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Bioprospecting: Creating value for biodiversity

Dr. Alka Sawarkar, Dr. RK Sharma and Dr. Vidhi Gautam

Abstract

Bioprospecting is a systematic and organized search for useful product derived from bio resources including plants, micro-organisms, animals etc, that can be developed further for commercialization and overall benefits of the society. Bioprospecting involves searching, collecting and deriving genetic material from samples of biodiversity that can be used in commercialized pharmaceutical, agricultural, industrial or chemical processing end product.

Historically, nature was the origin of all medicines and ethno pharmacology has provided some very notable past success, including morphine, quinine and digitoxin etc. Reserpine was isolated from the plant *Rauwolfia serpentina* and was turned into antihypertensive drug. Ephedrine isolated in 1923 from *Ephedra sinca* formed the basis for the synthesis of salbutamol and salmetrol which are the antiasthmatic drugs (beta agonist). Atropine comes from Belladonna and even aspirin was derived from salicin present in willow bark. Teprotide was isolated from the venom of the pit viper, *Bothrops jaraca* and this formed the skeletal framework of the captopril and enalapril, which are used in treating cardiovascular diseases. More recent developments in an association with traditional uses include artemisinin and its derivatives for malaria and prostratin as an anti-viral drug.

Bioprospecting with its potential as a rich source of new therapeutic agents is an important tool for drug discovery and research. However collaborations between pharmaceutical companies and the countries supplying the indigenous knowledge and medicinal resources should be regulated for mutually beneficial relationship.

Keywords: bioprospecting, *rauwolfia serpentina*, *bothrops jaraca*

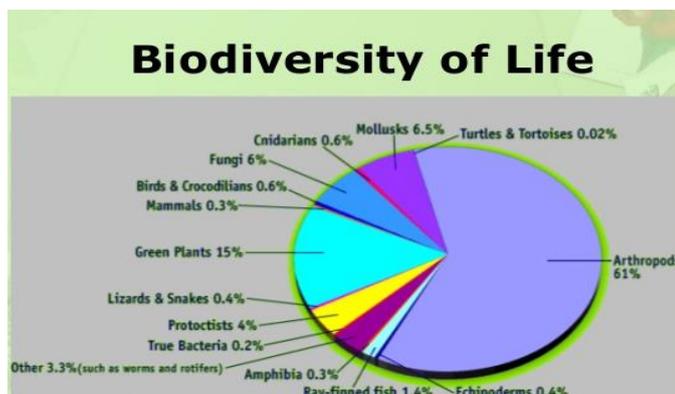
Introduction

Bioprospecting is a systematic and organized search for useful product derived from bio resources including plants, micro-organisms, animals, etc, that can be developed further for commercialization and overall benefits of the society. Bioprospecting involves searching, collecting and deriving genetic material from samples of biodiversity that can be used in commercialized pharmaceutical, agricultural, industrial or chemical processing end product (Bluestein, 2006) [6].

The bioprospecting of plant and living organism for pharmaceutical purpose is useful. The biological task can be achieved by the development of competitive mechanism such as production of toxins, enzymes, antimicrobial agent like antibiotics etc. Microbial derived enzymes are highly valued in many industrial applications, such as enzymes are usually used in the processing of food and beverage, paper, pulp, textile, animal feed, detergent, cosmetic and chemical synthesis processes (Butler, 2008) [7].

Bioprospecting involves searching, identifying and collecting appropriate biospecimen, in addition, bioprospecting uses various cutting technologies to process and develop genetic material from these specimen that exhibit desirable characteristic as commercial product (Cao and Kingston, 2009) [8]. It is a misconception that bioprospecting decimates an organism population to near extinction but there is a need of only a few specimens to extract the genetic material.

Historically, most of the active ingredients in medicines have been natural products and natural products continue to form a productive source of new drugs (Newman and Cragg, 2007; Butler, 2008) [27, 7]. It involves indigenous understanding about practices and characteristics of medicinal plants and animals as well as the search for formerly unidentified compounds in natural sources that have never been used in folk medicine. The aim of this is to give a rational approach to design a bioprospecting program development and is useful to initiate a search from biodiversity.



Biodiversity

Variability among living organism from all sources including terrestrial marine and other aquatic ecosystem and the ecological complexes of which they are a part. It measures the variety of animal and vegetable species in the biosphere and is the result of long evolution processes. This includes diversity within species, between species and ecosystem. The element that makes up biodiversity can be divided into three parts.

Types of biodiversity

- Genetic biodiversity
- Species biodiversity
- Ecosystem level biodiversity

Genetic biodiversity

Variation in the number and types of genes as well as chromosomes present in different species.

Species biodiversity

Variety in the number and richness of the species within a region.

Ecosystem biodiversity

Interaction of species living together in a physical environment of given area (Frisvold and Day, 2008) ^[13].

Importance of bioprospecting

However the age of blockbuster drugs seems to largely be over, and the pharmaceutical sector, as it is currently structured, is unable to deliver enough new products to market to generate revenues sufficient to sustain its own growth. Nearly all major drug developers are critically examining current R&D practices and, in some cases, considering a radical overhaul of their R&D models (Kaitlin, 2010) ^[20]. In order to deal in a comprehensive way with the opportunities and the potential drawbacks which bioprospecting encompasses, several countries have developed a national bioprospecting policy. To develop a comprehensive bioprospecting policy, it is necessary to coordinate, align and integrate policies and strategies across sectors; in the case of bioprospecting, this would comprise issues related to intellectual property rights, tenure of land and natural resources, Research & development, conservation and protection of biodiversity etc. The policy should provide for mechanisms where by its objectives can be translated into appropriate action and which can influence decision making on relevant issues. A comprehensive bioprospecting policy should contain at least the following, complementary elements as given below:

1. It creates an incentive to monitor and preserve biodiversity

in order to avoid the risk of losing economic opportunities from competitors or extinction.

2. It promotes technology and knowledge transfer among countries along with foreign direct investment; local populations will become increasingly aware of the potential economic value of natural habitats, providing incentives to the domestic population for biodiversity conservation.
3. It promotes innovation, helping countries to develop new pharmaceutical products.
4. It also favours employment opportunities related to natural products.
5. It helps to preserve traditional culture and habits by rediscovering ancient native practices.

Bioprospecting benefits

Bioprospecting defined common perception is that bioprospecting is a new science linked to modern biotechnology. The fact is that humankind has been studying, manipulating, and exploiting natural diversity, for obtaining commercially valuable products.

Early bioprospecting lead to the improvement of methods for growing food, building shelters, and maintaining health. Modern-day bioprospecting is simply an extension of our long history of exploring nature to improve our quality of life, as the exploration of biodiversity for commercially valuable genetic and biochemical resources and result in the protection of wild lands and wildlife, through funding of conservation activities. Furthermore, bioprospecting should bolster economic and conservation goals underpinning medical and agricultural advances needed to combat disease and sustain a growing human population (Cox and Balick, 1994) ^[10].

The natural products like plants, animals, microorganisms, marine organisms have been important sources of the potential drug leads and this will continue for years to come. Many bioactive molecules have been isolated from these sources applying three main types of search strategies, bio-rational, chemo-rational and random approaches. For example, Conocurvone-an anti-HIV agent was discovered as a result of random approach of screening. Drugs such as artemisinin, morphine and quinine, were discovered using bio-rational approach (Rishton, 2008) ^[33].

Out of these three search strategies, bio-rational approach is the most effective one. Bio-rational approach is mostly guided by the ethno-medical information generated from the traditional medicines. More than 13,000 species of plants are being used in the traditional medicines and herbal cosmetics and about 8000 of these medicinal plants species are known in South Asia alone.

These natural products (Rausser and Small, 2000) ^[32] contain reservoir of ethno-medical and ethno-botanical information. Recently at the National Cancer Institute although random high throughout screening, method furnished a large number of testing extracts, out of 800 medicinal plant extracts collected from Vietnam and Laos, at least 25 biologically active compounds were isolated; of these 13 were new anti-HIV agents and 3 were anti-malarial agents. In the USA, out of 119 plant drugs available from 1959 to 1980, 74 per cent of these were discovered as a result of chemical studies directed at isolating the active substances from the plants used in traditional medicines.

The search for new drugs continues, but mostly using the ethno-directed bio-rational approach. In 1999, the NAPRALERT database recorded more than 88,000 natural

product isolates and many of them formed the skeletal framework of many renowned drugs available in the market today. For example, mevastatin (compactin) and lovastatin which were isolated from Penicillin species, became the cholesterol lowering drug (Pushpangadan, 2005) [30]. Drugs like Ivermectins isolated from the *Streptomyces* species became an anthelmintic and antiparasitic drug.

Reserpine was isolated from the plant *rauwolfia serpentina* and was turned into antihypertensive drug. Ephedrine isolated in 1923 from *ephedra sinca* formed the basis for the synthesis of salbutamol and salmetrol which are the antiasthmatic drugs (beta agonist). Atropine comes from belladonna and even aspirin was derived from salicin present in willow bark. Teprotide was isolated from the venom of the pit viper, *bothrops jaraca* and this formed the skeletal framework of the captopril and enalapril, which are used in treating cardiovascular diseases (Newman and Cragg, 2007) [27].

Bioprospecting processes

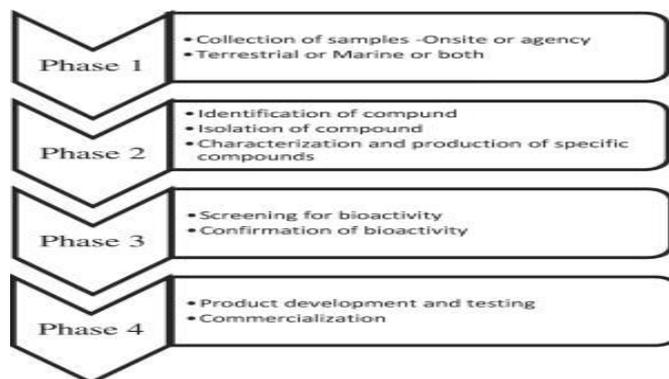
Bioprospecting is the search for plants, animals, and microbial species for academic, pharmaceutical, biotechnological, agricultural, and other industrial purposes. It serves as a means to commercialize biodiversity. In the forefront of this activity have been pharmaceutical corporations, biotechnology companies, and their intermediates, which have led expeditions into forests and scoured the fields and waters in tropical and subtropical countries in search of valuable genetic material. For instance, the US National Cancer Institute (NCI) screened over 35,000 plants and animals for anticancer compounds between 1956 and 1976 (Congressional Research Service Report for Congress, 1993). In 1993, the US National Institutes of Health devoted US\$ 60 million to biodiversity related research on drugs and medical products from natural sources. The NCI has collected over 50,000 samples derived from plants, microorganisms, and marine samples from over 30 tropical countries aimed at finding cures for cancer and AIDS. These samples are now held in NCI's Natural Product Repository and are available under material transfer agreements to researchers-

Phase I: On-site collection of samples.

Phase II: Culturing of organisms, isolation and characterization of specific compounds.

Phase III: Screening for specific uses.

Phase IV: Product development and commercialization, including patenting, trials, sales, and marketing, if well managed, bioprospecting can be advantageous for the developing countries since in addition to generating new products. It can generate income and provide an incentive for the, conservation of biodiversity. However, if not managed properly, it can create environmental problems due to over-exploitation or habitat destruction, and socioeconomic issues related to unfair sharing of benefits. Over the past couple of decades, large pharmaceutical companies have scaled back bioprospecting activities primarily due to the expenses involved and complex negotiations over intellectual property rights (IPR). The majority of natural products research is being done in laboratories in academic and government funded research institutes.



Historically, nature was the origin of all medicines and ethno pharmacology has provided some very notable past success, including morphine, quinine and digitoxin etc. More recent developments in an association with traditional uses include artemisinin and its derivatives for malaria and prostratin as an anti-viral drug (Kingston, 2011) [22]. A number of drugs had been developed through bioprospecting and they are classified in three categories:

1. Drugs from plants
2. Drugs from animals
3. Drugs from microorganisms

Drugs from plants



Papaver somniferum (Opium poppy)

Morphine

Morphine was first isolated in 1803-1805, from plant *Papaver somniferum* by Friedrich Sertuner. Mechanism of action of morphine is presynaptic action of opioids to inhibit neurotransmitter. Opioid receptors generally mediate neuronal inhibition. Opioid receptors couple to G_i or G_o , and produce inhibition of Ca^{2+} channels and opening of K^+ channels. They also inhibit adenylate cyclase. Through this and other downstream signaling pathways, opioid receptors modulate synaptic plasticity and gene expression. Like other G-protein-coupled receptors (GPCRs), opioid receptors may interact to form homodimers and heterodimers.

This generates receptors with different pharmacology, signaling ability and perhaps trafficking characteristics

compared to the cloned receptor subtypes. The significance of this finding is only beginning to be explored, but it may contribute to the pharmacological diversity of opioid receptors, and has exciting implications for drug design.

It is used primarily to treat both acute and chronic severe pain; it is also used for pain due to myocardial infarction mechanism of acute pulmonary edema. Morphine is effective in relieving cancer pain.

1. It belongs to the group of medicines called narcotic analgesics.
2. Morphine acts on the central nervous system (CNS) to relieve pain (Clark *et al.*, 2007)^[9].
3. It is used primarily to treat both acute and chronic severe pain; it is also used for pain due to myocardial infarction mechanism of acute pulmonary edema. Morphine is effective in relieving cancer pain.
4. Morphine is available in the form of Roxanol; (Morphine is sold under many trade names).



Spirea ulmaria (Meadow sweet)

Aspirin

The name Aspirin was derived from the name of the chemical ASA *Acetyl spir saure* obtained from meadow sweet plant, *Spirea ulmaria*, Salicylic acid is a main component of an herbal extract found in the bark of a number of trees, including the willow tree, and in a number of fruits, grains, and vegetables. As such, salicylic acid and related salicylates have long been common components of a normal human diet, The first recorded use of salicylates dates back about 4,000 years to the Sumerians, who noted the pain remedies of the willow tree on early clay tablets. Ancient civilizations in Mesopotamia used the extract from willow trees to treat fever, pain, and inflammation (Sudhof, 2001)^[38]. Both Chinese and Greek civilizations employed willow bark for medical use more than 2,000 years ago, and the Chinese also used poplar bark and willow shoots to treat rheumatic fever, colds, hemorrhages, and goiter. One of the most noteworthy reports of the use of salicylic acid comes from the father of modern medicine, Hippocrates recommended chewing of willow-tree bark to patients suffering from fever and pain, as well as the use of a tea brewed from willow bark given to women to lessen pain during child birth, the Greek physician dioscorides prescribed willow bark as an anti-inflammatory agent.

The mechanism of action of aspirin is to inhibit the activity of enzyme called as cyclooxygenase which leads to the formation of prostaglandins. The applicable parts of meadowsweet are the above ground parts. Meadowsweet has stomachic, mild urinary antiseptic, antirheumatic, astringent, and antacid activities. It contains tannins and salicin, a plant salicylate. In animals, the above ground parts of meadowsweet decrease motor activity, lower temperature,

induce muscle relaxation, and potentiate the effect of narcotics. In animals, the flower extract increases life expectancy; decreases vascular permeability; increases bronchial, intestinal, and uterine tone; and promotes uric acid excretion. In *vitro*, it has bacteriostatic activity. Meadowsweet aqueous extracts contain high concentrations of tannins with strong astringent effects (Berger *et al.*, 2011)^[5].

The aspirin is used for analgesic, anti-inflammatory and antipyretic properties. Aspirin prevent clotting diseases such as heart attacks and strokes. Aspirin is available in the form of Anacin, Acupri.

Quinine



Cinchona officinalis

Quinine was first isolated from the bark of *Cinchona officinalis* tree in 1820. Mechanism of action of quinine is theorized to be toxic to the plasmodium falciparum by interfering with the parasites ability to dissolve and metabolize haemoglobin and inhibit the spontaneous formation of beta- haematin. It has also been shown to inhibit the ATPase of *Plasmodium falciparum* food vacuoles; this mechanism appears to be independent of its alkalization of the food vacuole (Ballestero *et al.*, 2005)^[3].

It is used chiefly in the treatment of malaria, an infection caused by the protozoan parasite which is transmitted to humans by the bite of various species of mosquitoes. Quinine, acts by interfering with the growth and reproduction of the malarial parasites, which inhabit the red blood cells (erythrocytes).

This medication is used alone or with other medication to treat malaria. Quinine belongs to a class of drugs known as antimalarials. It was also used as a muscle relaxant (Ballestero *et al.*, 2005)^[3]. Quinine is available in the form of Quaaliquin.

Artemisinin

Artemisinin was discovered from the plant *Artemisia annua* in 1972. This herb has been widely exported, and today it commonly grows across Africa, together with several other indigenous species of the same genus. *Artemisia* species have been screened as a part of malaria drug discovery research.

The mechanism of action of artemisinin is haem mediated decomposition of the end peroxide bridge to produce carbon centred free radicals and has been used traditionally for the treatment of fevers. Mechanism is still being sorted out. A recent computational study suggested that artemisinin gets activated by iron, which in turn inhibits the calcium pump. The importance of artemisinin has led to several synthetic and semisynthetic approaches to its production to complement its isolation from *Artemisia annua*. The most fruitful current

approach is the chemical conversion of artemisinic acid, produced by engineered strains of *Saccharomyces cerevisiae* to artemisinin. Many analogues and derivatives of artemisinin have been prepared in attempts to improve its activity (White, 1997). Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria. Most widely accepted theory was that they are first activated through cleavage after reacting with haem and iron oxide, which results in the generation of free radicals that in turn damage susceptible proteins, resulting in the death of the parasite. It is available in the form of coartem.

Vinblastin

Vinblastin was originally isolated from the plant Madagascar periwinkle. *Catharanthus roseus* in 1958. Now a day's Vinblastin is synthesized by a series of cyclization and coupling reactions which create the required stereochemistry. The overall yield may be as great as 22 per cent which makes this synthetic approach more attractive than extraction from natural sources, whose overall yield is about 10 per cent. Stereochemistry is controlled through a mixture of chiral agents, and reaction conditions.

Vinblastin's utility as a chemotherapeutic agent was first suggested by its effect on the body when an extract of the plant was injected in rabbits to study the plant's supposed anti-diabetic effect (Graf *et al.*, 1996) [15]. A tea made from the plant was a folk-remedy. Mechanism of vinblastin is inhibition of mitosis at metaphase through its interaction with tubulin.

Vinblastin binds to tubulin in intact microtubules with two widely different affinities depending upon whether the tubulin binding site is located at the microtubule ends or is situated along the microtubule surface. The binding sites on the microtubule surface have low affinity for vinblastin 1-2 sites per molecule of tubulin dimer in microtubules. Binding of Vinblastin at high concentrations to these sites *in vitro* depolymerizes the microtubule at both ends by the peeling of protofilaments and leads to formation of tubulin-vinca alkaloid paracrystals in cells suppression of tubulin exchange at microtubule ends, which occurs at low vinblastin concentrations in the absence of significant microtubule depolymerization, appears to be due to the reversible binding of Vinblastin to high affinity binding sites located uniquely at one or both microtubule ends binding sites per microtubulin experiments with populations of microtubules in suspension, we found that Vinblastin inhibits tubulin exchange at microtubule ends by 50 per cent when an average of only one or two molecules of Vinblastin is bound per microtubule.

In addition, Vinblastin reduces the rate of tubulin loss from plus ends, kinetically capping these ends in the absence of significant microtubule depolymerization. Video microscopy of individual microtubules, both in living BSC-1 cells and *in vitro* with microtubules assembled from bovine brain tubulin, indicated that Vinblastin significantly suppresses dynamic instability at microtubule plus ends at Vinblastin concentrations that are below the concentrations required to reduce the microtubule polymer mass.

It is used in diabetes and to treat different types of cancer (Jain and Kumar, 2001). This includes Hodgkin's lymphoma, non-small cell lung cancer, bladder cancer, melanoma, and testicular cancer. Vinblastin is available in the form of Alkaban-AQ.

Paclitaxel

Paclitaxel is plant alkaloids and was first isolated from the pacific yew *brevifolia* and named it taxol in 1971. It was approved for medical use in 1993 (Koehen and Carter, 2005) [24].

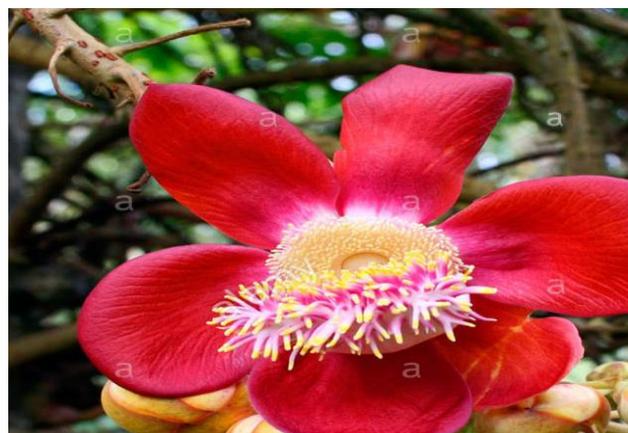
The mechanism of action paclitaxel is to block the cells in the G2/M phase of the cell cycle and such cells are unable to form a normal mitotic apparatus, Paclitaxel binds to beta-tubulin subunits of microtubules. The Paclitaxel is used in the treatment of breast, ovarian, and lung cancer, and Kaposi's sarcoma. Paclitaxel, an antitumor drug that is demonstrating encouraging activity in human malignancies, is likely to play a major role in cancer chemotherapy.

Paclitaxel has an unusual chemical structure--it is a complex diterpene having a taxane ring with a four-membered oxetane ring and an ester side chain and a unique mechanism of action. *In vitro*, paclitaxel enhances the polymerization of tubulin to stable microtubules and also interacts directly with microtubules, stabilizing them against depolymerization by cold and calcium, which readily depolymerize normal microtubules.

The fact that the drug has a specific binding site on the microtubule polymer makes it unique among chemotherapeutic agents, and the ability of paclitaxel to polymerize tubulin in the absence of cofactors like guanosine triphosphate and microtubule-associated proteins.

When paclitaxel and microtubule protein are irradiated with ultraviolet light, the drug preferentially binds covalently to the beta-subunit of tubulin. Paclitaxel binds to cells in a specific and saturable manner with a single set of high-affinity binding sites. The microtubule cytoskeleton is reorganized in the presence of paclitaxel and extensive parallel arrays or stable bundles of microtubules are formed in cells growing in tissue culture. Paclitaxel blocks cells in the G2/M phase of the cell cycle and such cells are unable to form a normal mitotic apparatus.

It may also be used to treat esophageal, head, neck, and bladder, uterine and cervical cancers. Paclitaxel belongs to a class of drugs known as plant alkaloids. These drugs are also known as antimicrotubule agents. Paclitaxel helps stop or slow the growth of cancer. It is available in the form of Abraxane, Taxol.



Reserpine

Reserpine was isolated from the dried root of *Rauwolfia serpentina* in 1952. It is also called as Indian snakeroot, which had been known as *Sarpagandha*.

The mechanism of action of reserpine is to irreversibly block the vesicular monoamine transporter, VMT normally transport intracellular norepinephrine, serotonin and dopamine in presynaptic nerve terminal for subsequent release into the synaptic cleft.

The applicable part of Indian snakeroot is the root. The properties of the whole root of *rauwolfia serpentina* differ from those of reserpine. The whole root contains over 50 alkaloids *Rauwolfia serpentina* demonstrates hypotensive, sedative, and tranquilizing effects. It also reduces heart rate, has anti-arrhythmic effects, and causes a general sense of euphoria. The active constituents of the whole root include the rauwolfia alkaloids, reserpine, rescinnamine, and deserpidine (11-desmethoxyreserine). Hypertensive effects are believed to be due to the depletion of both catecholamine and serotonin stores and prevention of reabsorption. The greater the proportion of alkaloids present, the greater the hypotensive activity. The sedative effects of Indian snakeroot can result from the depletion of amine stores in the central nervous system (CNS). It is used in fever and snakebite (Baumeister *et al.*, 2003). In some countries reserpine is still available as part of combination drugs for the treatment of hypertension, Reserpine is available in the form of diupres-2.

Digitoxin

Digitoxin isolated from plant *Digitalis purpurea*; it is also called as foxglove. The term digitalis is used for drug preparation that contains cardiac glycosides, digitoxin extracted from various plants.

Mechanism of digitoxin, digitoxin is cardiac glycosides it inhibits the Na-K-ATPase membrane pump, resulting in an increase in intracellular sodium and calcium concentration. Increased intracellular concentration of calcium may promote activation of contractile protein like actin, myosin (Shrivastav *et al.*, 2007) [37]. Cardiac glycosides are noncompetitive allosteric inhibitors of the α -subunit of the membrane-bound Na⁺K⁺-ATPase α , β -dimer and inhibit the exchange of intracellular Na⁺ for extracellular K⁺ to provide for a Ca²⁺ influx, which boosts the contractility of the myofibrils of the heart muscle. Na⁺/K⁺-ATPase have validated anticancer properties. Cardiac glycosides were also recently noted to inhibit the expression of certain genes overexpressed in prostate cancer cells, to provide protective effects against polyglutamine-based diseases and to inhibit TNF- α /NF- κ B signaling. Cumulatively, these fascinating molecules render a vast range of therapeutically important biological activities, the mechanisms of which remain under active investigation by many groups.

It is used, to increase the contractibility and as an antiarrhythmic agent to control the heart rate particularly in the irregular atrial fibrillation and also used in congestive heart failure. It is available in the form of lanoxin.

Warfarin

Discovery of warfarin originated in the 1920s from *Melilotus alba* and *Melilotus officinalis*. The mechanism of action of warfarin is disrupts to vitamin-K metabolism by inhibiting the enzyme peroxide reductase (Hirsh *et al.*, 2007). Warfarin is a vitamin K antagonist. It produces its anticoagulant effect by interfering with the vitamin K cycle. Specifically, it interacts with the KO reductase enzyme so that vitamin KO cannot be recycled back to vitamin K. This leads to a depletion of vitamin KH₂, thereby limiting the γ - carboxylation of the coagulation factors mentioned above. Factors like

prothrombin are not carboxylated, and cannot effectively bind to phospholipid membranes. Its activation by Factor Xa is not affected. Thus blood coagulation is limited.

Warfarin is used for blood clots in vein, arteries which can reduce the risk of stroke of the main advantages of warfarin were high oral bioavailability and high water solubility; it was more potent than dicoumarol, but its effect could still be reversed by vitamin- K.

Prostratin

Prostratin was obtained from plant Mamala tree derived from the *Homalanthus nutans*. Prostratin is also able to inhibit HIV infection because it induces down regulation from surface of target cell.

Mechanisms by which prostratin down-regulates HIV receptor and co-receptor surface expression in lymphocytic and monocytic cell lines. Our results indicate that prostratin induces down-regulation of surface expression of CD4 and CXCR4, but not CCR5, in various cell lines.

Down-regulation of CD4 and CXCR4 by prostratin is achieved by internalization through receptor-mediated endocytosis and macropinocytosis, which is then followed by degradation of these molecules. Because prostratin is a protein kinase C (PKC) activator, we next examined the potential contribution of distinct PKC isoforms to down-regulate CD4 and CXCR4 in response to prostratin stimulation. Although exposure of cells to prostratin or phorbol-myristate-acetate (PMA) induces the translocation of several PKC isoforms to the plasma membrane, the use of specific PKC inhibitors revealed that novel PKCs are the main mediators of the prostratin-induced CD4 down-regulation, whereas both conventional and novel PKCs contribute to CXCR4 down-regulation.

Altogether these results showed that prostratin, through the activation of conventional and novel PKC isoforms, rapidly reduces cell surface expression of CD4 and CXCR4, but not CCR5, by inducing their internalization and degradation. Prostratin used in the treatment of HIV (Paul *et al.*, 2008) [29].

For example, villagers in Samoa have a benefit-sharing agreement with US-based research institutions for shares of royalties from the use of prostratin, an anti-viral chemical derived from the bark of the native mamala tree. Their claim to compensation was thought to derive not just from their occupation of land containing the plant, but from their healers' prior knowledge of mammal's curative properties.

Drugs from microorganisms

Penicillin

Penicillin was discovered by Alexander Fleming in 1930s accidentally found a mould penicillin notatum. The amazing part of his work was that he found no microbial growth within a radius of 3–5 cm of the mould. Fleming named the drug penicillin, which was isolated from *Penicillium notatum*.

Mechanism of Penicillin is inhibiting the transpeptidase that catalysed the final steps in the cell wall biosynthesis, the cross linking of peptidoglycon. Penicillium; penicillin against gram positive bacteria, including *Streptococci*, *Clostridium*, *Neisseria*, and *Listeria*. Structurally, penicillins are β -lactam antibiotics. Bacterial cell walls are consisting of a protective peptidoglycan layer, which is continuously undergoing remodeling. The remodelling process involves the breaking of the β -(1,4) linked N-acetylmuramic acid and N-acetylglucosamine; as well as the breaking of the cross-linking peptide chains. This cross-linking peptide chains is

what provides the rigidity, to the otherwise fluid cell wall. The breaking of this peptide cross-linking is performed by an enzyme called transpeptidase.

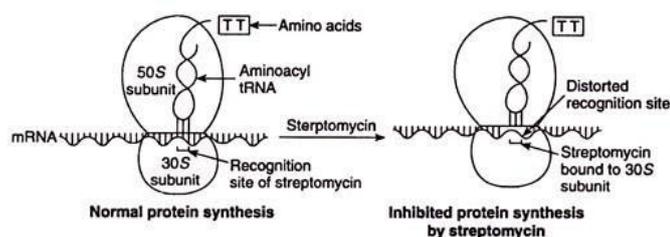
The transpeptidase also helps in reforming the peptide bonds once the restructuring of the cell wall is done. The penicillins act by inhibiting this particular enzyme. By inhibiting this enzyme the penicillin prevents the reformation of the peptide bonds.

This beta-lactam ring of the penicillin is generally not very stable and therefore it participates in the inactivation of bacterial cell enzymes which are essential for synthesis of peptidoglycan. Transpeptidase attacks the beta-lactam ring which opens up to give a more stable compound. When this happens the drug remains bound to the transpeptidase via covalent linkage and thereby inhibits the enzyme by acylation of the active site. The resistance to penicillin arises due to mutations in the active site of the transpeptidase enzyme. Thus there are many variants of the transpeptidase enzyme which need the use of newer penicillin antibiotics. It is available in the form of Benzylpenicillin (Grossman, 2008) [16].

Streptomycin

Streptomycin was discovered in 1943 from *Streptomyces griseus*, by Waksman (Kingston and Waksman, 2004) [23]. Mechanism of action streptomycin is a protein synthesis inhibitor. It binds to the small 16SrRNA to the 30s subunit of the bacterial ribosomes, interfering with the binding of formyl – mthionyl- tRNA to the 30s subunit.

Streptomycin is effective against both Gram positive and Gram negative bacteria, *Streptococci*, *Staphylococci* and *Pseudomonas* etc Streptomycin, like other aminoglycosidic antibiotics (e.g., gentamicin, neomycin, kanamycin, tobramycin), inhibits protein synthesis in bacterial cells by binding to the 30s subunit of ribosomes. The streptomycin causes a structural change which interferes with the recognition site of codon-anticodon interaction resulting in misreading of the genetic message carried by messenger RNA (mRNA). The mechanism of inhibition of protein synthesis. Streptomycin is available in the form of trobicin.



Chloramphenicol Pure chloramphenicol

Choramphenicol is Isolated from *Streptomyces venezuelae* in 1947. The mechanism of Choramphenicol is bacteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosomes. It specifically binds 50s ribosomal subunit preventing peptide bond formation (Glazko *et al.*, 1977) [17].

Chloramphenicol is a nonionized, highly lipophilic compound. It enters bacterial cells by passive or facilitated diffusion and binds primarily to the 50s ribosomal subunit but may also bind to the 30s subunit. As a result bacterial protein synthesis is inhibited. Chloramphenicol can also bind to the

mammalian ribosome (70s) that resembles bacterial ribosomes and interfere with mitochondrial protein synthesis. This is particularly relevant in erythropoietic cells. It is used for the treatment of a number of bacterial infections like, *Neisseria meningitis*, *Streptococcus pneumonia* and *Haemophilus influenza*.

Gentamicin

Gentamicin discovered in 1963. It was obtained from the bacteria *Micromonospora purpurea*. Gentamicin is a bactericidal antibiotic which irreversibly binding the 30s subunit of the bacterial ribosome, interrupting protein synthesis. Gentamicin “irreversibly” binds to specific 30s-subunit proteins and 16S rRNA. Specifically gentamicin binds to four nucleotides of 16S rRNA and a single amino acid of protein. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30s subunit. This region interacts with the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes. It is used to treat several types of bacterial infections like gram negative bacteria *Pseudomonas*, *Proteus*, *Escherichia coli*, *Klebsilla pneumoniae*, *Enterobacter aerogens* and Gram positive bacteria such as *Staphylococcus* (Weinstein, 1967) [40]. Gentamicin is sold under brand name Garamycin.

Drug from animals

Captopril

Captopril was isolated from venom of snake *Bothrops jarara*, Pit viper in 1977. The discovery of the ACE inhibitor and the creation of captorill was one of the really great advances in cardiovascular medicine. (Attoub *et al.*, 2008) [2]. Uses are based on its vasodilation and inhibition of some renal function activities. These benefits are most clearly seen in, Hypertension; Cardiac conditions such as congestive heart failure and after myocardial infarction; it is available in the form of capoten.

Marine bioprospecting

1. Bioprospecting in the terrestrial environment can be traced back centuries, collecting and screening commercially valuable samples from the marine environment.
2. Marine bioprospecting, also known as marine natural products research, is concerned with the exploration and exploitation of the rich biological and chemical diversity found in marine organisms which inhabit the oceans.
3. Numerous commercial products based on marine organisms have been brought to the market, including the following, (Molinsky *et al.*, 2009) [26].

Marine products

Cod liver oil

It is obtained from liver of cod fish, from different types of cod fishes like *Gadus callarius* and *Gadus morrhua* and other cods. It contains omega-3 fatty acids vitamin A and vitamin D. It is used for lowering blood fat, high blood pressure, osteoarthritis, kidney diseases, glaucoma and used for wound healing. In view of rich concentration of vitamin A and D with digestible fat, it is found to improve nutrition and calcification in patients with rickets and tuberculosis when used. It can also be used as supplement for children and can be applied to wounds and burns. The leading role of cod-liver

oil on rickets was a relevant factor in the knowledge of this disease. In 1922, the preventive and therapeutic value of cod-liver oil and sunlight against rickets in young infants was confirmed.

The seasonal variation in the incidence of rickets, the role of skin pigmentation, of diet and the fact that breast milk was not an adequate source of vitamin D were understood.

The discovery of essential fatty acids omega-6 and omega-3 have shown that deficiencies, mainly of omega-3 long chain polyunsaturated fatty acids, result in visual and cognitive impairment and disturbances in mental functions in infants and also in cognitive function in adults, as fatty acids are beneficial to vascular health and may forestall cerebrovascular disease and thus dementia.

An adequate ratio of fatty acids may promote a healthier balance of eicosanoids, which would protect membrane function with a nutraceutical function. Dietary lipids not only influence the biophysical state of the cell membranes but, via direct and indirect routes, they also act on multiple pathways including signalling, gene and protein activities, protein modifications and they probably play important role in modulating protein aggregation. Significant advances have been made in understanding the relation between dietary factors and inflammation, which is a central component of many chronic diseases, including coronary artery disease, rheumatoid arthritis, cancer prevention.

However, the identification of those who will or will not benefit from dietary intervention strategies remains a major obstacle. Adequate knowledge about how the responses depend on an individual's genetic background (Nutrigenetic effects), the cumulative effects of food components on genetic expression profiles through nutrigenomics mechanism, may assist in identifying responders and non-responders.

Thus, fish and fish oil consumption might encourage brain development and gene expression to brain maintenance during aging through nutrigenomic mechanism. It is available in the form of E-COD Omega-COD Plus (Rajakumar, 2003)^[31].

Shark cartilage

Derived from cartilage of shark from tough material of shark's skeleton. It is used for prevention of various diseases like cancer, It is available in varieties of brand name including Carticin, tiburon etc (Molinsky, 2009)^[26].

- **Shark cartilage:** Used for cancer, including a type of cancer called Kaposi's sarcoma, which is more common in people with HIV infection. Shark cartilage is also used for arthritis, psoriasis, wound healing, damage to the retina of the eye due to diabetes, and inflammation of the intestine (enteritis). Some people apply shark cartilage directly to the skin for arthritis and psoriasis (Ostrander, 2004)^[28].
- **Cancer:** Most research shows that taking shark cartilage by mouth does not benefit people with advanced, previously treated cancers of the breast, colon, lung, prostate, and brain or advanced previously treated non-Hodgkin's lymphoma. There are reports that shark cartilage might decrease tumors called Kaposi sarcoma (Davies *et al.*, 2012)^[11].
- **Osteoarthritis:** When applied to the skin, products containing shark cartilage in combination with chondroitin sulfate, glucosamine sulfate, and camphor reportedly reduce arthritis symptoms. However, any symptom relief is most likely due to the effect of camphor and not the other ingredients. Additionally, there is no research

showing that shark cartilage is absorbed through the skin.

- **Kidney cancer:** Taking a specific shark cartilage extract, used in advanced kidney cancer (renal cell carcinoma). This product has FDA "Orphan Drug status" for renal cell carcinoma. The Orphan Drug law gives drug makers special incentives to study drugs for rare conditions.
- **Psoriasis:** Developing research suggests that a specific shark cartilage extract might improve appearance and decrease itching of plaque psoriasis, when taken by mouth or applied to the skin (Arieta and Duarte, 2011)^[1].

Fish Protein

The proteins of the fish have high digestibility, biological and growth promoting value. Hence, it plays an important role in human nutrition. The available amino acids are more evenly balanced than the other proteins of animal origin. Amino acids like lysine and methionine are rich in fish protein, fish protein is somewhat superior to egg albumen, bean protein and casein and perhaps equal to chicken proteins. Fish muscle contains an excellent amino acid composition and is an excellent source of nutritive and easily digestible proteins. However, because fish is extremely perishable and because chemical composition can vary, the utilization of fish as a basic raw food material presents unique food processing problems. 15 - 25 per cent of protein is obtained from the fish muscle which forms the chief source (Ryan *et al.*, 2011)^[35].

Fish mea

Fish meal can be made from almost any type of seafood, but is generally manufactured from wild-caught, small marine fish that contain a high percentage of bones and oil, and are usually deemed not suitable for direct human consumption. The fish caught for fish meal purposes solely are termed "industrial" Other sources of fishmeal are from by catch and by products of trimmings made during processing (fish waste or offal) of various seafood products destined for direct human consumption. It is used as a protein source added in the poultry and pig feed; fishmeal is a excellent source of protein for poultry. It has high level of essential amino acid such as methionine and lysine, and it also has good balance of unsaturated fatty, certain minerals and vitamins (Rosendal, 2006)^[34].

Current practices in bioprospecting

Most bioprospecting is currently performed on a small scale by numerous academic groups throughout the world. There are some larger programmes based on multi-group collaborations (Hollway, 2006)^[18].

Table 1: Bioprospecting programme in India

1	CSIR Coordinated programme on drug Discovery (1996)
2	Department of biotechnology- bioprospecting and molecular taxonomy programme (1998)
3	New Millennium Indian Technology Leadership Initiative (NMITLI)-Planning Commission CSIR (2002)

CSIR coordinated programme on drug discovery

CSIR has initiated a coordinated programme on drug discovery with a network of 19 CSIR laboratories and other R&D institutions working in the area of traditional systems of medicines. The programme on "Bioprospecting" which was initiated in 1996 aims at discovering new bioactive molecules from plants, fungi, microbes, insects, etc. using new techniques of both synthesis and bio evaluation (Simpson *et*

al., 1996)^[36].

Department of Biotechnology

The Department of Biotechnology, Government of India, initiated the network programme on “Bio prospecting. Some of the significant achievements under the bio prospecting programme.

1. Prospecting of bio resources for agriculturally important compounds.
2. Construction of cDNA library for a mangrove species, *Avicennia marina*
3. Isolation of 24 genes with implication on stress tolerance for mangrove and cold desert plants
4. Identification of novel salt tolerant nitrogen fixing and phosphate solubilising bacteria from a wild rice (Farnsworth and Soejart, 1985)^[12].

New millennium Indian technology leadership initiative (NMITLI) on drug prospecting

The Planning Commission, Government of India and the Council of Scientific and Industrial Research (CSIR) in India has embarked on a few bio -prospecting programmes with some specific targets goals – such as the inter laboratory collaborative programmes on biomolecule drugs, drug prospecting. The Planning Commission sponsored the NMITLI, one of the most innovative bioprospecting programmes. NMITLI has major herbal drug development programme for developing effective herbal remedies for hepatic disorders, arthritis and diabetes, which has shown highly encouraging results within a short period of less than one year.

Four CSIR laboratories namely; National Botanical Research Institute (NBRI), Lucknow; Regional Research Laboratory (RRL), Jammu; Indian Institute of Chemical Biology, Kolkata; and Indian Institute of Chemical Technology, Hyderabad; and a large number of medical college hospitals like Kings Edward Memorial Hospital, Mumbai; Nizam Institute of Medical Science, Hyderabad; All India Institute of Medical Sciences, New Delhi; Bhartiya Vidyapeeth Deemed University, Pune; Bhartiya Vidya Bhavan’s Swami Prakashanand Ayurvedic Centre, Mumbai; are the research partners in this programme. Kottakkal Arya Vaidya Pharmacy, Arya Vaidya Pharmacy, Coimbatore, Dabur Pharmaceuticals etc. (Lindgreen *et al.*, 2009)^[25].

Future prospects

Bioprospecting has been proposed as a potential means to encourage the conservation and sustainable use of biodiversity. Natural products may only be available in small amounts: techniques for direct synthesis (Sunazuka *et al.*, 2008)^[39] or production by molecular biology (Kennedy, 2008)^[21] have been rapidly developing. While there is certainly no single “best” way to conduct drug discovery, just as there is not a single panacea for all ailments, it is surely time for a fresh look at the relatively unexplored opportunities provided by modern approaches to applying natural products in drug discovery.

Perhaps the lead will have to be provided by the numerous academic groups active in bioprospecting. However, these groups would stand more chance of success if they could pool resources and work towards finding validated lead compounds that are likely to be suitable for development into medicines for unmet therapeutic needs. The growth of translational research and the establishment of centers of

translational research will be academic groups to become essential partners.

Conclusion

Bioprospecting has been historically a dominant source for medicines and agricultural products. It remains a significant source for these, and a variety of other, economic goods. In the developing world, the vast majority of the population regularly relies on natural products as their main source of medicine. Even in the developed world, a majority of the most-prescribed prescription drugs are either natural products themselves or substantially derived there from. Genetic resources represent a rapidly growing and highly promising source of new drugs.

Bioprospecting is the process of discovery and commercialization of new products based on biological resources. Despite indigenous knowledge being intuitively helpful, bioprospecting has only recently begun to incorporate such knowledge in focusing screening efforts for bioactive compounds. Bioprospecting with its potential as a rich source of new therapeutic agents is an important tool for drug discovery and research. However collaborations between pharmaceutical companies and the countries supplying the indigenous knowledge and medicinal resources should be regulated for mutually beneficial relationship. Bioprospecting is advantageous in several ways; the methods and application adopted by pharmaceutical firms have been criticized in several forums.

The phenomenon of bioprospecting faces a typical situation where crucial raw material are primarily owned by the poor tropical countries while necessary biotechnology and R&D component are regulated by the pharmaceutical firms of the developed nation.

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