IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

FORMULATION OF MICROEMULSION BASED TRANSDERMAL PATCHES AND EVALUATION OF ANTICANCER AND ANTIOXIDANT ACTIVITY

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Abstract:

Uncontrolled cell proliferation can cause cancer, which is characterized by the growth of an unusually large tumour that starts off as a localized illness but has the potential to spread and affect other organs or vital functions.

Cancer is one of the worst diseases of our day, taking countless lives annually. The differences in the disease around the world, the influence of the medical facilities that are available, and other socioeconomic factors have all had an impact on the efficient care of this ailment.

Therapeutic applications are being found for numerous groups of phytochemicals found in herbal medicine. Herbal medicine treatment has been found to be especially beneficial for cancer patients, with many reporting a large increase in survivorship. The anti-oxidative and superoxide scavenging properties of particular active ingredients in herbal medicine have been demonstrated in recent studies, along with their inhibitory effects on lipid peroxidation and anti-cancer qualities. Certain herbal remedies have analgesic, antipyretic, anti-inflammatory, and anti-cancer properties. Apart from its numerous medicinal properties, herbal medicine finds application as a nutrient supplement with anti-inflammatory and anti-cancer properties. Herbal medicine has been the subject of several in vivo and in vitro investigations using various cell lines. The mechanisms of actions are still unknown, though.

Herbal anticancer medications have drawn a lot of attention lately because of their potential as complementary or alternative therapy to traditional cancer treatments. These medications, which are made from a variety of plants and other natural sources, show encouraging anticancer effects by inducing apoptosis, inhibiting angiogenesis, and having anti-proliferative effects. This abstract examines the state of the field, emphasizing the effectiveness, safety, and difficulties related to the creation and application of herbal anticancer medications in cancer treatment.

Keywords:

Angiogenesis, apoptosis, malignancy, MTT assay, DPPH scavenging assay, reducing power activity, zebra fish tumour induced model, Brine Shrimp Lethality Assay, metastasis, microemulsion, transdermal patches.

INTRODUCTION:

A malignant, unwanted, uncontrolled and damaging growth of cells that differ structurally & functionally from normal cells is "Cancer." Cancer is a term used for disease in which abnormal cells divide without control and are able to invade other tissues and can spread to other parts of body through the blood and lymph system. Cancer spread into surrounding, tissue can cause changes to DNA and it called genetic changes, it can cause by gene mutation.

Cancer cells become bizzare in size, shape and other features, it more bizzare they become, the more aggressive and malignant is their behaviour. Cancer is responsible for about 26-30-% of deaths.

Approximately 9.6 million deaths, or 1 in 6 deaths, were attributed to cancer in 2018, making it the second most common cause of death worldwide.

Denmark has the highest cancer rate in the world, affecting approximately 335 out of every 100,000 individuals, according to the World of Statistics. Ireland and Belgium are the next most affected countries.

China has the greatest rate of cancer mortality despite the United States having the highest cancer rate. Lung, liver, stomach, breast, and colon cancers are the top five causes of cancer-related fatalities in China. In 2020, there were 18,094,716 million cancer cases diagnosed worldwide. In 2020, the combined age-standardized rate for men and women for all malignancies (except from non-melanoma skin cancer) was 190 per 100,000. Men had a greater rate than women (178.1 per 100,000) at 206.9 per 100,000.

TYPES OF TUMOURS:

1) Non-malignant or benign tumours

2) Malignant tumours

Benign tumours-

- It stay together as a lump or swelling.
- Do not spread to other parts of body-removed easily by surgery.
- this type of tumour are not cancerous.

Malignant tumours-

- Cells in these tumours can invade nearby tissues and spread to other parts of body.
- The spread of cancer from one part of body to another is called metastasis.

Cancer cells differ from normal cells by:

- Uncontrolled proliferation
- De-differentiation and loss of function
- Invasiveness
- Metastasis

Substance that can cause cancer is called "**carcinogen**".

Examples of naturally occurring carcinogens are some viruses and UV light from the sun. Human generated carcinogens include cigarette smoke and exhaust fumes from moving vehicles. The majority of carcinogens cause mutations in cells by interacting with their DNA.

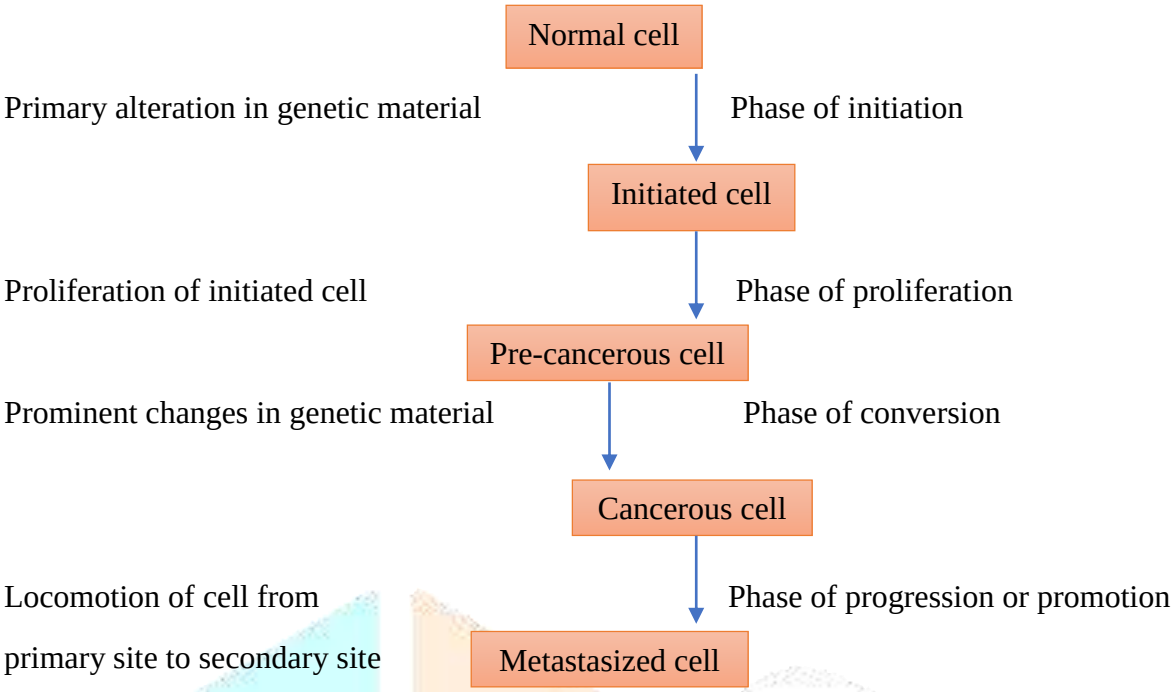
Three major categories are used by researchers to classify carcinogens. Among them are:

- **Chemical carcinogens:** These are carcinogens that people release into the environment through pollution, such as through Trusted Source car exhaust fumes, industrial by-products, and cigarette smoke.
- **Physical or environmental carcinogens:** The environment contains these carcinogens. Physical carcinogens include sunlight's UV rays and radiation from X-rays and other radioactive elements.
- **Oncogenic viruses** are viruses that can cause cancer. Examples Trusted Source include human papillomavirus (HPV), Epstein-Barr, and hepatitis B

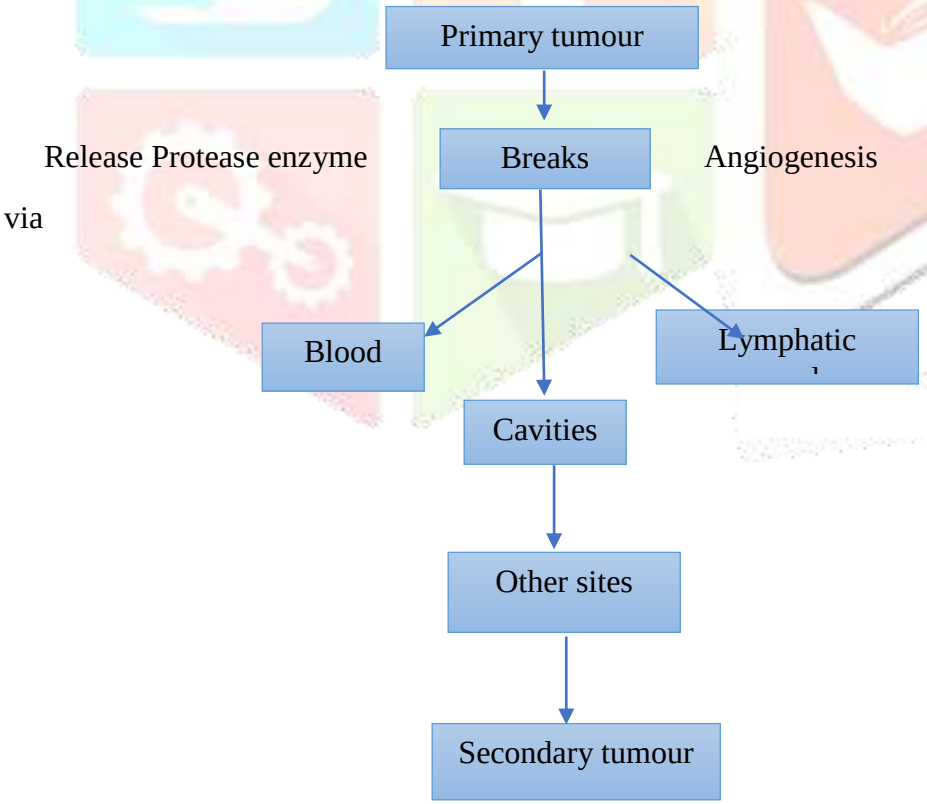
Carcinogenic Substances:

- Arsenic
- Asbestos
- Benzene
- Benzidine
- Beryllium
- Coal Tar and Coal-Tar Pitch
- Coke-Oven Emissions
- Crystalline Silica (respirable size)
- Erionite
- Ethylene Oxide
- Formaldehyde
- Hexavalent Chromium Compounds
- Nickel Compounds
- Radon
- Tobacco Smoke
- Soot
- Thorium
- Trichloroethylene
- Vinyl Chloride
- Mineral Oils: Untreated and Mildly Treated
- Strong Inorganic Acid Mists Containing Sulfuric Acid
- 1,3-Butadiene
- Cadmium

Mechanism of carcinogenesis:



Metastasize:



Steps of metastasis:

Tumour formed

↓

Separation from primary tumour

↓

Invasion through tissue around the initial lesion and penetration of their basement membrane.

↙ ↓ ↘

Blood Cavity Lymph

↓

Reaching distal organ

↓

Common site of metastasis

↙ ↙ ↓ ↓ ↘ ↘

Breast Lungs Prostate Colon Testicular Ovarian

↓

Formation of new lesion along with new blood vessels which feed the tumour – **ANGIOGENESIS**

Type of tumour:

1) Carcinoma:

Cancer that begins in the skin or in tissues that line or cover internal organs subtypes of carcinoma

a) Adenocarcinoma
b) Basal cell carcinoma
c) Squamous cell carcinoma
d) Transitional cell carcinoma

2) Sarcoma:

Cancer that starts in blood vessels, muscle, connective tissue, bone, cartilage, or another type of supporting or connective tissue.

3) Leukemia:

Large quantities of aberrant blood cells are created and released into the bloodstream when cancer begins in bone marrow or other blood-forming tissue.

4) Lymphoma and Myeloma:

Cancer originating from immune system cells

5) Central nervous system cancers:

Cancer that begins in the tissue of the brain and spinal cord

Introduction:

Chemotherapy –

Medication used to kill cancer cells; it does this by preventing the cancer cells from proliferating, dividing, and creating new cells.

Chemotherapy is a prescription medication used to kill your body's quickly multiplying cells with strong chemicals.

Chemotherapy is the preferred cancer treatment because cancer cells multiply and spread far more quickly than the majority of normal cells in the body.

There are many different chemotherapy drugs on the market. Chemotherapy drugs can be used alone or in combination to treat a wide range of cancer types.
Chemotherapy medications can have serious side effects. Not all drugs cause all negative effects, and each medicine has unique side effects

Chemotherapy medication side effects frequently include:

- Nausea
- Vomiting
- Diarrhoea
- Hair loss
- Loss of appetite
- Fatigue
- Fever
- Mouth sores
- Pain
- Constipation
- Easy bruising
- Bleeding

Chemotherapy treat cancer
Chemotherapy is systemic medication. Which means it travels through the bloodstream and reaches all parts of the body.

There are many different types of chemotherapy. Chemotherapy drugs are potent substances that target cancer cells at particular stages of the cell cycle. Every cell goes through the cell cycle, which produces new cells. Chemotherapy has a greater impact on cancer cells because they undergo this process more quickly than healthy cells and because their proliferation is unchecked.

Chemotherapy can harm healthy cells during their regular cell cycle since it affects every cell in the body. For this reason, among many other side effects, chemotherapy may induce hair loss and nausea.

Goals of chemotherapy

Chemotherapy objectives vary depending on the type and extent of your cancer. Chemotherapy can be administered as a stand-alone treatment or as a component of a multimodal treatment regimen. Here are a few examples of how chemotherapy is applied:

as the main course of action. Chemotherapy treatments can sometimes be used to eradicate cancer completely and prevent recurrence. We could refer to this as "curative chemotherapy." prior to other therapies. Chemotherapy might be used before radiation treatment or surgery to shrink the size of the tumor. This is known as "neoadjuvant chemotherapy." following additional therapies. Chemotherapy can be used to eradicate any cancer cells that remained following radiation therapy or surgery. We refer to this as "adjuvant chemotherapy."

To lessen cancer's symptoms and delay its spread. Chemotherapy can partially shrink tumors and stop tumor development and spread for varying periods of time, even in cases when the cancer is incurable. Chemotherapy can increase survival in some situations, reduce cancer-related symptoms, and enhance quality of life. These kinds of chemotherapy are commonly referred to as "palliative chemotherapy."

Chemotherapy is a treatment option for numerous cancer types. Additionally, metastatic and recurring cancers can be treated with it. Cancer that returns after therapy is known as recurrent cancer. Cancer that has spread to other body parts is known as metastatic cancer.

Factors that determine a chemotherapy plan

The drugs, dose, and treatment schedule depend on many factors, these include:

- The type of cancer
- The stage of the cancer. Cancer stage is determined by the size and location of the tumour and whether or not the cancer has spread. tumour size, its location, and if or where it has spread.
- Your age and general health
- Your body weights
- The possible side effects of each drug.
- Any other medical conditions you have
- Previous cancer treatments

Aim and objective:

The most common anticancer drug is alkylating agent.

We know the most of synthetic anticancer drug such as alkylating drug (mechlorethamine, chlorambucil, cyclophosphamide, melphalan, busulfan, platinum-based drugs like cisplatin) or etoposide can lead to the secondary cancer and can affect another organ.

Chemotherapy treatment have various side effects such as neutropenia, lymphedema, hair loss, nausea and vomiting.

Cost of anti-cancer drug is high and it requires a long term treatment to recover the tumour.

Aim:

The study of anticancer activity of herbal drug in microemulsion and performed its in-vitro and in-vivo screening test, as drug in microemulsion can be easy to transport and store, thermodynamically stable therefore it had increased shelf life. In-vivo Zebrafish is used because of its small size, chemical permeability, ease of observation and physiologic similarities to human.

Zebrafish used while cancer study because of its transparent body, rapid development, high genetic homology, and ease of genetic manipulation.

Objective:

- To formulate herbal drug having strong anticancer activity.
- Drug which can heal the tumour/ help to reduce to tumour, or stop the undamaging control and inhibit the tumour at initial stage.
- Drugs which have minimum side effects and which will not lead to secondary cancer after cure of disease or tumour.
- To give alternate drug for synthetic anticancer therapy which gives severe side effects.

DRUG PROFILE:

1)GREEN TEA

Synonym: Chinese Tea, Camellia sinensis, Japanese Tea

Biological source: It consist of fresh and dried leaves of camellia sinensis.

Family: Theaceae

Chemical constituents: Catechin, caffeine, epigallocatechin, theaflavin, gallic acid, aminobutyric acid

Uses: Antioxidant, reducing high blood pressure and cholesterol

2)TULSI

Synonym: Sacred basil, kali-tulsi, veranda

Biological source: Tulsi consists of the fresh and dried leaves of ocimum species like *ocimum sanctum* Linn.

Family: Labiatae

Chemical constituents: Linalol, eugenol, linolen,thymol, cineol

Uses: Immunomodulatory agent, spasmolytic, antiaging, antioxidant

3)TURMERIC

Synonym: Saffron Indian, haldi, Curcuma longa, curcumin, curcuma domestica.

Biological source: Turmeric is extracted from the dried rhizomes of *curcuma longa*

Family: Zingiberaceae

Chemical constituents: Sesquiterpenes like zingiberene, alpha- phellandrene, turmerone

Uses: Antioxidant, anti-inflammatory, blood purifier, anti-periodic, stimulant**.**

4) GINGER

Synonym: Rhizome zingiberis, zingibere

Biological source: Ginger is extracted from the dried rhizomes of the *zingiber officinale* Roscoe

Family: Zingiberaceae

Chemical constituents: Gingerol, zingiberene, shogaol, borneol, terpineol, citral

Uses: Antiemetic, antioxidant, spamolytic, stimulant, carminative

5) GARLIC

Synonym: Allium, lasan

Biological source: Garlic is the ripe bulb of allium sativum Linn

Family: Liliaceae

Chemical constituents: Allicin, diallylsulfide, sulfoxide, alliinase, allyl propyl trisulfide

Uses: Carminative, expectorant, stimulant, antitumor, antidiabetics

MATERIAL AND EQUIPMENT:

Material used:

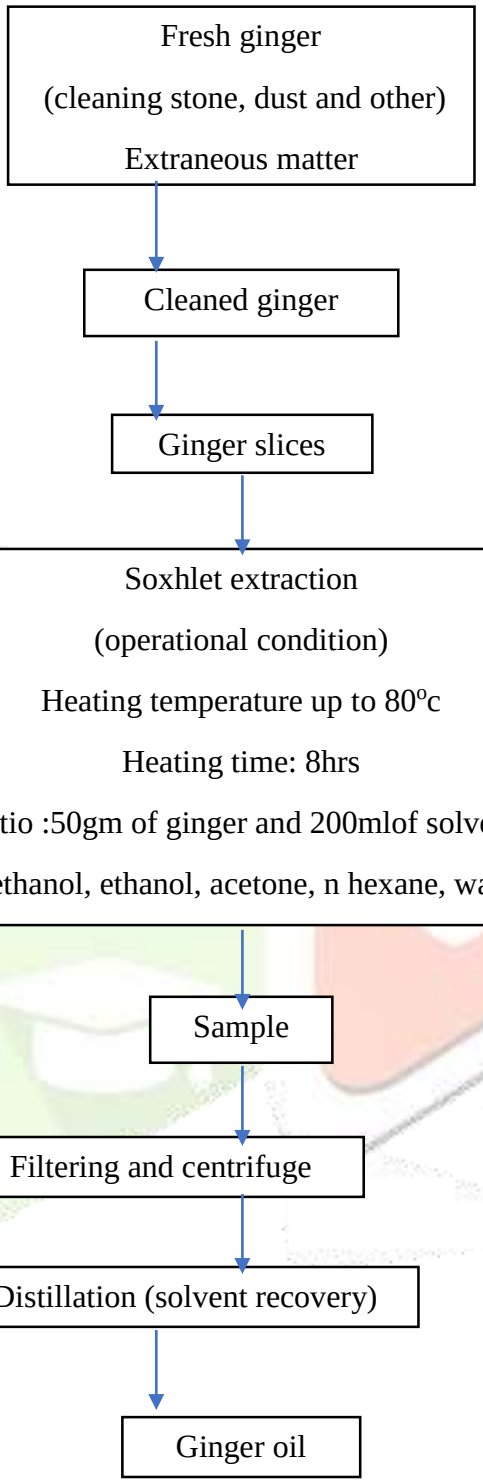
Sr. No	Material	Source
1	Green tea	Local market , Shirur
2	Turmeric	Local market , Shirur
3	Tulsi extract	BRM Herbals
4	Garlic	Local Market, Shirur
5	Ginger	BRM Herbals
6	Tween 80	STCOP,Shirur
7	Propylene glycol	STCOP,Shirur

Equipment:

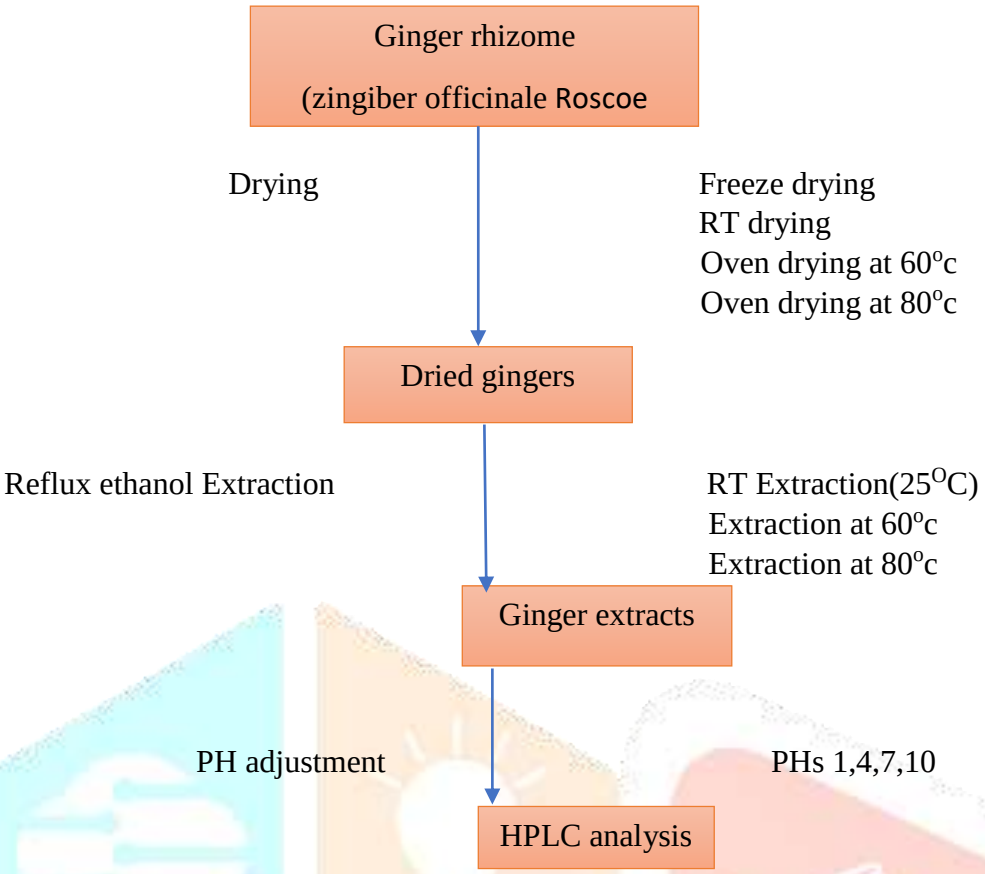
Sr. No	Equipment	Source
1	Magnetic stirrer	STCOP/ B. PH/-155/03/2022-23 GI -1109
2	Beaker	STCOP,Shirur
3	Measuring cylinder	STCOP,Shirur
4	Mortar pestle	STCOP,Shirur
5	Syringe	Medical Store,Shirur
6	Petri plate	STCOP,Shirur
7	Centrifuge	STCOP/ B. PH/1/114/1/2022-23
8	Band-Aids	Medical Store, Shirur
9	Aerator	STCOP, Shirur
10	Microscope	STCOP, Shirur
11	Electron Microscope	STCOP/ B. PH/D-3-18/01/22-23 GI-1109
12	Hot Air Oven	STCOP/ B. PH/1-156/1/2022-23
13	Digital PH Meter	STCOP/ B. PH/1-104/03/2022-23
14	Colorimeter	STCOP/ B. PH/1-16/01/22-23 GI-1109
15	Electronic Balance	STCOP/ B. PH/1-157/1/2022-23
16	UV Spectrophotometer	Jasco V 730

Methodology:

Extraction method of Ginger:



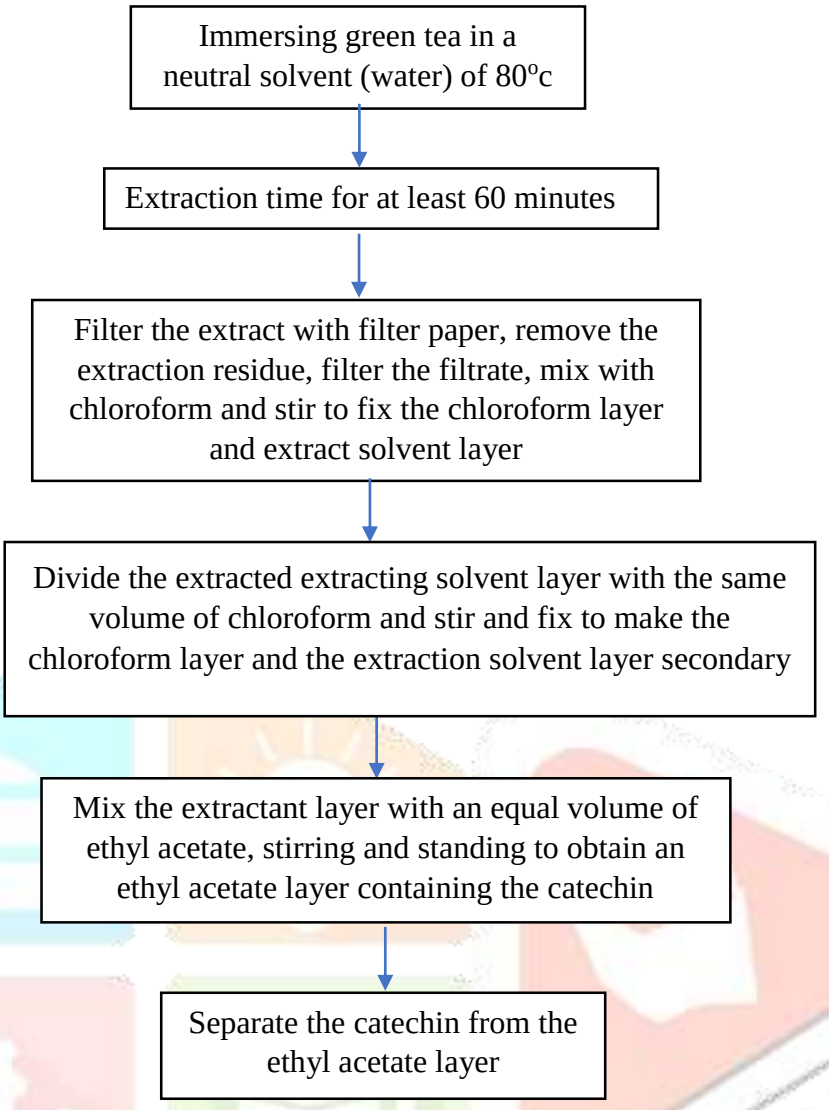
Isolation of shogaols from Ginger:



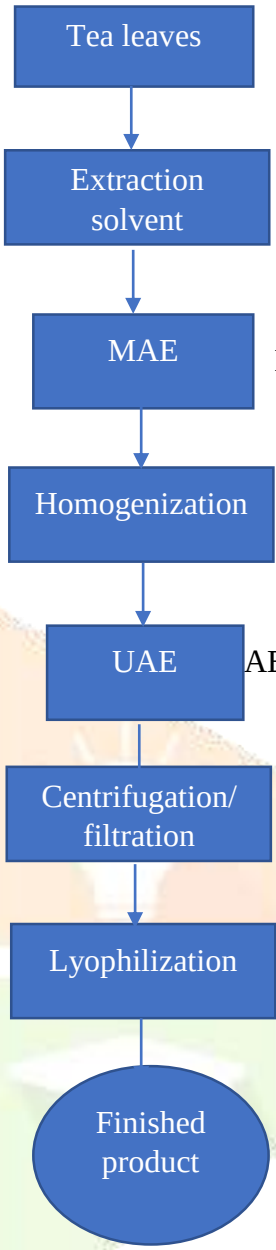
Identification test:

Sr.no	Active compound	Reagent type	Result of aqueous extract of zingiber officinale
1	Alkaloids	Mayer's reagent	+
2	Flavonoids	Sulphuric acid	+
3	Glycosides	Mulch reagent	+
4	Phenolic content	Chloride reagent	+
5	Tannins	Lead acetate reagent	+
6	Resins	Alcohol ethyl	-

Extraction of Green tea:



Isolation of Catechin:



MAE- Microwave assistant extraction

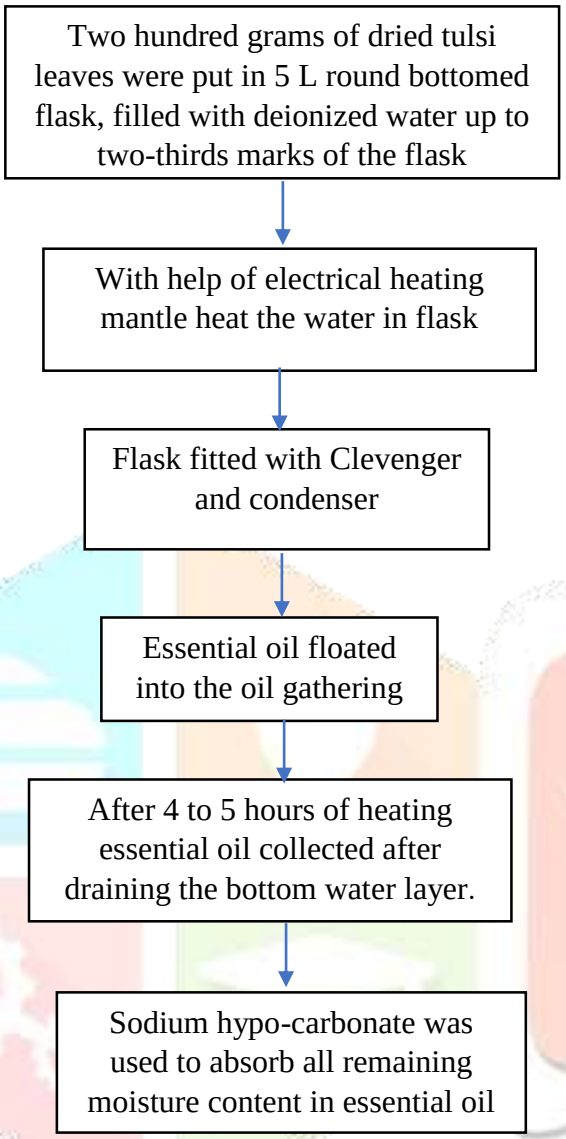
AE- Ultrasonic assistant extraction

Identification test for Green tea:

Sr.no	Phytochemical constituent	Intensity
1	Saponins	+
2	Flavonoids	+
3	Terpenoids	+
4	Glycosides	+
5	Alkaloids	+
6	Phenol	+
7	Tannins	+
8	Steroids	-

Extraction of Tulsi essential oil:

Essential oils were extracted by hydro-distillation using Clevenger's type apparatus



Isolation of Eugenol from Tulsi:

Solvent extraction

One of the most popular and widely used techniques for removing essential oils from plants is solvent extraction.

As a result, several solvents, including methanol, ethanol, petroleum ether, and N-hexane, have also been used to extract eugenol.

Unwanted flavour alterations in the meal and the addition of additional soluble residues are the main obstacles to solvent extraction. Nonetheless, there are still many uses for this technique in extracting essential oils like eugenol from other fragrant herbs.

The normal procedure for extracting eugenol from tulsi using a solvent involves grinding the clove buds, wrapping them in filter paper, running the filter paper through an extraction thimble, and then placing the filter paper into a 500 mL reflux flask.

The extraction process is then completed in a Soxhlet apparatus with an appropriate organic solvent. Using a rotary vacuum evaporator, the extracted materials are concentrated at 50 °C to complete the process. The typical solvent extraction process has undergone a number of changes that demonstrate increased efficiency over the original approach. For example, the batch extraction procedure is a desirable substitute for the Soxhlet extraction method. This technique uses a reactor that has a motor running at 1200 rpm and

an agitator with four blades. Garkal et al conducted a study on this technique recently, extracting eugenol from tulsi plant leaves using methanol as a solvent and reporting an acceptable extraction efficiency. They also said that the speed of agitation had no bearing on the effectiveness of the eugenol extraction process.

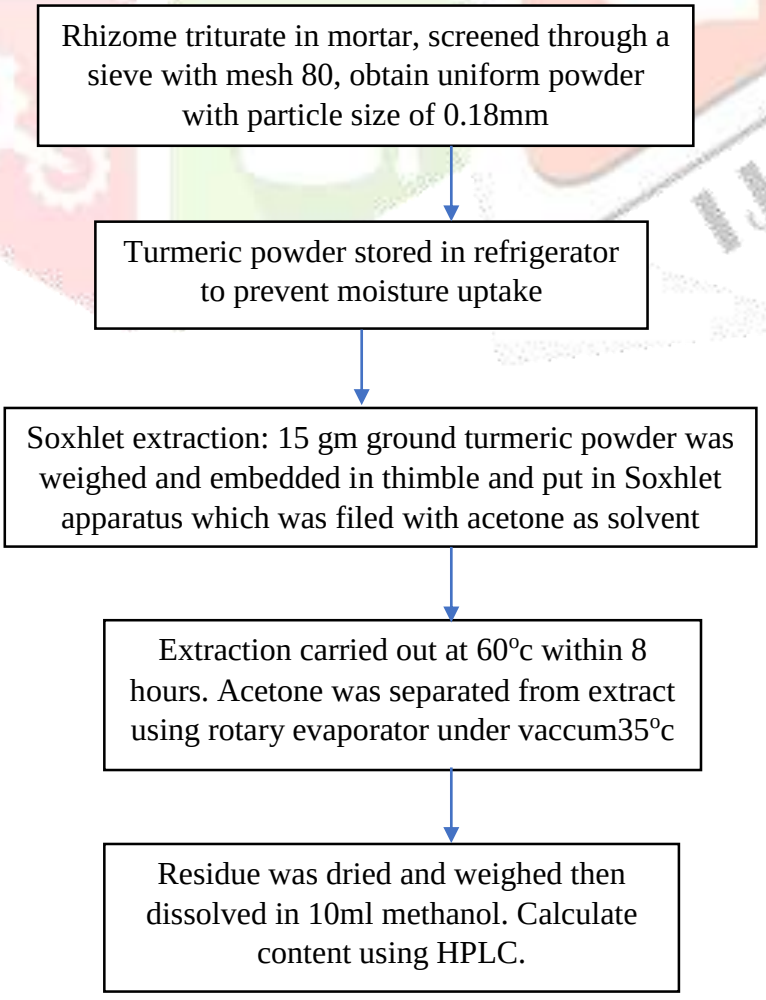
Identification test for tulsi:

Sr.no	Phytochemical constituent	Intensity
1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Saponin	-
5	Carbohydrates	-
6	Steroids	+
7	Terpenoids	+
8	Proteins	-

Fresh Tulsi extract's FTIR spectra revealed peaks at around 1636, 2132, and 3336 cm −1, which correlate to the groups C=C (approximately 1635 cm −1), C≡C (approximately 2100 cm −1), and amine N-H/O-H vibration stretch (approximately 3300 cm −1). Neat quercetin, on the other hand, revealed peaks at approximately 1639, 2105, and 3264 cm −1.

Extraction of Turmeric:

Conventional extraction using Soxhlet:



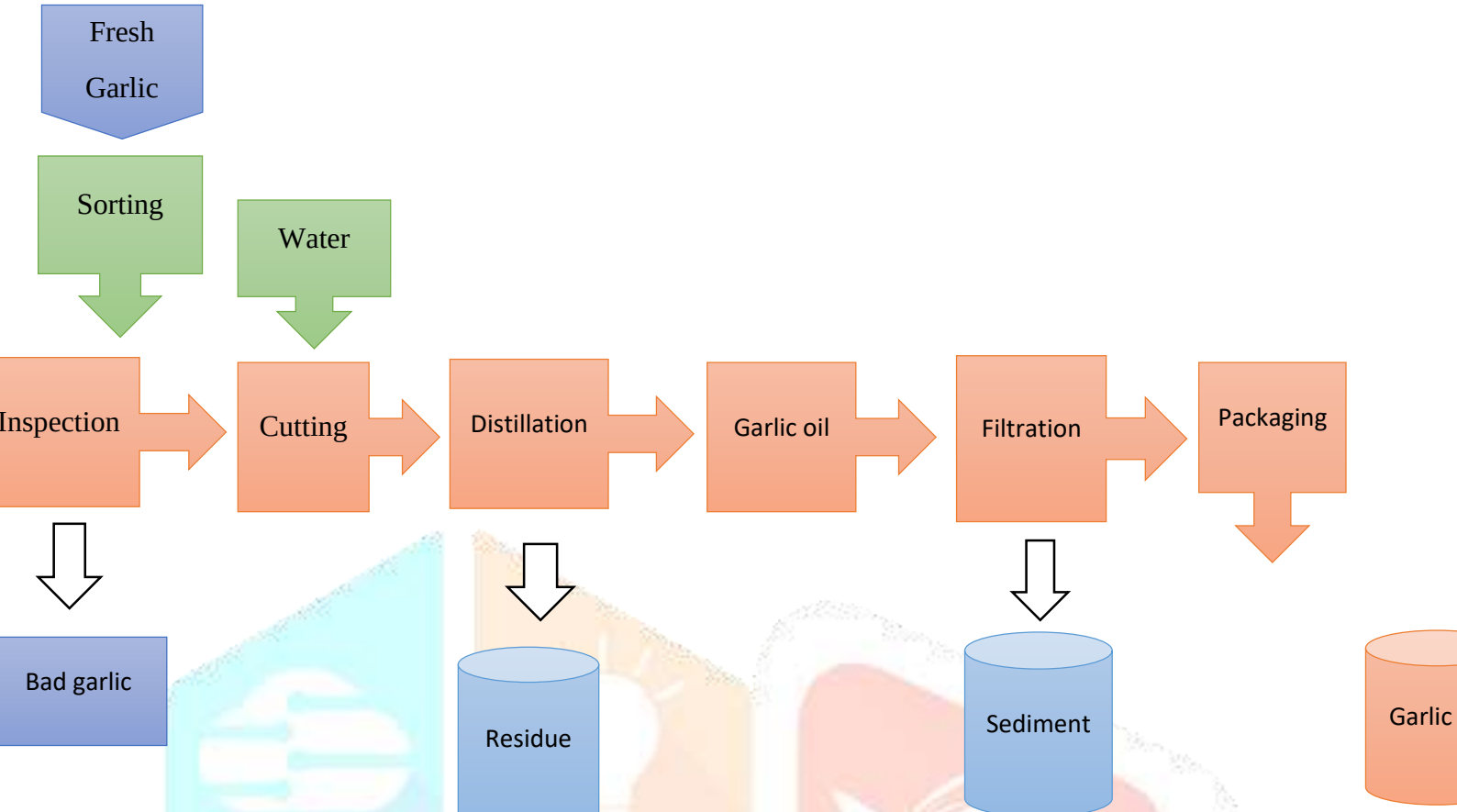
Isolation of Curcumin from turmeric:

The process of extracting a target component into a polar or nonpolar extraction solvent can be done continuously or in batches. This procedure contains the following steps: in the first stage, the solvent (usually ethanol, methanol, isopropanol, or acetone) penetrates the crushed plant solid sample; in the second stage, the solute of interest dissolves in the solvent; and in the third stage, it is released back out into the solid matrix. Gathering the solute is the final stage. Up until equilibrium is established, the phenomena of solute movement and solid matrix penetration happen concurrently. At the conclusion of the procedure, the solvent is eliminated by evaporation, yielding a concentrated product of superior quality.

Identification test for Turmeric:

Sr.no	Secondary metabolites	Intensity
1	**Saponin**	-
2	**Alkaloids**	+
3	**Phenolic content**	-
4	**Tannin**	+
5	**Steroid**	-
6	**Flavonoids**	+
7	**Glycosides**	+
8	**Carbohydrates**	+
9	**Terpenoids**	+
10	**Amino acid**	+

Extraction of Garlic:



Identification test for garlic:

Sr.no	Phytochemical	Intensity
1	Alkaloids	+
2	Flavonoid	+
3	Glycosides	+
4	Reducing sugar	-
5	Saponin	+
6	Steroids	+
7	Phenols	+
8	Terpenoids	+
9	Anthraquinones	+
10	Tannin	+

Microemulsion:

A dispersion of one liquid's droplets in another immiscible liquid is called an emulsion. The second liquid is referred to as the continuous phase, and the droplets as the dispersed phase. A surfactant or co-surfactant is added to an emulsion to stabilize it so that the droplets stay dispersed and don't split into two phases.
There are two forms of microemulsions: water-in-oil (w/o) and oil-in-water (o/w), depending on the phase.

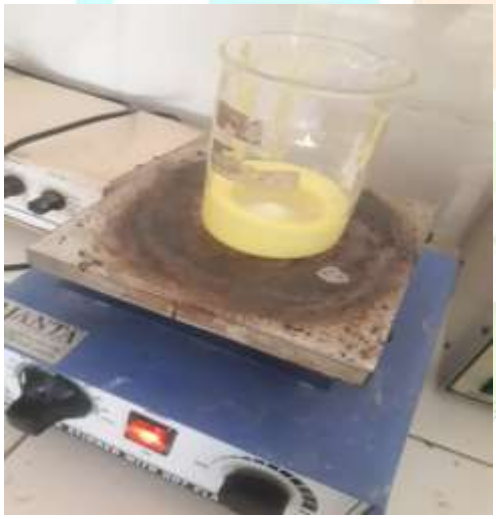
Water is the scattered phase in w/o emulsions, as the name suggests, whereas oil is the dispersed phase in o/w emulsions.

The size of the droplets in the dispersed phase of microemulsions ranges from 5 to 100 nm, whereas macroemulsions have droplets larger than 100 nm. This is one of the primary distinctions between macroemulsions and microemulsions.

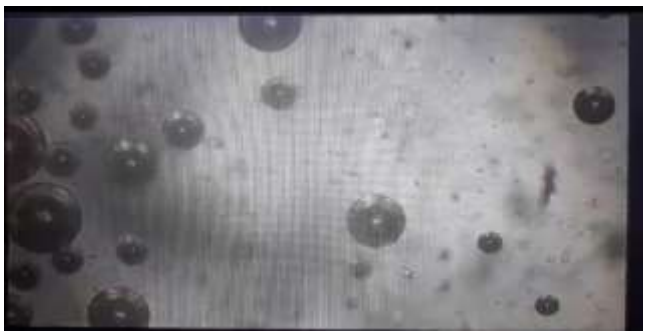
Procedure:

1) Oil and a mixture of surfactant and cosurfactant were combined to create microemulsion systems
2) Water was then carefully added to the oily phases while the mixture was being stirred magnetically at a speed of 300 rpm per minute at 37°C.
3) After 30 minutes of gentle magnetic stirring to bring the systems to equilibrium, the proper amount of PG was dissolved.
4) The same PG content was dissolved in purified water to create the aqueous solution.

Drug	F1	F2	F3	F4	F5
Ginger	3	3.5	4	4.5	5
Garlic	2	2	1.5	1.5	1.5
Turmeric	1.5	1.5	1.5	1.5	1.5
Green tea	1.5	1.5	1.5	1.5	1.5
Tulsi	q.s	q.s	q.s	q.s	q.s
	10	10	10	10	10



Evaluation of Microemulsion:



1) Visual inspection:

1) Phase Separation: Look for any signs of phase separation, such as the formation of distinct layers or regions within the microemulsion.

2) Clarity: Assess the clarity of the microemulsion. Any cloudiness or haze could indicate microbial contamination.

3) Colour Change: Check for any unexpected changes in colour, which could be indicative of microbial growth or chemical reactions.

4) Odour: Sniff for any unusual odours, which might suggest microbial contamination or degradation.

5) Visual Inspection under Microscope: If necessary, examine a sample of the micro emulsion under a microscope to detect any microbial growth or particulate matter.

6) Microbial Testing: Perform microbial testing, such as microbial enumeration or identification tests, to confirm the presence of any contaminants.

2) Stability studies:

A microemulsion's physical, chemical, and microbiological stability are usually evaluated during a stability study. Particle size, appearance, pH, viscosity, and droplet size distribution are some of the criteria that need to be examined. In order to replicate real-world settings and assess the microemulsion's stability under diverse scenarios, it also entails exposing it to a variety of stress conditions, including temperature fluctuations, freeze-thaw cycles, and centrifugation

3) Dye solubility test:

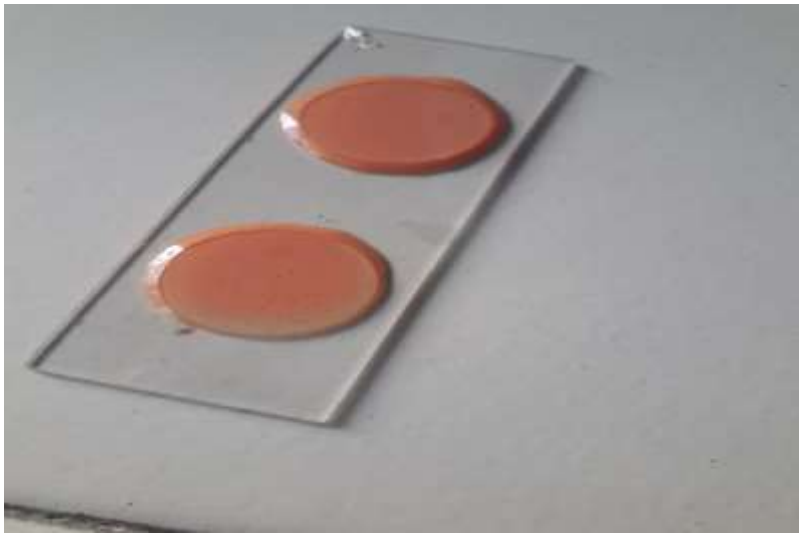
This test involves observing an emulsion under a microscope after mixing it with a water-soluble dye, such amaranth.
The emulsion is of the o/w type if the continuous phase is red in color. It is w/o type if the dispersed globules seem red and continuous phase colorless.

Methods to identify type of emulsion:

1)Dye test:

This test involves observing an emulsion under a microscope after mixing it with a water-soluble dye, such amaranth.

The emulsion is of the o/w type if the continuous phase is red in color. It is w/o type if the dispersed globules seem red and continuous phase colorless.



2)Fluorescence Test

An emulsion is classified as w/o type if, when exposed to UV radiation, it exhibits continuous florescence under a microscope.

If it shows only spotty fluorescence, then it is Oil in o/w type.

3) Dilution Test:

Water or oil can be added to the emulsion to perform this test. If the emulsion is combined with water and added, it is an oil-in-water (o/w) emulsion. The term "water-in-oil" (w/o) refers to an emulsion in which water does not mix with the mixture.

4)Conductivity test:

To determine the conductivity of the emulsion, a certain amount of electrolyte is supplied in this test. An increase in conductance indicates that the emulsion is an oil-in-water kind. Conversely, the emulsion is of the water-in-oil kind if the conductance remains unchanged.

Method of Making Transdermal Patches:

Sr.no	Substance name	F1	F2	F3
1	Starch(%w/v)	3	3.5	5
2	Gelatin (%w/v)	2.5	2.5	2.5
3	Propyl paraben (%w/v)	1	1	1
4	Green tea	0.5	0.5	0.5
5	Ginger	0.5	0.5	0.5
6	Garlic	0.5	0.5	0.5
7	Turmeric	0.5	0.5	0.5
8	Tulsi	0.5	0.5	0.5
9	Aqua	50	50	50

Weigh each ingredient and have ready the equipment and supplies needed. Subsequently, the potato starch is distributed using aqua distillate and mixed using a stirring rod to create a suspension. After dissolving propyl paraben in gelatin, it was combined with the starch suspension and the remaining water was blended until it was evenly distributed. Additionally, the mass that has been created is heated for about 50 minutes at a temperature between 55 and 65 degrees Celsius using a magnetic stirrer on a hot plate. After that, it is

poured onto a slab and let to sit at room temperature for three days. The film was taken out after three days and prepared for characterisation.
Characterization Edible Film

1. Organoleptic

Examination Organoleptic examination includes observing the shape, colour, and smell of the film produced

2. Thickness Film

Edible film thickness was measured using a micrometre screw using 001mm precision tool.

Measurements were carried out at 5 different places with three repetitions

3. pH test

pH measurement was carried out by of film dissolving in distilled water up to 10 ml. in a container. The electrode in dipped in. The electrode is dipped se container containing a solution of edible film, see until the number indicated by the phi meter is the pH value of the preparation.

4. Inspection of Moisture Content

The oven is conditioned at the temperature to be used, and then the porcelain crucible is placed in the oven for 30 minutes. The porcelain crucible was transferred so a desiccator and allowed to cool, and then the weight of the porcelain crucible was weighed. Edible film was weighed and then put into a porcelain crucible and put in an oven at a temperature of 105°C, the porcelain crucible was weighed and repeated heating until a constant weight was obtained

Measurement of water content using the formula Moisture content B-C/B-A 100%

5. Stability studies

Stability studies are to be conducted according to the ICH guidelines by storing the TDDS samples at 40±0.5°c and 75+5% RH for 6 months. The samples were taken out at0,30,60,90, and 180 days, and their drug content was appropriately analyzed.

ANTIOXIDANT ACTIVITY:

1)Antioxidant activity on potato:

When potato is peeled and cut, raw potatoes turn brown quickly. This process is called as oxidation, it happens because potatoes are a naturally starchy vegetable.
And when exposed to oxygen, starches turn grey, brown, or even black. Some part of potato was cut and patches were stick to potato. Region where patches are stick does not turn brown or grey. Which means patches or drug show antioxidant activity as it inhibits oxidation.

2)Brine Shrimp Lethality Assay:

The lethality assay using brine shrimp, also known as fairy shrimp, sea monkeys, or Artemia Salina, is frequently used to evaluate the cytotoxic potential of bioactive substances.
This is an initial toxicity screening for pesticides, heavy metals, cyanobacteria toxins, plant extracts, and dental material and nanostructures.
It is therefore advised that an animal model be used for its establishment. The about 22 mm length larvae (nauplii; singular nauplius) are large enough to examine without the need for a high magnification and tiny

enough to hatch in large quantities without requiring a great amount of workspace in a laboratory.

Michael et al. initially suggested this assay in 1956. Others went on to further improve it after that. In 1982, Meyer et al. used this lethality assay as a reference for bioassays including active cytotoxic and antitumor drugs.

This is a quick and thorough test for the bioactive compound, whether it is manufactured or natural. Because aseptic methods are not needed, this test is also affordable and straightforward. It requires relatively little sample (2–20 mg or less), no specialized equipment, and can readily use a large number of organisms for statistical validation.

Brine Shrimp Lethality Assay:

An significant technique for the preliminary cytotoxicity assay of plant extract and others is the brine shrimp lethality assay, which measures the assailant's capacity to kill a laboratory-cultured larva of (nauplii). For a full day, the nauplii are subjected to varying amounts of plant extract. The efficacy of the extract is determined by counting the motile nauplii.

Preparation of reagents:

Serial dilution of extract:

After cleaning, mark the test tubes. An analytical balance was used to weigh ten milligrams of plant extract. Subsequently, 10 mg of plant extract that was soluble in water was dissolved in 1 milliliter of water to create the stock solution. Through serial dilution from the stock solution, concentrations of 1 mg/mL, 100 µg/ml, 10 µg/mL, and 1 µg/ml were created. We labeled five test tubes 1 through 5. Next, one milliliter of the prepared solution and one milliliter of seawater were added to each of the test tubes that held ten nauplii. After a day, the number of dead nauplii was tallied.

Procedure:

Hatching brine shrimp:

1. Fill the rectangular jar with 3 liters of water after measuring it with a measuring cylinder.

2 Using a balance, weigh approximately 27 g of table salt and transfer it to the jar filled with water.

3. Use a spatula to mix the water.

4. To ensure adequate aeration, insert the airline tip from an air pump into the jar's bottom.

5. Fill the jar to the brim with water and add roughly 15 g of brine shrimp eggs.

6. Turn on a 60–100 watt lightbulb that is positioned a few inches away from the jar.

7.2 The nauplii will hatch in 20–24 hours.

8. Examine the nauplii and eggs.

9. After the following 24 hours, gather the nauplii.

10. The hatched nauplii and the empty egg need to be separated. You can accomplish this simply turning off the light and the air. While the brine shrimp focus in the water column, the empty egg will float.

11. Use a Pasteur pipette to transfer 10 nauplii to a test tube.

Toxicity testing:

• Vary the concentrations of the plant extract that the nauplii are exposed to.
• After a 24-hour period, determine the proportion of deaths and the number of survivors.
•For every test article, the dosage response parameters were determined. Results were expressed in terms of LC50, TGI, and G150 values. The experiment data were evaluated using the linear regression approach of plotting the cell viability against the molar drug concentration of the investigated compounds.

Summary of parameters:

• G150: Drug concentrations that decrease cell growth by 50%, as determined from

[(C-Tz) / (Ti-Tz)] x 100 = 50

• LC50: The medication concentration that results in a 50% cell death, determined by

[(Ti-Tz) /Tz] x100

• TGI: The concentration of medications that completely block cell development, determined using Ti = Tz.

3)Reducing Power Method:

Reducing power:

Radical scavenging activity and reducing power assays are two methods that can be used to quantify the antioxidant activity of plant extracts. The more antioxidant chemicals that convert iron (Fe) from its oxidation form in ferric chloride to ferrous (Fe) in the reducing power assay.
Technique for Power Reduction:
The Oyaizu (1986) approach was used to calculate the reducing power. 2.5 ml of different plant methanolic extract strengths, 2.5 ml of 200 mmol/l sodium phosphate butter (pH 5.5), and 2.5 ml of 1% potassium ferricyanide were combined. For twenty minutes, the mixture was incubated at 50°C.

Following the addition of 2.5 ml of 10% w/v trichloroacetic acid, the mixture was centrifuged for 10 minutes at 650 rpm. .. At 700 nm, the absorbance of the top layer (5 m2) was measured after being combined with 5 ml of deionized water and 1 ml of 0.1% ferric chloride. A greater absorbance implies a stronger reducing power.
The assays were run three times, and the mean values and standard deviations represent the results. The graph of absorbance at 700 nm against extract concentration was used to determine the extract concentration that provided 0.5 of absorbance (BC). As standards, a-tocopherol and BHA were employed.

When compared to tests for radical scavenging activity, the ECsa values obtained for reducing power were superior.

Plant extracts' potential to donate hydrogen may be the cause of their lowering power. Consequently, it is possible that L. dercoxus has larger concentrations of reductone, which may react with free radicals to stabilize and prevent radical chain reactions.

4) Total Phenolic content:

Procedure:

1. Prepare calibration curve of standard Gallic acid (10-100 ug/ml in water).
2. Prepare 1 mg/ml of extract solutions.
3. Mix 1 ml of each sample with 0.25 ml of Folin-Ciocalteu's reagent and 1.25 ml of 20% sodium carbonate solution.
4. Allow the mixture to react for 40 mins, at room temperature.
5. After the reaction period, mix the contents and measure the blue color at 725 nm in comparison with standards. Calculate the amount of total phenols from calibration curve as a Gallic acid equivalent by the following formula: T= C.V/M
6. Where, T total content of phenolic compounds, (milligram per gram of plant extract), C= the concentration of gallic acid established from the calibration curve (milligram per milliliter). V the volume of extract (milliliter), M = the gram weight of plant extract.

5) Zebra fish induced cancer model:

Zebrafish has gained attention as a effective model for cancer research mainly because of its ability to produce abundance of offspring, cost-effective maintenance, dynamic visualization of tumour growth in vivo, and the possibility of chemical screening in large numbers of animals at reasonable costs, easy to identify the growth of tumour as they are transparent.

It is known that benzene causes cancer. If exposed to benzene vapours for an extended length of time, it can result in cancer.

A highly promising experimental model for drug testing and cancer research is the zebrafish. Benzene is used as a carcinogen in our research of carcinogenic activity in zebrafish to see if it causes cancer in the fish. These fish (fish with cancer) may be utilized in drug discovery, cancer therapy, or cancer treatment and zebra fish were injected with varying doses of benzene water (1ppb,10ppb, 100ppb, 1ppm) and the fish were observed.

With its many special advantages, the zebrafish (Danio rerio) is widely utilized in research on human illness models, drug screening, and drug toxicity and safety assessments. First, the human and zebrafish genomes are approximately 70% identical, and over 80% of the genes linked to human diseases are also present in the zebrafish genome. Moreover, human and zebrafish proteins and disease processes are conservative. This indicates that medications that are effective for people are frequently also effective for zebrafish since they target the same organ. The zebrafish's embryos and larvae are transparent, which is a crucial feature that enables researchers to see the entire process of embryogenesis firsthand and to gain in vivo cellular and subcellular imaging using optical instruments. Zebrafish are also widely used as animal models because to their tiny size, external fertilization, quick development, high reproduction rate, and cheap maintenance costs.

An effective method for evaluating and creating anti-cancer medications derived from natural sources is the zebrafish model. Using high throughput phenotypic screening on live zebrafish, one can ascertain the bioactivity of natural compounds.

6) DPPH Radical Scavenging Assay:

• Blois (1958) first reported the DPPH radical scavenging assay, which was used to evaluate the extracts' antioxidant capacity.
• The paramagnetic action of its own electron (delocalization of the spare electron over the molecule as a whole) makes DPPH (1, 1-dipenyl-2-picryhydrazyl) a stable radical.

• The solution in pure ethanol exhibits a prominent absorption band at 520 nm and a rich violet color.
• The DPPH radical is a stable, diamagnetic molecule that is pale violet in color. It can take an electron or a hydrogen radical.
• The conventional approach for determining an ingredient's antioxidant activity is to combine it with DPPH solution; if the material becomes violet, it has the potential to scavenge free radicals.

Procedure:

1. Various extracts dissolved in water extract in pure ethanol and distilled water.
2. Diluted every sample for a minimum of five concentrations using two-fold dilutions.
3.In 100% ethanol, 3. 6×10^{5} M of DPPH was produced.
4. Each sample solution was placed in a tube containing 500µl. For every concentration, three duplicates were examined.
5. The sample solution was transferred and combined with 500 µl of DPPH solution.
6. Shake it well and leave it for 30 minutes to sit at room temperature.
7. The absorbance at 520 nm was determined using a combination of 500 µl sample solution and 500 ul 100% ethanol as a blank.
8. For each experiment, the standard solution and control were set up as follows:
The control ethanol is a mixture of 500 µl of absolute ethanol and six times the 10°M DPF3 in absolute ethanol.
The answer is 100% ethanol.
A 500 µl mixture of distilled water and 6x10M DPPH in 100% ethanol was used as the control.
Blank: 500 µl of distilled water and 500µí of pure ethanol combined.
9. A dosage response curve with the concentration and percentage inhibition plotted.
10. To determine the effective sample concentration needed to scavenge the DPPH radical by 50%, linear regression analysis is performed (ED50value).
11. A natural antioxidant serves as the reference point.

Calculation of % inhibition:

OD control-OD sample OD control % inhibition = × 100

In Vitro Methods:

1.Tetrazolium salt assay

When it comes to assessing the viability, proliferation, and activation of cells, this colorimetric assay is reliable, quantitative, and sensitive.

The assay's foundation is the capacity of mitochondrial dehydrogenase enzymes found in living cells to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), a yellow, water-soluble substrate, into a dark blue, water-insoluble formazan result.
The quantity of formazan produced is closely correlated with the number of cells in the range of cell lines.

The cell lines were generated and cryopreserved using substances such as DMSO, which preserves cells during freezing. At room temperature, DMSO poses a risk. The frozen ampoule is gradually thawed and brought back to room temperature by agitation.
The cryovials are quickly thawed until they become liquid by submerging them in a water bath.
Centrifuge the mixture with saline for ten minutes to eliminate the DMSO. After the saline is eliminated, an aliquot is taken for cell counting, cell viability testing, and subculturing.

The MTT assay is a quantitative colorimetric technique that measures cellular growth, survival, and proliferation based on the capability of living cells. In the assay, 3-(4, 5-dimethyl thiazol-2yl) 2. 5-diphenyl was employed.

Tetrazolium bromide, or MTT. Living cells' mitochondrial enzyme dehydrogenase cleaves MTT to produce formazan, a measurable purple result.

The generation of formazan is directly and inversely associated with the number of viable cells and the level of cytotoxicity.

ASSAY PROCESS:

The well's media was carefully aspirated and disposed of after incubation.

Fetal calf serum (FCS) was not utilized; instead, each was thoroughly cleaned using Eagle's Minimum Essential Medium (EMEM).

200 µl of MTT solution (5 mg MTT/ml of PBS, pH 7.2) will be added to each well.

The plate was incubated for 6-7 hours at 37°C in a CO2 incubator with 5% CO2. After the incubation period, each well received 1 milliliter of DMSO, which was then mixed using a pipette and let to stand at room temperature for 45 seconds. In the wells, purple formazan formation was present.

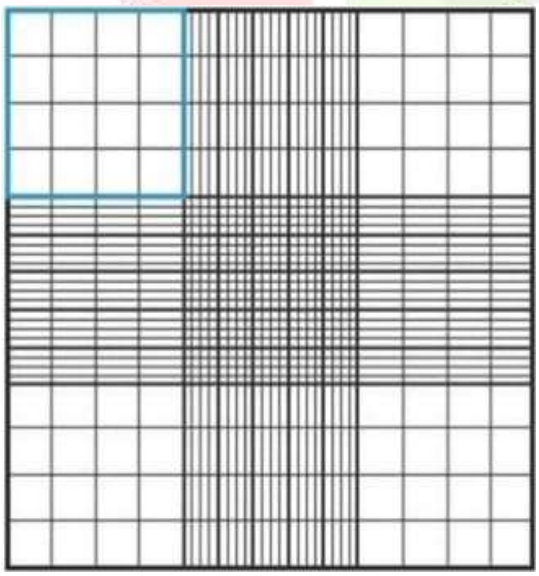
Solvent controls and cell controls were included in each experiment to evaluate the total cell viability in cytotoxicity and antitumor activity assessments.

The suspension was transferred into a cuvette of a spectrophotometer, and the optical density (OD) was measured at 540 nm using DMSO as the blank.

The cell viability % was calculated using the following formula: Mean OD of each plant extract dilution well divided by the mean OD of the control wells multiplied by 100 equals cell viability percentage.

HEMOCYTOMETER CELL COUNTS

Hemocytometer % cell viability (OD of treated cells/OD of control cells) 100 is the most widely used routine method for precise and efficient cell counting.



2) Sulphorhodamine B Assay

The Sulphorhodamine B assay is used to assess the whole-culture protein content, which should be proportionate to the number of cells.

The antiproliferative SRB test was employed to quantify growth inhibition. By staining every cellular protein with the SRB dye Skehan, this colorimetric technique provides an indirect estimate of the cell count. The cell lines were grown in RPMI 1640 medium that had 10% fetal bovine serum and 2 mM L-glutamine added to it. Taking into account the time it takes for each cell line to double, 90 µL of cells per well were seeded at appropriate densities into 96-well microtiter plates for the current screening study.

Before the experimental medications were administered, the microtiter plates were incubated for 24 hours at 37 °C, 5% CO2, 95% air, and 100% relative humidity after cell inoculation.
Cells from one plate of each cell line were fixed in-situ with trichloroacetic acid after 24 hours to represent a measurement of each cell line's cell population at the time of drug delivery (Tz).
The experimental extracts were frozen and solubilized at a 400-fold final maximum test concentration in the appropriate solvent before to use. Before adding medication, an aliquot of frozen concentrate was thawed and diluted with complete medium containing test material at concentrations of 100, 200, 400, and 800 µg/ml, ten times the required final maximum test concentration.
Aliquots of 10 µl of each of these dilutions were added to the appropriate microtiter wells, which were already containing 90 µl of cell suspension. This gave rise to the required final drug concentrations of 10, 20, 40, and 80 µg/ml.

Endpoint measurement:

Following the addition of the drug, the plates were incubated for 48 hours under standard conditions before the test was stopped with the addition of cold TCA. The cells were incubated for 60 minutes at 4°C after being gently fixed in-situ with 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA). After discarding the supernatant, the plates underwent five water washes and were allowed to air dry.

After adding 50 µl of a 0.4 percent (w/v) sulforhodamine B (SRB) solution in 1% acetic acid to each well, the plates were allowed to sit at room temperature for 20 minutes. Following staining, the unbound dye was extracted, and any leftover dye was washed away five times with 1% acetic acid. Plates were allowed to air dry.

After eluting the bound dye with 10 mM Trizma base, the absorbance was measured at 540 nm using a reference wavelength of 690 nm using an Elisa Plate Reader.

For each plate, the percentage growth between the test and control wells was computed.
The ratio of the test well's average absorbance to the control wells' average absorbance* 100 was used to express the percent growth. The percentage growth was computed at each drug concentration level using the six absorbance measurements: time zero (Tz), control growth (C), and test growth in the presence of the drug at the four concentration levels (Ti).

3) Morphological Assay:

Large-scale morphological alterations that occur in the cytoskeleton or at the cell surface can be used to estimate the viability of a cell.
Damage is indicated by significant volume decreases brought on by protein losses and intracellular ion losses as a result of altered permeability to potassium or sodium.
Necrotic cells exhibit nuclear enlargement, chromatin flocculation, and absence of nuclear basophilia.

Nuclear condensation, nuclear fragmentation, and cell atrophy in apoptotic cells.

4) Dye exclusion test:

The assay relies on the cells' structural integrity.
While dead cells would have lost their ability to withstand certain dyes, such as propidium, Eosin, or trypan blue, live cells have intact membranes that are impervious to these substances.
As a result, they would absorb the dyes whereas the living cells would not.

METHOD:

1. In 96-well plates, cell lines are counted, grown, and inoculated as previously described.
2. For four days, cells were cultured with various test substance doses.
3. Using a hemocytometer, the number of cultivated cells in each well was counted following appropriate dye staining.

%Cell Viability = [No. Of Viable Cell / Total No. Of Cells (Viable + Dead)] X100

5)Clonogenic test:

A colony formation assay is another name for the clonogenic assay, which is a test for in vitro cell survival. It assesses the ability of a single cell to endure and divide into colonies. Since it was first described in the 1950s, this test has been a vital resource in radiobiology, helping researchers understand how radiation impacts the growth and survival of cancer cells.

Clonogenicity can only be measured by planting cells at very low densities and letting them form colonies for one to three weeks. Subsequently, colonies undergo enumeration, preservation, and crystal violet staining. Analyzing data is made possible by plotting cell survival curves.

The clonogenic assay is an in vitro test for cell survival that evaluates all types of cell death by calculating the number of times a single cell can multiply into a colony. 50 cells or more are considered to be in a colony. Different cell lines have differing plating efficiencies (PE). Untreated cells seeded as a single-cell solution at low densities will form colonies in around 10–14 days. PE is calculated by dividing the total number of cells implanted by the number of colonies. The number of colonies that form after cell treatment, expressed in relation to the individual cell PE, is known as the surviving fraction (SF).

Single cells were sown onto 6-well plates, allowed to adhere for six hours, treated for 48 hours as described earlier, incubated for ten to fourteen days in a replacement medium free of medicines. The plates were stained with crystal violet, and colonies with fifty or more cells were manually counted. The vehicle control's colony-forming efficacy was utilized to standardize the outcomes. The survival fraction was calculated by comparing the number of colonies generated in drug-related cells to the untreated control.

Essentially, a clonogenic experiment can be carried out in two different ways:

Low densities of cells can be planted, and their clonogenicity can be tested by treating the cells thereafter. To examine clonogenic capacity, cells can be treated for a predetermined amount of time and then re-plated at low densities in treatment-free media.

6)Cell counting assay:

Numerous techniques exist for measuring cell proliferation, each with differing degrees of sensitivity, repeatability, and compatibility with high-throughput formats.
This procedure outlines the application of three distinct techniques for assessing in vitro cell growth, such as:

• The traditional counting chamber hemocytometer.
• A luminescence-based test that calculates the relative number of cells by measuring the variation in the metabolic activity of live cells.
• A multi-mode cell imager that counts cells through an algorithmic counting process.

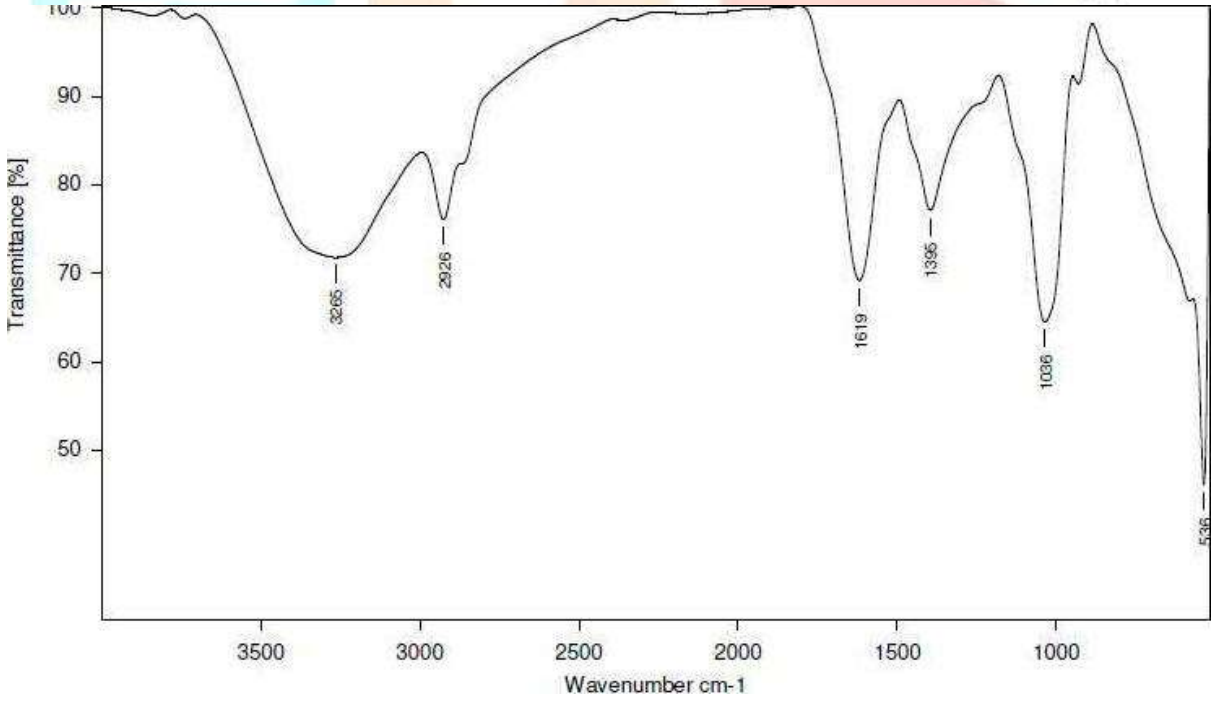
For the measurement of cell proliferation, each approach has pros and cons of its own, such as compatibility with high throughput, cost, and time.
This protocol shows that all of the methods were sensitive to growth at different densities of cells and could assess cell proliferation over time with accuracy.

Furthermore, confluence and morphology could be obtained by measuring cell proliferation with a cell imager, which also made it possible to track the growth of the cells over time. In conclusion, the technique selected depends on the user but all are capable of assessing cell growth.

RESULT AND DISCUSSION:

1) IR spectra of Ginger:

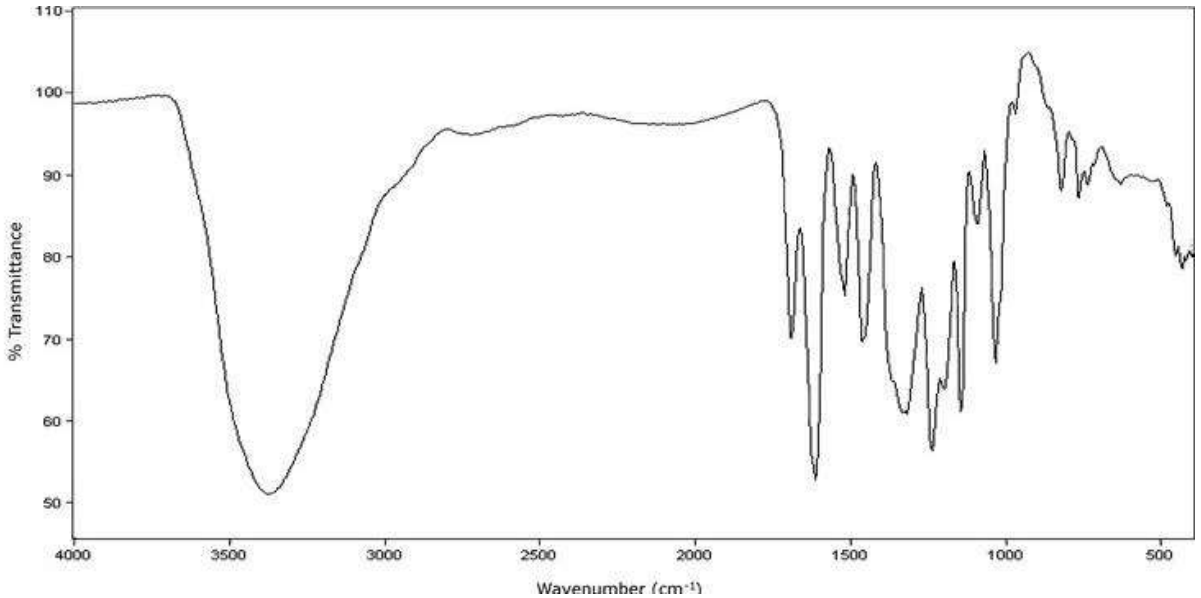
SR.NO	FUNCTIONAL GROUP	ABSORPTION
1	O-H	3400 -3100 cm^{-1}
2	C-O	1036 cm^{-1}
3	C=O	2926 cm^{-1}
4	C-H	800-500 cm^{-1}



2) IR spectra of Green tea:

The spectra display the distinctive absorption bands for the C = C group at around 1600 cm^{-1}, the O-H group (3400 -3100 cm^{-1}), and the C -O group (1150 -1010 cm^{-1}).

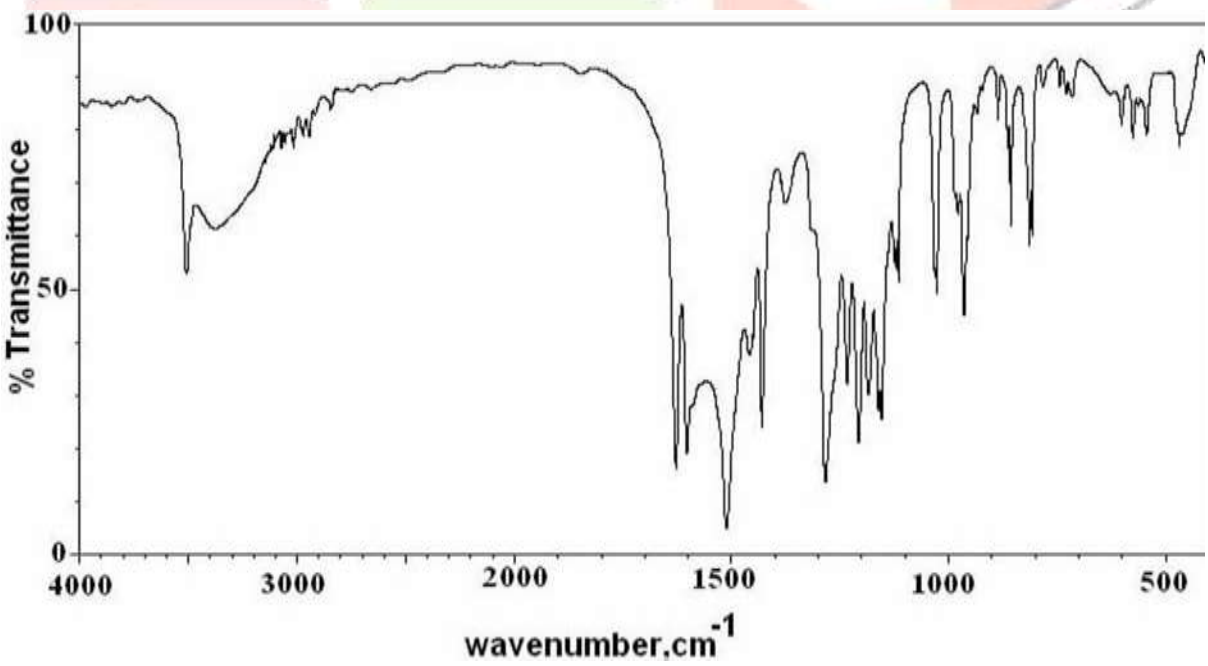
SR.NO	FUNCTIONAL GROUP	ABSORPTION
1	C = C	1600 cm^{-1}
2	O-H	3400 -3100 cm^{-1}
3	C -O	1150 -1010 cm^{-1}



3) IR Spectra of turmeric:

The FTIR spectrum of curcumin revealed that the aromatic C = C groups were stretched at 1487.40 cm $^{-1}$, the phenolic hydroxyl groups were stretching at approximately 3500.34 cm $^{-1}$, and the C = C (alkenes) and carbonyl (C = O) groups were vibrating at 1626.26 cm $^{-1}$.

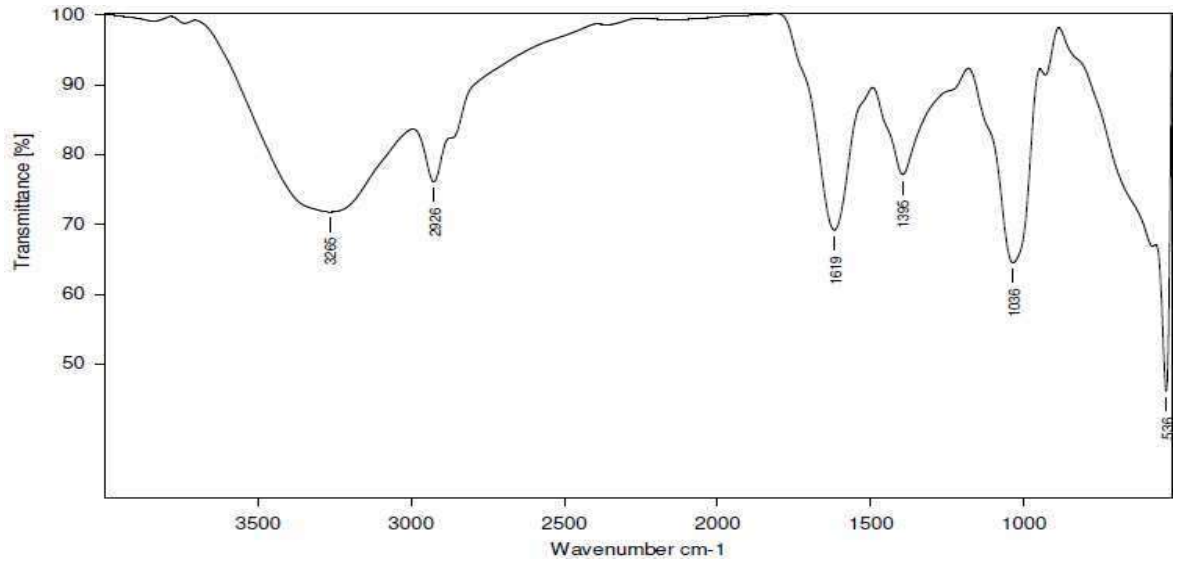
SR.NO	FUNCTIONAL GROUP	ABSORPTION
1	C = C (stretched)	1487 cm $^{-1}$
2	O-H (stretched)	3400 -3100 cm $^{-1}$
3	C = C	1600 cm $^{-1}$
4	C =O	1626 cm $^{-1}$



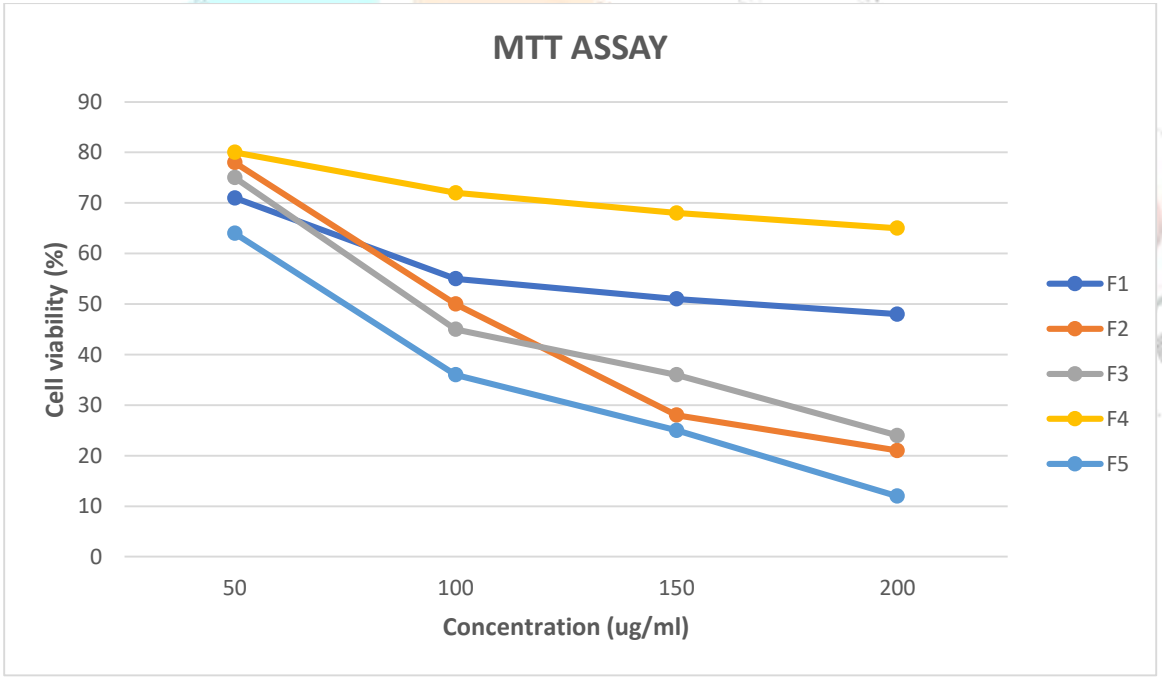
4) IR spectra of Garlic:

SR.NO	FUNCTIONAL GROUP	ABSORPTION
1	C-O	1619
2	C=O	1132-1032
3	S-O	1396

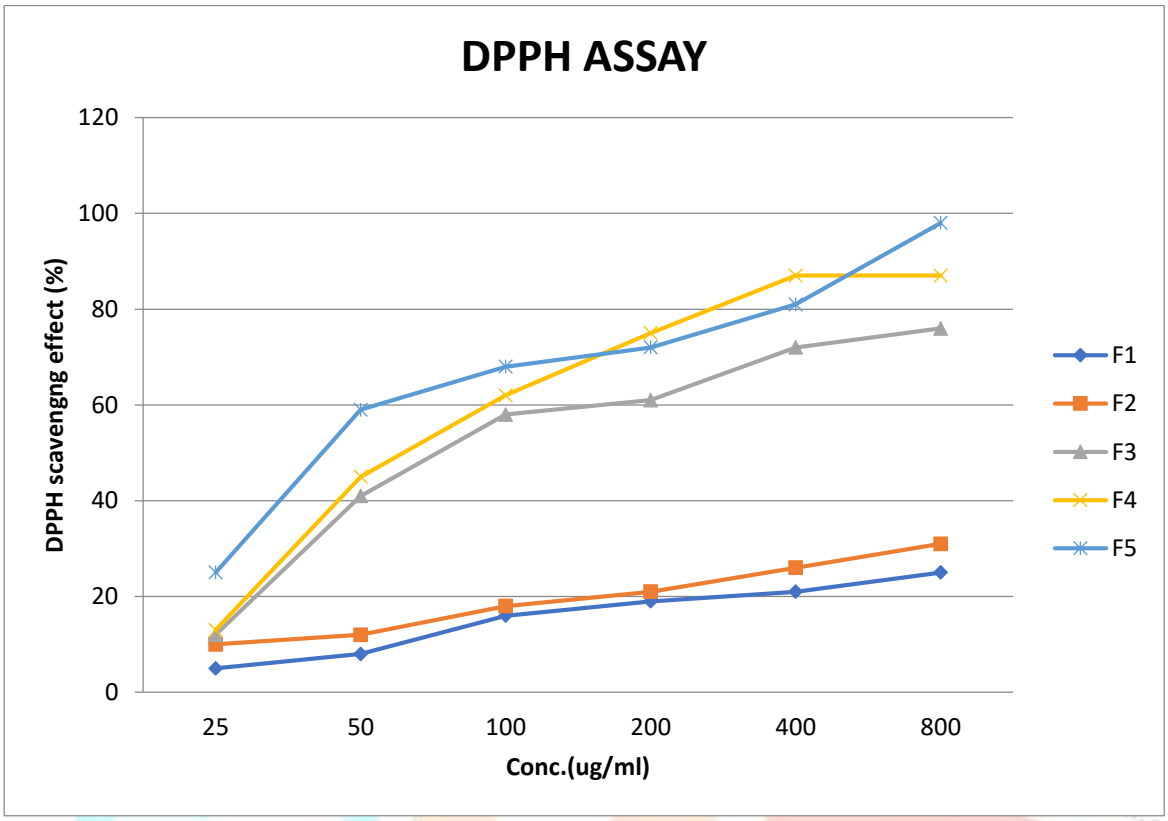
Allicin peak at 240 nm was observed Liquid Extraction



5) MTT ASSAY :



6) DPPH ASSAY:



Evaluation test:

1) Antioxidant activity on potato:

Fig a) Before oxidation process

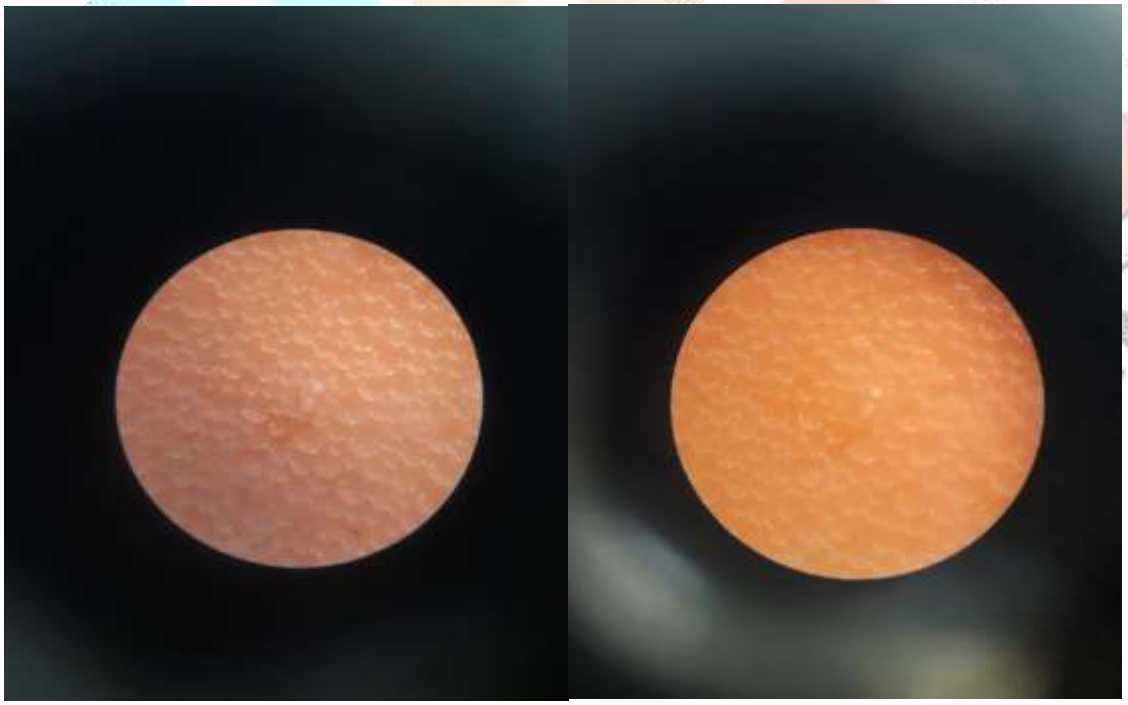
Fig b) After oxidation reaction and application of transdermal patches

2) Reducing power method:

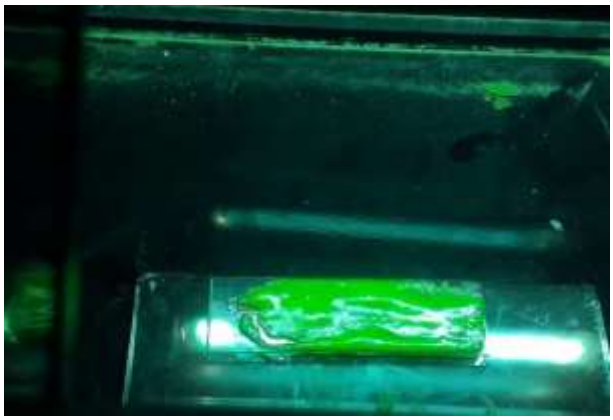
A) Standard

B) Drug Sample

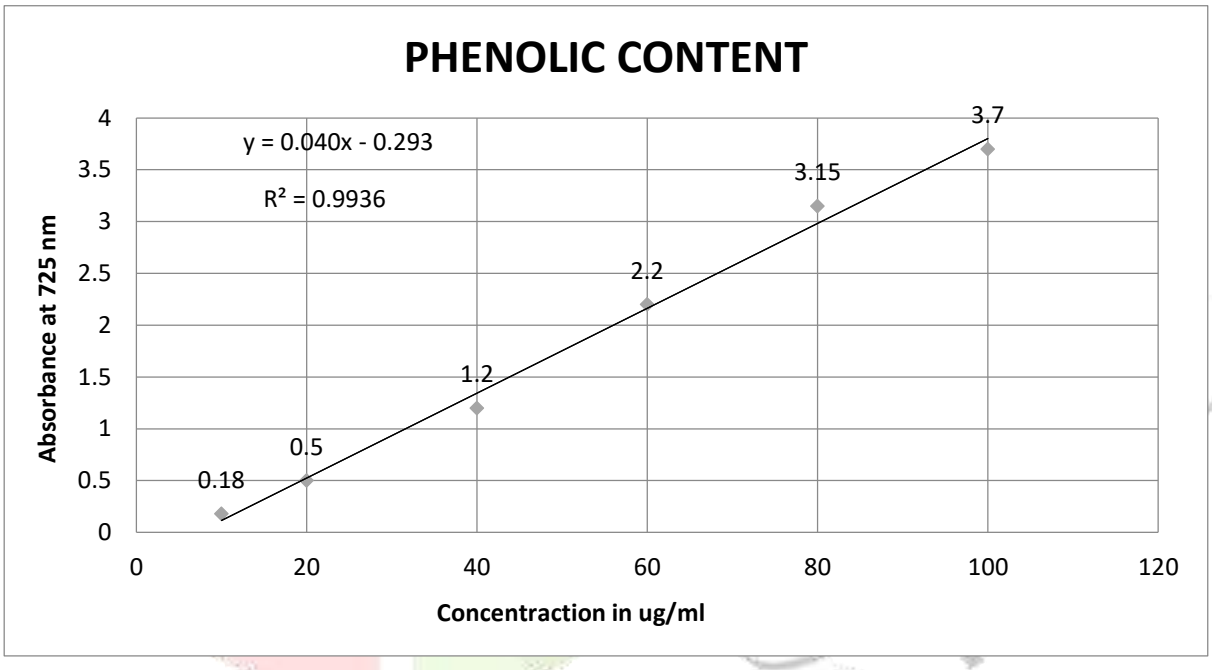
3) Dye test:



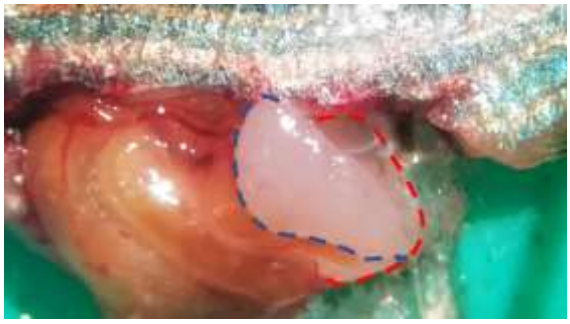
4) Fluroscence test:



5) Phenolic content:



6) Zebra fish induced cancer model:



REFERENCE:

1) Dr S.S Khandelwal, Dr S.L Deore, Dr B.A Baviskar, "Experimental phyto-pharmacognosy", Nirali Prakashan, Page no. 4.7.

2) Indian Pharmacopoeia 2010, Government of India Ministry of health and Family Welfare Indian Pharmacopoeia Commission (Volume II), 2010 Eighth edition, page no. 562.

3) C. K. Kokate, A. P. Purohit, S.B. Gokhale, " Pharmacognosy", 55th edition, Nirali Prakashan, Page no.14.8

4) Frederick O. Stephens, Karl Reinhard Aigner "Basics of Oncology" Springer publication, Page no. 3-16.

5) International Journal of Creative Research Thoughts | ISSN: 2320-2882, Review on "Screening methods for anti-cancer drug: in vitro and in vivo".

6) C. K. Kokate, A. P. Purohit, S.B. Gokhale, " Pharmacognosy", 55th edition, Nirali Prakashan, Page no.14.25

7) Prof.Mohammed Imran ,Prof. Tushar A. Deshmukh, Prof. Md.RageebMd, Usman, Prof. Sufiyan Ahmad, " Practical Book of Instrumental Method of Analysis", PV Publication, Page no. 232.

8) C. K. Kokate, A. P. Purohit, S.B. Gokhale, "Pharmacognosy", 55th edition, Nirali Prakashan, Page no.15.26

9) Mukta M. Abhyankar, Supriya G. Jagtap, "NATURAL DRUG TECHNOLOGY" Tech- Neo Publication, Page no 3.6-3.11.

10) A textbook of Herbal Drug Technology", Dr. Mrs. Varsha Tiwari, Nirali prakashan, Ist edition, Jan 2020, page no.- 1.14- 1.26 & 5.11-5.5.

11) Herbal Drug Technology", Dr. V. M. Shinde, Mrs. K. S. Bodas-Yadav, 2nd edition, Dec 2020, no. 5.13, 1.1-1.22, 7.15-7.16.

12) "Rang and Dale's Pharmacology" H. P. Rang, M. M. Dale, Relx India Pvt. Ltd., Nineth Edition, Jan. 2019, Page no.-203-285.

13) "Essentials of Medical Pharmacology", K. D. Tripathi, Jaypee Brothers Medical Publishers. Eighth edition, Jan. 2018, page no.- 857.

14) "Essentials of Medical Pharmacology", K. D. Tripathi, Jaypee Brothers Medical Publishers. Eighth edition, Jan. 2018, page no.- 886.

15) IP International Journal of Comprehensive and Advanced Pharmacology