Antibiotic natural products: Opportunities and challanges

Zafar Alam Mahmood[1] PhD and Saad Bin Zafar Mahmood[2] MBBS

[1]Colorcon Limited, Flagship House, Victory Way, Crossways, Dartford, Kent, DA2 6 QD, England
[2]Ziauddin Medical College, Ziauddin University, Karachi – Pakistan
[1]Corresponding author e-mail: zmahmood@colorcon.com

The biomedical and therapeutic potentials of Antibiotic Natural Products have been given a prominent and distinct position in the field of medical sciences in view of the novel molecules delivered by this discipline of science after extensive and exhaustive research investigations. Introduction of new or improved technology relating to isolation, extraction and structure elucidation has significantly contributed in the identification of new molecules from natural sources originating from higher plants, herbs and marines which can act as a lead compound. Consequently, various molecules from these natural sources have been discovered and classified under phytomedicines and phytopharmaceuticals for more extensive studies to explore the possibility of their use as antimicrobial agent, including HIV. The natural habitats from terrestrial and marine sources (such as marine algae, sponge, and humic acid sediments) because of the chemical diversity, have been observed to produce bioactive molecules through secondary metabolic pathways and are capable of acting against a wide range of microbial flora or pathogens. The antimicrobials from marine sources have been given considerable attention globally and as a result a number of drugs are under different phases (such as I/ II and III) of clinical studies and a few have already been marketed. The antimicrobial enzymes and peptide have been given distinct position because of their applications against resistant strains, while the essential oils from aromatic plants have been recognized as a highly effective way and tool for controlling microbial propagation by using in various personal and health care products. The secondary metabolites belonging to various groups, such as alkaloids, phenolics, tannins, and triterpenoids from natural sources have been extensively investigated and observed to possess potential antimicrobial activity to be used in modern system of medicine. Interestingly, the various natural samples collected and reported worldwide showed quite impressive results in terms of antimicrobial action. For more appropriate and fast results, bioautographic, genomic and proteomic methods can be applied in screening of antimicrobials from natural habitats using their crude extracts, while the introduction of more systematic research and investigation using standardized extract develop through advanced analytical procedure will certainly be helpful in establishing and commercializing natural products as potent antimicrobial agents. Further, design of a suitable pharmaceutical dosage form of the bioactive compounds, either as an immediate or modified release form along with pharmacokinetic studies are highly desirable.

Keywords Antibiotics; Plants; Herbs; Essemtial oils; Marine Algae; Antimicrobial Enzymes and Peptides Secondary Metabolites; Bioautography.

1. Introduction

The natural products labeled under secondary metabolites are synthesized through simple to complex mechanism in natural habitats and play a momentous role in the management of various disorders because of their biological activity. The magnitude of natural products as medicine in human healthcare is massive and is chronicled throughout the history of evolution. The world's two biggest system of medicine, the "Traditional Chinese Medicine" (TCM), based on *"Wu Shi Er Bing Fang*" (Prescriptions for fifty-two diseases) compiled in around 350 BC and the "Indian Ayurveda" written during 900 BC, encompass about 247 natural products and 341 plant derived medicines respectively [1,2]. One of the leading groups of such natural products has been identified as "Antibiotics", substances which have crafted insurgency in the microbial world in terms of management and therapy. The vast majority of research activities worldwide pertaining to the exploration of secondary metabolites from natural habitats have been undertaken under screening program from academic point of view only. However some studies have been organized not only for a better understanding of nature but also to discover and deliver the products with enhanced therapeutic value and its possible commercialization for the benefits of human beings. The official compendia of different regions and countries have certainly contributed well to incorporate various bioactive compounds, yet a large number of identified and well researched compounds are waiting for acceptance. The delay in such acceptance relates to global as well as regional regulatory issues in extending the prominent status of natural products for clinical use. At the global level, the factors which have fostered a division between mainstream drugs and botanical / natural therapies include the development of considerable number of pharmaceutical industries, with sophisticated facilities, well capable of producing highly purified and potent chemicals in bulk which are used as drugs as well as the lack of patent protection for botanical / herbal or natural products in absence of proper legislation along with the regulatory requirements by the US FDA. The regional factors are more operational in nature. In contrast to the existing FDA approved drugs (antibiotics or semisynthetic antibiotics) with well-established chemistry and pharmacology, the herbs and other natural sources contain a wide range of phytochemicals, the relative concentration of which varies considerably depending on genetics, growing conditions, parts used, time of harvesting, preparation, and storage and thus hampering in reproducing the antimicrobial activity at the end of clinical studies. Though, the present status of botanical drugs approval by FDA is

quite disappointing and only two pure botanical drugs, "Veregen" (polyhenone E, kunecatechins), a special extract of "Green Tea" and "Fulyzaq" (crofelemer) made from the sap of the *Croton lechleri* are available only by prescription. Veregen was approved in 2006, for the topical treatment of genital warts caused by the human papilloma virus (HPV), while, Fulyzaq, was given approval very recently in 2012, the first oral (and second botanical drug in history of FDA) for the management of HIV associated watery diarrhea [3].

Despite all the challenges, the approval of Fulyzaq by FDA is expected to boost the natural / botanical drug market in near future. Based on recent market reports the natural products industry which has now grown up to US$ 22.1 billion in 2012, is expected to reach US$ 26.6 billion in 2017 [4]. A new category or title "NB" to cover botanical drugs has now been given for "Natural Product Botanical", which is also recognized by FDA and other regulatory authorities [5]. There is hardly any doubt in communicating and documenting the benefits of natural antibiotic substances to mankind, even in presence of potent synthetic compounds. The modern knowledge of phytochemistry, scientific equipment and technology has had a great impact upon natural product chemistry, including isolation, extraction, purification and structure determination, but the discipline still demands more from the research investigators in terms of establishing the clinical significance of natural compounds to be recognized as a drug. The antibiotics from natural sources still needs considerable attention in terms of more organized studies to understand their behavior and targeted comparative studies to ascertain their clinical significance before highlighting any benefits over the existing antibiotics or the synthetic antimicrobials. Though, the international regulatory bodies and organizations are supporting such studies, but nevertheless it needs considerable funding to encourage scientist to work more and explore superior products of commercial value.

The historical background of antibiotic from nature is not only very interesting but also attractive. With the discovery of Penicillin in 1928 during World War II, then its purification in 1941, followed by its commercial production, around 70.762 MT per annum by the end of 1949 had revolutionized the concept of antibiotic therapy and the period was regarded as "Golden Age of Antimicrobial Therapy". The subsequent discoveries of a series of antibiotics and semi-synthetic antibiotics belonging to Penicillin and Cephalosporin groups, followed by the introduction of Macrolides and Quinolones provided a new vista in the management of a vast majorities of bacterial infection. The molds (Penicillium) and the Actinomycetes (Streptomyces) were on the top in the discovery of a series of antibiotics for at least five decades and finally after saturation, started declining and since 2009 microorganisms, including those isolated from marine sources are now more focused by the big pharma industries for new drugs. During the period when microorganisms were utilized for most antibiotics, the use of plant or marine based products (pure compound / extracts or derivatives) as antimicrobials was practically absent. The herb based antimicrobials have once again gained popularity during late 1990s and a number of research institutions started working on the subject because of the development of resistant strains of common pathogens worldwide with existing antibiotics. For more details on the existing antibiotics, it will be reasonable to read some comprehensive reviews and texts published elsewhere as it is really beyond the scope of this chapter to discuss in detail the history and development of existing antibiotics from molds and actinomycetes.

It will not be exaggerating to mention here that nature has been the prominent source of antibiotics since times immemorial. The role and importance of natural source available both at land & sea (such as higher plants, herbs, marine algae, sponges etc.,) in the management of infection & infectious disease cannot be overemphasized. Out of total area of the world (510.10 million sq. km), the water occupies around 70% (361.80 million sq.km), while land is only around 30%, equivalent to 148.30 million sq.km [6], showing great potential for marine habitats for the new drug discovery. Based on several studies, it is now well documented that the natural habitats from terrestrial and marine sources can provide everlasting foundation for vital active ingredients in the management of many intractable infectious diseases and next couple of decades will certainly be very interesting to watch how drugs from natural source can prove their superiority over the synthetic ones. The earlier studies on antibiotic from natural source are not very much promising, yet a number of plants, herbs and marine algae (seaweeds) were identified to possess reasonable antimicrobial activity against a wide range of pathogens in the crude extracts. However, elaborated or well controlled clinical studies are still lacking to authenticate their clinical significance. With the advancement in extraction, purification and structure elucidation techniques, scientists have been able to purify some potential pure compounds but still the large part of the scientific studies on natural antibiotics are based on crude or standardized extract. Further, with the introduction of biopharmaceutics, followed by advanced level of pharmacokinetics and pharmacodynamics information, researches started paying attention to study the effect of pure compounds or the standardized extracts on various clinical isolates and the behavior of compound in the animal modules, yet it needs more broadened studies to fulfil the criteria to be used as a drug for therapy. The advancement in isolation, extraction and structure elucidations techniques have greatly helped to expend the study of bioactive phytochemicals in its pure form both from terrestrial and marine sources. However, at the same time a great deal of research activities has also been noted using standardized herbal extract and some essential oils.

With the increasing prevalence of resistance with existing antibiotics against common pathogens, more focus has been driven to expand the spectrum of antimicrobials parallel to antibiotics available through terrestrial and marine sources. The theme of the World Health Day 2011 was very much in the same line and direction, i.e., *"Antimicrobial resistance: no action today, no cure tomorrow"*. Over the years it has now become evident that productive use of many antibiotics is haggled by the rapid development of resistance. On the other hand, the percentage of new antibiotic

discovery and its approval has not kept pace with the rising prevalence of drug resistance [7]. One of emerging strategy to overcome, the resistance issue is the introduction of antimicrobial enzymes and antimicrobial peptides, now brought into intense investigation with the aim to disrupt the bacterial cell and biofilm formation [8]. A series of antimicrobial enzymes, such as proteolytic enzymes (e.g., subtilisins, lysotaphin, bacteriophage lysins), polysaccharide-degrading enzymes (e.g., lysozymes, alginate lysases, dispersin B, amylases), oxidative enzymes (e.g., hydrogen peroxide–producing enzymes, hydrogen peroxide-responsive enzymes), anti-quorum sensing enzymes (e.g., acyl homoserine lactones) have now been studied and reported, Discovered in late 1980's, the antimicrobial peptides (APMs or APs) have been identified as novel broad spectrum antibiotics capable of eradicating both Gram negative and Gram positive organisms (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), protozoa, fungi (*Cryptococcus & Aspergillus*) yeasts (*Candida albicans*) and viruses (HIV and Influenza virus). A number of naturally occurring peptides and their derivatives have been developed for the treatment of conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), and topical skin infections [9,10].

The studies relating to natural products have been very well supported by WHO and other governing bodies worldwide. Some of the earlier reports indicated that more than 80% of the total population of the globe is still benefiting with the use of herbs and herbal products and more than 100 countries have regulations for herbal medicines [11,12]. The Alma-Ata Declaration in 1978 has greatly supported and accelerated the research activity not only in the Eastern side of the globe, but at the same time the US and EU also decided to fine-tune their laws on herbal products in 1994 and 2004 respectively [13]. The pharmaceutical industries are investing a lot to enter into the natural product business and trying hard to commercialize their research products. The research activities relating to antibiotics from natural source other than microorganisms has thus also occupied a prominent place and as a result few valuable products have been discovered and marketed very successfully. It has tremendous opportunities to bring new molecules and compounds which can fight against resistant organisms. However, the scenario is equally challenging in view of great regulatory issues, poor financial support for research and lack of coordination between academia and pharmaceutical industries in generating efficient, meaningful and constructive data.

2. The natural source for antibiotics

2.1. Antimicrobials from terrestrial source (higher plants, herbs, herbal extracts and essential oils)

For a long period of time, herbs and their preparations including the essential oils have been acknowledged as valuable source of natural products (lead compounds) for maintaining human health, both for prophylaxis and treatment. Most extensive studies relating to antibiotics from herbs have been reported in Indian Ayurvedic system and in Traditional Chinese System of Medicine. Yet, the higher plants, still largely seems to be unexplored for the identification of potent compounds active against microorganisms. Very few active compounds so far have been isolated and tested out of huge number of plant species (250,000 to 500,000) reported to be present in the globe, while the overall percentage of the plants so far been explored out of these huge numbers, the antimicrobials lies between 1 to 10% [12,14]. Even with this low percentage, some very useful plants have been identifed showing potential to explore the possibility to use as drugs. Classicial examples are present where plant extracts or plant based products have been used for some specific treatment when other resources were not available or remain unable to deliver the desired effect. For example, *Andrographis paniculata* (Kalmegh or Indian chiretta) was very successfully used to control influenza epidemic of Bengal (India) in 1919 using a tincture of *Andrographis paniculata.* During the same period it was also used to control cholera and dysentery in the Phhillippines, while China is reported to use this plant to treat fevers and as an anti-infectives for centuries [15]. This is a great plant which has been extensivly studied for its pharmacological and toxicological studies. The antimicrobial activity is reported against *Salmonella, Shigella, Escherichia coli, Streptococci, Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Plasmodium falciparum , Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania infantum and* viricidal against herpes simplex virus 1 (HSV-1) without having any significant cytotoxicity at viricidal concentrations [16]. The plant ceretainly needs attention but so far has not been recognized as drug. Other examples, include that of Allinine from *Allium sativum* (Garlic), Berberines from *Hydrastis canadenis*, phenolics and anthocyanins of *Vaccinium macrocarpon,* caftaric acid and cichoric acid *of Echinacea purpurea / Echinacea angustifolia* which certainly needs attention to explore as drugs.

Desipite, several examples, it is still believed that antibiotics from plants symbolize a massive unexploited source for medicine. Therefore a continued and in depth controlled studies for exploration of plant based antimicrobials are desirable in view of their enormous therapeutic potentials. Most in vitro studies only relates to the level of screening program to evaluate the phytochemicals and their antimicrobial activity. It is evident that herbal extracts or pure compounds, both with known antibiotic properties, can be of great magnitude in therapeutic treatments and in the last few years, a number of scientific papers have been published all over the world to prove the efficacy of a large number of herbal extracts or compound herbal formulations. Similarly, the essential oils (EOs) have long been recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties and have been very successfully used in cosmeceuticals and hosehold products [17]. PubMed search for the antimicrobial activity of medicinal plants generated

3328 articles from a period between 1961 to March 2013. The number of scientific paper published in each decade is quite interesting: only 45 papers during 1961 to 1971, 104 papers during 1972 to 1982, 360 papers during 1983 to 1993, 1500 papers during 1994 to 2004 and 1315 papers from 2005 till March 2013. The PubMed figures are reasonable to conclude that significant research activity has been done in the last two decades to explore antimicrobials from plant source. The new scientific approach has provided considerable support and as a result, large number of herbs are now being used in traditional system of medicines, due to their characteristic antimicrobial feature which relates to some bioactive compounds synthesized through the secondary metabolism. This approach has also extended considerable focus in evaluating the antimicrobial activity of some prominent compounds, such as alkaloids, flavonoids, phenolics, tannins, naphthaquinines, diterpenes, triterpenes and sesquiterpene.

A large number of herbs used in the form of crude or standardized extract, or even in some cases as active component(s) to combat infection or support in wound healing are available as herbal products or nutritional supplements for use in different countries. However, because of regulatory status, these products or the formulations containing active components from various herbs are not treated as drugs. Therefore it will be worth to focus more attention to find and collect best possible pharmacological and toxicological data to bring these products into main stream line of antimicrobials for their possible use as drugs in view of their antimicrobial activity and spectrum. There is a huge list of herbs and essential oils so far evaluated in different parts of the world to explore the antimicrobial activity and these have been summarized in some excellent reviews which reflect more in depth information on the subject [18-20]. The history of phytomedicines reflects three distinct phases of their uses and application as antimicrobial agents.

Some of the most prominent herbs and essential oils used over the counter products and are available as herbal drugs or nutritional supplements have been reported to possess broad spectrum antimicrobial activity. These include: Aloe (*Aloe barbadensis*), Basil (*Ocimum basilicum*), Barberry (*Berbaris vulgaris; Berbaris aristata*), Bay (*Laurus nobilis*), Chamomile (*Matricaria recutita / Chamaemelum nobile*), Cinnamon (*Cinnamomum verum),* Clove *(Syzygium aromaticum),* Cranberry (*Vaccinium macrocarpon*), Dandelion (*Taraxacum officinale*), Dil (*Anethum graveolens*), Echhinacea (*Echinacea purpurea / Echinacea angustifolia*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Ginseng (*Panax notoginseng*) Goldenseal (*Hydrastis Canadensis*), Gotu Kola (*Centella asiatica*), Grapefruit (*Citrus paradise*), Green Tea (*Camellia sinenis*), Lavender (*Santolina chamaecyparissus*), Lemongrass (*Cymbopogon citratus),* Lemon verbena (*Aloysia triphylla*), Licorice (*Glycyrrhiza glabra*), Marigold (*Calendula officinalis*), Olive (*Olea europaea*), Rosemary (*Rosmarinus officinalis*), Sweet Flag (*Acorus calamus*), Tea Tree (*Melaleuca alternifolia*), Thyme (*Thymus vulgaris*), Turmeric (*Curcuma longa*), Usnea (*Usnea longissima*) and Yarrow (*Achillea millefolium*). Some reasonable in vitro studies and in vivo results in animal modules and even in some cases well organized clinical studies to supports their uses are also available. Despite all these, the above are still waiting to be recognized as drug for more extended clinical applications worldwide [18, 21-34]. Combination of various essential oils has mostly been observed in synergism and in some cases producing additive effects against various microorganisms [17]. The antimicrobial activity of some very commonly used herbs and essential oils is depicted in Table – 1.

Table 1 Antimicrobial activity of some selected herbs and essential oils.

Herbs / Essential oils	Antimicrobial spectrum of activity
Aloe	*Corynebacterium, Staph. aureus, E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus niger.*
Basil	*Salmonella typhimurium*
Barberry	*E. coli, Staph. aureus, Aspergillus flavus, Bacillus cereus, Streptococcus pneumoniae, Candida albicans.*
Bay	*Staph. aureus, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Clostridium botulinum*
Chamomile	*Mycobacterium tuberculosis, Staph. aureus, Salmonella typhimurium*
Cinnamon	*Staph. aureus, Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa, E. coli, Aspergillus parasiticus.*
Clove	*Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa, E. coli Aspergillus sp.*
Cranberry	*E. coli, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, and Bacillus subtilis,*
Dandelion	*Erwinia carotovora, E. coli, Staph. Aureus, Pseudomonas aeruginosa, Candida albicans, Saccharomyces cerevisiae*
Dil	*Klebsiella pneumoniae* and one strain of *Pseudomonas aeruginosa*
Echhinacea	*Hemophilus influenzae, Influenza Viruses, Streptococcus pyogenes (Group A streptococcus, or GAS)*
Garlic	*Salmonella typhymurium, E. coli, Staph. aureus, Bacillus cereus, Bacillus subtilis, Aspergillus sp,*

	Candida albicans.
Ginger	*E.coli, Staph. epidermidis, Streptococcus viridans, Candia albicans, Aspergillus niger, Pseudomonas aeruginosa.*
Ginseng	*E. coli, Sporothrix schenckii, Staphylococcus sp., Trychophyton*
Goldenseal	*Giardia duodenale, Trypanosomes*
Gotu Kola	*E.coli, Pseudomonas aeruginosa,*
Grapefruit	*Staph. aureus, Staph. epidermidis, Bacillus subtilis, Streptococcus faecalis, Candida albicans.*
Green Tea	*Shigella, Vibrio, Streptococcus mutans, Viruses*
Lavender	*Proteus vulgaris, Pseudomonas aeruginosa, Candida*
Lemongrass	*Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis. Proteus vulgaris, Pseudomonas aeruginosa.*
Lemon verbena	*Ascaris, E. coli, Mycobacterium tuberculosis, Staph. aureus.*
Licorice	*Staph. aureus, Mycobacterium tuberculosis, Helicobacter pylori.*
Marigold	*E. coli, Salmonella typhi, Klebsiella pneumonia, Enterobacter aerogenes, Agrobacterium tumefaciens.*
Olive	*E.coli, Pseudomonas aeruginosa, Staph. aureus, Klebsiella pneumonia, Bacillus subtilis.*
Rosemary	*Bacillus cereus, Staph. aureus, Vibrio parahaemolyticus*
Sweet Flag	*Escherichia coli, Staph. aureus, Klebsiella pneumoniae, Salmonella typhi and Listeria monocytogenes*
Tea Tree	*E. coli, Propionibacterium acnes, Klebsiella pneumonia, Staph. aureus (MRSA), Strep. Pyogenes, Prevotella spp., Porphyromonas endodentalis, Bacteroids spp, Porphyromonas gingivalis.*
Thyme	*Vibrio parahaemolyticus, E. coli, Staph. aureus, Pseudomonas aeruginosa, Viruses*
Turmeric	*E. coli, Vibrio cholera, Staph. aureus, Pseudomonas aeruginosa, Enterococcus faecolis,*
Usnea	*E. coli, Staph. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella dysenteriae, Trichoderma viride, Candida albicans*
Yarrow	*Erwinia carotovora ,bacillus subtilis, Staph. aureus, Pseudomonas aeruginosa, Viruses, Helminths.*

2.2. Antimicrobials from marine organisms (seaweeds, sponges and humic acid sediments)

Marine algae, sponge and humic acid sediments have been evaluated in different regions of the globe where coastal area is reported with rich source of some novel algal and other marine species responsible to act against wide variety of microorganisms. It is really very difficult to predict the actual number of marine species, but most researchers believe it to be around 800,000 to several millions or even more [35,36]. Though use of various algal species for therapeutic effect has a long history and is documented in Chinese herbal and Ayurvedic system of medicine, however no such application example (as a drug) is available in modern pharmaceutical system. At present the algal derived products, e.g., agar, carrageenan and alginates are mainly used in the food industries and as excipients in pharmaceuticals. Some other products derived from microalgae, such as carotenoids (e.g., β-carotene and astaxanthin), and long-chain polyunsaturated fatty acid (LC-PUFAs), docosahexaenoic acid (DHA) have been commercially produced and used as nutritional supplements and nutraceuticals.

The systematic research to explore the possibility of using some algal species having antibiotic properties had started during 1950s and by the mid of 1980s some valuable information was available showing potential of various algal species to be used as an antimicrobial agent [37-47]. Comprehensive attention during the last two decades has been given to explore marine organisms for the isolation and identification of antimicrobial compounds. This has resulted in enormous scientific publications documenting some potent antiviral, antifungal and antibacterial activities, against both Gram positive and Gram negative organisms [48,49].

The advancement in natural product chemistry has certainly boosted the research activity to explore the potential of marine habitats for new and potent antimicrobials capable of inhibiting the resistant strains which are developing at a continuous pace. The major threat is of methicillin resistant *Staphylococcus aureus* and some *Enterococci* resistant to even vancomycin, thus demanding more attention towards the possibility to explore some new molecules to counter these resistant strains in managing the infectious diseases. It is now well accepted in view of some published reports that marine based organisms can be helpful in introducing novel antibiotics with broad spectrum activity to counter such issues. The marine algae (seaweeds) have been paid special attention and all the three classes, Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) have been reported to possess antiviral, antifungal and antibacterial components [50]. During earlier studies, brown seaweeds have been observed to have broader antibacterial activity than the green and red algae [47,51]. A large number of original research articles as well as reviews are available, showing antimicrobial activity of marine algae against a wide range of microorganisms as a potential to explore further for new antibiotics [50].

Some studies on marine sponges relating to isolation of potent antibiotics have been performed. The sponges showed potential of such products which are synthesised naturally or because of symbionts of chemicals that may be used to control viruses, bacteria and fungi [52, 53. Marine sponges have been reported as sessile invertebrates with a wide variety of colours, shapes and consistencies. Highly diversified group of active compounds, such as alkaloids, peptides, sesquiterpenes, purine derivatives, fatty acids, macrolides, lectine, glycolipids etc., have been identified from various species of sponges and reported to possess antibacterial activity against resistant strains of *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Neisseria gonorrheae, Streptococcus pneumoniae*, and antibacterial activity against some species of *Mycobacterium tuberculosis and Helicobacter pylori*, thus showing excellent potential to explore the possibility to identify and extract novel compound responsible for such activity. Some important antibiotics (antibacterial, antifungal and antiprotozoal) identified from marine sponges include: Agelasines and agelasimines, Caminosides A-D, Discorhabdin R, Lectine, Psammaplin A, Macrolides, Eurysterols A-B, Discobahamin A-B, Manzamine A, Naamine D, Secomanoalde, Plakortide, Pachymatismin, Mirablin B, Kalihinol A etc. In addition some antiviral (anti HIV), such as Ara-A, Avarol, Dragmacidin F, Microspinosamide, Papuamides A-D A-B, along with Hamigeran B active against herpes and polio viruses have also been reported [54].

2.3. Antimicrobial enzymes and peptides

One of emerging strategy to overcome, the resistance issue with the common pathogens is the introduction of antimicrobial enzymes and antimicrobial peptides, now brought into intense investigation with the aim to disrupt the bacterial cell and biofilm formation [8]. The incidence relating to multiple resistances against various pathogens due to unsystematic use of antibiotics during treatment has significantly increased and the situation has perhaps, now become a global concern and challenge for the researchers to find a reasonable solution. A series of antimicrobial enzymes, such as proteolytic enzymes (e.g., subtilisins, lysostaphin, bacteriophage lysins), polysaccharide-degrading enzymes (e.g., lysozymes, alginate lysases, dispersin B, amylases), oxidative enzymes (e.g., hydrogen peroxide–producing enzymes, hydrogen peroxide-responsive enzymes), anti-quorum sensing enzymes (e.g., acyl homoserine lactones) have now been studied and reported.

Some of the antimicrobial enzymes are now successfully being used by healthcare, food and pharmaceutical industries to overcome the formation of biofilms with their products. These antimicrobial enzymes are quite effective in degrading the complex extracellular substances (such as, polysaccharides, proteins, lipids glycopeptides and nucleic acid) secreted by different types of microflora and are associated with formation of biofilm which is of great concern with respect to human health and environmental protection. A large number of products are now available commercially, containing suitable concentration of antimicrobial enzymes capable of acting against the formation of biofilm as well as the disruption of microbial cells [55]. The products used in healthcare (such as, catheters, contact lenses, dental plaques, endotracheal tubes, mechanical cardiac valves etc.) which are quite sensitive and vulnerable to biofilm attack are excellent examples where antimicrobial enzymes are used to avoid hospital-acquired infections which usually develop due to colonization of microorganisms in these medical devices. The pharmaceutical manufacturing facilities, including machinery, equipment, working area and production line all can provide enough opportunity for the colonization of microorganism and thus represents another sector for the application of these antimicrobial enzymes. The food, consumer and household cleaning product industries also find application of antimicrobial enzymes.

Subtilisins are the most widely used antimicrobial enzymes in different industries [56]. It has been successfully used in detergents and household cleaning products, to remove proteinaceous deposits and stains. [57]. Lysostaphin finds its applications in cellulose bandages, porcine biomesh, hernia repair meshes, catheters and carbon nanotubes to control biofilms and especially biofilms formed by the methicillin resistant *Staphylococcus aureus* on these products [58-62] and is also being successfully used in dairy industry to prevent mastitis, caused by several species of [63]. The bacteriophage endolysins are also gaining their applications in health, food, agriculture and in environmental maintenance. It has been successfully applied topically for the treatment of erythromycin and clindamycin resistant strains and in animal module to prevent colonization of *Streptococci* through oral application [64]. Lysozyme is commonly used since long to treat sore throats and has applications as preservatives to increase shelf life of fruits, vegetables, meat and cheese. It is also used in some chewing gums to treat periodontitis in children [65,66]. Amylases are quite useful in eliminating the biofilm from surface developed due to *Pseudomonas* and *Staphylococci* [67,68]. Some good results have been observed when alginate lyase enzymes were used to treat colonization of Pseudomonas aeruginosa in patients with cystic fibrosis. Combination with gentamycin has potentiated the treatment [69-71]. Studies to utilize the benefits of oxidative enzymes, glucose oxidase and hydrogen peroxide-responsive enzymes, haloperoxidases (e.g., myeloperoxidase and lactoperoxidase) in food, environment and personal and healthcare products are under investigation. In addition, another emerging group of antimicrobial enzymes, given the name anti-quorum sensing enzymes are also under investigation and expected to play important role in healthcare and agriculture products [8].

Discovered in late 1980's, the antimicrobial peptides (APMs or APs) have been identified as novel broad spectrum antibiotics capable of eradicating both Gram negative and Gram positive organisms (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), protozoa, fungi (*Cryptococcus & Aspergillus*) yeasts (*Candida albicans*) and viruses (HIV and Influenza virus). A number of naturally occurring peptides

and their derivatives have been developed for the treatment of conditions as diverse as oral mucositis, lung infections associated with cystic fibrosis (CF), and topical skin infections, inflammation and sepsis. In addition, the antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators [9,10,72]. For many years, antimicrobial peptides have been used to inhibit or manage infections, e.g., the peptides polymyxin B, gramicidins, and bacitracin extend prominent antibacterial effect in many topical applications. Omiganan pentahydrochloride, or MBI 226, a topical antimicrobial peptide prevents central venous catheter-related infections due to colonization of a catheter by skin bacteria. Lantibiotics, such as nisin produced by *Lactococcus lactis* is another type of antimicrobial peptide which has also been given consideration by researchers as a food preservative. The use of nisin has now been approved as a preservative for cheeses and other food products. In addition, nisin has also been approved for use in ready-to-eat meats where it has most important and promising function, i.e., to control the growth of *Listeria monocytogenes* [73].

Antimicrobial peptides have also been reported in bacteria, frogs, some plants and in mammals, including humans [74,75]. Most findings suggest that antimicrobial peptides are protected evolutionally in their innate immune response, which provides a natural first-line of defence system for the majority of living organisms. The innate immunity peptides have been reported as upcoming source of new antibiotics with extended clinical applications[76]. During the last two decades extensive research work has been performed and reported by a large number of investigators on antimicrobial peptides. Earlier reports published in 2004, indicated the presence of 525 peptides, out of which 498 were identified as antibacterial, 155 antifungal, 28 antiviral and 18 as antitumor[77]. The figure is now significantly increased to 1228 in 2009, with 944 antibacterial, 327 antifungal, 76 antiviral (53 anti-HIV), and 65 antitumor antimicrobial peptides. The new peptides have been identified both at the gene and protein levels [78]. With the introduction of second version of peptide database (PDA2), it's now very convenient to check and identify the peptide family, source, post-translationally modified peptides and peptide binding targets. Very recently, cysteine-containing antimicrobial peptides of plant origin have been reported to be very active against Gram negative bacteria, such as *Salmonella enterica* and *Helicobacter pylori* with very low hemolytic activity, indicating potential for the design of new antimicrobial peptides [79].

Plant antibacterial peptides are of significant importance as components of barrier defence and as constitutive defence response induced upon infection in a wide variety of plants. Purothionin is the first antibacterial peptide isolated from plant species*, Triticum aestivum* (wheat flour), capable of inhibiting the growth of some phytopathogens such as *Pseudomonas solanacearum, Xanthomonas campestris and Corynebacterium michiganense*. In addition several antimicrobial peptides with antibacterial activity have been reported. These include: thionins, now named defensins, but also by other groups of proteins such as cyclotides, glycine-rich proteins, snakins, 2S albumins, and hevein-type proteins. Various parts of the plants, such as roots, seeds, flowers, stems, and leaves and have been demonstrated to produce antimicrobial peptides. Antimicrobial peptides have now become a remarkable tool for the elaboration of new techniques in the management of crop losses and in the production of new antibiotics for the treatment of various human infections [80].

3. Antimicrobial screening procedures

The in vitro antibiotic screening methods include, diffusion, dilution and bioautographic assays against designated ATCC (American Type Culture Collection) cultures. However, some clinical isolates can also be used to detect the antimicrobial activity against resistant strains. The initial screening tests are followed by the determination of minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration able to inhibit any visible microbial growth. The diffusion assays can be performed by disc diffusion assay, i.e., by using paper disc (of specific diameter) impregnated with a suitable concentration of natural extract or by well diffusion assay, where extract sample is poured into a well (of specific diameter). In both cases suitable solid medium, such as Tryptic soy agar, or Mueller-Hinton agar containing appropriate concentration (around 10^4 – 10^8 CFU) of 24 hours old bacterial or 18 hours fungal culture (inoculum) is used to spread on the agar surface or mixed into agar media. The plates are incubated for 18 to 24 hours at 35^{o}C to 37^{o}C depending upon the type of microorganisms used and the sensitivity or spectrum of activity is recorded by measuring the zone of inhibition. In case of dilution assays, which are usually applied to determine the MIC, it is usually carried out in liquid media, such as Tryptic soy broth or Mueller-Hinton broth. In some cases, solid media (agar dilution assay) can also be used where the microbial cell suspension is spread over the surface of the solid medium, inoculated in the centre of the plate by streak method or by mixing with the medium as performed in the broth dilution assy. The natural products are dissolved in DMSO (10% of the final volume) and usually diluted with culture broth at a concentration of 2 mg/ml. The determination of MIC is followed by the estimation of minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFG) by plating out samples completely inhibited dilution cultures and assessing growth after incubation[47,81,82].

The bioautographic technique is an excellent and very fast way to detect the antimicrobial activity of natural extracts or compounds. The method utilises the application of samples on TLC plates prepared from silica gel G60 F_{254}. The plates are run with the suitable solvent system and after drying, the plates can be irradiated with UV light to detect the florescent spots. The chromatogram is then transferred to a sterile petri dish and suitable solid medium (as described under diffusion assays) containing required concentration of the test organism (similar to diffusion assays) is poured

over the chromatogram and incubated in a similar way as described above. At the end of the incubation period, the growth inhibiting zones yielded by the active substance are difficult to visualize on bioautographic plates because of the opacity. Therefore, indicator solutions, such as 0.2% triphenyltetrazolium chloride and 0.5% glucose are required to spray over the plate. The plates are further incubated for one hour and then the inhibitory zones can be visualized as light coloured areas against dark coloured growth of organisms [83].

4. Pharmaceutical dosage form and presentation

The successful commercialization of any research product finally depends upon how the product is delivered to achieve the desired therapeutic effect and this leads to design of appropriate dosage form suitable for the patient. The history of pharmaceutical dosage form for human application is quite old and a gradual improvement has been observed with the advancement in pharmaceutical technology and engineering. The design of a suitable dosage form, especially of natural antibiotics is of considerable importance as the desired level of the drug is required to be present in the blood stream to achieve antimicrobial activity. The solid oral dosage form is perhaps the most suitable and effective way to deliver natural antibiotics from plant and marine sources. However, in view of vast majority of compounds active against skin, oral flora, creams, ointments and lotion for dermatological application along with mouth washes should also be considered effective way of presentation. The activity of some compounds against bacterial conjunctivitis, also justifies the preparation of sterile ophthalmic dosage form. The injectable form should also be investigated, if the drug is degradable in the GIT because of the enzymatic interaction or if the oral absorption is limited. In such cases more sophisticated techniques can be used to ascertain the safety profile.

The best possible way to deliver natural products will be through the immediate release film coated tablets, masking the bitter taste and strong smell. This may be followed by oral suspension for pediatric use in palatable form. The role of excipients used to formulate the drug will have a great impact on the bioavailability of the drug and a pharmacokinetic study will be required to observe the pattern of drug absorption, distribution, followed by biotransformation and elimination. In most cases the killing curve will be highly useful to ascertain the activity of the drug and to compare with known drugs. In the advanced level of investigation, bioequivalence test can also be performed to compare two dosage formulations with respect to pharmacokinetic profile. With recent advancement in pharmaceutical dosage form, an extended release form can also be considered to formulate solid oral dosage form to achieve a sustained effect over a period of time, based on the drug solubility and absorption pattern. It will be suitable to use the freezdried form of the drug to formulate the immediate or extended release product in view of their hygroscopic nature. The final presentation of the tablets can be made depending upon stability of the product and the climatic zone, accordingly suitable packing material, such as high density bottle, amber colored glass bottle or blister packing with Alu-Alu on sides or a PVC / PVDC packing can be used.

5. Proposals and conclusion

There is hardly any doubt in concluding and proposing the natural products as one of the most leading sources to explore and find new antibiotic molecules with broad spectrum activity and at the same time least side effects to fight against pathogens. This may require some immediate measures both on global and regional levels and funding to encourage the academic researchers, whereas commercial research organizations should also make a liaison with academic institution to support their new findings in designing and establishing well organized study of antimicrobials from terrestrial and marine sources. The antimicrobial enzymes and peptides are targeted as future hopes in creating unique, operative, safe and effective broad spectrum antibiotics capable of countering the resistant strains. So far WHO has done remarkable job to promote natural products, including herbs and herb based products. The use of new techniques relating to isolation, identification, extraction and commercialization of the products should be given preferences to better, faster and more reliable economical products. More authentic scientific data is highly essential in the recognition of natural antibiotics. Thus, along with the in vitro studies, more controlled in vivo studies on animal modules, followed by phase wise clinical trials are proposed. The inclusion of the behaviour of the natural products or the active components in the body, i.e., pharmacokinetics, followed by mechanism of action, toxicological and histopathological studies are highly desirable. In addition, focus has to be given to determine the most suitable dosage form for stable, persistent and reproducible effect.

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