Bioactive Peptides From Plants: A Promising Area of Therapeutics

A project submitted

by

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ID: 13146011

Session: Spring 2013

to

the Department of Pharmacy

in partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

August, 2017

Certification statement

This is to certify that this project titled bioactive peptides from plant source: a promising area of therapeutics' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University, constitutes my own work under the supervision of Dr. HasinaYasmin, Associate Professor, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project paper itself.

Signed,

Countersigned by the supervisor

.....

Acknowledgement

First of all, I express my greatest gratitude to Almighty Allah for endowing me with health, patience, protection and faith in all aspect of my life.

I would like to express my sincere gratitude to my supervisor Dr. HasinaYasmin, Associate Professor, Department of Pharmacy, BRAC University, for her continuous support, patience, motivation, enthusiasm, immense knowledge and guidance in this project work. Her guidance, positive attitude and scholastic knowledge helped me during the research and writing of this project paper. Without her guidance, my study and research would not be complete.

I would also like to thank Dr. Eva Rahman Kabir, Chairperson of Department of Pharmacy, BRAC University, for providing me with an opportunity and necessary support to carry out the project at an individual level.

Finally, I would like to thank the faculty members of Department of Pharmacy at BRAC University, my friends and my family who constantly encouraged me and pushed me to get through and complete my project successfully.

Md.Fazle Rabbi

Abstract

Bioactive peptides play an important role as a source of therapeutic drugs. Peptides are found from both food and plant sources. This review article focused on the plant source of bioactive peptide. The objective of this research was to find out different therapeutic activity of bioactive peptides isolated from plant source. Methodology of this review article was literature review from different authentic sources of journals. Bioactive peptides from plants have some important therapeutic activity such as; anticancer, antitumor, antimicrobial etc. The rationale of this research is to establish peptides from plant source which can be a promising area of therapeutics in near future. The purpose of the study was to find an alternative source of drugs which will help to reduce the resistance problems of many drugs, especially antimicrobial resistant. Bioactive peptide has a wide range of antimicrobial activity. Additionally this will not cause antimicrobial resistance. The result of the study is that bioactive peptide from plant can be a promising area of therapeutics. This source can be used to develop different therapeutics drugs along with antimicrobial drugs which is a burning question of now a days.

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Abbreviations

AMPAntimicrobial peptides

AMR Antimicrobial resistance.

ACE Angiotensin converting Enzyme

CRP Cysteine-rich peptides

FPLC Fast performance liquid chromatography.

SPPS Solid phase peptide synthesis.

HPLC High performance liquid chromatography.

FDAFood and drug administration.

CNSCentral nervous system.

1.1 Plants as source of drugs

From the ancient time plant is the most trustworthy source of therapeutic drugs. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age which is about 60,000 years ago (Solecki 1975). There are plenty of examples of plants which work as a source of therapeutic drugs. Such as *Azadirachtaindica*is a well-known plant and used to treat different diseases including skin diseases, stomach pain etc. It also shows antimicrobial activity (Kawshik*et al.* 2002). *Ocimumtenuiflorum*is used to treat sore throat, cough and common cold, asthma, heart disease, lung disorder etc. *Aloebarbadensis Mill*is another plant having great therapeutic effects. It has proteolytic enzyme which help to repair or replace the dead cells on scalp. It has also some beneficial effects on skin. A list of some clinically important drugs along with the sources is shown in Table 1.1

Table 1.1 List of drugs from plant sources along with clinical activities (Marjorie*et al.* 1999)

Drugs	**Therapeutic activity**	**Sources**
Valepotriates	Sedative	Valerianaofficinalis
Senosides	Laxative	Cassia spp.
Vincamine	Cerebral stimulant	Vinca minor
Theophyline	Diuretic, bronchodilator	Camellia sinensis
Scopolamine	Sedative	Datura metal
Teniposide	Antitumor agent	Podophyllumpeltatum
Tevioside	Sweetener	Stevia rebaudianabertoni

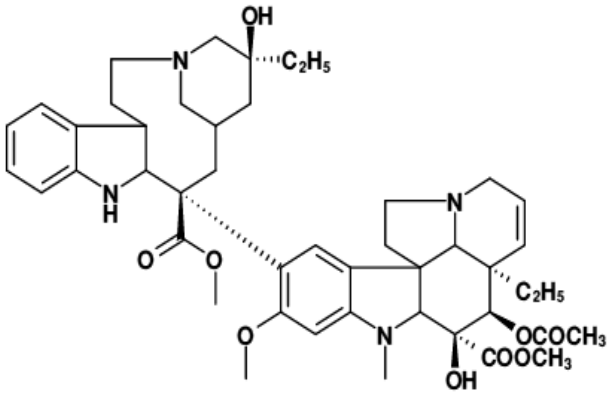
ValerianaofficinalisVinca minor

Camellia sinesisPodophyllumpeltatum

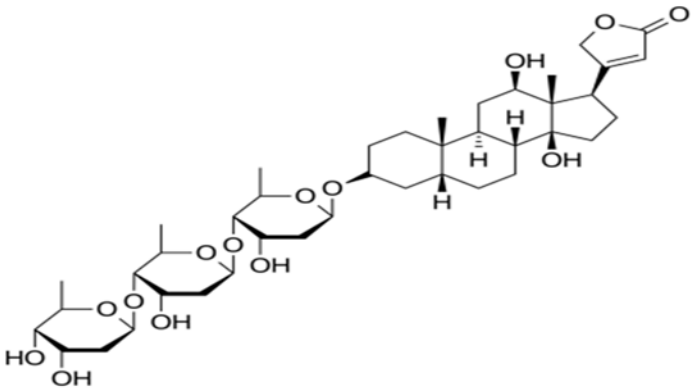
Figure 1.1Some important medicinal plants

There are some reasons why plants are used as a trusted source for therapeutic agents such as,

a) Some important bioactive compounds can directly isolated from plants which can be used as drugs for example, vinblastine, digoxin, vincristine, digitoxin, morphine are isolated directly from plants.



Vinblastine



Digoxin

Figure 1.2 Drugs directly isolated from plants (Norman, 1985)

b) Bioactive compounds which structures are known we can be used them to synthesize these derivatives.

c) The total plant or the fragment of the plant can be used as a herbal remedy such as- cranberry, echinacea, feverfew, garlic, ginkgo biloba etc. (Norman, *et al*. 1985)

Plants are also used in aurvedic and unani medicine system from ancient time and even in this age too. Availability of plants in nature is also a great reason to choose plants as a source. Nature has given us a vast resource of plants which we can use to treat various diseases. Recently a lot of new moleculesare derived from plants(Table 1.2).

Table 1.2Sources of plant products and their derivatives (Patricia *et al.* 2011)

Source plant	**Plant product**	**Derivative**
Cannabis sativa	9-tetrahydrocannabinol n cannabadio	Nabilone (Eililly)
Artemesiaannua	Artemisinin	Artemether
Camptotheca acuminate	Camptothecin	Topotecan
Podophyllumpeltatum	Podophyllotoxin	Etoposide
Taxusbrevifolia	Taxol	Docetaxel (Aventis)

1.2 Bioactive peptides

The term ‘bioactive” is a combination of two different words. These are ‘bio’ and ‘active’. These two different words came from two different origins. One is ‘bio’ which came from Greek word ‘bios’ and the other one is ‘active’ which came from Latin word ‘activus’. This term can be expressed in an alternative way that is biologically active. In a broader sense it is a compound which has an effect on any form of living cells or organisms (Guaadaui*et al.* 2014).

Bioactive compounds are molecules or compounds which are active in living organisms, cells or tissues. These compounds contain different kinds of essential molecules which may cure different kinds of diseases of living cells as well as they can supply proper nutrition to the living organisms (Kepiniski *et al.* 2006). Most popular source of bioactive peptide is food supplements and some microorganisms. These bioactive peptides are used for both healing and nurturing the body. Applications of bioactive peptides are covering different areas. There

are some specific functions which are triggered by peptides such as; antioxidative, antimicrobial, antihypertensive, cytomoduatory, immunomodulatory. Proteins and peptides play critical role in living body. From a dietary point of view, peptides are more bioavailable than proteins or free amino acids (Shimuzu*et al*. 2005).Further, peptides with low sub-atomic weight have been known to be less allergenic than their local proteins. Many physiological activities are instigated by bioactive peptides and these bioactivities are controlled by the number, arrangement and properties etc of amino acids display in the peptide. A few proteins, for example, lysozyme and α-lactalbumindo not exert this bioactivities until proteins are hydrolyzed to discharge physiologically dynamic peptides. Table 1.3 shows some bioactive peptides along with applications (Danquah et. al 2012).

Table 1.3 Some food proteins and their therapeutic application (Danquah 2012).

Protein source	**Microorganism and enzyme used**	**Amino acid sequence**	**Therapeutic application**
Caprine milk	Pepsine	α- casein(203-208)	Antimicrobial Antioxidant
Bovine milk	Lb. helveticus	Lle-pro-pro: Val-pro-pro	Antihypertensive
Egg white	Alcalase	Arg-val-pro-ser-leu	ACE Inhibitory

The market of therapeutic peptide is an open market and distributed throughout the world. Day by day demands of peptide derived drugs are increasing. It is representing 52% nearly over all the market share. There are three leaders in market which are leading in the market they are Teva Pharmaceuticals, Takeda pharmaceuticals co. ltd and Novo Nardisk. The global peptide therapeutics market was valued at US$ 19.98 billion in 2015 and is estimated to reach US$ 23.70 billion in 2020(Albany 2016).

1.3 Sources of bioactive peptides

Bioactive peptides can be attained from both nature and synthetic sources. However there is no difference in activities between naturally and synthetically obtained bioactive compounds.Bioactive compounds can be also obtained from the marine sources, like marine organism and plants (Aneiros 2008). Examples of some marine organisms which are a source of bioactive peptides are given in the table 1.4.

Table 1.4 Marine organisms as a source of bioactive peptides (Bargos 2012)

Compounds	**Sources**	**Organisma**	**Bioactivity**
Aplidine	ascidian	Aplidiumalbicans	Antitumor Antileukemc
Arenastatin A	Sponge	Dysideaarenaria	Antitubilin
Aurilide	Tunicate	Dolabellaauricularia	Antitumer
Didemnin	Tunicate	Tridemnum sp.	Antitumor
Dolastatin	Mollusk	Dolabellaauricularia	Antineoplastic
Geodiamolide H	Sponge	Geodia sp.	Antiproleferative
homophymines	Sponge	Homophymia	Antitumor
Jaspamide	Sponge	Jaspis sp. Hemiastrrella	Antiproleferative
Khalalide F	Molusk	Elysiarufescens, spisulapolynyma	Antitubiln
Keenamide A	Mollusk	Pleurobranchusforskalii	Antitumor
Mollamide4	Ascidian	Didemnummolle	Antiproliferative
Phakellistatins	Sponge	Phakelliacarteri	Antiproliferative
Tamandarins A and B	Antiproleferative	Didemnum sp.	Antitumor

1.4 Peptides

The simple science of amino acids linked together, via peptide bonds, brings to life this now common word – "Peptides". Used for a variety of health, fitness and anti-ageing concerns, Peptides can help in many ways to promote fat loss, build lean muscle, assist with injury recovery, increase agility, and many other anti-ageing benefits. Peptides are amino acid chains that act as messengers in the cellular world by telling your cells what to do. One of the world's best kept secrets for optimal health is Human Growth Hormone (HGH). HGH is so instrumentally important that its decline as we age is directly related to our health and quality of life. Modern medicine is now recognizing its decrease as a clinical syndrome called "Growth Hormone Deficiency Syndrome". By stimulating your Growth Hormone levels back to a level that is clinically predicted for your age will allow your body to perform at its peak which allows you to feel youthful and rejuvenated. (Godkin*et al.* 1997). Peptide having antimicrobial activity is called antimicrobial peptide. It is also known as host defense peptides.

There are some definite characteristics of peptide bonds such as.

1. Peptides are solid with incomplete two fold bond character
2. This bond is not possible to break by applying general or normal method which is known almost as an example heating or use of high concentration of salt.
3. In spite of having these properties acid or base can destroy this attachment. Just the duration of working should be prolonged. Moreover high temperature can do this work and the condition is same it has to be for an extended time duration.
4. To break peptide bond some specific enzymes can also be used.
5. In spite of having these properties acid or base can destroy this attachment. Just the duration of working should be prolonged. Moreover high temperature can do this work and the condition is same it has to be for an extended time duration.
6. To break peptide bond some specific enzymes can also be used.

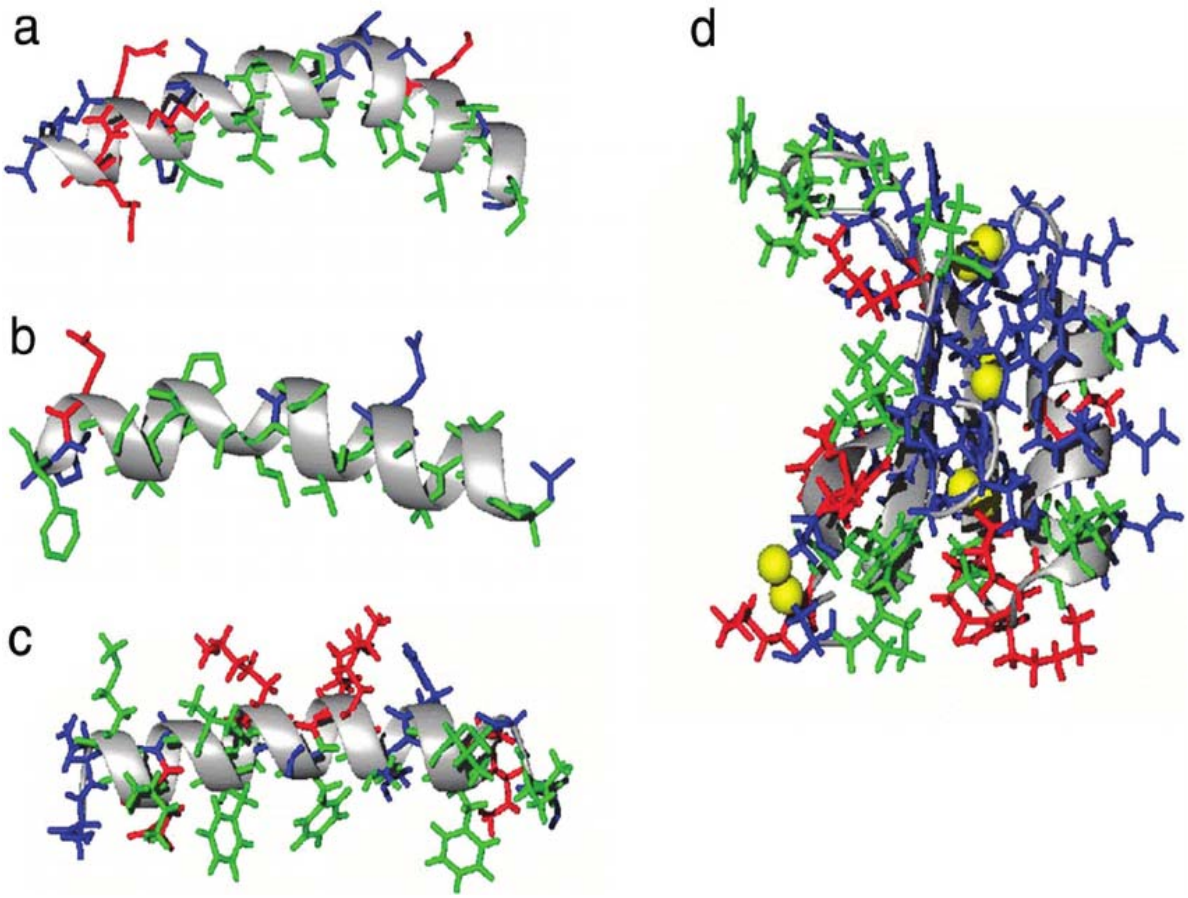


Figure 1.3 The structures of selected antibiotic peptides (Koradi et al. 1996).

In figure 1.3 the charged, predominantly cationic residues are shown in red, polar amino acids in blue, and, hydrophobic side chains in green: (a) Melittin (2MLT), (b) alamethicin (1AMT), (c) magainin (2MAG), and (d) the plant defensin RS-AFP1 (1AYJ). The sulfates forming cystine bonds within the defensin structure are shown in yellow. The illustration was created using the MOLMOL software (Koradi et al. 1996).

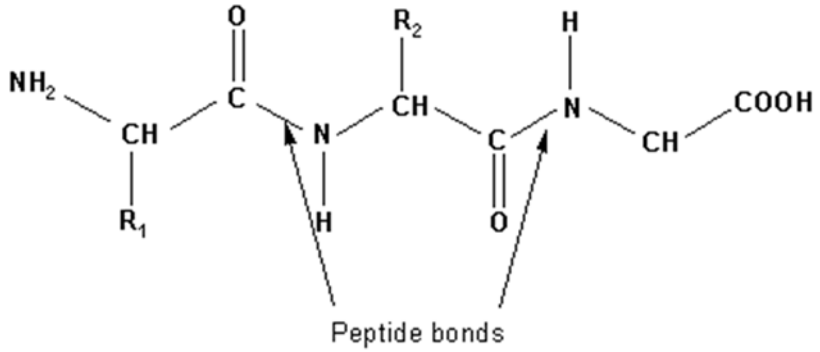


Figure 1.4 Peptide bonds

There are many peptides with different activities such as carnosine a non-peptide protein and dipeptide. This is found in muscle and brain. Anersine is a peptide which chemical name is N-methyl carnosine. This is 5X stronger than carnosine and found in skeletal muscle. Aspartame this is a synthetic dipeptide and an artificial sweetener. Glutathione it is a tripeptide (glutamate, cystine, glycine). This works as antioxidant defense system. One of the most important examples of peptide is insulin (Edward *et al.* 2005).

1.5 How plant produces peptides

Plants are a great source of peptides. Peptides play a great role in plants defense system. To protect itself plant produce different kinds of antimicrobial molecules. Among these molecules peptide is one of them. These are also called antimicrobial peptides (AMP). This is a barrier defense system of plants. These peptides can be isolated from plant's seeds, roots, flowers or fruits etc. peptides are secreted out from cell and act on neighboring cell.That neighbor cell will act like defense cell. This is call non cellular autonomous mane. In which a cell impact other cell to show same action like itself.

The first identification of plant functional peptide was done in tomato. It was an18 amino acid polypeptide genes which play the role of rapid expression of defense genes via cellular communication.Plant peptides are a communicator of cell to cell through which it works out its defense system. After that many more peptides were identified and isolated from plant source.

1.6 Synthesis of bioactive peptides

Synthesis of peptides from plant source is done by a series of procedures. At first literature review is done for knowing the presence of the peptides of interest. Then in silico genome screening is done to identify the desired functional peptide in plant source.Theflow chart is given below describes the process more perfectly (Grunewald, *et al* 2006).

1.7 Antimicrobial agents

Bioactive peptide has a wide range of antimicrobial activity. Bioactive peptides from plant source has both bacteriostatic and bactericidal characteristics which work against microbe. Some specific bioactive peptide have binding affinity with the surface of bacteriophage or microbe which help them to bind worth them. After binding with the microbe they can easily affect the microbe to grow or show their activity in the host cell. That's how bioactive peptides work as antimicrobial drug (Rehman *et al.* 2011).

For example Glycyrrhizaglabra can be used. This is a traditional medicinal plant which contains bioactive peptides. This is well known by 'yasti-madhu' generally. Elements of these plants are followings: (Hawkward, 2009)

a. Glycyrrhizin
b. Glabridingly
c. Glycyrin
d. Liquiritigenin
e. Amino acid (peptides)

Traditionally this plant part or extract is used to treat cold, cough, cold sore etc. this can also be used to treat microbe. This can be a effective and useful antimicrobial drug in near future. So research on this can be proceeding. So bioactive peptide can be work against antimicrobial.

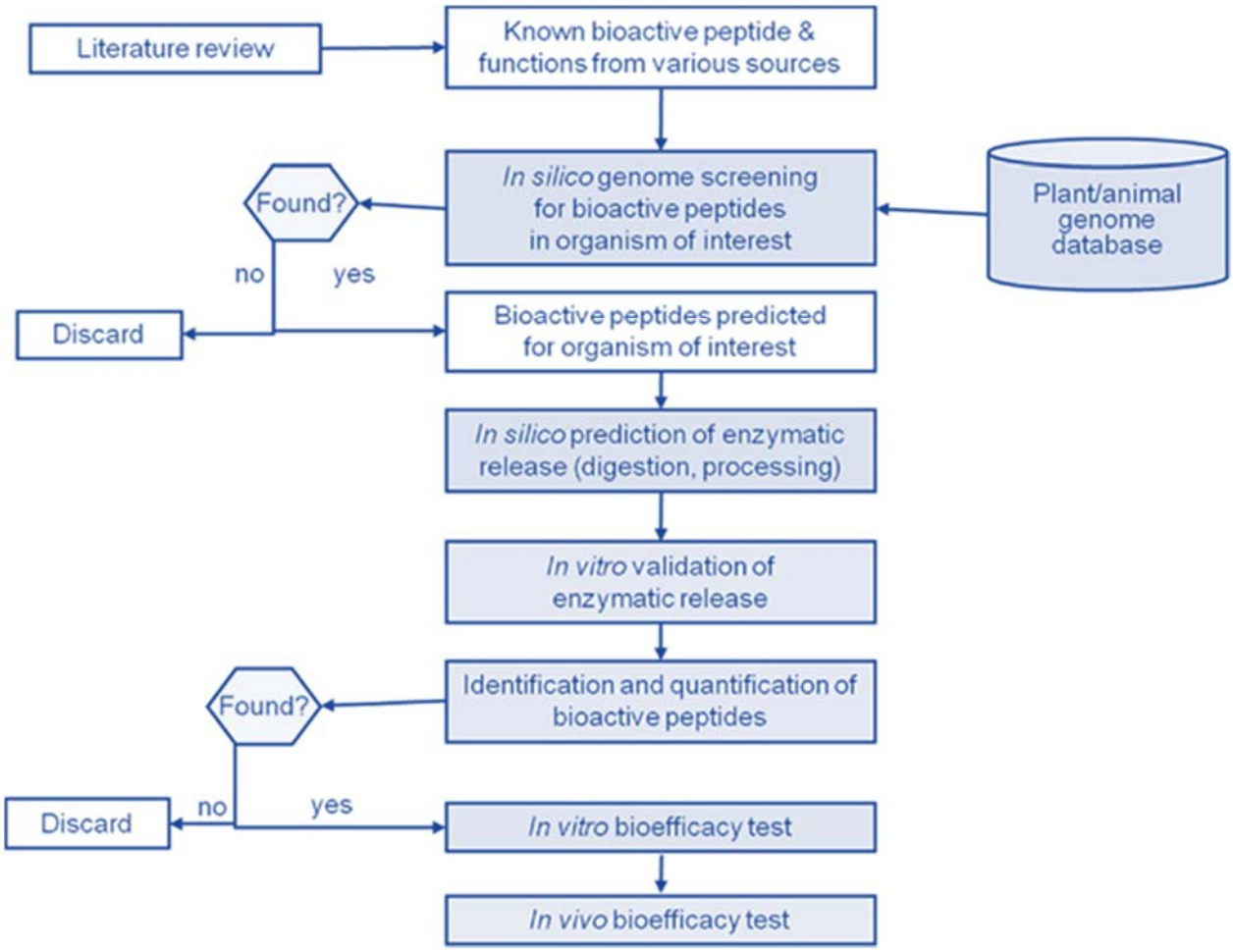


Figure 1.5 Bioactive peptide synthesis(Grunewald, *et al* 2006).

1.8 Resistance of antimicrobial agents

Antimicrobial drugs are those who work against the microbe or pathogens accommodate in the host cell. These pathogens can be resistant to the antimicrobial drugs. This is known antimicrobial resistance. This resistance help the microbe to fight against our immune system which can causes great harm to us. When a microbe become resistant to a specific microbe that can be strong enough to make harm to our health. In another word it can be said that if a microbe is resistance to a specific drug or drug component it's not so easy to remove or eliminate this (WHO, 2014).

1.9Reasons of antimicrobial resistance

At inception the antimicrobial agents were like magic bullets which were rapid and specific in treating infectious diseases with minimum side effects. This lead to their widespread usage in all types of diseases. Ultimately antimicrobial resistance developed.

Over prescription is a clinical practice which is done by applying or administrating antimicrobial agents randomly. It becomes more dangerous when the infection or disease is not properly diagnosed. Without proper diagnosis applying antimicrobial agent do the opposite which means it makes the microbes stronger against that specific microorganism.

The period of treatment with antimicrobial agents is usually 5-7 days, in some cases it is extended to 7-14 days to eradicate the infecting pathogens. However patients used to cease the treatment regimen as soon as symptoms reduces which ultimately speed up the overall microbial adaptive response. In many countries antimicrobials are availablewithout prescription and leads to over use of antimicrobials.

1.10Rationale of the study

Plant peptides are used as antifungal and antiviral agent. Now a day's people are becoming resistant to different antibiotics (WHO, 2014) .in this case a new source of antibiotic or antifungal peptides can be useful for us. They can be useful in antitumor therapy too. Various plant derived peptides are using in skin care to promote rejuvenation and skin tightness. Now a day's people are becoming more resistant to the antibiotics. As an example a graph is presented here (WHO, 2014).

In figure 1.6 shows that people are becoming resistant to some specific antibiotics which are mostly used to treat different types of diseases. This graph is also showing the rate of antimicrobial resistance in case E.coli bacteria which causes urinary infection.This is an alarming question for all of us. To get rid of this situation we need some other alternative to treat diseases. In this regard plant peptides can be a better source. As it has higher therapeutic effect with less toxicity (WHO, 2014).

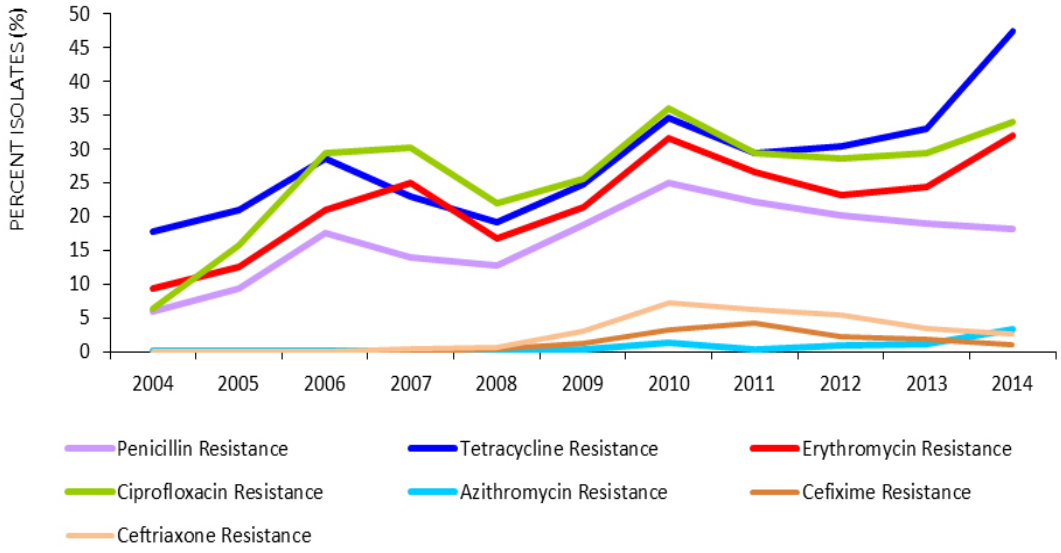


Figure 1.6 Graph showing antimicrobial resistance rate over time

1.11 Aim of the study

The aim of this review is to present an overview of what is currently known about bioactive peptides from plants, focusing on their biological activity and their potential application as therapeutics.

1.12 Objective of the study

There are several objectives of this review paper which are given below.

a. Find out specific plants which produce peptides
b. Find out the chemical structures of the peptides
c. Find out the useful peptides which have clinical importance
d. Find out their biological activity
e. Find out their prospect as therapeutic agents

2.1 Sources of bioactive peptides

Bioactive peptides can be found from different sources. It can be both from food derivatives and plants. Food is a wide spread source of bioactive peptide. Food containing protein is a great source of bioactive peptide. These peptides can be used in therapeutic drug to cure different diseases. Among the food products milk is one of the biggest sources of bioactive peptides. Following is the description of some food or sources of bioactive peptides (Turek*et al.*2014).

2.1.1 Milk

Milk or milk derivatives is one of the biggest source of bioactive peptides. Milk contains protein and it is a source of bioactive peptides. Drain proteins are forerunners of a wide range of organically dynamic peptides. These peptides are dormant inside the arrangement of the antecedent proteins yet can be discharged by enzymatic proteolysis. It is clear that bioactive peptide pieces starting from proteins ought to be considered as potential modulators of different administrative procedures in the body. Immunomodulating casein peptides animate expansion of human lymphocytes and phagocytic exercises of macrophages. Antimicrobial peptides execute touchy microorganisms. Antithrombotic peptides restrain fibrinogen official to a particular receptor district on the platelet surface and furthermore repress total of platelets.

Caseinophosphopeptides can frame solvent organophosphate salts and may work as bearers for various minerals, particularly calcium. In connection to their method of activity, bioactive peptides may achieve target locales (e.g., receptors and catalysts) at the luminal side of the intestinal tract or, after assimilation, in fringe organs.

Determined peptides can be delivered on a modern scale, and as a result, these peptides have as of now been considered for application both as dietary supplements in utilitarian sustenance’s and as medications. Consequently, these peptides are guaranteed to be wellbeing upgrading pharmaceuticals for sustenance and pharmaceutical arrangements.

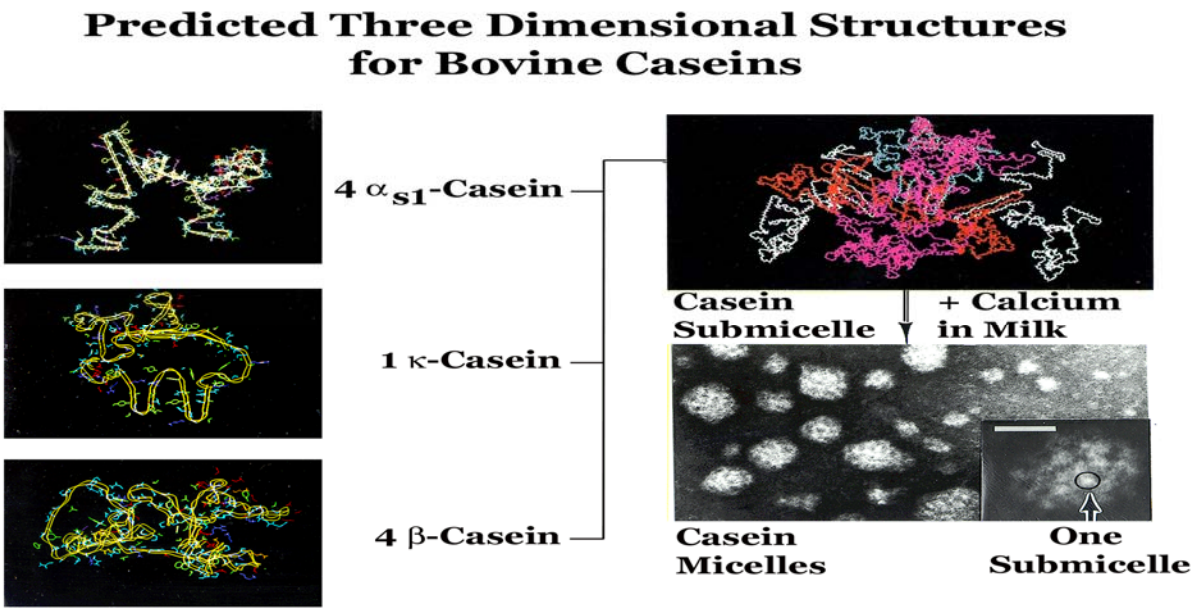


Figure 2.1 Bovine caseins(Turek*et al.*2014).

In the figure 2.1 it is shown that, casein from bovine milk is a phosphoprotein. There are four main types of Caseinwhich make up approximately 80% of the total protein in bovine milk: α-s1 Casein, α-s2 Casein, β-Casein, and κ-Casein. Casein is proposed to be the main protective constituent in milk.

2.2 Other food sources:

There are some other food sources containing bioactive peptides. They are following

I. Eggs: after milk egg is the wealthiest wellspring of peptides. It was demonstrated in an exploration of North American menopause society that egg yolk is contain a water dissolvable peptide which go about as defensive layer of bone. It is useful to the point that specialist is recommending it another option for the time being a day's osteoporosis treatments.

II. Grains: Corn, rice and wheat all contain peptides. In a 2002 report in "Science," researchers recognized the invulnerable framework trigger in wheat gluten that impels celiac ailment as a peptide. A 2011 report in the "Worldwide Journal of Biological Sciences" found that a reasonable "agreeable immunizer" against Alzheimer's contamination may be gotten from a peptide contained in rice. Beginning 2011, more than 80 particular peptides known

as plant defensins have been identified with antifungal properties, fusing a couple in corn and rice.

III. Soybeans: Soybeans and diverse beans and seeds contain peptides moreover. Different late audits have recognized diverse peptides in soybeans and related them with specific restorative favorable circumstances, for instance, a current report announced in "Test and Molecular Medicine" that insisted the development reckoning and tumor-smothering effects of the soybean peptide known as isoflavone-denied soy peptide.

2.3 Plant source

Plant is a great source of bioactive peptides. Plants are likewise utilized as a part of aurvedic and unani solution framework from antiquated time an even in this age as well. Accessibility of plants in nature is likewise an extraordinary motivation to pick plants as a source. Nature has given us a huge asset of plants which we can use to treat different maladies. (Morvic, 2003).

Table 2.1 Bioactive peptide from plant source (Morvic, 2003).

Source plant	**Plant product**	**Derivative**
Cannabis sativa	9-tetrahydrocannabinol n cannabadio	Nabilone (Eililly)
Artemesiaannua	Artemisinin	Artemether
Camptotheca acuminate	Camptothecin	Topotecan
Podophyllumpeltatum	Podophyllotoxin	Etoposide
Taxusbrevifolia	Taxol	Docetaxel (Aventis)

3.1 Bioactive peptides from plants

According to Sharma *et al.* (2012), bioactive peptides are specific protein fragments that have a positive impact on body functions and may influence health condition. Plants are good source of bioactive peptides and proteins and these are found in seeds, leaves, stems, flowers, and roots of the plants. Those bioactive peptides produced in plants show therapeutic activity and most of them possess antimicrobial properties and also play important role in cellular signaling (Salas *et al*., 2014). Besides these, many researchers have been conducted about the therapeutic activity of peptides in vitro and from the results of these researches, it has been invented that bioactive peptides produced in plants, have a wide range of therapeutic activities including cholesterol-lowering ability,blood pressure-lowering(ACE inhibitory) effects, antimicrobial properties, antioxidant activities, cyto- or immune modulatory effects, enhancement of mineral absorption and opioid-like activities (Malagut*iet al.,* 2014).For example, potato tuber is a good source of proteins and these proteins are classified into three major groups such as protease inhibitors, patatins, and other proteins. It is proved that patatin possess antioxidant or antiradical activity and also an allergen for some people (Pihlanto&Mäkinen, 2013). The low molecular weight protease inhibitor possesses enzymatic and inhibitory effectand protease is considered as the second major potato tuber storage protein. They can inhibit a variety of enzymes such as protease, invertaseetc and also show defense mechanisms against pathogens (Pihlanto&Mäkinen, 2013). Different types of plants of leguminosae family have been identified as a source of bioactive peptides which can show their activity as ACE-inhibitor, anti-oxidative agents and so on.For example, peptides from soybean, chickpea and pea are ACE-inhibitor. Some plants can show a systemic defense response because of the concomitant accumulation of the respective defense proteins, for example tomato plants can respond to local injury by herbivorous insects (Schaller, 2001).

3.2 Classification

In accordance with their characteristics they are classified on following classes (Sharma, *et. al* 2012)

a. Antimicrobial
b. Antithrombotic
c. Antihypertensive

d. Opioid
e. Immunomodulatory
f. Mineral binding
g. Antioxidative

3.3 Effect of bioactive peptides on different systems of human

Bioactive peptides have different kinds of actions or effects on different organs or systems of human. Effect of peptides varies with the classification of peptides.

Table: 3.1 Classifications of peptides along with effects (Korhonen&Pihlanto 2006)

Cardiovascular system	Nervous system	Gastrointestina system	Immune System
• Antihypertensive • Antioxidative • Antithrombroitc • Hypocholesteromic	• Opioid	• Mineral Binding • Anti-Apitizin	• Immunomodulatory

3.4 Therapeutic class

Peptides and proteins are utilized as a part of numerous signs also, can be utilized as a part of different helpful classes.

3.5 Antimicrobial activity of plant peptides

Antimicrobial peptides work as the first line defense system against plant pathogens and also show a significant role in the immunity of plants (Salas *et al*., 2014). Bioactive peptides form plantsdisplayed antibacterial, antifungal, antiviral and/or antiparasitic activities (Salas *et al*., 2014). To combat different stress conditions and attacks from pathogens such as fungi, bacteria and viruses, higher plants have a broad range of defense mechanisms. By releasing secondary

Table 3.2 Therapeutic classes of peptides (Kussman&Bladeren 2011).

1. Analgesia Antivirals	9. Gastrointestinal
2. Arthritis	10. Allergy and asthma
3. Baldness	11. Growth
4. Calcium metabolism	12. Impotence
5. Cancer	13. Vaccines
6. Cardiovascular	14. Obesity
7. CNS	15. Pain
8. Diabetes	16. Epilepsy

metabolites like tannins, phytoalexins and polyphenolic compounds, and the generation of pathogenesis-related (PR) proteins, plants response to the infections caused by a variety of pathogens. In the early 1970s, PR proteins were first discovered in tobacco leaves, in response to tobacco mosaic virus infections. Later, it was defined as the induced proteins that are released during pathogenic attacks. In a recent review, it has been estimated that there are at least 17 families which have been detected and isolated that possess a wide range of defense-related properties including antifungal,antibacterial, antiviral, chitinase and proteinase inhibitory activities, anti-oxidative activity and the families are defensin (PR-12 family), thionin (PR-13 family), knottin, α-hairpinin, hevein-like peptide, lipid transfer protein (PR-14 family) and snaking. .

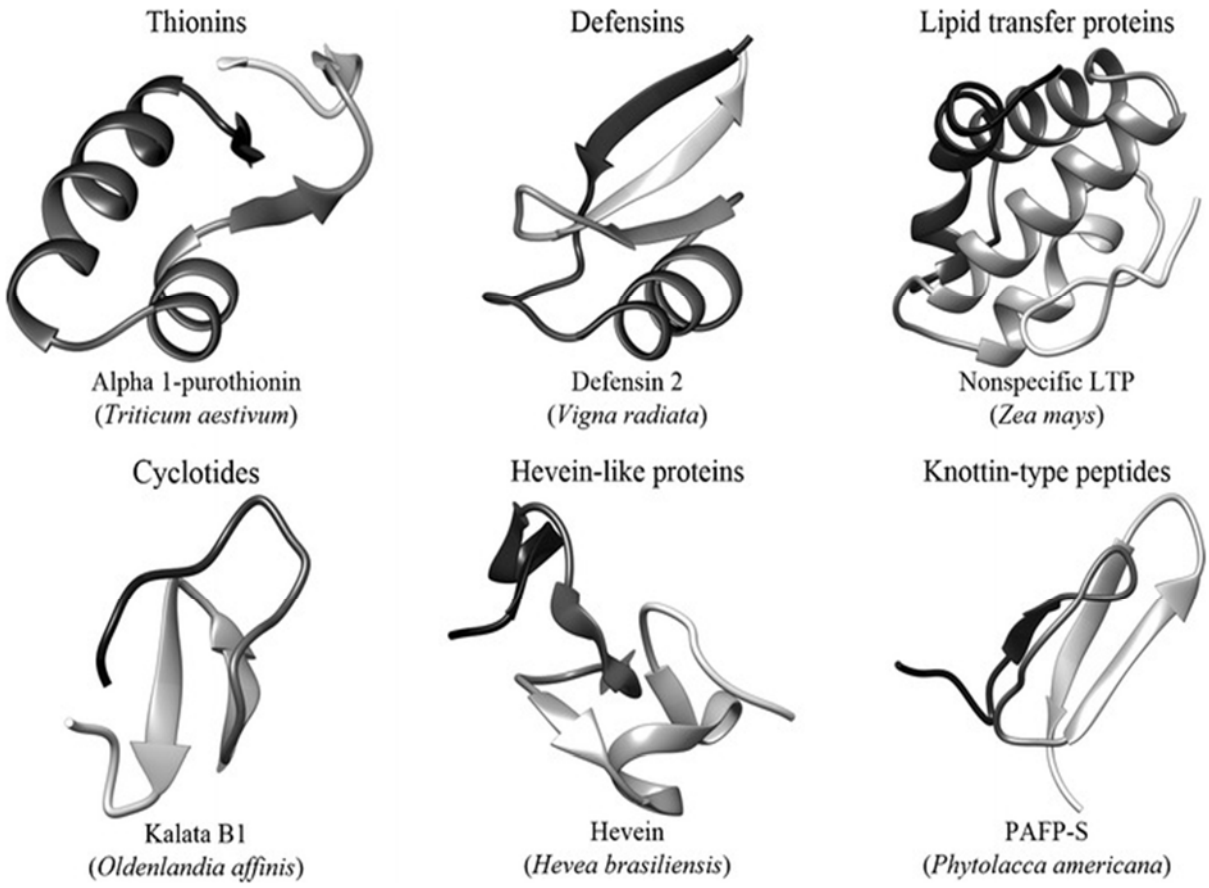


Figure 3.1 Examples of antimicrobial peptides of plants

Different types of vegetables, fruits and legumes are good sources of protein and legumes have a higher content of protein than vegetables and fruits. Examples of different plant peptides and proteins which have antimicrobial effect are mentioned in Table 3.3.

Table 3.3Sources of different plant peptides and proteins having antimicrobial effect (Nawrot*et al.*2014).

Source of Peptides	Activity
Thionins (types I–V)	Antibacterial
Thionein: alpha-1-purothionin (*Triticumaestivum)*	Antibacterial
Cyclotides: kalata B1 and B2 (*Oldenlandiaaffinis*)	Antibacterial, antifungal,
Lipid transfer proteins (LTPs) (*Zea mays*)	Antibacterial
Knottin-peptides: PAFP-S (*Phytolaccaamericana*)	Antibacterial
Puroindolines: PIN*A* and PIN*B* (*Triticumaestivum*)	Antibacterial
Snakins (*Solanumtuberosum*)	Antibacterial
Heveins (*Heveabrasiliensis*)	Antibacterial and antifungal
Peptides (*Phaseolus vulgaris*)	Antibacterial and antifungal
Peptide PvD1 (*Phaseolus vulgaris*)	Antibacterial and antifungal
Defensins	Antibacterial and antifungal

(*Triticumaestivum*and*Hurdeumvulgare*)	
Lunatusin **(*Phaseoluslunatus*)**	Antibacterial and antiviral
Vulgarinin **(*Phaseolus vulgaris*)**	Antibacterial, antifungal, and antiviral
Hispidulin **(*Benincasahispida)***	Antibacterial and antifungal
Lc-def **(*Lens culinaris*)**	Antifungal
Cicerin **(*Cicerarietinum*)**	Antifungal and antiviral
Arietin **(*Cicerarietinum*)**	Antifungal and antiviral
Peptide So-D1 **(*Spinaciaoleracea*)**	Antifungal and antibacterial
Shepherins **(*Capsella bursa-pastoris*)**	Antibacterial and antifungal
Peptides **(*Brassica napus*)**	Antiviral

4.1 Peptide and protein extraction techniques from plants

Different types of extraction techniques for proteins and peptides have been established according to the physicochemical and structural characteristics of the peptides and proteins such as molecular weight, solubility, hydrophobicity, isoelectric point. Some extraction techniques are given below:

4.1.1Centrifugation

Among the methods which are used for the isolation and fractionation of peptides and proteins, centrifugation method is one of the simplest and easiest methods. In order to isolate proteins, the first step is to separate different cell substructures because the proteins that we want to isolate are locally concentrated on membrane, mitochondria, or nucleus of the cell. Multiple centrifugation steps are involved in this process because it will cause separation of each component into different layers based on the molecular weight, size, and shape. After that, solubilization steps should be carried out prior for isolating the protein fraction from the selected layer (Jiang *et al.,* 2011).

4.1.2 Precipitation

Ammonium sulphate is used to make aggregation and precipitation of protein and this salt is the most widespread precipitants. Firstly, a large amount of this salt is added into a protein solution and other salts such as sodium chloride can also be used instead of ammonium sulphate. By increasing protein interaction, it causes protein aggregation and precipitation which is known as salting out process. This process allows selective protein separation because the salt concentration needed for protein precipitation varies from one protein to another (Bodzon-Kulakowska*et al*., 2007).

4.1.3 Electrophoretic method

This technique is mainly used as an analytical and preparative tool. Proteins are separated from the mixtures on basis of their charge/mass ratio, size, charge, or shape in electrophoresis method (Guttman*et al*., 2004). Sometimes, one-dimensional gel electrophoresis (1DE) has been used for protein purification. Bioactivity of the protein has been maintained in the solution.

4.1.4 Chromatographic method

Nowadays, liquid chromatography (LC) techniques are used for theseparation of different proteinsaccording to their hydrophobicity, charge, size, or specificity. In some cases, to eliminate some interference substances (e.g., salts), chromatographic methods can also be used (Schmidt *et al.*, 2007)

4.1.5 Aqueous solutions

Mainly, water is preferable because it is non-flammable and neither explosive nor toxic and that is why, water is advantageous over alcohols. Water can be used for the extraction of proteins because maximum proteins are solubilized in water at acid, neutral, or alkaline pH. The type of extraction process influence the extraction yield and properties of proteinandit is also influenced by different factors such as salts concentration, pH, the ionic strength of the medium, electrostatic repulsions and net charge (Tan *et al*., 2011).

4.1.6 Aqueous enzymatic extraction

It is an environmentally friendly, safe, and cheap alternative to extract oil and protein simultaneously (Latif and Anwar, 2009) and this process avoids serious damage to the proteins which can be produced by the refining steps and improving their nutritional and functional properties (Moure *et al.,* 2000). Moreover, in case of protein extraction, enzymes can aid in several ways, for example, carbohydrases is an enzyme which can attack the cell wall components, may liberate more protein from the matrix source and also increase the protein yield (Tang *et al*., 2002).

4.1.7 Low-pressure liquid chromatography

It is a process that can fractionate peptides on the basis of their molecular size and a bed of porous beads are used in this technique to separate the analytes (Ly and Wasinger, 2011). Several resins of different pore sizes are mostly used and polyacrylamide or divinylbenzene polymers are also used as stationary phase. Peptides are eluted with water, organic acids, ammonia, or ammonium salts depending on the resin composition.

4.1.8 Quantitative determination of proteins

Many methods have been established for determining the concentration of protein and among these methods, some are depended on the reactions of reagents with peptide bonds or amino acid side chains of the protein and others are depended on the binding of a reagent (dye) to the protein. Some of the methods are described below:-

4.1.9 The biuret method

A dilute copper sulfate solution is used in this method. At alkaline pH, it reacts with peptides or proteins and it creates a blue to violet color transformation that requires five minutes to complete.Then, protein is quantitated at 540 nm and for detection; this method requires relatively large quantities of protein (1 - 20 mg protein / mL).

4.1.10 The Lowry test

Actually, the Lowry test is themodification of the biuret method and it is the most sensitive quantitative colorimetric assay for protein detection. It can detect only only 0.005 to 0.3 mg protein per mL and an intense blue-green color is formed which comes from the reaction of the phosphomolybdotungstate in the Lowry reagent with the **W** and **Y** residues in the protein.

4.1.11 The Bradford method

In this method, a negatively charged dyenamedcoomasie Brilliant Blue G-250 has been used, which binds to positive chains of the protein and give a blue complex and it can detect from 0.2 to 1.4 mg of protein per mL. The color intensity depends on the concentration of the protein; the more concentrated the protein, the more intense the blue color. The time needed for the color formation is only 2-5 minutes and is stable up to 24 hours and for these reasons, it is the most popular method of protein quantitation.

4.2 Characterization

Once the peptides have been isolated they are usually characterized SDS-PAGE. Depending of the complexity of the sample one dimensional (1D) or two dimensional (2D) analyses are used. Digestion is an important step in characterization of peptidesandtwo types of digestion is commonly used: “in-gel” digestion and “in-solution” digestion. Identification of proteins from polyacrylamide gels offers a number of important advantages compared to gelfree approaches,

such as higher dynamic range of analysis of protein mixtures (ratio of lowest to highest abundance protein detectable) or removal of low molecular weight impurities before the MS analysis. In-gel protein digestion was first established by (Rosenfeld et al. 1992).

5.1 Introduction

The primary engineered peptide was set up by Emil Fischer a century back, however the therapeutic utilization of manufactured peptides begun after the Second World War, and just when peptides could be readied unadulterated by du Vigneaud's gathering in the USA, and by the Swiss mechanical gatherings of Robert Schwyzer (Ciba) and Huguenin (Sandoz). This was the season of oxytocin and vasopressin, cyclic nonapeptides with one disulphide connect, and of the angiotensins. The combination of peptides was a long and troublesome errand, a solitary peptide taking 1–2 years to create by customary techniques. It was the virtuoso of Bruce Merrifield, in 1963, who hurried and computerized this long procedure utilizing the technique he named Solid Phase Peptide Synthesis (SPPS). The acknowledgment of the technique was somewhat quick, helped by the advancement of new purging strategies (HPLC) which permitted sanitization of some incredible blending (loffet 2002)

From the 1960s to the finish of the Millennium, peptides were regularly considered as the medications of what's to come. Their principle disadvantage was their low bioavailability. Further, some real pharmaceutical organizations, which had put vigorously in peptide ventures, needed to drop some of these in the late clinical stage. This drove them to build up the idea of peptidomimetics with working standards, for example, 'no auxiliary amide gathering' or 'no sub-atomic weight more than 600', which practically murdered the field. Be that as it may, numerous Biotech organizations found new peptides withfascinating pharmacological properties, and SPPS was streamlined, permitting the normal combination of expansive polypeptides or little proteins from 30–100 amino corrosive deposits: peptides were spared (Loffet, 2002).

5.2 Marketed products

There are different kinds of marketed products of peptides in different form for different therapeutic sectors. Some classes of drugs in the market are following:

5.2.1 Monoclonal antibodies:

Monoclonal antibodies are important drugs derived from peptides are also available in the market. In accordance with (Loffet, 2002) following numbers of drugs are available in the market or in different phases of the market.

Marketed products – 20

Pre-registration and phase III- 20

Products in phase II- 45

This is the quickest developing portion of the peptide and protein showcase, as new adapted monoclonal antibodies are being tried in nearly each conceivable helpful application. The first potential "blockbuster" in this classification is the GpIIb/IIIa inhibitor Reopro (Eli Lilly 1998). Despite the fact that the quantity of promoted items seems, by all accounts, to be Or maybe little, the pipe-line is truly noteworthy, particularly at the early phases of improvement, as there are more than 100 items on which clinical testing has begun.

5.2.2Synthetic peptides

According to (Loffet, 2002) synthetic peptides are available in market. Besides this many other products are in different phases to present in the market.

Marketed products – 40

Preregistration and phase III- 20

Phase II- 60

5.2.3 Gonadorelin Super-agonists

These classes of peptides are used to treat endocrine cancer a breast cancer following products are the main component (Loffet 2002).

i. Leuprolide
ii. Abbott TAP

iii. Buserelin
iv. Aventis
v. Zoladex
vi. AstraZeneca
vii. Triptorelin
viii. IpsenBeaufour
ix. Nafarelin
x. Roche

5.2.4 Somatostatin Analogues

This peptide is also used to treat cancer. It is connected to the counter development impact of these somatostatin agonists. These are some marketed products which are available.

i. Octreotide
ii. Novartis
iii. Somatuline
iv. Ipsen

5.2.5 ACE inhibitors

More often than not the angiotensin changing over compound inhibitors have not been considered as peptides, indeed, even by peptide scientific experts, as they are basically substituted dipeptides which have been created in extensive volumes by ordinary natural amalgamation. The best known individuals from this family are Enalapril and Lysinopril, both from Merck and both blockbusters. Regardless of the possibility that their transcendence is currently blurring with the presentation of the new non-peptide angiotensin II receptor adversaries, they are still generally utilized items. Other surely understood items in the class are Ramipril, Trandolapril and Perindopril. The aggregate class of ACE inhibitors has around 15 propelled items with offers of over USD 4 billion, with amounts more than 100 tons, in immense difference of scale to the gonadorelin super-agonists.

5.2.6 HIV Protease Inhibitors

The grouping of the HIV protease waswhich had to some degree less peptidic character, concerning, obviously, derived from cDNA arrangements however it was the combination by Steve Kent and Dan Veber which permitted the exact assurance of its enzymatic action and the plan of manufactured substrates for testing. The precious stone structure was additionally settled from manufactured material, and this permitted, right off the bat, the outline of peptide inhibitors, and afterward of altered peptides, which could it might be said be considered as peptidomimetics regardless of the possibility that despite everything they consolidated peptide themes. The main HIV protease inhibitor to achieve the market was Roche's Saquinavir, in the blink of an eye taken after by Merck's Indinavir and Abbott's Ritonavir. This original was trailed by a moment illustration Agouron's (presently Pfizer) Nelfinavir. The deals of this class of item is over USD 1.5 billion with amounts more than 200 tons. The third era now under review is truly non-peptidic, yet still can't seem to be propelled, and will touch base available more than six years after the "peptidic" items.

5.3 Newly launched products

Integrilin: It is a cyclic heptapeptide created by Cor Pharmaceuticals and showcased by ScheringPlough, which is the principal GpIIb/IIIa inhibitor of its class. It contends specifically with Lilly's Reopro refined monoclonal immune response which has the favorable position of being propelled a couple of years prior Integrilin, however is a great deal more costly. The number of signs in which Integrilin has been endorsed is expanding, and regardless of the possibility that it is not a 'blockbuster', its deals, which are required to achieve USD 250 million in 2001, are noteworthy.

Atosiban: It is an oxytocin enemy propelled by Ferring on the European market after it had fizzled FDA endorsement taking after clinical trials driven by Johnson furthermore, Johnson. It is utilized to smother untimely withdrawals in pregnancy. The accessibility of other shabby treatments don't enable Atosiban to take a noteworthy piece of the market

Bivalirudin: It is the hirudin simple initially created in the mid-1990s by Biogen, yet was halted for financial reasons, the action of the item having never been in uncertainty. It was

assumed control a couple of years back by The Medicine Company, who have prevailing with regards to enlisting the item in New Zealand. Bivalirudin was affirmed a year ago by the FDA and propelled in the USA; its endorsement in Europe is pending. Its blood coagulation dissolving movement is of awesome significance when crisis treatment must be given.

Taltirelin: It is a TRH simple without endocrine movement yet dynamic on the CNS; it was as of late propelled by Tanabe in Japan.

Conclusion

Bioactive peptides can be found from both plant and food products. In case of food product milk and milk derived food is a great source of peptides. Plant peptides are preferred for therapeutic drugs for its great availability. Components of peptides may impact directly or it can stimulate receptor inside the organism so that they can prevent diseases. Peptides from plant source has effect on different types of system on our physiology. It can affect our cardiovascular system, nervous system etc. Most importantly it has a wide range of antimicrobial effect. Now a day'smany antimicrobial drugs are becoming resistant so that they cannot be used to cure any kind of microbial infections. In this case peptides from plant source could be the potential antimicrobial agent.

In conclusion in this era microbial resistance is a burning issue as it is becoming life threatening for us. To solve this problem new antimicrobial drugsare needed. Antimicrobial plant peptides can be extracted from plants. This source is natural and also plenty in nature, so this can be used to develop new drugs which will open a new era of therapeutics for us.

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