

Preparation of Anticancer Extract

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Abstract: *In India, the hibiscus rosa-sinensis (HS) flower is well-known and is used to worship Lord Ganesha. China rose, or Hibiscus rosa sinensis, is a member of the Malvaceae family. In numerous tropical nations, this plant has a wide range of significant medical benefits for treating wounds, inflammation, fever and coughing, diabetes, infections caused by bacteria and fungi, hair loss, and gastric ulcers. According to phytochemical analysis, flavonoids, tannin, terpenoids, saponins, and alkaloids are the principal bioactive substances in charge of its therapeutic benefits. Various pharmacological properties, including anti-pyritic, analgesic, anti-inflammatory, anxiety study, and anti-depressant, were demonstrated by experiment from recent study. These reviews seek to provide information on the different pharmacological and pharmaceutical uses of Hibiscus rosa sinensis. The article discusses a few literary works that are based on the studies conducted on Hibiscus rosa sinensis.*

Background: *Infectious diseases are the most common causes of morbidity and mortality in developing countries. Wound and wound infections are also major health problem. Nowadays, medicinal plants play a major role in treatment of infectious diseases and wound healing and they are easily available and more affordable as compared to synthetic compounds. The aim of this study is therefore, to investigate the antibacterial and wound healing activities of 80% methanol extract of Hibiscus micranthus leaves using disc diffusion methods and rat excision model respectively. Methods: In vitro antibacterial screening was carried out against S. aureus, S. pneumoniae, S. pyogenes, E. coli, P. aeruginosa, K. pneumoniae and P. mirabilis bacterial strains using disc-well diffusion assay. Wound healing activity was done in rats divided into four groups each consisting of six animals. Group I was served as a negative control (ointment base), Group II served as a positive control Nitrofurazone (NFZ 0.2% ointment), Groups III and IV were treated with 5 and 10% extracts respectively. The acute oral toxicity test and skin sensitivity test were also performed before conducting the actual study. The extract was analyzed for secondary metabolites using.*

Keywords: Hibiscus micranthus Anticancer Activity, Antibacterial activity, Wound healing activity

I. INTRODUCTION

Extraction is the method of removing active constituents from a solid or liquid (plant or animal tissues) using a liquid solvent.

Extraction defined as the treatment of the plant or animal tissues with solvent, whereby the medicinally active constituents or API are dissolved & most of the inert matter remain undissolved.

Menstruum, solvent used for extraction.

Marc, the insoluble material that remains after extraction.

Types of Extraction methods

Liquid-Liquid Extraction:

It is also known as Solvent Extraction refers to an operation in which the components of the liquid mixture are separated by contacting it with a suitable insoluble liquid solvent which preferentially dissolves one or more components. In this type of operation, the separation of the components of solution depends upon the unequal distribution of the components between two immiscible liquids. In liquid extraction the feed solution is one phase and the solvent used for extraction is another phase. In solvent extraction both the liquids i.e. the feed and solvent forms a homogeneous mixture and are separated by contacting it with one another which separates out one of the two liquids preferentially.

Eg : Extraction of Methanol from LPG With Water .

Solvents for Liquid – Liquid Extraction:

Aqueous solvents	Water – Immiscible organic solvent
Basic Solution	Dichloromethane
Acidic Solution	Diethyl ether
Water	Hexane, Petroleum Ether
High Salts	Chloroform

Leaching (Solid – Liquid Extraction)

Solid -Liquid Extraction (Leaching) means the removal of constituents from a mixture of solids by bringing the solid material into contact with a liquid solvent that dissolves this particular constituents . Leaching may either be used for production of concentrated solution of a valuable solid material, or in order to free an insoluble solids from a soluble material with which it is contaminated. An everyday example of leaching is making coffee. In this case the soluble constituents i.e. the ground coffee is separated from insoluble grounds by solution in water Mechanism of Leaching. Leaching involves two steps which are as follows:

Contacting step

Contacting the solid with the selective solvent - the liquid in order to dissolve the soluble solute in the solvent. The solute is first dissolved from the surface of the solid, then passes into the main body of the solution by diffusion. This process may result in formation of pores in the solid material which exposes new surfaces to subsequent solvent penetration to such surface .

Separation step

Separation of insoluble phases, i.e. separation of liquid phase from solid physically by settling

Introduction of Cancer:

Cancer is a disease in which some of the body’s cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn’t. These cells may form tumors, which are lumps of tissue. Tumors can be cancerous or not cancerous (benign). Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors. Many cancers form solid tumors, but cancers of the blood, such as leukemias, generally do not. Benign tumors do not spread into, or invade, nearby tissues. When removed, benign tumors usually don’t grow back, whereas cancerous tumors sometimes Benign tumors can sometimes be quite large, however. Some can cause serious symptoms or be life threatening, such as benign tumors in the brain.

How Does Cancer Develop

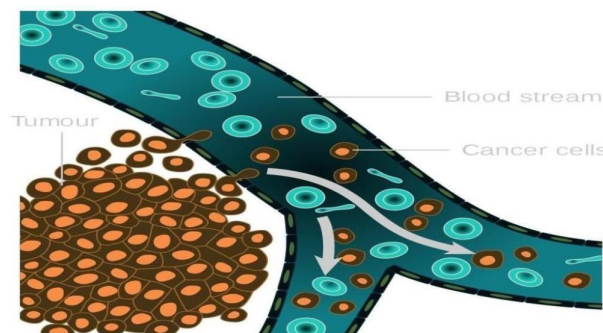


Fig 1: figure of how cancer are formed

Types of Genes that Cause Cancer :

The genetic changes that contribute to cancer tend to affect three main types of genes—proto- oncogenes, tumor suppressor genes, and DNA repair genes. These changes are sometimes called “drivers” of cancer.

Proto-oncogenes are involved in normal cell growth and division. However, when these genes are altered in certain ways or are more active than normal, they may become cancer-causing genes (or oncogenes), allowing cells to grow and survive when they should not.

Tumor suppressor genes are also involved in controlling cell growth and division. Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner.

When Cancer Spreads:

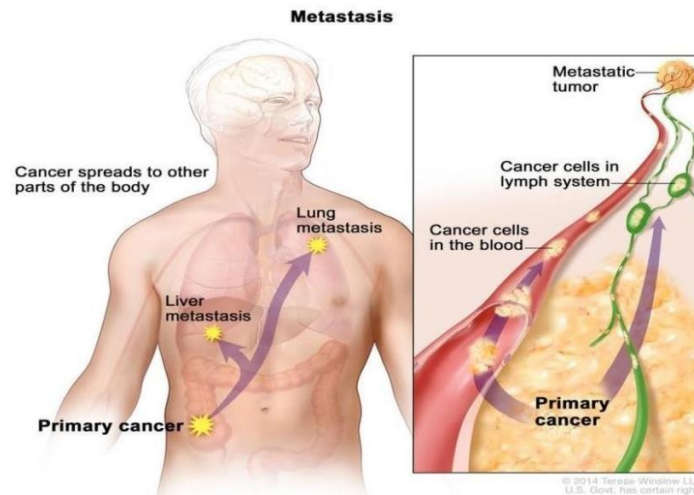


Fig 2: figure of when cancer spread

In metastasis, cancer cells break away from where they first formed and form new tumors in other parts of the body.

A cancer that has spread from the place where it first formed to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis.

Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer.

Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes.

In some cases, treatment may help prolong the lives of people with metastatic cancer. In other cases, the primary goal of treatment for metastatic cancer is to control the growth of the cancer or to relieve symptoms it is causing. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.

How can we preserve cancer?

- Preventing Cancer Don't smoke.
- Maintain a healthy weight.
- Exercise regularly
- Eat a healthy diet
- Drink alcohol in moderation, if at all Protect yourself from the sun.
- Protect yourself from infections.
- Get screening test regularly

Introduction Of Plant :

Secondary metabolites are organic compounds found in many plants that are not typically involved in the growth and development of organisms but frequently play a crucial role in plant defence . One of the most significant sources of medications comes from plants. The traditional medical system known as "Ayurveda" makes considerable use of medicinal plants worldwide Given that it is mostly found in south-east China and a few islands in the Pacific and Indian Oceans, the lovely flowering plant *Hibiscus rosasinensis* is frequently referred to as the "Queen of the Tropics" or the "China rose." One of Hawaii's beloved national plants, the hibiscus, is frequently worn in the hair for ceremonial purposes . This plant belongs to the subkingdom Magnoliophyta and to the class Magnoliopsida, meaning that it is a vascular plant that produces seeds. It belongs to the family Malvaceae, and it is one of the 300 species of the hibiscus.



Fig No 3 Hibiscus

Classification:

Kingdom : *Plantae*
 Division :- *Magnoliophyta*
 Class :- *Magnoliopsida*
 Order :- *Malvales*
 Family :- *Malvaceae*
 Genus :- *Hibiscus L.*

Botany

The shrubby species of *Hibiscus rosa-sinensis*, which typically grows to a height of 4 metres, is evergreen and has oval branches with stalks that measure 10 cm broad by 15 cm long. The majority of flowers are located on long stalks, are about 20 cm wide, and have whorled oval petals (egg-shaped), smooth edges, and are joined at the base to the central staminal column. A style with five lobes at the tip and numerous yellow anthers is part of this central column. Moving on to the flower's outer layer, we can see that its cup-shaped calyx measures 2.5 cm long, and its epicalyx is made up of 5 or 7 bracteoles that are each 1 cm long . Typically , flower are borne in single shapes .

Traditional Uses of *Hibiscus rosa sinensis*

- The roots of *Hibiscus rosa sinensis* Linn can be used as a cough suppressant.
- Leaves and flowers can be used as a hair growth promoter and to prevent premature graying and to treat scalp disorders.
- Leaves possess emollient properties, it can be used in the treatment of Dysentery and Diarrhea.
- The flowers is reported to be good for the treatment of heart diseases, diabetes, epilepsy, leprosy.

Content Of Hibiscu

Raw hibiscus contains some carbohydrates, calcium, magnesium, potassium, vitamin C, and B vitamins. Hibiscus tea tends to be lower in nutrients but still offers many beneficial compounds. In fact, these bioactive compounds appear to be primarily responsible for the purported health benefits of the plant.

Hibiscus has good nutritional value, which is given below:

Nutritional Value	Calyx	Seeds	Leaves
Carbohydrate (g)	10.2	25.5	8.7
Fat (g)	0.1	21.4	0.3
Protein (g)	2	28.9	3.5
Vitamin C (mg)	17	9	2.3
Calcium (mg)	150	350	240
Iron (mg)	3	9	5
Thiamine (mg)	0.05	0.1	0.2
Riboflavin (mg)	0.07	0.15	0.4
Niacin (mg)	0.06	1.5	1.4

Properties of Hibiscus :

- It may have anticancer activity . It may have antiseptic properties.
- It may have anti-spasmodic properties (relieves muscle spasms). It may have blood pressure-lowering properties.
- It may have a mild laxative effect (help constipation).
- It may have a diuretic effect (increase urine production). It may have antioxidant activity.
- It may have an antipyretic effect (reduce fever). It may have sedative properties.
- It may have blood sugar-lowering properties.

Introduction of Vincristine and Vinblastine :

In present study we are reviewing the use medicinally important plant *Cantharantus roseus* in cancer therapy. *Cantharantus roseus* is a traditional medicinal plant which possesses alkaloids known to cure many health problems like cancer, diabetes, blood pressure, asthma etc. The anticancerous compounds of *Cantharantus roseus* namely vinblastin and vincristine are broadly used as medicine to cure various cancers.

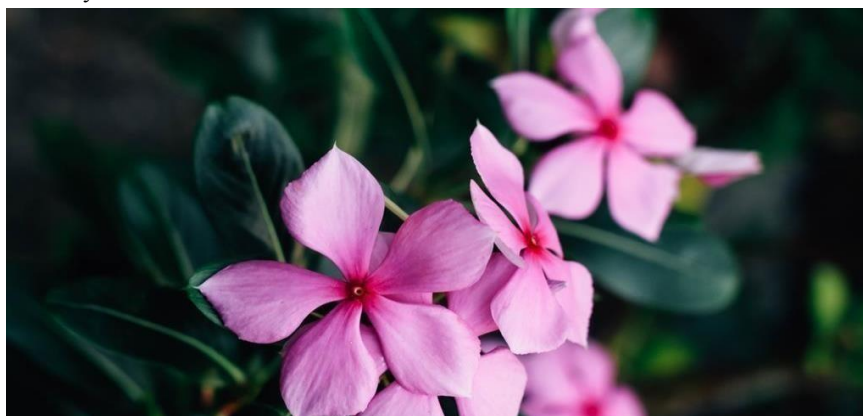


Fig 4 : Vincristine and Vinblastine plant

Scientific classification

Botanical Name(s): *Vinca Rosea* (*Catharanthus roseus*)

Family Name: Apocynaceae

Kingdom: Plantae

Division- Magnoliophyta (Flowering plants)

Class- Magnoliopsida (Dicotyledons)

Order- Gentianales Family- Apocynaceae

Genus- *Catharanthus*

Species: *Catharanthus roseus*

Morphology

Catharanthus roseus is an evergreen herb. The presence of oval leaves and white to dark pink flowers makes it unique Traditional and centre of the attraction. A corolla is about 2-5 cm in diameter with five petals like lobes. The fruit is a pair of follicles about 2-4 cm long and 3 mm broad7 . Phytochemical constituents and their therapeutic usesThe alkaloids isolated from this plant are known sedative, hypotensive and anti-cancerous. *C. roseus* is traditionally used by folklore to get rid from various health problems like disorders related to central nervous system, body pain and bleeding nose. It is also well established remedy for gastritis, cystitis and diarrhea. The book of pharmacognosywritten by Rang et al describes the strong antioxidant properties of this plant which was also proved by many studies.

Anticancerous compounds of *C. roseus*The most potent and commercially available molecules characterized from *C. roseus* are Vincristine and Vinblastin. In vitro studies of these molecules have shown significant anti-tumour activity as elaborated below.

Vincristine and Vinblastin

According to the reports shown by The American Society of Health System Pharmacists in 2015, vincristine and vinblastin are known for inhibiting mitosis i.e it stops the division of the cells and results in killing of the cells.

Vincristine formulation helps in binding to the tubulin protein and stops the cells from separating its chromosomes at the time of metaphase which is responsible for cell death . It binds tubulin which results in inhibiting the microtubule assembly. According to a survey conducted on the 13 children suffering from acute leukemia showed remission rate of 54% after the treatment with vincristine sulfate12. On the other hand vinblastine results in the arresting of M phase specific cell cycle by inhibiting the microtubule assembly by improper mitotic spindle formation and kinetochore, which is important for the separation of chromosome at the time of anaphase.

Medicinal Properties

Plants contain alkaloids, terpenoids, coumarins and many other phytochemicals which are responsible for various therapeutic uses. The Vinblastine and Vincristine are two alkaloids of *C. roseus*, which are responsible for the anticancer activity . The growths of some human tumours are suppressed by these alkaloids of *C. roseus*. The use of Vinblastine is recommended for chorio carcinoma and Hodgkins disease. The other alkaloid namely Vincristine is used for leukemia in children. The Vinblastine is available under the name of Velban in the market, whereas Vincristine is sold as oncovin. The various extracts of *C. roseus* are known for curing many diseases, like the ethanolic extract of leaves and flower of *C. roseus* showed anti-diabetic property because it helps lowering of blood

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observed to have anti-oxidant property . The anti- helmenthic property is also shown by this plant to cure chronic illness caused by the helminthes infections. Vincamine and Vindoline alkaloids of the plant have anti ulcer property to great extent. The ethanolic leaf extracts shows the anti diarrheal activity which was tested in the wistar rats with castor oil as an experimental diarrhea inducing agent in addition to the pretreatment of the extract. The anti diarrheal effect of ethanolic extracts *C. roseus* showed the dose dependant inhibition of the castor oil induced diarrhea . This plant also used in phyto remediation by bioaccumulates heavy metal like cd etc.sugar16,17. The extracts of *C. roseus* leaf were known for its anti-bacterial activity against like *Salmonella typhimuruim* NCIM2501, *Pseudomonas aeruginosa* NCIM2036, *Staphylococcus aureus* NCIM502118. The ethanolic root extract of *C. roseus* were observed to have anti-oxidant property . The anti- helmenthic property is also shown by this plant to cure chronic illness caused by the helminthes infections. Vincamine and Vindoline alkaloids of the plant have anti ulcer property to great extent. The ethanolic leaf extracts shows the anti diarrheal activity which was tested in the wistar rats with castor oil as an experimental diarrhea inducing agent in addition to the pretreatment of the extract. The anti diarrheal effect of ethanolic extracts *C. roseus* showed the dose dependant inhibition of the castor oil induced diarrhea . This plant also used in phyto remediation by bioaccumulates heavy metal like cd etc.

Extraction Procedure :

Firstly the extraction procedure is more important for us. We have use the soxhlet appratus for this extraction procedure. The motive of to use soxhlet appratus is that the it take more active ingredient from the plant.

Soxhlet appratus :

- a) The Firstly the flowers are taken from the botonical garden of rashtriya college of pharmacy hatnoor .
- b) In these the hibiscus flowers are collected and wash property.
- c) In the thimble of appratus the flowers are kept.
- d) Ethanol is add upto 20 to 30 ml in the which are the part of assembly of the soxhlet appratus.
- e) After started heating there is extract run 2 -3 times.
- f) There is a extract of hibiscus has formed and we stand out for cooling of hibiscus extract.
- g) After cooling the extract we remove the ethanol by heating the extract in bound bottom flask.
- h) Therefore there is a pure formation of hibiscus extract.



Fig 5: Extraction of hibiscus via soxhlet appratus

Extraction of Vincristine and Vinblastine by Soxhlet appratus :

- a) Firstly the Vincristine and Vinblastine leaves are collected from the botanical Gardens of Rashtriya college of Pharmacy hatnoor.
- b) The leaves of Vincristine and Vinblastine are wash properly.
- c) In the thimble of appratus the leaves are kept.
- d) Ethanol is add upto 20 to 30 ml in the which are the part of assembly of the soxhlet appratus.
- e) After started heating there is extract run 2 -3 times.

- f) There is a extract of Vincristine and Vinblastine has formed and we stand out for cooling of hibiscus extract.
- g) After cooling the extract we remove the ethanol by heating the extract in bound bottom flask.
- h) Therefore there is a pure formation of Vincristine and Vinblastine extract.



Fig 6 :Extraction of Vincristine and Vinblastine via soxhlet apparatus

Information of Extracted product :

Vincristine is known for binding to the tubulin dimmers which results in the inhibition of microtubule assembly and restricts mitosis at metaphase. The side effect associated with vincristine is that it targets all frequently dividing cell types which can also shows effects on the intestinal epithelium and bone marrow. Vinblastine at very low concentration is able to suppress the microtubule dynamics, whereas at high concentration it reduces the polymer mass of microtubule. It also causes the microtubule fragmentation¹³. C. roseus contains carbohydrates, flavonoids, alkaloids and saponins. Among all the most important constituents are alkaloids are used in pharmaceutical, pesticides and food industry. The anti- cancerous compound vinblastine is analkaloid by nature whereas vincristine is formed by the coupling of indole alkaloids namely vindoline and canthranthine present in the C. roseus.

The active constituents of hibiscus for anticancer activity :

Delphindin3 -Sambubioside, is a potent apoptosis inducer in leukaemia cells

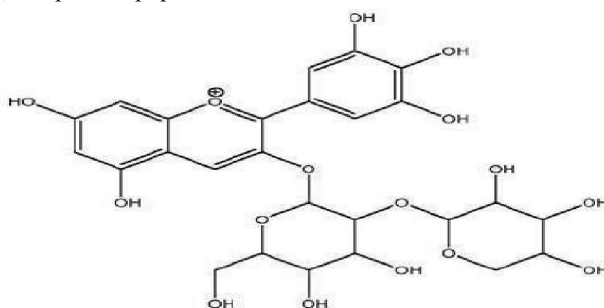
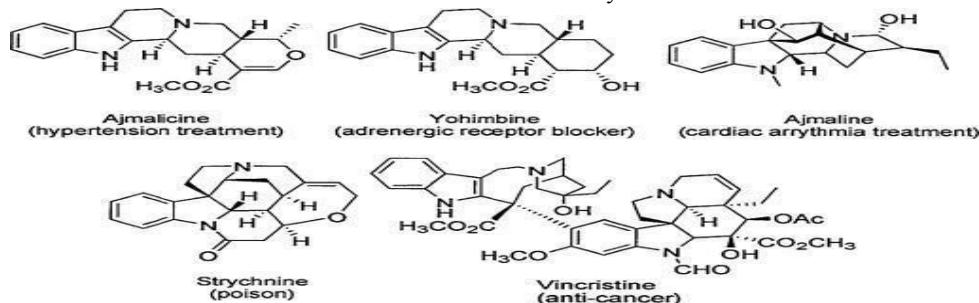
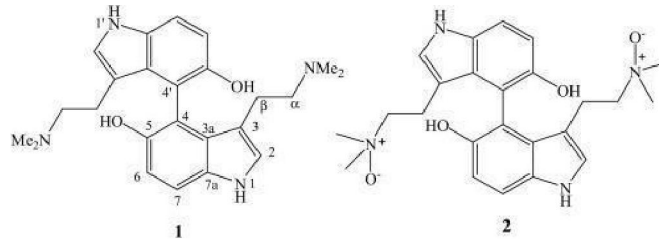


Fig no 6 : Delphindin3 -Sambubioside,

Active constituents of Vincristine and Vinblastine for anticancer activity :



Indole base dimeric alkaloids :



Test of Extracted product :

1) Uv

One of the earliest instrumental techniques for analysis is UV-VIS spectroscopy. Many different types of materials can be characterized using UV-Vis spectroscopy. The UV-Vis delivers details based on the degree of absorption or transmittance of a varied wavelength of beam light and the various responses of samples. Radiant energy absorption by materials can be quantitatively described using the general law known as Beer's law. The UV-VIS spectrometer is simple to use and handle. Both qualitative and quantitative analyses can make use of it. The metal hot wand metal oxide nanoparticles are typically characterized using wavelengths between 200 and 700 nm.

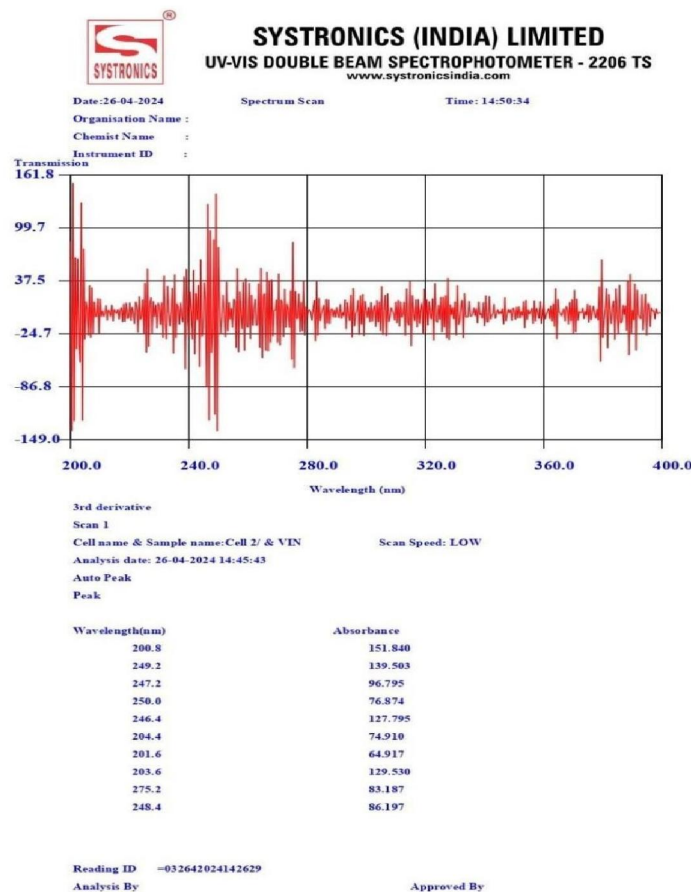


Fig 7 : The uv of Vincristine and Vinblastine

Test of extracted product like solubility :

The hibiscus extract and Vinblastine and Vincristine extract are mixed in same amount in a measuring cylinder. Then we add solubility enhancer like a peppermint, raspberry etc. Then we add peppermint on the mixed extract. Shake the extract till they dissolve.

Therefore this test has we performed and dissolve easily.

Test of extracted product like Stability :

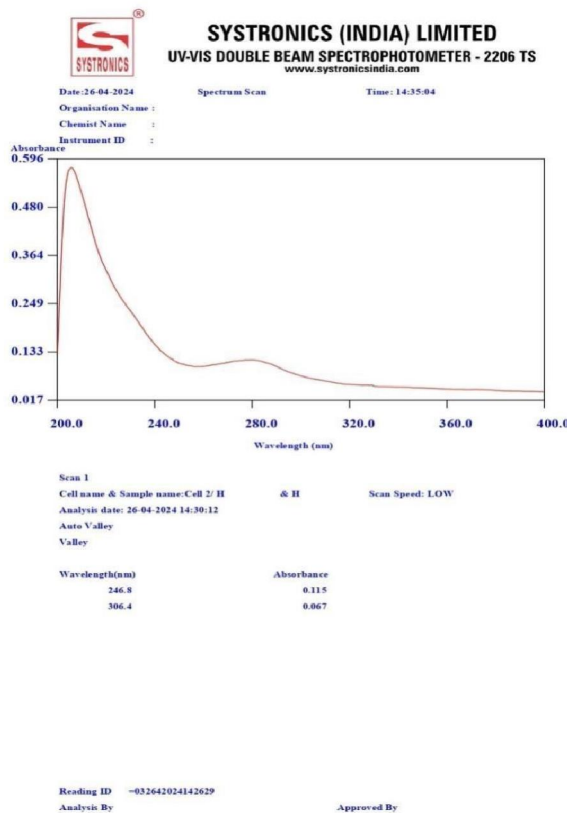
The stability of extracted product should be able to good. We check to maintain stability like :

Stability studies Methods:

- ❖ **Accelerated Testing:** Product subjected to high temperature, humidity, light, etc. 40°C/ 75% RH. At 3rd and 6th month.
- ❖ **Real Time (long term) Testing:** Longer duration. 25-30°C & 35- 75% RH (depending on climatic zone), for 3rd, 6th, 9th, 12th, 18th, 24th, and 36th month
- ❖ **Intermediate Testing:** Conducted when accelerated studies fail. At 25°C for longer duration of time
- ❖ **Stress Testing:** Includes effects of temperature, i.e., above 40°C and 275% RH
- ❖ **Forced Degradation Testing:** Performed to provide intrinsic stability assessment of drug

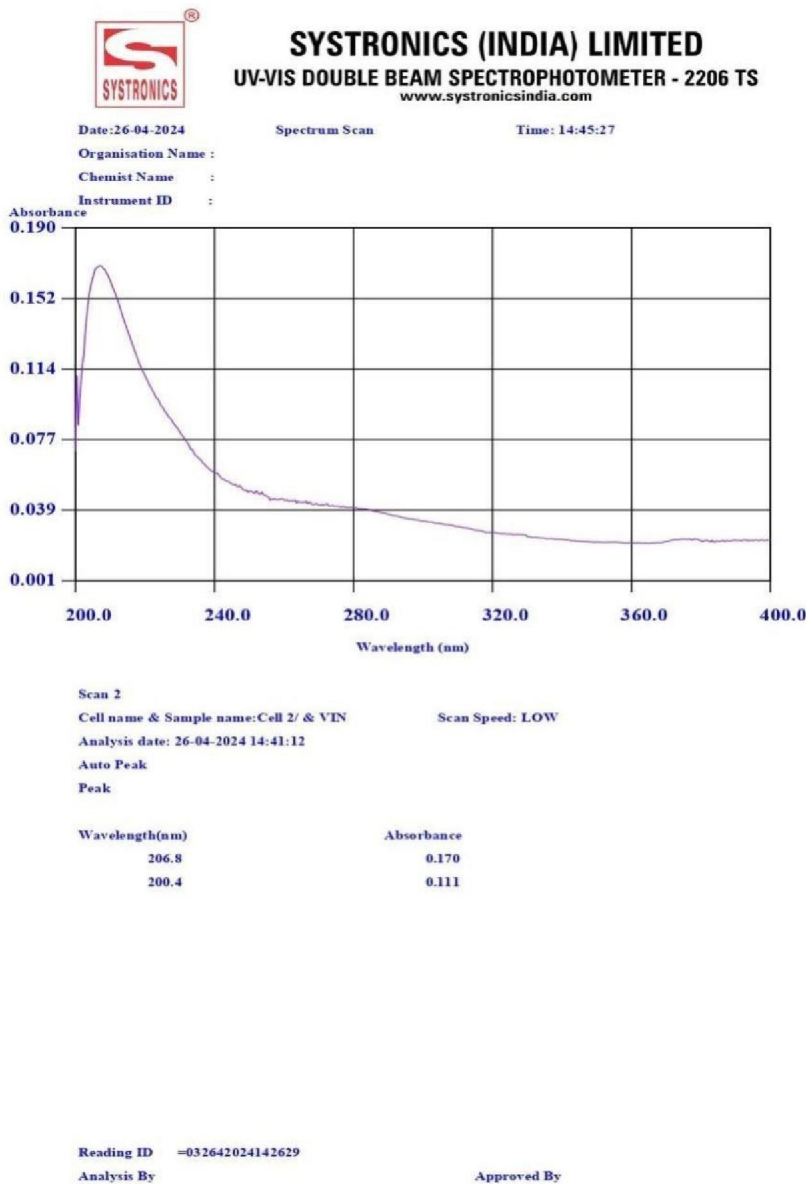
Addition on Graph :

The graph of hibiscus extract and Vinblastine and Vincristine are following :



Graph : of Hibiscus extract

DOI: 10.48175/IJAR SCT-19101



Graph : of Vincristine and Vinblastine

II. CONCLUSION

Plan for the diagnosis and treatment of cancer is a key component of any overall cancer control plan. Its main goal is to cure cancer patients or prolong their life considerably, ensuring a good quality of life. In order for a diagnosis and treatment programme to be effective, it must never be developed in isolation. It needs to be linked to an early detection programme so that cases are detected at an early stage, when treatment is more effective and there is a greater chance of cure. It also needs to be integrated with a palliative care programme, so that patients with advanced cancers, who can no longer benefit from treatment, will get adequate relief from their physical, psychosocial and spiritual suffering. Furthermore, programmes should include a awareness-raising component, to educate patients, family and community members about the cancer risk factors and the need for taking preventive measures to avoid developing cancer.

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