FORMULATION AND EVALUATION OF *Alpinia galanga* EXTRACT LOADED NIOSOMAL PATCH FOR THE TREATMENT OF BREAST CANCER

A dissertation submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

A.ANANTHALAKSHMI

REG.No: 261920652

Under the guidance of

DR. P. MUTHUSAMY, M.PHARM., Ph.D., B.L.

ASSISTANT PROFESSOR

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600 003

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DEPARTMENT OF PHARMACOGNOSY
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMIL NADU

CERTIFICATE

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EXAMINERS

Place: Chennai-03

Date:

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600 003

TAMIL NADU

DR. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A.,
Principal,
College of Pharmacy,
Madras Medical College,
Chennai- 600003.

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DR.A.JERAD SURESH, M.Pharm., Ph.D., M.B.A.,

Place: Chennai-03

Date:

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600 003

TAMIL NADU

DR. R. RADHA, M.Pharm., Ph.D., M.B.A.,
Professor & Head,
Department of Pharmacognosy,
College of Pharmacy,
Madras Medical College,
Chennai - 600003.

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DR.R.RADHA M.Pharm., Ph.D., M.B.A.,

Place: Chennai-03

Date:

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600 003

TAMIL NADU

DR. P. MUTHUSAMY, M.Pharm., Ph.D., .B.L.,

Assistant professor,

Department of Pharmacognosy,

College of Pharmacy,

Madras Medical College,

Chennai - 600003.

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DR.P.MUTHUSAMY, M.Pharm., Ph.D., .B.L.,

Place: Chennai-03

Date:

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A.ANANTHALAKSHMI

Reg.No. 261920652

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INTRODUCTION

1. INTRODUCTION

Plants are an integral part of our lives and have been so since the time immemorial, due to their omnipresence.

"Medicine heals doubts as well as diseases" - Karl Marx

Since its birth, they have served mankind by providing food, shelter, healthy, beautiful and pleasing environment, and adding colors and rhythms to the canvas of our lives. A guess estimate shows that there are about 4,00,000 plant species, though the worldwide plant lists holds over one million names under 642 plant families out of which many are considered to be synonyms.[1]

HERBAL MEDICINE

Chemical substances derived from animals, plants, and microbes have been used to treat diseases since the dawn of medicine, while plant-derived products have dominated the human pharmacopoeia for thousand of years and have provided endless source of medicine.

Natural product is extracted from its source, then concentrated, fractionated and purified, yielding essentially a single biologically active compound. It is already well known that sometimes complex mixtures of compounds in herbal medicines have greater effects than isolated compounds.[2]

HERBAL MEDICINE AND ITS COMPLEX COMPOSITION

Classical knowledge-based approach is the ethnopharmacological approach, where the traditional medicinal use of plants constitutes the basis for the selection of the test material and the pharmacological assay. Ethnopharmacology involves the observation, description, and experimental investigation of traditionally used drugs and their bioactivities.

As like the modern allopathic medicine which is described as evidence-based medicine, treatment with plants was also based on various ethnobotanical, ethnopharmacological evidence-based, though the evidence or criteria on which justifications were arrived at apparently different. Those observations might not be considered 'scientific' in modern lexicon, but they utilized the human senses to the

fullest, and served as the basis for the emergence of the organized traditional folk medicines that were also intertwined with faith.

Evaluating the action of whole plant extracts versus the action of purified preparation showed that, in many cases, the potency of the later declines as the purification of extract continues into more isolated fractions or single compounds. Thus, one of the advantages of herbal medicines is their complex composition. Their components have multiple activities that result in a greater total activity. Possible explanations include synergy, enhanced bioavailability, cumulative effects, or simply the addictive properties of the constituents, but further research is required.[3,4]

MULTI-TARGETED APPROACH OF HERBAL DRUGS

WHO estimated that 80% of the world's population use medicinal plants in the treatment of diseases, and in African countries this rate is suggested to be even higher. Modern medicine advocates that an ideal therapeutic intervention should act on single or at specific targets.

Use of herbal medicines was described as an "herbal shotgun" approach, due to the high number of chemical compounds present within the extracts. Although the use of a single molecule as treatment may fit the pathophysiology of some diseases, it may not for many others.

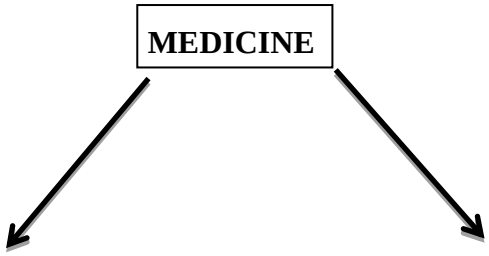
Table 1: Wild and cultivated medicinal plants: advantages and disadvantages[5]

Factor considered	**Wild MP**	**Cultivated MP**
Availability	Decreasing	Increasing
Agronomic manipulation	No	Yes
Adulteration	Likely	Relatively safe
Botanical identification	Not always reliable	Not questionable
Fluctuation of supply	Unstable	Constant quality
Genetic improvement	No	Yes
Quality control	Poor	High
Post-harvest handling	Poor	Poor Usually good

Diseases with a multifactorial cause or those with a high incidence of resistance, or variable response to treatments, were usually treated using a combination of different drugs, aiming different targets.

It is reasonable to assume that a mixture of phytochemicals or synthetic components would have greater bioactivity than a single compound because a mixture of bioactive compounds has the ability to affect multiple targets.

Thus, the combination of several therapeutic agents or drugs, aiming at different therapeutic targets is also the most claimed advantage of herbal medicines.[6]



HERBAL	CONVENTIONAL
● Several active principles (in low concentration)	● One active principle (in high concentration)
● Other substances (Salts of organic acids, bitter and tannins, saponins, mucilages, minerals, pectic substance, etc.)	● Other substances (excipients)
● Complex Medicine - (Promoted for several divergent uses.)	● Simple Medicine - Singular indication

Table 2: Herbal medicine vs conventional medicine

MECHANISM OF ACTION OF HERBAL CHEMOPREVENTIVE AGENTS[7]

- Antioxidant action
- Antimutagenic
- Arachidonic acid metabolism inhibition
- Polyamine synthesis inhibition
- Ornithine Decarboxylase Inhibition
- Radioprotection
- Angiogenesis modulators
- Apoptosis modulation

DISEASE PROFILE[8-17]

MALIGNANT NEOPLASMS OF THE BREAST:

Cancer of the breast is the most common malignant neoplasm in women, accounting for as many as one in five of all female cancer deaths in the UK. The disease is rare under the age of 25, and increases steadily in incidence with age, reaching its peak incidence in elderly women.

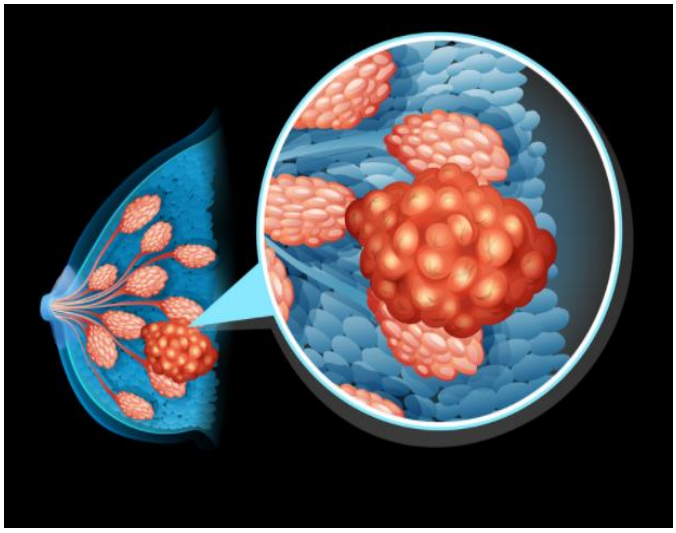


Figure 1: Breast cancer

TYPES

Breast cancer can begin in different areas of the breast — the ducts, the lobules, or in some cases, the tissue in between.

- Ductal carcinoma In Situ (DCIS)
- Invasive Ductal Carcinoma (IDC)
- Inflammatory Breast Cancer
- Lobular Carcinoma In Situ (LCIS)
- Male Breast Cancer
- Molecular Subtypes of Breast Cancer
- Triple-Negative Breast Cancer
- Paget's Disease of the Nipple

- Phyllodes Tumors of the Breast
- Recurrent Breast Cancer
- Metastatic Breast Cancer

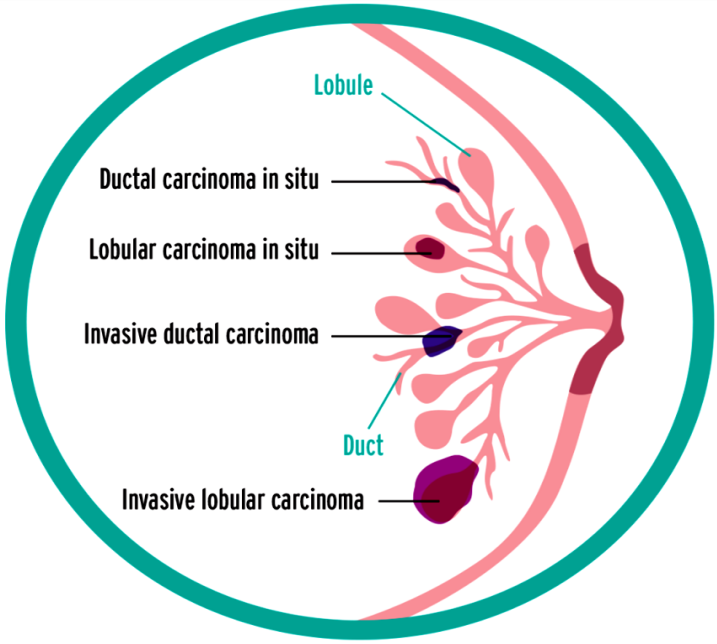


Figure 2: Types of Breast cancer

3 MAIN TYPES OF RECEPTORS

1. Estrogen Receptor (ER)

2. **Progesterone Receptor (PR)**

3. Human Epidermal Growth Factor Receptor 2 (HER2)

Triple-negative

Breast cancer that is ER-negative, PR-negative, and HER2-negative is called **triple-negative breast cancer**. This type of breast cancer grows and spreads faster than other types of breast cancer.

Factors: Patient age and menopausal status, tumour size and grade, involvement of axillary lymph nodes or skin, and presence of hormone receptors within the tumour may be a guide to the extent and aggressiveness of disease, and hence have prognostic significance for treatment.

SYMPTOMS

- New lump(s) or area(s) of thickened tissue in the breast(s)
- Swelling in the breast(s)
- Pain in the breast or nipples
- Skin rash, scaly or patchy skin around the breast or nipples
- Discharge from the nipple (other than breast milk) or change in the shape of nipples, like inverted or sunken nipples
- Dimpling or puckering around the nipple(s) or on the breast skin.

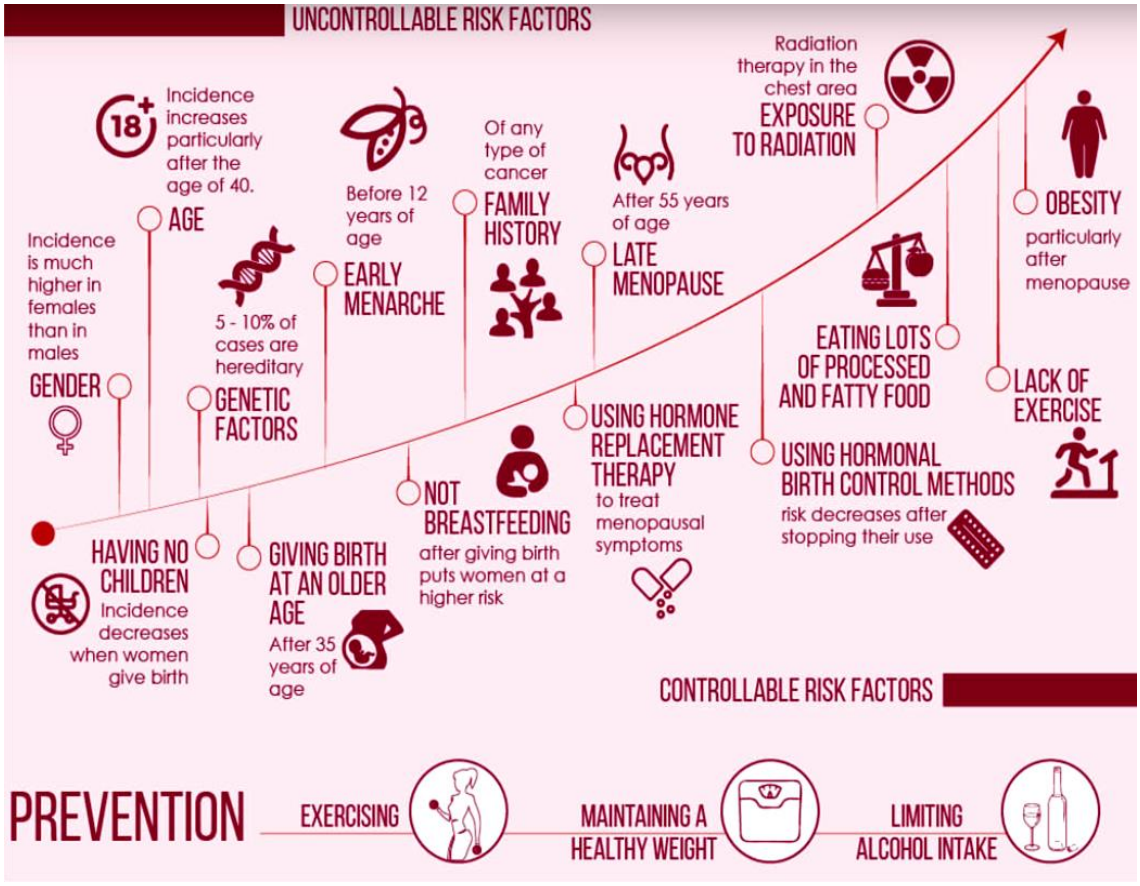


Figure 3: Risk factors of breast cancer

STAGES OF BREAST CANCER

If diagnosed with breast cancer, it is important to determine the stage of cancer so that appropriate medical treatment can be provided by doctors. Breast cancer is usually classified into 4 stages. These stages are further divided into sub categories within them and are classified using alphabets.

- **Stage 0**

  It is called 'situ', means "in the original place", referring to the fact that the cancer has stayed in the milk glands or ducts of the breast where it has started.

- **Stage I**

  This level onwards the cancer becomes invasive, or to say it breaks free and can attack healthy tissue.

  - ✓ IA: The cancer spreads to the fatty breast tissue
  - ✓ IB: Very small amounts of cancer cells may be found in few lymph nodes

- **Stage II**

  The cancer grows and/or spreads

  - ✓ IIA: If there is a tumor in the breast then it is still small. Up to 3 lymph nodes may be affected.
  - ✓ IIB: The tumor may be the size of a walnut and may not be in the lymph nodes.

- **Stage III**

  The cancer is harder to fight at this stage though no organs or bones have been affected.

  - ✓ IIIA: Cancer is present in up to 9 lymph nodes between the collarbones and underarm, or it spreads to or enlarges the lymph nodes in the breast. In some cases there may also be a tumor.
  - ✓ IIIB: Even if it has not spread to the lymph nodes, the tumor has grown into the chest walls or skin around the breast.
  - ✓ IIIC: The cancer spreads to 10 or more lymph nodes, has spread above or below the collarbone or even if fewer lymph nodes are affected outside but those inside the breast are enlarged or cancerous.

- **Stage IV**

  When the cancer spreads far away from lymph nodes. The most common sites are the bones, lungs, liver and brain

TREATMENT OF BREAST CANCER BY STAGE

- **Stage 0 -** Stage 0 cancers are limited to the inside of the milk duct and are non-invasive (does not invade nearby tissues). Ductal carcinoma in situ (DCIS) is a stage 0 breast tumor.Lobular carcinoma in situ (LCIS) used to be categorized as stage 0, but this has been changed because it is not cancer. Still, it does indicate a higher risk of breast cancer. See Lobular Carcinoma in Situ (LCIS) for more information.

- **Stages I-III- Treatment for stages I to III breast cancer usually includes**

  - **Chemotherapy** Chemo can be given before and/or after surgery.

  - **Hormone therapy** If the cancer is hormone receptor-positive, hormone therapy (tamoxifen, an aromatase inhibitor (AI), or one followed by the other) is typically used. It can be started before surgery, but because it continues for at least 5 years, it needs to be given after surgery as well.

  - **HER2 targeted drugs:** Some women with HER2-positive cancers will be treated with adjuvant (after surgery) chemotherapy with trastuzumab with or without pertuzumab for up to 1 year. Treated first with trastuzumab (with or without pertuzumab) followed by surgery and then more trastuzumab (with or without pertuzumab) for up to a year. If after neoadjuvant therapy, residual cancer is found at the time of surgery, the targeted drug, ado-trastuzumab emtansine, may be used instead of trastuzumab. It is given every 3 weeks for 14 doses.

  - **Targeted drug therapy**: **Targeted drugs**, such as trastuzumab (Herceptin), pertuzumab (Perjeta), or abemaciclib (Verzenio).

  - **Immunotherapy:** Women with TNBC might get the immunotherapy drug, pembrolizumab, before surgery and then again after surgery. See Treatment of Triple-negative Breast Cancer for more details.

- **Treatment of Stage IV (Metastatic) Breast Cancer**

These may include: Hormone therapy, Chemotherapy (chemo), Targeted drugs, Immunotherapy or some combination of these. **Surgery and/or radiation therapy may be useful in certain situations.**

- **Recurrent breast cancer**

Cancer is called **recurrent** when it comes back after primary treatment. Recurrence can be local (in the same breast or in the surgery scar), regional (in nearby lymph nodes), or in a distant area. Treatment for recurrent breast cancer depends on where the cancer comes back and what treatments you've had before.

CERTAIN DIETARY COMPOUND USED FOR CHEMOPREVENTIVE AND CHEMOTHERAPEUTIC PURPOSE FOR BREAST CANCER

- **Green tea catechin -** Antioxidant, anti-mutagenic, cell arrest
- **Resveratrol -** Antioxidant, anti-inflammatory, anti-angiogenic, anti-metastatic, pro-apoptotic effects.
- **Curcumin -** Antioxidant, anti-inflammatory, induce apoptotic effects, anti-proliferative.
- **Soya protein**- Decrease activation of carcinogen, competes with estrogen in binding to estrogen receptors.
- **Dietary carotenoids (Lycopene)-** Involve in apoptosis, cell communication, cell cycle regulation.
- **Onion and garlic** - Increase antioxidant level, anti-proliferative, inhibit promotion of tumors.

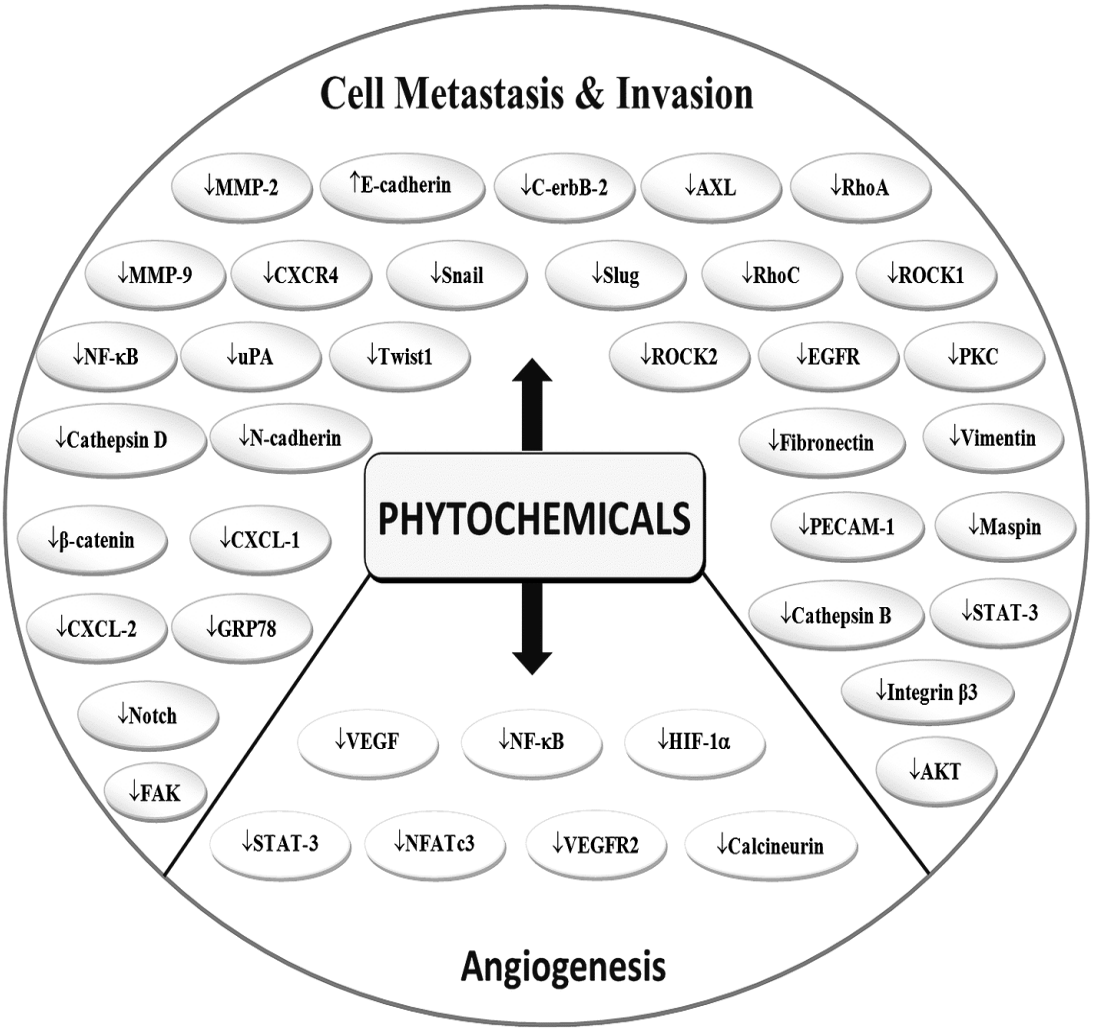


Figure 4: Mechanism of phytochemicals in anticancer activity

NIOSOMES

Niosomes are vesicular nanocarriers and are receiving much attention as potential transdermal drug delivery systems due to properties such as enhanced drug penetration, local depot for sustained drug release, and a rate-limiting membrane for modulation of systemic absorption of drugs via the skin.

Niosomal carriers are suitable for the transdermal delivery of numerous pharmacological agents, including antioxidant, anticancer, anti-inflammatory, antimicrobial, and antibacterial molecules. [18,19]

❖ COMPOSITION OF NIOSOMES

They have lamellar (bilayer) structures composed of amphiphilic molecules surrounded by an aqueous compartment. These amphiphilic molecules, known as surfactants, contain both hydrophobic groups (tails) and hydrophilic groups (heads) and show self-assembling properties, aggregating into a variety of shapes like micelles or into a planar lamellar bilayer.

Surfactants that could be used as potential drug delivery systems include sorbitan esters and analogs, sugar-based, polyoxyethylene-based, polyglycerol, or crown ether-based surfactants, sometimes in addition to membrane additives, such as cholesterol or its derivatives.

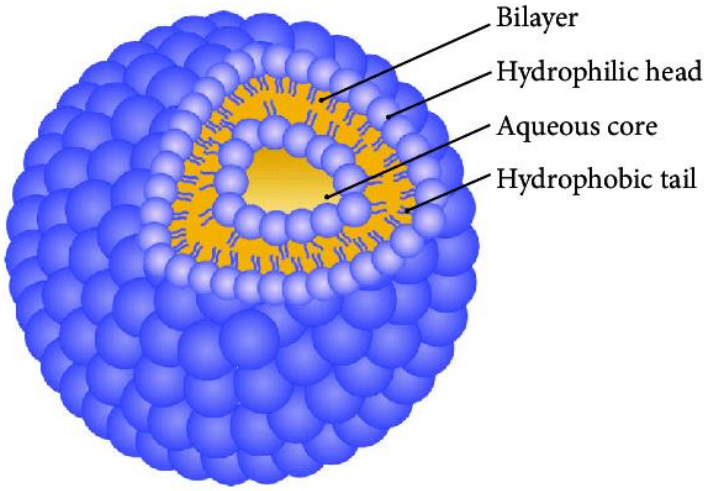


Figure 5: Structure of niosome

Nonionic surfactants are preferred because they have less potential to cause irritation, which decreases in order of cationic > anionic > nonionic.

Table 3: Components of niosomes

S.No	Components of niosomes[20]	Uses
1.	**Nonionic surfactants**	More stable and biocompatible and less toxic compared to their anionic, amphoteric, or cationic counterparts.
2.	**Cholesterol**	Influences the structures of niosomes and physical properties such as entrapment efficiency, long time stability, release of payload, and biostability. Cholesterol improves the rigidity of vesicles and stabilizes niosomes.
3.	**Charged molecules**	Increasing the amount of charged molecules can inhibit niosome formation

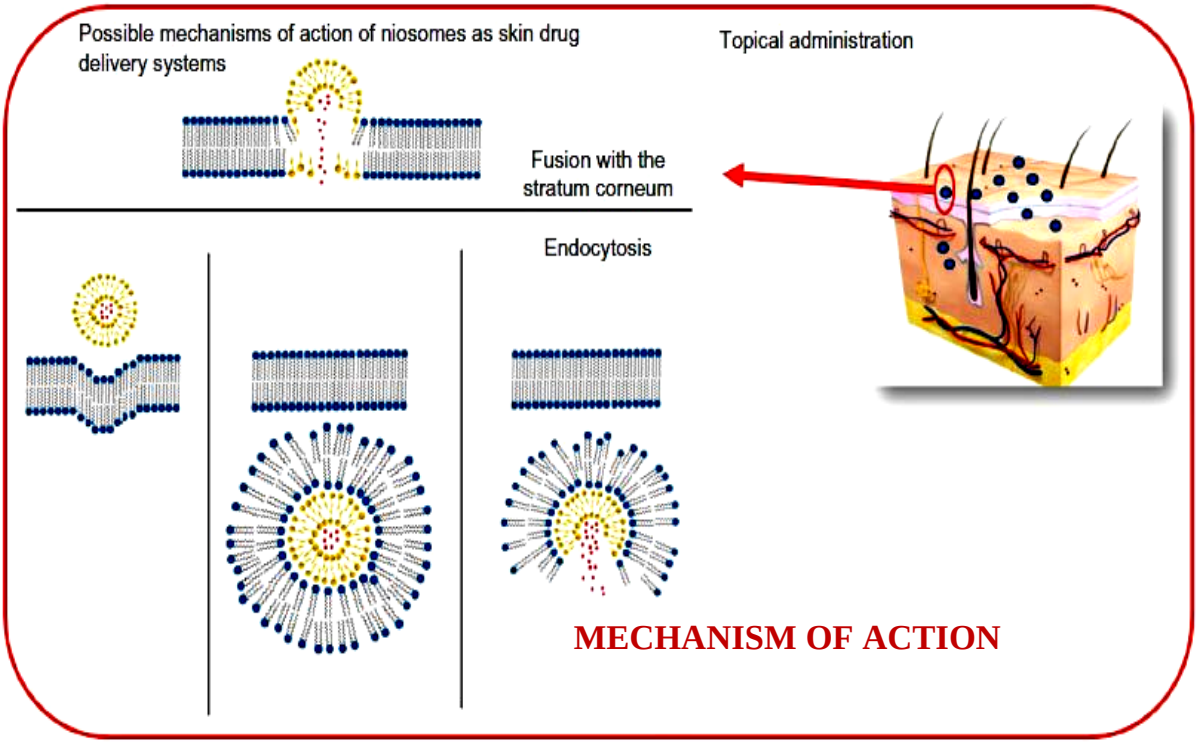


Figure 6: Niosome- Mechanism of action

ADVANTAGES-[21-22]

- Surfactants used to prepare niosomes are biodegradable, biocompatible, and not immunogenic.
- The method used for routine and large-scale production of niosomes does not involve use of unacceptable solvents.
- Due to the chemical stability of their structural composition, the handling and storage of niosomes does not require any special conditions.
- The physicochemical properties of niosomes, such as their shape, fluidity, and size, can be easily controlled by changing their structural composition and the method of production.
- Niosomes are able to encapsulate a large amount of material in a small vesicular volume.
- The structure of niosomes protect drug ingredients from heterogeneous factors present both inside and outside the body, so niosomes can be used for the delivery of labile and sensitive drugs.
- Niosomes improve the therapeutic performance of drug molecules by delaying clearance from the circulation and restricting effects to target cells.
- Niosomes can be administered via different routes, such as oral, parenteral, and topical, and using different dosage forms such as powders, suspensions, and semisolids, improving the oral bioavailability of poorly soluble drugs and also enhancing the permeability of drugs through the skin when applied topically.
- The aqueous vehicle-based suspension formulation results in better patient compliance when compared with oily dosage forms; in addition, niosomal dispersion, being aqueous, can be emulsified in a nonaqueous phase to regulate the drug release rate.
- Niosomes have been reported to achieve better patient adherence and satisfaction and also better effectiveness than conventional oily formulations.

DISADVANTAGES

- Decrease their shelf life.
- Include physical and chemical instability.
- Aggregation, fusion of vesicles.

- Leaking or hydrolysis of the encapsulated drug.
- Moreover, the methods required for preparation are time-consuming and may require specialized equipment for processing.

Structural components of Niosomes

1. Surfactants:

A wide range of surfactants and their combinations in different molar ratios have been used to entrap many drugs in niosomes of varying features such as size. Non-ionic surfactants are the most common type of surface active agent used in preparing vesicles due to the superior benefits they impart with respect to stability, compatibility and toxicity, compared to their anionic, amphoteric or cationic counterparts.

They are generally less toxic, less hemolytic and less iritating to cellular surfaces and tend to maintain near physiological pH in solution. They are also strong P-glycoprotein inhibitors, a property useful for enhancing drug absorption and for targeting to specific tissues.

The various types of non- ionic surfactants are

a. Ether linked surfactants:

These are polyoxyethylene alkyl ethers which have hydrophilic and hydrophobic moieties Iinked with ether. The general formula of this group is C_nEO_m, where n can be 12-18 and m can be 3-7. Surfactants with polyhydroxyl head and ethylene oxide units are also reported to be used in niosomes formation. Single alkyl chain surfactant Cl6 mono alkyl glycerol ether with an average of three glycerol units is one of the examples of this class of surfactants used for the preparation of niosomes. Polyoxyethylene cetyl ethers (Brij58) and Polyoxyethylene stearyl ethers (Brij72 and 76) are also used in preparation of niosomes.

b. Ester linked surfactants:

These surfactants have ester linkage between hydrophilic and hydrophobic groups

and have been studied for its use in the preparation and delivery of sodium stilbogluconate to the experimental marine visceral leishmaniasis.

c. Sorbitan Esters:

These are most widely used ester linked surfactants especially in food industry. The commercial sorbitan esters are mixtures of the partial esters of sorbital and its mono and di-anhydrides with oleic acid. These have been used to entrap wide range of drugs viz doxorubicin.

d. Alkyl Amides:

These are alkyl galactosides and glucosides which have incorporated amino acid spacers. The alkyl groups are fully or partially saturated C_{12} to C_{22} hydrocarbons and some novel amide compounds have fluorocarbon chains.

e. Fatty Acids and Amino Acid Compounds:

These are amino acids which are made amphiphilic by addition of hydrophobic alkyl side chains and long chain fatty acids which form "Ufasomes" vesicles formed from fatty acid bilayers.

2. Cholesterol:

Steroids bring about changes in fluidity and permeability of the bilayer and are thus important components. Cholesterol a waxy steroid metabolite is usually added to the non-ionic surfactants to provide rigidity and orientational order. It does not form the bilayer itself and can be incorporated in large molar ratios. Rigidization is provided by alternative positioning of rigid steroidal skeleton with surfactant molecules in the bilayer by restricting the movement of carbons of hydrocarbon. Cholesterol is also known to prevent leakage by abolishing gel to liquid phase transition.

NIOSOMES VS LIPOSOMES

- Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation, and thus entrapment efficiency.

METHOD OF PREPARATION

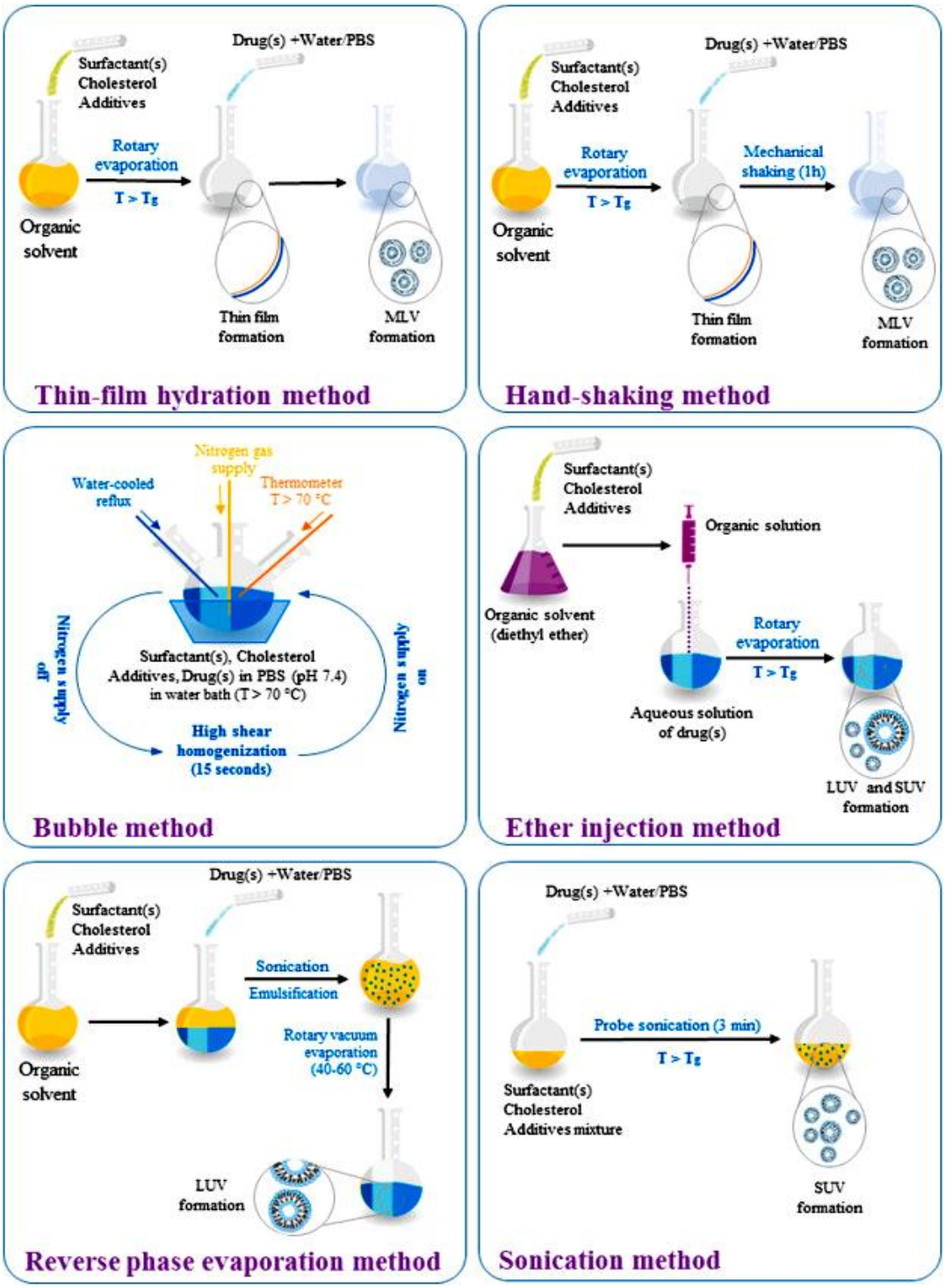


Figure 7: Method of preparation of of niosomes

- However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged).
- The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling and purity of natural phospholipids is variable.

APPLICATION

- Niosomal carriers are suitable for the delivery of numerous pharmacological and diagnostic agents, including antioxidants, anticancer, anti-inflammatory, antiasthma, antimicrobial, anti-Alzheimer's, and antibacterial molecules, oligonucleotides, and others.
- Various routes of administration exist for niosomal drugs, i.e, intravenous, intramuscular, oral, ocular, subcutaneous, pulmonary, and transdermal. Several other routes have been used to administer niosomal drugs, including the intraperitoneal and vaginal routes.
- Niosomes have been used for successful targeting of drugs to various organs like the liver and brain or to pathological districts such as tumor, enhancing drugs pharmacological activities while reducing side effects.
- In particular, targeted niosomal systems have been designed with different mechanisms of action, including active, passive, and magnetic targeting, leading to more advanced and specific macromolecular drug carriers.

Eg- For liver targeting, in cancer therapy, localized psoriasis, leishmaniasis, carrier for haemoglobin and in diagnostic imaging.

- For controlled release of drugs

1. To prolong the release rate
2. In ophthalmic drug delivery.

- To improve the stability and physical properties of drugs

  1. To increase oral bioavailability.
  2. For improvement of stability of peptide drugs from proteolytic enzymes.
  3. For improvement of stability of immunological products.

- Usefulness of niosomes in cosmetics.

Table 4 : Examples of Phyto-niosomes

S.No	Phyto-niosomes	Application
1.	*Fumaria officinalis*	Antidiabetic, antineuropathic, anti-inflammatory activity.
2.	*Annona squamosa*	For dermal delivery of the antioxidant.
3.	*Gymnema sylvestre*	Antihyperglycemic activity.
4.	Marigold extract, *Psidum guajava*	Wound healing.
5.	Propolis extract	Oral recurrent aphthous ulcer
6.	*Withania somnifera*	Enhance topical drug delivery with anti-melanoma properties.
7.	D-limonene	Cancer therapy
8.	*Volvariella volvacea*	Topical anti-aging application
9.	*Manihot esculenta*	Antioxidant, anti-inflammatory, analgesic, and antidiabetic benefits.
10.	Curcuminoids	Antioxidant, anti-inflammatory, antimicrobial and anticancer properties.

TRANSDERMAL DRUG DELIVERY SYSTEMS

Transdermal delivery is a term that should be restricted to the situation in which a solute diffuses through the various layers of the skin and into the systemic circulation for a therapeutic effect to be exerted, e.g., treatment of withdrawal symptoms using nicotine.

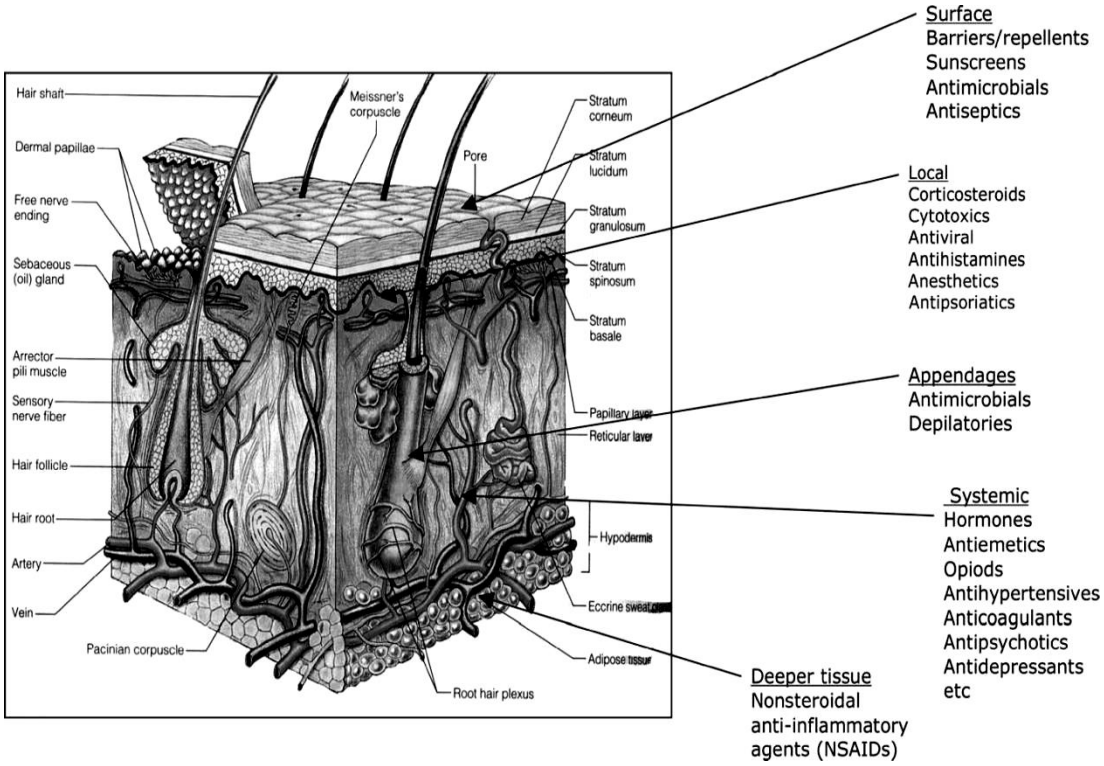


Figure 8: Transdermal delivery system

Benefits

- The avoidance of first pass metabolism and other variables associated with the GI tract such as pH, gastric emptying time.
- Sustained and controlled delivery over a prolonged period of time.
- Reduction in side effects associated with systemic toxicity i.e., minimization of peaks and troughs in blood-drug concentration.
- Improved patient acceptance and compliance
- Direct access to target or diseased site, e.g., treatment of skin disorders such as psoriasis, eczema, and fungal infections.
- Ease of dose termination in the event of any adverse reactions either systemic or local.

- Convenient and painless administration.
- Ease of use may reduce overall health care treatment costs.
- Provides an alternative in circumstances where oral dosing is not possible (in unconscious or nauseated patients).

Figure 9: Breast patch

Limitations

- A molecular weight less than 500 Da is essential to ensure ease of diffusion across the subcutaneous membrane, since solute diffusivity is inversely related to its size.
- Sufficient aqueous and lipid solubility, a Log P (octanol/water) between 1–3 is required for the permanent to successfully traverse the SC and its underlying aqueous layers for systemic delivery to occur.
- Intra-and intervariability associated with the permeability of intact and diseased human skin. This implies that there will be fast, slow and normal skin absorption profiles resulting in varying biological responses.
- The barrier nature of intact subcutaneous membrane ensures, that this route is only applicable for very potent drugs that require only minute concentrations (e.g. 10–30 ng/ml for nicotine) in the blood for a therapeutic effect.
- Pre systemic metabolism; the presence of enzymes in the skin such as peptidases and esterases might metabolize the drug into a form that is therapeutically inactive, thereby reducing the efficacy of the drug.
- Skin irritation and sensitization; referred to as the "Achilles heel" of dermal and transdermal delivery. The skin as an immunological barrier may be provoked by exposure to certain stimuli, this may include drugs, excipients, or components of delivery devices resulting in erythema, oedema, etc.[23-24]

DOCKING STUDIES:[25]

Molecular docking studies are used to find out the interaction between a ligand/ drug and a protein at the atomic level which allows us to characterize the behavior of drugs in the binding site of targets as well as explains fundamental biochemical processes.

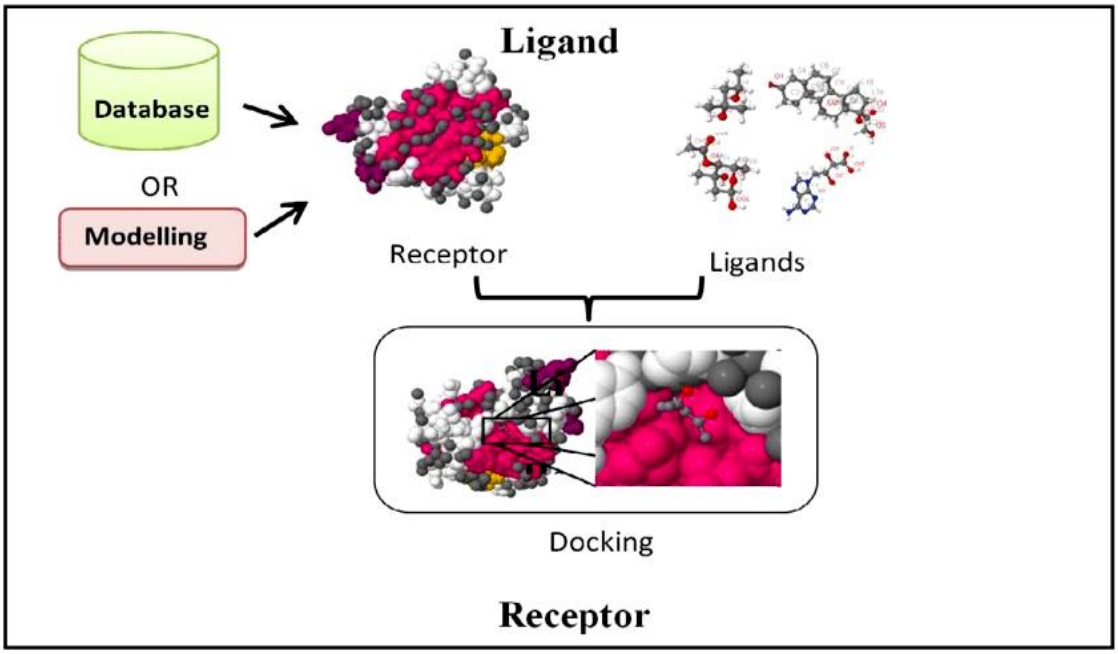


Figure 10: Molecular docking

TYPES OF DOCKING:

Rigid docking (lock & key):

In rigid docking, the internal geometry of both the receptor and ligand are treated as rigid.

Flexible docking (induced fit):

An enumeration on the rotations of one of the molecules is performs. Every rotation the energy is calculated, later the most optimum pose is selected.

Key stages in docking:

- Target/ Receptor selection and preparation
- Ligand selection and preparation
- Docking
- Evaluating docking results

SOFTWARE USED IN MOLECULAR DOCKING

- **SANJEEVINI**-IIT Delhi

  (www.scfbio.iitd.res.in/sanjeevini/sanjeevini.jp)

- **GOLD-** University of Cambridge, UK
  (www.ccdc.cam.ac.uk/solutions/Goldsuite/Pages/GOLD.asp)
- **AUTODOCK-** Scripps Research Institute, USA

  (autodock.scripps.edu/)

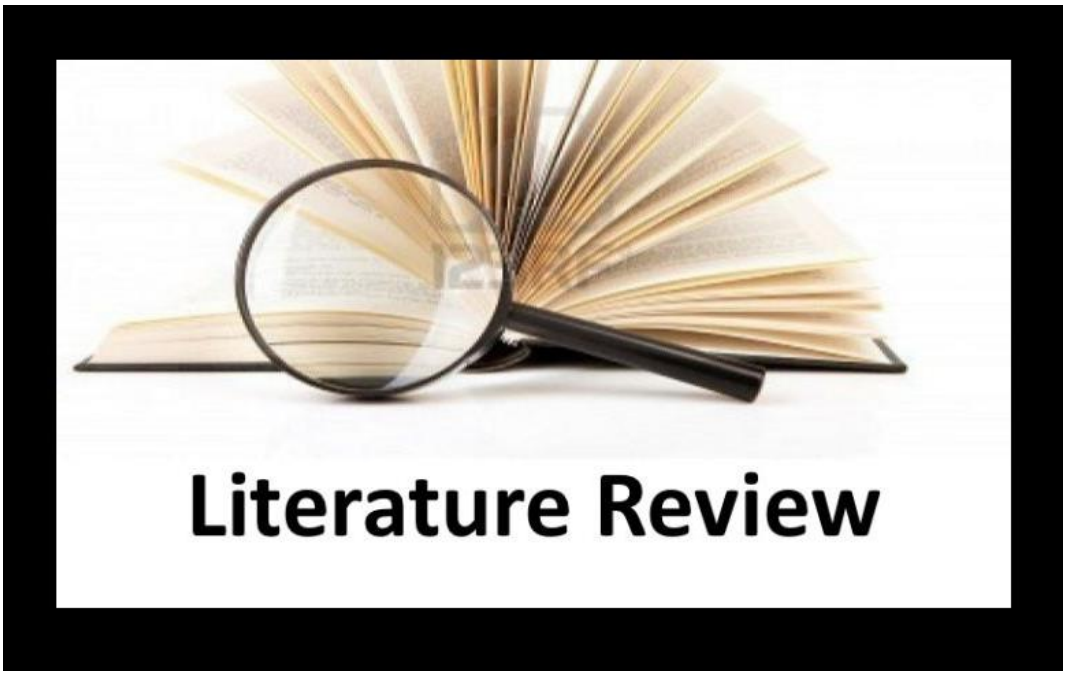
- **GemDock** (Generic Evolutionary Method for Molecular Docking)-

  National Chiao Tung University, Taiwan (gemdock.life.nctu.edu.tw/dock/)

- **Hex Protein Docking –** University of Aberdeen, UK

  (hex.loria.fr/)

- **GRAMM** (Global Range Molecular Matching) Protein docking- A centre for Bioinformatics, University of Kansas, USA
  (www.bioinformatics.ku.edu/files/vakser/gramm/)


Literature Review

2. REVIEW OF LITERATURE[26-41]

PHARMACOGNOSTICAL STUDIES

- **Sanjay Kumar Chauhan *et.al* (2015)** developed the Pharmacognostic profile of *Alpinia galanga* willd. (Zingiberaceae). Morphological, macroscopic and microscopic characteristics of leaf and rhizomes were done. Physico-chemical studies like ash values, acid insoluble ash value, extractive values and loss on drying were performed. Phytochemical and fluorescence analysis of plant powder were done according to the methods as prescribed in WHO guidelines and Ayurvedic Pharmacopoeia of India on quality control methods for medicinal plants materials. These studies were used as taxonomic tools for species level identification.[26]

- **Chitra *et.al* (2008)** reported the Pharmacognostical evaluation on the rhizome of *Alpinia galanga* Linn. (Willd) which provide the major diagnostic characters, qualitative chemical and physical tests responsible for the pharmacognostic identity of the rhizomes.[27]
- **Malode UG *et.al* (2017)** studied the microscopic characters of rhizomes, petiole and leaf of *Alpinia galanga* Linn. (Willd) which showed the drug mainly consists of stomata, starch grains, fibers, vessels, volatile oils, resins, tannins and glycosides. Due to the presence of these characters the plant can hold many secondary metabolites.[28]

PHYTOCHEMICAL STUDIES

- ✓ **Kaushik D *et.al* (2011)** carried out pharmacological and phytochemical studies of the plant *Alpinia galanga* which revealed the presence of essential oils, tannins, phenol, glycosides, monoterpenes and carbohydrates in more quantities than other bioactive components.[29]

- ✓ **Subhashree Singh *et.al* (2020)** screened the Chemical constituents analysis of methanolic extract of *Alpinia galanga* and *Alpinia calcarata indicated that t*he major phytochemical constituents found in leaf extracts of *A. galanga* were benzenepropanal and 3-phenyl-2-butanone whereas, rhizome extract contain carotol, Eucalyptol, 5-hydroxymethylfurfural as major constituents.[30]

- ✓ **Tabarak Malik *et.al* (2016)** carried out an evaluation of Phytochemicals, Antioxidant, Antibacterial and Antidiabetic Potential of *Alpinia galanga* and *Eryngium foetidum* Plants of Manipur (India) which showed that the flavonoids, phenols, saponins, tannins, anthocyanines, sterols, triterpenoids and anthraquinones were the most common phytochemicals found in *Alpinia galanga*. Further evaluated the antioxidant, antibacterial and antdiabetic potential of the plant. These findings suggest that these plants could be a promising source of bioactive compounds having therapeutic potentials.[31]

- ✓ **S Mitsui et al (1976)** extracted the Constituents from seeds of *Alpinia galanga* Wild, by using various fractionation steps. Further the constituents were tested for invivo anti-ulcer activity using shay rats.[32]

PHARMACOLOGICAL STUDIES

ANTICANCER ACTIVITY

- **Riris Jenie *et.al* (2021)** investigated the antiproliferation activity of galangal extract (GE) on HER2- overexpressing breast cancer cells by analyzing GE (Galangal Extract) cytotoxicity and its mechanism of action. Cytotoxic test indicated that GE has modest cytotoxicity toward HCC1954 (IC50 >200 μg/mL) and GE increased cell accumulation at the G2/M phase. Although GE showed no induction on apoptosis, it increased the number of senescent cells. This effect correlated with the increasing intracellular ROS level on treatment with GE. Furthermore, when combined with doxorubicin (Dox), GE increased the Dox's cytotoxicity. *Alpinia galanga* produced antiproliferation activity against HER2-overexpressing breast cancer and was associated with its capability to induce cell senescence and intracellular ROS level, causing halted cell cycle progression.[33]

- **Khozaymeh *et.al* (2018)** investigated the inhibitory and cytotoxic effect of Kouchner (Alpinia galanga L) extract on cell growth with a potential of selective effect on malignant cells of squamous cell carcinoma (SCC) compared with normal cells. The results showed that the percentage of viable cells is significantly correlated with different concentrations of Kouchner (*A. galanga* L) extract ($p > 0.001$) and is

significantly correlated with the type of cell in the extract concentrations above 20% ($p = 0.023$). However, there is no significant relationship on the extract concentrations below 20% ($p = 0.123$).The results of this study showed that cytotoxicity of the Kouchner extract at concentrations above 20% on malignant cell line (SCC) has been higher than normal cell lines, and, in concentrations below 20%, they are similar. According to this study, the Kouchner extract may be used as a natural substance with targeted antimalignancy properties and with less side effects against SCC.[34]

- **Andi suhendi *et.al* (2017)** determined Acetoxy Chavicol Acetate (ACA) concentration and cytotoxic activity of Alpinia galanga extract (AGE) from three local markets on HeLa, MCF7 and T47D cell lines. The galangal used from three local markets namely Pasar Legi Surakarta, Beringharjo Yogyakarta, and Wonogiri. The extraction was performed by maceration using 96% ethanol as solvent. ACA quantitation using UV spectrophotometer at $\lambda = 208.5$ nm. Samples were prepared by liquid-liquid extraction using an ethyl acetate. Cytotoxic activities were performed by MTT assay. The result showed that the concentration of ACA of AGE from the three local markets were 3.798; 0.035; and 0.009 % w/w, respectively. Cytotoxic activity, describes as IC50 value, on HeLa cell line of AGE from three local markets, in order were 13.26; 36.32 and > 100 µg/ mL. Meanwhile, AGE from Pasar Legi on MCF7 and T47D cell lines have IC50 value of 15.80; 12.50 µg/ mL, respectively. In contrast, two other samples have IC50 values of greater than 100 µg/ mL. The highest activity was from the highest concentration of ACA on the samples.[35]

- **Saeed Samarghandian *et.al* (2014)** investigated the potential of galangal rhizomes to induce cytotoxic and apoptotic effects in the cultured human breast carcinoma cell line, (MCF-7) in compare with the non-malignant (MRC-5) cells. The results showed that the ethanolic extract of galangal rhizomes decreased cell viability of the malignant cells in concentration- and time- dependent manner. The IC50 values against MCF-7 were determined at 400.0 ± 11.7 and 170.0 ± 5.9 µg/ml after 48 and 72 h respectively. The morphology of MCF-7 cells treated with the ethanolic extract confirmed the cell proliferation assay results. *Alpinia galanga* induced apoptosis in MCF-7 cells, as determined by flow cytometry. We concluded that the extract of *Alpinia galanga* exerts

pro-apoptotic effects in a breast cancer-derived cell line and could be considered as a potential chemotherapeutic agent in breast cancer.[36]

- **Muhammad Da'I *et.al* (2019)** determined the selectivity of ethanol extract, ethyl acetate fraction, and methanol fraction of of galangal, and ACA on cancer cell lines. Cytotoxic activity was carried out using the MTT method on T47D breast cancer, WiDr colon cancer, HeLa cervical cancer, and Vero normal cell lines. The results showed that galangal ethanol extract and its fractions had selectivity index equal to or less than 2 on cancer cells. Meanwhile, ACA had selectivity index more than 3 on T47D cell and HeLa cell. ACA showed a strong cytotoxic activity against cancer cells T47D, HeLa, and WiDr with IC50 values of 3.14, 7.26, and 12.49 μg/ml, respectively. Based on data, it could be concluded that ACA was the most selective to inhibit T47D cell with a selectivity index of 6.6.[37]

ANTI-INFLAMMATORY ACTIVITY

Subash KR *et.al* (2016) studied the ethanolic extract of *A.galanga* rhizome was scientifically validated for anti-inflammatory screening technique on rats by carrageenan induced pleurisy. Results suggested that the extract reduced the inflammation in rats at 400mg.[38]

Satish R *et.al* (2003) investigated the evaluation of anti-inflammatory activity for total alcoholic and aqueous extracts of rhizome of *A.galanga* Linn using an acute rat model and subacute rat model. At the dose level of 100 mg/kg both exhibited significant anti-inflammatory activity, which was comparable to that of phenylbutazone. [39]

MinHJ *et.al* (2009) Acetoxychavicol acetate (ACA) exhibited significant antioxidant activity and hydroxychavicol acetate (HCA) suppressed T-bet expression, which inturn suppresses IL-2 and IFN gamma induction in Th cells and inhibited T-bet-mediated Th1 cell differentiation. This research suggested that HCA can be beneficial for inflammatory immune disorders.[40]

IMMUNOMODULATOR

Bendjeddou D *et.al* (2003) studied in hot water polysaccharide extracts of *A.galanga* (L.) Willd. were tested for their immunostimulating activity in mice. It is concluded that there was a marked increase in phagocytic activity and high mitogenic effect. 25 mg/kg of polysaccharide extract of *A.galanga* had the most activity with a stimulation index higher than 2.7 and the half life of carbon was 4.30 min.[41]

HEPATOPROTECTIVE ACTIVITY

Hemabarathy B *et.al* (2009) investigated the hepatoprotective effect of the crude extract of *A.galanga* at 200 and 400 mg kg^{-1} were tested against paracetamol induced hepatotoxicity in male Sprague-Dawley rats. Rats with paracetamol induced hepatotoxicity showed significantly decreased serum protein levels and an increased serum AST, ALT and liver MDA levels. While *A.galanga* extract supplemented group maintained serum protein and liver SOD levels with significant decrease in liver MDA levels as compared with the group treated with 3000 mg kg-1 paracetamol.[42]

ANTI-DIABETIC ACTIVITY

Akhtar MS *et.al* (2002) investigated the hypoglycaemic invivo activity in rabbits to know the effects of *A.galanga* rhizome on blood glucose levels. In normal rabbits methanol and aqueous extracts significantly lowered the blood glucose levels while there was no effect in diabetic rabbits after treatment with rhizome powder, methanolic or aqueous extract. [43]

Heera P *et.al* (2014) studied the *in vitro* antidiabetic activity in the methanolic extracts of *A.galanga* shows a considerable inhibition of the haemoglobin glycosylation, α -amylase and α-glucosidase activity in a concentration dependent manner.[44]

ANTI-HIV ACTIVITY

Ye Y *et.al* (2006) investigated the Rev an regulatory HIV-1 protein that binds and mediates the transport of mRNAs from the nucleus into the cytoplasm for translation into viral proteins. Maximal inhibition of virus production by In the HIV-1 X4-tropic reporter NL4-3-infected or R5-tropic reporter JR-CSF-infected cells, ACA inhibited virus production at 4 μM. In addition ACA and didanosine synergistically inhibited HIV-1 replication. [45]

ANTIPLATELET ACTIVITY

Jantan *et.al* (2005) studied the inhibitory effects of the methanol extracts of *A.galanga* on PAF receptor binding to rabbit platelets was demonstrated which shows that they significantly inhibited PAF at 73.9% and IC_{50} was found to be 5.5 μgml^{-1}. Thus it is a potential source of new PAF antagonist and for treatment of immunological and inflammatory disorders.[46]

ANTI-ULCER ACTIVITY

Matsuda H *et.al* (2003) investigated the effects of 1'S-1'-Acetoxychavicol acetate and 1'S-1'-acetoxyeugenol acetate the phenylpropanoids from the rhizomes of *A.galanga* that demonstrated the inhibition of ethanol-induced gastric mucosal lesions in rats and the mode of action involved in this effect were the increment of glutathione levels of gastric mucosa.[47]

ANTIALLERGIC ACTIVITY

Matsuda H *et.al* (2003) studied the effects of 1'S-1'-acetoxychavicol acetate and 1'S-1'-acetoxyeugenol acetate on ear PCA reaction in mice showed that it dose dependently effective for the immediate phase reactions in type I allergy. Results proven that aqueous acetone extract of the rhizomes of *A.galanga* was found to inhibit release of β-hexosaminidase, as a marker of antigen-IgE-mediated degranulation in RBL-2H3cells.[48]

ANTIMICROBIAL ACTIVITY

Taechowisan *et.al* (2009) studied the root tissues of *Z. officinale* and *A.galanga* contains 59 endophytic actinomycetes in which 10 isolates could produced the substances antagonistic against *Colletotrichum musae* and *Fusarium oxysporum* and 9 isolates against *Candida albicans.* Endophytic *S.aureofaciens* isolate (CMUAc130) was fermented to get more broth, the ethyl acetate extracts of which exhibited a broad antifungal spectrum.[49]

Janssen AM *et.al* (1985) studied the *A.galanga* extract for the following micro organisms i.e. *Trichophyton mentagrophytes, T.rubrurn, T.concentricum, Epidermophyton floccosum, Rhizopusm tolonifer, Penicillium expansum, Aspergillus niger*; yeast, *Candida albicans*; bacteria, *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus* for testing its anti-microbial potency. The results show that the essential oils and

acetoxychavicol acetate from the rhizomes of *A. galanga* showed some activity especially against the gram-positive bacteria, the yeast and the fungi.[50]

Rao K *et.al* (2010) investigated the antibacterial activity of methanol extracts of root at acidic pH (5.5) were excellent than solvent of low polarity. The inhibition zone of methanol root extract towards *S. typhimurium* was more, compared to positive standard where as *K. pneumoniae* and *P. aeruginosa* were more susceptible to acetone and diethyl ether extracts. The leaf extracts of *A. galanga* in acetone were more efficient in inhibiting *K. pneumoniae* and *P. aeruginosa*.[51]

ANTIOXIDANT ACTIVITY

Mahae N *et.al* (2009) studied the Galangal extracts obtained by using 50 and 75% ethanol at 30°C and 50°C exhibited high inhibition percentage (33.58-36.63%) as they act as most effective radical scavenger, followed by the water extract and the essential oil which was calculated from DPPH test. IC50 values of the galangal ethanolic extract (10.66 mg/ml), water extract (55.48 mg/ml) and essential oil (455.43 mg/ml) were higher than those of α-tocopherol and butylated hydroxyanisole. Similarly ethanolic extract have high ORAC values.[52]

Melanathuru V *et.al* (2017) studied the 1'-acetoxychavicol acetate isolated from the extract of this plant showed good antioxidant property.[72] Total phenol and flavonoid content of aqueous extracts of rhizome *A.galanga* were found to be higher. DPPH assay, nitric oxide scavenging assay, Fe^{3+} reducing power assay and phosphomolybdenum reduction assay proved that aqueous extract at 120µg/ml has high antioxidant activity.[53]

Padma S Vankar *et.al* (2006) investigated the ethanolic extract of *A.galanga* showed the potent scavenging activity by DPPH method with the IC_{50} value of 69.5±1.375 µg/ml, by lipid per oxidation method with the IC_{50} value of 77±1.876 µg/ml, hydrogen peroxide radical scavenging activity with the IC_{50} value 55±1.59 µg/ml, ABTS radical scavenging method with the IC_{50} value 0.086±1.10 µg/ml.[74] Compared to dichloromethane and methanol crude extracts, methanolic extract showed greater antioxidant activity.[54]

CARDIOPROTECTIVE ACTIVITY

Ravichandra V *et.al* (2014) studied the cardioprotective effect of Galangin on Doxorubicin induced cardiotoxicity in rats. Rats treated with doxorubicin exhibit abnormal ECG pattern while galangin significantly normalized ECG pattern and also reduced the serum biomarkers with increase in the tissue antioxidant level.[55]

DOCKING STUDIES

Manzano *et.al* (2022) investigated *Alpinia galanga* among the Philippine native medicinal plants with extensive studies on its phytopharmacological properties yet reports on its human placental aromatase inhibitory activity remain rudimentary. Thus, a total of 119 database-derived *A. galanga* secondary metabolites was molecularly docked onto the catalytic site of human placental aromatase. Drug-likeness was assessed *in silico* using Swiss ADME. Of the screened compounds, galanolactone **(1)**, 4-(3,4-dimethoxy-*trans*-cinnamoyl)-*trans*-cinnamic acid **(2)**, isocoronarin D **(3)**, quercetin **(4)**, β-sitosterol **(5)**, (*E*)-8ß,17-epoxylabd-12-ene-15,16-dial **(6)**, galangin **(7)**, labda-8(17),12-diene-15,16-dial **(8)**, 7-(4-Hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one **(9)**, and 3,5,7-trihydroxy-4-methoxyflavanone **(10)** conferred highest binding affinities against aromatase ranging from binding energies of -8.7 to -8.0 kcal/mol with notable formed hydrogen bonds and interactions against key amino acid residues. Top-ranked compounds exhibited druggability with at most one violation of the Lipinski Rule of Five (LRo5). Overall, the study indicates the potential of top *A. galanga* secondary metabolites as promising drug pharmacophores in developing therapeutics against breast cancer.[56]

Reetuparna Acharya *et.al* (2019) studied certain phytochemicals which has potent actions on ERα, PR, EGFR and mTOR inhibition. The current study is performed by the use of molecular docking as protein-ligand interactions play a vital role in drug design. The 3D structures of ERα, PR, EGFR and mTOR were obtained from the protein data bank and docked with 23 3D PubChem structures of furanocoumarin compounds using FlexX. Drug-likeness property was checked by applying the Lipinski's rule of five on the furanocoumarins to evaluate anti-breast cancer activity. Antagonist and inhibition assay of ERα, EGFR and mTOR respectively has been performed using appropriate *in-vitro* techniques. The results confirm that Xanthotoxol has the best docking score for breast

cancer followed by Bergapten, Angelicin, Psoralen and Isoimperatorin. Further, the *in-vitro* results also validate the molecular docking analysis. This study suggests that the selected furanocoumarins can be further investigated and evaluated for breast cancer treatment and management strategies.[57]

FORMULATION STUDIES

Balakrishnan *et.al* (2009) formulated minoxidil niosomes using thin film-hydration method and characterization was also made to improve low skin penetration and bioavailability in the hair loss treatment.[58]

Manosroi *et.al* (2013) compared the penetration of papain from gel formulations containing niosomes and nanospheres loaded with papain. This study demonstrated that niosomes showed the enhancement of rat skin transdermal absorption of papain and the improvement of scar reduction in rabbit ear model.[59]

Kamble *et.al* (2013) developed and evaluated *Gymnema sylvestre* extract-loaded niosomes which demonstrated significant increase in antihyperglycemic activity and was comparable with glibenclamide.[60]

Arafa, M.G. *et.al* (2018) formulated and evaluated propolis-based niosomes as oromuco-adhesive films and further randomized clinical trial of a therapeutic drug delivery platform was performed for the treatment of oral recurrent aphthous ulcers in which this films produced very satisfactory outcomes in patients with RAUs in terms of ulcer size reduction, prolonged duration of pain relief and reduced ulcer healing time.[61]

Shefrin *et.al* (2019) formulated and characterized midazolam loaded niosomal transdermal patches prepared by thin film hydration method for overcoming the frequent dosing and lower bioavailability complications associated with conventional therapy. This formulation showed better entrapment, release with permeation profile for the daily management of epilepsy with decreased dosing frequency.[62]


PLANT
PROFILE

3. PLANT PROFILE

Alpinia galanga[63-73]

Synonym : *A.alba, A.bifida, A.carnea*

Family : Zingiberaceae

Scientific Name : *Alpinia galanga* (L.) Willdenow

Common name : Galangal, Greater galangal, Java galangal, Siamese ginger

Parts used : Rhizome and fruit.

Figure 11 : *Alpinia galanga* Plant

VERNACULAR NAMES

Sanskrit : Dharnula tikshra mula, kulanjana, Mahabhara vacha

English : Galanga Major, Great galanga, Java galangal

Hindi : Bara khulanjan, Khulanjan

Tamil : Perarattai, Peria-reta

Arabic : Khulanjan-e-kabir

Telugu : Pedda-dhumpa, Dumpa rastramu

PLANT TAXONOMY

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Subclass : Zingiberidae

Order : Zingiberales

Family : Zingiberaceae

Subfamily : Alpinioideae

Tribe : Alpinieae

Genus : Alpinia

Species : *Alpinia galanga*

DISTRIBUTION

Throughout India, often cultivated in konkan, North Kanara, South India, Bengal and also cultivated Ceylon-Malay Islands. Wild distribution occurs from Southeast Asia (Indonesia, Malaysia, Myanmar, Thailand, Laos and Vietnam) to Southern China (Fujian, Guangdong, Guangxi, Hainan, Yunnan) and Taiwan. Rhizomes and fruits can be easily available in markets and are said to be used for calico-printing in U.P.

AGROECOLOGY

Galangal grows best in warm climates in shaded open areas and is extremely sensitive to frost and drought and will rot easily when left exposed to cold, wet conditions. They can be grown up to an altitude of 1000 m. A rich organic soil, such as sandy loam, provides roots with ample space to grow, and it also allows water to drain properly. The land should be prepared with farmyard manure, well decomposed humus or vermicompost. The crop is usually propagated by rhizome splits. In southern India, the rhizomes should be sown from April to May. In the north-eastern hilly tracts, February to mid-April is the optimal period to cultivate the crop. It can be stored in refrigerator for two to three weeks and also frozen without losing any of its flavor. Flowering and fruiting occurs during the rainy season.

Habitat

It usually grows in forests, scrub or grasslands, at an altitude from 100 to 1300m. Prefers a rich, well-drained soil which contain high organic matter and grows well in shade or partial shade. Not tolerable to waterlogged soils or drought.

BOTANICAL DESCRIPTION

Alpinia galanga is tillering perennial herb with tuberous underground, much-branched rhizome.

TASTE: Bitter, Acrid.

Table 5: Description of each parts of *Alpinia galanga* plant

S.NO	PARTS	DESCRIPTION
1.	**Rhizomes**	Subterate, 3-5 cm in diameter, fibrous, hard, shiny pink, pale yellow and aromatic.
2.	**Pseudostem**	Erect formed by rolled leaf shealth.
3.	**Leaves**	1 cm long hairy petiole, leaf blade-oblong lanceolate, 25-60 long by 6-15 cm wide, glabrous pubescent, base attenuate, apex acute or acuminate.
4.	**Flowers**	Yellowish-white to greenish-white and fragrant with tubular calyx and corolla.
5.	**Capsule**	Globose to ellipsoid, 1-1.5 cm, contains 3-6 seeds.

MEDICINAL USES

Treatment of cough, Indigestion, Arthritis, Fever, Joint pains, Hypothermia.

PHYTOCONSTITUENTS

Rhizome- Essential oil (0.3%) - α - pinene(7.1%), β - pinene (11.9 %), linalool, methyl cinnamate (48%), methyl eugenol, limonene((4.3%), cineol (33%), a-thujene (6.2%), terpinen-4-ol (6.0%) and α - terpineol.; quercetin, kaempferol, isorhamnetin, kaemferide, quercetin-3-methyl ether, galangin, l-acetoxychavicol acetate, 1-acetoxyeugenol acetate, galangal A & B, Galanganal and galanolactone.

Seeds- Caryolane- 1,9b-diol and galaganin were produced by the seeds of A. galanga (L.) Willd which suppressed the proliferation of cancer cell lines.

Leaf- Essential oil of *A. galanga* leaf is rich in 1,8-cineole (28.3%), camphor (15.6%), beta-pinene (5.0%), (E)-methyl cinnamate (4.6%), bornyl acetate (4.3%) and guaiol (3.5%).

Stem- contains essential oil such as 1,8-cineole (31.1%), camphor (11.0%), (E)-methyl cinnamate (7.4%), guaiol (4.9%), bornyl acetate (3.6%), b-pinene (3.3%) and a-terpineol (3.3%).

Root contains essential oil such as a-fenchyl acetate (40.9%), 1,8-cineole (9.4%), borneol (6.3%), bornyl acetate (5.4%) and elemol (3.1%).

ETHNO MEDICINAL USES

- Antibacterial, Anti-fungal, Anti-inflammatory, Anti-hepatotoxic, Antioxidant, Immunodulator, Anti-ulcerative, Anti-tumor, and Anti-allergic activities.
- Used to treat stomach pain, back pain, rheumatism, asthma, diabetes, heart disease, disorders of the liver, kidney disease, and to increase the appetite.
- As a substitute for antibiotics, disinfectants, and food seasonings.
- **Seed -** gastric therapy, treat cardiotonic lesions, diuretic, antiplatelet, antifungal, and anti-tumor activities.
- **Tuber -** asthma, fever, dyspepsia, bronchitis, diabetes mellitus, and irritations.

PREPARATIONS

Powder (dose 5 to 10 grains). Tincture (1 in 10), dose - half to 1 drachm. Paste made with any bland oil to apply locally in skin diseases.

ADULTERATION

Alpinia– *A.galanga*, *A.calcarata,* and *A.officinarum*–are all treated as galanga in the world market as they have both similar look and medicinal properties. Sometimes its rhizomes were adulterated with other species such as *A.calcarata, A.conchigera, A.mutica, A.nigra, A.rafflesiana* and *A.scabra.* Dry fruits of *A.galanga* were used as medicine and are easy to adulterate with other species because of the similarity in odour, morphology, chemical constituents and anatomical characters and they are difficult to distinguish.

EXCIPIENT PROFILE

EXCIPIENT PROFILE[74]

1. SPAN 60

Synonym

Sorbitan mono octadecanoate, Sorbitan stearate

Chemical name

Sorbitan mono stearate (Span60)

Molecular formula

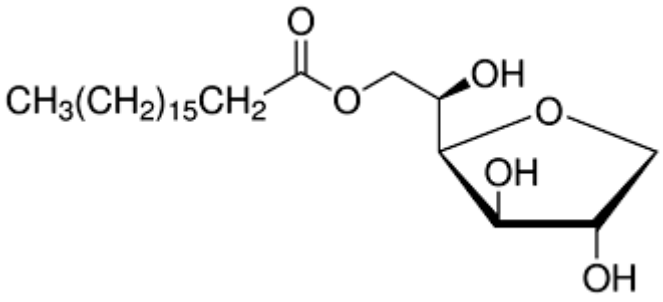
$C_{24}H_{46}O_6$

Molecular weight

430.62

Structural formula

Figure 12 : SPAN 60



Description

Pale yellowish waxy solid, Slightly odour, Bland taste

Physical property

Density - 0.98 - 1.031

Melting point - 57^0C

HLB value - 4.7

Flash point - > 110^0C

Category

Emulsifying agent, Nonionic surfactant, Solubilizing agent,Wetting agent and dispersing agent.

Applications

It is used for cosmetic product, food products, and pharmaceutical formulations.

It is mainly used as an emulsifing agent in the preparation of creams, ointment and emulsion.

Stability and Storage Condition

Gradual soap formation occurs with strong acid or bases, sorbitan esters are stable in weak acids or bases. Keep container tightly closed. Keep container in a cool, well ventilated area. Do not store above 24^0C (72.2^o F).

Solubility

Insoluble in cold water, soluble in ethanol, isopropanol, mineral oil, and vegetable oil. Insoluble in propylene glycol.

Method of manufacture

Sorbitol is dehydrated to form hexitan (1,4- sorbitan), which is then esterified with the desired fatty acid.

Handling Precaution

1. Keep away from heat and sources of ignition.

2. Avoid contact with eyes.

3. Wear suitable protective clothing, in case of insufficient ventilation.

4. Wear suitable respiratory equipment.

2. CHOLESTEROL

Synonym

Cholesterin

Chemical name

Cholest 5-en3β-ol

Molecular formula

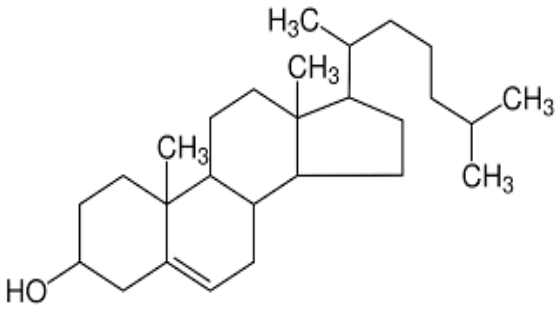
$C_{27}H_{46}O$

Molecular weight

386.67

Structural formula

Figure 13 : CHOLESTEROL



Description

White or faintly yellow colour, almost odourless, pearly leaftlets, Needles, powder granules.

On prolonged exposure to light and air, it acquires a yellow to tan colour.

Solubility

Insoluble in water, soluble in vegetable oil, acetone and chloroform:methanol mixture.

Physical property

Boiling point : 360^0C

Melting point: 147^0C-150^0C

Density :1.052g/cm2

Dielectric constant: 5.41

Stability

It is stable, and should be stored in a well-closed container and protected from light.

Category

Emollient, emulsifying agent.

Application

It is mainly used in cosmetic and pharmaceutical formulations.

It is used in the ointment preparation for emollient activity. Cholesterol additionally has a physiological role.

3. HYDROXY PROPYL METHYL CELLULOSE

Nonproprietary names:

Hypromellose (BNF), Methyl hydroxyl propyl cellulosum

Synonyms:

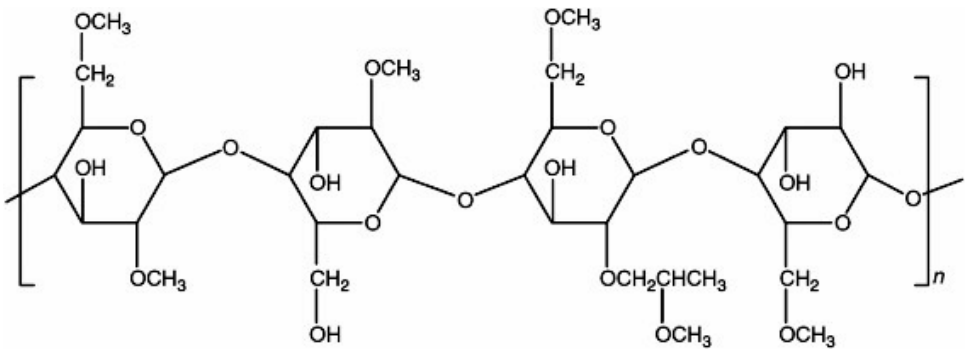
Cellulose hydroxyl propyl methyl ether, Metolose, Methyl cellulose propylene glycol ether,Methyl hydroxypropyl cellulose, Methocel.

IUPAC name:

(2R,3R,4S,5R,6R)-2,3,4-trimethoxy-6-(methoxymethyl)-5- [(2R,3R,4S,5R,6R)-3,4,5-trimethoxy-6-(methoxymethyl)oxan-2-yl]oxyoxane.

Chemical structure:

Figure 14 : HYDROXY PROPYL METHYL CELLULOSE



Molecular formula:

$C_{56}H_{108}O_{30}$

Molecular weight:

1261.45 g/mol

Description:

HPMC absorbs moisture from the atmosphere. HPMC as a partly O methylated and O-(2-hydroxypropylated) cellulose. It is available in several grades which vary in viscosity and extent of subscription. HPMC defined in the USP XXII specifies the substitution type by appending a four digit number to the non proprietary name, e.g. HPMC1828 . The first two digits refer to the approximate percentage content of the methoxy group & the second digits refer to the approximate percentage content of the hydroxyl propyl group calculated on a dried basis.

Appearance:

White to creamy- white fibrous or granular powder

Odor:

Odorless and tasteless

Melting point:

Browning temperature 190–200°C; charring temperature 225–230°C.

Acidity / alkalinity:

pH= 5.5–8.0 (1% w/v aqueous solution)

Nominal viscosity:

15000 mPa s

Density :

0.25-0.70 g/cm3

Solubility:

Soluble in cold water forming a viscous colloidal solution, a mixture of ethanol & DCM, a mixture of water and alcohol and a mixture of methanol and DCM and practically insoluble in ethanol (95%), chloroform and ether.

Different grades:

HPMC K4M, HPMC K15M, HPMC K100

Storage:

HPMC powder should be stored in well closed container, in a cool, dry, place.

Functional category:

- Coating agent
- Film-former
- Rate controlling polymer for sustained release
- Stabilizing agent
- Suspending agent
- Tablet binder
- Viscosity increasing agent

Applications in pharmaceutical formulations and technology:

HPMC is widely used in oral and topical pharmaceutical formulations. In oral products, HPMC is primarily used as tablet binder, in film coating and as an extended release tablet matrix. Depending upon the viscosity grades, concentration between 2 -10%w/w are used as film forming solutions to film coat tablets. Lower viscosity grades are used in aqueous film coating solution while higher viscosity grades are used with organic solvents. HPMC is also used as suspending agent and thickening agent in topical formulations especially in ophthalmic preparations. Compared with MC, HPMC produces solutions of greater clarity, with fewer undispersed fibers present, and is therefore preferred in formulation for ophthalmic use. Concentration of between 0.45%-1.0% w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solution. HPMC is also used as an emulsifier, suspending agent and stabilizing agent in topical gels and ointments. In addition, HPMC is used as adhesive in plastic bandages and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products

4. ETHYL CELLULOSE

Nonproprietary names: Ethyl-cellulose, Ethocel

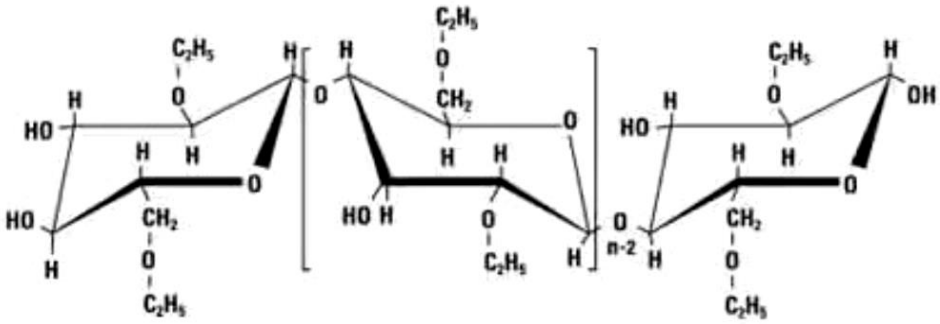
Synonyms: Aquacoat, Aqualon, E462, Ethocel, Surelease

IUPAC name:

2-[4, 5-diethoxy-2-(ethoxymethyl)-6-methoxyoxan-3-yl]oxy-6- (hydroxymethyl) -5-methoxyoxane-3,4-diol

Chemical structure:

Figure 15 : ETHYL CELLULOSE



Molecular formula:

$C_{20}H_{38}O_{11}$

Molecular weight:

454.513 g/mol

Description:

Ethyl cellulose is a hydrophobic ethyl ether of cellulose. It is a derivative of cellulose in which some of the hydroxyl groups on the repeating glucose units are converted into ethyl ether groups.

Appearance:

Free-flowing white to tan powder

Odor:

Odorless and tasteless powder

Melting point:

160-210°C

Viscosity:

18.00 – 24.00 cp

Density:

1.07-1.18 g/cm3

Solubility:

Practically insoluble in water, in glycerol and in propane-1, 2-diol but soluble in varying proportions in certain organic solvents depending upon the ethoxyl content. Ethyl cellulose containing 46 - 48% or more of ethoxyl groups is freely soluble in chloroform, in methanol, in toluene and in ethyl acetate. Freely soluble in mixture of aromatic hydrocarbons with alcohol.

Storage:

Stored between 700C-30°C in a dry area away from all sources of heat and away from bright light. Store in a well closed container.

Functional category

- Coating agent
- Flavouring
- Tablet binder and filler
- Film former
- Rate controlling polymer for sustained release
- Stabilizing agent
- Suspending agent
- Viscosity increasing agent

Applications in pharmaceutical formulations and technology:

Ethyl cellulose is a non-toxic, stable, compressible, inert, hydrophobic polymer that has been widely used to prepare pharmaceutical dosage forms. It is a Polymer Profile stabilizer and thickener for foods. It is currently used in pharmaceutical applications for micro encapsulation of actives, controlled-release matrix systems, taste masking, solvent and extrusion granulation, tablet binding, and as a controlled-release coating for tablets and beads. It is directly extracted from plant fiber and is then chemically modified. It is a kind of cellulose ether and it shows good thermo stability and electric properties. The film made from EC has quite good permeability; it has been used widely as industrial air filter.

5. GLYCERIN

Non proprietary Names

BP : Glycerol

PhEur : Glycerolum

Synonyms

Croderol; E422; glycerine; Glycon G-100; Kemstrene; Optim; Pricerine; 1,2,3-propanetriol; trihydroxypropane glycerol.

Chemical name

Propane 1, 2, 3-triol.

Empirical Formula

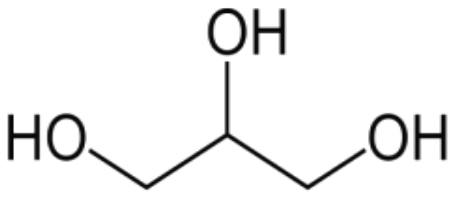
$C_3H_8O_3$

Molecular Weight

92.09

Structural Formula

Figure 16 : GLYCERIN



Description

Its a clear, colorless, odourless, viscous, hygroscopic liquid, it has a sweet taste, approximately 0.6times as sweet as sucrose.

Functional category

Antimicrobial, preservative, emollient, humectant, plasticizer, solvent. Typical properties

Melting point :

17.8^{o}C

Solubility :

Soluble in water, methanol

Applications in Pharmaceutical Technology

- It is used in a wide variety of pharmaceutical formulations including oral, ophthalmic, topical & parenteral preparations. In topical pharmaceutical formulations and cosmetics, it is used primarily for its humectant & emollient properties.
- In parenteral formulations it is used mainly as a solvent.
- In oral solutions, glycerin is used a solvent, sweetening agent, antimicrobial preservative and viscosity increasing agent.
- It is also used as a plasticizer and in film coatings.
- It is additionally used in topical formulation such as creams and emulsions. It is used as a plasticizer of gelatin in the production of soft-gelatin capsules and gelatin suppositories.
- It is employed as a therapeutic agent in a variety of clinical applications, and is also used as a food additive.

Stability and storage conditions

It is hygroscopic. Pure glycerin is not prone to oxidation by the atmosphere under ordinary storage conditions but it decomposes on heating, with the evaluation of toxic acrolein. Mixture of glycerin with water, ethanol & propylene glycol are chemically stable.It may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20^{o}C. It should be stored in an airtight container, in a cool, dry place.

RATIONALE FOR SELECTION

RATIONALE FOR SELECTION OF THE STUDY

Among females, breast cancer, followed by cervix, uteri and ovary cancer were the most common cancer sites. It is reported that, breast cancer accounts for 14% of cancers in Indian women and for every four minutes, an Indian woman is diagnosed with breast cancer.

- The plant chosen is ***Alpinia galanga*** belonging to the family Zingiberaceae was selected for the present work based on ethnomedical uses, ease availability and contains antitumour phytoconstituents such as 1-acetoxychavicol acetate, 1-acetoxy eugenol acetate, flavanoids and galanal-A & B.

- Previous researches revealed that the alcoholic extracts of *A.galanga* showed significant anticancer cancer activity. The anti-tumor constituents being highly water soluble gets excreted easier and has less bioavailability. But **niosome formulation** possesses biphasic features, diversity in design and composition; thus, they are used as carrier for its improved drug solubility, greater entrapment efficiency, bioavailability, stability and targeting.

- Therefore, the main purpose of this study was to prepare phyto-niosomal patch. Invitro drug release, cytotoxicity studies, docking studies were done to prove its bioavailability, cytotoxic potential and their mechanism of action.


Aims &Objectives

AIM AND OBJECTIVES OF THE PRESENT STUDY

AIM

The main aim of the study is to formulate niosomal patch from *Alpinia galanga* extract so as to increase the bioavailability of its therapeutic constituent at the targeted site and to evaluate its cytotoxic activity.

OBJECTIVES

- Collection and Authentication of *Alpinia galanga.*
- Raw materials analysis.
- Extraction of bioactive constituents from *Alpinia galanga*.
- Phytochemical analysis of bioactive constituents of *Alpinia galanga*.
- To predict the molecular mechanism of bioactive constituents of *Alpinia galanga* on the receptor present in the breast by using autodock studies.
- Invitro evaluation of cytotoxic activity of *Alpinia galang*a extract by cell line study.
- Formulation and evaluation of *Alpinia galanga* extract loaded Niosomes.
- Formulation and evaluation of Niosomal patch.
- Screening of niosomal patch for cytotoxic activity.


work
plan

PLAN OF WORK

The present work was planned to be carried out as per the following steps given below:

1. SELECTION OF PLANT

2. COLLECTION AND AUTHENTICATION OF PLANT

3. PHARMACOGNOSTICAL STUDIES

- Organoleptic studies
- Macroscopic studies
- Microscopic studies
- Histochemical studies
- Physicochemical studies

4. PHYTOCHEMICAL STUDIES

- Extraction of bioactive compound
- Preliminary phytochemical evaluation of extract
- Fluorescence study

5. CHARACTERIZATION OF ACTIVE CONSTITUENTS

- TLC Studies
- HPTLC Studies
- IR Studies

6. PHARMACOLOGICAL STUDIES

- Molecular docking studies
- Cytotoxicity analysis of *Alpinia galanga* extract.

7. PREFORMULATION STUDIES OF NIOSOMES

- Physical compatibility
- Solubility
- Compatibility study of extract and excipients by FT-IR

8. FORMULATION OF NIOSOMES

9. EVALUATION PARAMETERS OF NIOSOMES.

- Determination of Percentage yield
- Morphological study of niosomes by scanning electron microscopy (SEM)
- Determination of Particle size
- Determination of Zeta potential
- Determination of drug Content

10. FORMULATION OF *ALPINIA GALANGA* EXTRACT LOADED NIOSOMAL PATCH

11. EVALUATION OF *ALPINIA GALANGA* EXTRACT LOADED NIOSOMAL PATCH

- Weight variation
- Surface pH
- Folding endurance
- % Moisture content
- Moisture uptake
- Drug content study
- Film thickness
- Percentage elongation
- Tensile strength
- Invitro drug release study


MATERIALS &
METHODS

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF PLANTS:

The dried rhizomes of the plant *Alpinia galanga* was collected from Chennai, Tamil Nadu, India, on December 2021 and it was botanically identified and authenticated by Dr.K.N.Sunil Kumar, Research officer and HOD Pharmacognosy Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India.

PHARMACOGNOSTICAL STUDIES [75-83]

Pharmacognostical studies mainly deals with the authentication and standardization of herbal medicinal plants through macroscopical, microscopical characters and physicochemical observations as prescribed by an authoritative source such as World Health Organization (WHO) and these observations must be done before any tests are undertaken, because lack of proper identification leads to the usage of improper drug or adulterated drugs.

ORGANOLEPTIC, MACROSCOPIC AND CYTOMORPHIC EVALUATION:

The color, odor, taste, appearance, nature, smell of the plant material was to be analyzed as per the Pharmacopoeia specification. Macroscopical and microscopical characters of raw materials were studied and are compared with the details mentioned in standard reference books and Ayurvedic pharmacopoeia of India (volume 1 to 4) for their perfect identity. Histochemical studies were also performed using various reagents and observed.

DETERMINATION OF PHYSIOCHEMICAL CONSTANTS [84-87]

LOSS ON DRYING

The test for loss on drying determines both water and volatile matter in the crude drug. The loss on drying test is important when the herbal substances are known to be hygroscopic. An excess of water in herbal materials will encourage microbial growth, presence of fungi, insects and deterioration. In modern pharmaceutical technology, the water content provides information concerning the shelf life and the quality of the drugs.

Loss on drying is the loss of mass expressed as % w/w. About 5 g of drug was weighed in a tarred flat weighing bottle previously dried and again dried at 110°C for 2 hrs and cooled in a suitable dessicator and weighed. The drying was continued and weighed to a constant weight at one hour interval.

It is the loss of mass expressed as percent w/w.

$$\%\ Loss\ on\ drying = \frac{Final\ weight}{Initial\ weight} \times 100$$

DETERMINATION OF ASH VALUES

The ash content of crude drug is generally taken as the residue remaining after incineration. It usually represents the non-volatile inorganic salts like metallic salts and silica naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration, contamination and substitution. This is important parameter for the evaluation of crude drugs. The ash value can be determined by three different methods like total ash, acid insoluble ash and water soluble ash. Sulphated ash is also ash value used to find out the sulphated residue.

TOTAL ASH

Total ash method is designed to measure the total amount of material remaining after ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface. Percentage of total ash content was calculated with reference to the air-dried drug.

Procedure-

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators. Incinerate about 2 to 3 g accurately weighed, ground drug in a tarred silica dish at a temperature not exceeding 450°C until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

$$\%\ Total\ Ash = \frac{Weight\ of\ Residue}{Weight\ of\ Sample\ (Crude\ drug)} \times 100$$

ACID INSOLUBLE ASH

Boiled the ash obtained from the total ash for 5 minutes with 25 ml of dilute hydrochloric acid collected the insoluble matter in an ashless filter paper, washed with hot water and ignited at 450ºC to constant weight. Percentage of acid insoluble ash content was calculated with reference to the air-dried drug.

$$\%\ Acid\ insoluble\ ash = \frac{Weight\ of\ Residue}{Weight\ of\ sample} \times 100$$

WATER SOLUBLE ASH

The difference in weight between the total ash and the residue after treatment of the total ash with water.

Determination of Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450^0C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material.

$$\%\ water\ soluble\ ash = \frac{Weight\ of\ residue}{Weight\ of\ sample} \times 100$$

DETERMINATION OF EXTRACTIVE VALUES

The method determines the amount of active constituents in a given amount of crude drugs when extracted with the solvents. The extraction process of crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents provides the preliminary information on the quality of a particular drug sample.

WATER SOLUBLE EXTRACTIVE VALUE

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml (95 mL distilled water and 5 mL of chloroform)of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air-dried drug was calculated.

$$\%\ Water\ soluble\ extractive = \frac{Weight\ of\ Residue}{Weight\ of\ sample} \times 100$$

ALCOHOL SOLUBLE EXTRACTIVE VALUE

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air- dried drug was calculated.

$$\%\ Alcohol\ soluble\ extractive = \frac{Weight\ of\ Residue}{Weight\ of\ sample} \times 100$$

ETHER SOLUBLE EXTRACTIVE

Ether soluble extractive values determined for evaluation of crude drugs which are volatile and non volatile ether soluble extractives. The volatile ether soluble represents volatile oil content of the drug, while non volatile ether soluble represent resin, fixed oils or colouring matter present in drugs. The percentage of ether soluble extractive was calculated.

$$\%\ Ether\ soluble\ extractive = \frac{Weight\ of\ Residue}{Weight\ of\ Sample} \times 100$$

PHYTOCHEMICAL STUDIES

Presence of phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, anthroquinones, proteins and tannins were qualitatively analyzed. The steps involved in the qualitative phytochemical analysis are given below:

- Extraction
- Preliminary qualitative tests
- Fluorescence test

PREPARATION OF EXTRACT [88-91]

Step 1: The rhizomes were collected, washed with distilled water thoroughly and shade dried then powdered.

Step 2: The coarse powder was defatted by using petroleum ether at 50ºC in soxhlet extractor.

Step 3: Then the powder was removed and dried until no trace of pet.ether was observed.

Step 4: The powder was packed again in soxhlet apparatus and extracted with ethanol.

Step 5: The extract was concentrated using rotary evaporator and used for further studies.

PHYTOCHEMICAL SCREENING [92,93]

The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds and subsequently may lead to the drug discovery and development. Phytochemical test were carried out adopting standards procedure.

PRELIMINARY PHYTOCHEMICAL SCREENING

1. TEST FOR ALKALOIDS

Extract of small quantity was treated with few drops of dilute hydrochloric acid and filtered .The filtrate was used for the following tests,

Mayer's reagent (Potassium Mercuric Iodine solution)

Alkaloid gives cream color precipitate with Mayer's reagent.

Dragendroff's reagent (Potassium Bismuth Iodide solution)

Alkaloids give reddish brown precipitate with Dragendroff's reagent.

Hager's test (Saturated solution of Iodine in Potassium Iodide)

Alkaloid gives yellow colored precipitate with Hager's reagent.

Wagner's test:

Alkaloids yielding reddish brown precipitate with Wagner's reagent.

2. TEST FOR CARBOHYDRATE:

Small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate subjected to the following tests to detect the presence of carbohydrates

Molish's test:

The filtrate treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated sulphuric acid was added along the sides of the tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrate.

Benedict's test:

Filtrate were treated with Benedict's reagent and heated gently, orange red ppt indicates the presence of reducing sugar.

Fehling's test:

The filtrate was treated with 1 ml of Fehling's solution A and B, heated on a water bath. A reddish precipitate is obtains shows the presence of carbohydrate.

Barfoed's Test:

Take 1ml of test solution add 1ml of Barfoed's reagent in a test tube, then keep this test tube in boiling water bath, brick red colored precipitate is formed at the bottom indicating carbohydrate.

3. TEST FOR PROTEIN AND FREE AMINO ACID:

Millon's test:

Few drops of Millon's reagent added to the sample, Appearance of red color shows presence of protein and free amino acids.

Ninhydrin test:

Few drops of Ninhydrin reagent was added to the sample, Appearance of purple colors shows the presence of protein and free amino acid.

Biuret test:

Take equal volume of 5 % sodium hydroxide solution and 1 % copper sulphate were added to the sample, appearance of pink color or purple color shows the presence of protein and free amino acid.

4. TEST FOR FIXED OILS AND FATS:

Spot test:

Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test:

Few drops of 0.5 % alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap with the alkali indicates the presence of fixed oils and fats.

5. TEST FOR STEROID:

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then chloroform layer was subjected to the following tests to detect the presence of steroid.

Libermann- Burchard's test:

The above prepared chloroform solution was treated with drops of concentrated sulfuric acid followed by few drops of dilute acetic acid and 3 ml of acetic anhydride. Appearance of bluish green color indicates the presence of steroid.

Salkowski reaction:

To 1 ml of the above prepared chloroform solution, few drops of concentrated sulfuric acid was added. red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

6. TEST FOR GLYCOSIDES:

Legal's test:

To the filtrate, 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides.

Borntrager's test:

The filtrate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Development of pink color in the ammonia layer a shows the presence of glycosides.

7. TEST FOR FLAVONOIDS:

With aqueous sodium hydroxide solution:

Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow color indicates the presence of flavonoids.

With concentrated sulphuric acid:

To a small portion of extract, concentrated sulphuric acid was added. Development of yellow orange color shows the presence of flavonoids.

Shinoda test:

Small quantity of sample was dissolved in alcohol and then added magnesium pieces followed by hydrochloric acid were added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

8. TEST FOR PHENOLS

- **Ferric chloride test:**

  A small quantity of substance was dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or green colour.

9. TEST FOR SAPONINS:

A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

Foam test:

The extract was diluted with 20 ml of distilled water and it is agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

10. TEST FOR TANNINS:

The powdered drug was mixed with basic lead acetate solution. Formation of white precipitate, which indicates the presence of Tannins.

11. TEST FOR TERPENOIDS:

Chloroform test:

The 5 ml of the extract add few drops of chloroform and concentrated sulphuric acid was added along the sides of the test tube to form a layer. The formation of reddish brown colour indicates the presence of terpenoids.

FLUORESCENCE ANALYSIS [94-96]

Fluorescence analyses were carried out in day light and in UV light. The fluorescence was observed in day light and in short, 254nm and long UV light 365nm respectively. Many crude drugs shows fluorescence when the sample is exposed to UV radiation.

Evaluation of crude drugs based on fluorescence in day light was not much used, as it was usually unreliable due to the weakness of the fluorescent effect.

Fluorescent lamps are fitted with suitable filter, which eliminate visible radiation from the lamp and transmits UV radiation of definite wavelength, several crude drug show characteristic fluorescence useful for their evaluation. Drug powder is treated with various reagents and thus visualized in UV light and fluorescence is thus observed.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS

To the ash of the drug material 50%v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.

Arsenic: Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (Scheele's green) which on boiling gives a red precipitate of cupric oxide.

Borate: The mixture obtained by the addition of sulphuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Calcium: Solution of calcium salts, when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Carbonate: Carbonate, when treated with dilute acid effervescence, liberating carbon dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.

Copper: An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute HCL.

Lead: Strong solution of lead salts, when treated with HCL, yield a white precipitate. Which is soluble in boiling water and is re deposited as crystals when the solution is cooled.

Magnesium: Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

Mercury: Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.

Phosphate: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.

Potassium: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream-coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

Sulphates: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide.

CHARACTERIZATION OF ACTIVE CONSTITUENTS

The concentrated extract was subject to the following analysis

1. UV STUDIES

Estimation of Quercetin content in alcoholic extract

The amount of Quercetin present in the alcoholic extract *Alpinia galanga* was estimated by using calibration curve method. First λ_{max} of the Quercetin was determined by dissolving the substance in buffer solution (pH 5.8) and spectrum was obtained in the range of 200-800 nm. Two peaks were observed for Quercetin at 265nm and 373 nm. The other components of the alcoholic extract were showing many peaks at 373 nm, it was observed that 256 nm was more suitable for the estimation of Quercetin in the extract. Patel et al., (2020) has also estimated the Quercetin using the 256 nm and the method was validated. Hence, 256 nm was used for the estimation of Quercetin present in the alcoholic extract AG. A calibration curve was plotted both concentration vs absorbance value of more than five standard dilutions at the λ_{max} of the Quercetin.

Procedure:

Preparation of standard solution of Quercetin

A standard plot of quercetin was made by dissolving 10 mg in 10 ml of Buffer solution, pH5.8 (A). From this solution 1 ml was diluted to 100 ml of distilled water in volumetric flask (B). From the solution B, different concentrations 1, 2 3, 4 and 5 µg/ml were prepared by transferring 1ml, 2ml, 3ml, 4 ml and 5 ml solution to 10 ml volumetric flask and make up with Buffer solution (pH5.8). The absorbance of these dilutions was measured at 256 nm using buffer solution as blank.

Estimation of Quercetin content in alcoholic extract

10 mg of alcoholic extract was weighed accurately and dissolved in 10 ml Buffer solution (pH 5.8) in volumetric flask (A). From this solution, 1 ml was transferred to another volumetric flask and made up the volume up to 10 ml with Buffer (B). Absorbance of the resulting solution (B) was measured at 256 nm against blank solution. After observing the absorbance, with the help of standard plot of pure Quercetin, the concentration of quercetin present in the extract was determined.

2. **TLC STUDIES:**

The plates were prepared using Stahl TLC spreader. Using silica gel G TLC plates was prepared, air dried until the transparency of the layer disappeared and the plates were then dried at 110°C for 30 minutes and kept in desiccators. Mobile phase Single or mixture of solvent was selected based on the phytoconstituents present in the extracts. Factors such as nature of the components, stationary phase, mobile phase, polarity influence the rate of separation of constituents. From various trials, best solvent was selected which showed good separation with maximum number of components.

Stationary phase - Silica gel 60 F 254

Mobile phase - Toluene: chloroform: acetone (4:2.5:3.5) and Methanol: Glacial acetic acid: Formic acid : Water (3:0.9:0.9:0.5)

Detection - UV light

R_f value was calculated by using the formula

$$\mathbf{R_f} = \frac{\textbf{Distance travelled by solute from the base line}}{\textbf{Distance travelled by solvent from the base line}}$$

3. HPTLC STUDIES:[100]

High Performance Thin Layer Chromatography has become a routine analytical technique due to its advantages of reliability in quantification of analytes at micro and even in Nanogram levels and is cost effective. The *Alpinia galanga* extract was taken and they are subjected to HPTLC study.

HPTLC FINGERPRINT PROFILE

HPTLC is one of the advanced and versatile chromatographic technique which helps in the identification of compound and thereby authentication of purity of herbal drugs. It is very quick process. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of the phytoconstituents which is unique to each plant. The finger print obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extracts.

PROCEDURE

The extracts of *Alpinia galanga* were subjected to phytochemical fingerprinting using High Performance Thin Layer chromatography (HPTLC). The extract were spotted on pre-coated Silica gel G60 F254 TLC plates (Merck, Germany) along with the reference standard viz., quercetin using spotter which were spotted in the form of bands of width 6 mm with a 100 µL syringe on precoated silica gel aluminum plate. The mobile phase used was toluene: ethyl acetate : formic acid (7:3:0.5).

The samples (10 µL) of extract fractions and standard compound quercetin were spotted in the form of bands of width 6 mm with a 100 µL Hamilton syringe on precoated silica gel aluminum plate. Linear ascending development was carried out in a 20 cm x 10 cm twin through glass chamber saturated with the mobile phase. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was placed in UV chamber and observed at 254 nm. R_f values and finger print data were recorded.

FT-IR STUDIES:

The Fourier Transform Infrared Spectroscopy (FTIR) allows the analysis of a relevant amount of compositional and structural information in plants. They are used to characterize and identify the functional group in the compound. FT-IR spectra were taken for the dried sample of extract on the sample area of the FTIR instrument. The extract was taken and mixed with KBr. The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into disk.The scanning range was set to 450–4000 cm^{-1} at a spectral resolution of 4 cm^{-1} before analysis.

PHARMACOLOGICAL STUDIES

MOLECULAR DOCKING STUDY [56-57, 106]

Docking is a computational procedure of searching for an appropriate ligand that fits both energetically and geometrically the protein's binding site. In other words, it is a study of how two or more molecules e.g. ligand and protein, fit together.
It is commonly known that molecular binding of one molecule (the ligand) to the pocket of another molecule (the receptor), which is commonly a protein, is responsible for accurate drug activity.

Molecular docking has been proved very efficient tool for novel drug discovery for targeting protein. Among different types of docking, protein-ligand docking is of special interest, because of its application in medicine industry. Protein-ligand docking refers to search for the accurate ligand conformations within a targeted protein when the structure of proteins is known.

The most popular and commonly used softwares for molecular docking are AutoDock, AutoDock/Vina, GOLD, FlexX, FRED, DOCK and ICM.

AUTODOCK

Auto dock 4.2.5.1 is an automated procedure for predicting the interaction of ligands with biomolecular targets. In any docking scheme, two conflicting requirements must be balanced; the desire for a robust. Current version of Auto dock, using the Lamarckian Genetic Algorithm and empirical free energy scoring function, typically will provide reproducible docking results for ligands with approximately 10 flexible bonds. The quality of any docking results depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results.

Following are the steps employed

- ✓ Protein preparation
- ✓ Ligand preparation
- ✓ Receptor grid generation
- ✓ Ligand docking (Screening)

DOCKING IN CANCER STUDY

Different in vitro, in vivo, and computational methods were employed to assess the anticancer potential of drugs or chemicals. Among these methods, docking has been used widely in drug designing for cancer.

A variety of compounds from plant sources have been reported to possess substantial anticancer properties; however, their modes of action have not been clearly defined. Selected plant-derived compounds that exhibit anticancer activity were subjected to docking simulations using AutoDock 3.0.5.

PREPARATION OF RECEPTOR PROTEIN:

3D crystallographic structures of proteins were obtained from protein data bank (PDB) and those of small molecules were retried from PubChem compound database. Read molecule from the file (allowing reading of PDB coordinate files)

Edit- Charges – compute Gasteiger (for arbitrary molecules)

Edit – Hydrogen – Merge non polar

Saves as.pdb in Auto dock folder

LIGAND PREPARATION:

Ligand – input from file

Ligand – Torsion – Chosen Torsion: Rotatable bonds are shown in green, and nonrotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as

amide bonds and bonds that are made rigid by the used are shown in magenta. Ligand – Torsion- set number of torsion; set the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable

Ligand – output- save as .pdbqt in Auto Dock folder

GRID PREPARATION:

Grid – Macromolecule – Open (open the pdb file that has been saved and then save it pdbqt extension in AutoDock folder.

Grid – Set map types – Open ligand: tools to define the atom types for the grids that will be calculated.

Grid – Grid box – Launches interactive commands for setting the grid dimensions and centre (set dimension of 60:60:60 – centre: centre on macromolecule.)

File – Close saving current.

Grids – Output – save as .gpf (grid parameter file).

Open command prompt [cd AutoDock cd 4.2.5.1 autogrid4.exe –p a.gpf –l a.glg]

PREPARATION OF DOCKING PARAMETERS:

Docking – macromolecules – set rigid file name

Docking – ligand – open

Docking – search parameters- genetic algorithm parameters; this command open a panel file setting the parameters used by each of the search algorithms

Docking – docking parameters: open a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.

Docking – output- Lamarkian GA – save as .dpf (docking parameters file) Open command prompt [autodock4.exe –p a.dpf –l a.dlg]

VISUALIZATION/ INTERPRETATION OF DOCKING:

Analysis – docking – open.dlg (docking log file) file

Analysis – macromolecule open

Analysis – confirmation- play and play ranked by energy: play will use the order of confirmation as they were found in the docking calculations and play ranked by energy will order the confirmation from lowest energy to highest energy.

Analysis – load- information on the predicted interaction energy in shown at the top and individual conformations.

Analysis – docking – show interactions, specialized visualization to highlight interaction between the docked conformation of the ligand and the receptor

IN-VITRO ASSAY FOR CYTOTOXICITY STUDIES [101-102]

For MTT assay, serial two fold dilutions were prepared.

Cell lines and culture medium

The cell lines of MCF7 seeded in 96-well microplates (1×10^6 cells/well) and incubated at 37°C for 24 h in 5% CO 2 incubator and allowed to grow 90% confluence. Then the medium was replaced and the cells were treated with the sample at different concentration such as 25, 50, 100, 200 and 400 µg and incubated for 24 h. The cells were then washed with phosphate-buffer saline (PBS, pH- 7.4) and 20 µL of (MTT) solution (5 mg/mL) was added to each well. The plates were then stand at 37ºC in the dark for additional 4 h. The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrometrically at 570nm.

$$\text{\% of viability} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The percentage of cell viability was expressed as above formula . The concentration that inhibited 50% of cell growth was referred as IC 50 value, which was used as a parameter for cytotoxicity study. The morphological changes of untreated (control) and the cells treated at IC_{50} were observed under bright field microscope after 24 h and photographed. Graphs were plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

PREFORMULATION STUDIES [103, 104]

At various stages during development, it is essential to understand the physiochemical characteristics of compounds or biological entities that can affect their development into final products. Data acquired from such preformulation studies forms an important basis for understanding the potential pharmacokinetics of a drug in humans and animals and the opportunities and limitations for process change as the product is scaled up in manufacture. Preformulation studies are also performed to predict the stability of the formulation during manufacture, transport and storage and thus determine the shelf life of the marketed product.

Definition

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

Before beginning the formal preformulation programs, the manufacturing scientist must ensure the following factors:

- The amount of drug available
- The physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.
- The nature of information, a formulation should have or would like to have.

A) Physical Compatibility study:

Physical admixture of the drug and excipients so as to reflect those expected to be present in the final product will be taken in 2 ml glass vials and sealed. Keep these glass vials at room temperature and 40℃/75 % RH for 20 days. At the end of 10 days, withdraw the sample and analysed for color change.

B) Solubility

Solubility of *Alpinia galanga* extract in water, ethanol and phosphate buffer pH 7.4 was determined at room temperature with the help of magnetic stirrer.

C) Compatibility study of extract and excipient by FT-IR:

The Fourier Transform Infrared Spectroscopy (FTIR) allows the analysis of a relevant amount of compositional and structural information in plants. They are used to characterize and identify the functional group in the compound. FT-IR spectra were taken for the dried sample of extract of *Alpinia galanga* combined with non-ionic surfactant, cholesterol, were taken using FT-IR. The extract and the excipients were taken and mixed with KBr. The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into disk. The disk was applied to the centre of the sample holding device and scanned from 4,500 to 400 cm-1 using FT-IR spectrophotometer.

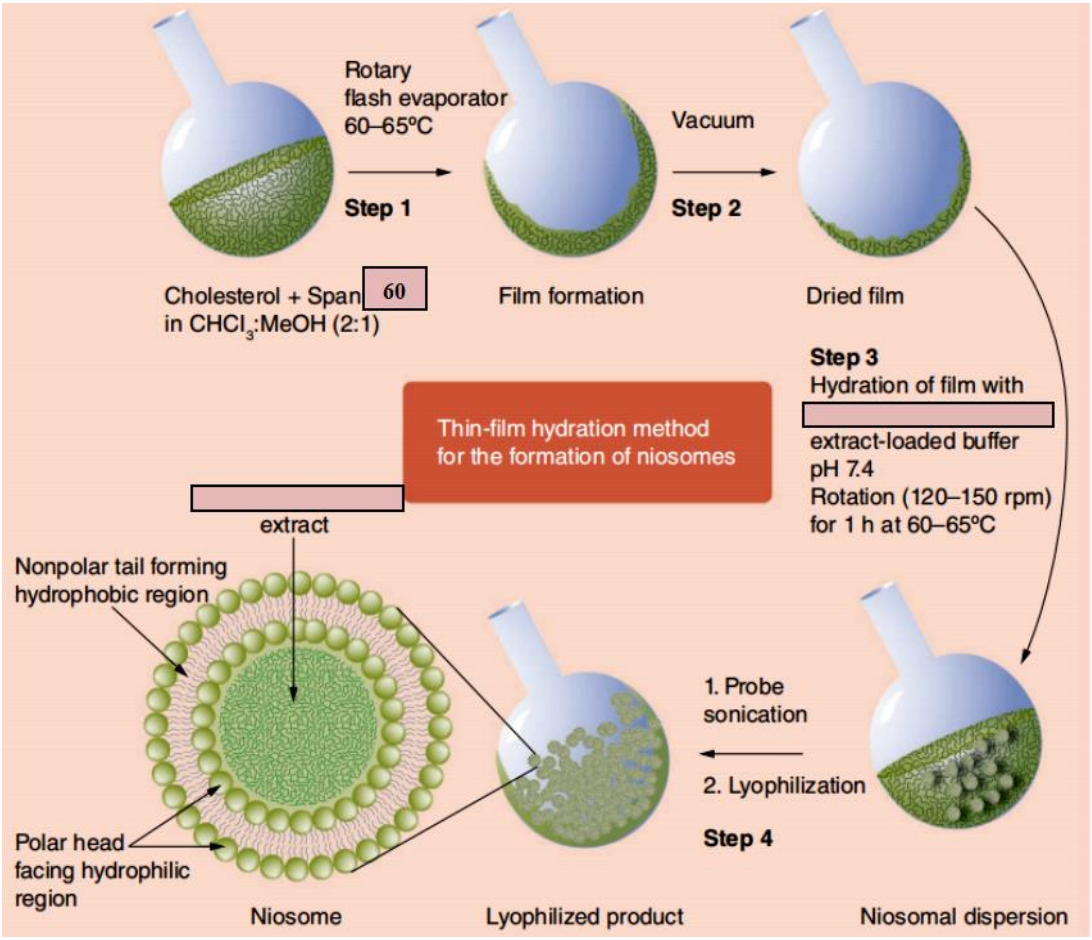
FORMULATION OF NIOSOME[58-60]

Preparation of *Alpinia galanga* loaded niosome by thin-film hydration method

i) Thin-film hydration method was employed for the preparation of *A.galanga* loaded niosomes. Nonionic surfactant and cholesterol were dissolved in 10 ml of chloroform:methanol (2:1) mixture in a round-bottom flask.

ii) The flask was allowed to rotate for 60 min at 120 rpm in a rotary flash evaporator at 60°C under reduced pressure (435 mbar) to obtain a dry film.

Figure 16: Formation of niosomes (Thin film hydration method)



iii) The organic solvents were evaporated slowly using a rotary evaporator at 60◦C under reduced pressure.

iv) This film was hydrated with 10 ml of phosphate buffered saline (PBS, pH 5.8) containing extract and was allowed to rotate further for 60 min under similar conditions.

v) The niosome dispersion was sonicated for 30 min using a probe sonicator in three cycles of 10 min each.

vi) The unentrapped drug was removed by centrifugation at 13,000 rpm, and the obtained niosomal sediment was redispersed in double-distilled water.

vii) The dispersion was again subjected to sonication for 5 min, followed by lyophilization to obtain the free-flowing dry powder.

Table 6: Composition of Galangal extract loaded niosomal (GN) formulations

Formulation No.	**Galangal extract**	**Span 60**	**Cholesterol**
GN 1	1	1	1
GN 2	1	1	2
GN 3	1	2	1

EVALUATION OF NIOSOMES

The evaluation of Niosome includes

A) DETERMINATION OF PERCENTAGE YIELD

Determination of Percentage yield of niosome complex was calculated by the following formula:

$$\textbf{Percentage Yield} = \frac{\textbf{(Practical yield)}}{\textbf{(Theoretical yield)}} \times 100$$

B) SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy was used for surface morphologic and topographic evaluation of developed niosomal formulation. Morphological characteristics will be observed by environmental scanning electron microscope (SEM) -quanta 200. Spread the phytoniosome on a circular aluminium stub precoated with silver glue (for enhancing conductivity of electrons) and placed observation area of the instrument. It will be then observed under the SEM in varying magnifications and record the micrographs. Analyze the sample in SEM at low vacuum. The detector used was secondary electron detector.

C) MEASURING PARTICLE SIZE

The size of phytoniosomes was measured by laser light scattering method in a particle size analyzer, 24 h after preparation for all formulations. Particle size of niosome was measured by particle size analyzer (Microtrac). For the measurement, 1 ml of the formulation was diluted with an appropriate volume of PBS pH 5.8 and the vesicle diameter was determined.

D) DETERMINING THE ZETA POTENTIAL VALUE

The zeta potential values is the most important parameter for physical stability of niosome. ZP value more than +20 mV or less than 20 mV predicts good physical stability of dispersion. For the measurement, dilute 1ml of the sample to 10ml with water, transfer 5ml of this diluted sample to a cuvette and measure the zeta potential.

E) DETERMINATION OF DRUG CONTENT

11 ml of niosome dispersion was extracted with 10 ml of petroleum ether in separating funnel successively three times. Then this mixture was diluted with 10 ml of buffer solution (pH 5.8). After discarding the organic layer containing cholesterol, the aqueous layer was separated, absorbance measured. The amount of Quercetin present in niosome dispersion was estimated from the linearity equation of pure Quercetin.

F) DETERMINATION OF DRUG ENTRAPMENT EFFICIENCY

Entrapment efficiency of the constituents was determined using a centrifugation method. Aliquots (4ml each) of the niosome dispersion were centrifuged at 13,000 rpm for a period of 90 minutes. The clear supernatant solution was collected and analyzed for unentrapped constituents using a UV spectrophotometer at 256 nm (λ_{max}). The UV method was validated for its linearity, accuracy and precision, and the calibration curve was used for the determination of entrapment efficiency. The percentage of entrapped drug was calculated by applying the following equation:

The percentage of entrapped drug was calculated by applying the formula:

Percentage entrapment = (total amount of quercetin in niosome dispersion - amount of quercetin un-entrapped/total amount of quercetin in niosome dispersion) × 100

METHODS FOR PREPARATION OF PATCHES[61-62, 105]

Transdermal patches were prepared by solvent evaporation technique. The polymer (HPMC) and GN were weighed. PEG which acts as plasticizer and permeation enhancer, was used in the concentration of 30%v/v, 10ml distilled water was used as a solvent. PEG 0.3 ml was dissolved in distilled water with stirring. The calculated amount of HPMC (10 mg) was dispersed in distilled water. Galangal niosomes was dispersed uniformly and this solution was then added to polymer base and stirred continuously to get uniform solution. This solution was poured into petriplate coated with liquid paraffin and then dried a room temperature. Then the patches were removed and cut into required sizes and used for further studies. The formulation was done in triplicates.

EVALUATION OF MEDICATED TRANSDERMAL PATCHES

Formulations were evaluated for the following tests:

1) WEIGHT VARIATION TEST:

The study was carried out on 3 films in both type of formulation. The mean weight of the film as well as deviation from the mean was obtained.

2) THICKNESS OF THE PATCH

The thickness of the patch was assessed by using digital vernier caliper at different points of the 3 patch. The average value for thickness of a single patch was determined.

3) DETERMINATION OF FOLDING ENDURANCE:

A patch was repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

4) PERCENTAGE MOISTURE UPTAKE:

The patch were weighed accurately and placed in desiccators containing aluminium chloride. After 24 hrs, the film were taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight. With respect to initial weight. It is calculated by using following formula

$$\text{Percentage moisture content} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

5) PERCENTAGE MOISTURE CONTENT:

The prepared films were weighed individually and kept in a desicators containing fused calcium chloride at RT for 24 hrs. After 24 hrs the films were reweighed and the percentage moisture content was calculated by the given formula,

$$\text{Percentage moisture content} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

6) DETERMINATION OF SURFACE PH

The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min.

7) PERCENT ELONGATION

When stress is applied, a patch sample stretches and this is referred to as strain. Strain is basically the deformation of patch divided by original dimension of the sample. Generally elongation of patch increases as the plasticizer content increases. It is calculated by using following formula.

$$\text{Percentage Elongation} = \frac{\text{Increase in length of patch}}{\text{Initial length of patch}} \times 100$$

8) DRUG CONTENT STUDY:

30 mg of extract was used to prepare 11 ml of solution. This was used to prepare the film and it gave the area of 49 cm (7 x7 cm) and 4 cm area (2x 2cm) of film was used for the dissolution profile. Hence 4cm area of film was taken and dissolved in 10 ml of ethanol. After filtering this solution, it was made up to volume of 10 ml with ethanol in volumetric flask and the resulting solution's absorbance was estimated using ethanol as blank. The amount of Quercetin present in film was estimated from the linearity equation of pure Quercetin.

IN VITRO EVALUATION STUDY OF TRANSDERMAL PATCHES

Cellophane Membrane Treatment:

Cellophane membrane was boiled in the distilled water for 1 hr and washed with fresh distilled water for three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60°C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study.

Drug Permeation Studies:

The *in-vitro* release rate of *Alpinia galanga* niosomal patch were evaluated by open ended tube using PB pH 5.8 as diffusion medium.

Procedure:

1. A beaker containing 100 ml of dissolution medium was placed on a hotplate and warmed the dissolution medium to 37 ± 0.5 °C. The medium was agitated by using magnetic stirrer rotating at 50 rpm.
2. The specified liquid used was phosphate buffer pH 5.8 as the dissolution medium.
3. Film size of 2 x2 cm was tied along with dialysis membrane lower at the lower end of the tube and placed in a beaker and time is noted. Aliquots of 5 ml solution were withdrawn from dissolution medium and the same volume of the dissolution medium was replaced to maintain a constant total volume. The time intervals selected were 0, 1, 2, 3, 4, and 5 hour.
4. Two samples are assayed for each dissolution profile. The withdrawn samples were filtered and the absorbance of the sample dilution was measured at 256 nm (λ max).
5. The cumulative Quercetin release and the % Quercetin release were calculated from the linearity equation.

Identification of Quercetin in the extract and formulation by TLC

THIN LAYER CHROMATOGRAPHIC STUDY:

Procedure:

The chromatographic plates were activated in the oven at 110º C for 30 minutes. Two different solvent systems toluene, ethyl acetate, formic acid & ethanol (5:5:3.1:0.5) and toluene, ethyl acetate, formic acid in the ratio of (4.5:5:4:0.1) and respectively for quercetin was used. The two solvent systems were poured in the TLC chamber separately and allowed to saturate for 45 minutes. The test solutions (plant extract and formulated film) were prepared by extracting the plant extract and formulated film with 5 ml of ethanol and filtered. The tests and standard solutions were applied as spot at a distance of 2 cm from each other with the help of capillary tube and air dried and these were developed in two different solvent system. The chromatogram was allowed to develop $3/4^{th}$ of its size. The plates were withdrawn from the chamber and the solvent front was noted and dried at the room temperature for 15 minutes and in oven for 3 to 5 minutes.

The plates were placed in UV chamber at short and long wavelength. Also the plates were kept in iodine chamber (yellowish brown spot) for 2 to 5 mins or alternatively sprayed with 5% HCL (prepared in methanol) and heated at 100º C for 2-5 minutes until a dark spot is developed. The spots were marked and calculated R_f value for sample and standard solutes. Presence of quercetin in extract and formulation (Film) was identified by thin layer chromatography by comparing solutes R_f value with that of pure quercetin marker compound.

RESULT AND DISCUSSION

RESULTS & DISCUSSION

PHARMACOGNOSTICAL STUDIES OF *Alpinia galanga* RHIZOME

1. MACROSCOPY

Organoleptic characters

Colour **:** Externally reddish Brown, Internally orange yellow

Taste **:** Spicy and sweet

Odour **:** Aromatic

Macroscopical features

Figure 16 : Macroscopy of *Alpinia galanga* rhizome

Length **:** 2 – 10 cm

Diameter **:** 6 - 12 cm

Shape **:** Cylindrical and branched, base of the aerial shoot swelling

and becoming subglobose.

Texture **:** Longitudinally ridged with prominant rounded warts, marked with fine annulations of leaf bases..

Fracture **:** Hard and fibrous; and is rough.

2. MICROSCOPY

Figure 17 : Endodermis

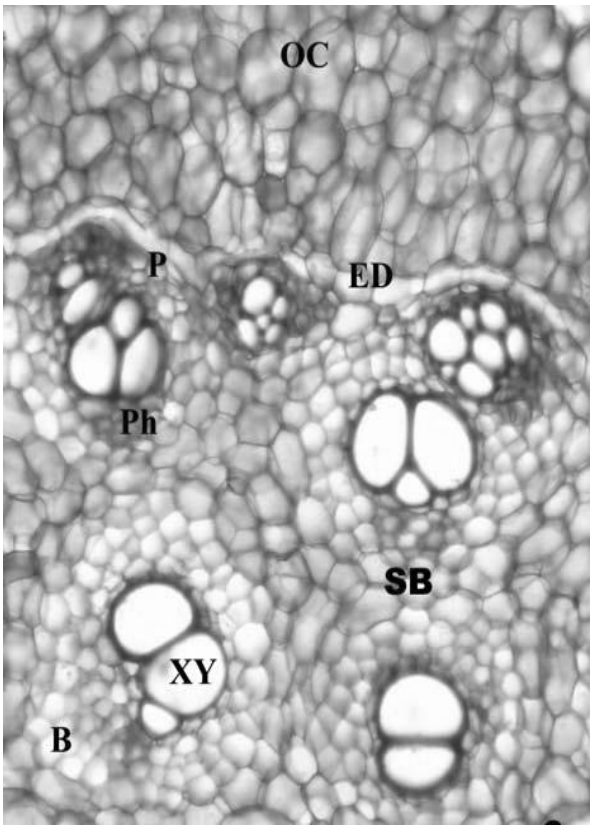


Figure 18 : Outer cortex

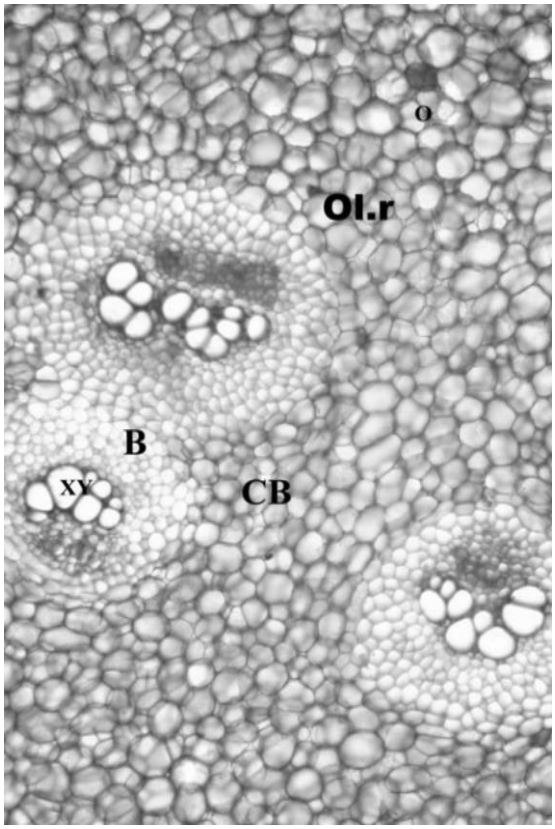
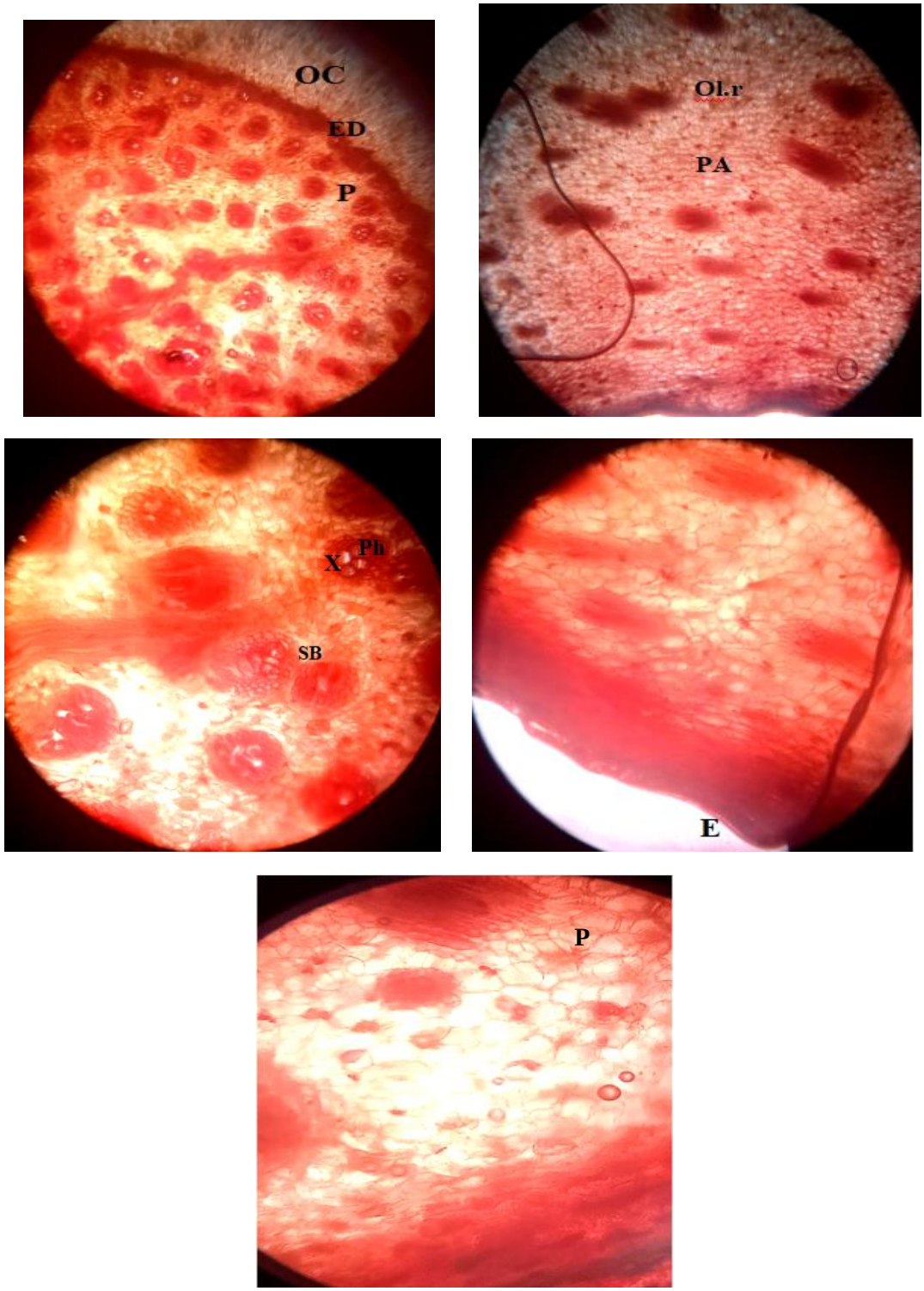


Figure 19 : Microscopy of *Alpinia galanga* rhizome



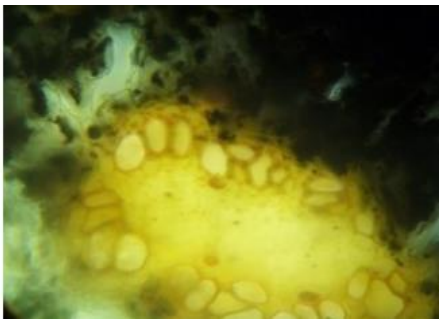
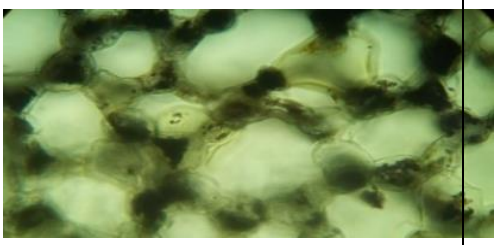
B:Bundle sheath, CB:Cortical bundle, ED:Endodermis, F: Fibres, H: Hypodermal cells, OC: Outer cortex, Ol.r: Oleo resin cells, P: Pericycle, Ph: Phloem, S: Spots of *A.galanga*, SB: Stelar bundles, t: Tracheids, XY: Xylem Vessels

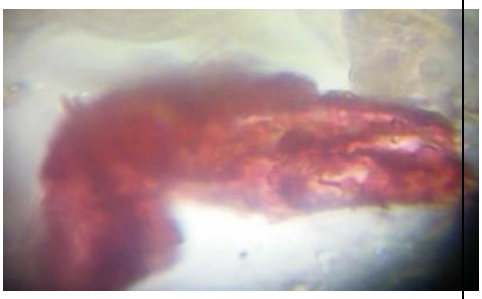
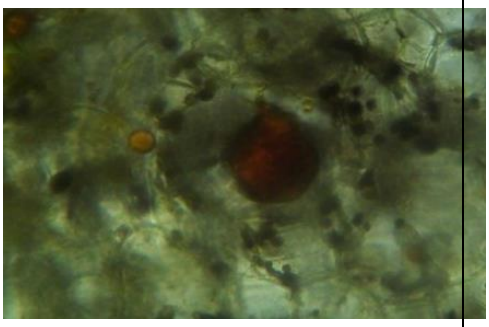
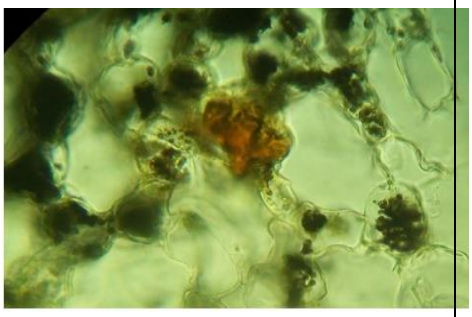
Transverse sections of the rhizome of *Alpinia galanga* reveals the presence of epidermis which are tangentially elongated, single layered oval to rounded; thin walled parenchymatous cells with very thick outer walls. Cortex region of the rhizome possess numerous oleoresin cells, long simple rod shaped starch grains and thin walled polygonal cells with numerous vascular bundles in them. Transport beams and vascular bundles are present in abundance in parenchymatous tissue.

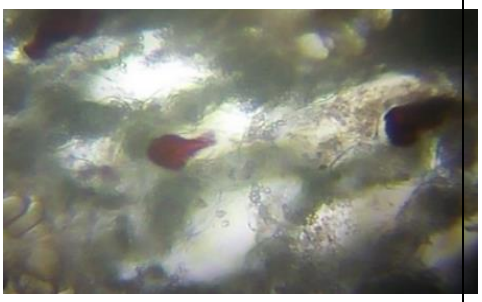
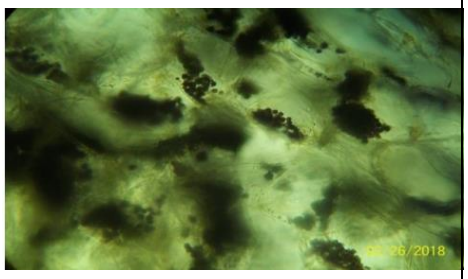
Vascular bundles are almost circular in shape and are composed of xylem and phloem which are of closed collateral type. Several starch grains were found clearly visible within the parenchyma tissue, the narrow beak-like end become black when stained with dilute iodine but the remaining part become light blue or brown. The ground tissue is mostly made of polygonal, rounded, thin walled parenchymatous cells. A row of thin walled cells can be found at the endodermis region.

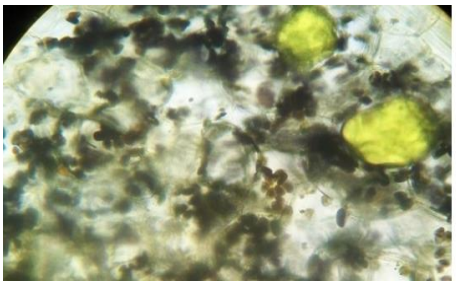
3. HISTOCHEMICAL STUDIES

Table 7: Histochemical studies of *Alpinia galanga* rhizome

S.NO	CHEMICAL	METHOD	INFERENCE
1.	**CALCIUM**	**Alkaline-Pyrogallol method:** Fresh plant sectioned and were treated in the Alkaline Pyrogallol reagent for 5 minutes and washed thoroughly until the sections become destained. Further, the sections were allowed to rest in water for several hours so that the colour developed slowly.	**Figure 20 : Calcium**  Yellowish brown in colour.
2.	**STARCH GRAINS (**Cortex & Stele **)**	**Iodine-Potassium iodide method:** Fresh plant sectioned and mounted in Iodine-Potassium iodide solution.	**Figure 21: Starch grains**  Blue to black in colour.

3.	**CALLOSE**	**Soda method:** The fresh plant materials were sectioned and placed in a 4% aqueous solution of soda for 10 minutes. Later the sections were transfered to glycerin and mounted.	**Figure 22: Callose**  Bright red in colour.
4.	**CHITIN**	**Potassium hydroxide-Iodine- Potassium iodide method:** Fresh plant materials were treated in Potassium hydroxide. Then it was autoclaved at 15 Psi, washed and stained in Iodine- Potassium iodide solution in 1% Sulphuric acid.	**Figure 23: Chitin**  A violet or red-violet colour.
5.	**LIGNIN** (Xylem vessel)	**Maule's reaction:** Fresh plant and treated in 1% neutral potassium permanganate solution for about 5 to 20 minutes. Later it was washed with distilled water and decolourized with 2% dilute hydrochloric acid.	**Figure 24: Lignin**  Lignin stained red.

		Then repeatedly washed thoroughly with water and treated with few drops of ammonium hydroxide solution.	
6.	**PHENOLICS**	**Nitroso reaction:** Fresh plant material were sectioned and 10% sodium nitrite, 10-20% urea and 10% acetic acid were added in equal volumes. Later after 3 - 4 minutes and to this sodium hydroxide were added.	**Figure 25: Phenolics**  Cherry - red colour
7.	**TANNINS**	**Ferric chloride method:** Fresh plant materials were sectioned and placed in 10% formalin solution containing 2% ferric chloride.	**Figure 26: Tannins**  Blue or blue green precipitate.

8.	**PROTEIN**	Fresh plant materials were sectioned and treated with picric acid.	 Protein secretory cells stained in yellow colour.

4. POWDER MICROSCOPY

ORGANOLEPTIC CHARACTERISTICS

Nature : Coarse powder

Colour : Dark brown

Odour : Aromatic odour

Taste : Characteristic taste

MICROSCOPICAL CHARACTERS

Colour : Brown colour powder

Odour : Aromatic agreeable odour and

Taste : Pungent taste.

Powder microscopy of the rhizomes shows the presence of epidermal cells, parenchymatous and oleoresin cells. Elongated, lignified fibres with scalariform/reticulately thickened vessels, parenchyma cells with oil cell, starch grains, xylem vessel with spiral thickening, simple trichome, pitted tracheid, scalariform tracheid and prismatic crystals of calcium oxalate can be seen.

Figure 26: Xylem vessel with reticulate thickening

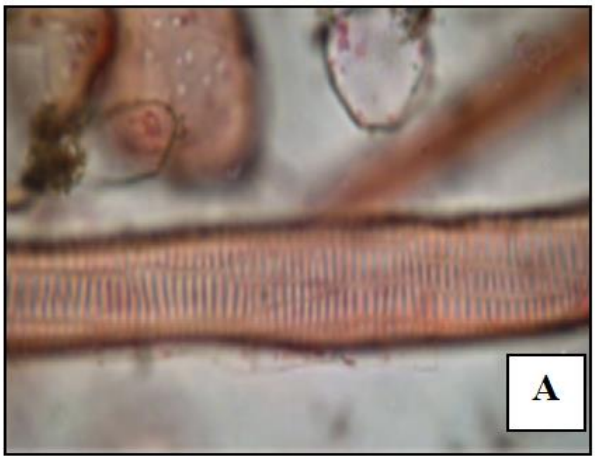


Figure 27: Oil cells

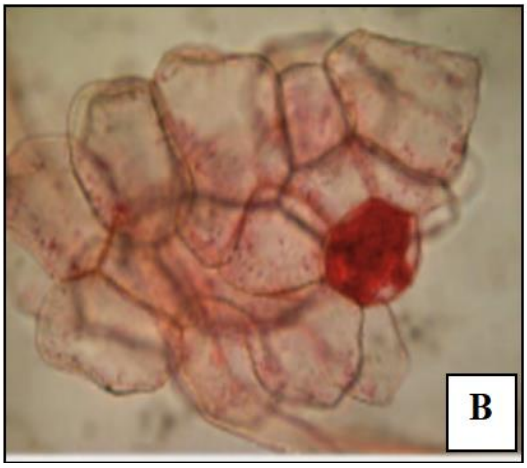


Figure 28: Starch grains

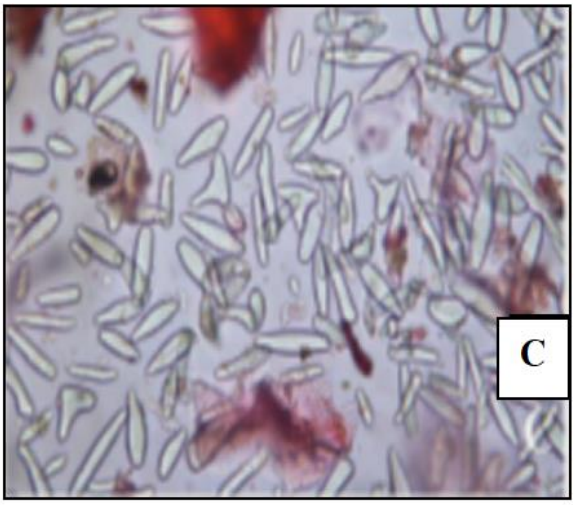


Figure 29: Xylem vessel with reticulate thickening & Tracheid

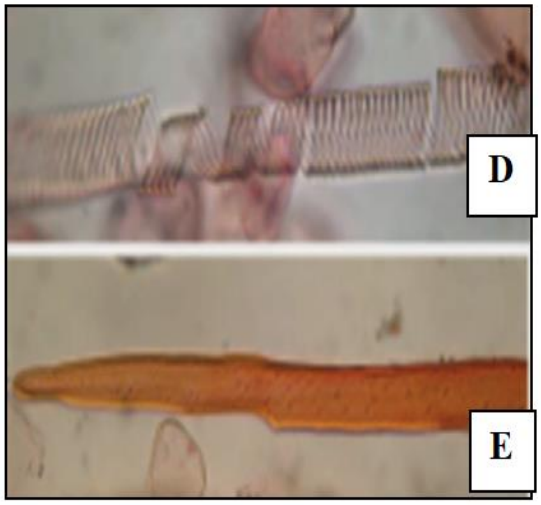


Figure 30: Sinple Trichome

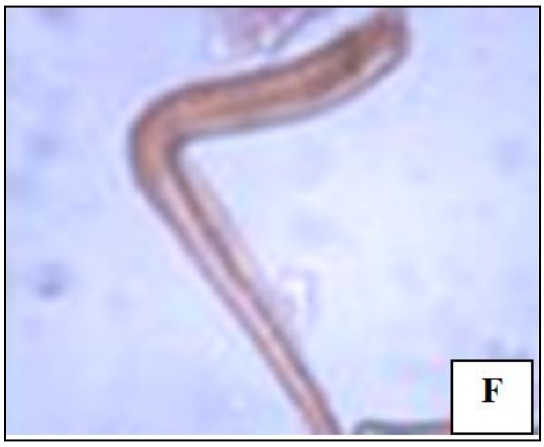


Figure 31: Pitted tracheid

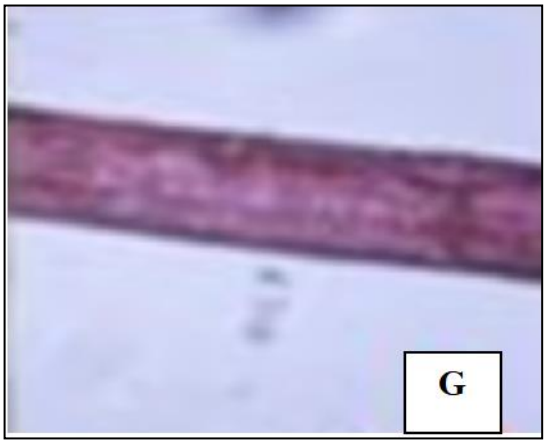


Figure 32: Scalariform tracheid

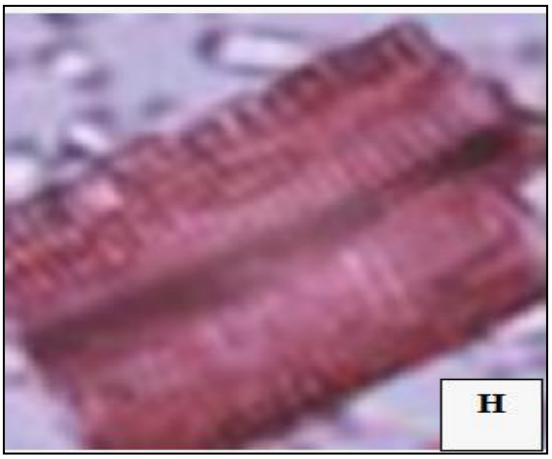


Figure 33: Spiral tracheid

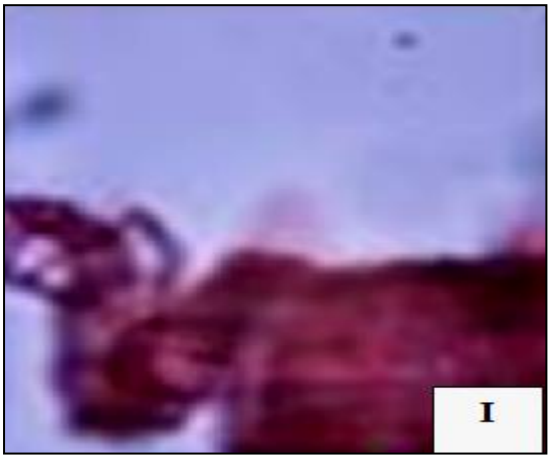
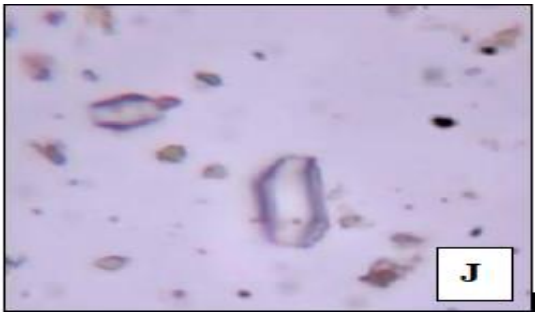


Figure 34: Prismatic Crystals of Calcium oxalate



A. Xylem vessel with reticulate thickening, B .Parenchyma cells with oil cell, C. Starch grains, D. Xylem vessel with spiral thickening, E. Tracheid, F. Simple trichome, G. Pitted Tracheid, H. Scalariform tracheid, I. Prismatic Crystals of Calcium oxalate

PHYSICOCHEMICAL CONSTANTS

The rhizomes of *Alpinia galanga L. Willd* was evaluated for physicochemical constants like total ash values, acid insoluble ash, water soluble ash, extractive value, loss on drying and foreign organic matter and were reported. The results of the physicochemical parameters are given in Table 8.

Figure 35: Ash value

Figure 36: Extractive value

Table 8: Determination of physicochemical parameters

S.NO	PARAMETERS	PERCENTAGE (% W/W)
I	**Ash values**	
1	Total ash	6.22±0.2
2	Acid insoluble ash	2.89±0.2
3	Water soluble ash	3.11±0.3
II	**Extractive values**	
1	Alcohol soluble extractive	8.4±0.2
2	Water soluble extractive	12.4±0.2
3	Ether soluble (volatile) extractive	3.92±0.8
III	**Loss on drying**	4.60±0.5

Values are expressed as Mean ± Standard Deviation, n=3

Qualitative analysis of heavy metals and inorganic elements of *Alpinia galanga L. Willd*

Table 13: Determination of heavy metals and inorganic elements

S.NO	ELEMENTS	OBSERVATIONS
1	Aluminium	-
2	Borate	-
3	Calcium	+
4	Carbonate	-
5	Copper	+
6	Iron	+
7	Magnesium	+
8	Potassium	+
9	Silver	-
10	Sulphates	-

+ indicates presence, - indicates absence

PERCENTAGE YIELD OF EXTRACT

Figure 37: Soxhlet extraction

Figure 38:

Ethanolic extract of *Alpinia galanga*

Table 9: Percentage yield of ethanolic extract of the rhizome of *Alpinia galanga*

S.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD % w/w
1	Ethanol	Soxhlet extraction	Semisolid	Dark brown	7.4

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF OF *A.galanga* RHIZOME

Table 10: Phytochemical analysis of powder and ethanolic extract of *Alpinia galanga* rhizome

PHYTO CONSTITUENTS	PRESENCE + (OR) ABSENCE - IN THE EXTRACT OF RHIZOMES	PRESENCE + (OR) ABSENCE - IN THE POWDERED RHIZOMES
Alkaloid	-	-
Glycoside	+	+
Phenols	+	+
Carbohydrates	-	-
Tannins	+	+
Steroids	+	+
Flavonoids	+	+
Saponins	-	-
Proteins and aminoacids	+	+
Terpenoids	+	+

+ indicates presence, - indicates absence

FLUORESCENCE ANALYSIS

Table 11: Fluorescence analysis of powder and ethanolic extract of *Alpinia galanga* rhizome

Reagent with Powder(P)	Normal Light	UV 254nm	UV 365nm
Dry powder	Light Brown	Green	Yellowish Green
P+ 5% NaOH	Light Brown	Green	Orange
P+ 5% KOH	Light Brown	Green	Black
P+ Dil. Ammonia	Brown	Red	Dark Red
P+ HCl (Conc.)	Dark brown	Red	Dark Red
P+ Iodine	Blue	Dark blue	Dark Red
P+ Nitrocellulose	Brown	Light Brown	Light Brown
P+ Diluted nitric acid (1:1)	Orange	Light yellow	Bright yellow

Fluorescence analysis with extract	Normal Light	UV 254nm	UV 365nm
Extract	Light Brown	Brown	Brown

CHARACTERIZATION OF ACTIVE CONSTITUENTS

1. UV STUDIES

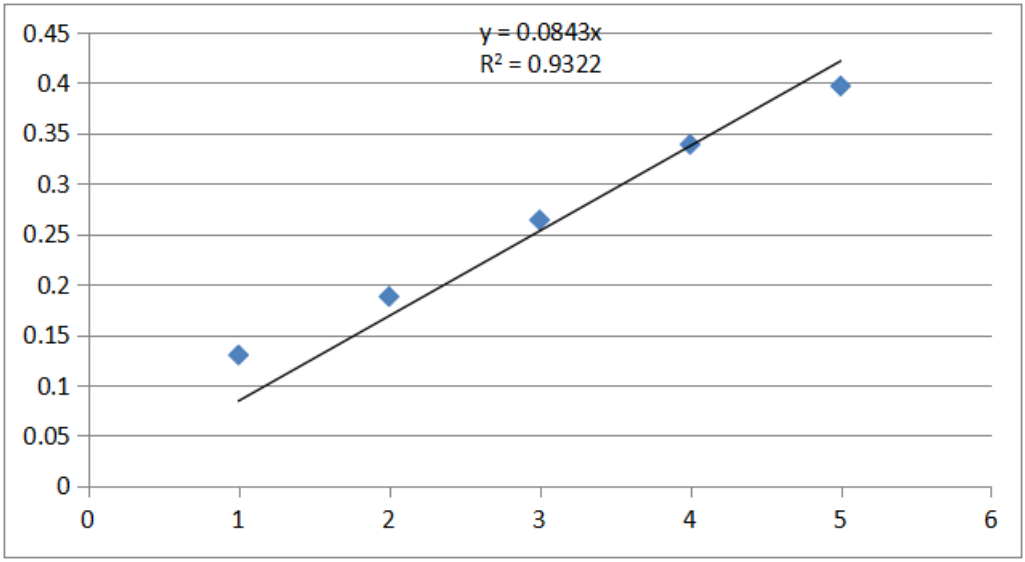
The calibration curve was made by plotting absorbance vs concentration for both quercetin in ethanol and buffer respectively.

QUERCETIN IN ETHANOL

Table 20: UV spectrum at various concentration

Conc. in µg/ml	Absorbance
1	0.13
2	0.188
3	0.264
4	0.339
5	0.397

Figure 64 : Graph for linearity of UV spectrum

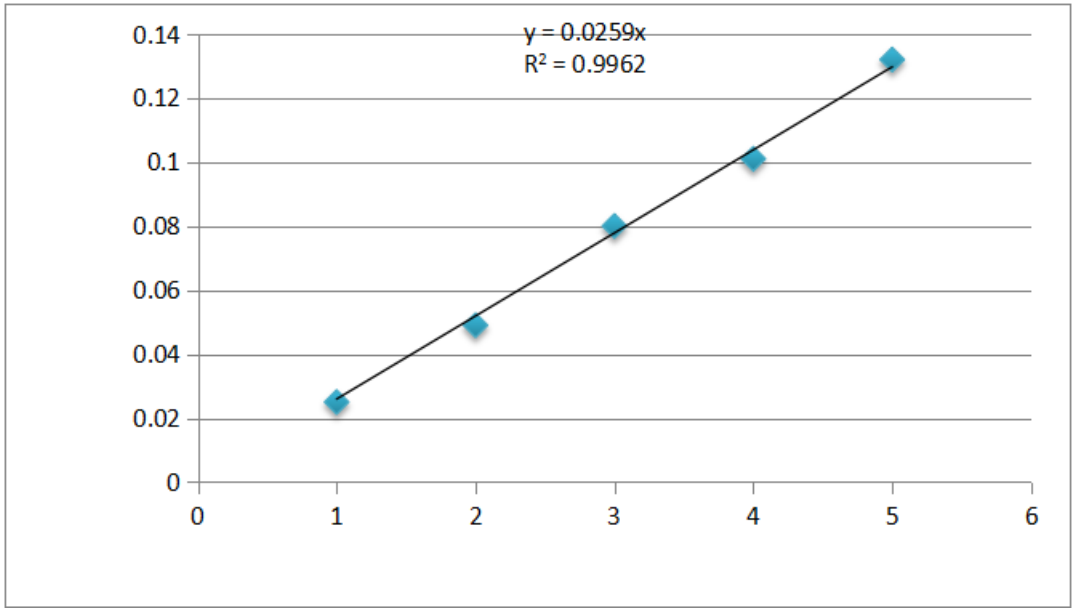


QUERCETIN IN BUFFER

Table 21: UV spectrum at various concentration

Conc. in µg/ml	Absorbance
1	0.025
2	0.049
3	0.08
4	0.101
5	0.132

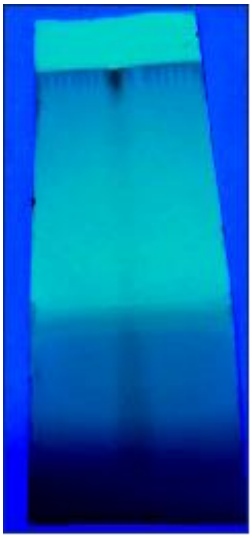
Figure 65: Graph for linearity of UV spectrum



The linearity of different concentration of quercetin was plotted. From this curve the amount of quercetin in the ethanolic extract and the same in buffer can be calculated. Thus 10 mg of *Alpinia galanga* extract in ethanol and buffer contains 1.293 mg and 2.308 mg of quercetin respectively.

2. THIN LAYER CHROMATOGRAPHY STUDIES

Figure 40: TLC of *Alpinia galanga*



Stationary Phase : Silica gel 60 F 254

Solvent system : Toluene: Ethyl acetate: Formic acid (7:3:0.5)

Detection : UV light

Identification mark : Green colored spot

R_f value : 0.52, 0.83, 0.90

3. HPTLC STUDIES

The HPTLC studies of *Alpinia galanga* extract was shown in figure 40, 41, 42 and the R_f values were given in table 12 indicating the various phytoconstituents present in the extract. Peak obtained at 256 nm.

Width : 84

Bands : 6

Volume : 537826839

Displayed Volume : 5358.22

Figure 40: Peaks obtained at 254 nm

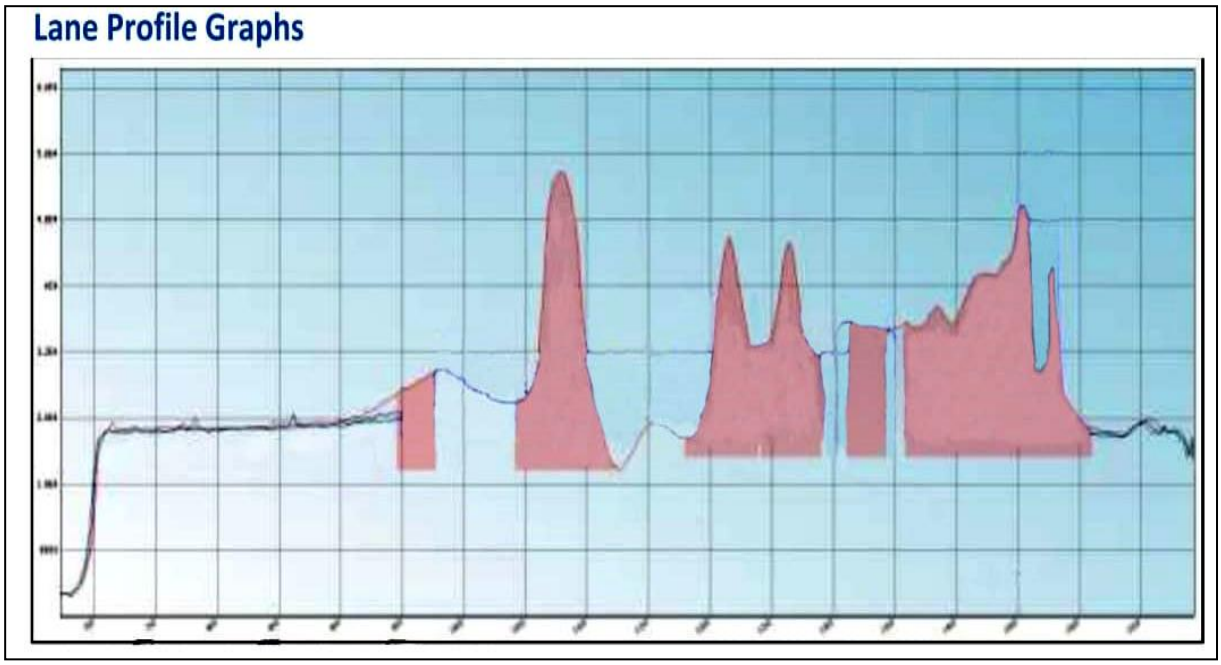


Table 12: Bands obtained at 256 NM

Lane ID	Band ID	Rf	Area	Volume	Displayed Volume
1	1	0.681	2629	27294051	279.36
1	2	0.562	6844	118472154	1234.8
1	3	0.413	4859	67421739	628.97
1	4	0.369	3992	68999812	660.74
1	5	0.283	2743	41075902	410.52
1	6	0.180	1320	207827990	2090.9

6 peaks were obtained in *Alpinia galanga L. Willd extract* with different R_f values and different percentage of areas indicating the presence of various phytoconstituents.

Figure 41: HPTLC at 366 nm

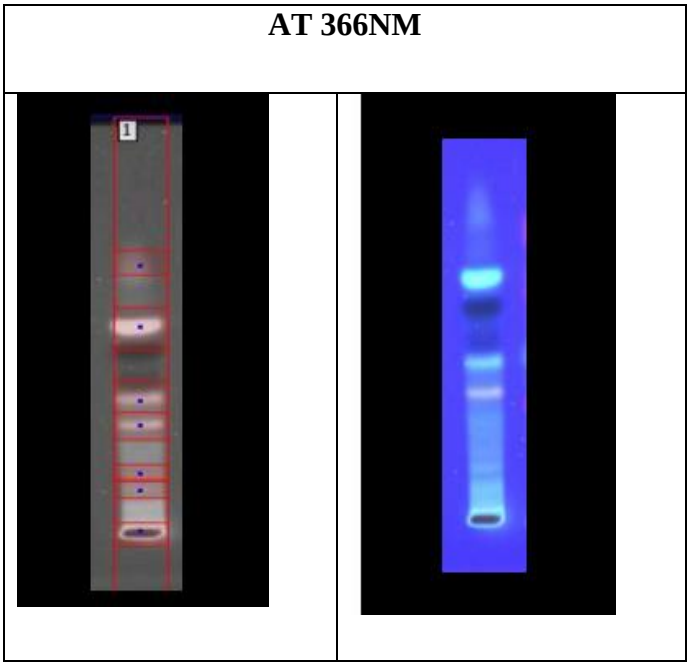
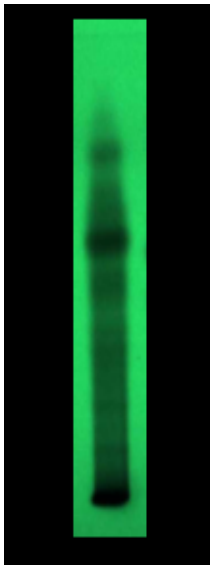
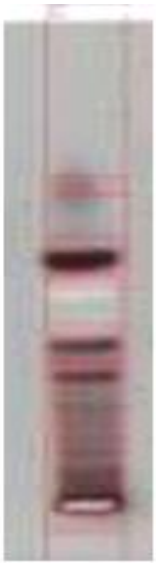


Figure 42: HPTLC at 254 nm

AT 256NM	
	

3. FT-IR Studies:

The FT-IR spectroscopy of the ethanolic extract of *Alpinia galanga* was taken. The results obtained were shown in Figure 43. The interpretation of the FT-IR was given in the table 14.

Figure 43: FTIR spectrum of *Alpinia galanga* extract

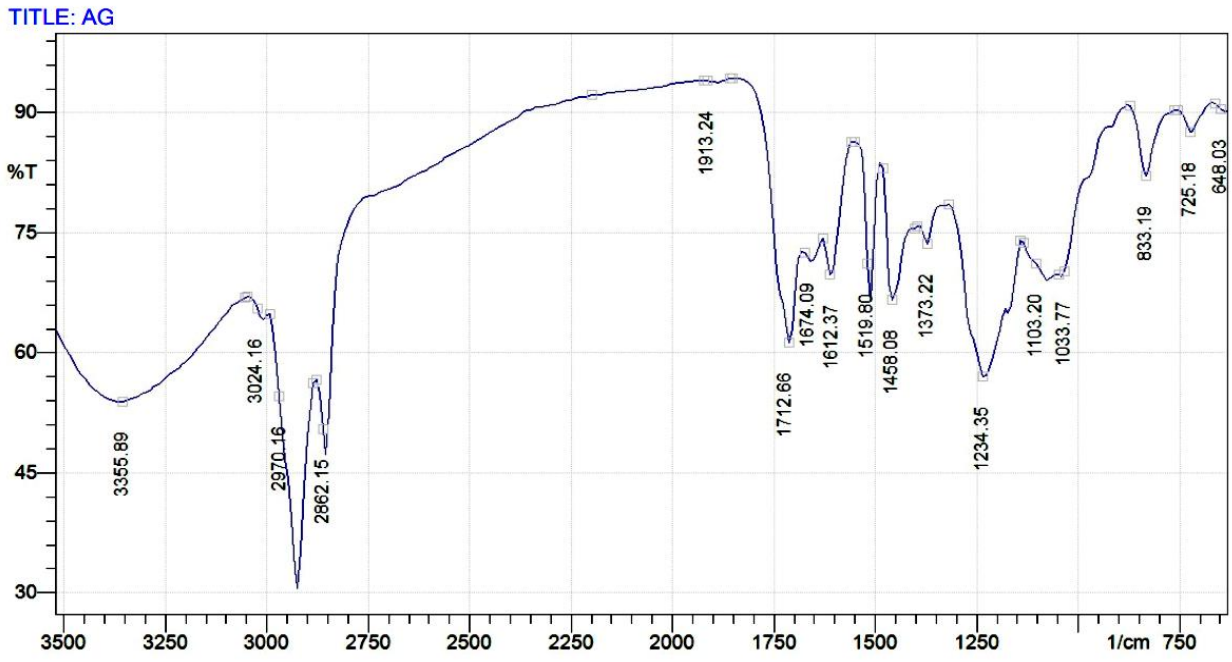
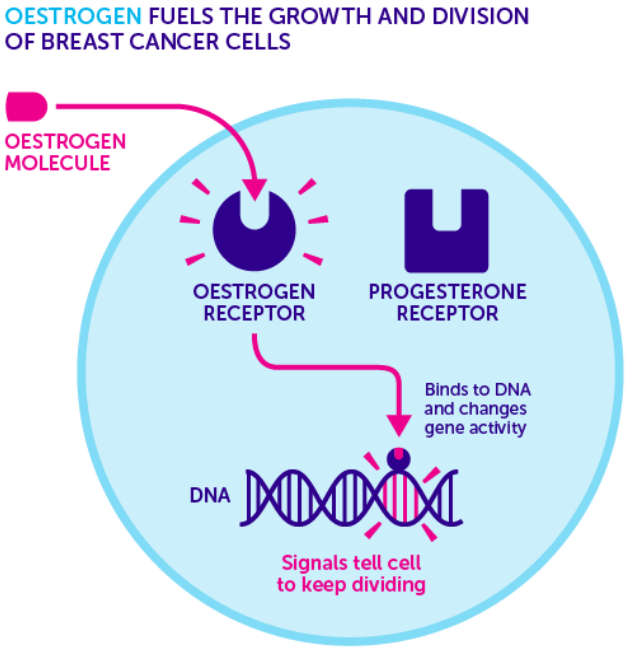


Table 14: FTIR interpretation of *Alpinia galanga* extract

S.NO	WAVE NUMBER cm^{-1}	INTERPRETATION
1	3355.89	O-H stretching
2	3024.16	Aromatic C-H stretching
3	2970.16	CH_3 Symmetric stretching
4	1712.66	C=O Stretching
5	1674.09	C=C Stretching
6	1458.08	C-H asymmetric bending
7	1234.35	C-O Stretching

ESTROGEN RECEPTOR AND BREAST CANCER

Figure 44: Estrogen receptor and its effects in breast cancer



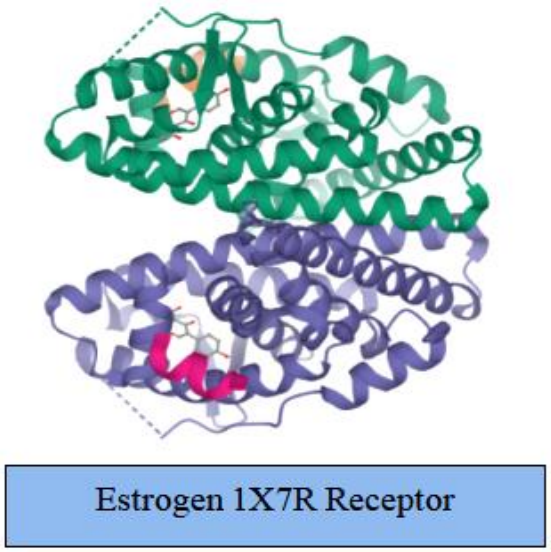
INSILICO EVALUATION OF *Alpinia galanga* CONSTITUENTS AT THE ESTROGEN RECEPTOR 1XR7

The molecular docking studies were done for important constituents present in the *Alpinia galanga* rhizome against estrogen receptor 1XR7 .

Macromolecule Content

- Total Structure Weight: 29.91 kDa
- Atom Count: 2087
- Modelled Residue Count: 244
- Deposited Residue Count: 258
- Unique protein chains: 2

Figure 45: Estrogen 1X7R Receptor



The binding energy of the compounds were analysed using Autodock software and the results were tabulated.

Table 15: Estrogen receptor binding energy of the bioactive components of *Alpinia galanga* rhizome vs standard letrozole

S.NO	COMPOUNDS	BINDING ENERGY (Kcal/mole)
1	Quercetin	-3.9
2	Kaempferol	-3.59
3	1-Acetoxychavicol acetate	-3.48
4	1- Acetoxyeugenol acetate	-2.86
5	Galangal	-4.99
6	Galangin	-4.73
7	Galanolactone	-4.52
8	Isorhamnetin	-4.74
9	Kaempferide	-4.41
10	Quercetin-3-methylether	-3.12
11	Rutin	-1.73
12	Letrozole	-4.41

Most of these bioactive components from *Alpinia galanga* rhizome possess good binding capacity with the estrogen receptor like letrozole (standard) in which these components may have potential to inhibit estrogen binding at the ER-1X7R and thus inhibits further growth of breast cancer cells.

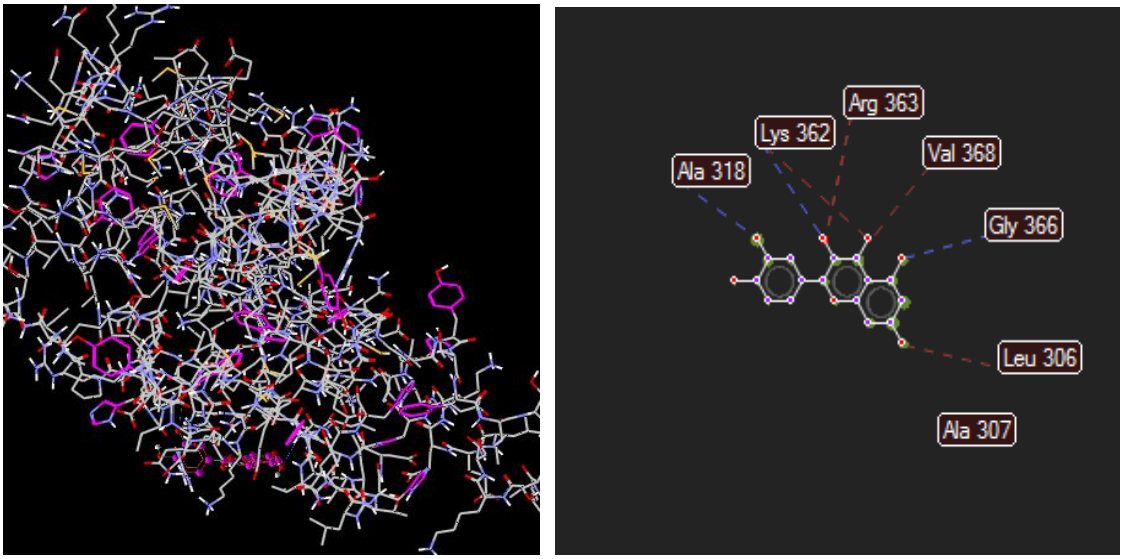


Figure 46 : DOCKING OF QUERCETIN WITH 1XR7

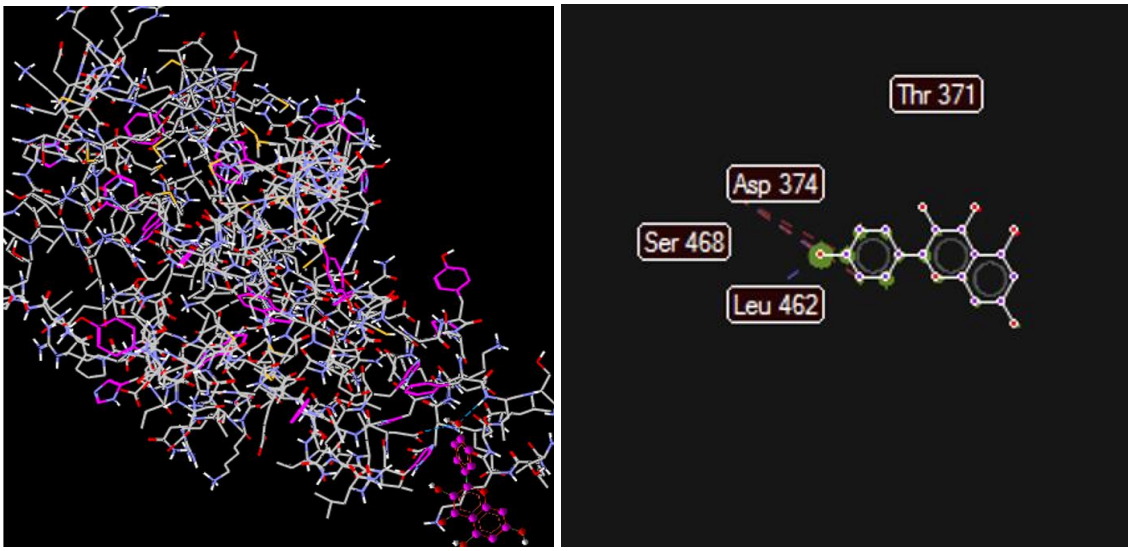


Figure 47 :DOCKING OF KAEMPFEROL WITH 1XR7

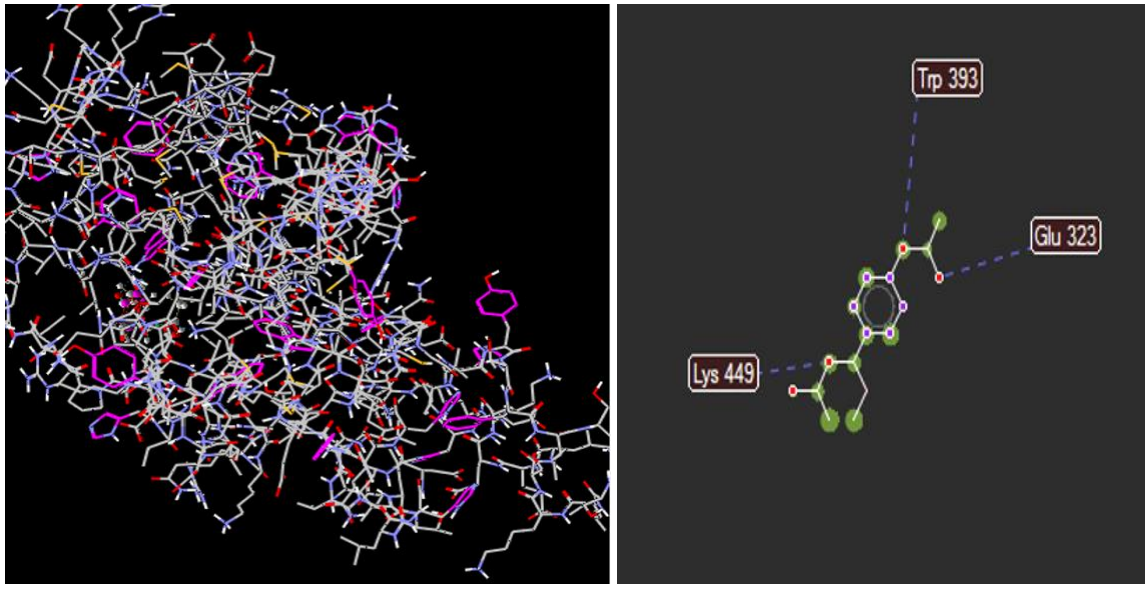


Figure 48 :DOCKING OF 1-ACETOXY CHAVICOL ACETATE WITH 1XR7

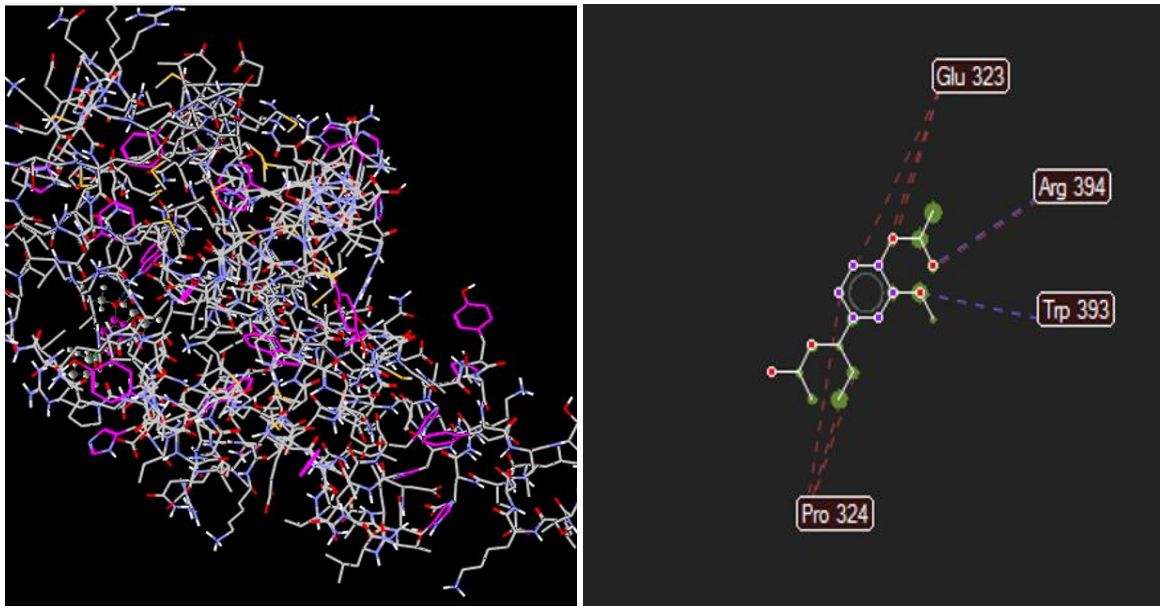


Figure 49 :DOCKING OF 1-ACETOXY EUGENOL ACETATE WITH 1XR7

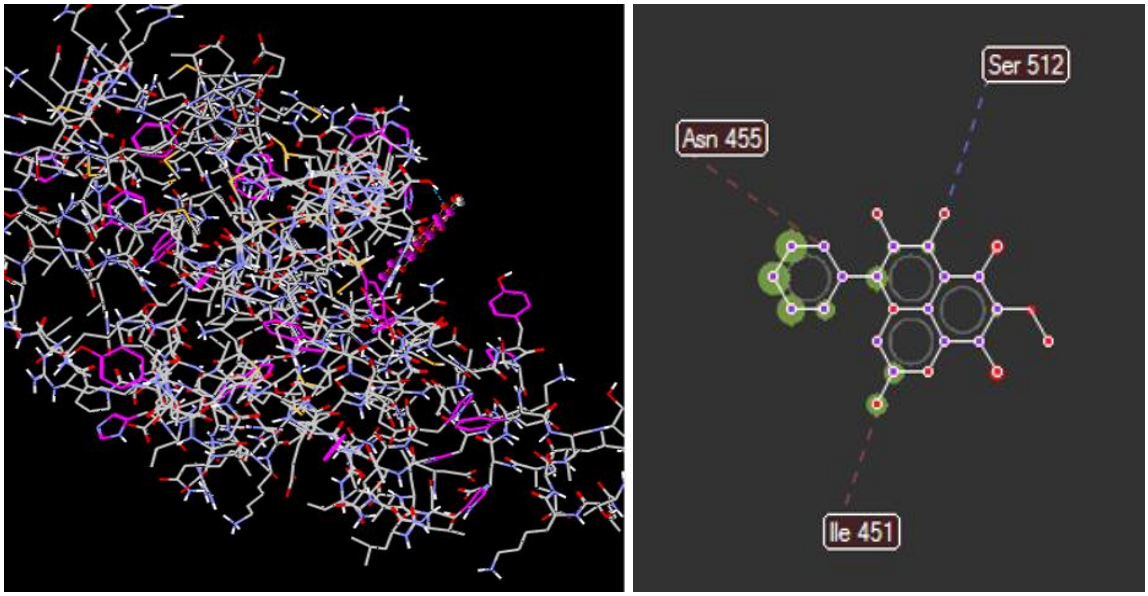


Figure 50: DOCKING OF GALANGAL ACETATE WITH 1XR7

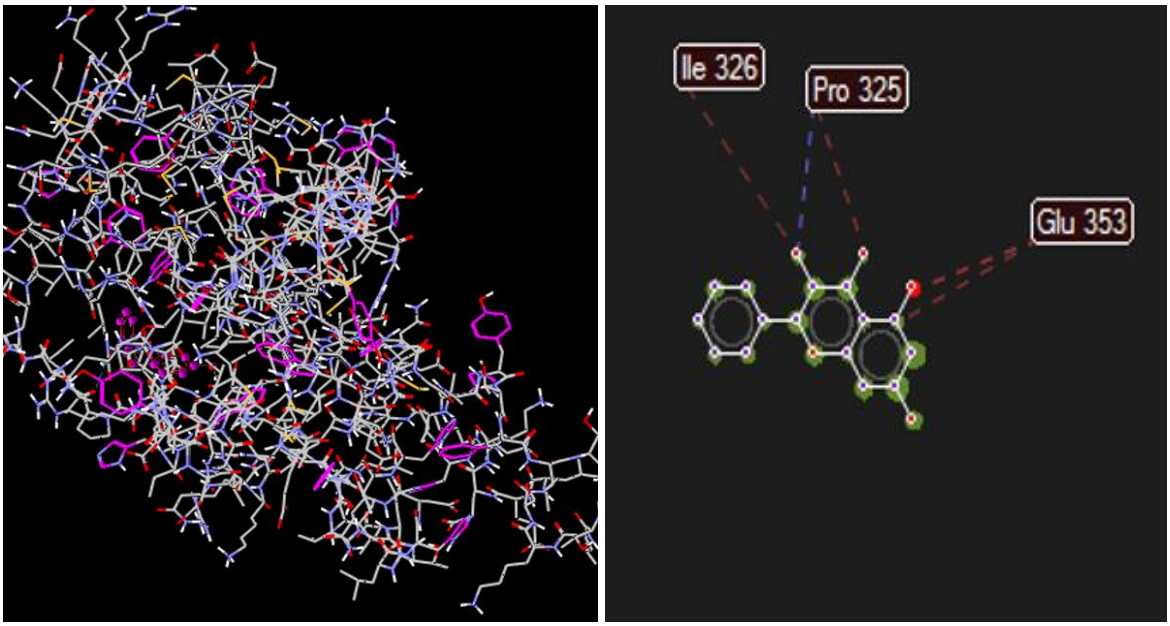


Figure 51: DOCKING OF GALANGIN WITH 1XR7

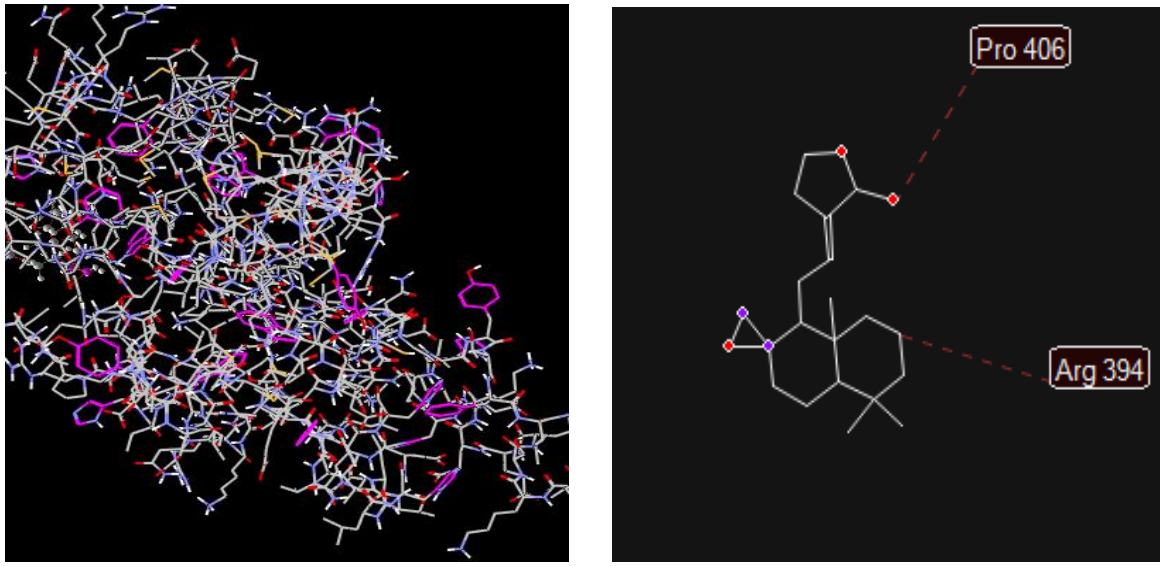


Figure 52: DOCKING OF GALANOLACTONE WITH 1XR7

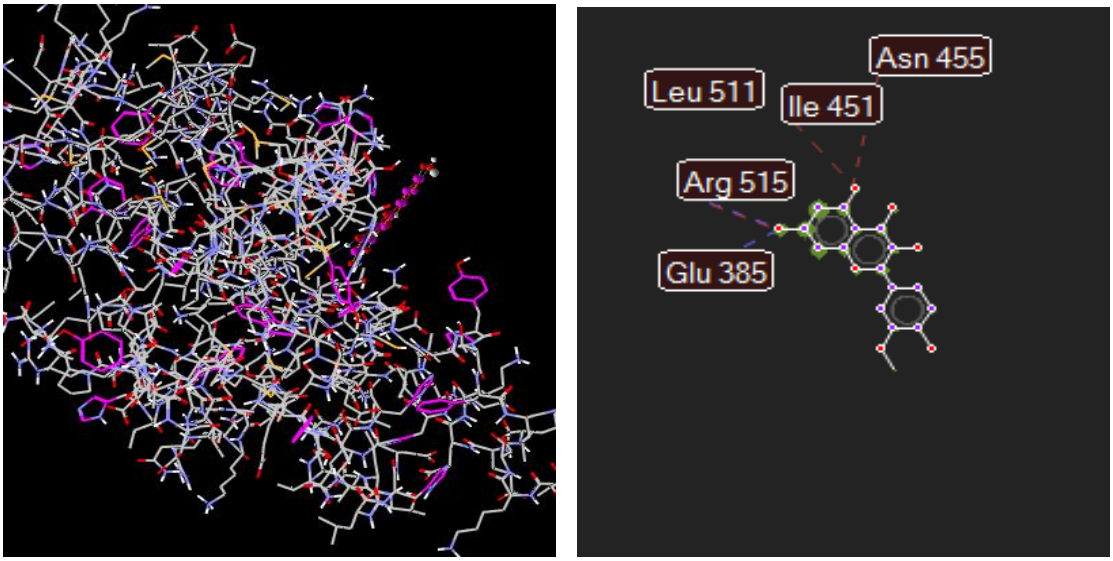


Figure 53: DOCKING OF ISORHAMNETIN WITH 1XR7

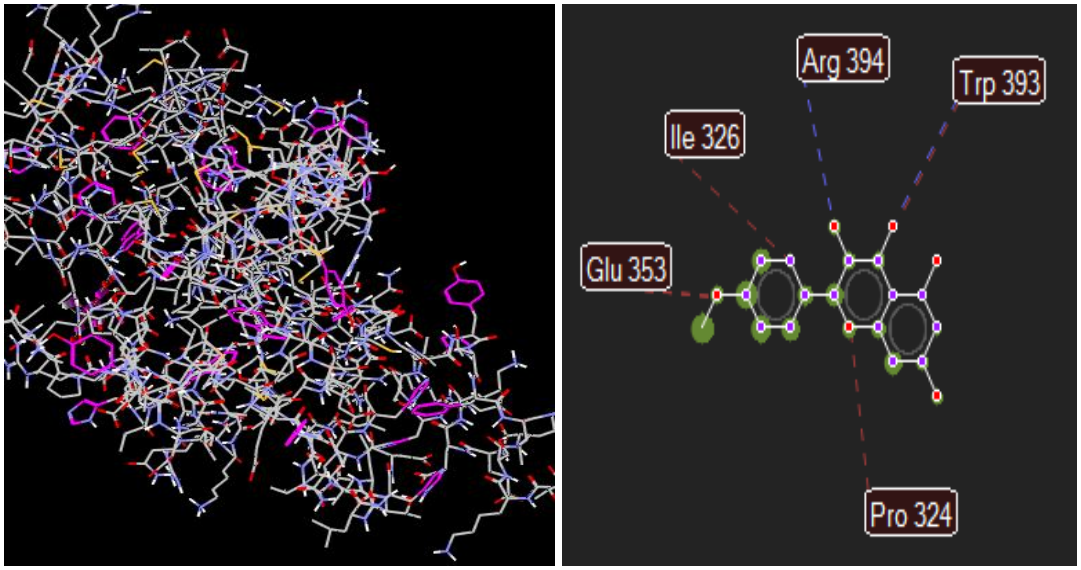


Figure 54: DOCKING OF KAEMPFERIDE WITH 1XR7

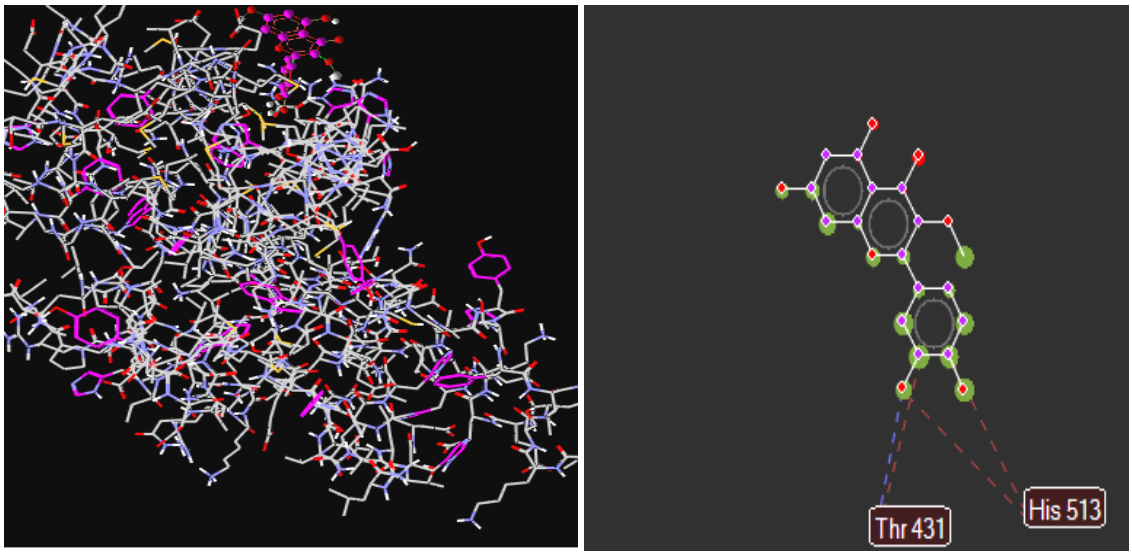


Figure 55: DOCKING OF QUERCETIN 3-METHYL ETHER WITH 1XR7

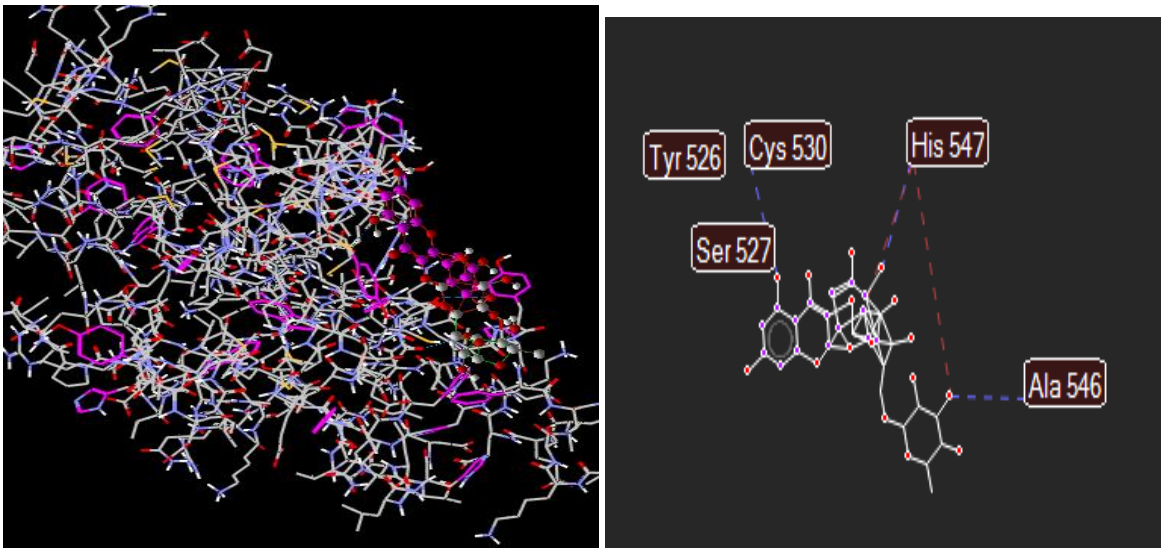


Figure 56: DOCKING OF RUTIN WITH 1XR7

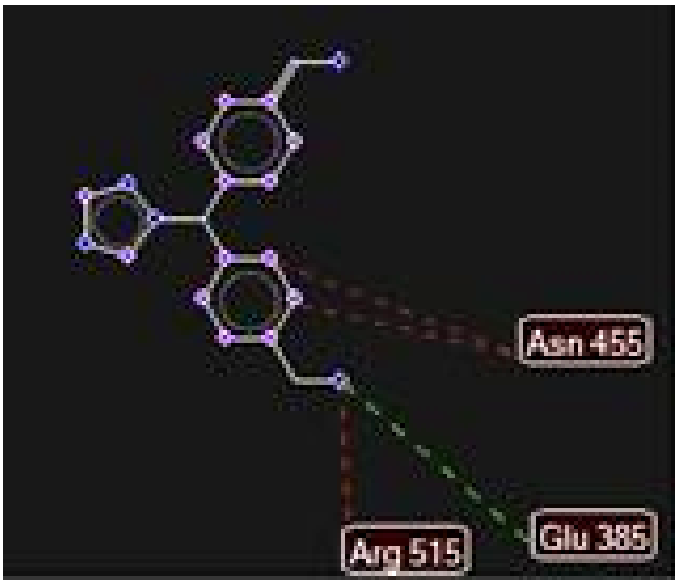


Figure 57: DOCKING OF LETROZOLE WITH 1XR7

PHARMACOLOGICAL EVALUATION

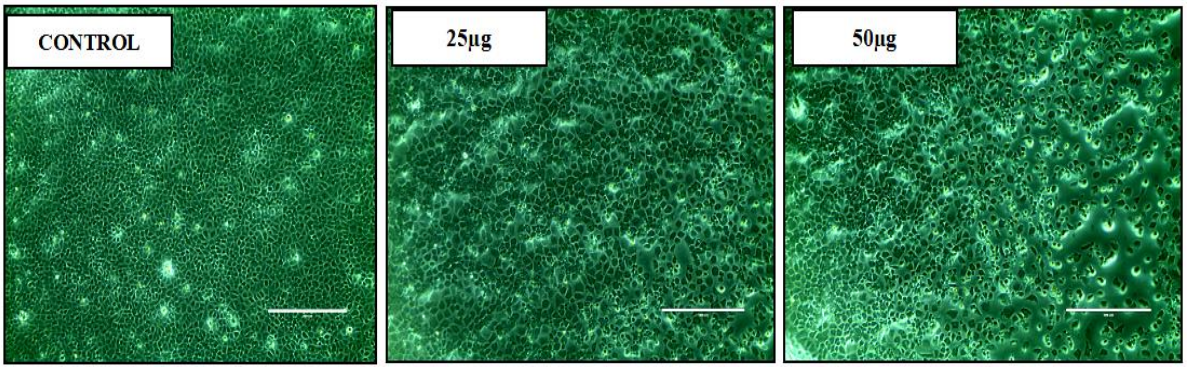
MTT ASSAY -Cell line study :

The cytoxicity study by MTT assay was performed on breast cancer (MCF7) cell line. The result was shown in the table given in Table 16, 17 and figure 58.

Table 16: CYTOTOXICITY STUDY ON MCF7 BREAST CANCER CELL LINE OF *Alpinia galanga* EXTRACT

		ABSORBANCE			
S.No	**CONCENTRATIONS (µg/ml)**	**I**	**II**	**AVERAGE**	**CELL VIABILITY (%)**
1.	**Control**	0.711	0.718	0.7145	100
2.	**25**	0.678	0.686	0.682	95.45136459
3.	**50**	0.541	0.545	0.543	75.99720084
4.	**100**	0.389	0.377	0.383	53.60391882
5.	**200**	0.265	0.259	0.262	36.6689993
6.	**400**	0.111	0.119	0.115	16.09517145

Figure 58: Microscopical observation- Cytotoxicity study on breast cancer cell line of *Alpinia galanga* extract



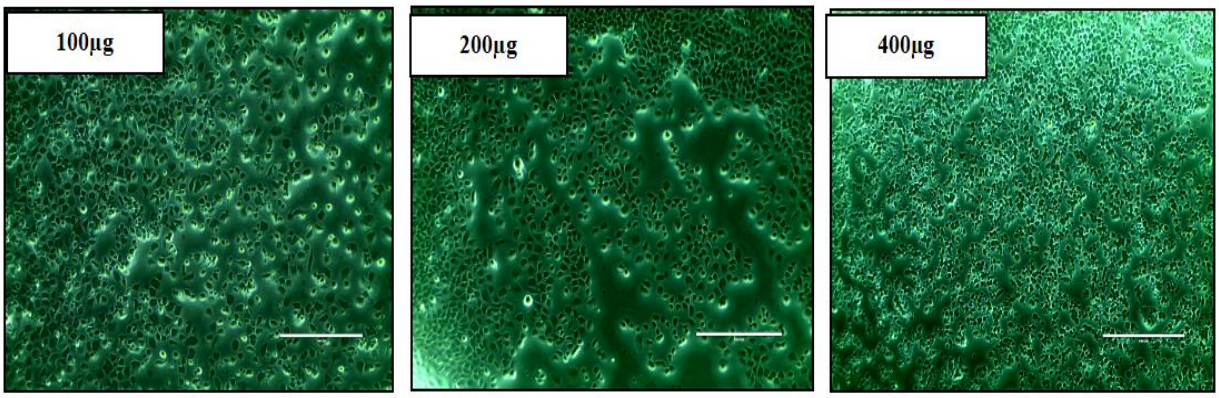


Figure 59: Cytotoxicity IC50 value of *Alpinia galanga* extract on MCF7 cell lines

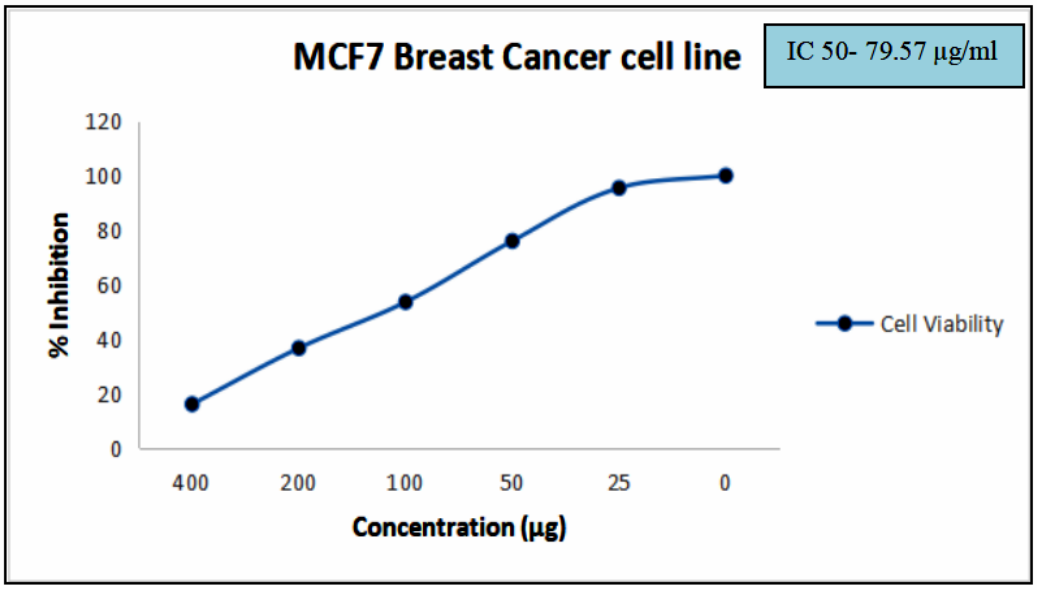
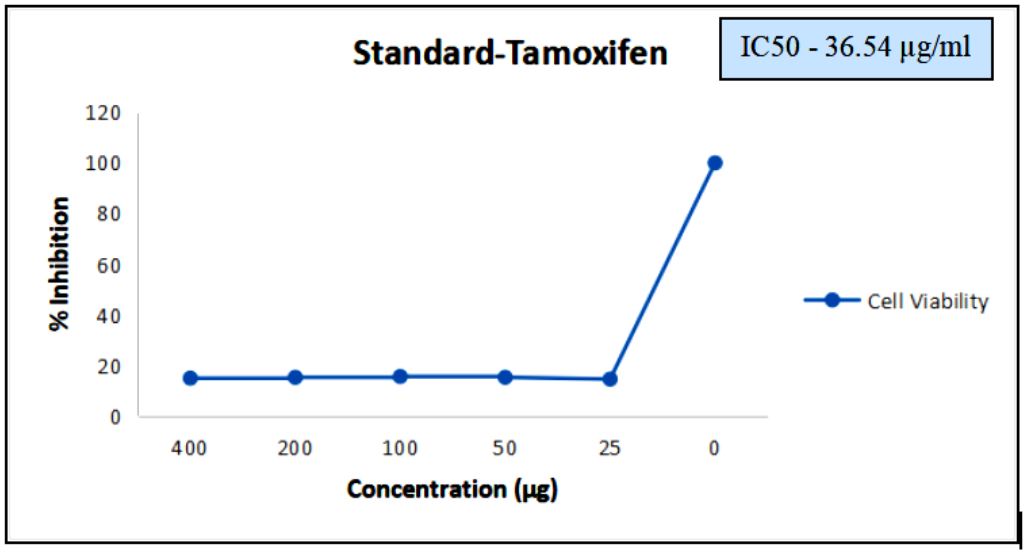


Table 17: CYTOTOXICITY OF TAMOXIFEN ON MCF-7 BREAST CANCER CELL LINE

S.No	CONCENTRATIONS (µg/ml)	CELL VIABILITY (%)
1.	Control	100
2.	25	14.58
3.	50	15.29
4.	100	15.60
5.	200	15.28
6.	400	14.94

Figure 60: Cytotoxicity IC50 value of tamoxifen on MCF7 cell lines



In-vitro anticancer study of *Alpinia galanga* extract was evaluated by MTT assay. The cytoxicity study was done in MCF7 breast cancer cell line by using different concentration to determine the IC50 value. The IC50 value of the extract and the standard was found to be **79.57** and **36.54 µg**, respectively.

FORMULATION OF NIOSOMES

PREFORMULATION STUDIES:

A. Physical Compatibility of the drug and excipient:

In order to obtain a stable and efficient dosage, the excipient must be carefully selected hence physical compatibility study was performed and the result was given **in table 17.**

Table 18: Physical compatibility study of extract and excipient

S.No	Extract + Excipient	Description before test	RT 40°C / 75 % in days	
			10	20
1.	Extract	Dull Brown colour	NC	NC
2.	Extract+span 60	Dull Brown colour	NC	NC
3.	Extract+Cholesterol	Dull Brown colour	NC	NC

B. Solubility

Table 19: Solubility study of extract

S.No	Solvent	Solubility
1.	Ethanol	Easily soluble
2.	Water	Sparingly soluble
3.	Phosphate buffer pH	Sparingly soluble

C. Compatibility study of extract and excipient by FT-IR studies:

This study is performed to know the interaction between the extract and excipient.

Figure 61: Compatibility Study of Extract and Span 60

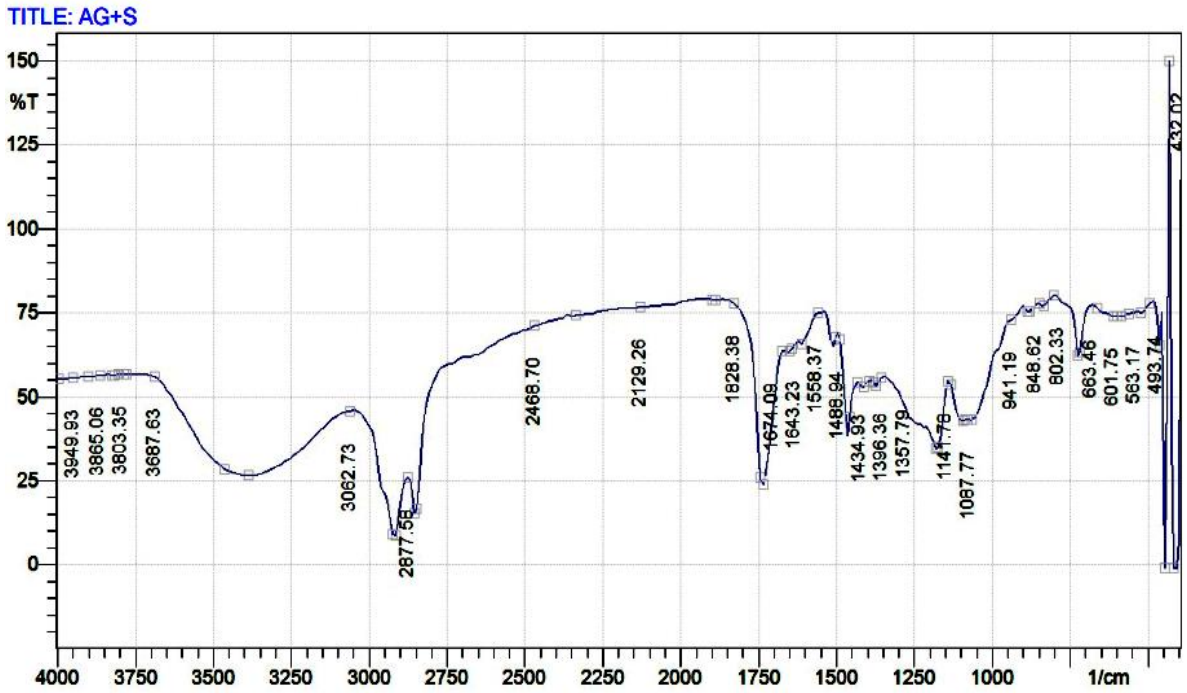


Table 20: FTIR interpretation of *Alpinia galanga* extract and span 60

S.NO	WAVE NUMBER cm^{-1}	INTERPRETATION
1	3687.63	O-H stretching
2	1674.09	Amide C=O Stretching
3	2877.58	Alkane C-H Symmetric stretching
4	1434.93	CH_2 bending
5	1396.38	C-O-H bending
6	1141.70	C-N Stretching
7	663.46	C-H deformation

The Peak obtained in the FT-IR spectrum for extract and **Span 60 was** shown in **Figure 61 and table 14.** It shows no shift and no disappearance of characteristic peak of drug. This suggests that there is no interaction between the drug and Span 60.

Figure 62: Compatibility Study of Extract and Cholesterol

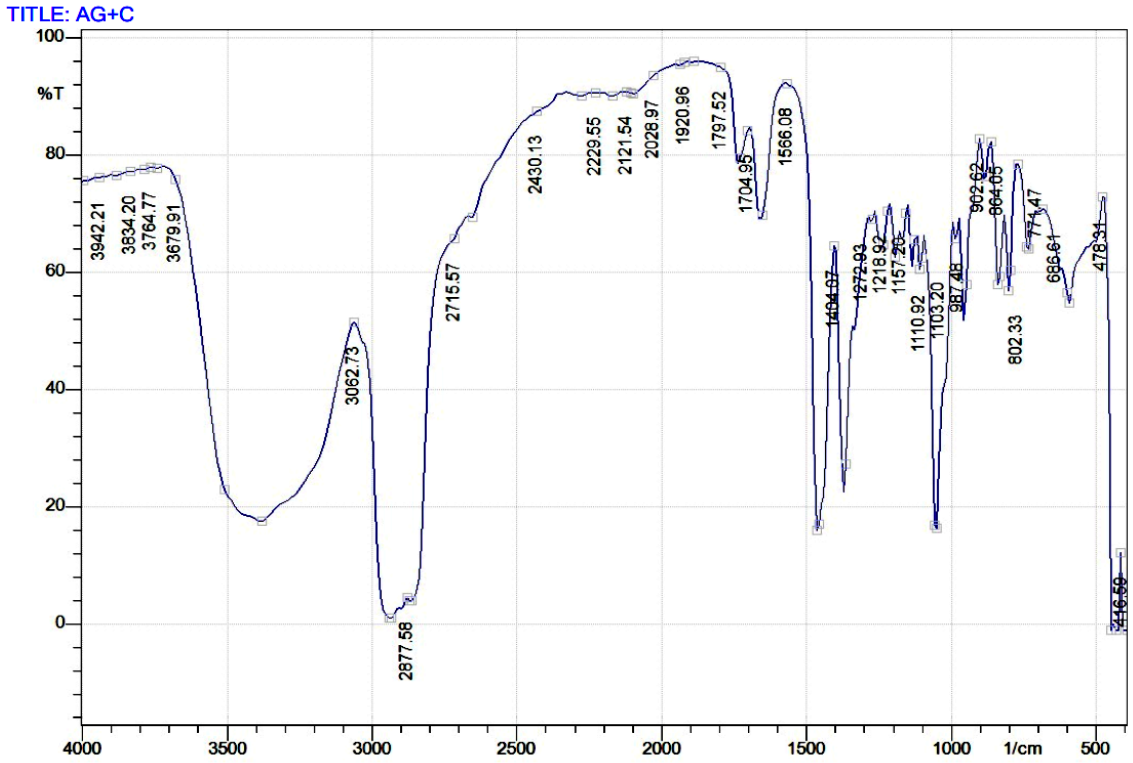


Table 21: FTIR interpretation of *Alpinia galanga* extract and cholesterol

S.NO	WAVE NUMBER cm^{-1}	INTERPRETATION
1	3679.91	O-H stretching
2	1704.95	Carboxylic acid C=O Stretching
3	2877.58	Alkane C-H Symmetric stretching
4	1404.07	CH_2 bending
5	1272.03	C=O bending
6	1218.92	C-O Stretching

The Peak obtained in the FT-IR spectrum for extract and cholesterol was shown in **Figure 62 and table 15.** It shows no shift and no disappearance of characteristic peak of drug. This suggests that there is no interaction between the drug and Cholesterol.

FORMULATED NIOSOMES:

From 3 trial batches of different proportions were formulated, Galangal niosome formulation 2 is good which is used for further studies.

Figure 63: Formulation of niosomes by thin film hydration method

EVALUATION OF NIOSOMES

A. Determination of Percentage yield:

The percentage yield of the formulated niosome was found to be 80 % .

B. Scanning Electron Microscopic studies :

The scanning electron microcopy studies was shown in figure 39.

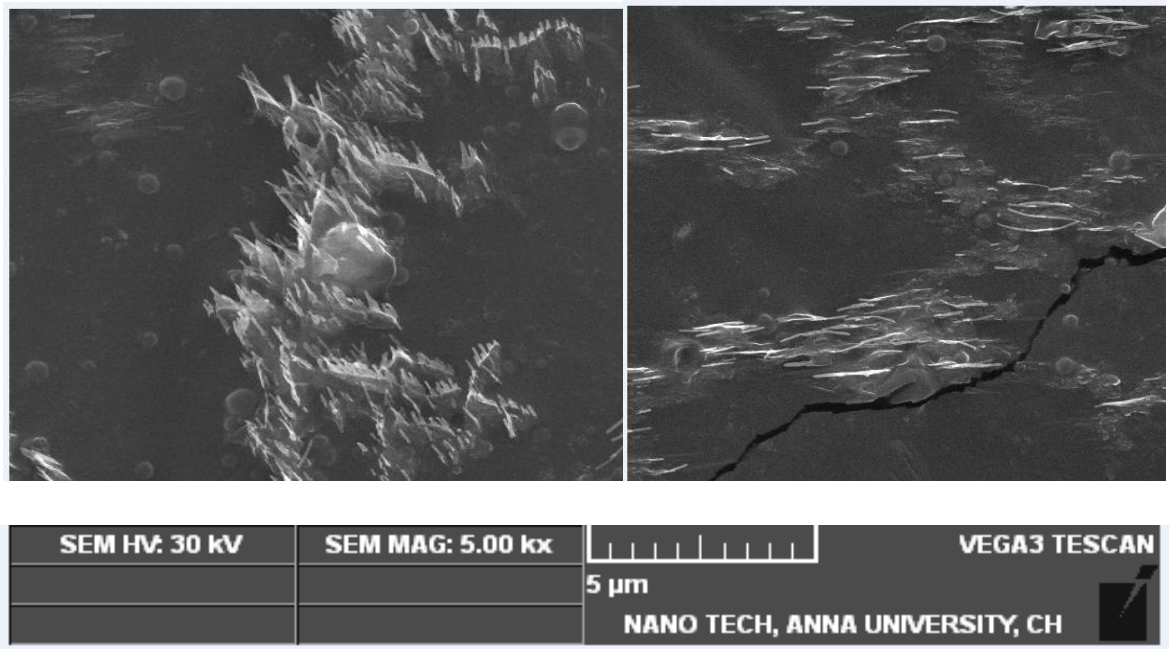
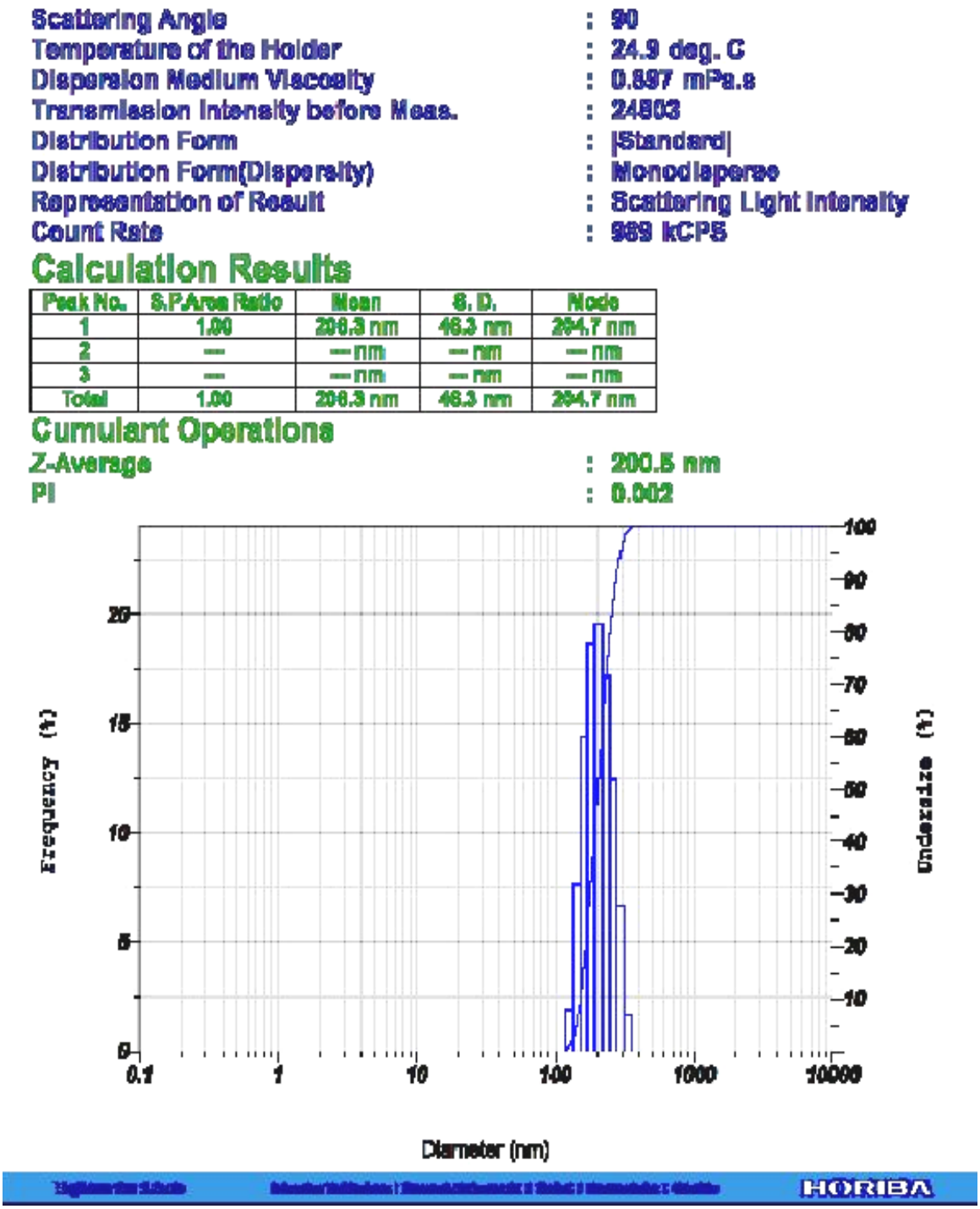


Figure 66: Scanning Electron microscopic studies

C. Measurement of Particle size:

The particle size of the formulated niosome wa**s** found to be **206.3 nm**

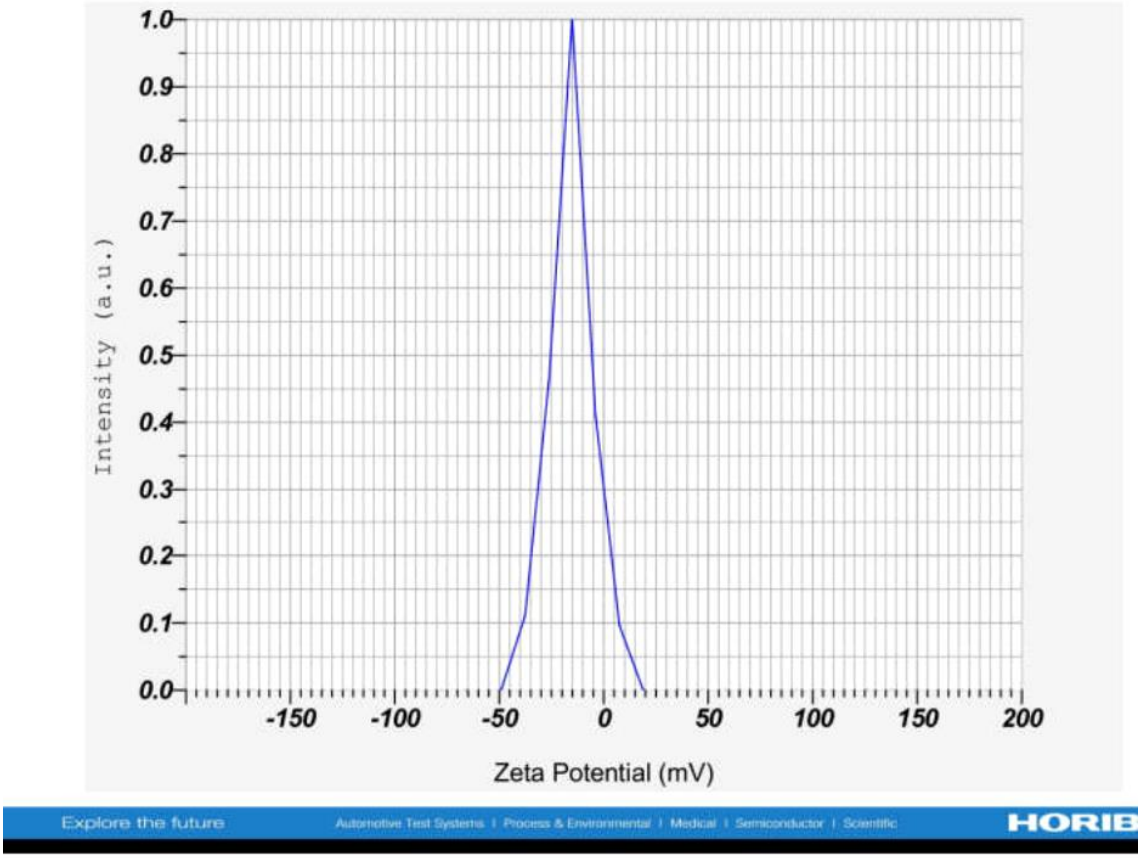
Figure 67: Particle size of niosome



D. Measurement of Zeta Potential:

The Zeta potential of the niosome was found to be **-18.6 mV.**

Figure 68: Zeta potential of niosome



E. DETERMINATION OF DRUG CONTENT:

The drug content of the formulated niosome was carried out by using UV spectrophotometric method. 11 ml of niosome dispersion solution contains 3.8845 mg of quercetin.

F. DETERMINATION OF DRUG ENTRAPMENT EFFICIENCY

The percentage entrapped in the niosomal formulation was found to be 96.91%

PREFORMULATION STUDIES OF OPTMIZED NIOSOMES:

A. Physical Compatibility of the drug and excipient:

In order to obtain a stable and efficient dosage, the excipient must be carefully selected hence physical compatibility study was performed and the result was given in table 24.

Table 24: Physical compatibility study of extract and excipient

S.No	Extract + Excipient	Description before test	RT 40°C / 75 % in days	
			10	20
1.	Extract	Dull Brown colour	NC	NC
2.	Extract + HPMC	Dull Brown colour	NC	NC
3.	Extract + PEG	Dull Brown colour	NC	NC

B. Compatibility study of extract and excipient by FT- IR studies:

Figure 69 : Compatibility Study of Extract and Hydroxypropyl methylcellulose:

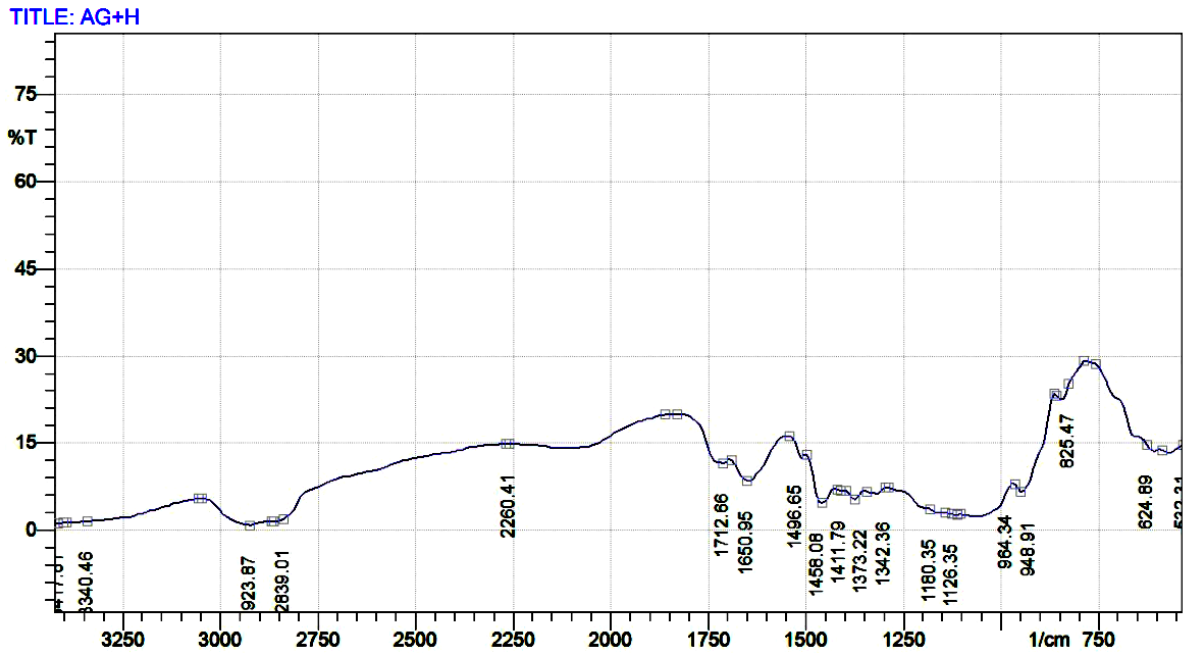


Table 25: FTIR interpretation of *Alpinia galanga* extract and Hydroxypropyl methylcellulose

S.NO	WAVE NUMBER cm^{-1}	INTERPRETATION
1	1650	C=C stretching
2	1712	C=O Stretching
3	2839	Aldehyde C-H stretching
4	1458	C-H bending
5	1373	CH_3 bending
7	948	Alkene C-H bending

The Peak obtained in the FT-IR spectrum for extract and Hydroxypropyl methylcellulose shown in Figure 69 and table 24. It shows no shift and no disappearance of characteristic peak of drug. This suggests that there is no interaction between the drug and Hydroxypropyl methylcellulose.

Figure 70: Compatibility Study of Extract and Polyethylene glycol

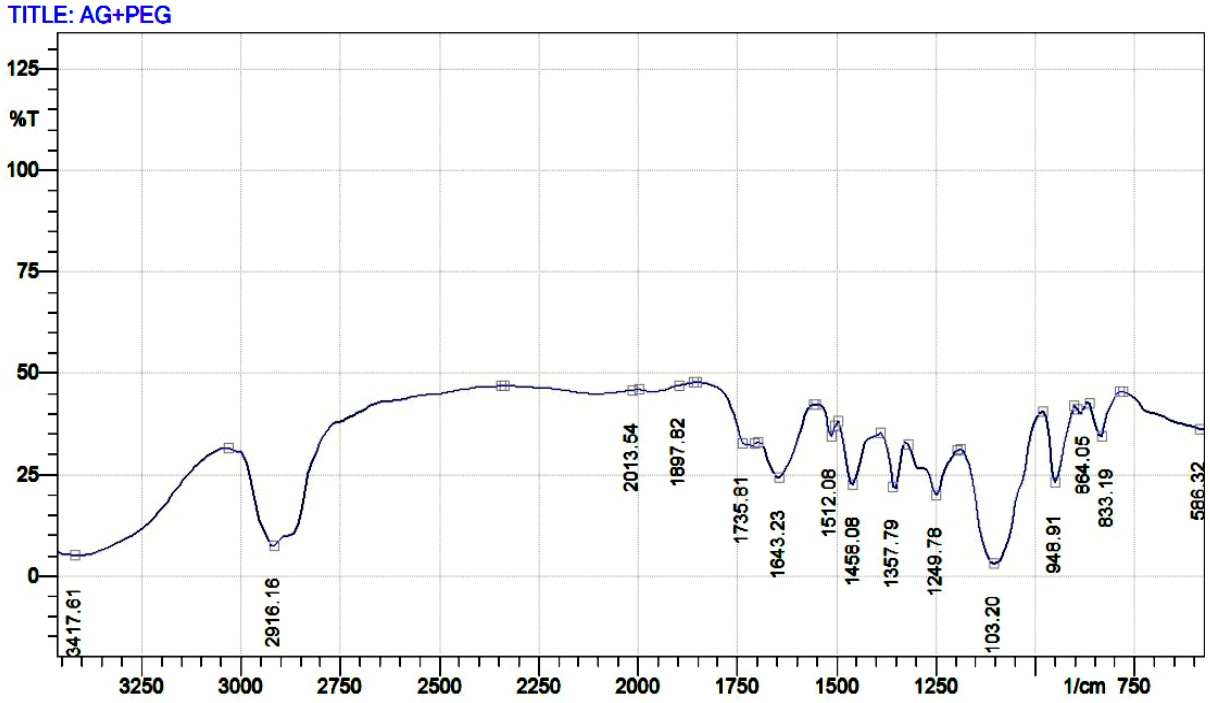


Table 26: FTIR interpretation of *Alpinia galanga* extract and Polyethylene glycol

S.NO	WAVE NUMBER cm^{-1}	INTERPRETATION
1	1643	Alkene C=C stretching
2	1735	Ester C=O Stretching
3	2916	Alkane C-H stretching
4	1458	C-H asymmetric stretching
5	1249	Alcohol C-O bending

The Peak obtained in the FT-IR spectrum for extract and Polyethylene glycol shown in Figure 60 and table 26. It shows no shift and no disappearance of characteristic peak of drug. This suggests that there is no interaction between the drug and Polyethylene glycol.

PHYSICOCHEMICAL EVALUATION OF SELECTED FORMULATIONS

The three (H1,H2,H3) batches of drug loaded films were subjected to various physicochemical evaluations.

Table 27: Evaluation of transdermal patch

Formulation code	Uniformity of weight (g)	Thickness (mm)	Drug content (%)	Folding Endurance e(no's)
H1	0.32	0.18	91.3	233
H2	0.35	0.143	95.3	242
H3	0.41	0.139	94.7	251

Formulation code	Moisture Uptake (%)	Moisture Content (%)	Surface pH	Percent Elongation (% mm)	Tensile strength (Kg/mm^2)
H1	2.43	2.542	6.3	66	2.120
H2	2.14	2.429	6	83	2.824
H3	2.56	2.705	6.4	79	2.281

Based on thickness, uniformity of weight, folding endurance, percentage moisture uptake, the formulation H2 was selected for further studies.

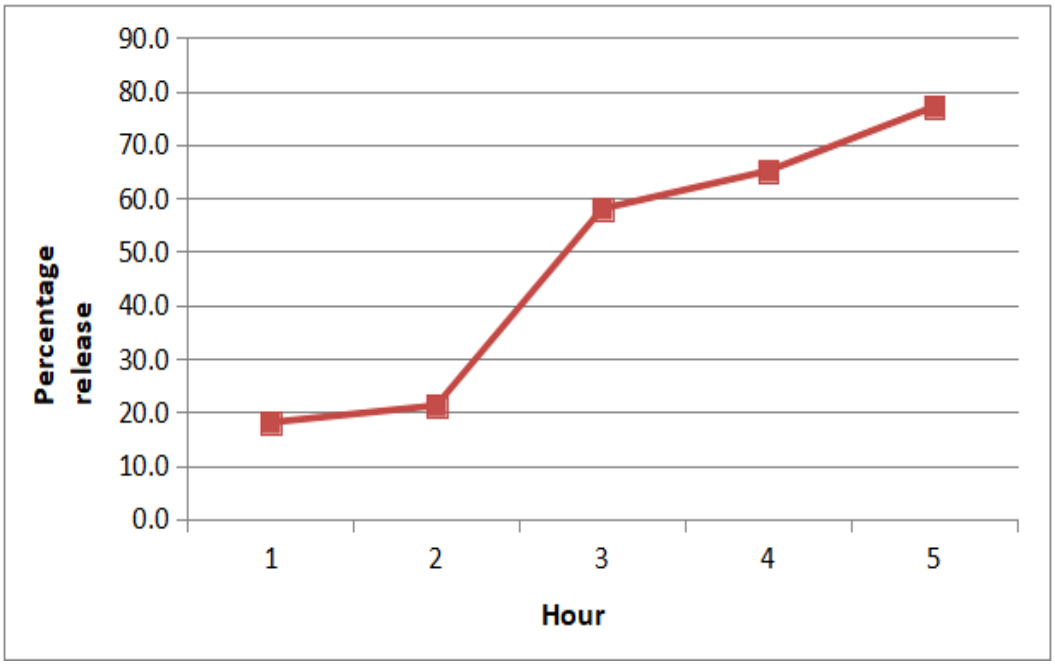
Figure 71: Niosome loaded transdermal patch

IN VITRO EVALUATION STUDY OF TRANSDERMAL PATCHES

Table 28: % Drug Release study

S.No	Time (min)	% Drug release
1.	0	0
2.	1	18
3.	2	21.2
4.	3	58.0
5.	4	65.1
6.	5	77.1

Figure 72: Curve based on %drug release

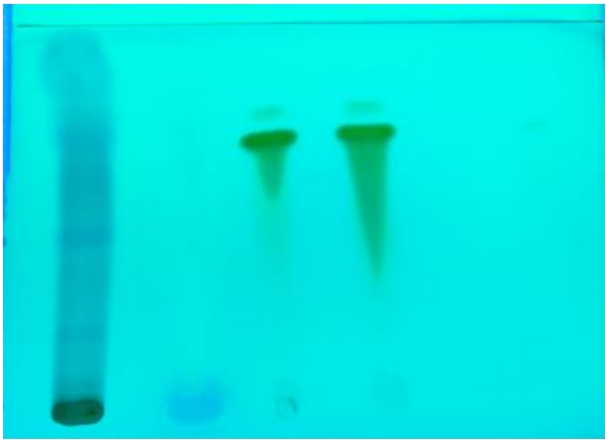


Identification of Quercetin in the extract and formulation by TLC

Table 29 : Identification of quercetin by TLC

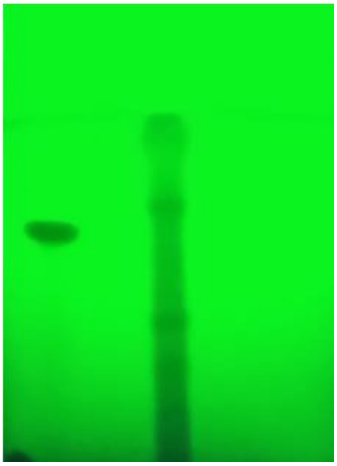
R_f Value	Extract	Niosomes	Film	Quercetin
R_f Value for solvent system 1	0.70	0.76	0.75	0.74
R_f Value for solvent system 1	0.73	-	0.69	0.66

Figure 72 : Identification of Quercetin in the extract and formulation by TLC



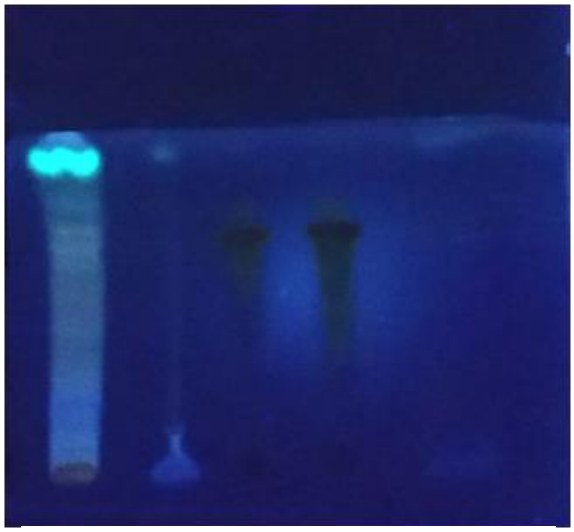
Ext Nio Q Q Film

Solvent System 1, at long wavelength



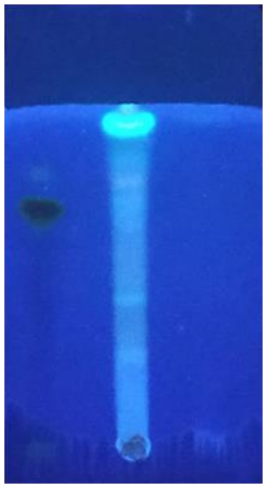
Q Ext Film

Solvent system 2 at long wavelength



Ext Nio Q Q Film

Solvent system 1 at short wavelength



Q Ext Film

Solvent system 2 at short wavelength


Summary And
Conclusion

SUMMARY AND CONCLUSION

Cancer of the breast is the most common malignant neoplasm in women, accounting for as many as 25.8 (12.7 mortality) per 100,000 women in the India. The disease is rare under the age of 25, and increases steadily in incidence with age, reaching its peak incidence in elderly women.

Plants have been and still being used by people throughout the world for healthcare and for treatment of various acute and chronic diseases. Lot and lots of medicinal properties was possessed by each plants but still the drawback exist due to lack of proper scientific validation and standardization procedures.

Therefore this present study is an attempt to formulate and evaluate the patch containing niosomal formulation of *Alpinia galanga* for the treatment of breast cancer.

Based on the extensive review of literature, the plant with more medicinal properties and also has increased amount of bioactive molecules were selected for the study.

The plant was collected and authenticated. Pharmacognostical studies such as macroscopy, microscopy of plant part, powder microscopy and histochemical analysis were done for identification of the plant as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India.

The Physicochemical parameters like loss on drying, ash values and extractive values and qualitative analysis of heavy metals and inorganic elements which prove the drugs were of good quality and safe.

The extraction of active constituents and Preliminary phytochemical investigation were carried out revealed the presence of various phytoconstituents such as steroids, glycosides, flavonoids, phenols, tannins, and terpenoid in the raw materials.

Fluorescence analysis was carried out to detect the presence of any chromophore present in the powder and extracts.

Characterization of active components by UV spectrophotometric studies, TLC studies, HPTLC studies, FT- IR studies analysis of ethanolic extract was carried out to showed various

phytoconstituents present in the ethanolic extract. Amount of bioactive constituent present in the extract is evaluated with the standard drug by using UV spectrophotometry.

Docking studies were performed to know the Binding energy which showed good inhibition effect to the estrogen binding.

In vitro anticancer activity of the ethanolic extract was done by using MCF7 breast cancer cell line and the IC_{50} value was evaluated.

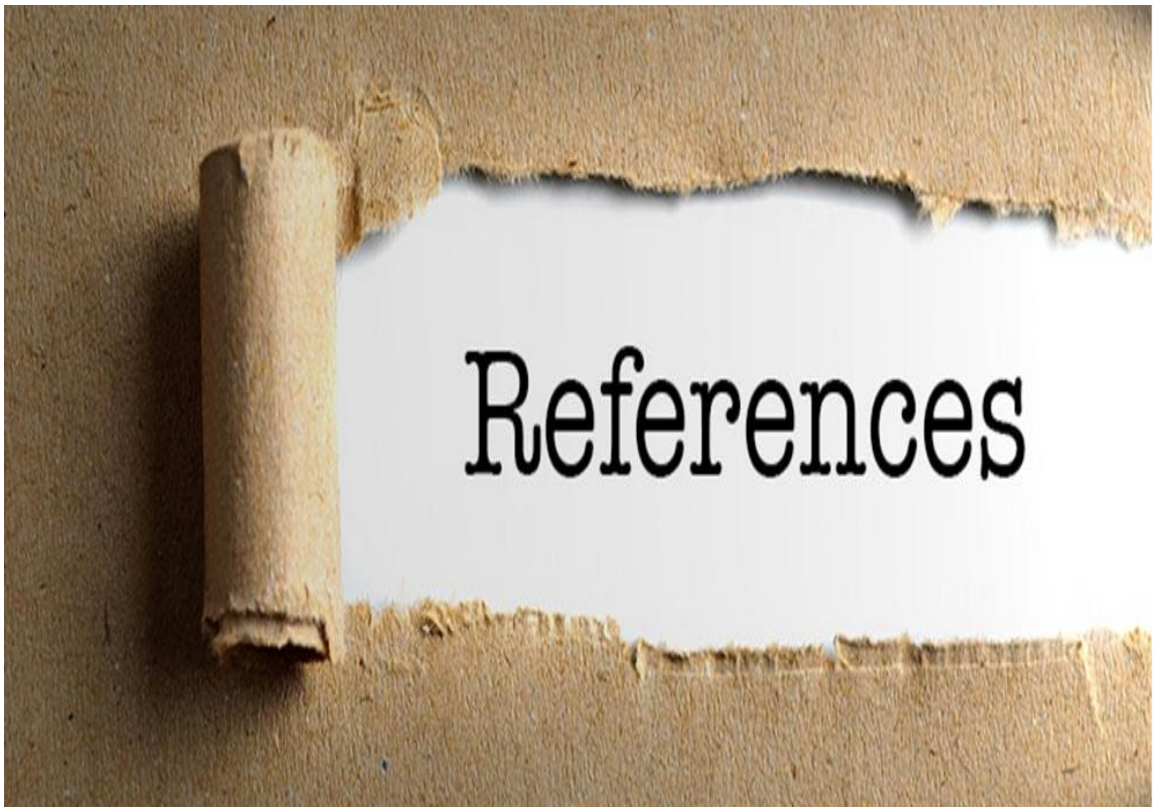
Niosomal formulation is one of the novel drug delivery system which has several advantages such as encapsulating both hydrophilic and hydrophobic drugs, high entrapment efficiency, more stablility and less toxic than liposomes. Another advantage of this type of formulation is that they improve the bio-availability than other conventional dosage forms. They also has high elasticity to penetrate into the skin and hence the niosome was selected to make the extract loaded patch for targeted delivery to the cancer cells.

Preformulation studies for compatibility of extract and excipient, Solubility were performed and identified before formulating the niosomes. The 3 trial batches of niosomes were taken and the optimized formulation selected. Evaluation of niosome formulation such as percentage yield, morphology by SEM studies, particle size, zeta potential, drug entrapment efficiency and drug content was estimated and the results showed that the formulation complies the limits.

Preformulation studies of optimized niosomes were done to study the interaction between the niosome and the excipient using IR spectroscopy showed that there was no interaction. Hence triplicates of patch loaded with niosomes of *Alpinia galanga* extract were formulated. The formulated patches were evaluated for the parameters like weight variation, surface pH, folding endurance, % moisture content, moisture uptake, drug content study, film thickness, percentage elongation, tensile strength and invitro drug release study were evaluated which complies with the pharmacopeial limits.

Further the presence of bioactive molecule which is responsible for pharmacological activity was identified and was compared between the extracts and its formulations using thin layer chromatographic technique..

Hence, from these scientific studies it is concluded that the formulation proves effective in the treatment of breast cancer.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annexure

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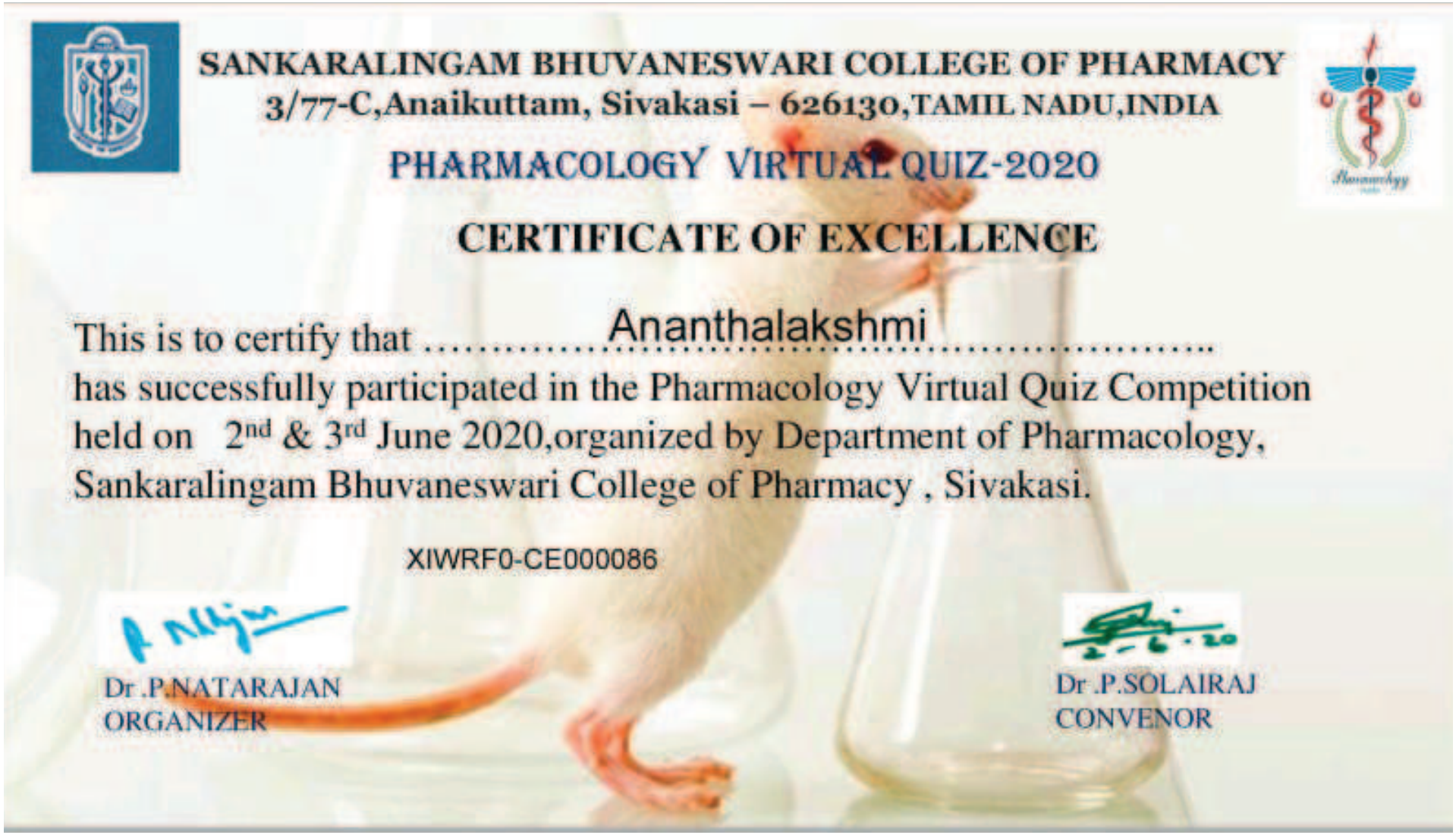

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PHARMACOLOGY VIRTUAL QUIZ-2020

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3-5-2020

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May 07, 2020, Thursday @ 05.30PM – 7.00 PM

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This is to certify that Dr./ Mrs./ Mr./ Ms.

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Dr. S. SHOBA	Dr. A. S. K. SANKAR	Dr. T. VETRICHELVAN
Co-ordinator.	Vice-Principal.	Principal

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MOTHER THERESA POST GRADUATE AND RESEARCH INSTITUTE OF HEALTH SCIENCES

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DEPARTMENT OF PHARMACOGNOSY - COLLEGE OF PHARMACY

SHORT TERM COURSE

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This is to certify that Ms/Mr. ANANTHALAKSHMI A from

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has actively participated in the online short term course on *"Analytical and Molecular Strategies in Herbal Drug Research including Herbal Patenting"* conducted from 22nd to 29th July 2021, organized by Department of Pharmacognosy, College of Pharmacy, MTPG & RIHS, Puducherry -605006, India.

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Mr/ Ms/Dr Ananthalakshmi

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Dr. JKK. MUNIRAJAH. M.Tech (Bolton)
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HEALTHINESS THROUGH INNOVATION

CERTIFICATE OF PARTICIPATION

This is to certify that

Ananthalakshmi A

has successfully completed the session on
"Designing of Novel Inhibitors of COVID 2019/SARS COV 2"
on 12th June 2020 organized by Institute Academic Cell (IAC) of SVCP
in association with Antiviral Research Society.

Dr.S.Sarojini,
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CERTIFICATE FOR PARTICIPATION

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Mr/ Ms/Dr Ananthalakshmi

has participated in National Webinar on "HEALTHY QUARANTINE - COMBAT COVID-19 WITH NUTRITION" organized by

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This is to certify that ... Ananthalakshmi .....
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B.EDWIN JOSE
ORGANIZING SECRETARY
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VANDALUR, CHENNAI- 600048, Tamil Nadu, India

Certificate of Participation

Ananthalakshmi

has participated in the Webinar on "Life Cycle of a Drug" on 5th June 2020 organized by CRESCENT SCHOOL OF PHARMACY, Vandalur, Chennai-600048.

Dr. S. M. Shaheedha
Convener

Dr. M. Vijaya Vara Prasad
Dean, Crescent School of Pharmacy

For further information: deanpharmacy@crescent.education

Date: 05-06-2020

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08-June-2020
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Department of Pharmacognosy, College of Pharmacy, Puducherry - 605 006

Theme: Harmless Herbs Heal

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