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ANTICANCER ACTIVITY OF CATHARANTHUS ROSEUS AND MURRAYA KOENIGII

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ABSTRACT: Bio-informatics is an interdisciplinary field of science involved in collecting and analyzing biological data. It is processed with the help of software tools thereby to increase the understanding of biological processes. The Anti-cancer activity is done to arrest or prevent the growth of cancer cells. It can be done either by using drugs or by extracting proteins from plants. Defining the benefits of using drugs as an anti-cancer agent is difficult. As it may harm the patient due to the presence of chemotherapeutic agents and are cost effective .So, the protein extracts of Catharanthus roseus and Murraya Koenigii are the best anti-cancer agents that overcomes the disadvantages of drugs. Further MCF-7 cell lines are cultured and the plant extracts are swapped. In this phase the cell death is observed and compared, and thereby Catharanthus roseus has the best anticancer activity.

INDEX TERM: Anticancer, Genomics, Drugs, Plant.

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I. INTRODUCTION

Bio-informatics is n interdisciplinary field of science involved in collecting and analyzing biological data. The main work of Bio-informatics is to make sense of complex processes taking place inside living organisms. The basic task of Bio-informatics involves searching for similarities, or homologies, storing, retrieving, modelling, organizing biological data. (1) There are several applications of Bio-informatics that led to development of biological software. Some of the main applications of Bio-informatics is in the field of Medicine, Microbial Genome Applications, and Agriculture.

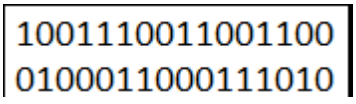
II. AREAS OF BIO-INFORMATICS

The areas of Bio-informatics aim to research on biological composition, structure, function, and evolution of cells, and organisms using informatics, and computer science. The Bio-informatics is often used to integrate data, protein predictions from sequence, comparision of sequences. The Bio-informatics pathway focuses on three areas namely

- Computational biology
- Genetics and genomics
- System biology

Computational biology

It is an interdisciplinary field that involves application of data-analytical and mathematical modeling and computational Techniques for the study of biological, behavioral. This is similar to that of evolutionary computing. Computational biology is being used to sequence the human genome, create accurate models and in modelling biological systems. The development algorithm and tools enable solving of problems.



ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

Genetics and genomics

Genetics is the study of genes and how the traits are passed to the upcoming generation. Genes are the unit of heredity. These hold the information about the molecule of DNA. Humans have thousands of genes that are packed into 23pairs of chromosomes. Genetics also play role on organism's health and disease. Genetics is inherited. Genomics is the study of whole organism, which includes or incorporates the elements of Genomics. This is done with the help of genome. The genome is the entire DNA content of any organism.

Fig. 1

Systems biology

System Biology is an interdisciplinary field of computing that focuses on interactions within biological systems, this mainly includes the study of complex interactions between the components of biological systems, function and behavior of the data. System Biology can be in multi-disciplinary fields like Engineering Principles, Non Linear system Analytics, Network Theory, Physics, Biology, Chemistry etc., The system biology in reproductive medicines coveres the system approaches like genomic, cellular, proteomic, metabolic, BioInformatic, molecular, and biochemical, to solve questions in reproductive biology.

Fig. 2

Cancer

When a cell is multiplies out of control due to alterations it results in cancer. A tumor is composed of a cluster of such abnormal cells. Cancer, also called malignancy, Cancer is a major health problem in the India and many other parts of the world. Currently, one in four deaths in the India is due to cancer. There are more than 100 types of cancer. Some of the major types of cancer are carcinoma, sarcoma, lymphoma, melanoma and leukemia. Out

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

of these, Carcinoma is the most commonly diagnosed cancer which multiplies. Cancer is considered as a genetics fields of Bio-informatics.

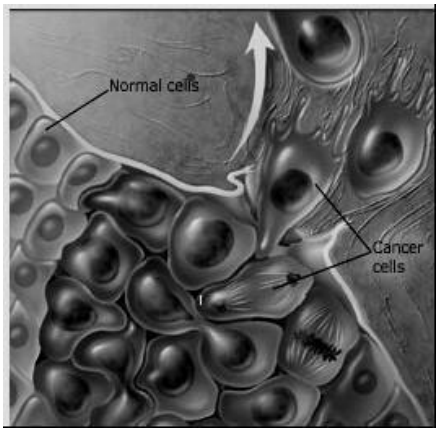


Fig. 3

Anticancer activity

The process of arresting or preventing the multiple growth of cancer cells are called anticancer activity. To prevent its growth either drugs can be used or the medicinal extract of plants can also be used. Cancer has been a constant battle across the world with a lot of development aids in cures, preventative therapies and treatments.

Drugs

Anti-cancer drugs are called chemotherapeutic agents. They act rapidly upon cancer cells by dividing and destroying them. The different kinds of anti-cancer drugs are alkylating agent's cisplatin, chlorambucil, procarbazine, carmustine, methotrexate, cytarabine, gemcitabine. The main drawbacks of using drugs are hair loss, nausea and vomiting, anaemia etc.

Plant extract

Extracts from medicinal plants has played an important role in the treatment of cancer and it is the clinical applications of cancer therapies. Natural antioxidants, such as vitamins and polyphenols, have high antioxidant capacities that are present in many seeds, leaves, fruits and vegetables, whose consumption is considered to be inversely related with some of the cancers. Some of the plants with anticancer activity are Polygonum tinctorium Lour, Leaves of Catharanthus roseus (cures breast cancer), Seeds of Murraya Koenigii, Taxus Brevifolia (cures refractory ovarian cancer), Podophyllum Peltatum (cures both lung and testicular cancer).These are some of the plants that has anti-cancer activities in it. The plants, which has anti-cancer activities have protein characterization to arrest the growth of multiplying cells. Some of the proteins, are longation factor 1-γ, glyceraldehydes-3-phosphate dehydro-genase, heat shock protein truncated nucleolar phosphoprotein B23, and tubulin-β chain.

Breast cancer

Breast cancer is the formation of cancer cells in the breasts. It is found that 5-10 percent of the Breast cancer may be inherited linked through gene mutation. The first breast cancer cell line was BT-20 BRCA1 (BReast CAncer gene-1), BRCA2 (BReast CAncer gene-2), MCF-7 (Michigan Cancer Foundation-7), T-47D these were some of the cells found later from studies. There are several tests to screen breast cancers and it is highly treatable at an early stage, there are many reasons to develop breast cancer.

Anticancer activity

1. Catharanthus roseus

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

Catharanthus roseus is an important medicinal plant throughout the world. It contains more than 120 terpenoid indole alkaloids (TIA's). A number of alkaloids are isolated from this plants for its medicinal use. Some of them are ajmalacine-an anti-hypertensive alkaloid, and vincristine and vinblas-tine – the antineoplastic bisindole alkaloids. The leaves of Catharanthus roseus are used as an anticancer agent. The leaves are about 3cm oval and oblong. The anticancer compounds present in the plant makes it a lifesaver. Vinorelbine is active against treatment of breast cancer.

Fig. 4

2. Murraya koenigii

Therapy which targets apoptosis and inflammation can be very effective for the treatment of cancer. Murraya Koenigii is an edible herb that is used for cancer treatment. The current study reports the anticancer effects of girinimbine, a carbazole alkaloid is from isolated from Murraya Koenigii.

Fig. 5

Preparation of plant extract

Plant extraction is a process that aims to extract medicinal components present in plants. It is a solid/liquid separation operation: the plant is placed in contact with the solvent (fluid).

Protein estimation

The Lowry protein assay is used to determine the total level of protein content in a solution. The total protein concentration can be noticed by a color change of the sample in proportion to protein concentration, which can then be measured using colorimetric techniques.

Total antioxidant activity

The total antioxidant activity is done to evaluate the antioxidant response against the cells. Example: proteins, lipids in the body.

Antibacterial activity

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

Antibacterial activity is performed the zone of inhibition against suitable microorganisms such as Escherichia coli, Bacillus subtilis.

Preparation of plant extract

Collect the leaves of Catharanthus roseus and seeds of Koenigii Murraya. Place it in sunlight and let them dry. Take 2g of leaves (Catharanthus roseus) and 2g of seeds (Koenigii Murraya). Crush them separately by dissolving 20ml of Distilled water. Filter the mixture and collect the extracting two different conical flasks. Place the extract in the orbital shaker at 40° with 50-60 Rpm for 24-48 hours.

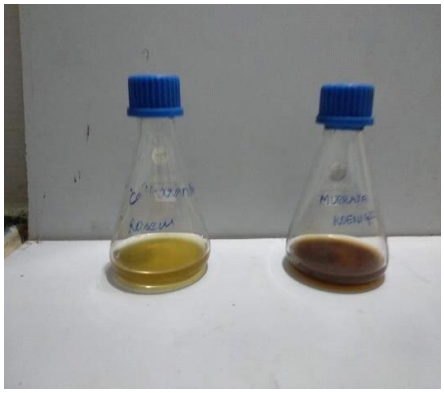


Fig. 6

Protein estimation

The prepared plant extract is used for Protein estimation. The Protein estimation method is also called Lowry's Method. Take 0.5ml of the extract in tow different eppendorf tube. Add 2.5ml of SOLUTION C

Preparation of solution C

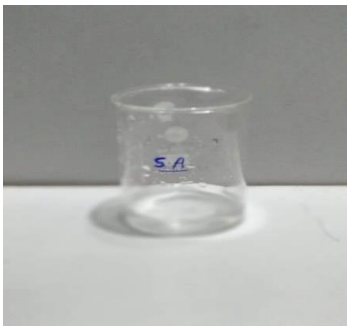
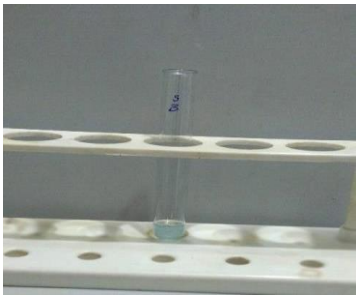


Fig. 7

Solution A= 0.1N of NaOH in 2% Na2CO3



ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

Fig. 8

Solution B= 0.5% CuSo4

Solution C= Solution A 50ml + Solution B 1ml

Mix well and incubate at room temperature at 10 mins. Add 0.20ml of Folin's phenol reagent.

Fig. 9

Optimal Density at 660nm Note the optimal Density values using Microprocessor UV-VIS Spectrometer in single beam.

Catharanthus Roseus (Leaves)	Koenigii Murraya (Seeds)
O.D VALUE:1.424	**O.D VALUE:1.321**

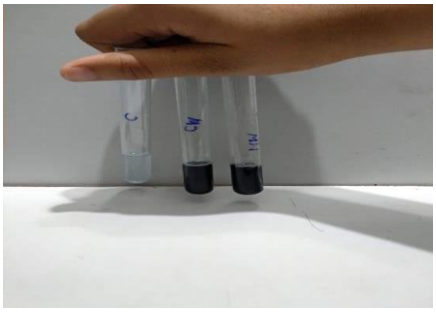


Fig. 10

Antioxidant activity

This process is done to find the Total Antioxidant Activity of the extract. Take 0.5ml of the extract in two different test tube. Add reaction mixture to the 0.5ml of the extract.

Preparation of reaction mixture

This is done by mixing 0.6M H2SO4 with 28Mm Sodium phosphate and 4Mm Ammonium Molybdate. Mix 90minutes water bath. Find the Optimal Density at 695 nm. Note the Optimal Density values for the extract and control (Distilled Water) using Microprocessor UV-VIS Spectrometer.

Catharanthus Roseus (Leaves)	Koenigii Murraya (Seeds)
O.D VALUE:0.225	O.D VALUE:0.283

Antibacterial activity

Prepare Muller-Hinter Agar in a petidish. Add two different bacteria's (E.coli and Bacillus subtillus) to the upper layer. Make 4 well layers to it using spanners. Add 40/20µl of plant extract in the first two wells. In the

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

remaining wells add distilled water in one well and antimicrobial disk in the other well. Incubate it at 37° for 24 hours. By following the steps, the antibacterial activity is observed.

Anticancer activity

Growth of cancer cells

Preparation of plant extract

- Collect the leaves of *Catharanthus roseus* and seeds of *Koenigii Murraya*.
- Place it in sunlight and let them dry.
- Take 2g of leaves (*Catharanthus roseus*) and 2g of seeds (*Koenigii Murraya).*
- Crush them separately by dissolving 20ml of Distilled water.
- Filter the mixture and collect the extract in two different conical flasks.
- Place the extract in the orbital shaker at 40℃with 50-60 Rpm for 24-48 hours.

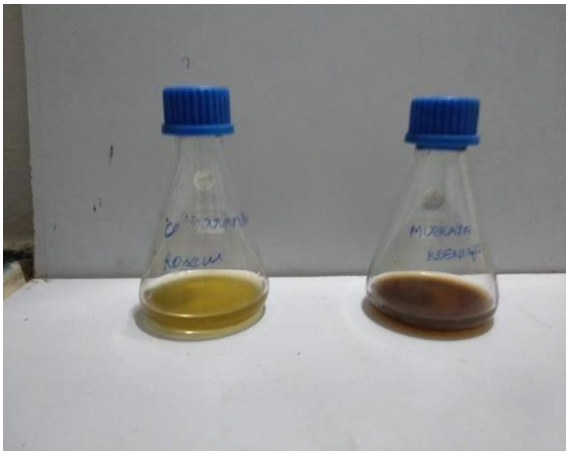


Fig. 11

Preparation of cancer growth medium

The medium used to grow cancer cells is DMEM (Dulbecco's Modified Eagle Medium) medium.

Fig. 12

Add 20ml of Sterile distilled water to the prepared DMEM medium. Add cancer cells to it. Place it in the Co2 Incubator – 5% Co2-37°

Anticancer (killing cells)

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

Take microplate reader with 96 wells. Consider three wells Control, extract1 and extract2 for 100µl and add 10µl and 20µl of medium to note cell death.

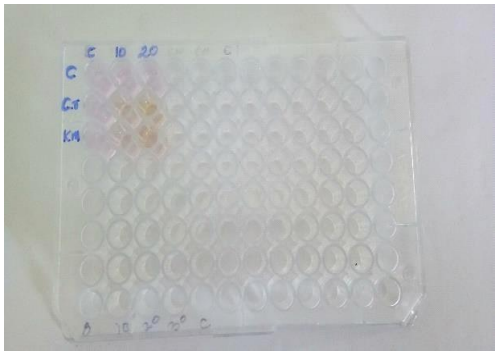


Fig. 13

Keep it in Co2 incubator for 24 hours. Add 10µl MTT dye to it and incubate it for 1 hour. Finally using ELISA Reader, note the readings and notice cell death.

	Control	10µl	20µl
Control	0.869	-	-
Catharanthus Roseus	-	0.762	0.726
Koenigii Murraya	-	0.735	0.698

Sequencing

MSQFKRQRINPLPGGRNFSGTASTSLLGPPPGLLTPPVATELSQNARHLQGGEKQRVFTGIVTSLHDYFG
VVDEEVFFQLSVVKGRLPQLGEKVLVKAAYNPGQAVPWNAVKVQTLSNQPLLKSPAPPLLHVAALGQ
KQGILGAQPQLIFQPHRIPPLFPQKPLSLFQTSHT

LHLSHLNRFPARGPHGRLDQGRSDDYDSKKRKQRAGGEPWGAKKPRHDLPPYRVHLTPYTVDSPICDF
LELQRRYRSLLVPSDFLSVHLSWLSAFPLSQPFSLHHPSRIQVSSEKEAAPDAGAEPITADSDPAYSSKVL
LLSSPGLEELYRCCMLFVDDMAEPRETPEHPLK
QIKFLLGRKEEEAVLVGGEWSPSLDGLDPQADPQVLVRTAIRCAQAQTGIDLSGCTKWWRFAEFQYLQ
PGPPRRLQTVVVYLPDVWTIMPTLEEWEALCQQKAAEAAPPTQEAQGETEPTEQAPDALEQAADTSRR
NAETPEATTQQETDTDLPEAPPPPLEPAVIARPGCVNLSLHGIVEDRRPKERISFEVMVLAELFLEMLQRD
FGYRVYKMLLSLPEKVVSPPEPEKEEAAKEEATKEEEAIKEEVVKEPKDEAQNEGPATESEAPLKEDGL
LPKPLSSGEEEEKPRGEASEDLCEMALDPELLLLRDDGEEEFAGAKLEDSEVRSVASNQSEMEFSSLQD
MPKELDPSAVLPLDCLLAFVFFDANWCGYLHRRDLERILLTLGIRLSAEQAKQLVSRVVTQNICQYRSL
QYSRQEGLDGGLPEEVLFGNLDLLPPPGKSTKPGAAPTEHKALVSHNGSLINVGSLLQRAEQQDSGRLY
LENKIHTLELKLEESHNRFSATEVTNKTLAAEMQELRVRLAEAEETARTAERQKSQLQRLLQELRRRLT
PLQLEIQRVVEKADSWVE KEEPAPSN

3D Structure of cancer cell

ISSN- 2394-5125 VOL 7, ISSUE 8, 2020

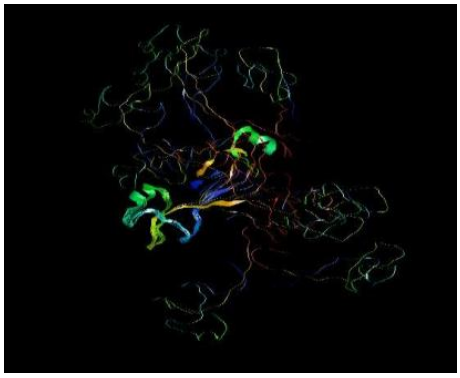


Fig. 14

Phylogenetic analysis

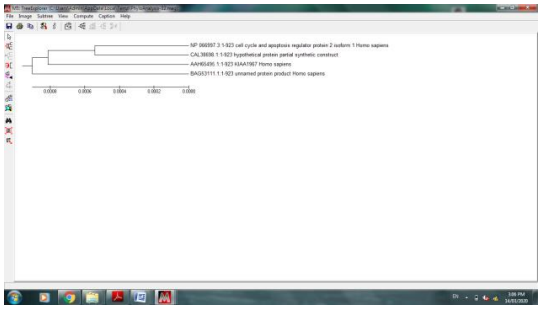


Fig. 15

Table 1

REFERENCE	SCIENTIFIC NAME	PARTS USED	MECHANISM
A Systematic Review of Iran's Medicinal Plants With Anticancer Effects - Majid Asadi-Samani, Candidate1,Wesam Kooti, Elahe Aslani, and Hedayatollah Shirzad	Ferula assa-foetida	Shoot, resin	Inhibition of DNA destruction and cancer cells proliferation
Curcuma longa and curcumin: A Review Article - M. Akrami, Shahab-uddini, AfzalAhmed2,Khan,Usmanghani3,Abdul Mohiuddin4, M.Asif	Curcuma longa	Rhizome	Inhibition of cancer cells proliferation
Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review- A.B. Sharangi	Camellia sinensis	Leaf	Inhibition of cancer cells proliferation (by inhibit of 5-a reductase enzyme activity)
Anticancer activity of guava (Psidium guajava L.) branch extracts against HT- 29 human colon cancer cells-Sang-Bong Lee1 and Hae-Ryong Park2	Psidium guajava L.	Branches	Apoptosis-genetical cell death
Anticancer Activity of Nigella sativa (Black Seed)- Mohammad Akram Randhawa and Mastour Safar Alghamdi	Nigella sativa	Seed	Alpha-hederi used as cytotoxic drug
Study of phytochemical, anti-microbial, anti-oxidant, and anti-cancer properties of Allium wallichii-Jaya Bhandari, BushraTaj Muhammad, Pratiksha Thapa & Bhupal Govinda Shrestha	Allium wallichii	Whole Plant	Separation of pure compounds-showing cytotoxicty

Selective killing of cancer cells by leaf extract of Ashwagandha: Components, activity and pathway analyses Author links open overlay panel, G.Shrestha , TetsuroIshii , Sunil C.Kaul RenuWadhwa	Ashwagandha	Leaf	Selective killing of cancer cells
Antimicrobial and anticancer activities of entophytic fungi from Mitrajyna javanica Koord and Val-Thirawatthana Pharamat1, Tanapat Palaga2, Jittra Piapukiew3, Anthony J. S. Whalley2, 4 and Prakitsin Sihanonth.	Mitrajyna javanica Koord and Val	Leaf	Apoptic Cell death is observed

III.RESULT AND DISCUSSION

Research on natural products has been used as a strategy to discover new drugs with potential to applications in complementary medicines because of their lower side effects than conventional drugs. The aim of this study is to make a drug for a malignant disease, thereby to reduce its side effects and increase the medicinal value of the plant. One of the significant development in the field of medicine is to use cytotoxic drugs made of chemical components as an agent for cancer chemotherapy, which may not only kill the tumor cells but may also harm the non-tumor cells thereby harming the patients. These side effects of chemotherapeutic agents aim in killing the target malignant cells. Therefore, the search for alternative drugs which are effective in killing cancer cells while showing minimal toxicity to normal cells is an active area of research nowadays, Moreover these researches were to find a plant-based medicine. According to WHO 80% of the world's population depend on plant-based medicine. As natural drugs can overcome the disadvantage of the chemical cytotoxic drugs, Catharanthus roseus was selected after going through several medicinal plants, which acts as an anti-cancer agent. This plant contains more than 120 alkaloids which is more effective than other drugs. The plant parts like Leaf, flower etc are used as a drug. (8) Presence of alkaloids like vinblastine motivated to know more about its extract. After the cell death is noticed the Catharanthus roseus has the optimal density value of 0.762 for 10µl and 0.726 for 20µl similarly Murraya koenigi has the optimal density value of 0.735 for10µl and 0.698 for 20µl with the help of above values it is noticed that Catharanthus roseus has the best anticancer activity. Thus the result is analyzed (2)

IV.CONCLUSION

Catharanthus roseus is a powerful plant drug that inhibits the growth of MCF-7 cells, this indicates the prevention of tumor cells within the organ and has all the required proteins within its parts. Due to the presence of vinblastine, it makes it more effective. Catharanthus roseus is available throughout the world. Thus to conclude the hope for apoptosis in the case of using Catharanthus roseus as a drug is more. The National Cancer Council of Malaysia (Majlis Kanser National, MAKNA) uses Catharanthus roseus as a logo- Symbol of hope for cancer patients.

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