennaceus* (Fainzilber et al., 1998) and $\mu$-PnIVA from *C. pennaceus* (Fainzilber et al., 1995) target molluscan pacemaker channels and voltage gated sodium channels, respectively. Conorfamide from *C. spurius* venom may be an agonist for neuropeptide FMRFamide gated ion channels, as well as a potent modulator of $\text{E Na C}$ channels in mammalian systems (Maillo et al., 2002). Thus, the range of conopeptide families that target ion channels is likely to be significantly greater than indicated in the preceding sections. Representatives of a new family of four-cystine, three-loop conotoxins (designated framework 14) have been isolated from the venom of *C. floridanus floridensis* and *C. villepini*, two worm-hunting species (Möller et al., 2005). They adopt $\alpha$ helix– loop–helix structure similar to that of Cs a/a scorpion toxins such as the $\kappa$-hefutoxins. Recently, two new O-superfamily conotoxins, vx6a and vx6b, were isolated from the worm-hunting snail *C. vexillum* (Jiang et al., 2006). They elicited behavioral responses upon i.c.v. injection but their molecular target is unknown. Several conopeptides also target G-protein coupled receptors. Two of these are agonists, conopressin-G for the vasopressin
receptor (Cruz et al., 1987), and contulakin-G for the neurotensin receptor (Craig et al., 1999b). The only antagonist characterized to date is ρ-conotoxin TIA, which targets the α1 adrenergic receptor (Sharpe et al., 2001). Peptide tx5a (also called TxIX) reduces Ca\textsuperscript{2+} flux in presynaptic termini, but the actual molecular target (possibly a Ca\textsuperscript{2+} channel or a G-protein coupled receptor that affects Ca\textsuperscript{2+} channels) has not been identified (Rigby et al., 1999; Walker et al., 1999).

A number of peptides have been characterized biochemically but have either different or unknown targets, for example χ-conotoxin MrIA (or mr5a), a member of the T-superfamily (McIn tosh et al., 2000), which may target the norepinephrine transporter (Sharpe et al., 2001), and the spasmodic peptides (Jacobsen et al., 1998). We have determined the structure of a spasmodic peptide, gm9a, from C. gloriamaris (Miles et al., 2002). This member of the P-superfamily adopts the well-known ICK motif structure but because the P-conotoxins have no cysteines directly adjacent to one another, the scaffold underlying this superfamily has the greatest scope for exhibiting structural, and therefore probably functional, diversity amongst all of the 6-Cys containing conotoxins. Several larger polypeptides from Conus venoms have also been reviewed recently by Terlau and Olivera (2004).

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RECOMBINANT LACTIC ACID BACTERIA FOR THE TREATMENT OF CALCIUM OXALATE STONE DISEASE

Prof. G.S.Selvam *

Abstract:

Lactic Acid Bacteria (LABs) are being used as a probiotic very often for various enteric problems. Many genetically modified LABs are created by different workers for various novel applications. In this study, we examine the expression of heterologous oxalate decarboxylase (oxdc) in Lactobacillus plantarum NC8. Generally, this enzyme is not present in Lactobacillus spp. OxdC gene from Bacillus subtilis was polymerase chain reaction-amplified and cloned in a shuttle vector pSIP400 series, downstream of the inducible promoter, Porfx. In the presence of an inducing peptide, Sakacin-P, the expression of OxdC was observed in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The cell-free extract and the purified protein from the recombinant LABs showed the presence of OxdC activity. The above recombinant LABs, with desired modifications, can be used as a possible probiotic for the degradation of intestinal dietary oxalate for preventing enteric hyperoxaluria.

Introduction:

Dietary lactic acid bacteria (LABs) are mostly known for their widespread use in the production and preservation of fermented foods and as such have obtained the “generally regarded as safe” (GRAS) status within the food industry. Some members of this diverse group of bacteria are components of the indigenous gut microbiota of both animals and humans and have long been recognized for their health promoting properties. Indeed, specific strains of LABs, and in particular lactobacilli, have been used as a probiotic because they

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are thought to play a crucial role in maintaining a healthy microbiota as well as contributing to an expanding list of health-promoting activities.

Furthermore, evidence suggests that LABs play a role in controlling intestinal microbiota, restoring intestinal barrier function, and alleviating inflammatory responses, which has laid to their proposed use as therapy aid management of immunological disorders such as Crohn’s disease and Pouchitis. Over the last few years, a coordinated effort involving a number of research strategies has set out to provide experimental evidence for the efficacy of different prototype health products based on the mucosal administration of recombinant LAB. Genetically modified LABs such as *L. lactis* has been used for delivery of interleukin-2, -6, and -10 to combat murine colitis. *Lactobacillus casei* has been used for the delivery of b-lactoglobulin in the digestive tract of mice. Similarly modified *Lactobacillus* was shown to protect against dental caries in rat. The future of recombinant LABs as novel therapy for the human can be foreseen when the potential benefits are significant, particularly when the absence of satisfactory treatments for a particular disease necessitates the development of new and innovative therapies. Oxalic acid is known to be a nonessential end product of human and animal metabolism and is excreted unchanged in urine. Under normal conditions, the daily load of oxalate deriving from endogenous production and intestinal absorption is fully excreted by the kidneys. Elevated oxalate load results in hyperoxaluria, a major risk factor for recurrent nephrolithiasis. In addition, several pathological conditions, including Crohn’s disease, steatorrhea, and cystic fibrosis, or medical procedures such as jejunileum bypass surgery are associated with enteric hyperoxaluria due to enhanced oxalic acid absorption in the colon.

Intestinal oxalate degrading bacteria are capable of degrading oxalate to CO2 and formate, the latter being further metabolized and excreted via feces. Commonly, oxalate-degrading microbes use oxalyl-CoA decarboxylase and
formyl-CoA transferase enzymes to degrade oxalate in the gut. These enzymes are involved in the activation of oxalate to oxalyl-CoA and further decarboxylation to give formyl-CoA and CO2. It is believed that the oral administration of antibiotic abolishes the oxalotrophic microbes in the gut, leaving the individual with the risk (hyperoxaluria) of kidney stone formation by hyperabsorption of dietary oxalate. It is speculated that both endogenously produced as well as dietary oxalate can be significantly removed via the intestinal tract.

Manipulation of the gut flora with the right probiotic bacteria might have a positive impact on gastrointestinal tract oxalate levels and might decrease oxalate absorption. Although Oxalobacter formigenes is under study for its application to mitigate hyperoxaluria, it is important to introduce alternative strategies such as delivering modified probiotic strains into the gut that can degrade dietary oxalate for tackling the problem. In the present work, we attempt to study the possibility for the overexpression of oxalate decarboxylase (oxdc) from Bacillus subtilis in Lactobacillus plantarum NC8, which can be further modified for future application, such as mitigation of enteric hyperoxaluria by degrading dietary oxalate in the gut and reducing the absorption of the oxalate.

Materials and Methods:

Bacterial Strains and Media: The bacterial strains used in this study are listed in Table 1. Escherichia coli strains were grown in Luria–Bertani media at 37°C with shaking; Lb. plantarum was grown aerobically in MRS media (Himedia) at 30°C without shaking. When required, erythromycin was added as follows: 200 μg/ml for E. coli and 10 μg /ml for Lb. plantarum. Preparation of Plasmid, Cloning, and Transformation Plasmids used and modified in this study are listed in Table 1. Plasmids were modified using standard molecular cloning
techniques. Primers used in polymerase chain reactions (PCR) are listed in Table 2. Restriction enzymes were purchased from Fermentas and New England Biolabs. Primers were procured from Microsynth.

PCR was performed with the GeneAmp PCR System 9700 (Applied Biosystems) and recombinant Taq DNA polymerase (Fermentas) using standard procedures. Plasmid DNA from *E. coli* was isolated using a miniprep spin kit (Qiagen) and from *Lb. plantarum*, plasmid was isolated by a modified alkaline lysis method. *E. coli* DH5α (Novagen) was used as the host strain for the construction of plasmids. *E. coli* DH5α was transformed using the calcium chloride method. *Lb. plantarum* was electrotransformed according to Aukrust and Blom and personal communication with Dr. Lars Axelsson. Briefly, *Lactobacillus* was grown overnight in MRS broth without glucose, inoculated to an optical density of 0.25 at 600 nm in MRS with 1% glycine, and incubated at 30°C. At an optical density of 0.6 at 600 nm, the cells were chilled on ice and harvested, washed once with ice-cold 1 mM MgCl2 and once with ice-cold 30% polyethylene glycol 1500 (PEG–1500) and resuspended in 1/100 culture volume of 30% PEG-1500. Forty microliters of the cell suspension and 1–2 µg of DNA in 2–5 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) were added to a 0.2-cm cuvette for the transformation. Pulse was applied at settings of 1.5 kV, 400 X and 25 IF (Gene pulser and pulse controller; Bio-Rad). Immediately after the pulse, cells were transferred to the 460 µl of MRS broth containing 0.5 M sucrose and 0.1 M MgCl2 and incubated at 30°C for 2 h.

The cells were plated on MRS agar containing the required antibiotic and incubated for 24 h at 30°C until visible colonies were observed. Cloning Strategy, the oxalate decarboxylase gene (*B. subtilis*) was amplified from the plasmid pLB36, using primer set oxdcF2 and oxdcR2 (Table 2). Thirty-six cycles of PCR was performed using 0.5 µM of each primer with an annealing
temperature of 58°C for 1 min. The amplicon was digested with NdeI and made blunt by end filling at the 50 termini of the gene using a klenow fragment. Subsequently, the gene was digested with XhoI to give a sticky end at 30-termini.

The plasmid vector pSIP409 was digested with NcoI and made blunt using S1nuclease treatment, followed by digestion with XhoI to get respective compatible ends. Purification of the gene fragment and the linearized plasmid were carried out using a gel extraction kit (Qiagen). The plasmid vector and the gene was ligated using a Quick ligation kit (Roche) and transformed into *E. coli* DH5α. The clones were confirmed by DNA sequencing using the primer PorfXF. Oxdc Induction and Protein Analysis *Lactobacillus plantarum* harboring vector with oxdc was induced with 50 ng/mL of sakasin-P (SppIP)-inducing peptide at OD600 * 0.3 and grown to OD600 * 1.8. MnCl2 to final concentration of 5 mM was added along with the peptide inducer.

The induced cells from the culture was harvested every hour and suspended in 50 mM phosphate buffer and disrupted by sonication. The cell-free extract was analyzed on 12% sodium dodecyl sulfate– polyacrylamide gel electrophoresis (SDS-PAGE). An assay for OxdC was performed by a stopped assay as described by Tanner et al. In brief, the harvested cells were suspended in sodium citrate buffer and disrupted with sonication.

The cell-free extract was obtained by centrifugation at 16,000 g for 30 min at 2°C. The enzyme activity was examined in the presence of 150 mM potassium oxalate and 10 mM sodium citrate (pH 4.0) at 25°C. After 2 min of incubation, the mixture was neutralized with phosphate buffer (pH 9.5) to increase the pH to 7.5 to stop the decarboxylation reaction. Subsequently, NAD was added and was monitored for its reduction at 340 nm in the presence of formate dehydrogenase (Sigma Aldrich). One unit of enzyme activity was defined as the amount of enzyme required to reduce 1 μmol of NAD per minute.
### Table 1: Bacterial strains and plasmid vectors used in this study.

<table>
<thead>
<tr>
<th>Plasmid or Strains</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLB36</td>
<td>pET 32a with oxdc from <em>B. subtilis</em></td>
</tr>
<tr>
<td>pSIP409</td>
<td>p256rep/pUC(pGEM)ori; P_{orfX::gusA}; Em^r</td>
</tr>
<tr>
<td>pLdh-oxdc</td>
<td>p256rep/pUC(pGEM)ori; P_{ldhl::oxdc}; Em^r</td>
</tr>
<tr>
<td><em>E. coli</em> DH5a</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> BL21(DE3)</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> NC8</td>
<td>Host strain, Plasmid-free, silage isolate</td>
</tr>
</tbody>
</table>

### Table 2: Primers and peptide sequences used in this study.

<table>
<thead>
<tr>
<th>Primer/peptide Name</th>
<th>Primer/peptide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxdcF2</td>
<td>5’ GGATATCCATATGAAAAAACAAAATGACATTCCG C 3’</td>
</tr>
<tr>
<td>P1dhL-Fa</td>
<td>5’ GAG AGT CGA CTC AAT CTT CTC ACC GTC TTG -3</td>
</tr>
<tr>
<td>P1dhL-Ra</td>
<td>5’ GAG ACC ATG GCA TAT GTC AAT AAG TCA TCC TC T CG T-3</td>
</tr>
</tbody>
</table>
Cloning and expression of oxalate decarboxylase (oxdc) from *Bacillus subtilis* in Lactic Acid Bacteria


Fig: 2 FPLC profile of Histagged recombinant OxdC from *Lactobacillus plantarum* NC8

Fig: 3 Protein profile on SDS-PAGE after inducing the expression of OxdC in *L.plantarum*. Numbers at extreme left are molecular size of protein markers in kilodaltons. Lane 1: Protein marker; Lane 2: uninduced; Lane 3, 4 and 5: 1st, 2nd and 3rd hour after induction with SppIP inducible peptide (50ng/ml); Lane 6: His-tagged OxdC purified with Ni-NTA column using FPLC.
Development of Lactic acid bacteria expressing oxalate decarboxylase constitutive promoter PLdh1 signal sequence

Development of Lactic acid bacteria expressing extra cellular oxalate decarboxylase under constitutive promoter, Pusp consisting secretory signal sequence.

*******************************************************************************
In biology, statistics is widely applied to derive conclusions and meaningful interpretation of data. Indeed many manuscripts are returned by the Journal Editor for lack of application of statistical tools and data interpretation. The purpose of this presentation is to elucidate the situations which demand the application of a statistical tool. Nowadays one need not go for the laborious calculation of volumes of data. There are statistical software to do the function. Some of the common software applied include Statistica, SPSS, COSTAT, sigmastat, sigmaplot etc. Application of the software gives the result immediately with accuracy. The common softwares are updated periodically and improved versions are released from time to time. Research papers, while being submitted for publication, should mention the version of the software used for the analysis of the data. The softwares can be purchased.

While the software application is easy, the researcher is often confused by the procedure to be followed to enter and analyze the data. The investigator is also often confused about the method to be applied for a given situation.

In laboratory investigations, the researcher conducts an experiment and collects the data. Once the data are collected, he should know which method is to be applied. For this the best guide often is similar tests carried out by an earlier researcher in articles published in reputed journals. A thorough knowledge of literature helps an investigator in many ways including data analysis, statistical tools applied and interpretation of the results often in line

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with the earlier results. Now let us briefly find out the situations and the statistical tool to be applied.

**Single mean:**

Suppose we measure the height of 100 boys out of 1000 boys in an hostel, the 1000 boys become the population or Universe. This means that only 1000 members are being considered for the study and out of that, we take a sample of 100 boys. We measure the height of 100 boys and find out the mean, standard deviation, and standard error. Suppose we measure all the 1000 boys we can get the actual value which is called as the population value. But we have a sample value only. Can we find out how this value is related to the population value? Or can we give a range of values with a maximum and minimum and say confidently that the population value should lie within this? Yes, that we call as the Confidence Interval.

**Two means:**

Often we conduct a study which involves the comparison of two means. For example, we want to compare the height between boys and girls. Or we want to find out the effect of two diets on growth of fish, or the effect of two nutrient media on the growth of a callous, or the effect of a hormone on the growth, or two levels of protein on the weight gain of two groups of Tilapia. Or we have a control and we want to compare the treatment with the control. These are only a few examples of two mean comparisons. In such cases, we have to apply student t test.

In this regard, we should know the types of t tests when we use a software. The software asks whether it is a paired t test? The answer usually is unpaired. Paired t test means the same individual is used twice in the study. Such studies involve the before and after effect. For example, in a study on the effect of a drug in blood pressure in man, the BP can be measured before the
administration of the drug and again after its administration. Thus we use the same individual for the measurement to study the before and after effect. Such a study that involves pairing or using the same individual twice for an observation involves a paired t test.

**One tail and two tail tests:**

If we study the effect of the drug on the blood pressure of man, it is called a two tail test. That is the drug may increase or decrease the blood pressure. But if we already know that the drug will increase the BP, then we will be interested to know only how far will the drug increase the pressure. This becomes a one tail test.

**Comparison of more than two means:**

If we are interested in comparing more than two means, then we should apply analysis of variance abbreviated as ANOVA. Anova partitions the total variation into: a. within group variation (which is the variation that exists in all the groups), b. between groups variation (the variation because of the experiment/treatment).

The method of partitioning the variation is explained in the following study.

**Application of ANOVA:**
Crop (ponni variety) yield (Bags of paddy/plot) with three fertilizers.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>120</td>
<td>130</td>
<td>180</td>
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<td></td>
<td>70</td>
<td>140</td>
<td>130</td>
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---------------------------------------------------------------
610 730 870
---------------------------------------------------------------
\[
\text{Bet var.:} \quad \frac{(510)^2 + (730)^2 + (870)^2}{6} - \text{CT} = 5644.4
\]

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>18169.11</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>5644.40</td>
<td>2</td>
<td>2822.20</td>
<td>3.382</td>
</tr>
<tr>
<td>Within</td>
<td>12524.71</td>
<td>15</td>
<td>834.56</td>
<td></td>
</tr>
</tbody>
</table>

Once the test tells us that there is a significant difference between the means, the next step will be to find out which means are different from each other. Or which are not different. Perhaps two means are the same. Here the test is called post hoc test. There are various tests applied like: duncan, tukey and SNK. The test is also called as multiple mean comparisons. The following table gives an idea about SNK test:

**SNK test to compare means:**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1322.82</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between</td>
<td>1077.32</td>
<td>4</td>
<td>269.33</td>
<td>49.33</td>
</tr>
<tr>
<td>Within</td>
<td>245.50</td>
<td>45</td>
<td>5.46</td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05} (4,45) = 2.58; \; 0.01(4,45) = 3.77; \; 0.001 (4,45) = 5.57 \]

\[ \text{SE of group mean : } \sqrt{\text{MS within/10}} = 0.7389 \]

Array group means: 58, 58.2, 59.3, 64.1, and 70.1

\[ \text{LSR : } Q\alpha (kv) \times SE \]

2 3 4 5
\[
\begin{align*}
Q &= 2.85 \quad 2.43 \quad 2.77 \quad 4.02 \\
\text{LSR} &= 2.10 \quad 2.53 \quad 2.79 \quad 2.97 \\
\text{Compare:} \\
58, 58.2, 59.3, 64.1, 70.1 \\
\text{Compare K5} & \quad 70.1-58 = 12.1^* \quad \text{Significant since: LSR = 2.97} \\
\text{Compare K4} & \quad 64.1-58. = 6.1^* \quad \text{Significant} \quad \text{LSR = 2.79} \\
\quad & \quad 70.1-58.2 = 11.9^* \quad \text{-do-} \\
\text{Compare K3} & \quad 59.3-58 = 1.3^{\text{NS}} \quad \text{Not significant} \quad \text{LSR = 2.43} \\
\quad & \quad 64.1-58.2 = 5.9^* \quad \text{Significant} \\
\quad & \quad 70.1 - 59.3 = 10.8^* \quad \text{Significant} \\
\text{Compare K2} & \quad 64.1-59.3 = 4.8^* \quad \text{Significant} \quad \text{LSR = 2.10} \\
\quad & \quad 70.1-64.1 = 6^* \quad \text{Significant} \\
\text{Ranked means:} & \quad 58, 58.2, 59.3, \quad 64.1, \quad 70.1
\end{align*}
\]

The procedure for student t test is given below:

\[t'\text{ test for small samples:}\\
\bar{X}_1 = \frac{\sum X_1}{n_1} = \frac{182}{8} = 22.75 \\
\bar{X}_2 = \frac{\sum X_2}{n_2} = \frac{238}{12} = 19.83 \\
S^2 = \frac{\sum X^2 - \left(\frac{\sum X}{n}\right)^2}{n} = \frac{4202 - \left(\frac{182}{8}\right)^2}{8} + \frac{5108 - \left(\frac{238}{12}\right)^2}{12} \div 18 = 24.95 \\
t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S^2}{n_1} + \frac{S^2}{n_2}}} = \frac{22.75 - 19.83}{\sqrt{\frac{24.95}{8} + \frac{24.95}{12}}} = 2.917 \div \sqrt{5.2} = 1.279 \\
\sqrt{24.95 \times (\frac{1}{8} + \frac{1}{12})}
\]
Table value: $t_{0.05, df18} = 2.01$.

**Hence accept null hypothesis.**

**Another example for t test:**

Levels of enzymatic antioxidants in the liver mitochondria of control and DEN (Diethyl nitroso amine) induced rats after 60 and 90 days of treatment.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>treatment I</th>
<th>treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>78.49±8.94</td>
<td>66.08±6.8*</td>
<td>47.94±4.8*</td>
</tr>
<tr>
<td>Superoxide</td>
<td>6.59±0.08</td>
<td>5.09±0.49*</td>
<td>4.19±0.46*</td>
</tr>
<tr>
<td>Dismutase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>10.08±1.06</td>
<td>7.33±0.7*1</td>
<td>6.92±0.78*</td>
</tr>
<tr>
<td>Peroxidase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is $\text{mean±SD}$ of 6 observations each.

Body weight and liver weight of control and DEN induced Hepatic carcinoma experimental rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>treatment I</th>
<th>treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight</td>
<td>3.82±0.46</td>
<td>4.92±0.68</td>
<td>7.56±0.97</td>
</tr>
<tr>
<td>Body weight</td>
<td>100.02±10</td>
<td>82.6±8.51*</td>
<td>72.8±7.4*</td>
</tr>
</tbody>
</table>

Each value is $\text{mean±SD}$ of 6 observations.

**Reference:**

“The integrity and maintenance of genome is very important for the survival of living system on the planet. The genome is under threat by various factors which directly or indirectly damages the genome. Even though the genome is damaged by various factors for millions of years, the genome maintains its integrity. For the genome integrity, checkpoint signal transduction pathways, the structural advantage of DNA and the DNA repair proteins are the key factors. Because of the three factors, the cell divides with successful duplication of chromosome and existence of living organism on the planet”.

Life on the planet earth is a mystery. Scholars and philosophers through the centuries have sought to understand the living system, its origin and its propagation. Despite man’s endeavors to probe into the secrets of the universe, the universe remains a mystery. The universe is commonly defined as the totality of everything that exists, including all physical matter and energy, the planets, stars, galaxies, and the contents of intergalactic space. All matter in our universe is made up of atoms. The atom is a basic unit of matter that consists of a dense, central nucleus surrounded by a cloud of negatively charged electrons. Atoms make every tangible material in the universe. The earth contains approximately 1.33×10⁵⁰ atoms (Weisenberger and Drew, 2008). Solids, liquids, gases - all matter - are made up of atoms. In the planet's atmosphere, small number of independent atoms of noble gases exist, such as argon and neon. The remaining 99% of the atmosphere is bound in the form of molecules, including carbon dioxide, diatomic oxygen and nitrogen. At the surface of the Earth, atoms combine to form various molecules, such as water, salt, silicates and oxides. Atoms combine together to form molecules. A molecule is an electrically neutral

* Associate professor and Head, Dept. of Biotechnology, Manonmaniam Sundaranar University, Alwarkurichy – 627 412, E-mail: sudhakarmsu@yahoo.com
group of at least two atoms held together by covalent chemical bonds. Molecules can be much bigger. For example, one molecule of vitamin C is made up of 20 atoms (6 carbons, 8 hydrogens, and 6 oxygens - that's C\textsubscript{6}H\textsubscript{8}O\textsubscript{6}).

![Diagram](image)

**Figure 1. Formation of molecules from atoms**

The atomic and molecular evolution start from so small to so big complex system as follows:

Atoms $\rightarrow$ Molecules $\rightarrow$ Macromolecules $\rightarrow$ Cell organelles $\rightarrow$ Cells $\rightarrow$

Organisms $\rightarrow$ Populations $\rightarrow$ Ecosystems $\rightarrow$ Biospheres

The Periodic Table provides a great deal of information about various elements. As of 2011, the table contains 118 chemical elements whose discoveries have been confirmed. The first 94 are found naturally on Earth, and the rest are synthetic elements that have been produced artificially in particle accelerators. The living system has been constituted by selected atoms as shown in the following table. There are three groups of atoms which have been selected to constitute the cells. Group I has Na, K, Mg and Ca; group II has Mn, Fe, Co, Cu and Zn; Group III consists of C, N, O, F, P, S and Cl. There are something in the selection of the atoms to constitute cells. The secret is yet to be solved.
Biological macromolecules

Atoms group together to form molecules. Molecules group together to form macromolecules. When small molecules are joined together, giant molecules are produced. These giant molecules are known as macromolecules. Macromolecules are formed by dehydration reactions in which water molecules are removed from the formation of bonds. By varying the sequence, an incredibly five different macromolecules were being produced. The variation in the form of macromolecules is largely responsible for molecular diversity. The five main types of macromolecules control all activities of cells. They are DNA, RNA, proteins, carbohydrates, and lipids. These are composed of different monomers and serve different functions.

Table 1. Composition and function of macromolecules present in living cells.

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Composition of monomers</th>
<th>Function of macromolecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Proteins</td>
<td>C, H, O, N, S Amino acid monomers</td>
<td>Molecular transport and muscle movement</td>
</tr>
<tr>
<td>2. Carbohydrates</td>
<td>Sugar monomers</td>
<td>Storage of energy</td>
</tr>
<tr>
<td>3. Nucleic acids (DNA and RNA)</td>
<td>C, H, O, N, P</td>
<td>Protein synthesis and to transfer genetic information from one</td>
</tr>
<tr>
<td>RNA)</td>
<td>generation to the next.</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td>4. Lipids</td>
<td>Fatty acids, phospholipid and steroid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Store energy, cushion and protect organs, insulate the body and form cell membranes.</td>
<td></td>
</tr>
</tbody>
</table>

### The cells

Life is marvelous and wonderful. The cell is nothing but the arrangement of the five different macromolecules in a water medium. Otherwise it is micron size machine created by the five different macromolecules. Understanding of the macromolecules and their functions in the cell is called Molecular Biology. All organisms are composed of the fundamental unit of life, the cell. The cell is the simplest unit of matter that is alive. From the unicellular bacteria to multicellular animals, the cell is one of the basic organizational principles of biology. It has been reported that our bodies contain 75 to 100 trillion cells. Cells do everything in an individual’s life. It provides structure and stability, energy and a means of reproduction for an organism. There are two primary types of cells. They are prokaryotic and eukaryotic cells.

Prokaryotes are single-celled organisms. They were the earliest and most primitive forms of life on earth. Prokaryotes inhabited the Earth from approximately 3–4 billion years ago. Prokaryotes can live in environments that would be deadly to most other organisms. They are able to live and thrive in various extreme habitats. Archaeans for example, live in areas such as hydrothermal vents, hot springs, swamps, wetlands, and even animal intestines. Until next few billion years, no major changes occurred in morphology or cellular organization of these organisms.

The eukaryotic cells emerged between 1.6–2.7 billion years ago. Eukaryotic cells are called so because they have a true nucleus. Plants, animals and fungi are examples of organisms that are composed of eukaryotic cells. The next major change in cell structure came when bacteria were engulfed by
eukaryotic cells, in a cooperative association called endosymbiosis. The engulfed bacteria and the host cell then underwent co-evolution, with the bacteria evolving into either mitochondria or hydrogenosomes. Another engulfment of cyanobacterial-like organisms led to the formation of chloroplasts in algae and plants.

The fossil record until today says that, the most salient feature of life has been the stability of the bacterial mode and into all future time so long as the earth endures. This is truly the "age of bacteria" - as it was in the beginning, is now and ever shall be.

**Evolution:**

```
<table>
<thead>
<tr>
<th>Life on Earth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hadean Eon</td>
</tr>
<tr>
<td>(4567.17-3800 million years ago)</td>
</tr>
<tr>
<td>Paleozoic Era</td>
</tr>
<tr>
<td>(542-251 million years ago)</td>
</tr>
</tbody>
</table>
```

The basic timeline of Earth is 4.5 billion years old.
<table>
<thead>
<tr>
<th>Years ago</th>
<th>Origin of life</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8 billion years</td>
<td>Prokaryotes (Simple cells)</td>
</tr>
<tr>
<td>3 billion years</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>2 billion years</td>
<td>Eukaryotes (Complex cells)</td>
</tr>
<tr>
<td>1 billion years</td>
<td>Multicellular life</td>
</tr>
<tr>
<td>600 million years</td>
<td>Simple animals</td>
</tr>
<tr>
<td>570 million years</td>
<td>Arthropods (ancestors of insects, arachnids and crustaceans)</td>
</tr>
<tr>
<td>500 million years</td>
<td>Fish and proto-amphibians</td>
</tr>
<tr>
<td>475 million years</td>
<td>Land plants</td>
</tr>
<tr>
<td>400 million years</td>
<td>Insects and seeds</td>
</tr>
<tr>
<td>360 million years</td>
<td>Amphibians</td>
</tr>
<tr>
<td>300 million years</td>
<td>Reptiles</td>
</tr>
<tr>
<td>200 million years</td>
<td>Mammals</td>
</tr>
<tr>
<td>150 million years</td>
<td>Birds</td>
</tr>
<tr>
<td>130 million years</td>
<td>Flowers</td>
</tr>
<tr>
<td>65 million years</td>
<td>Non avian dinosaurs died out</td>
</tr>
<tr>
<td>2.5 million years</td>
<td>Appearance of genus Homo</td>
</tr>
<tr>
<td>200, 000 years</td>
<td>Humans started looking like they do today</td>
</tr>
</tbody>
</table>

Homo Sapiens appeared on the earth recently. Evolution in organisms occurs through changes in heritable Traits. Heritable traits are known to be passed from one generation to the next via DNA, a molecule that encodes genetic information. Humans arose as a result of thousands of linked events.

**Cytochrome ‘c’:**

Cytochrome c (a small heme protein) is heart of life in the oxygen-rich world. Cytochrome c is an ancient protein, which continues its presence along with the evolution of life. An interesting fact is, Cyt c is a highly conserved protein sequence across wide variety of species from unicellular organisms which originated 4 billion years ago to the higher animals which originated recently. There may be possibilities for similar cytochrome c in closely related species. But, even the distantly related species have similar sequences of
cytochrome c. The existence of cyt c in so many different kinds of living things, from bacteria to man, might be the result of evolution.

Cyt c is found loosely associated with the inner membrane of the mitochondrion. Its primary structure consists of a chain of about 100 amino acids. Many higher order organisms possess a chain of 104 amino acids (Strahler, and Arthur. 1997). Cytochrome c is a component of the electron transport chain in mitochondria. The transition of cytochrome c between the ferrous and ferric states within the cell, makes it an efficient biological electron transporter and it plays a vital role in cellular oxidations in both plants and animals. It is regarded as a universal catalyst of respiration, forming an essential bridge between the respirable substrates and oxygen. Its main function in cellular respiration is to transport electrons from cytochrome c reductase to cytochrome oxidase.

Cytochrome c is also an intermediate in apoptosis, a controlled form of cell death used to kill cells in the process of development or in response to infection or DNA damage. Upon release of cytochrome c to the cytoplasm, the protein binds apoptotic protease activating factor. Release of cytochrome c from mitochondria has been established by determining the distribution of cytochrome c in subcellular fractions of cells treated or untreated to induce apoptosis (Bossy-Wetzel, E. et al., 1998; Kharbanda, S. et al., 1997; Kluck, R.M et al., 1997; Yang, J. et al., 1997).

The sequence, structure and function of cytochrome c are similar from bacteria to human. The data suggest that the DNA segment which carries the genetic information of the protein cyt c evolved in bacteria 4 million years ago sustains the genetic information in the living system till today.

**DNA damage**

In contrast to the cytochrome c genetic information stability, DNA damage occurs inside the cell at a rate of 10 lakh molecular lesions per cell per
Each human body contains about trillions of cells. Massive damage will be caused in the cells each day in human system. These lesions cause structural damage to the DNA molecule and can alter the cell’s ability to transcribe the gene that the affected DNA encodes and the lesions can causes harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis.

**Common sources causing DNA damage**

<table>
<thead>
<tr>
<th>Sources of DNA damage</th>
<th>Exogenous sources</th>
<th>Endogenous sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV radiation from sunlight</td>
<td>1. UV radiation from sunlight</td>
<td>1. Oxidative damage by free radicals (Oxygen metabolism)</td>
</tr>
<tr>
<td>Pollution</td>
<td>2. Pollution</td>
<td>2. Replicative errors</td>
</tr>
<tr>
<td>Foodstuffs</td>
<td>4. Foodstuffs</td>
<td>4. Alkylation agent (malondialdehyde)</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>5. Radiation therapy</td>
<td></td>
</tr>
<tr>
<td>i) Ionizing radiation</td>
<td>i) Ionizing radiation</td>
<td></td>
</tr>
<tr>
<td>ii) X-rays</td>
<td>ii) X-rays</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>6. Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>i) Cisplatin</td>
<td>i) Cisplatin</td>
<td></td>
</tr>
<tr>
<td>ii) Mitomycin C,</td>
<td>ii) Mitomycin C,</td>
<td></td>
</tr>
<tr>
<td>iii) Cyclophosphamide</td>
<td>iii) Cyclophosphamide</td>
<td></td>
</tr>
<tr>
<td>iv) Psoralen</td>
<td>iv) Psoralen</td>
<td></td>
</tr>
<tr>
<td>v) Melphalan</td>
<td>v) Melphalan</td>
<td></td>
</tr>
</tbody>
</table>

DNA damage affects the primary structure of the double helix. Therefore the bases themselves are chemically modified. These modifications disrupt helical structure of DNA by introducing non-native chemical bonds that do not fit in the standard double helix. DNA is, however, supercoiled and wound around "packaging" proteins called histones (in eukaryotes), and both superstructures are vulnerable to the effects of DNA damage.
DNA damage can occur in either of the DNA strand. The replication of damaged DNA before cell division can lead to the incorporation of wrong bases complementary to the strand. Therefore, the daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable.

**Types of DNA damage**

DNA damages are caused either spontaneously via deamination, depurination, tautomerism called as spontaneous mutation or induced by certain chemicals such as hydroxyl amine, alkylating agents, intercalating agents (ethidium bromide) or by radiations called as induced mutations.

![DNA damage diagram]

<table>
<thead>
<tr>
<th>DNA damage</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td></td>
</tr>
</tbody>
</table>

i. Depurination (Loss of purine base)  
ii. Deamination  
iii. Tautomerism  
iv. Slipped strand mispairing nitrosourea  

i. Chemicals  
a. Hydroxyl amine  
b. Base analogs (eg. BrdU)  
c. Alkylating agents (N-ethyl-N-  
d. Intercalating agents (Ethidium bromide)  
e. DNA cross linkers  

ii. Radiations  
a. Ultraviolet radiation  
b. Ionizing radiation  
c. Radioactive decay (\(^{14}\)C)  

iii. Viral infections

In this hazardous environment, the DNA persists and attains the greatest stability. Hence the studies of DNA integrity and maintenance are more interesting.
Sickle cell anemia

Sickle Cell Anemia was the first genetic disease to be characterized at the molecular level. It occurs due to the mutation of only one nucleotide DNA out of the three billion in each human cell. Yet it is enough to change the chemical properties of hemoglobin, the iron and protein complex that carries oxygen within red blood cells.

![Figure 3. Normal and sickle shaped RBCs.](image)

Figure 3. Normal and sickle shaped RBCs.

There are approximately 280 million hemoglobin molecules in each red blood cell (RBC). The protein portion of hemoglobin consists of four globin subunits: two alpha and two beta chains. In normal Haemoglobin A, glutamic acid is on the 6th position of the beta chain while in sickle-cell disease, this glutamic acid is replaced by valine leading to the formation of sickle cells. Haemoglobin A with this mutation is referred to as Haemoglobin S (HbS). The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GTG codon mutation, becoming a GUG codon by transcription. Normal red blood cells are disc-shaped and can move easily through blood vessels. Sickle hemoglobin causes the cells to develop a sickle, or crescent, shape. The sickled red blood cells are fragile and prone to rupture. They tend to block blood flow causing tissue and organ damage and pain. Healthy red blood cells typically live 90–120 days, but sickle cells only survive 10–20 day. This results in short supply of RBCs, leading to anemia. The single mutation in the gene causes a very big chances in the physiology of human body. Here DNA damage occurs inside the cell at a rate of 10 lakh molecular lesions per cell per day. Human body contains
trillions of cells. Therefore, the sum of DNA damage in total body per day is unimaginable. But even though the chance of DNA damage is huge, the mutations are rare events. In the living cell, DNA undergoes frequent chemical change, especially S phase of the eukaryotic cell cycle during replication. It has been estimated that in humans and other mammals, uncorrected errors i.e., the mutations occur at the rate of about 1 in every 50 million (5 x 10^7) nucleotides added to the chain. Then how the cells maintain the genomic integrity is a big question.

The reason for such a higher stability of DNA is due to the following factors. 1. The double helix nature of DNA molecule; 2. The cell cycle arrest mechanism which is otherwise called as Checkpoint System; 3. The DNA repair pathways.

Due to the complementary nature of DNA, if any one of the strand is damaged, it has the ability to copy the information from the other strand. The copying of information from one strand to another is done by the DNA repair proteins. There are about 200 of them in the living systems from bacteria to human. For example, the nucleotide exchange repair pathway consists of several proteins to repair the UV induced TT dimer lesions. The pathway and the most of the protein in the pathway are conserved from bacteria to human. In addition, the copying of genetic information is possible only from the complementary strands but also from the sister chromatides by homologous recombination. For example, the protein, Rad51 is a key protein which coats the single strand DNA. The formation of Rad51 protein coated single strand DNA is important for the homologues recombination process which helps to copy the loss of DNA base/or bases from sister chromatides. The protein Rad51 is conserved from bacteria to human. In the bacteria, the protein, is called as Rec. The homologue recombination is the ultimate choice for the cell to repair the DNA damage. Thus complementary strands in the double helix nature of DNA is acting as backup to
copy the lost or damaged bases by DNA repair proteins. In addition, the sister chromatides provides one more backup chance to copy the lost or damaged bases in both of the DNA strands to repair by the recombination.

The structural advantage of DNA molecule with the help of DNA repair proteins provides magnificent stability. In fact, the structural advantage of DNA molecule and the function of DNA repair protein is meaningful if the DNA damage is sensed and the cell cycle is arrested. The cell has facility to sense and arrest the cell cycle upon DNA damage. The process of cell cycle arrest upon the DNA damage is called as Checkpoint System also called as Cell Cycle Arrest System.

The Checkpoint Systems which are present in the cell, have the ability to arrest the cells at different cell cycle stages. If the cells are irradiated by X-ray or UV rays, the DNA is damaged and broken. Then immediately, the cells sense the damage and arrest the cells in various stages of the cell cycle. The ability of cells arrested at G1 is called G1 checkpoint. The arrest of cells during DNA synthesis is called intra-S-Checkpoint. Three more checkpoints have been identified: G2 checkpoint, Spindle Checkpoint and Density arrest Checkpoint. The available time duration during the cell cycle arrest is utilized to repair the damaged DNA and to recruit repair proteins to the damaged DNA. Then the DNA damage is repaired by the DNA repair proteins. After clearing the DNA damage by the help of repair proteins, the Checkpoint would be relaxed and cells starts its journey successfully with corrected genetic material.

If the extent of DNA damage is too heavy and beyond repairable level, then, the Checkpoint System triggers apoptosis which is also called as ‘programmed cell death’. If the cell couldn’t sense the DNA damage, or if the cell couldn’t stop cell cycle for repair process, then the damage DNA bases cannot be
repaired. The cell cycle progression with damaged DNA ultimately results in mutations which cause the disease called cancer.

**Mechanism of Checkpoint**

The important function of checkpoints is to provide sufficient time to repair the DNA damage, which is detected by sensor mechanisms. To arrest the cell cycle, the checkpoint proteins group together. The group of the proteins are called Pathway. Since the proteins in a Pathway pass the signals among them by post translational modification (mostly by phosphorylation and dephosphorylation), Pathways are called as Signal Transduction Pathways. Depending upon the function, the proteins in the Pathways are classified as follows: 1. Sensor, 2. Mediators, 3.Transducer, and 4.Effector. The different functions of Checkpoint Proteins are listed in Table 1. The sensor senses the damage in the DNA molecules. Example for sensor in the ATM signal transduction pathway is ATM, Mre11-NBS1-Rad50. The four proteins as a complex are responsible for sensing the DNA damage. In intra-S-checkpoint, Rad17, Rad1, Rad9, Rad26, and Hus1 (Abraham, 2001) proteins senses the damaged DNA. Rad1, Rad 9 and Hus 1 form a trimeric complex in intact cells . These three protein complex encircles the DNA at or to the nearer site of damage to form a Checkpoint Sliding Clamp (CSC) (Venclovas and Thelen, 2000).These protein complex play a role to recruit DNA polymerase also to signal the activation of downstream DNA damage Checkpoint Proteins. Apart from CSC and Rad 17, checkpoint protein Rad 26 has also been implicated as DNA damage sensor. It senses the damage upstream of the DNA. Recently it was found out that for the activation of both G1/S andG2 DNA damage checkpoints requires hRad 17 which undergo phosphorylation by ATR (Bao et al.,2001) . Another one protein ATRIP which is a cloned human protein also act as sensor and has a role in G2/M checkpoint (Cortez et al., 2001).
The sensed DNA damage by the protein complex is mediated by chromatin remodeling proteins. For example, H2AX, MDC1 and Cep164 are mediators in the pathway (Sudhakar et al., 2008). Then, the DNA damage signal are propagated by group of proteins which are called as Transducers. Examples for the transducers are Chk2 and Cdc25A in the ATM signal transduction pathway. Then, the propagated signals were executed by groups of proteins which are called as effectors. In the pathway, the cyclin and p53 are the example for the effectors. The effectors completes the function. Upon DNA damage, the Cyclin stops the cell cycle (Johnson and Walker, 1999). If the damage is heavy, then P53 triggeres the apoptosis process.

Table 1: The different functions of checkpoint proteins

<table>
<thead>
<tr>
<th>Sensors</th>
<th>Transducers</th>
<th>Effectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. ATR, ATRIP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Flow chart 1: The ATM signal transduction arrests cell cycle at G1 phase

Radiation causes double strand break

Sensor proteins (ATM, Mre11-NBS1-Rad50)

Mediators (H2AX, Mdc1 and Cep164)

Transducers (Chk2 and Cdc25A)

Cyc/cdk → P53

Cell Cycle arrest → Apoptosis
Table 2: Cell cycle checkpoint proteins

<table>
<thead>
<tr>
<th>Replication factors and associated proteins</th>
<th>Humans</th>
<th>Budding Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polα-Primase</td>
<td>Polç-Primase</td>
<td></td>
</tr>
<tr>
<td>Polε(^a)</td>
<td>Pole</td>
<td></td>
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<tr>
<td>RFCs2-5</td>
<td>RFCs2-5</td>
<td></td>
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<tr>
<td>RPA</td>
<td>RPA</td>
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<tr>
<td>TopBP1(^a)</td>
<td>Dpb11</td>
<td></td>
</tr>
<tr>
<td>ATR (ATM and Rad 3-related)</td>
<td>MEC1</td>
<td></td>
</tr>
<tr>
<td>ATM (Ataxia Telangiectasia Mutated)</td>
<td>Tel 1(^a)</td>
<td></td>
</tr>
<tr>
<td>ATRIP (ATR-interacting Protein)</td>
<td>Ddc/Pie1/Lcd1</td>
<td></td>
</tr>
<tr>
<td>Rad 17</td>
<td>Rad24</td>
<td></td>
</tr>
<tr>
<td>Rad9</td>
<td>Ctf18/Chl 12</td>
<td></td>
</tr>
<tr>
<td>Rad1</td>
<td>Rad17(^a)</td>
<td></td>
</tr>
<tr>
<td>Hus1</td>
<td>Mec3(^a)</td>
<td></td>
</tr>
<tr>
<td>Claspin</td>
<td>Mrc1</td>
<td></td>
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<tr>
<td>BRCA1,53BP1(^a)</td>
<td>Rad9(^a)</td>
<td></td>
</tr>
<tr>
<td>Chk2</td>
<td>Rad53</td>
<td></td>
</tr>
<tr>
<td>Chk1</td>
<td>Chk1(^a)</td>
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</tbody>
</table>

The above Table gives the list of proteins which play a role in Cell Cycle Checkpoint Pathways. These proteins are conserved from yeast to humans. Hence for the studies of Checkpoint, researchers use yeast as model. Any finding in checkpoint area in the yeast system, can be directly useful to understand the Human Checkpoint System because the Checkpoint System are very conserved from yeast to human. Complete understanding of checkpoint system is the only way to find out remedy for cancer.
The cell cycle can be arrested at G0 stage by density arrest. The primary cell grows in tissue culture plate as a monolayer. When the cells occupy the entire area of the plate, the cells face the space problem to grow further. The space problem causes stress to the cells and the cells activates the checkpoint which is called G0 checkpoint or density arrest checkpoint. Here the cell density triggers the over expression of p53 and also triggers the phosphorylation of p53 at serine 15 and 20 by ATM. The phosphorylation at p53 molecule disturbs the binding of p53 with Mdm2 molecule. The disassociation of the p53 from mdm2 protein triggers the protein p53 sequestration from cytoplasm to nucleus. In the nucleus, the protein p53 forms teramer which can bind with DNA and triggers expression of more than 200 genes. Among them, p21 is important protein. The protein p21 blocks the function of cyclin D and cdk complex which can phosphorylate retinoblastoma (Rb) protein. Rb phosphorylation is important to move cell cycle from G0 to G1 stage. Since the cyclin D/Cdk complex is inhibited by p21 upon density stress, Rb phosphorylation is not possible and hence the cells are arrested at G0 stage. The Rb is the first tumor suppressor reported in the field of cancer biology. The flow chart of the Rb pathway has been illustrated in the chart 2.

**Flow chart 2: Cell Cycle Checkpoint Pathways**

1. **G0 arrest**

   ![Flow chart of G0 arrest](image-url)
In addition, to explain the checkpoint system, the ATR signal transduction pathway can be taken. Here the protein ATR phosphorylates its binding partner ATRIP upon the DNA damage induced by UV light, Then, the complex phosphorylates varieties of following proteins to arrest the cells at intra-S phase and G2/M phase of cell cycle: H2AX, Mdc1, Cep164, RPA subunits, Rad17, Hus1, Rad10, and Chk1. The signal transduction pathway of ATR protein has been illustrated in the flow chart 3. The phosphorylation of Mdc1, H2AX and Cep164 triggers the chromatin remodeling by which the cell unwind the chromatin region where the DNA is damaged. Then, the phosphorylated RPA subunits coats the single strand DNA. Then the Protein Chk1 is phosphorylated. The Chk1 protein then phosphorylates the protein Cdc25c which is a phosphotase. Usually the phosphotase removes the phosphate from the protein complex of Cyclin b/cdc2. Upon DNA damage, the phosphorylated Cdc25C is sequestered to cytoplasm from nucleus. Then the protein is degraded in cytoplasm. In the absence of the protein, Cdc25C, the dephosphorylation of Cyclin b /Cdc2 is possible. The phosphorylated Cyclin b/Cdc2 complex arrest the cell cycle at G2/M phase and intra-S phase.

2. **S phase delay**

\[
\text{ATR/ATRIP activation} \\
\downarrow \\
\text{Mdc1}^P, \text{H2AX}^P \text{and Cep164}^P \\
\downarrow \\
\text{RPA coated Single strand DNA} \\
\downarrow \\
\text{Chk1}^P \\
\downarrow \\
\text{degrade} \\
\downarrow \\
\text{Cdc25A}^P \\
\downarrow \\
\text{Cdk1/Cyclin A} \\
\downarrow \\
\text{S phase delay}
\]
Mutation in Checkpoint Genes

Mutation in gene occurs due to many factors like radiation, chemicals or due to certain endogenous sources. These agents induce mutation either by modifying the DNA or cause chromosomal damage. Mutation occurs anywhere across the genes and results in cancer. For example, a mutation may alter the conformation of a cyclin protein and result in unregulated progression of the cell cycle. Also cell cycle checkpoint defects result in genomic instability and have been associated with cancer predisposition. The accumulation of mutation in the somatic cell causes cancer and in embryonic stages, the mutations causes the defective child birth.

1. Somatic mutation

Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. Somatic mutation plays a key role in transforming normal cells into cancerous cells. This accumulation of alterations can cause cancer or other diseases. The spread of somatic mutations depends on the mutation rate, the number of cell divisions in cellular lineage, and the nature of competition between different cellular lineages. A good example for somatic mutation is breast cancer which is associated with two abnormal genes: BRCA1 (BReast CAncer gene one) and BRCA2 (BReast CAncer gene two). BRCA 1 and BRCA2 proteins has major role in the activation of the checkpoint pathway and also in repairing DNA damage. The genes don't function normally and then normal cell cycle arrest is not possible upon DNA damage. Then there is a risk for getting breast cancer.

2. Embryonic mutation

Mutation occurs in germ cells and it passes to children. The mutation in the embryonic stage causes defective child birth. The mutation in the checkpoint protein at embryonic stages triggers the aborting by dissolving the embryo. For
example, the genes Chk1, Chk2, ATR etc are important for the normal embryogenesis. The functional defect in the gene triggers apoptosis of cells at the embryonic stage. Thus the checkpoint pathways help to maintain the integrity of genome with the help of DNA repair proteins and structural advantages of DNA molecule.

References:


ECOLOGICAL DEMOCRACY FOR SUSTAINABLE LIVELIHOODS IN RURAL AREAS

Dr. G. Poyya Moli*

Background:

Two-thirds of India’s people depend on agriculture for a living, 65% of Indian population in rural areas & 55% of the population rely on agri based livelihood - very limited external support, technological/financial/market/information access- 42-72 % of BPL live in rural India (<Rs 447-600/month).

Multi-Functionality Farming “not only produces food but also sustains rural landscapes, protects BD, generates employment, and contributes to the viability of rural areas

Ecosystem Services (ESS) = Benefits People Obtain from Ecosystems

- Provisioning Services – Food, Freshwater, Wood Fuel, Timber, Fiber
- Genetic Resources
- Regulating Services, Climate Regulation, Flood Regulation, Disease Regulation, Water Purification
- Cultural Services- Aesthetic, Spiritual, Educational, Recreational, Social Relations
- Supporting Services- Soil Regeneration, Photosynthesis, Primary Production, Nutrient Cycling

There are closer /inseparable bio-cultural linkages between Agriculture, ESS & Rural Livelihoods. But GDP growth was around 8 to 9% per annum in the period 2004-05 to 2007-08. Investment and savings rates were quite high - 32 to 36%. GDP did not measure Environmental Degradation. Rapid developments in Industrialization, FE, IT, and stock market & improvements in telecommunications etc. did not benefit the rural poor. However, exclusion continued in terms of low agriculture growth, low quality employment growth, low human development, rural-urban divide

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gender and social inequalities, and regional disparities etc, hunger, unemployment & poverty traps and suicides.

**Indian Agriculture- emerging problems**
- Urbanization, industrialization, SEZ, STZ...break down of local VLIs →
- Shrinking land resources, declining water resources & degraded CPrs/env. → Declining agri. Prodn. - migration to peri-urban/urban areas
- Desertification, water logging … → Soil health problem - shortages of plant nutrients, scarcity of chemical fertilizers & demand for org. farming
- Higher/escalating costs of Inputs (energy & materials) Vs stagnant prices for agricultural products vs perverse subsidies
- Fragmentation of land holdings – 81% small farmer landholdings + 12% medium; 40%/20% area under SF /MF
- Climate change - most vulnerable areas- Western Rajasthan, Southern Gujarat, MP, Maharashtra, Northern Karnataka, Northern A.P. & Southern Bihar
- Market imperfection (stagnating/fluctuating market ) & inadequate credit availability & middlemen – debt burden/poverty traps/ suicides.

Technological requirements of resource-poor farmers

Innovation characteristics important to poor farmers- Input saving & cost reducing, Risk reducing, Expanding toward marginal-fragile lands, Congruent with peasant farming systems, Improving nutrition, health & environment

Criteria for developing technology for poor farmers- Based on indigenous knowledge or rationale, Economically viable, accessible & based on local resources, Environmentally sound, socially /culturally sensitive, Risk averse, adapted to farmer circumstances, Enhance total farm productivity & stability.
Principles of Ecological Democracy (ED) - Humility, Reversibility, Precautionary /Prevention, Polluter-pays, Endogenous - self reliance, Public access & participation, Human rights pro-poor approaches & Environmental justice - eco-restoration; thus, by adopting the principles of ED in rural areas, can achieve food/water/energy/health/livelihood security

Goals for ED in rural India

- Food security – through the principles of Agro-Ecology
- Energy security – through RE resources/technologies energy efficient designs
- Water security – through water harvesting/re-use, and co-management of water resources
- Livelihood security – through sustainable livelihood strategies (organic farming, AEIPs, creative cultural industries, micro-enterprises, etc.)
- Health security – through Agro-ecology (quality food), Ecological sanitation...

The connecting links are the Eco-technologies & ICTs through - ED, community participation, self reliance, good governance, etc...International instruments such as Agenda 21, CBD, PRSP, MDGs, etc. strengthen ED

Food Policy Concepts – all are related to ecological democracy

- Food security - “Food that is available at all times, that all persons have means of access to it, that is nutritionally adequate in quantity, quality and variety and it is acceptable within the given culture” (FAO)
- Right to Food – “the right to have regular, permanent and unrestricted access, either directly or by means of financial purchases, to quantitatively and qualitatively adequate and sufficient food corresponding to the cultural traditions of the people …and dignified life free of fear.” (A/HRC/7/5, para 17)- hunger, food insecurity & malnutrition must be prevented by the states.
- Food Sovereignty - “…the right of peoples, communities, and countries to define their own agricultural, labor, fishing, food and land policies which are ecologically, socially, economically &culturally appropriate to
their unique circumstances”-Local autonomy/ governance- local production/consumption

Thus, these principles support ED & Agro Ecology

**The Role of Agrobiodiversity:**

Increase productivity, food security, & economic returns, Reduce the pressure of agriculture on fragile areas & endangered species, Make farming systems more stable, robust, & sustainable, Contribute to sound pest & disease management, Conserve soil & increase natural soil fertility & health, Contribute to sustainable intensification, Diversify products & income opportunities by reducing/spreading risks to individuals & nations, Reduce dependency on external inputs by optimal use of local resources, Improve human nutrition & provide sources of medicines & vitamins, Conserve ecosystem structure & stability of sp. Diversity- ESS. About 7,000 plant species have been cultivated/collected for food by humans since agriculture began c.12,000 years ago. Today, only c. 15 plant species & 8 animal species supply 90% of our food. This can be changed by restoring the lost traditional crops/cropping systems.

Why alternative approaches?

**Green Revolution Agriculture**

- Hybrid monocultures- loss of traditional germ plasm- GMOs?
- Chemicalization -pollution- fertilizers & pesticides – Soil quality/ biota?
- Over-exploitation/degradation of water/aquifers-Hydrology?- Salinization
- Heavy reliance on fossil fuels & mechanisation– Environmental impacts
- Socio-economic problems- loss of self-reliance– increasing debt burden, poverty spirals/traps, suicides?

To come out of these vicious cycles - organically/ symbiotically link innovations, investment & enterprises -Agenda 21, CBD, PRSP, MDGs.. etc. - Agro-Ecology, LEISA, SRI, Zero budget natural farming (Subash Palekar, Nammalvar…) etc

Technological requirements of resource-poor farmers

- Innovation characteristics important to poor farmers- Input saving & cost reducing, Risk reducing, Expanding toward marginal-fragile lands, Congruent with peasant farming systems, Improving nutrition, health &
environment

- Criteria for developing technology for poor farmers - Based on indigenous knowledge or rationale, Economically viable, accessible & based on local resources, Environmentally sound, socially /culturally sensitive, Risk averse, adapted to farmer circumstances & Enhance total farm productivity & stability.

**Agro-ecology (AE) is an alternative systems of production aimed at:**

- Self reliance, spatial/temporal diversity & continuity
- Sustainable intensification/diversification of products at the farm level to minimize risks & increase synergism; Integrated farming systems to enhance the family nutritional intake/health as well as ecosystem health
- promoting an efficient use of local resources (i.e. land, water, labor, agricultural sub-products, etc. ) by upgrading LKMS
- improving the natural resource base through water, soil and local germ-plasm conservation/Eco-restoration;
- reducing the use of external inputs (INM/ISFM & IPM)) to reduce dependency, but sustaining yields with appropriate technologies
- ensuring that alternative systems have an overall beneficial effect not only on individual families, but on the entire community.

AE will improve the productivity, stability, resiliency & sustainability – Bio-regionalism –natural way of farming based on healthy ESS.

**Application of Panchagavya & Amuthakaraisal**

5 products (dung, urine, milk, curd and ghee) from the desi cow, fermented overnight and 21 days respectively - bio-fertiliser/bio-pesticide for agricultural/horticultural crops Salt affected soils-Panchagavya and Amuthakaraisal for seed /seedling treatment/ storage, foliar spray, soil application along with irrigation water as per the standard suggestions / recommendations.

3 liters of fortified Panchagavya can be mixed in 100 liters of water (3%) or 300ml fortified Panchagavya can be filled in a hand pump of capacity 10 l and can be applied in the morning or in evening.

The main advantages of LEISA based SRI - Yield increase (2 to 3 fold), Reduced number of irrigations (25 – 40 %), Reduced inputs (25 – 30 %), Improved soil quality (no inorganic inputs)- micronutrients, enzymes,, Improved self reliance, Improved seed /yield quality
**Genetically Modified Crops?**

Genetically Modified Crops are modified by Genetic Engineers to produce higher crop yields & increase resistance to drought, cold, heat, toxins, plant pests & diseases. The gene to cause the plant to become sterile after the first year in the genetically modified crop is called the terminator gene.

Environmental Impacts of Genetic Modifications - Large environmental risks because changes are novel (new to nature); novel changes are more likely to have negative impact than changes similar in kind & frequency to those occurring in nature.; Impacts on other organisms, ESS & resilience? Sheep death after eating BT cotton stalk – 7 EU countries banned GM food.

**Is Agro ecology /organic farming/Natural farming really feasible?**

Refer - http://www.i-sis.org.uk/MTWS.php

- 2- to 7-fold energy saving on switching to org. LEISA [17, 25]
- 5 to 15% global fossil fuel emissions offset by sequestration of carbon in organically managed soil [26]
- 5.3 to 7.6 tonnes of carbon dioxide emission disappear with every tonne of nitrogen fertilizer phased out [27]
- 2- to 3-fold increase in crop yield using compost in Ethiopia, outperforming chemical fertilizers [32]
- Organic farms in Europe support more birds, butterflies, beetles, bats, and wild flowers than conventional farms [36]
- Organic foods contain more vitamins, minerals and other micronutrients, and more antioxidants than conventionally produced foods [37-40]
- Reduced leakage due to localised production /consumption- multiplier effect

**Capitalism and Socialism: Two sides of the same materialistic coin**

- The two great modern economic systems, capitalism and socialism, agree on many fundamentals. In fact, the Father of Socialism, Karl Marx said, “the capitalism was good but not good enough”.
- Both capitalism and socialism agree on a basic materialistic view and on the importance of economic growth, large industry, mechanization & unlimited progress.
- They disagree on who controls the means of production in a society. One system preferring to leave the production and distribution in private hands and the other making the state the owner and distributor of wealth on a needs basis.
• Once again increasing centralization and concentration of wealth or power or both are common features of capitalism and socialism. **Both are predatory and violent economies, resulting in imperialism** (of the Anglo-American or the Soviet sort).

• While capitalism makes a virtue out of selfishness and extreme individualism and materialism, actually-existing socialism (as opposed to theoretical Marxist utopias) subordinates the individual to the state by fiat thereby robbing him/her of all individuality.

• But humans do not live by bread alone. **Our economic decisions are embedded in a socio-cultural matrix** that imparts certain values which one may call spiritual or simply human. Indeed, all major religions have something to say about the economic life of man. For example, the injunctions against taking usurious interest in Islam and Christianity.

• Humans can put their community’s interest above their own but the size of the community becomes critical. The nation state is too large for this purpose as the socialist countries discovered. Therefore, **several thinkers have recognized the importance of keeping economies local**. These include Gandhi, Kumarappa and Schumacher. – Imperatives for ED

**Replacing greed with need: Gandhi and Kumarappa**

“The earth provides enough to satisfy every man’s need but not for every man’s greed.” - M.K. Gandhi

JC Kumarappa was advocating “Economy of Permanence”. From the economic point of view, the central concept of wisdom is permanence. We must study the economics of permanence. Nothing makes sense unless its continuance for a long time can be projected without running into absurdities. There can be growth towards a limited objective but there cannot be unlimited, generalized growth = the so called modern principles of SD are borrowed from our forefathers!

The economics of permanence implies a profound reorientation of science & technology, which have to open their doors to wisdom and in fact have to incorporate wisdom into their very structure.

**Rangasamy Elango and the Kuthambakkam Gram Swaraj Project**

Kuthambakkam in the Thiruvallur District, Tamil Nadu, is located about 40 km from Chennai. Of the 1000 families (5000 villagers) living in 7 hamlets, 55% are Dalits living in 2 hamlets. Eight years ago, the village suffered under
riotting between the dalit and non-dalit communities. Infrastructure and sanitary conditions were poor. Kuthambakkam ranked 22nd among 12,619 villages in Tamil Nadu in illicit liquor brewing, employing about 35% of the population.

Rangaswamy Elango was born into a dalit, farmer's family in Kuthambakkam in 1960. A chemical engineer by training, he worked as a scientist at the Central Electro Chemical Research Institute (CECRI). His involvement in a rural reconstruction project, took him back to face rural reality and inspired him to read Gandhian literature. As Elango's life's calling turned stronger, he finally left his job in 1994 and returned to Kuthambakkam for good. He is currently working on establishing a land/agriculture-based local economy in Kuthambakkam, along the lines of the model of J.C. Kumarappa, the Gandhian Economist.

Village Economy in Kuthambakkam: Kumarappa's Model

Elango's plan for his village is inspired by Dr. Kumarappa’s "Economy of Permanence" with a few suitable alterations in keeping with the times. In Kumarappa's words, the objective is "to bring together the consumer and the producer into such intimate relationship as to solidify society into a consolidated mass, which alone can lay claim to permanence". In this model, the villagers who are producers are consumers themselves. By bringing together six neighboring villages into a cluster, many products that are consumed by the villagers can be produced by themselves. Around 50% of the people in a cluster will be producers of these consumables using sustainable technologies.

The rest will be earners by virtue of their skills, age, tradition and interest producing products (mainly handicrafts) to be exported out of the cluster and earn money from outside. Elango has estimated, through a detailed door-to-door survey by his team, that Kuthambakkam consumes Rs.60 lakh worth of commodities every month. The survey covered 50 most commonly used items. He also identified that as much as Rs.50 lakh worth of commodities can be produced within the village and traded among themselves. The objective is to minimize the outflow of money from, and maximize the inflow of money into the Village Cluster Economy.

Plans to revive village economies are always met with skepticism with one of the most immediate responses being "How can our products compete
with those of big corporations?" Elango challenges the foundation of modern economics which says "A healthy economy is all about competition, and being more strategic and faster than the rest", and to relearn that it is more about cooperation and pride.

The rural industries will, to the extent possible, be land- (agro-) based, use locally available raw-materials and indigenous knowledge, cater to the local market; meet the basic needs of villagers - food, clothing and shelter - to attain self-sufficiency, be diverse; work on a cooperative model through men and women SHGs, be heavily dependent on human power & be environment-friendly. Elango has traveled across the country identifying low-cost and appropriate technologies, and is well on his way towards a vibrant, self-reliant village.

**Ecological Democracy – Constraints**

Social, Economic and Institutional Factors, Non authoritative role of environmental activists, Near-total criminalization of power, discretion and administration, Increasing privatization of the Indian States- neocolonialism, Responsibility without power, Overlapping of powers , inadequacy of personnel

**Ecological Democracy - The future**

- Endogenous knowledge creation/revival by and for the people – PLA based action research on farm ‘living campuses’ for sustainable livelihoods- facilitating institutions for horizontal ToT -socio-cultural networks linking with North-South/South-South Agro-ecological/organic food networks, through ED
- Using citizen panels, citizen juries, future scenario workshops and referendums to capture the full diversity of interests in deciding on strategic research and funding priorities, allocating resources and assessing technological risks.
- Opening-up decision-making bodies & governance structures of a wider representation of different actors; greater transparency, equity and accountability in budget allocation &decisions on R&D priorities.
- Reorganising conventional scientific and technological research to encourage participatory knowledge creation and innovations that combine the strengths of farmers and scientists in the search for locally adapted solutions and food systems.
- Ensuring that knowledge, genetic resources and innovations remain accessible to all as a basic condition for ED.
Introduction:

Computer-Assisted Drug Design (CADD), also called Computer-Assisted Molecular Design (CAMD), represents more recent applications of computers as tools in the drug design process. Drug Design is an iterative process that begins when a chemist identifies a compound that displays an interesting biological profile and ends when both the activity profile and the chemical synthesis of the new chemical entity are optimized. Drug design, also sometimes referred to as rational drug design, is the inventive process of finding new medications based on the knowledge of the biological target. The drug is most commonly an organic small molecule which activates or inhibits the function of a biomolecule such as a protein which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the biomolecular target to which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on topology and computer modeling techniques. This type of modeling is often referred to as computer-aided drug design.

What is really meant by drug design is ligand design. Modeling techniques for prediction of binding affinity are reasonably successful. However, there are many other properties such as bioavailability, metabolic half life, lack of side effects, etc. that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to optimize using rational drug design techniques. Traditional approaches to drug discovery rely on a step-wise synthesis and screening program for large number of compounds to optimize activity profiles. Nobody could design a drug before knowing more about the disease or infectious process than past. For "rational" design, the first necessary step is the identification of a molecular target critical to a disease process or an infectious pathogen. Then the important prerequisite of "drug design" is the determination of the molecular structure of target, which makes sense of the word “rational”.

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There are two major types of Drug Design. The first is referred to as ligand-based drug design and the second, structure–based drug design. Structure-Based (SBDD) and Ligand-Based (LBDD) Drug Design are extremely important and active areas of research in both the academic and commercial realms.

**Ligand-based design:**

The Ligand-Based Approach is applicable when the structure of the receptor site is unknown, but when a series of compounds have been identified that exert the activity of interest. To be used most effectively, one should have structurally similar compounds with high activity, with no activity, and with a range of intermediate activities. In recognition site mapping, an attempt is made to identify a pharmacophore, which is a template derived from the structures of these compounds. It is represented as a collection of functional groups in three-dimensional space that is complementary to the geometry of the receptor site. Alternatively, a Quantitative Structure-Activity Relationship (QSAR) in which a correlation between calculated properties of molecules and their experimentally determined biological activity may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs. QSAR model can be used to predict which members of a series of proposed compounds are likely to be active and therefore should be synthesized and tested. As new compounds are assayed, the additional experimental data are used to refine the model. This general approach has been used in the pharmaceutical industry for many years to guide medicinal chemistry efforts.

**Pharmacophore:**

The official 1998 IUPAC definition is as follows: A pharmacophore is the ensemble of steric and electronic features that are necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response. A pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capabilities of a group of compounds towards their target structure. A pharmacophore model can also be derived from a set of known ligands for the target. Traditionally, a pharmacophore is the set of features common to a series of active molecules, where features can include acceptors, donors, ring centroids, hydrophobes etc. A 3D pharmacophore specifies the spatial relationship
between the groups or features, often defining distance ranges between groups, angles between groups or planes and exclusion spheres.

**Structure-based design:**

Structure-Based Drug Design (or **direct drug design**) requires the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or spectroscopy. It is also possible to design ligands for and screen against a homology model for which there is a high degree of confidence. Structure-based design attempts to use the 3D protein structure to predict which ligands will bind to the target. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

**Binding site Analysis:**

Understanding the structure and function of protein binding site is a cornerstone of Structure-Based Drug Design. Developing this understanding requires knowledge of both the location and physical properties of the binding site. It analyzes the protein to find the binding pocket, derives key interaction sites within the binding pocket, and then prepares the necessary data for Ligand Fragment Link. The basic inputs for this step are the 3D structure of the protein and a pre-docked ligand in PDB format, as well as their atomic properties.

**Rational Drug Discovery:**

In contrast to traditional methods of drug discovery which rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value. In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will have therapeutic value. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease states. The second is that the target is "drugable". This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule.

Once a suitable target has been identified, the target is normally cloned and expressed. The expressed target is then used to establish a screening assay. In addition, the three-dimensional structure of the target may be determined.
The search for small molecules that bind to the target is begun by screening libraries of potential drug compounds. This may be done by using the screening assay (a "wet screen"). In addition, if the structure of the target is available, a virtual screen may be performed of candidate drugs. Ideally the candidate drug compounds should be "drug-like", that is they should possess properties that are predicted to lead to oral bioavailability, adequate chemical and metabolic stability, and minimal toxic effects. Several methods are available to estimate drug likeness such Lipinski's Rule of Five and a range of scoring methods such as Lipophilic efficiency. Several methods for predicting drug metabolism have been proposed in the scientific literature, and a recent example is SPORCalc. Due to the complexity of the drug design process, two terms of interest are still serendipity and bounded rationality. Those challenges are caused by the large chemical space describing potential new drugs without side-effects.

Lipinski’s Rule of Five:

*Lipinski’s Rule of Five* is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules. The rule does not predict whether a compound is pharmacologically active. Lipinski's rule says that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular weight not greater than 500 daltons
- An octanol-water partition coefficient \( \log P \) not greater than 5.

These properties can differentiate drugs from other chemicals.

**ADME/T**:

*ADME* is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism, and excretion, and describes the disposition of a pharmaceutical compound within an organism. The four criteria influence the drug levels and kinetics of drug exposure to the tissues and hence
influence the performance and pharmacological activity of the compound as a drug.

**Absorption/Administration:**

For a compound to reach a tissue, it usually must be taken into the bloodstream - often via mucous surfaces like the digestive tract (intestinal absorption) - before being taken up by the target cells. Factors such as poor compound solubility, gastric emptying time, intestinal transit time, chemical instability in the stomach, and inability to permeate the intestinal wall can reduce the extent to which a drug is absorbed after oral administration. Absorption critically determines the compound's bioavailability. Drugs that absorb poorly when taken orally must be administered in some less desirable way, like intravenously or by inhalation (e.g. zanamivir).

**Distribution:**

The compound needs to be carried to its effectors’ site, most often via the bloodstream. From there, the compound may distribute into tissues and organs, usually to differing extents. After entry into the systemic circulation, either by intravascular injection or by absorption from any of the various extracellular sites, the drug is subjected to numerous distribution processes that tend to lower its plasma concentration.

Distribution is defined as the reversible transfer of a drug between one compartment to another. Some factors affecting drug distribution include regional blood flow rates, molecular size, polarity and binding to serum proteins and forming a complex. Distribution can be a serious problem at some natural barriers like the blood-brain barrier.

**Blood-Brain Barrier:**

Drugs that act in the CNS need to cross the Blood-Brain Barrier (BBB) to reach their molecular target. By contrast, for drugs with a peripheral target, little or no BBB penetration might be required in order to avoid CNS side effects.

**Metabolism:**

Compounds begin to break down as soon as they enter the body. The majority of small-molecule drug metabolism is carried out in the liver by redox enzymes, termed cytochrome P450 enzymes. As metabolism occurs, the initial (parent) compound is converted to new compounds called metabolites. When
metabolites are pharmacologically inert, metabolism deactivates the administered dose of parent drug and this usually reduces the effects on the body. Metabolites may also be pharmacologically active, sometimes more so than the parent drug.

**Excretion/Elimination:**

Compounds and their metabolites need to be removed from the body via excretion, usually through the kidneys (urine) or in the faeces. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism. There are three sites where drug excretion occurs. The kidney is the most important site and it is where products are excreted through urine. Biliary excretion or fecal excretion is the process that initiates in the liver and passes through to the gut until the products are finally excreted along with waste products or faeces. The last method of excretion is through the lungs e.g. anesthetic gases.

**Toxicity:**

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ (organotoxicity), such as the liver (hepatotoxicity). Toxicity is responsible for many compounds failing to reach the market and for the withdrawal of a significant number of compounds from the market once they have been approved. It has been estimated that approximately 20-40% of drug failures in investigational drug development can be attributed to toxicity concerns.

**Molecular Docking:**

In the field of modeling, Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular Docking is a key tool in structural biology and computer–assisted drug designing.

It is usually performed between a small molecule and a target macromolecule. Molecular Docking can be thought of as a problem of “lock-and-key”, where one is interested in finding the correct relative orientation of the “key” which will open up the “lock” (where on the surface of the lock is the key hole, which direction to turn the key after it is inserted, etc.). Here, the
protein can be thought of as the “lock” and the ligand can be thought of as a “key”. Molecular Docking may be defined as an optimization problem, which would describe the “best-fit” orientation of a ligand that binds to a particular protein of interest.

Understanding the interactions between proteins and ligands is crucial for the pharmaceutical and functional food industries. The experimental structures of these protein/ligand complexes are usually obtained, under highly expert control, by time-consuming techniques such as X-ray crystallography or NMR. These techniques are therefore not suitable for routinely screening the possible interaction between one receptor and thousands of ligands. To overcome this limitation, computational algorithms (i.e. docking algorithms) have been developed that uses the individual structures of the receptor and ligand to predict the structure of their complex.

**Conclusion:**

A very important part of Drug Design is prediction of small molecule binding to target macromolecules. De novo drug design is an iterative process in which the three dimensional structure of the receptor is used to design a newer molecules. It involves structure determination of lead target complexes and the design of the lead modification using different tools. It can also be used to design new chemical classes of compounds that present similar substituent to the target. Pharmaceutical drug development using computer design technique inherently requires complex drug design.
THE BARCODE OF LIFE INITIATIVE

Prof. S. Ajmal Khan*

The Barcode of Life Initiative is based on a simple, but powerful premise. It argues that sequence diversity in short, standardized gene regions (also known as DNA barcodes) can provide a sophisticated tool for both the identification of known species and the discovery of new ones. Furthermore, by shifting the activity of species identification from the analogue character arrays used by traditional taxonomy to a system based on digital DNA characters, barcoding promises automated identifications. This advance will massively improve our capacity to monitor and manage biodiversity with profound societal and economic impacts. Additionally, DNA barcoding promises newly detailed insights into important biological processes as a direct result of its survey of sequence diversity in a standard gene region across broad swaths of life.

Since its inception at the University of Guelph in 2003, the Barcode of Life Initiative has gathered considerable momentum, gained extensive international participation, and captured the attention of the scientific community, government agencies, and the general public. Reflecting the deployment of human resources and research infrastructure, barcode data have now been obtained from more than 235,000 specimens (representing nearly 27,000 species). In short, we are witnessing a rapidly rising tide of barcode data that promises a major advance in our ability to identify and discover species and to manage their economic and societal impacts. We emphasize that a barcode region has been designated for the animal kingdom (a 648 bp region of the mitochondrial COI gene) and that studies on varied animal groups have established its effectiveness in species identification. While work on other kingdoms of life is less advanced, early results on protists and fungi have shown

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that the same region of COI shows high promise as a barcode target. Because of their slowed rates of mitochondrial evolution, a different region for plants will be required. However, work on selection of target gene(s) is well advanced.

The primary goal is to develop an accurate, rapid, cost-effective, and universally accessible DNA-based system for species identifications. Once fully implemented, this system will revolutionize access to biological information and will exert broad impacts on research, policy, pest and disease control, food safety, resource management, conservation, and many other areas in which society interacts with wild biodiversity. We emphasize that ecosystem health has critical impacts on all national economies. At this time, increasing globalization of trade and climate change mean that, nations are facing unprecedented exposure to new species which threaten their agriculture, forestry and fisheries. A DNA barcoding system will enable the prompt diagnosis of invasive species, thereby allowing quarantine and eradication efforts to begin years earlier with massive reductions in cost and increased chances of success. The same strategy extends to the selection of optimal control strategies for pest species impacting the varied natural resource sectors. Barcoding can, as well, play a critical role in regulating trade in endangered or protected species or products. It will also contribute importantly to the implementation of the recognized need for management at an ecosystem rather than species-level. In the latter context, as massively parallel sequencing technologies rise, the sequence libraries assembled by barcoding will enable newly sophisticated environmental monitoring that will exploit living organisms as integrators of environmental change or as early warnings of damage. For example, we can look forward to a time when large-scale, automated monitoring of species presence and abundance in the world’s oceans, inland waters, agro-ecosystems, and plantations is routine. In a very real sense, biotic monitoring will move from the canaries that probed air quality in 19th century mines to global biotic surveys that will register planetary health in the 21st century.
The Alfred P. Sloan Foundation has granted $669,000 to the Smithsonian Institution to establish the Consortium for the Barcode of Life (CBOL), an international initiative devoted to promoting the growth and use of DNA barcoding. CBOL’s Secretariat is located in the National Museum of Natural History in Washington, DC. Since CBOL’s launch in May 2004, more than 125 institutions from 40 countries on six continents have become Member Organizations by signing a Memorandum of Cooperation and agreeing to deposit their barcode data in a public repository. The members include museums, herbaria, zoos, biodiversity research institutes, universities, conservation organizations, government agencies and private companies. CBOL has held one international conference and supports several Working Groups that improve the scientific and technological basis for barcoding. Several regional meetings in South America, Africa and Asia are being planned to increase the participation of developing countries in DNA barcoding activities. CBOL’s Working Groups are exploring research issues linked to barcoding: biodiversity database architecture, protocols for sequence acquisition, data analysis, and technology advancement.

The Global Taxonomy Initiative works to overcome “the taxonomic impediment” — the lack of data concerning Earth’s biodiversity which limits our ability to manage living resources in a sustainable and responsible manner. The shortage of trained taxonomists and access to the essential information resources (especially museum and herbarium collections, taxonomic publications, databases on the Web) are most acute in developing countries where biodiversity is highest. DNA barcoding has the potential to increase access to taxonomic knowledge in all regions of the world. Databases of reference barcodes are connecting specimens to their correct species names, providing a direct route to species information associated with those names. CBOL is working with GenBank and its partner DNA repositories (European Molecular Biology Laboratory (EMBL) and DNA Data Bank of Japan (DDBJ))
to construct a global library of reference barcode sequences. Each barcode record is linked to a voucher specimen in a collection, a valid species name, and the associated taxonomic literature. Connections are being built to the Global Biodiversity Information Facility (GBIF) and other biodiversity data portals. Through these efforts, an integrated information infrastructure for taxonomy is growing rapidly.

The barcode research does not simply promise an upgraded understanding of biodiversity; it will also provide important insights into evolutionary processes. The barcode region for animals, a segment of the mitochondrial cytochrome c oxidase I (COI) gene, is a sentinel for shifts in nucleotide composition and in rates of evolution across the mitochondrial genome. Measures of sequence divergence in the barcode region further correlate with those in the nucleus, meaning that barcode data provide contextual information that is valuable in selecting taxa for other investigations. As a result, the immense horizontal survey of sequence diversity executed by DNA barcoding will deliver new insights into the factors modulating rates of molecular evolution. It will also provide the most densely parameterized record of sequence information for any gene, enabling delicate investigations into pathways of COI protein evolution. The barcode protocol involves the storage and curation of DNA extracts, creating an invaluable resource for studies that seek to investigate evolutionary divergence in other gene regions.

The analytical chain — Methods of barcoding assembly

Barcoding research requires the procurement of numerous DNA sequences from a wide array of taxa. In order to examine the ever-growing number of the world’s species that are included in this survey, a variety of methods and techniques have been employed.

Because the aim of DNA barcoding is to provide reference sequences from expert identified voucher specimens the act of collecting should always be
accompanied by thorough documentation. This can take many forms, and not every form of documentation is desirable or even possible for each collecting event.

The following data elements are recommended for BOLD:

- Collectors
- Collection Date (dd-mmm-yy)
- Locality: Latitude and longitude using the World Geodetic System 1984 (WGS 84), and coordinates are in degree decimal-degree format (e.g. 72.098, -114.84), and the FA0 region.
- Elevation/Depth in meters
- If possible collection gear, (sampling method/effort), vessel (notes field)
- Notes on habitat, microhabitat, and associations (notes field)
- Sex of specimen
- Life stage (adult, juvenile)
- Comprehensive information on the institution where specimens are vouchered and accession/catalogue number
- ID
- Identifier

Identification

Error rates in museum collections and catalogues can be significant. Records in BOLD include an ‘identified by:’ field and wherever possible, the name of the person associated with a given identification should be captured. To be thorough, the taxon concept used by the identifier can also be recorded if available (i.e. original description, field guide, etc). The notes field provided by BOLD can host this and other additional information.

Taxonomy

If the species is unknown, please type in genus only (in the “Genus” field) and leave the “Species’ field blank; If the species is not in FishBase/ITIS,
but is a valid name accepted by alternative taxonomic authorities/checklists, you may wish to include it as is (along with the authority/reference for that name), however, this will have to be discussed with your Regional Chair and also the BOLD campaign manager, as a conduit to FishBase in order to maintain uniform naming throughout BOLD and the community. If a species does not have a valid name (e.g., under description or provisional morphospecies) the species epithet, ideally, should be composed of the author’s initials, “sp.” and an alphabetic or numeric index. An example: “Hyla SL sp. A”. This format follows the recommendations for provisional species names of NCBI GenBank:

For tentative ID’s please use “cf.” (=conformis) between genus and species epithets, e.g., “Hyla cf. arborea”. Please note that (aside from the cases specified above) the “Species” field should contain a full binomen or trinomen, if applicable.

**Specimen Imaging**

BOLD encourages image submission. It can be helpful in sorting out misidentifications and should be included whenever possible particularly for large or small specimens that are not easily vouchered morphologically. Because bright colors of many specimens fade rapidly after death, photographs should be taken as soon as possible after collection and prior to fixation if possible. While imaging dorsal, lateral and ventral views are critical, knowledge of the diagnostic features of a particular taxon facilitate capture of additional, more detailed photographs that are often critical for identification purposes. For example, for some specimens, close-up views of the body parts are also useful.

Appropriate prerequisites for a digital image associated with a DNA barcode:

- Image format 4x3 to ensure optimal representation in most databases (640 x 480 pixels).
- The initial resolution can be higher (600-1200 dpi) to provide a good source for additional, more detailed views. Images submitted to BOLD
will be automatically reduced to screen resolution. Though submission of high resolution images are encouraged.

- References to scale (ruler) and color (color bar or color wheel) are required in every image.
- Save the high-quality images of the specimen in jpg format.
  - The high-quality images of a specimen should be submitted to BOLD in a package (compressed file, e.g. zip-format) consisting of all image files and a spreadsheet with the file names and ancillary data. Make sure that all images in the package are accounted for in the spreadsheet. The submission spreadsheet should be named ImageData.xls and contain the following columns:
    - Image file name including extension
    - Original file (yes/no)
    - View Metadata (nature of image)
    - Notes (additional info)
    - Measurement (including unit), e.g. body length
    - Measurement type (measured item)
    - Sample Id (specimen number given by the submitter)
    - Process Id (specimen number given by the BOLD system). To get this process ID
    - specimen data have to be submitted prior or parallel to images.

**DNA Sampling**

The current best practice in genetic resource collection involves a system of redundancy: in an ideal situation, two archival quality tissue samples will be immediately collected from each specimen, one frozen to preserve the broadest array of molecular characters possible and one placed into a preservation fluid, such as ethanol, to serve as a back up in case of a meltdown or loss of the frozen
specimen. Several tissues are suitable for DNA extraction from fishes. These include the following.

- **Musculature**: remove one or more cubes (5-7 mm) of lateral muscle
- **Gill tissue**
- **Insect or crustacean leg or leg parts**
- **Eye**: remove one eye from extremely small specimens such as larvae.
- **For species with small body size**, entire specimens can be placed in preservative in lieu of sub-sampling. This should be avoided unless a series of conspecifics are available for fixation in formalin for standard morphological analysis.

Tissues samples for DNA extraction should be frozen or preserved in fresh 95% ethanol and stored in a cool place, preferably in a freezer. Large pieces of tissue should be cut into small pieces (<5-7 mm) to permit adequate fluid penetration. Concerns exist about whether ethanol-preserved collections more than 10-20 years old have suffered DNA degradation. Freezing EtCH preserved tissues might minimize this problem. However, alcohol varies in several ways, (e.g. hydration levels, possible contaminants). For shipment airtight seals on containers are critical for minimizing evaporation and/or hydration of the sample. The volume of EtCH to specimen is also an important consideration with a threefold or higher relative volume of EtCH to tissue desirable. Ultimately, EtCH is flammable and difficult to transport. Other preservatives are DNA-friendly such as RNA Later (Ambion), lysis buffer (Seutin et al. 1991), and FTA® databasing paper.

Surplus DNA extracts from barcoding could be archived at participating sequencing facilities using this platform to voucher the sequence run. However, this does not overcome the need for archiving morphological voucher specimens or tissue samples required for further comparative genetic analysis to
confirm the authenticity of a suspect DNA extract and/or associated barcode sequence.

**DNA Barcoding**

**DNA Extraction**

Currently, two standard protocols are recommended - a simple proteinase K digestion for specimens in good condition with extraction utilizing silica-based columns such as PALL plates and an extraction kit (GenElute Mammalian Genomic DNA Miniprep Kit - Sigma Genosys,) protocol for old or degraded specimens. Additional information on automated high throughput protocols is available at [www.dnabarcoding.ca](http://www.dnabarcoding.ca).

A standard protocol for COI amplification includes a 25 u1 PCR reaction mix:

- 18.75 u1 of ultrapure water,
- 2.25 u1 of 10x PCR buffer,
- 1.25 u1 of MgCl2 (50 mM),
- 0.25 u1 of each primer (0.01 mM),
- 0.125 u1 of each dNTP (0.05mM),
- 0.625 U of Taq polymerase,
- 0.5—2.0 u1 of DNA template.

Approximately 655 bp can be amplified from the 5’-region of the COI gene from mitochondrial DNA using an appropriate primer combination.

The standard thermal regime consists of:

- an initial step of 2 min at 95°C
- followed by 35 cycles of
  - 0.5 min at 94°C,
  - 0.5 min at 54°C
  - 1 min at 72°C
- followed in turn by 10 min at 72°C and then held at 4°C
An alternative protocol involves the use of a multiplex primer cocktail with M13 tailed primers.

PCR products are visualized on 1.2% agarose gels and visible products are selected for sequencing. Sequence information can be uploaded on BOLD for several specimens at once in FASTA format. The FASTA header line must conform to the following format: it should begin with a ‘>’ followed by the Process ID, followed by either a bar (‘|’), an underscore (‘_’) or a space (‘ ‘), followed by any other information the user wishes to add. There can be no spaces before the end of the Process ID. Trace files and phred scores can be uploaded in ab 1 and phd format using a routine similar to the image submission.

**The Barcode of Life Database (BOLD)**

The Consortium for the Barcode of Life (CBOL) was launched in May 2004 and now includes more than 120 organizations from 45 nations. CBOL is fostering development of the international research alliances needed to build, over the next 20 years, a barcode library for all eukaryotic life. It has already initiated the first campaigns with a global sweep; they seek to deliver barcode coverage for all species of birds and fishes by 2012. Although these two projects will generate some 0.5 million records, a comprehensive barcode library for the animal kingdom will be much larger, ca. 100 million records — almost twice the current size of GenBank (52 million sequence records as of 7 March 2006). This potential volume of data makes clear the need for enterprise-scale software to support novel aspects of DNA barcoding, a realization that motivated development of the Barcode of Life Data System. Key features include the requirement for a persistent linkage between a barcode sequence and its source specimen and a secure environment that stores, organizes and queries these records, accessible to the entire biodiversity community. There is also a need to establish and enforce data standards. To meet these challenges, CBOL
initiated dialogue with the major genomics repositories [e.g. National Center for Biotechnology Information (NCBI)], biodiversity organizations [e.g. Global Biodiversity Information Facility (GBIF)], major barcoding centres and the multiple taxonomic communities. These joint consultations have now led to the establishment of formal guidelines that must be met for records to gain barcode designation. Gene sequences must derive from a designated gene region, they must meet quality standards and they must derive from a specimen whose taxonomic assignment can be reviewed, ordinarily through linkage to a specimen that is held in a major collection.

The Barcode of Life Data System (BOLD) — [www.barcodinglife.org](http://www.barcodinglife.org) provides an integrated bioinformatics platform that supports all phases of the analytical pathway from specimen collection to tightly validated barcode library. First, it is a repository for the specimen and sequence records that form the basic data unit of all barcode studies. Second, it is a workbench that aids the management, quality assurance and analysis of barcode data. Third, it provides a vehicle for collaboration across geographically dispersed research communities by coupling flexible security and data entry features with web based delivery.

BOLD was initially developed as an informatics workbench for a single, high-volume DNA barcode facility. It has evolved into a resource for the DNA barcoding community, as evidenced by its adoption for the first major barcode campaigns (birds, fishes, Lepidoptera). It has, as well, been selected by the Canadian Barcode of Life Network ([www.bolnet.ca](http://www.bolnet.ca)) for its campaign to barcode all eukaryotic life in that nation. Although BOLD aids the assembly of barcode data and maintains these records, a copy of all sequence and key specimen data also migrate to NCBI or its sister genomic repositories [DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL)] as soon as results are ready for public release. As such, BOLD is one of a spectrum of ancillary sites that provide the biological science community with
specialized services that cannot be delivered by the global sequence databases, which were designed for a different mission — the assembly and organization of all sequence records. Access to BOLD is open to any researcher with interests in DNA barcoding; computational resources and personnel are available to sustain its primary site until 2011. The prospects for extended support, as well as funding to establish regional data nodes and mirror sites, will strengthen as usage grows.

Some features on BOLD, such as access to data in public projects and use of the Identification System, are available to any visitor. However, system registration (which only involves the provision of contact details) grants additional privileges, such as the ability to create private projects and to share access to password-protected data. The core data element in BOLD is a biphasic record consisting of a specimen page and a sequence page. The specimen page assembles varied collateral data, including the date and location of capture, as well as from one to several image(s) of each specimen. When spatial coordinates are available, they are plotted on a geographic information system with flexible scaling. This page also records both the taxonomist responsible for a specimen’s identification and the holding institution. Each specimen page is coupled to a companion page that records the barcode sequence and the PCR primers used to generate the amplicons that were sequenced. The primer registry allows the deposition of information on both standard PCR primer sets and more complicated cocktails, but it does not record amplification conditions. Currently, BOLD is exclusively populated with COI data, but it can support other single-gene or multigenic barcodes. As a result, it is positioned to deal with the additional data storage requirements created when supplemental barcode regions gain registration for the animal kingdom or as alternate barcode regions are designated for the other kingdoms of life. Regardless of the genetic target(s), each barcode record is placed into a project
that can accommodate data for up to 999 specimens. This size constraint was imposed as an aid to quality control; single massive projects compromise both internal and peer review of results. However, there is no barrier to investigations that require the submission of very large volumes of data; some current studies include more than 10,000 specimen records. In such cases, specimen records are placed in a series of projects that sit within a single ‘container’ whose structure mirrors that of a standard Windows/Mac folder.

Because DNA barcoding projects often involve the analysis of large numbers of specimens, an interface is needed to both monitor analytical progress and compliance with data standards. The project management console serves this function. It reports progress in the submission of those data fields (trace files, images, GPS coordinates) that are needed for a record to gain barcode status. It also registers the number of specimens lacking sequences within a project and it monitors the sequence lengths themselves. BOLD includes several tools for routine data analysis. Among these, the taxon ID tree, which employs varied distance metrics to generate a neighbour-joining (NJ) tree based on nucleotide or amino acid sequences, is particularly valuable. This module supports the labelling of terminal branches with taxonomic information, locality data and/or sequence lengths and provides results in a PDF format to aid transmission to collaborators. The ID tree can also be colourized in various ways to highlight, for example, recently collected data or members of a specific taxonomic category (e.g. genus or family). In addition, when specimen photographs are available within a project, an image library can be generated which matches the ordering of specimens in the ID tree. Other analytical tools within the MAS deliver synoptic views that aid detection of data anomalies that can then be probed in detail. For example, the nearest-neighbour analysis highlights both cases where individuals assigned to different species show barcode congruence and those where deep divergences occur among conspecifics. Other analytical modules summarize information on nucleotide
composition, assemble image libraries, or display aggregate information, such as the collection points for specimens in a project.

Unknown specimens are identified by pasting their sequence record into the window on the ID page. The query sequence needs to satisfy just one criterion — it must include at least 300 bp from the barcode region of COI. BOLD also has two identification functions that operate within single projects. One tests the validity of existing identifications, recording both cases of confirmation and conflict with those generated by the ID System. The other function assigns identifications to specimens lacking prior taxonomic placements, essentially carrying out a project-wide implementation of the ID function. In all three cases, the ID System employs a linear search to collect nearest neighbours from a global alignment of all reference sequences). A query-optimized search library is maintained outside the main database with new records added weekly. This rapid access ensures that all data are available to guide identifications (although the sequence records themselves remain private until moved into the public realm), but this comes at a cost. Some barcode records that have not been through full validation will derive from misidentified specimens or will reflect analytical errors. Because of this fact, barcode records in the search library are placed into two categories. Species with a minimum of three representatives and a maximum con-specific divergence of two percent are classified as verified barcodes and these records are used as the default search library. All other records are stored in another data partition where they can be used for a full screen of the data, but this analysis is risky because the records are unvalidated. Any query sequence is aligned very quickly to the global alignment through a Hidden Markov Model (HMM) profile of the COI protein, followed by a linear search of the reference library. BLAST methods were tested, but users had difficulty interpreting results because scores are influenced by sequence length as well as by sequence
similarity. Moreover, the best BLAST hit is of little value when no closely related taxa are in the reference database. Finally, benchmark studies demonstrated that linear searches supported by HMM alignments are faster than BLAST.

**Conclusion**

Mangroves are repository of biodiversity. Endemism is also found in mangroves. There is a very good need to barcode the organisms living in mangroves. When large number of mangrove species is barcoded, a separate portal for barcode of mangrove species can be created.
SCREENING METHODS FOR PHYTOCHEMICALS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS

Dr.S.Maneemegalai*

Since the civilization of human beings, plants are utilized as therapeutic agents. Mother Nature has been a source of medicinal plants. The healing properties of many herbal plants have been recognized by Siddha, Ayurveda and Unani medicines. Being nontoxic, having no side effects and easily affordable, there has been a resurgence in the consumption and demand for medicinal plants. With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. (1).

It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties, this has made traditional medicine relatively cheaper than modern medicine (2). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (3). Systematic screening of them may result in the discovery of novel effective compounds (4).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (5). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (6).

In recent times, emphasis is placed on use of natural materials in the control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable side effects. It has been proved that

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oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer and many other diseases. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (7).

To evaluate and for the preliminary screening of the phytochemicals, antimicrobial and antioxidant activities of plant extract the following methods can be used.

**Plant material and preparation of extract**

The leaves and flowers or any part of the plant material may be collected and authenticated by a Botanist and a voucher specimen was deposited. The plant materials were examined carefully and old, infected, and fungus damaged flowers or leaves (plant part) were removed. Extracts were prepared from dried and also from fresh material. Healthy plant parts were spread out and shade dried at room temperature for about ten days and ground into fine powder using electric blender. 100 g of powdered material was taken.

**Methanol extract:**

50 g of powder was taken in a separate container. To this 250 ml of methanol was added and kept for 24 h in a shaker. Then filtered through eight layers of muslin cloth and the extract were collected. Again 250 ml of methanol was added to the filtered plant material and again kept for 24 h, then filtered. Then the collected filtrates (extract) were pooled.

**Ethanol extract:** It was prepared in the same way as methanol extract.

After filtration, pooled extracts were concentrated in vacuo using rotary vacuum evaporator and reduced to 10 ml volume (8) and stored at 4°C in air tight bottle.

**Extract from fresh plant material**

10g of plant material was taken and washed in running tap water and sterilized distilled water, then ground with 100 ml ethanol separately and kept in
a shaker for 24 h and filtered. The filtered extract was evaporated to make a final volume of 1 ml and used.

Likewise any solvent may be added and extracts can be prepared.

Phytochemical screening of plant extract was carried out. (3,9,10).

1. **Test for Terpenoids:** 5.0 ml of each extract was mixed with 2.0 ml of chloroform, and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

2. **Test for Tannins:** About 0.5 g of the dried powdered sample was boiled in 20 ml of distilled water in a test tube and then filtered. Few drops of 0.1\% ferric chloride was added and observed for brownish green or a blue black coloration.

3. **Test for Flavonoids:** 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulphuric acid. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

4. **Test for Saponin:** About 2.5 g of the plant material was extracted with boiling water. After cooling, the extract was shaken vigorously to froth and was then allowed to stand for 15-20 minutes and classified for saponin content as follows: no froth = negative; froth less than 1 cm = weakly positive; froth 1.2 cm high = positive; and froth greater than 2 cm high = strongly positive.

5. **Test for Cardiac Glycosides:** 5.0 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1.0 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxy sugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in acetic acid layer, a greenish layer may form gradually throughout the thin layer.

6. **Test for phlobatanins:** When an aqueous plant extract was boiled with 1% hydrochloric acid, deposition of red precipitate indicates the presence of phlobatanins.
GC / MS Analysis:

GC / MS Analysis of plant extract was determined by GC - MS. Shimadzu Model QP-2010 Mass Spectrometer under the following conditions: DB - Polyethylene Glycol coated fused silica capillary column (30m length x 0.25mm ID x 0.25µm film thickness) : Helium Carrier Gas (1.34 ml/minute). 250°C injector temperature; 240°C interface temperature. 200°C Ion Source Temperature. Column temperature programmed at 60°C with 10°C / minute rise to 230°C. For GC/MS detection an ionization energy of 70ev was used.

50mg of plant extract sample were taken separately and made up to 10ml with methanol, from which 1µl of sample was injected (split mode) in the column. The components were identified based on NIST Library / Wiley Library.

In vitro determination of antimicrobial activity

Stock culture were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4h at 37°C. Antibacterial activity was determined by agar disc diffusion method (11). Standard suspension of bacteria was inoculated on the surface of Muller-Hinton (Himedia) agar plates. DMSO and Methanol (1:1) was used to dissolve the plant extract. Sterilized filter paper discs (5mm)containing 30µl of each extract (varied concentration/ml) were arranged on the surface of the inoculated plates and incubated at 37°C for 18-24h. Along with this 30µg tetracycline disc (Himedia standard) was studied for antimicrobial activity as a positive control whereas the solvent used for preparing extract was used as a negative control. At the end of incubation, inhibition zones formed around the disc were measured with Himedia zone scale. The study was performed in triplicate and the mean values were presented.

Plant extracts were assayed for antifungal activity against the fungal strain This fungus was grown on Potato Dextrose Agar (PDA) plate at 28°C and maintained with periodic sub-culturing at 4°C.
Antifungal activity

The extracts of different plant materials were screened for antifungal activity by agar well diffusion method (12) with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of extracts of different plant materials were introduced serially after successful completion of one plant analysis. Incubation period of 24-48 hours at 28ºC was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration values were determined by broth dilution assay. Varying concentrations of the extracts (200µg/ml, 150µg/ml, 100µg/ml, 50µg/ml and 25µg/ml) were prepared. 0.1ml of each concentration was added to each 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. The tubes were incubated at 37°C for 24h. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration (13).

Determination of Minimum Bactericidal and Fungicidal Concentration:

A 1ml sample from the tubes used in MIC determination which did not show any visible growth after the period of incubation were streaked out on Nutrient agar and Potato Dextrose agar to determine the minimum concentration of the extract require to kill the organisms. These concentrations were indicated by the failure of the test organisms to grow on subsequent transfer to Nutrient agar and Potato Dextrose agar plates. The lowest concentration of the extract indicating a bactericidal effect after 24h of aerobic incubation was regarded as the Minimum Bactericidal Concentration (MBC) while the lowest concentration
that prevent fungal growth after 7 days of aerobic incubation was recorded as the Minimum Fungicidal Concentration (MFC) (13).

**Effect of temperature on stability of extracts**

The extracts were heated in a water bath at 30°C, 60°C, 80°C, 100°C, for 30 minutes and in an autoclave at 121°C for 15 minutes. After cooling, the extracts were tested for antimicrobial activity. (13)

**Antioxidant activity (DPPH free radical scavenging activity)**

**Determination (7)**

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free radical activity. Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

The bioactive compounds from the crude extract can be identified and isolated by chromatographic methods and the isolated compounds could be tested against the microbes by the above method and used for the benefit of living beings.
References


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There are several components to a strong grant application. First, the subject must be creative, exciting, and worthy of funding. Then, the project must be developed through a rigorous, well-defined experimental plan. Finally, ensure that the information is presented in clear language and that the application follows the rules and guidelines detailed in the grant application.

The following will help to prepare a research project and to desand the study. The Reviewers shall assess the study on the criteria men tired of below.

Eight Basic Questions Reviewers Ask

- How high are the intellectual quality and merit of the study?
- What is its potential impact?
- How novel is the proposal? If not novel, to what extent does potential impact overcomes this lack? Is the research likely to produce new data and concepts or confirm existing hypotheses?
- Is the hypothesis valid supported by relevant references.
- Are the aims logical?
- Are the procedures appropriate, adequate, and feasible for the research?
- Are the investigators qualified? Do they possess competence, credentials, and experience?
- Does the organization has basic adequate facilities and the environment conducive to research?

Writing a grant application is a major undertaking.
Before You Begin

Before you start writing the application, make sure you have done your homework: know the field, choose an excellent idea to pursue, and equally important, read the entire grant application very carefully.

Begin by focusing on the big picture. It is critical that you are intimately familiar with the field you apply for funding. You must be aware of the field's directions, knowledge gaps, and research already being done. Your application will be reviewed by your peers, investigators who are knowledgeable about the research area of your proposal.

Developing the Hypothesis

- Most reviewers feel that a good grant application is driven by a strong hypothesis. The hypothesis is the foundation of your application. Make sure it's solid. It must be important to the field, and you must have a means of testing it.
- Provide a rationale for the hypothesis. Make sure it's based on current scientific literature. Consider alternative hypotheses. Your research plan will explain why you chose the one you selected.
- A good hypothesis should increase understanding of biologic processes, diseases, treatments and/or preventions.
- Your proposal should be driven by one or more hypotheses, not by advances in technology (i.e., it should not be a method in search of a problem). Also, avoid proposing a "fishing expedition" that lacks solid scientific basis.
- State your hypothesis in both the specific aims section of the research plan and the abstract.

Developing Your Research Plan

A top-quality research plan is the most important factor determining your application's success in peer review. As with a scientific publication, developing your ideas is key.

- Your application should be based on a strong hypothesis.
- Be sure your project has a coherent direction.
• Keep the sections of the plan well coordinated and clearly related to the central focus.

• Emphasize mechanism: A good grant application asks questions about biological mechanisms.

• Don't be overly ambitious - your plan should be based on a feasible timetable.

• Specific aims and experiments should relate directly to the hypothesis to be tested.

A. Specific Aims

• Your specific aims are the objectives of your research project, what you want to accomplish. The project aims should be driven by the hypothesis you set out to test. Make sure they are highly focused.

• Begin this section by stating the general purpose or major objectives of your research. Be sure all objectives relate directly to the hypothesis you are setting out to test. If you have more than one hypothesis, state specific aims for each one. Keep in mind your research methods will relate directly to the aims you have described.

• State alternatives to your hypothesis and explain why you chose the one (or more) you selected.

• Choose objectives that can be easily assessed by the review committee. Do not confuse specific aims with long-term goals.

B. Background and Significance

• Keep the statement of significance brief. State how your research is innovative, how your proposal looks at a topic from a fresh point of view or develops or improves technology.

• Show how the hypothesis and research will increase knowledge in the field. Relate them to the longer-term, big picture scientific objectives and to the betterment of public health.

• Justify your proposal with background information about the research field that led to the research you are proposing. The literature section is very important because it shows reviewers you understand the field and have a balanced and adequate knowledge of it.
• Use this opportunity to reveal that you are aware of gaps or discrepancies in the field. Show familiarity with unpublished work, gained through personal contacts, as well.

• Identify the next logical stage of research beyond your current application.

C. Preliminary Studies/Progress Report

By providing preliminary data, this extremely important section helps build reviewers' confidence that you can handle the technologies, understand the methods, and interpret results.

• Preliminary data should support the hypothesis to be tested and the feasibility of the project.

• Explain how the preliminary results are valid and how early studies will be expanded in scope or size.

• Make sure you interpret results critically. Showing alternative meanings indicates that you've thought the problem through and will be able to meet future challenges.

• Preliminary data may consist of your own publications, publications of others, unpublished data from your own laboratory or from others, or some combination of these.

• Include manuscripts submitted for publication. Make sure it's clear which data are yours and which others reported.

D. Research Design and Methods

Describe the experimental design and procedures in detail and give a rationale for their use. Organize this section so each experiment or set of experiments corresponds to one of your specific aims and is stated in the same order. Even holding to this structure, the experiments still must follow a logical sequence. They must have a clear direction or priority, i.e., the experiments should follow from one another and have a clear starting or finishing point.

Convince reviewers that the methods you chose are appropriate to your specific aims, that you are familiar with them, and that, unless innovative, they are well established. If your methods are innovative, show how you have changed
existing, proven methods while avoiding technical problems. Also, describe why the new methods are advantageous to the research you propose to do.

More and more applicants are including colored charts, graphs, and photographs in their applications. If you must use color to get your point across, it is wise to also place a copy of the item in an appendix, noting this in the body of the text. (However, do not put important figures only in the appendix, or overly-reduced figures in the body of the application with enlargements in the appendix. The Research Plan must be self-contained. The appendix should not be used to circumvent the Research Plan page limits.) Many applicants are not aware that most of the study section members receive only black and white photocopies of their original application. However, assigned reviewers do receive originals of the appendices and usually receive original copies of the application as well.

**Approach**

- State why you chose your approach(es) as opposed to others.
- If you are choosing a nonstandard approach, explain why it is more advantageous than a conventional one. Ask yourself whether the innovative procedures are feasible and within your competence.
- Call attention to potential difficulties you may encounter with each approach. Reviewers will be aware of possible problems; convince them you can handle such circumstances. Propose alternatives that would circumvent potential limitations.
- Consider the limitations of each approach and how it may affect your results and the data generated.
- Spell it out in detail. While you may assume reviewers are experts in the field and familiar with current methodology, they will not make the same assumption about you. It is not sufficient to state, "We will grow a variety of viruses in cells using standard in vitro tissue culture techniques." Reviewers want to know which viruses, cells, and techniques; the rationale for using the particular system; and exactly how the techniques will be used. Details show you understand and can handle the research.
- Make sure any proposed model systems are appropriate to address the research questions and are highly relevant to the medical problem being modeled.
Results

- Show you are aware of the limits to - and value of - the kinds of results you can expect based on current knowledge of the subject. State the conditions under which the data would support or contradict the hypothesis and the limits you will observe in interpreting the results.
- Show reviewers you will be able to interpret your results by revealing your understanding of the complexities of the subject.
- Many applications benefit from statistical analysis. The early involvement of a statistician to determine the amount of data to collect and the methods for analyses will favorably impress reviewers.
- Describe your proposed statistical methods for analyzing the data you plan to collect. Define the criteria for evaluating the success or failure of a specific test.

Other pointers

- Estimate how much you expect to accomplish each year of the grant and state any potential delays you can anticipate.
- Describe sources of reagents, animals or equipment not generally available. If collaborators will provide them, include letters from the sources in your application.
- Describe any procedures, situations, or materials that may be hazardous and precautions you will take.
- Include supporting data. Where appropriate, include well-designed tables and figures. Use titles that are accurate and informative. Label the axes and include legends. Reviewers will look for discrepancies between your data and text.
- Include relevant publications. If you (or your collaborators) have publications showing your use of the proposed methods, put them in the appendix.

E. Vertebrate Animals

If applicable, your application should include:

- A detailed description of the proposed use of animals.
• A justification for the choice of species and number of animals to be used (describe any statistical methodology used for this determination).

• Information on the veterinary care of the animals.

• An explanation of procedures to ensure that the animals will not experience unnecessary discomfort, distress, pain, or injury.

• Justification for any euthanasia method to be used.

F. Literature Cited

Refer to the literature thoroughly and thoughtfully but not to excess. The publications you cite need not be exhaustive but should include those most relevant to your proposed research.

Research proposals typically do not fare well when applicants fail to reference relevant published research, particularly if it indicates that the proposed approach has already been attempted or the methods found to be inappropriate for answering the questions posed.

Each citation must include the names of all authors (not et al.), name of the book or journal, volume number, page numbers (not first page only), and year of publication.

G. Consortium/Contractual Arrangements

This section should briefly describe any consortium and contractual arrangements you have made with regard to the proposed research plan. The roles of individuals or organizations with whom you have made such arrangements should be noted and reference made to any letters from them that are included in the application. Letters should describe the individual's or organization's understanding of the consortium or contractual arrangements.

H. Consultants

Careful selection and addition of consultants can add credibility to your application and greatly improve its quality. A letter describing the willingness of an investigator to participate as a consultant to your project should be included in your application.
Problems and Concerns Commonly Cited by Reviewers

- Lack of significance to the scientific issue being addressed.
- Lack of original or new ideas.
- Proposal of an unrealistically large amount of work (i.e., an over ambitious research plan).
- Scientific rationale not valid.
- Project too diffuse or superficial or lacks focus.
- Proposed project a fishing expedition lacking solid scientific basis (i.e., no basic scientific question being addressed).
- Studies based on a shaky hypothesis or on shaky data, or alternative hypotheses not considered.
- Proposed experiments simply descriptive and do not test a specific hypothesis.
- The proposal is technology driven rather than hypothesis driven (i.e., a method in search of a problem).
- Rationale for experiments not provided (why important, or how relevant to the hypothesis).
- Direction or sense of priority not clearly defined, i.e., the experiments do not follow from one another, and lack a clear starting or finishing point.
- Lack of alternative methodological approaches in case the primary approach does not work out.
- Insufficient methodological detail to convince reviewers the investigator knows what he or she is doing (no recognition of potential problems and pitfalls).
- Most experiments depend on success of an initial proposed experiment (so all remaining experiments may be worthless if the first is not successful).
- The proposed model system is not appropriate to address the proposed questions (i.e., proposing to study T-cell gene expression in a B-cell line).
- The proposed experiments do not include all relevant controls.
- Proposal innovative but lacking enough preliminary data.
- Preliminary data do not support the feasibility of the project or the hypothesis.
• Investigator does not have experience (i.e., publications or appropriate preliminary data) with the proposed techniques or has not recruited a collaborator who does.

• The proposal lacks critical literature references causing reviewers to think that the applicant either does not know the literature or has purposely neglected critical published material.

• Not clear which data were obtained by the investigator and which others have reported.

WHERE TO APPROACH FOR RESEARCH FUNDS?

(1) University Grants Commission (UGC)

Contact Address

The Secretary, University Grants Commission, Bahadur Shah Zafar Marg New Delhi – 110002, Tel. No: (011) 23234019, 23236350, Fax. No.: (011) 23239659. Website: www.ugc.ac.in

(2) All India Council for Technical Education (AICTE)

Contact Address:

Adviser-II RID Bureau, All India Council for Technical Education NBCC Building, East Wing, 4th Floor, Pragati Vihar, Bhisham Pitamah Marg, New Delhi – 110 003, Telefax No: (011) 24369632, E-mail: rid@aicte.ernet.in, Website: www.aicte.ernet.in

Contact Address:

The Head, Human Resource Development Group Council of Scientific and Industrial Research, CSIR Complex, Library Avenue, Pusa New Delhi – 110 012, Tel. Nos: (011) 25748632, 25721585 Fax. No: (011) 25840887, 25860595, E-mail: csircx@nda.vsnl.net.in, Website: http://csirhrdg.res.in

Contact Address:

The Director, Directorate of Extramural & Intellectual Property Rights Defence Research & Development Organisation, West Block 8, Wing 5, 1st Floor, R.K. Puram New Delhi – 110066, Telefax: 011-26170928, E-Mail: erip_er@drdohq.res.in, Website: www.drdo.com
Contact Address:
Secretary, AR&DB, Defence Research & Development Organization
332, 'B' Wing, Sena Bhawan, New Delhi – 110 011, Tel. No: (011) 23014034, Fax. No.: (011) 23793004,
E-mail: ardb@drdo.com, Website: www.drdo.com/boards/ardb/default.htm

Contact Address:
The Scientific Secretary / Programme Officer, BRNS Secretariat
Department of Atomic Energy, 1st Floor, Central Complex,
BARC, Trombay, Mumbai-400 085. Tel. No: (022) 25505223/25593946/25595331/25595386
Fax: (022) 25505151/25519613,
E-mail: drppc@barc.ernet.in/trehan@barc.ernet.in/gnpandey@barc.ernet.in

Contact Address:
Scientist In-charge, Project Registry Cell, Department of Biotechnology
Block 2, 7th Floor, C.G.O. Complex, Lodi Road, New Delhi – 110 003
Website: www.dbtindia.gov.in, www.btisnet.gov.in,
www.dbtindia.gov.in/organistion/nodal.htm

Contact Address:
The Director, Ocean Research & Manpower Development Programme
Department of Ocean Development, Block 12, CGO Complex, Lodi Road
New Delhi – 110 003, Tel. No.: (011) 24306839, 24362278, Fax No.: (011) 24360336, 24360779; E-mail: venkat@dod.delhi.nic.in,
Website: www.dod.nic.in

Contact Address:
Department of Science & Technology, Technology Bhawan,
New Mehrauli Road, New Delhi – 110 016, Telefax No: (011) 26963695
E-mail: venkatesh@nic.in, Website: www.serc-dst.org

Contact Address:
Department of Scientific & Industrial Research, Ministry of Science & Technology,
Technology Bhavan, New Mehrauli Road, New Delhi – 110016
Tel. No : (011) 26960629, Fax : (011) 26516078
E-mail : srv@nic.in, Website : www.dsir.gov.in
Contact Address:
Director General, Indian Council of Medical Research, V. Ramalingaswami Bhawan Post Box No. 4911, Ansari Nagar, New Delhi-110029,
Tel.No: 91-11-26588895, 91-11-26588980, 91-11-26588707, 91-11-26589794, 91-11-26589336, Fax: 91-11-26588662, E-mail: icmrhqs@sansad.nic.in

Contact Address:
Adviser, Research Division, Ministry of Environment and Forests Paryavaran Bhavan, Block No. 2, CGO Complex, Lodi Road New Delhi – 110003, Tel.No: (011) 24362840, Fax: (011) 24368654 E-mail: rmehta52@yahoo.com, Website: www.envfor.nic.in

(3) Science and Technology Application for Rural Development (STARD) Science and Society Related Programmes
The Head, Sci.& Society Division, Dept. of Sci. & Technology, Technology Bhavan, New Mehrauli Road, New Delhi – 110 016,
e-mail: sunilag@alpha.nic.in, web: www.scienceandtechnology-dst.org.
Fax: 26864570, 26863847, 26862418, Tel: 011-26567373 Extn. 298

(4) Science & Technology for Weaker Sections (STAWS). Science and Society Related Programmes
The Head, Sci.& Society Division, Dept. of Sci. & Technology, Technology Bhavan, New Mehrauli Road, New Delhi – 110 016,
e-mail: sunilag@alpha.nic.in, web: www.scienceandsociety.dst.org.
Fax: 26864570, 26863847, 26862418, Tel: 011-26567373 extn. 298

(5) Indian National Science Academy (INSA)
The Chairman, Indian National Science Academy, Bahadur Shah Zafar marg, New Delhi –110002, e-mail: insa@giasd101.vsnl.net.in, insa@delnet.ren.nic.in, web: insal@ndf.vsnl.net.in, Fax: 91-11-23235648/23231095, Tel: EPBAX No. 23221931 to 23221950

INTERNATIONAL FUNDING AGENCIES

(6) International Foundation for Science
Director, International Foundation for Science, Grev Turegatan 19, S.114 38, STOCKHOLM, SWEDEN, WEB: www ifs.se, e-mail: info@ifs.se., Tel: 46 545 81800
(7) Third World Academy of Sciences (TWAS)

Executive Director, Third World Academy of Sciences (TWAS), c/o the Abdus Salam, International Centre for Theoretical Physics, (ICTP), P.O. Box 586 – Via Beirut 6 – 34100 Trieste – Italy.
e-mail: info@twas.org, web: http://www.org/activities.html
Fax:+39 040 224559 ,Tel: +39040 2240387

(8) Third World Network of Scientific Organizations

The Third World Network of Scientific organizations, (TWNSO), c/o The Abdus Salam International Centre for Theoretical Physics (ICTP). Strada Copstoera 11- 340 14 Trieste – Italy: e-mail: info@twnso.org, web: www.twnso.org, Fax: +39 040 2240 689 ,Tel: +39 040 2240-683