IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
PP 71-76

Cancer Biology with Molecular Docking

T.S. Anushya Devi M.Sc., M.Phil. (Ph.D)
Research scholar
PSGR Krishnammal College for Women

Abstract: - *"CANCER BIOLOGY WITH MOLECULAR DOCKING" is carried out to determine the anticancer compounds present in the plant. The software used is BioJava. Molecular structure is viewed through Rasmol. The software BioJava used for molecular study and docking purpose of elements present in the extract.The compounds in the extract are determined by using Rf value of the process using paper, TLC, and column chromatography. Antibacterial activity of the extract is studied against E-coli, BacillusSubtilis, and pneumonia. The Anticancer is activity carried out against HeLaCellines, and studied using MTT Assay [1]. The percentage of cell death is determined using ELISA reader. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterisation of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.*
Keywords: - *Ocimum basilicum, Extraction, Chromatography, Anticancer, Phytochemicals*

I. Introduction

Ocimum basilicum L. (sweet basil) a member of Lamiaceae family, is native popular for its culinary and ornamental uses. Various parts of the plant of sweet basil have been widely used in traditional medicine. The leaves and flowers of basil are used in folk medicine as a tonic and vermifuge. Basil [2] tea is good for treating nausea, flatulence and dysentery. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, colds, spasm, rhinitis, and as a first Studies showed that basil possesses central nervous system (CNS) depressant, anticancer, cardiac stimulant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulation, analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, chemomodulatory and larvicidal activities Biological and Pharmacological Properties of the Sweet Basil (Ocimum basilicum) .Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) religious and medicinal purposes. Basil was originated in Asia and Africa. Basil is used in both Ayurvedic and Unani system of medicine and is also of a ligand with a protein of known three-dimensional structure.

II. Extract Preparation

Extractions are a way to separate a desired substance when it is mixed with others. The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble. It includes Liquid-liquid extraction, and Solid phase extraction [3]. The distribution of a solute between two phases is an equilibrium condition described by partition theory. This is based on exactly how the analyte move from the water into an organic layer. The types of extraction are:

- Methanol extract
- Water extract

Fig: 1

III. Phytochemical Assay

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from the Greek word phyton, meaning plant. Some phytochemicals have been used as poisons and others as traditional medicine.

As a term, phytochemicals [4] is generally used to describe plant compounds that are under research with unestablished effects on health and are not scientifically defined as essential nutrients. Regulatory agencies governing food labeling in Europe and the United States have provided guidance for industry limiting or preventing health claims about phytochemicals on food product or nutrition labels.

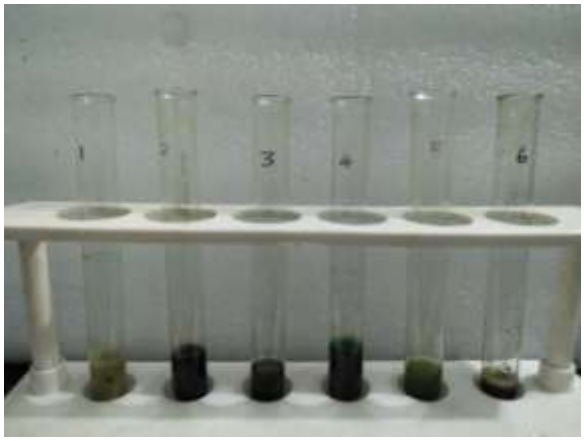

Fig: 2

Phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals. Challenges in that field include isolating specific compounds and determining their structures, which are often complex and identifying what specific phytochemical is primarily responsible for any given biological activity.

IV. Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography [5] may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

4.1 PAPER CHROMATOGRAPHY:

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper [6]. The paper is placed in a container with a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

4.2 TLC:

Thin layer chromatography (TLC) is a widely employed laboratory technique used to separate different biochemical on the basis of their size and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. TLC [7] is very versatile; multiple samples can be separated simultaneously on the same layer, making it very useful for screening applications such as testing drug levels and water purity. Possibility of cross-contamination is low since each separation is performed on a new layer. Compared to paper,

it has the advantage of faster runs, better separations, better quantitative analysis, and the choice between different adsorbents. For even better resolution and faster separation that utilizes less solvent, high-performance TLC can be used. An older popular use had been to differentiate chromosomes by observing distance in gel**.**

4.3 COLUMN CHROMATOGRAPHY:

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

The technique is very similar to the traditional column chromatography [8], except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

1 ANTIBACTERIAL ACTIVITY

Mueller Hinton Agar [9] (MHA) – Composition, Principle, Uses and Preparation Mueller and Hinton developed Mueller Hinton Agar (MHA) in 1941 for the isolation of pathogenic Neisseria species.

Fig: 3

Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique.

Five percent sheep blood and nicotinamide adenine dinucleotide may also be added when susceptibility testing is done on Streptococcus species. This type is also commonly used for susceptibility testing of Campylobacter.

1.1 Principle of MHA:

Mueller Hinton Media contains Beef Extract, Acid Hydrolysate of Casein, Starch and Agar. Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential

nutrients. Starch is added to absorb any toxic metabolites produced. Starch hydrolysis yields dextrose, which serves as a source of energy. Agar is the solidifying agent.

The use of a suitable medium for testing the susceptibility of microorganisms to sulfonamides and trimethoprim is essential. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller inhibition zones and innerzonal colonies, is demonstrated on unsuitable Mueller Hinton medium possessing high levels of thymidine. Both the PABA and thymine/thymidine content in Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antimicrobics.

Uses of MHA:

The major use of Mueller Hinton Agar is for antimicrobial susceptibility testing. It has become the standard medium for the Bauer Kirby method and its performance is specified by the NCCLS. It can be used to cultivate Neisseria. It is specified in FDA Bacteriological Analytical Manual for food testing, and procedures commonly performed on aerobic and facultative anaerobic bacteria.

Fig: 4

It is a non-selective, non-differential medium. This means that almost all organisms plated on here will grow. It contains starch. Starch is known to absorb toxins released from bacteria, so that they cannot interfere with the antibiotics. It also mediates the rate of diffusion of the antibiotics through the agar.

It is a loose agar. This allows for better diffusion of the antibiotics than most other plates. A better diffusion leads to a truer zone of inhibition. MHA shows acceptable batch-to-batch reproducibility for susceptibility testing.

MHA is low in sulfonamide, trimethoprim, and tetracycline inhibitors (i.e. concentration of inhibitors thymidine and thymine is low in MHA).

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2 ANTICANCER ACTIVITY

Anticancer drug [10], also called **antineoplastic drug**, any drug that is effective in the treatment of malignant, or cancerous, disease. There are several major classes of anticancer drugs; these include alkylating agents, antimetabolites, natural products, and hormones. In addition, there are a number of drugs that do not fall within those classes but that demonstrate anticancer activity and thus are used in the treatment of malignant disease. The term chemotherapy frequently is equated with the use of anticancer drugs, although it more accurately refers to the use of chemical compounds to treat disease generally.

3 ELISA READER

Enzyme Linked Immunosorbent Assay (ELISA) is a very sensitive immunochemical technique which is used to access the presence of specific protein (antigen or antibody) in the given sample and it's quantification. It is also called solid-phase enzyme immunoassay as it employs an enzyme linked antigen or antibody as a marker for the detection of specific protein. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and B-galactosidase.

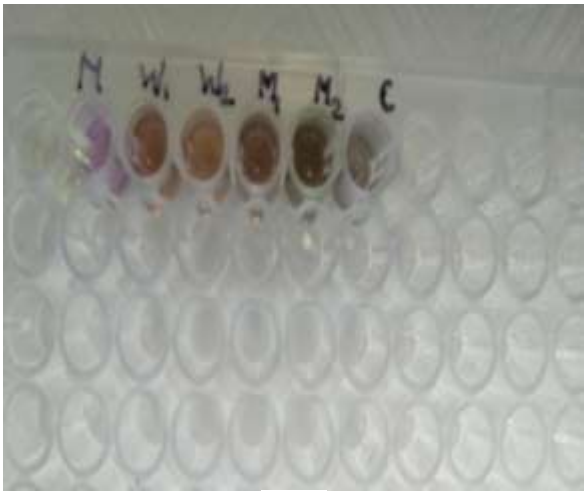

Fig: 5

3.1 Principles of ELISA:

ELISA [11] is a plate-based assay technique. Along with the enzyme-labeling of antigens or antibodies, the technique involves following three principles in combination which make it one of the most specific and sensitive than other immunoassays to detect the biological molecule:

- An immune reaction i.e. antigen-antibody reaction.
- Enzymatic chemical reaction i.e. enzyme catalyzes the formation of colored (chromogenic) product from colorless substrate.
- Signal detection and Quantification i.e. detection and measurement of color intensity of the colored products generated by the enzyme and added substrate.

1) 3.2 Advantages

- A wide variety of labeled secondary antibodies are available commercially.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Maximum immune reactivity of the primary antibody is retained because it is not labeled.
- Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification.

V. Conclusion

Cancer is an abnormal growth of cells that grows and spreads through uncontrolled cell division. These 'malignant' cells may invade other tissues and spread (metastasize) to more distant parts of the body. Cancer is not one disease but a group of more than 100 distinct disorders. It is the world's second biggest killer after cardiovascular disease and was responsible for the death of 7.6 million people in 2005. Globally the number of people diagnosed with cancer is estimated at around 11 million people, a figure that is set to rise to 16 million by 2020. Of all new cancer cases, it is estimated that one third could be cured if they were adequately diagnosed and treated.

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