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Formulation and Evaluation of Liposomes from Vinca Flowers Extract for Anti Cancer

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ABSTRACT: -
The traditional Indian medical system known as Ayurveda lays a lot of emphasis on the medicinal properties of plants. Catharanthus roseus is one such plant that is used in Ayurvedic medicine. Originally from the island of Madagascar, Catharanthus roseus (L.) G. Don, a member of the Apocynaceae family, is now widely distributed in India. This species has been grown as a garden plant and for use in herbal medicine. It has a lot of alkaloidal components. The anti-tumor, anti-diabetic, antibacterial, antioxidant, and antimutagenic properties of this plant are widely recognized. Approximately 130 alkaloids, including as vincristine and vinblastine, are synthesized by Catharanthus roseus. Hodgkin's disease, breast cancer, skin cancer, and lymphoblastic leukemia are some of the cancers that are treated with vincristine and vinblastine.
Cancer is a hereditary disease characterized by uncontrolled cell division that can spread to other body areas. It is caused by genetic or epigenetic changes in the somatic cells. 19 million cases of cancer were reported worldwide in 2023, including 9.5 million cases in males, 8.5 million cases in women, and 9.6 lakh deaths. Prostate, breast, lung, stomach, colorectal, and non-melanoma skin cancers are the cancers that spread the fastest worldwide, although there are 100 different kinds of cancer that impact people. The effects of cancer are becoming more and more apparent each passing day. Cancer is caused by tobacco use 22% of the time, infections such as HIV, Hepatitis B, and Epstein 15% of the time, and poor diet, obesity, and excessive use of alcohol 10% of the time.
In this study, two drugs were extracted from Vinca flowers, which come in two colors - pink and white and were kept at room temperature in the lab. Thus, we measured the solvent concentration for each of the two drug extractions from pink and white flowers.
Objective: Clearly state the purpose of the study, emphasizing the development and evaluation of liposomal formulations containing vinca flowers extract for anti-cancer applications.
Purpose: Liposome and immunoliposome formulations of two vinca alkaloids, vincristine and vinblastine, were prepared using liposomal egg yolk and cholesterol examined for their ability to stabilize the drug for targeted drug delivery in vitro study.
Result: Vinca alkaloid was extracted from vinca plant by using Soxhlet apparatus. Vinca extract shows identification test for vinca alkaloid was positive. Liposomes are novel drug delivery system. Ultrasonication methods gives good result in formulation of liposomes. Phospholipid used for formulation of liposomes. Shape of liposomes is spherical and have 45nm size of liposomes.
Conclusions: These results demonstrate that active targeting of tumors with liposomal formulations of Vincristine and vinblastine is possible when the resulting immunoliposomes are sufficiently stabilized.
Keywords: Alkaloids, Vincristine, Vincristine, lymphoblastic leukemia, somatic cells, colorectal cancer, non-melanoma skin malignancies, HIV, hepatitis b, Epstein, liposomal egg yolk, cholesterol, Liposomes.

I. INTRODUCTION: -

Hippocrates, a Greek physician, is associated with originating the term cancer. Loss of control over cellular growth and development, resulting in uncontrolled cell multiplication and spread, is a characteristic of cancer. Cancer is the result of inappropriate or uncontrolled cell growth and spread from the place of origin to another site (tumor/clumping of cells). The uncontrolled growth of cells in a group known as a tumor or neoplasm; these cells might be dispersed widely and create a lump or mass. All of the body's cells that are alive, regardless of age or gender, are susceptible to this illness. Therefore, cells from lumps or masses of extracted tissues that multiply when new cells are not required might be either benign or cancerous.

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Normal tumor cells do not spread to other areas of the body or permeate other tissues. Benign tumors rarely represent a threat to life and are not cancerous. Malignant, on the other hand, indicates the presence of cancerous cells inside the human body. A malignant tumor has the ability to penetrate nearby tissue and spread throughout the body. This is how cancer spreads, creating new tumors in various organs. Cancer spreads by a process known as metastasis, that the human body is unable to stop. Cancer is leading cause of mortality and morbidity all over the world. It is the outcome of uncontrolled cell death and proliferation without differentiation and apoptosis of cells. Usually, there are two types: benign (where the cancerous cells form a single mass and are unable to spread to other organs) and malignant (when the cancerous cells penetrate nearby normal cells and travel throughout the body through the cardiovascular system). The aging population, environmental contaminants, genetics, unhealthy lifestyles, obesity, tobacco product usage, virus infections, radiation, and other variables are some of the main risk factors for cancer. (1).

History of Cancer: -

Hippocrates, the Greek physician, was the first to use the term to the illness as cancer (460-370 BC). He is known as the "Father of Medicine." Hippocrates used both carcinosis as well as carcinoma to define non-ulcer developing and ulcer-forming tumors. This refers to a crab in Greek. The earliest documented incident of cancer in history was discovered on papyrus, or ancient Egyptian documents, going to approximately 1500 BC. Theodor Boveri, a scientist and professor at Munich and subsequently Würzburg, was discussed. He found a way to make cells that had several copies of the structure he known as the centrosome. Famous German chemist Paul Ehrlich began creating medications to treat infectious disorders in the early 1900s. He was she who first used the word "chemotherapy" to refer to the use of chemicals for the treatment of medical conditions. Chemotherapy's father is Paul Ehrlich. India's "father of chemotherapy" is Yellapragada Subba Rao. Global demographic statistics projected that by 2025, there will be around 420 million new instances of cancer year, indicating an increase in the rate of cancer in years. Around 18 million cases of cancer were reported globally in 2018; around 9.5 million of these cases occurred in men & 8.5 million occurred in women.

It was projected that cancer killed 9.6 million people worldwide. The most prevalent cancers are non-melanoma skin cancers (1.04 million), colon cancer (1.1 million), stomach cancer (1,03 million), prostate cancer (1.28 million), and female breast cancer (2.09 million). The most common causes of cancer-related mortality are lung cancer (1.76 million cases), colorectal cancer (862,000 cases), stomach cancer (783,000 cases), and liver cancer (782,000 cases). (3).

Different types of carcinomas are shown in a tabular form (1)

Table 1

Sr. No.	Carcinoma	Risk factors	Complication/ symptoms
1.	**STOMACH** (Developing from the lining of stomach)	Helicobacter pylori (65-80%), smoking	Dysphagia, heartburn, during meals sensation of being full, anemic, weight loss
2.	**LUNG** (Uncontrolled cell growth in tissues of lung)	Tobacco smoking (80%), genetic, radon gas, air pollution	Coughing including (blood), chest pain, shortness of breath

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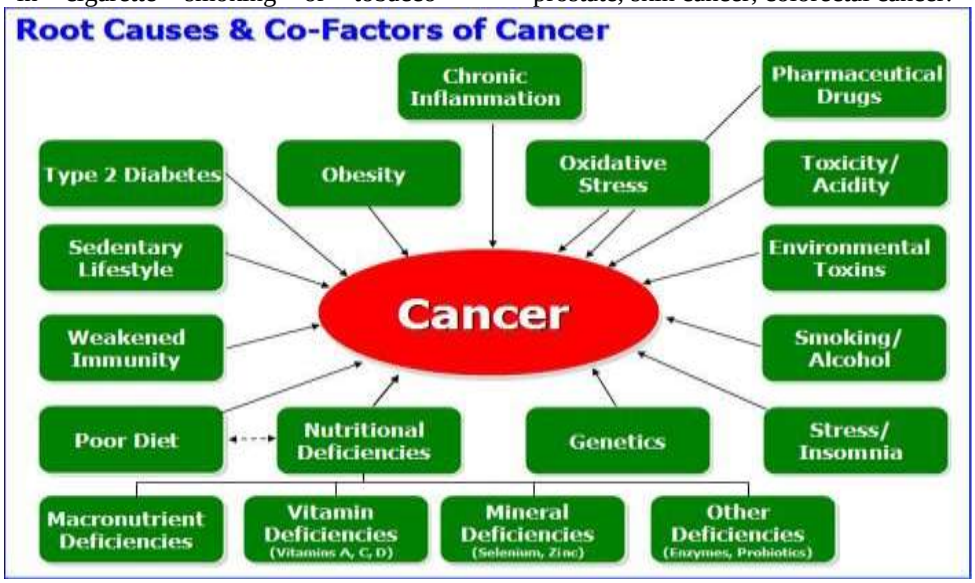
3.	**BLOOD** (starts from bone marrow)	Family history, smoking, down syndrome, ionizing Radiations	Bleeding and bruising, fever, feeling tired
4.	**BREAST** (Develops from breast tissue)	Obesity, alcohol consumption, hormone replacement therapy, ionizing radiations	Lump in breast, breast shape changed, fluid coming from nipple
5.	**COLORECTAL** (cancer that affects colon and rectum)	Unhealthy diet, obesity, smoking, alcohol use	Changes in bowel habits, diarrhea or constipation, pain and bloating in abdomen
6.	**OVARIAN** (Cancer that forms in ovary)	Never having children, obesity, genetic factors, fertility medication	Vague, bloating, pelvic pain, loss of appetite, abdominal swelling
7.	**SKIN** (arise from skin)	Light skin, poor immune Function	Ulceration, hard lump with a scaly top, mole that changed in size, shape, color

Causes of Cancer: - Three types of foreign agents that we consume interact with genetic factors to cause cancer: - (3).

I. **Physical Carcinogens:** Ionizing radiation such as radon, ultraviolet rays from sunlight, uranium, radiation from alpha, gamma, beta, and X-ray-emitting sources.

II. **Chemical Carcinogens:** Compounds like nitrosamines, asbestos, cadmium, benzene, vinyl chloride, nickel, and benzidine and contains about 60 known potent cancer-causing toxins or chemicals in cigarette smoking or tobacco consumption, a drinking water contaminant (arsenic), a food contaminant (aflatoxin).

III. **Biological Carcinogens:** Infections from certain bacteria, viruses, or parasites and Pathogens like human papillomavirus (HPV), EBV or Epstein-Barr virus, hepatitis B and C, Kaposi's sarcoma-associated herpesvirus (KSHV), Markel cell polyomavirus, Schistosoma spp., and Helicobacter pylori. Aging is also the cause of cancer. Age is the common incidence of cancer, which dramatically rises. Genetic is the commonest cause for cancer or tumor-like Ovarian, breast, prostate, skin cancer, colorectal cancer.



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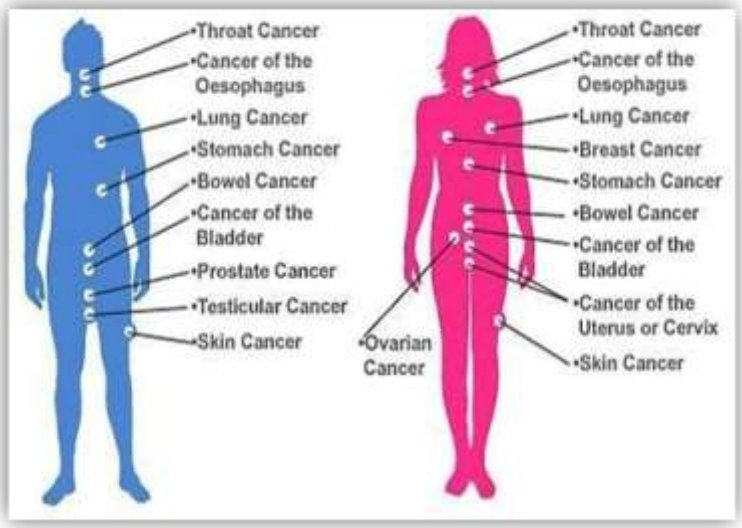

- **Types of Cancer:** Cancers are divided into various types that are- (3)

A. Carcinomas
B. Sarcomas
C. Leukemia's
D. Lymphomas
E. Central Nervous System Cancers
F. Multiple Myeloma
G. Melanoma
H. Germ Cell Tumors
I. Neuroendocrine Tumors

- **Symptoms and Signs of Cancer:** (3)
- **Early Symptoms:** Cancer does not exhibit any signs or symptoms in its early stages, making it impossible to diagnose the illness. Furthermore, the indications or symptoms are displayed in a harm condition. The following are a few typical signs of cancer that may appear:

1. Persistent Cough or Blood-Tinged Saliva
2. A Change in Bowel Habits
3. Blood in the Stool
4. Unexplained Anemia
5. Breast Lump or Breast Discharge
6. Lumps in the Testicles
7. Change in Urination
8. Persistent back pain, Unexplained weight loss, Stomach pain, nausea and Bone pain.

- **Late Symptoms:** These symptoms alter based on the type of cancer, its location, and the extent to which its cells have spread.

1. Change in bowel or bladder habits
2. Obvious change in the size, color, shape, or thickness of a wart or mole
3. Indigestion or difficulty in swallowing
4. Change in size, shape, color or thickness of mole.
5. A sore throat that does not heal.
6. Hoarseness
7. Thickening or lump in the breast and testicles.

- **Other signs or symptoms: -** These include the following:

1. Unexplained loss of weight or loss of appetite
2. Nausea
3. Vomiting
4. Fatigue
5. Unexplained low-grade fevers may be either persistent or not.
6. Recurring Infections
7. Pain in the bones and other body parts.

LIPOSOMES: - (7)

Colloid, vesicular structures known as liposomes are made up of one or more lipid bilayers encircling an equivalent number of aqueous compartments. Biocompatible liposomes have the ability to ensnare and shield water-soluble molecules within their own internal aqueous compartment as well as aqueous-insoluble molecules inside the membrane. To create liposomes, a variety of amphipathic compounds have been employed. The medication molecule may incorporate into the lipid bilayer or be enclosed in an aqueous space. Liposomes are vesicles with an aqueous compartment surrounded

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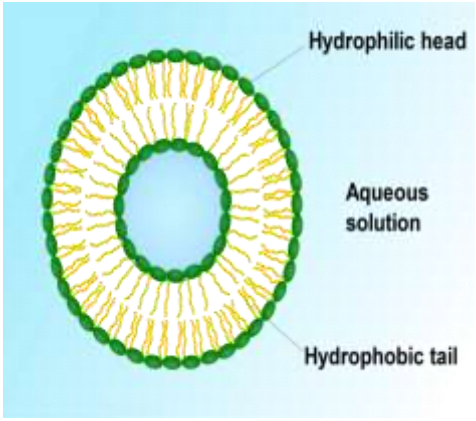
by bilayers or multilayers of cholesterol and phospholipids. The medication is encapsulated in a liposome and then released to be absorbed at the surface of the intestinal mucosa.



To deliver the medication to a specific site, a variety of carriers including liposomes, polysaccharides, lectins, and nanoparticles can be employed. Liposomal drug delivery is becoming more and more popular because of its benefits to a variety of fields, including drugs delivery, cosmetics, and biological membrane structure. Because they may carry a wide range of medications with possible therapeutic effects or additional properties, liposomes can be particularly helpful. Liposomes are colloidal carriers with a diameter ranging from 0.01 to 5.0 μm. In fact, phospholipids are hydrated in excess of aqueous medium or aqueous solution to create these bilayer vesicles. One potential benefit of liposomes is their ability to encapsulate both hydrophilic and hydrophobic drugs as well as target them to phospholipids that are too hydrated in aqueous media. One potential benefit of liposomes is their ability to encapsulate both hydrophilic and hydrophobic medications and deliver them to the specific illness site in the human body. (7)

- **GENERAL METHODS OF PREPARATION: -** (7)

There are four fundamental steps in all liposome preparation and formulation techniques:

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Drug loading and liposome preparation methods: -

The liposomes are prepared using one of the following methods:

a. passive loading techniques;
b. active loading techniques.

a. Passive loading techniques: -

Passive loading techniques include three different methods:

1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of non-encapsulated material).

1. Mechanical Dispersion Method: -

The following are types of mechanical dispersion methods:

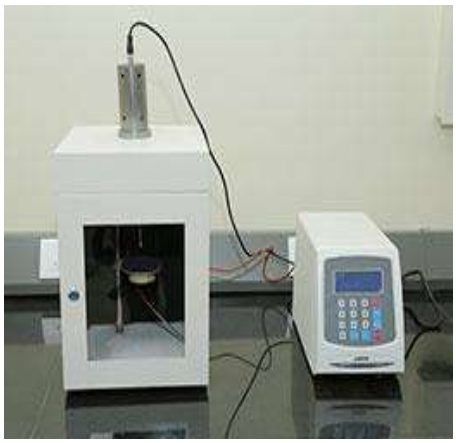
1.1. Sonication.
1.2. French pressure cell: extrusion.
1.3. Freeze-thawed liposomes.
1.4. Lipid film hydration by hand shaking, non-hand, shaking or freeze drying.
1.5. Micro-emulsification.
1.6. Membrane extrusion.
1.7. Dried reconstituted vesicle

1.1 SONICATION: -

The technique that is most frequently used to prepare SUVs is sonication, which is useful. There, MLVs are sonicated in a passive environment using a probe sonicator or a bath-type sonicator. The primary drawbacks of this technique are its extremely limited internal volume and encapsulation efficiency, potential phospholipid and chemical degradation, removal of big molecules, metal contamination from the probe tip, and existence of MLV in addition to SUV.

There are two Sonication techniques:

1. Probe Sonication:



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A sonicator tip is inserted directly into a liposome dispersion. This approach has a very large energy consumption for lipid dispersion. The specific heat produced by the energy coupling at the tip necessitates submerging the vessel in a water/ice bath. Over 5% of the lipids can be esterified during the sonication process for up to one hour. Additionally, titanium will slough off and contaminate the solution when using the probe sonicator.

2. Bath sonication:

The cylinder containing the liposome dispersion is put into a bath sonicator. Monitoring the operating temperature of a lipid dispersion or spread typically simple in this technique, in contrast with Sonication by dispersion through the tip. The substance that is being sonicated may be shielded by an inert environment, a sterile vessel, or similar probe equipment.

A. PLANT PROFILE: - **(8)**

- **VINCA : -**

Vinca Rosea is a significant medicinal plant that is a member of the Apocynaceae family. grows is entire India over 500 meters. It is readily grown in South India and the northeastern Indian states in tropical and subtropical climates.

Biological Source: The dried whole plant of Catharanthus roseus.

Geographical Source: Madagascar is where it originated. Vinca plants are grown for their decorative qualities and may be found in tropical locations such as Africa, Australia, Taiwan, Thailand, and Eastern Europe. Madagascar, an island in the Indian Ocean, is the natural home of Catharanthus roseus. This plant is considered threatened in the wild, and habitat damage by cutting and burning operations is the primary factor contributing to its decline. Still, agriculture has become widespread in many tropical and subtropical areas of the world, including the Southern United states.

Morphology :- The evergreen sub herb or herbaceous plant known as Catharanthus roseus can reach a height of one meter. The leaves are grouped in opposite pairs and are oval to oblong, 2.5–9.0 cm long, 1–3.5 cm wide, glossy, green, and hairless. They have a light midrib and a short, 1-1.8 cm long petiole. White and dark pink in color, the blooms have a centre, a basal tube that is around 2.5–3 cm long, and a corolla that is roughly 2–5 cm in diameter and has five lobes that resemble petals. The fruit consists of two follicles that are around 2-4 cm long and 3 mm wide. (8)

Fig 6. Periwinkle Vinca plant (pink and white)

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PINK FLOWER VINCA	WHITE FLOWER VINCA
Scientific Classification:	
Botanical Name: Catharanthus roseus	**Botanical Name:** Catharanthus roseus
Family Name : Apocynaceae	**Family Name :** Apocynaceae
Synonym: Vinca rosea	**Synonym:** Vinca rosea
Genus: Catharanthus	**Genus:** Catharanthus
Species: C. roseus	**Species:** C. roseus
Kingdom:	
Kingdom: Plantae	**Kingdom:** Plantae
Division: Magnoliophyta (Flowering plants)	**Division:** Magnoliophyta (Flowering plants)
Class : Magnoliopsida (Dicotyledons)	**Class :** Magnoliopsida (Dicotyledons)
Subclass: asteridae	**Subclass: Tracheophytes**
Order : Gentianales	**Order :** Gentianales

Table 2 Botanical identity

- **Common Vernacular Names:**

English: cayenne jasmine, old maid, periwinkle
Hindi: Sada bahar, sadabahar
Kannada: batla hoo, bili kaasi kanigalu, ganeshana hoo, kempu kaasi kanigalu
Malayalam: banappuvu, nityakalyani, savanari, usamalari
Marathi: sadaphool, sadaphul, sadaphuli
Sanskrit: nityakalyani, rasna, sadampuspa, sadapushpi
Tamil: cutkattu malli, cutukattu malli, cutukattuppu
Telugu: billaganneru
Gujarati: Barmasi
Bengali: noyontara

- **Chemical constituent of whole vinca plants: -**

C. roseus posse's carbohydrate, flavonoid, saponin and alkaloids. Alkaloids are the most potentially active chemical constituents of Catharanthus roseus. More than 400 alkaloids are present in the plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance, ingredients, food additives and pesticides.

1. actinia blastomeric
2. Vinblastine
3. Vincristine
4. Vindoline
5. Tuberculin
6. ajmalicine
7. vicenin
8. vincamine
9. raubasin
10. reserpine
11. catharanthine
12. Rosindin

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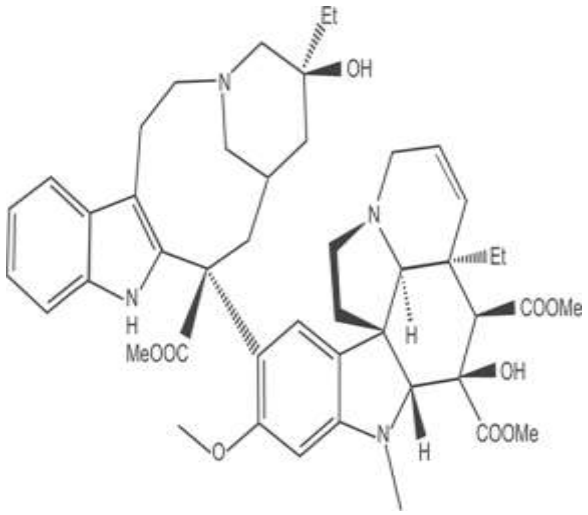
B. DRUG PROFILE: - (6)

- **CHEMISTRY: -**

The vinca alkaloids are large, closely related compounds having two distinct indole nuclei—the catharanthine component and the dihydro vindoline portion—joined by a carbon-carbon chain that can have different substituents attached to it. Vincristine is distinct from vinblastine, vindoline, and vinorelbine because it contains an acetaldehyde group rather than a methyl group at position one of the vindoline nucleus' nitrogen atom. At carbon 3 in the vindoline nucleus, vindesine contains an amide bond, whereas vincristine, vinblastine, and vinorelbine all have a methyl ester moiety attached. Vincristine, vinblastine, and vinorelbine have acetylation at carbon 4, but vindesine possesses a hydroxyl group. Vinorelbine's catharanthine component of the molecule has an altered structure as well; carbon has been removed, resulting in the replacement of the 11-membered ring with a 10-membered ring.

Vinblastine (VLB): - The alkaloid that used to be known as vincaleuko-blastine is currently referred to by its generic name, vinblastine (Fig. 7). It consists of a colourless compound. The sulphate derivative, or VLB, is a crystalline chemical that is hygroscopic, white to slightly yellow, and soluble in methanol and water. It can be used in the clinic. This anti-mitotic medication is often used in medicine to treat a variety of malignancies, including testicular, breast, cell pulmonary, head and neck, and Hodgkin's lymphoma. (6)



Generic Name: -Vinblastine
Weight: - 810.9741
Chemical Formula: - $C_{46}H_{58}N_4O_9$
Protein binding: - 98-99%
Metabolism: - Metabolism of vinblastine has been shown to hepatic cytochrome P450 3A isoenzymes.
Route of elimination: -route of excretion may be through the biliary system.
Half-life: - Triphasic: 35 min, 53 min, and 19 hours

- **Vincristine (VCR): -** There is another term for vincristine, which is also referred to as leurocristine (Fig. 8). VCR gets its name from the Vinca (Catharanthus roseus) alkaloid. The majority of commercial formulations of VCR show up as a clear liquid. It is a mitosis inhibitory that is widely used in chemotherapy treatments for cancer. The US FDA (Food and Drug Administration) authorized vincristine as Oncovin in July 1963 (Farnsworth, 1985). (6)

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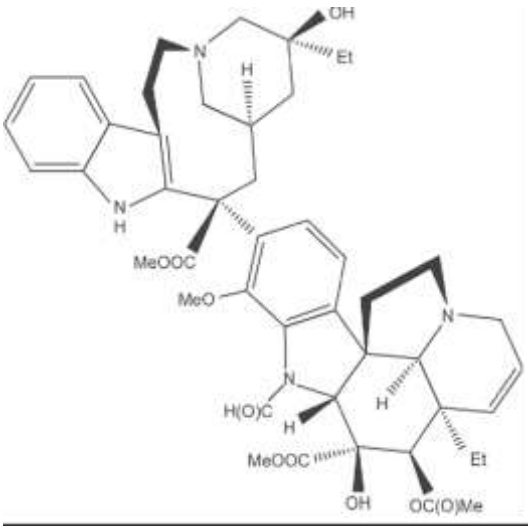
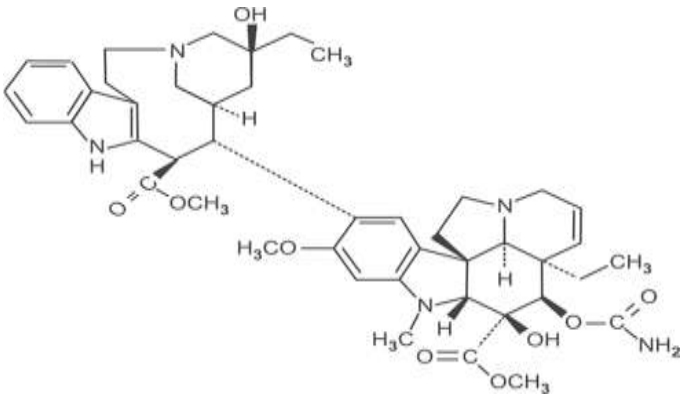


Generic Name: - Vincristine
Weight: - 824.972
Chemical Formula: - $C_{46}H_{56}N_4O_{10}$
Protein binding: - ~75%
Metabolism: - Cytochrome P450 isoenzymes of the CYP3A subfamily facilitate the metabolism.
Route of elimination: - 80% of an injected dose is excreted via feces. 10 - 20% is excreted via urine.
Half-life: -The initial, middle, and terminal half-lives are 5 minutes, 2.3 hours, 85 hours respective.

- **Vindesine (VDS):** - Vindesine is a type of anti-mitotic Catharanthus alkaloid utilized in chemotherapy (Foye, 1995; Fig. 9). The powder vindesine, which is sold commercially, dissolves into a colourless liquid. Vindesine is used in the treatment for various cancers, including as lung, breast, melanoma, lymphoma, and leukaemia. Vinblastine, or and vindesine contain equal toxicity as well as adverse effects. Vindesine is sold under the brand names Eldisine and Fideism. Its primary uses are in the treatment of melanoma, lung malignancies (carcinomas), and cancers of the uterus when combined with other drugs. (6)



- **Vinorelbine (VNLB, VRL):** - The first 5´NOR partially synthetic Catharanthus alkaloid is (Fig. 10). It is produced by semi-synthesis using the Polonovski fragmentation method from Catharanthus alkaloids such as vindoline and catharanthine. The Pierre Fabre Company discovered it in the middle of the 1990s. One anti-mitotic chemotherapy medication called vinorelbine (which is additionally known as nor anhydro vinblastine) was used to treat some cancers, such as lung cancer and breast cancer. It looks like a transparent liquid. Compared to other Catharanthus alkaloids, it appears to have a broader spectrum of anticancer action at present. Compared to vindesine, it is thought to be less of a nerve toxin. (6)

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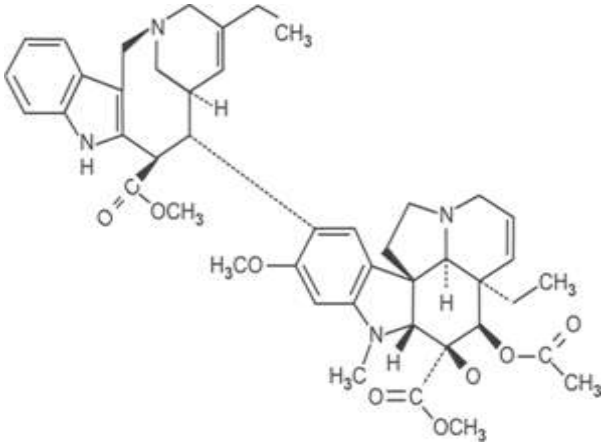
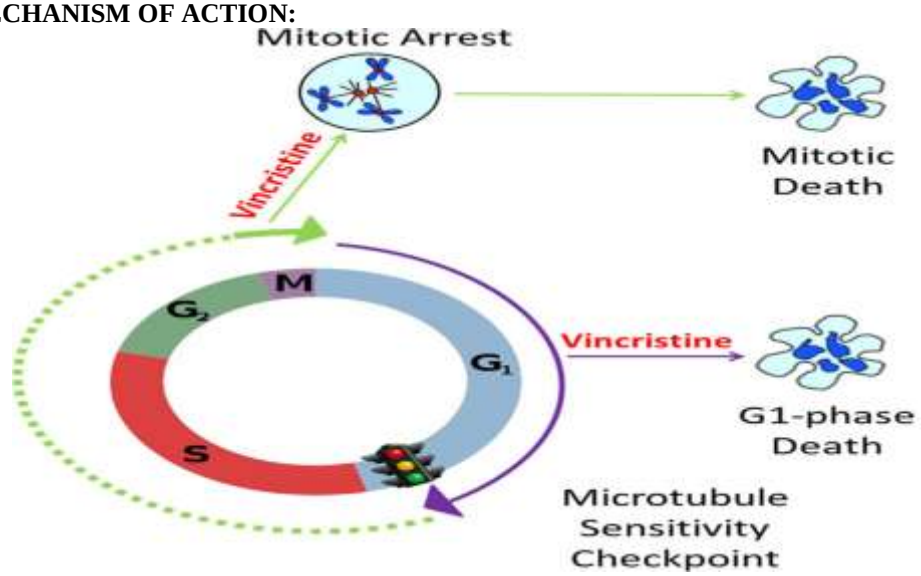


- **MECHANISM OF ACTION:**



- **Pharmacological Effect: -** (8)

1. Anticancer Property
2. Memory Enhancement Property
3. Wound Healing Property
4. Hypolipidemic Property
5. Hypotension Property
6. Antidiabetic Property
7. Anti-microbial property
8. Antioxidant Property
9. Anti-helminthic Property
10. Anti- ulcer property
11. Anti-diarrheal property

PHYTOCHEMICALS ANALYSIS: -(9)

A simple and rapid HPLC-DAD method: -

1. Chemicals: - Anhydro vinblastine, vinblastine, vincristine, ajmalicine, serpentine, tryptamine, tryptophan, anhydro vinblastine, and loganin are among the analytical reagents that are present in methanol and ethanol.

2. HPLC conditions: - The Agilent Technologies 1200 series vacuum degasser, G1310A pump, G1329A auto-sampler, and G1315D diode array detector made up the chromatographic system. The column was paired with a Safety Guard TM column and an Agilent Eclipse XDB-C18 (4.6 x 250 mm, 5 μm particle size). The mobile phase was made up of 1.5 ml per minute containing methanol (solvent B) and 5 mM Na2HPO4 (pH adjusted to 6 with HCl) (solvent A). The solvent A and solvent B eluent profile was adjusted to a linear gradient at 0–26 min, an isocratic mode at 26–30 min, a linear gradient at 30–35 min, and an isocratic elution at 14–86 (v/v).

3. Identification and quantitation: - There were developed standard solutions. Six layers of external

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standards were used for the quantification process. Level 6 involved diluting the stock solution (5 mg/mL) with methanol at a ratio of 1:50, v/v. Level 6 was diluted by a ratio of 2, 4, 8, 16, and 32, respectively, to obtain levels 5 to 1. The obtained ranges varied from 2-4 µg/ml to 64-128 µg/ml, contingent upon the stock solution's concentration of each chemical. Alkaloids were identified from the plant extracts by comparing their UV spectra and retention times to those of reliable standards. By producing and measuring each concentration in triple quantities, each calibration curve was created. For quantification, test samples were examined in triplicate using the standards' calibration curves.

4. Plant materials: - C. roseus seeds were bought. The seeds were surface-sterilized for two minutes in 75% (v/v) ethanol and five further minutes in 5% (v/v) NaClO2. The seeds were then placed on Petri dishes with MS (Murashige and Skoog) basal medium, and they germinated after being rinsed five times with sterile distilled water. The cultures were cultivated at 25 ± 2 °C with a 16-hour light and 8-hour dark photoperiod. Following a week of germination, seedlings were placed in soil and raised in a greenhouse with controlled air temperatures and humidity levels of 25°C and 55%. For the ensuing studies, the plants' blossoms, leaves, stems, and roots were all removed individually and immediately frozen in liquid nitrogen. At 4:00 pm on the 30th, 44th, 62th, 79th, and 99th days following germination, a few plants from a different batch were gathered to track the variation in alkaloid content as the plants grew. All observation group's samples were measured three times.

5. Sample preparation: - Using a mortar and pestle, freshly acquired specimens were crushed in nitrogen solution. The samples were then lyophilized for a duration of 72 hours. The 30 mg dried substance was placed in a micro-tube and extracted using 1 ml methanol during a 30-minute sonication and vertexing process in an ultrasonic bath (DL-60D). After centrifuging the samples for 10 minutes at room temperature at 12,000 rpm, the supernatant was filtered using a 0.45 µm needle type PTFE membrane filter (purchased from Sigma-Aldrich) before being subjected to HPLC analysis.

6. Data statistical analysis: - Three duplicates of each experiment were run. One-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used for statistical analysis. For each of the groups, there are three samples, and the data are mean ± SD. Significant p values were defined as those < 0.05. With all of the data, SPSS carried out an ANOVA. (9)

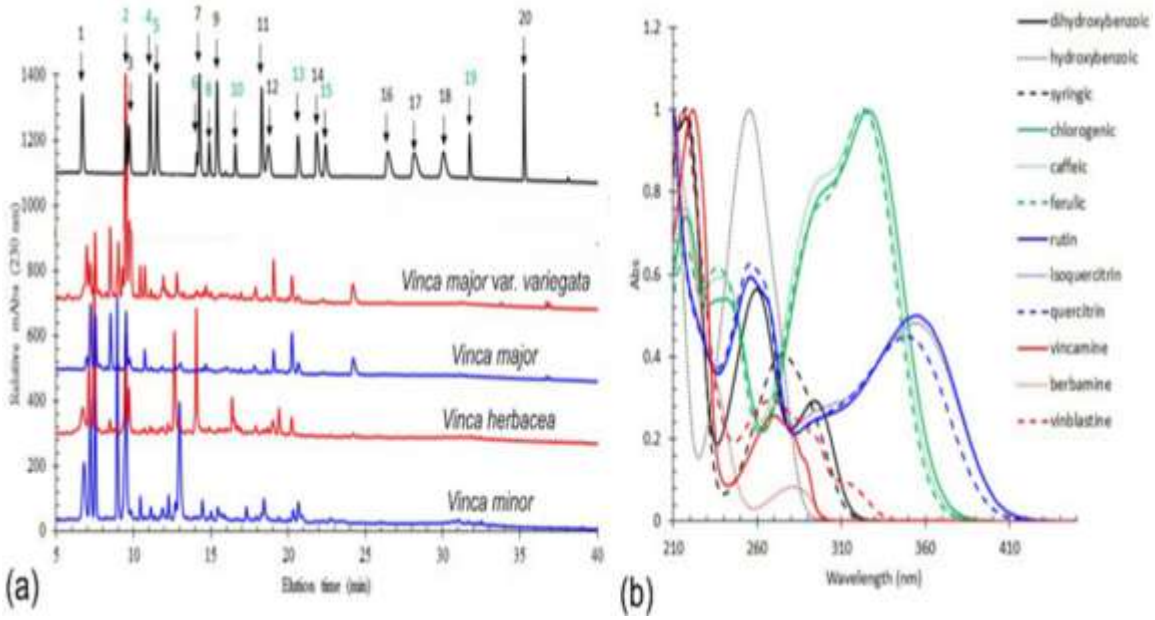

Figure 12. (a): - Vinca plant extract HPLC-DAD chromatograms recorded at 230 nm. (1) 3,4-dihydroxybenzoic acid; (2) chlorogenic acid; (3) 4-hydroxybenzoic acid; (4) caffeic acid; (5) syringic acid; (6) rutin; (7) p-coumaric acid; (8) isoquercitrin; 9) ferulic acid; 10) quercitrin; (11) myricetin; (12) berbamine; (13) vincamine; (14) jatrorrhizine; (15) quercetin, (16) palmatine; (17) berberine; (18) kaempferol; (19) vinblastine; and (20)

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galangin are among the analytical standards included in the chromatogram. In the upper chromatogram, the discovered chemicals in the investigated Vinca species are shown in green.
(b): - UV molecular absorption spectra for representative standards from each major phytochemical category (red alkaloids, green citric acids, blue flavonoids, and grey hydroxybenzoic acids) as recorded by the DAD detector. (10)

Table 3 - Compound Concentration Expresses in µg/g: -

COMPOUND	PINK	WHITE
Chlorogenic acid	**932 ± 260**	**1538 ± 200**
Caffeic acid	**182 ± 23**	**13 ± 1**
Rutin	**94 ± 10**	**2528 ± 160**
Isoquercitrin	**38 ± 4**	**87 ± 4**
Quercitrin	**45 ± 8**	**109 ± 10**
Vincamine	**31 ± 2**	**N.D.**
Quercetin	**28 ± 2**	**20 ± 3**
Vinblastine	**101 ± 6**	**195 ± 9**

Table 4. Peak Purity Test Results of Catharanthus Alkaloids Using Photodiode-Array Detection

Peak purity				
No.	**Alkaloid**	**Up**	**Down**	**Similarity**
1	Vincristine	0.98	0.99	0.99
2	Vinblastine	0.99	0.99	0.99
3	Catharanthine	0.99	0.99	0.99
4	Vindoline	0.99	0.99	0.99

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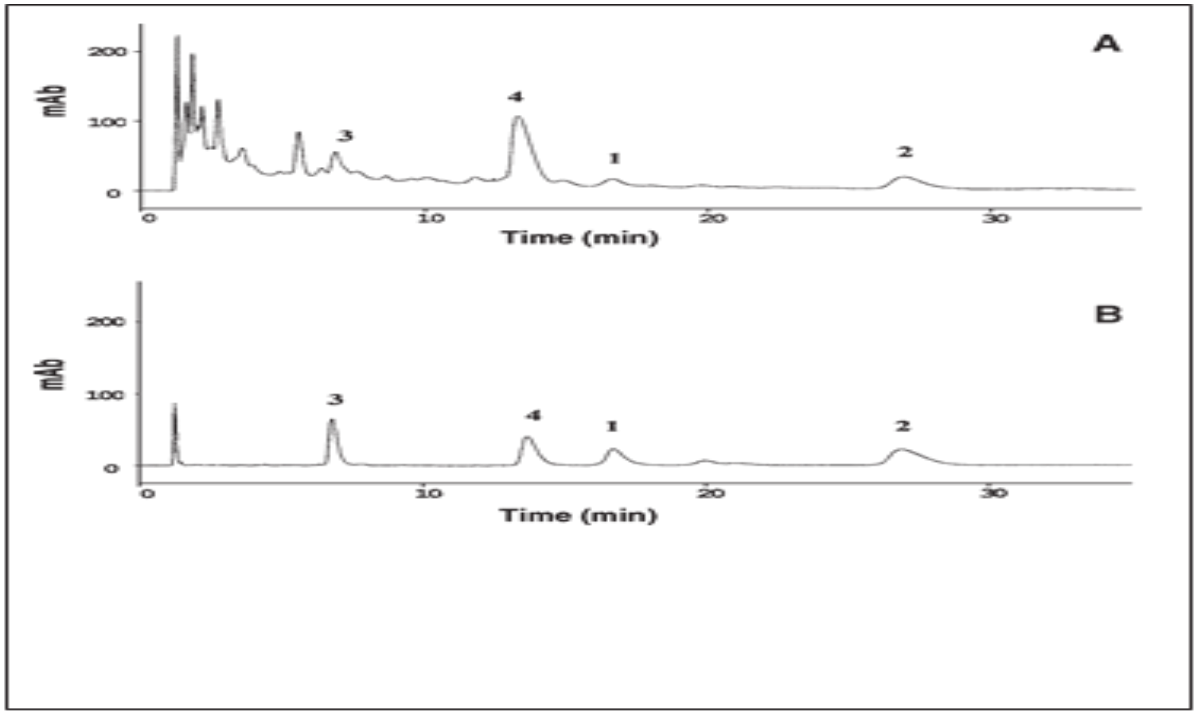

Graph. HPLC separation of a standard mixture of the four alkaloids (0.25 mg/mL) (A) and C. roseus extract (B). Condition: Chromolith per- formance RP-18e column; mobile phase, acetonitrile–0.1M phosphate buffer containing 0.5% glacial acetic acid (21:79, v/v); flow rate, 1.2 mL/min; and UV detection at 254 nm. Vincristine (1), vinblastine (2), catharanthine (3), and vindoline (4). (11)

II. MATERIAL AND METHOD: -

MATERIAL: -

Apparatus: - Beaker, Soxhlet apparatus, Test tubes, test tube holder, Glass rod, Funnel, flask, round bottom flask, Conical flask, Measuring cylinder etc.

Chemicals: - Cholesterol, Ethanol, Egg Yolk, Potassium iodide + Mercuric Chloride (Mayers reagents), Picric acid, Vanillin, Van Urks reagents etc.

Instruments': - Ultrasonicate, Weighing balance, Microscope, Heating mantle etc.

- **SELECTION OF METHOD: -**
- **SONICATION (Bath Sonication): -**

The cylinder containing the liposome dispersion is put into a bath sonicator. Monitoring the operating temperature of a lipid dispersion or spread typically simple in this technique, in contrast with Sonication by dispersion through the tip. The substance that is being sonicated may be shielded by an inert environment, a sterile vessel, or similar probe equipment. (7)

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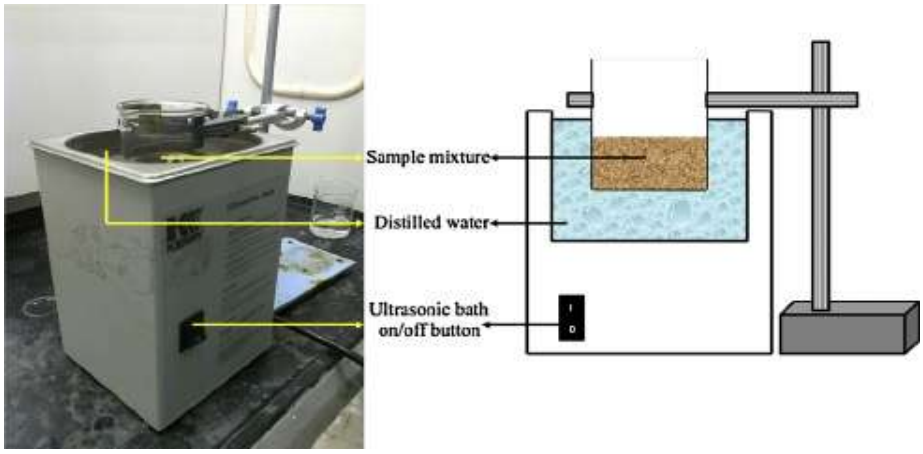

- **EXTRACTION: -**

1. Vinca plant was collected from garden.
2. Dried the leaves by sun drying method**.**
3. Prepared the powder of leaves by using grinder.
4. Weigh 30g of vinca powder and packed in filter paper.
5. Set up the Soxhlet apparatus and maintain temperature at 60°C.
6. Transfer/filled 271 ml of the ethanol in Soxhlet.
7. 5-15 Hrs. required for extraction.
8. Final extract was evaporated by rotary evaporator at 42- 47°C to obtain 3.07g alcohol-free residual extract of Catharanthus roseus.
9. Repeat the extraction process for the periwinkle flower extraction.
10. Prepare a two sample of extract and evaporate for the further formulation. (13)

- **FORMULATION OF LIPOSOME: -**

There is various method of formulation of Liposomes.

Ultrasonication method: Ultrasonication of an aqueous dispersion of phospholipids with a strong bath sonicator or probe sonicator will usually yield SUVs with diameters down to 15-25nm.

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Table 5: Formulation table: -

Sr no.	Chemical	Uses	F1 (pink flower)	F2 (White flower)	F3 (Mixed flower)
1	**Cholesterol**	Enhance the stability and fluidity of the liposomal membrane	10g	10g	10g
2	**Egg yolks**	Utilized in liposome formulation as a natural source of phospholipids.	10g	10g	10g
3	**Vinca Extract**	Active Pharmaceutical Ingredient	30ml	30ml	30ml (15ml pink+15ml White)
4	**Distilled Water**	Quantity makeup	50ml	50ml	50ml

Egg yolk, are commonly used in liposome formulation for several purposes:

1. **Structural Integrity:** Phospholipids serve as the primary building blocks of liposomal membranes, contributing to their bilayer structure. This structural integrity is crucial for encapsulating and protecting substances within the liposomes.
2. **Biocompatibility:** Phospholipids are biocompatible, making liposomes suitable for various biological applications, including drug delivery. The body recognizes phospholipids, reducing the likelihood of an immune response.
3. **Drug Delivery:** Liposomes can be used as carriers for drug delivery. By incorporating phospholipids into liposomes, it is possible to encapsulate both hydrophobic and hydrophilic drugs, improving their solubility and bioavailability.
4. **Cell Membrane Mimicry:** Phospholipids in liposomes mimic natural cell membranes, facilitating interactions with cells and tissues. This is especially advantageous in targeted drug delivery, where liposomes can fuse with specific cells, releasing their payload.
5. **Stability:** Phospholipids contribute to the stability of liposomes, helping them resist degradation and maintain their structure during storage or transportation.

- **PROCEDURE: -**

1. Weigh and measure the whole ingredient.
2. Dissolved cholesterol and egg yolk lecithin in distilled water.
3. Add vinca extract in above solution and stir it.
4. This solution placed in sonicator bath for dispersion of solution in Phospholipid. For 15-30 min.
5. Liposomes which is SUVs was formulated.
6. Filter the solution.
7. Dry the Liposomes. (18)

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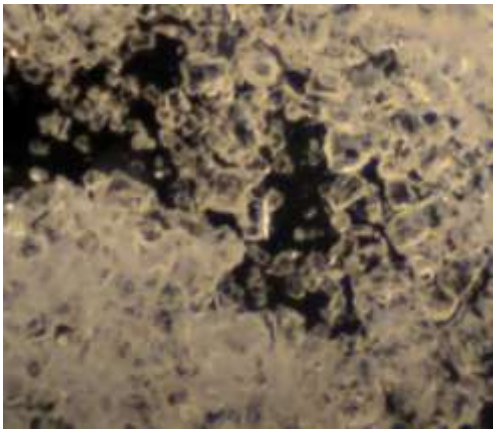
EVALUATION OF LIPOSOMES (IN VITRO STUDY): -

1. Particle Size: - **Both particle size and particle shape of distribution of liposomes influence their physical stability. These can be determined by the following method:**

a. **Laser light scattering**
b. **Transmission electron microscopy**

2. Particles Shape: - **Particles Shape was evaluated by using optical microscopy of 150 micron.**
3. Color: - **Color was evaluated by visual Inspection.**
4. PH: - **The pH of human blood is typically around 7.35 to 7.45.**



III. RESULT: -

Identification test of Vinca Alkaloids: -

Vinca alkaloid was extracted from powder of vinca flowers by using **Soxhlet apparatus** with ethanol solvent.

Identification test for vinca alkaloid in Vinca extract was performed which are Mayer reagent test, Hager's Test, Wagner's Test, Dragendroffs Test and Tannic Acid Test.

Sr. No.	Identification test	Procedure	Observation	Inference
1.	**Mayer reagent test**	3ml extract + few drops of Mayer's reagent	**Yellowish or white ppt.**	Vinca alkaloids present
2.	**Hager's Test**	3ml extract + few drops of Hager's reagent	**Yellow colored ppt**	Vinca alkaloids present
3.	**Wagner's Test**	3ml extract + few drops of Wagner's reagent	**Redish - Brown ppt.**	Vinca alkaloids present
4.	**Dragendroffs Test**	3ml extract + few drops of Dragendroffs reagent	**Orange colored ppt**	Vinca alkaloids present
5.	**Tannic Acid Test**	3ml extract + few drops of Tannic Acid reagent	**Turbid ppt**	Vinca alkaloids present

Table 6: -Identification Test

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Liposomes was prepared by Ultrasonication method. Liposomes of vinca extract was prepared by Ultrasonication method. Liposomes was prepared by using cholesterol and egg yolk as a phospholipid**.**

Table 7. Evaluation Parameter: -

PARAMETERS	**RESULT**
Particle size	**45nm**
Shape of Liposomes	**Spherical**
Visual Inspection	**Whitish colored particles**
PH	**7.40**

IV. DISCUSSION: -

Vinca alkaloid was extracted from vinca plant by using Soxhlet apparatus. Vinca extract shows identification test for vinca alkaloid was positive. vinca alkaloid such as vincristine, vinblastine and vinorelbine are widely used cytotoxic drugs that elicit their effect through disruption of microtubules, resulting in metaphase arrest. Liposomes are novel drug delivery system. Ultrasonication methods gives good result in formulation of liposomes. Phospholipid used for formulation of liposomes. Shape of liposomes is spherical and have 45nm size of liposome. PH of liposomes is 7.40.

V. SUMMARY: -

Cancer is an unregulated abnormal cell growth and spread of cells from sites of origin to other site (clumping of cells/ tumor). The unregulated growth of cells in group called neoplasm or tumor and they form a lump or mass and may be distributed diffusely. There are various types of cancer such as breast cancer, lung cancer, oral cancer, leukemia etc. Vinca bas anticancer property. Vincristine and vinblastine are vinca alkaloid which shows anticancer effect

Vinca alkaloid was extracted from vinca plant by using Soxhlet apparatus. Identification test for vinca alkaloid in Vinca extract was performed which are Mayer reagent test Hager's Test, Wagner's Test, Dragendroffs Test and Tannic Acid Test, Vinca extract shows positive test which means presence of vinca alkaloid in extract.

Liposomes are colloid, vesicular structure composed of one or more lipid bilayer surrounding an equal number of aqueous compartments. Liposomes are biocompatible and can both entrap and protect water-soluble molecule in their internal water compartment and water-insoluble into the membrane. Various amphipathic molecules have been used to form liposomes. The drug molecule can either encapsulated in aqueous space or intercalated into lipid bilayer. Liposomes are prepared by various methods.

For formulation of liposomes, sonication method was referred. Cholesterol and egg yolk is used as a phospholipid for preparation of liposomes. Extract, phospholipid was dispersed in distilled water. Formulated liposomes shows spherical shape and has 45nm in size. PH of liposomes is 7.40.

VI. CONCLUSION: -

There is different type of cancer in different countries. Vinca has anticancer property. Vinca alkaloid like vincristine and vinblastine shows anticancer effect. It inhibits microtubule formulation formation in mitotic spindle, resulting in an arrest of dividing cells at metaphase stage. Vinca alkaloid extracted by using Soxhlet apparatus. Identification test for vinca alkaloid was performed. Based on of plant bio factory for the production of highly potent anticancer compound in pharmaceutical industries. Liposomes was

prepared by using sonication method. This method was gives good result**.**

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