Nano technological approach to improve bioavailability and efficacy of phytotherapeutics for cancer treatment

By

Kunal kapoor(101707)

Namrata gautam (101728)

Under the Supervision

of

Dr. Maneesh jaiswal

to

Submitted in partial fulfillment of the requirement for the degree of

Bachelor of Technology in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,

WAKNAGHAT

TALBLE OF CONTENTS

TABLE OF CONTENTS

Certificate

This is to certify that the work entitled **"Nano technological approach to improve bioavailability and efficacy of phytotherapeutics for cancer treatment"** submitted by Kunal kapoor (101707) and Namrata gautam(101728) in partial fulfilment for the award of the bachelors degree B.tech programme in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or institutes for the award of this or any other degree or diploma.

Dr Maneesh Jaiswal

Assistant professor

JUIT, Waknaghat

Solan,HP

Acknowledgement

This project in this report is the result of the continues work done over the period of last one year and immense support from various sources. With immense relief and satisfaction at the successful partial completion of the project we would like to express our gratitude and acknowledge all those who made this work possible.

We would like to express our sincere gratitude to Dr Maneesh Jaiswal, our project advisor. Without his expert guidance, support and motivation the project would not have reached at this milestone. His constant effort, involvement and constructive criticism have helped us to work efficiently and precisely. He has enabled us to think logically and rationally. His specification about every little thing and emphasis on doing thing properly without wasting resources is now inscribed in us. He has helped us to grow in a better individual and better professional.

We take this opportunity to thank Dr R.S. Chauhan Dean , Department of Biotechnology and Bioinformatics, JUIT , Waknaghat for providing us with opportunity and facility to carry out our project. We are also very grateful to the teacher of the department for their guidance during mid-semester presentation and viva .

We also thank Mr Arun Kumar PhD scholar for his guidance and help during this complete course. Without his help the project wouldn't have been able to reach its current milestone

Also we would specially like to thank Dr Simran Tandon , Associate Professor JUIT and her PhD scholar Miss Shagun Arora for providing full support and giving access to their lab to complete one of the essential milestone of the project.

To all individual who have helped us in completing our experiment on time again we express our appreciation. We also extend our apologies to anyone we have failed to mention.

Signature of Students…….

Date……

Summary

Epidemiology studies have shown that the antioxidant and active component extracted from plant play a vital role in preventing the risk of some of the chronic ailment such as cancer. But when it comes to stability and efficacy of these antioxidant and active component reaching to the epicentre the numbers are very less. Hence the current study deals with the encapsulation of these components inside the nanocarrier or we may say it target specific nanoparticle which not only increase the stability of the extract but also increase its bioavailability and efficacy. The aim is to prepare the nanoparticle loaded with phytochemical and get alternative therapy to treat cancer.

Kunal kapoor (101707)

Namrata gautam (101728)

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

LIST OF SYMBOLS AND ACRONYMS

TC	Tinospora Cordifolia
DCIS	Ductal carcinoma in situ
DEN	Diethylnitrosamine
HCC	Hepatocellular carcinoma
GIT	Gastrointestinal tract
LBDDS	Lipid based drug delivery system
DLS	Dynamic Light Scattering
GRAS	Generally regarded as safe
EO	Ethylene Oxide
$NaNo_2$	Sodium nitrite
FICI	Fractional inhibitory concentration index
MIC	Minimum inhibitory concentration
AST	Aaverage survival time
PDI	Polydispersity index
PTX	Paclitaxel
BCG	Bromocersol green

INTRODUCTION

CHAPTER 1

1.1 Cancer

Cancer is class of diseases in which a group of cell display "uncontrolled growth" (division beyond the normal limits, "invasion" (intrusion and destruction of adjacent tissue) and sometimes "metastasis" (spread to other location by the mean of lymph or blood). These three malignant properties of cancer separate them from the benign tumor, which are self-limited and do not invade or metastasize. Most of the cancer form tumor but some like leukemia, do not. The branch of medicine concerned with the study, diagnostic, treatment and prevention of cancer is known as oncology.

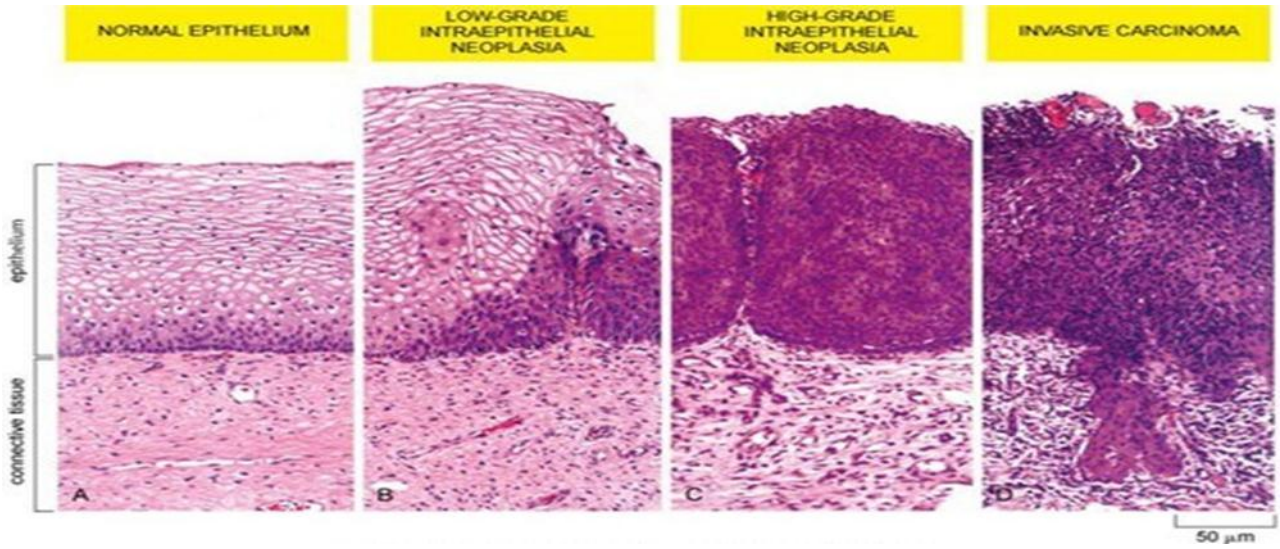


Fig 1. Progression of cancer from normal epithelium to invasive carcinoma adapted from Albert B et.al *2008*

Cancer can affect the people of all ages but the risk increases from with the age. Cancer itself causes about 13% of all human deaths. According to the American cancer society, 7.6 million people died as a result of cancer in the world during 2007.

Cancer are mostly caused due to the abnormality in genetic material of the transformed cells. The abnormalities may be due to the effect of carcinogens, such as tobacco, smoke, radiation, radiation chemical or the infectious agents. Other cancer-promoting genetic abnormalities may be randomly acquired through error in DNA replication, or are inherited, and thus present in all cell from the time of birth. The hereditary cancer is usually affected by complex interaction between carcinogens and host`s genome. New aspects of the genetics, such as DNA methylation and microRNAs are increasingly recognized as the important tool to understand the pathogenesis of cancer cell. Genetic abnormalities found in caner typically affect two general classes of genes. First is the cancer

promoting “oncogene” which is typically activated in the cancer cells giving the cell new properties such as the hyperactive growth and division, and the ability of the cancer cell to be protected against the programmed cell death, loss of respect for the normal tissue boundary, and ability to become self-established in diverse tissue environment. The other class is “tumor suppressor genes” are then inactivated in the cancer cells resulting in the loss of normal functioning of those cells such as accurate DNA replication, control over the cell cycle, orientation and adhesion within the tissue and interaction between the protective cells of the immune system.

Diagnosis of cancer usually requires the histologic examination of a tissue biopsy specimen by a pathologist although the initial indication of malignancy can be symptomatic or radiographic or radiographic imaging abnormalities. Most cancer can be treated or cured, depending on the specific type, location and stage. Once diagnosed can be treated by the combination of radiotherapy, chemotherapy and surgery. As the technique develops the treatment become more specific to different varieties of cancer. There has been significant increase in the development of the targeted therapy drugs for different varieties if cancer. These targeted therapies act specifically on some of the molecular abnormalities in certain tumor cells which minimize the damage to the normal cells. The prognosis of the cancer is most influenced by the type of the cancer, as well as the stage and the extent of the disease. In addition, histological grading and presence of some of the specific molecular markers can also be useful in establishing prognosis, as well as in determining individual treatment (GLOBOCAN,2007 factsheet).

Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. These types include:

- **Carcinoma**: Cancers derived from epithelial cells. This group includes the most common cancers, particularly found in the aged, and include nearly all those developing in the breast, prostate, lung, pancreas, and colon.
- **Sarcoma**: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develop from cells originating in mesenchymal cells present just outside the bone marrow.
- **Lymphoma and leukemia**: These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30%mortality rate.(1)

- **Germ cell tumor**: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- **Blastoma**: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

1.2 Colon cancer

Colon cancer is a disease of the large intestine in which cancer cells grow in colon or rectum. Colon is the longest part of large intestine (5 feet long) and is found in the lower part of gastrointestinal tract where some of the undigested food (fiber) and other nutrients are further absorbed. Colon cancer is a result of cancer cells that form the lining of colon. Nearly all colon cancers begin as noncancerous benign (polyps) which slowly develop into cancer. The incidence rate was found to be 50 times higher in persons aged between 60 to 79 years than those who were below 40 years of age (American cancer society, facts and figures 2011-2013).

1.3 HT-29 cell line

Human Colorectal Adenocarcinoma cell line was established in 1964 from primary tumor of 44 year old Caucasion female having colorectal adenocarcinoma.

1.3.1 Description

HT-29 cell line is having an epithelial morphology. Chemotherapeutic drugs such as 5-fluorouracil and oxaliplatin are used as standard treatment options for colorectal cancer. These cells grow as nonpolarized, undifferentiated multilayer under standard culture conditions. However altering the conditions and using inducers can result in differentiated and polarized morphology. (2-3)

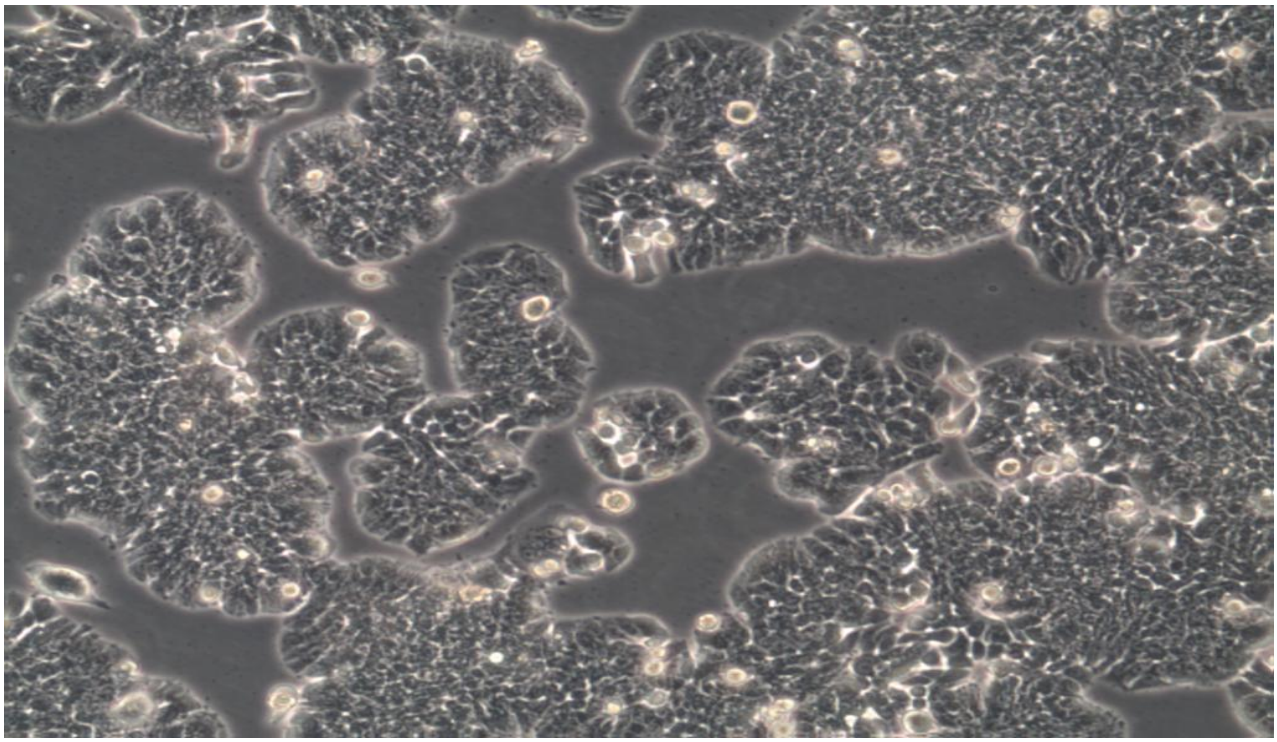


Fig 2: HT-29 cell line in culture (source: Cohen E et al. (1999)

1.4 How free radicals damage cell

Human cells are exposed to approximately 10^5 hits of oxidative damage per day (4-6). Permanent modification of the genetic material due to such oxidative damage results in mutagenesis and the rate of such mutagenesis are directly proportional to the oxidative DNA lesions which somehow escape the DNA repair mechanism (6). The ability of such oxidants to initiate DNA damage may be contributing to carcinogenesis in which DNA base changes occur in oncogenes and tumor suppressor genes (7). Such oxidative DNA damage causes wide chromosomal abnormalities.

1.4.1 ROS (Reactive Oxygen Species)

ROS stand for reactive oxygen species. Several reactive species that contain oxygen such ashydroxyl radical ($OH^{\cdot}$), superoxide radical anion ($O2^{\cdot}-$), peroxynitrite ($ONOO-$), hypochlorous acid (HOCl), etc. are formed inside living cells during normal metabolic activities (8). For example, sometimes mitochondrial electron transport chains consisting of flavoproteins, iron sulphur proteins, ubiquinone and cytochromes produces $O2^{\cdot}$ due to leakage of electrons to molecular oxygen (O2) from (9). There can be dismutation of $O2^{\cdot}-$by superoxide dismutase which produces

H_2O_2 (10). Enzymes such as oxidases can also produce H_2O_2 in cells. Though catalase and glutathione peroxidase enzymes can convert H_2O_2 into water and scavenge it, but reaction of H_2O_2 with $O2^{\cdot}-$ can yield OH radical following Haber–Weiss mechanism. Similarly, in cells, heme myeloperoxidases help in the formation of HOCl which is a powerful oxidizing and halogenating (11).Fenton reactions also occur in the presence of UV-induced photolysis of H_2O_2 and transition metals which can generate OH radicals.

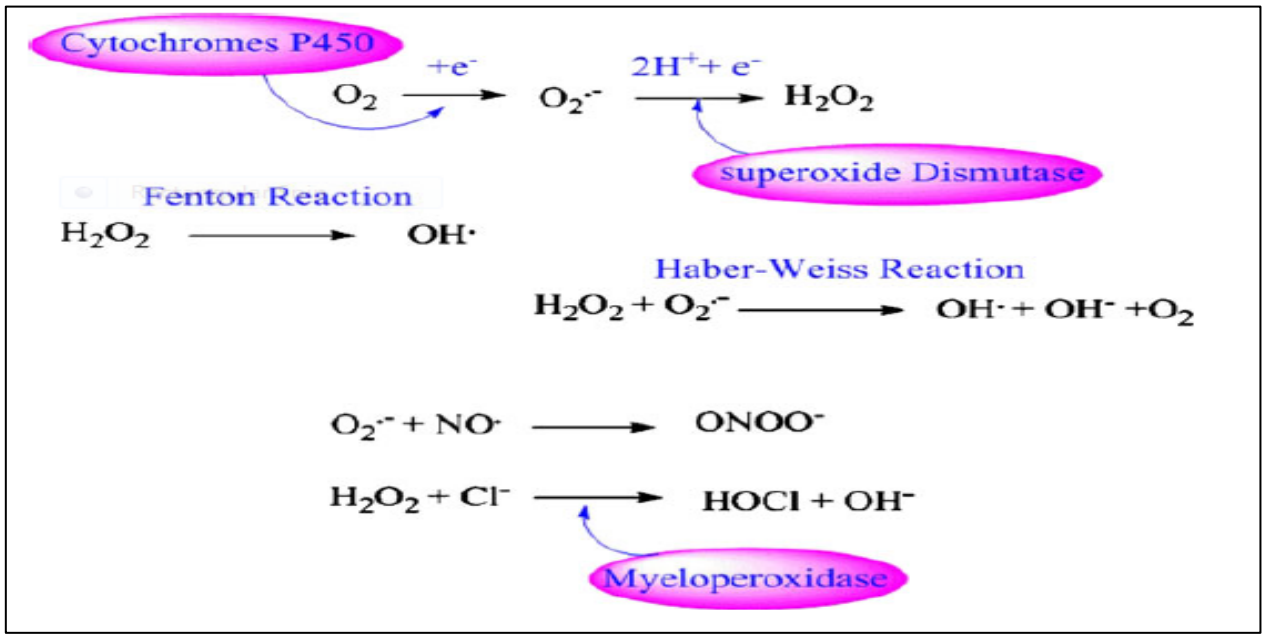


Fig 3. Reactive oxygen species (source: *Gungor et al. 2010*)

1.4.2 Lipid peroxidation and DNA damage

Cell membrane phospholipids are frequent targets for free radical-induced damage as they are very sensitive to oxidation and this enables them to participate in free radical induced chain reactions. Most of them occur between double bonds which include polyunsaturated fatty acids. So if high concentration of such fatty acids is present they can result in free radical chain reactions (12).

1.5 Available treatment for cancer

Treatment of cancer depends on many things, including the stage of the cancer. In general treatment may include method such as surgery, chemotherapy and radiotherapy

- **Surgery**: It is done to remove cancer cell, more extensive surgery is needed to remove the part of the organ that is cancerous.
- **Chemotherapy:** The chemotherapy drug such as 5-flurouracil has been shown to increase the chance to cure certain patients. Some of the monoclonal antibodies such as cetuximab ,

pantiumumab, bevacizumab, and other drug has been used alone or in combination with chemotherapy for the treatment of the affected organ

- **Phytotherapy:** Treatment of the cancer cells by various components derived from fruits and vegetables which affect
- **Radiation Therapy:** Generally it is the use of the ionizing radiation which is used for the treatment of malignant cell Radiation therapy is synergistic with chemotherapy, and has been used before, during, and after chemotherapy in susceptible cancers.(13)
- **Burning the cancer(ablation):** Specifically for liver help in treatment of colon cancer

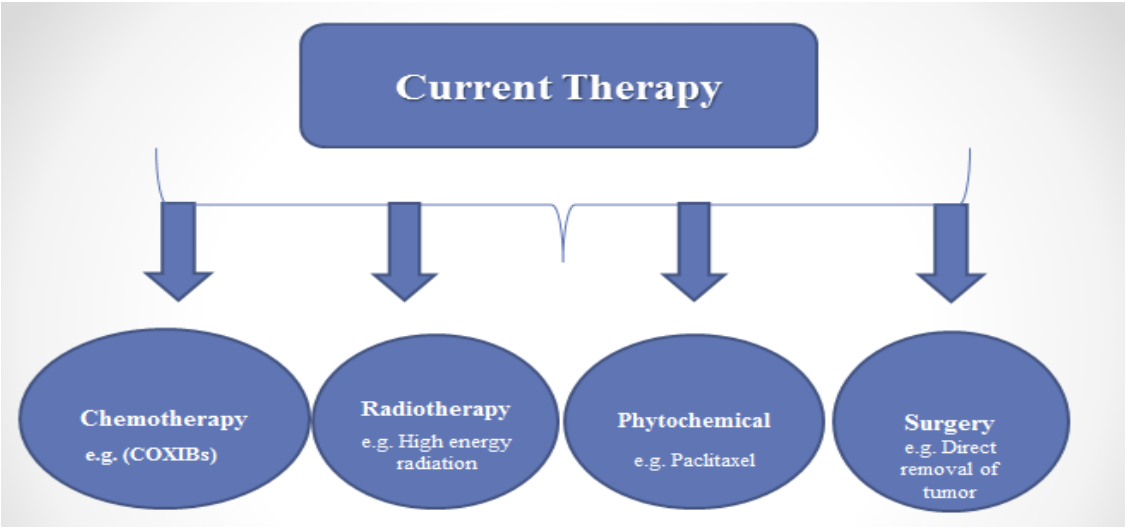


Fig 4. Current therapies available for cancer

1.6 Problem associated with current therapy

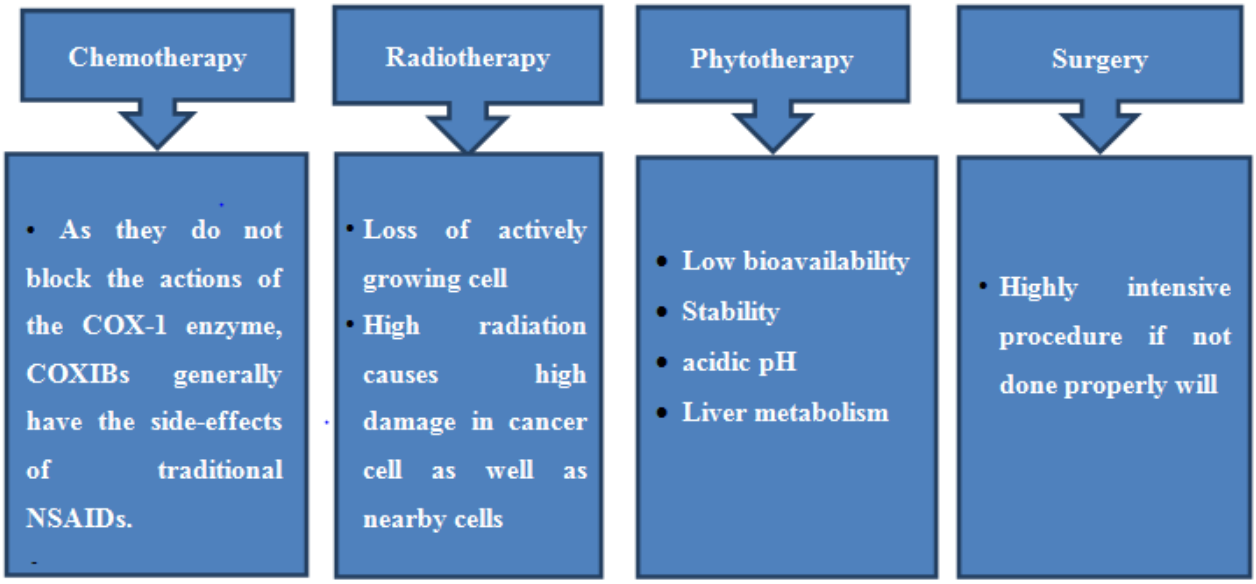


Fig 5. Problem associated with current therapy

1.7 Phytotherapy

Phytotherapy is the study of the use of extracts of natural origin as medicines or health-promoting agents. Phytotherapy medicines differ from plant-derived medicines in standard pharmacology. Where standard pharmacology isolates an active compound from a given plant, phytotherapy aims to preserve the complexity of substances from a given plant with relatively less processing (14).Phytotherapy is distinct from homeopathy and anthroposophic medicine, and avoids mixing plant and synthetic bioactive substances. Traditional phytotherapy is a synonym for herbalism and regarded as alternative medicine by much of Western medicine. Although the medicinal and biological effects of many plant constituents such as alkaloids (morphine, atropine etc.) have been proven through clinical studies, there is debate about the efficacy and the place of phytotherapy in medical therapies.

1.8 Phytochemical

Phytochemicals are a large group of plant-derived components hypothesized to be responsible for much of the protection against the disease conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Each phytochemical come from different plant sources and has different proposed effect on as well as many benefits for the body. Some of the scientist proposes that there are about 4000 of the phytochemical that are present in nature [15]. Although thousands of other phytochemical have been discovered by the scientist but out of that only a small fraction of the phytochemical have been studied closely. There are some of phytochemical present such as lycopene, lutein, resveratrol, anthocyanidine, isoflavones and beta carotene which not only help in the regulation of the immune system but also effect help in preventing some of the major diseases such as cancer. Phytochemicals hold a huge potential which include usage of the product produced from these components to the usage of these components as therapeutic agent. Unlike widely used allopathic system, the herbal remedies have thousands of constituents that all work simultaneously against the diseases. Epidemiological studies suggest that consumption of a diet high in fruits and vegetables is associated with a reduced risk of some of the chronic disease. Therefore hundreds of phytochemical compounds, with several different biological functions, have been identified in plant-based foods. Therefore, consuming a variety of plant-based foods helps to ensure that individuals receive the optimum benefits from the fruits and vegetables consumed. Unfortunately very little is known about the complete procedure of absorption and

utilization of the phytochemical hence, more research is needed to fully explain the actions of phytochemical compounds in the human body [16]. Table given below show some of the foods with the phytochemical present in them and their potential benefit

Food	Phytochemical	Possible Benefits	Ref.
Soy Beans, Soy Milk, and Tofu	Isoflavones (Genistein and Daidzein)	A reduction in blood pressure and increased vessel dilation [8]	[17]
Strawberries, Red Wine, Blueberries	Anthocyanins	Improvement of vision, inhibition of nitric oxide production, induction of apoptosis, decreased platelet aggregation, and neuroprotective effects [8]	[17]
Red Wine, Grape Juice, Grape Extracts, Cocoa	Proanthocyanidins and flavan-3-ols	Inhibition of LDL oxidation, inhibition of cellular oxygenases, and inhibition of proinflammatory responses in the arterial wall [8]	[17]
Garlic, onions, leeks, olives, Scallions	Sulfides, thiols	Decrease in LDL cholesterol [9]	[18]
Carrots, tomatoes, and tomato products, and various types of fruits and vegetables	Carotenoids such as lycopene, beta-carotenes	Neutralization of free radicals that cause cell damage [9]	[18]
Broccoli and other cruciferous vegetables such as kale, horseradish	sothiocyanates (sulforaphane)	Neutralization of free radicals that cause cell damage [9]	[18]

Table 1. Various sources of phytochemical and their potential benefits

1.9 Limitations or the issue associated with the phytochemicals

In spite of having such a huge potential for treating the diseases like cancer, there are many problem or we may say it as the limitation associated with the phytochemical. The limitation which hampers with its overall efficacy of these phytochemicals includes the poor bioavailability, biological

instability and liver metabolism (19). These issues led to the diminished oral bioavailability of these phytochemicals which required a higher dose regimen in order to meet therapeutic requirement which ultimately caused side effects and related complications as well.

1.10 Nanotechnology

Nanotechnology offers a means of providing novel formulation for the existing drugs (phytochemicals). Nanoparticles have been used as drug delivery vehicles due to their high bioavailability, relative lack of toxicity and good encapsulation properties. Furthermore technology of nanoencapsulation has been extended to natural products to protect them from chemical damage and product degradation and therefore increasing product shelf life before its final application. Bioavailability refers to the rate at which any drug enters the blood circulation and becomes available at the site of action. Nanobiotechnology (sometimes referred to as nanobiology) is best described as helping modern medicine progress from treatments of symptoms to generating cures and regenerative medicines. The most important objectives that are frequently found in nanobiology involve applying nanotools to relevant medical/biological problems and refining these applications (20).Nanotechnology is a field of applied science and technology which aims to develop devices and dosage forms in the range of 1 to 100 nm. The applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems have recently been referred to as nanomedicine. The nanocarriers have been made of safe materials, including synthetic biodegradable polymers, lipids, and polysaccharides. (21)

1.10.1 Protein nanoparticles

Proteins are a class of natural molecules with unique functionalities with applications in both biological as well as material field (22). Protein nanoparticles possess certain advantages such as they are biodegradable, non- antigenic, metabolizable and are easily amenable for surface as well as covalent attachment of drug and ligand due to defined primary structure of protein (23).Various protein nanoparticles such as gelatin, albumin, gliadin and legumin have been prepared.

1.10.2 Albumin

Albumin is an attractive macromolecular carrier which has advantages of biodegradability, nontoxicity and non-immunogenicity (24) and is used in the form of BSA (bovine serum albumin) or HAS (human serum albumin).Moreover albumin is the major plasma protein constituting 60% of it. This provides it distinct edge for nanoparticle preparation over other materials. A number of studies have reported that albumin accumulates inside solid tumor (25) which makes it potential

macromolecule carrier that can be used as effective targeted drug delivery vehicle of chemoprotective drugs. Abraxane which is albumin bound paclitaxel is the first nanodrug approved by FDA in January 2005.

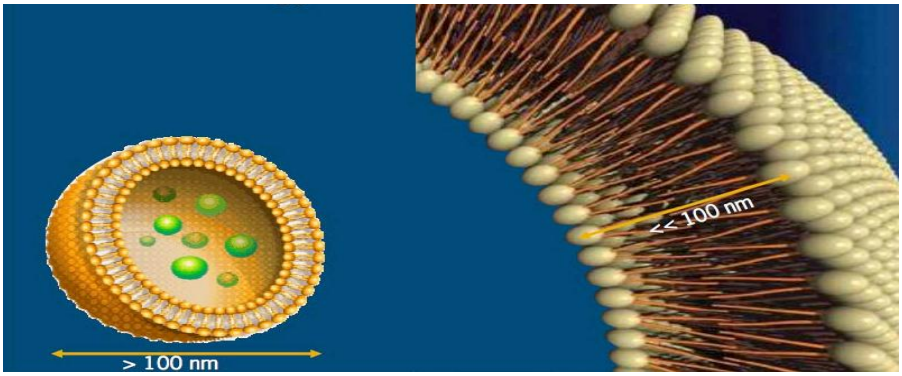


Fig 6. Nanoparticle (source: Wagenigen ur et.al)

1.11 How does nanotechnology help with the problem associated with the phytochemical?

Before reaching to the blood, many constituents of the phytochemical are degraded in the highly acidic pH of the stomach and the remaining gets metabolised by the liver. Resulting, the appropriate quantity of the phytochemicals may not reach the blood. If the drug does not reach in the optimum amount to the infected region at "threshold level," then there will be no means to show the therapeutic effect of the drug. Whereas with the help of Nanotechnology optimum amount of the drug will be carried to their site of action with the help of the nanocarrier, bypassing all the barriers such as acidic pH of stomach, liver metabolism and increase the prolonged circulation of the drug into the blood due to their small size. (21, 26) .Hence the nanoparticle provide us a great way to tackle some of the major issue with the usage of phtyochemicals.

Other reasons due to which the NDDS path is highly preferred for the herbal remedy are;

1. They appear to be able to deliver high concentrations of drugs to disease sites because of their unique size and high loading capacities. [21]
2. Deliver the drug in the small particle size that enhances the entire surface area of the drugs allocating quicker dissolution in the blood.
3. The concentration seems to persist at the sites for the longer periods. [21]

4. Shows EPR (enhanced permeation and retention) effect, i.e., enhanced permeation through the barriers because of the small size and retention due to poor lymphatic drainage such in tumor. [21]
5. Exhibits passive targeting to the disease site of action without the addition of any particular ligand moiety. [21]
6. Decrease in the side effects. [21]
7. Decrease in the dose of the drug formulation.

1.12 Rubus (raspberry)

Raspberry is an edible fruit which grows on thorny plant and belong to genus Rubus in the rosaceae family. Raspberries can be yellow, red, purple or black depending on the type of species. Due to substantial health benefits of black raspberry it has been widely studied (27). Fruits from Rosacea family are rich source of antioxidants (28). Phytochemicals from raspberries are found to be effective inhibitors of oxidative DNA damage invitro in immortalized human colon cells (29).Anthocyanins present in these berries show antiproliferative activity invitro on colon cancer cells (30). Polyphenols present in them also prevent metastasis and invasion of cancer (31).

Fig 7: Black raspberry (source: *Ozgen et al., 2008*).

1.12.1 Constituents of black raspberry

Various phytonutrients are present that provide antioxidant and anti-inflammatory health benefits (31). Earlier studies focused on ellagic acid as the major bioactive compound present in raspberries (32)

1.12.2 Molecular mechanism involved in chemopreventive action of black raspberries

Huang et al. demonstrated the inhibitory effect of black raspberries on transactivation of activator protein-1 (AP-1) as well as NF-κB (26). Overexpression of these transcription factors lead to transactivation of genes such as cyclooxygenase-2 (COX-2) and Vascular Endothelial growth factors (VEGF) which are responsible for cancer cell proliferation, tumor invasion, metastasis and angiogenesis. The major pathway which is involved in the activation of AP-1 transcription factor is the MAPK pathway or mitogen activated protein kinase pathway and that involved in the activation of NF- κB is IKK pathway. When growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factor (IGF) binds to the receptor tyrosine kinase it leads to the activation of three kinases which include ERK, JNK, P38K which translocate into the nucleus causing activation of many transcription factor including AP-1. However in case of cancer due to mutation in receptor or protein leads to the overexpression of AP-1 (34). Black raspberry affect the activity of ERK, JNK and p38 kinase pathway which ultimately downregulate the activity of COX-2 and VEGFs (35).

The other pathway which is IKK pathway involves the binding of TNF-α or bacterial product to toll like receptor or interleukin-1 which leads to degradation of inhibitor of NF-κB. Degradation of this inhibitor causes release of NF-κB which translocate into the nucleus and activates COX-2 and VEGF. Mutation in IKK pathway leads to overexpression of NF-κB which

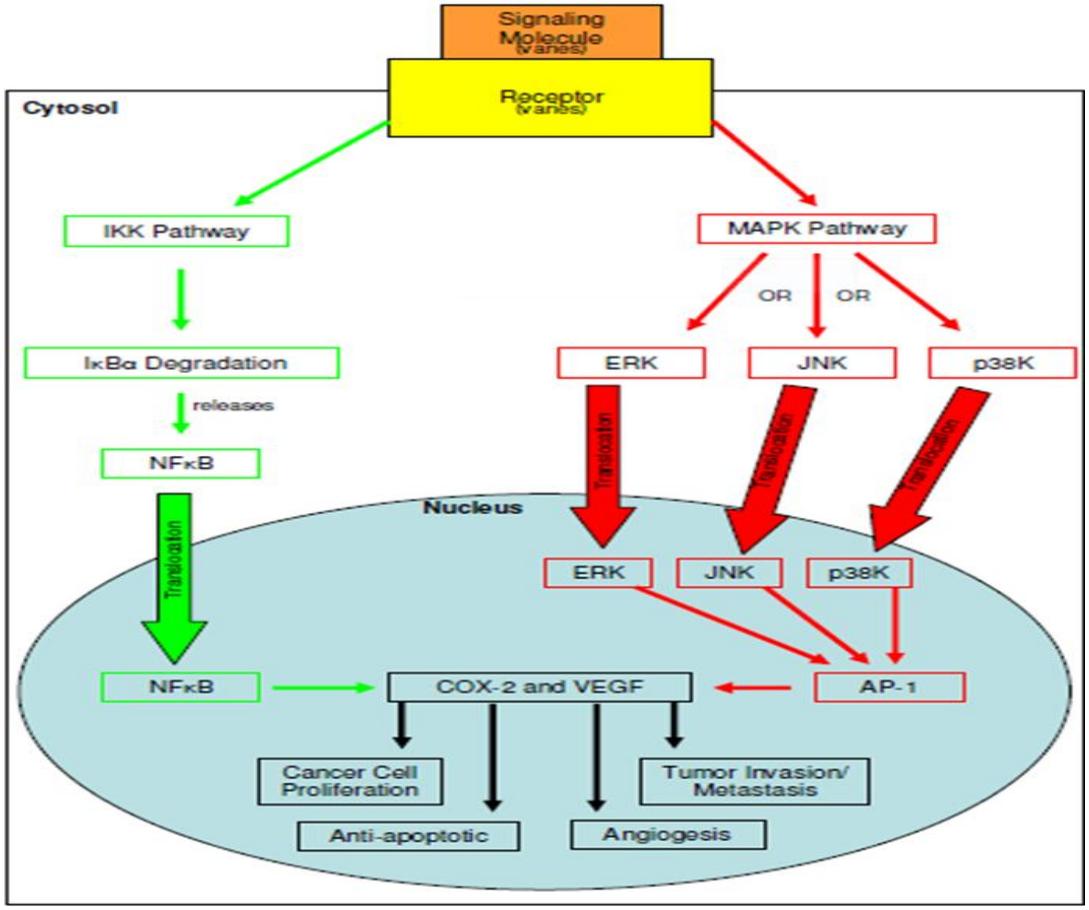


Fig 8. Pathways in cancer adapted from *Lodish et al., 2007*

1.13 Lantana camara

Lantana camara L. belongs to verbanaceae family also known as wild or red sage. It is regarded as a notorious weed and a popular ornamental plant (36). It grows at a height of 2000 m in tropical, sub-tropical and temperate regions. The plant is aggressive and has invaded vast expenses of forests, pastures and orchards in tropical and subtropical areas and is an obligate breeder weed (37). L. camara is known by different name in in India namely Raimuniya (Hindi), Tantani and Ghaneri (Marathi), Pulikampa (Telegu), Kakke and Natahu (Kanada), Chaturangi and Vanacehdi (Sanskrit), Arippu and Unnichedi (Tamil), Aripoov, Poochedi, Konginipoo and Nattachedi (Malayalam), Thirei, Samballei and Nongballei (Manipuri). Ethanolic extract of Lantana camara leaves has shown high antioxidant activity in both nitric oxide free radical scavenging assay as well as DPPH radical scavenging assay (38).

Fig9. *Lantana camara (adapted from Ghisalberti EL.et al.2000)*

1.13.1 Phytonutrients

Many triterpenes are present in the leaves of Lantana camara which include oleanonic acid, lantadeneA, lantadene B, lantanilic acid, icterogenin, camaroside, lantoic acid and lantadene D (39). Anti-inflammatory activity is shown by certain pentacyclic triterpenes present (40). For example oleanolic acid and ursolic acid show such activity (41). Primarily inhibitors of cyclooxygenase enzyme are of importance since they exhibit anti-inflammatory effects with minimum side effects compared to non-steroidal anti-inflammatory drugs. The levels of COX-2are found to be very low under normal conditions in most cells, but a 10-80 fold increase of COX-2 occurs after induction by growth factors, oncogenes, cytokines, serum and tumor promoters. The overproduction of prostaglandins, observed at acute and chronic inflammation sites, is thought to be due in part to up-regulation of COX-2 as when activated COX-2 triggers the release of prostaglandins. Ursolic and oleanolic acid possess inhibitory effects on various stages of tumor development and inflammation.

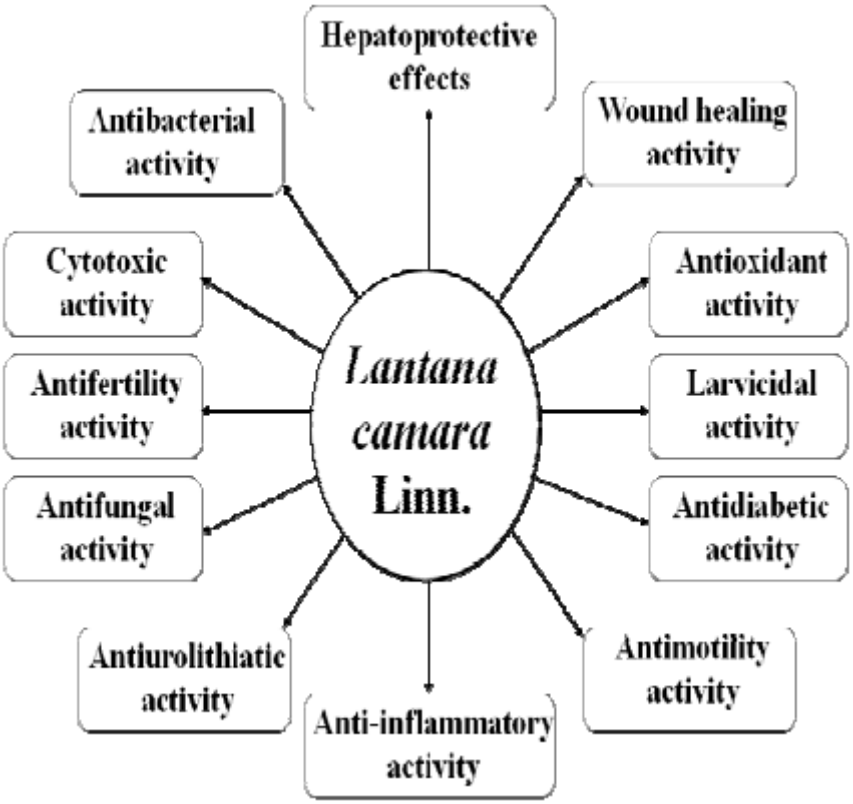


Fig10. Medicinal properties of *L.camara* (*Adapted from Sanjeev et.al 2007*)

1.13.2 Molecular mechanism involved in chemopreventive action of Lantana

Activation of Cox-2 which leads to excessive release of prostaglandins by catalyzing arachadonic following its release from the plasma membrane by the action of phospholipase-A2 (42). Major pathways affected in colon cancer are PI3K/AKT-signalling pathway Wnt pathway and Ras-MAPK pathway (43, 44). On receipt of extracellular signals various pathways are activated which leads to suppression of apoptosis (45). Upon engagement of the EP2 receptor, PGE2 stimulates a dual-signalling cascade involving the association of the G-protein αs subunit with Axin and activation of the PI3K/AKT pathway by the EP2-associated G-protein βγ subunits (79). All these pathways ultimately result in cell survival, angiogenesis, cell proliferation and anti-apoptosis.

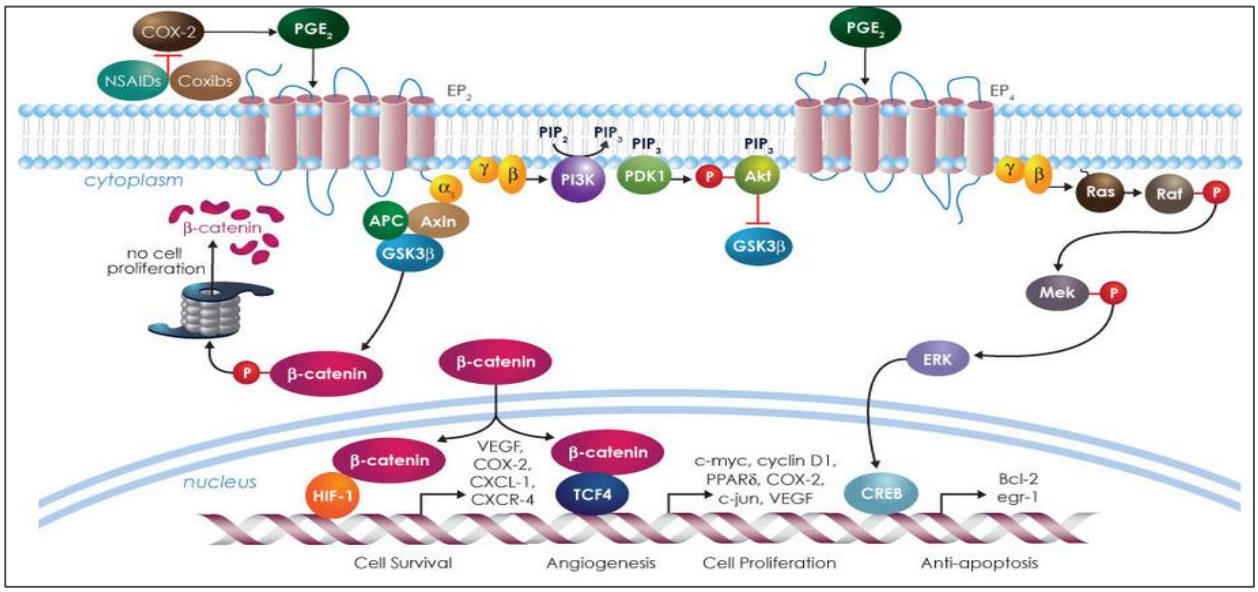


Fig 11. Signalling in colon cancer (source: COX-2/PGE2 Signalling: A Target for Colorectal Cancer Prevention by Williams et al., 1997)

1.14 Gp-60 mediated transcytosis

gp60 (albondin) is a 60-kDa glycoprotein which binds to native albumin present in a nanomolar range and is present on the endothelial surface (Schnitzer 1992). The transcytosis of albumin across endothelium blood vessel is mediated by gp-60 and caveolae. When albumin binds to gp-60 it results in gp-60 clustering and its association with caveolin-1 which is an intracellular protein. This results in the formation of transcytotic vesicles caveolae which carry gp-60 bound and fluid phase albumin or albumin bound drug and the vesicle content are released into sub endothelial space. Moreover, osteonectin lung, colon, liver which is a secreted protein acidic and rich in cysteine [SPARC] has been recognized as albumin binding protein which helps in accumulation of albumin to tumor site. SPARC protein is found to be overexpressed in variety of tumours including prostate, oesophagus, breast, liver, head and neck, skin melanoma and brain tumours for tumor invasion and metastasis. Abraxane has achieved 33% higher intratumoral paclitaxel concentration as compared to taxol. It shows that gp-60 transport in tumor blood vessel and accumulation of albumin to tumour specific site can enhance the accumulation of albumin bound drug to tumour specific site thereby improving the efficacy by targeting to tumour specific site. This mechanism increases the retention in the tumour interstitium.

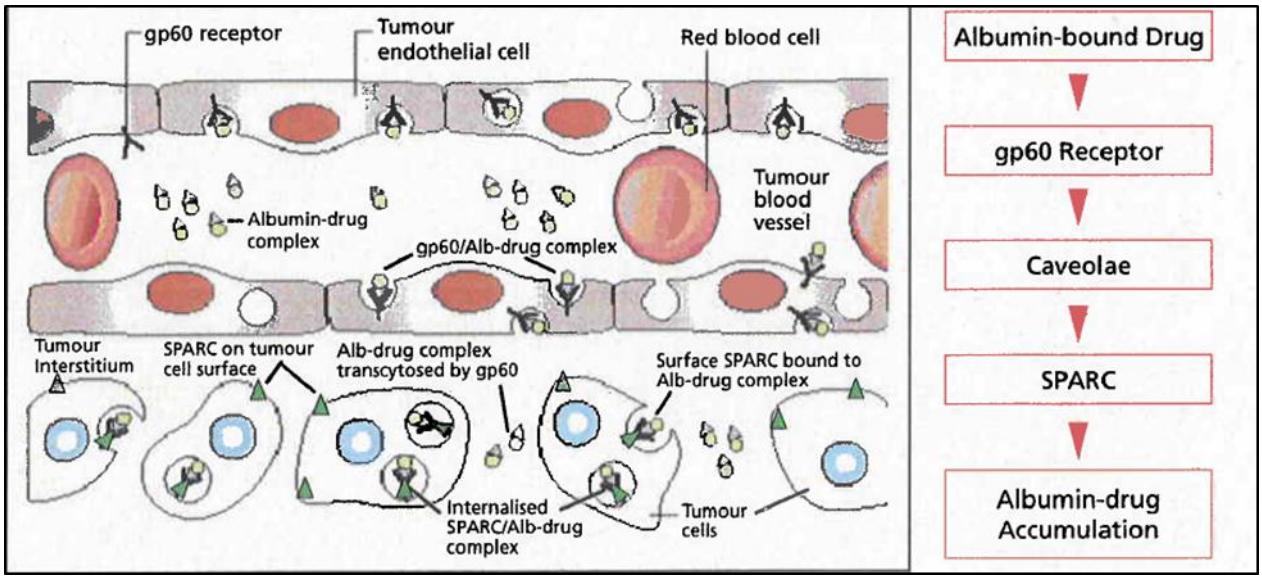


Fig 12. Mechanism for the transport and accumulation of albumin-bound drug in cancer (source: Drug Delivery Report Winter 2007/2008).

1.15 Assay Performed

- Phytochemical assay
- DPPH analysis
- Anthocyanin analysis
- Solubility assay
- Nanoparticle preparation
- Characterisation of nanoparticle
- Cytotoxicity studies

1.16 Objective

To evaluate the effect of phytochemical loaded nanoparticles on tumor growth and cancer cell multiplicity in colon cancer HT-29 cell line using Nano technological approach through phytotherapeutics. This is to be achieved by:

- Extraction and evaluation of (*Lantana camra (l*eaves*) & Rubus niveus (b*erries)
- Preparation and evaluation of the albumin based nanoparticle.
- Characterization of Extract loaded albumin nanoparticles
- Cytotoxicity evaluation

REVIEW OF LITERATURE

CHAPTER 2

Review of literature

.

- Globally colon cancer is the third most common cancer. According to GLOBOCAN factsheet, 2012 mortality is found to be lower (694,000 deaths, 8.5% of the total) but 52% of it occurs in less developed countries, reflecting poor survival in these regions The most common cancers (the type called adenocarcinoma) of large intestine arise from mucosa which is the inner lining of large intestine according to data provided by American Cancer Society, 2009. The incidence rate was found to be 50 times higher in persons aged between 60 to 79 years than those who were below 40 years of age.
- Epidemiological studies by Block et al. and others have indicated that person who consumes diets rich in fruits and vegetables have comparatively lesser risk of developing cancer (48-50). The hypothesis is supported by epidemiological as well as experimental data provided by Eberhardt et al. and others which indicate antioxidants are responsible for reducing cancer risk (51-52). Stoner, G.D. et al. reported that antioxidants are provided mainly by the flavonoid and phenolic acid content present in plants (53).These antioxidants have radical scavenging effects on reactive oxygen species involved in initiation and progression of colon cancer (54-55).
- Phytochemicals from raspberries are found to be effective inhibitors of oxidative DNA damage invitro in immortalized human colon cells (56).Olsoon.et al. reported that anthocyanins present in these berries show antiproliferative activity invitro on colon cancer cells (57-59). Lansky.et al. however reported that polyphenols present in them also prevent metastasis and invasion of cancer (60).
- Ozgen M.et al. reported that ellagic acid is the major bioactive compound present in raspberries (61). Due to the ability of ellagic acid to act as chemoprotective agent it has been widely studied (62). As the polyphenolic anthocyanins are found in large quantities in black raspberry it is being studied more for its chemoprotective activity (63).

- Following table provides list of various phytonutrients found in black raspberry.

S.no	phytochemical	Examples
1	anthocyanins	Cyanidins , Pelargonidins, Delphinidins , malvidins
2	flavonols	Quercetin, kaempferol
3	flavanols	Catechins,epicatechins
4	flavonoid glycosides	tiliroside
5	tannins	Ellagitannins, Gallotannins, proanthocyanidins
6	hydroxybenzoic acids	ellagic acid, lambertianin, sanguiin, vanillic acid, gallic acid chlorogenic acid
7	hydroxycinnamic acids	caffeic acid, coumaric acid, ferulic acid
8	stilbenoids	resveratrol

Table 2. Phytonutrients in black raspberry

- In an invitro studies by Emma M Coates and others (64) a colon available raspberry extract that contained phytochemicals surviving the digestion procedure. This colon available raspberry extract had reduced content of anthocyanin and ellagitannins compared to the original extract of raspberry .They significantly inhibited several important stages in colon carcinogenesis.
- Also invitro study was done by Rodrigo KA.et al. for antiproliferative effects on human oral squamous, cell carcinoma tumors was determined using freeze dried ethanolic extract of black raspberry (65). The cells were treated with 10, 50, or 100 µg black raspberry ethanol extract/mL of media and at 24, 48 and 72 hr cells were harvested and counted. The results showed that the freeze-dried black raspberry ethanol extract treatments inhibited the growth of the oral cancer cells in a dose-dependent manner.
- Kresty et al. reported that male mice fed with 5% and 10% lyophilized black raspberry extract for about 14 days followed by single injection of esophageal carcinogen, N-nitrosomethylbenzylamine (NMBA), showed a significant decrease in level (73%, 80%) of O6-methylguanine adducts in the DNA of their esophagus (66).

- A recent study by Stoner.et al. showed that an anthocyanin rich fraction, derived from LBRs and an alcohol: water (80:20) extract of LBRs, were equally as effective as a 5% LBR diet in reducing NMBA-induced tumors in the rat esophagus (67). Also a recent study shows that it also contains high levels of ellagitannins (S.S. Hecht, S. Carmella, L-S Wang and G.D. Stoner, unpublished data).Thus both anthocyanins and ellagitannins contribute towards chemopreventive effects of berries.
- The experiment conducted by Borges.G. et al. and others on uptake of anthocyanins and ellagitannins from berry juices in rodents have shown that these compounds are poorly absorbed and their plasma levels decline rapidly (68-69). These findings indicate that raspberry anthocyanins are poorly absorbed and most of them are degraded by colonic bacteria in the large intestine. There are many therapies available which show significant reduction in tumor but have certain toxicities associated with normal cells as well (70). So it is vital to find nontoxic and effective treatments to reduce the tumor growth in cancer.
- Huang et al. demonstrated the inhibitory effect of black raspberries on transactivation of activator protein-1 (AP-1) as well as NF-κB (71). Overexpression of these transcription factors lead to transactivation of genes such as cyclooxygenase-2 (COX-2) and Vascular Endothelial growth factors (VEGF) which are responsible for cancer cell proliferation, tumor invasion, metastasis and angiogenesis. The major pathway which is involved in the activation of AP-1 transcription factor is the MAPK pathway or mitogen activated protein kinase pathway and that involved in the activation of NF- κB is IKK pathway. Black raspberry affect the activity of ERK, JNK and p38 kinase pathway which ultimately downregulate the activity of COX-2 and VEGFs (71). Mutation in IKK pathway leads to overexpression of NF-κB which suppresses apoptosis (72). Lu H. et al. and others reported that black raspberry is found to downregulate the phosphorylation of IκBα (73, 74).

- Keppler K. et al. reported that anthocyanins are much less stable in the neutral Ph of small intestine and large intestine system (75, 76). About 60% of ingested amount reaches colon where it is degraded by its microbial microflora (77). Anthocyanin is measured as maximum blood plasma concentration or percentage of anthocyanins excreted in the urine. A major limitation of anthocyanins is that its bioavailability is very low, often the amount excreted in urine is found to be less than 0.1% of ingested one as reported by Carlton PS. et al. . So, the intact anthocyanins are not able to reach all parts of body in sufficient amount (78).
- Ethanolic extract of Lantana camara leaves has shown high antioxidant activity in both nitric oxide free radical scavenging assay as well as DPPH radical scavenging assay as reported by Bhakta D et al. (79, 80).Many triterpenes are present in the leaves of Lantana camara which include oleanonic acid, lantadeneA, lantadene B, lantanilic acid, icterogenin, camaroside, lantoic acid and lantadene D (81). Anti-inflammatory activity is shown by certain pentacyclic triterpenes present (82). For example oleanolic acid and ursolic acid show such activity as reported by Sharma VS (83, 84).
- In a recent study by Ringbom et al. Oleanolic acid was shown to have IC 50 of 295 mM and a COX-2/COX-1 selectivity ratio of 0.8. Ursolic acid was shown to have COX-2 inhibitory effect with an IC50 value of 130 mM and a COX-2/COX-1 selectivity ratio of 0.6 (85). Primarily inhibitors of cyclooxygenase enzyme are of importance since they exhibit anti-inflammatory effects with minimum side effects compared to non-steroidal anti-inflammatory drugs. Greater than 80% of colon cancers have increased COX-2 levels when compared to normal tissue present inside the body.(86). Major pathways affected in colon cancer are PI3K/AKT-signalling pathway Wnt pathway and Ras- MAPK pathway (87, 88, 89). All these pathways ultimately result in cell survival, angiogenesis, cell proliferation and anti-apoptosis.

- Nanotechnological approaches provide effective mean for sustained release of chemopreventive drug critically improving its bioavailability.

phytochemical	problem	Nanotechnological approach	reference	Result
curcumin	Low water solubility and bioavailability	polymer nanoparticle encapsulated curcu Emulsions	(Tsai YM et al., 2012) (Ucisik MH et al.2013)	40 fold increase due to high MW polymer 10,000 fold increase
colchicine	bioavailability	nanoemulsion	(Shen et al., 2011).	2.1 fold increase
EGCG	Low bioavailability	Polymer nanoparticle	(Imtiaz A. Siddiqui et al.2010)	10 fold dose advantage in inhibiting tumor cell growth
paclitaxel	Water insoluble but potent anticancerous drug	PLGA nanoparticles	(Feng, S.S et al.2004)	13 times more cell mortality in HT-29 cell line

Table 3. Nanotechnological approaches to improve bioavailability of drug

- All these results show that bioavailability is significantly enhanced by using a nanoformulation of these phytochemicals which increase the cell mortality in cancer cell line. Cancer nanotechnology offers nanoparticles which can target tumor and increase the bioavailability of drug to administer novel therapies as reported by Mc Neil (90). National Cancer Institute also considers cancer nanotechnology can make significant advances in cancer treatment (91).
- Protein nanoparticles possess certain advantages such as they are biodegradable, non- antigenic, metabolizable and are easily amenable for surface as well as covalent attachment of drug and ligand due to defined primary structure of protein as reported by Weber C (92).Various protein nanoparticles such as gelatin, albumin, gliadin and legumin have been prepared.

- Kratz F et al. reported that Albumin is an attractive macromolecular carrier which has advantages of biodegradability, nontoxicity and non-immunogenicity (93) and is used in the form of BSA (bovine serum albumin) or HAS (human serum albumin).Moreover albumin is the major plasma protein constituting 60% of it. This provides it distinct edge for nanoparticle preparation over other materials. A number of studies have reported that albumin accumulates inside solid tumor for instance those by Takakura Y (94) which makes it potential macromolecule carrier that can be used as effective targeted drug delivery vehicle of chemoprotective drugs. Abraxane which is albumin bound paclitaxel is the first nanodrug approved by FDA in January 2005.Abraxane has achieved 33% higher intratumoral paclitaxel concentration as compared to taxol in SPARC-positive MX-1 tumour xenografts as reported by Desai et al. (95). Trieu reported enhanced response to Abraxane in SPARC over-expressing line PC3/SP compared with wild type PC3 xenograft which clearly demonstrates its role in targeting to tumor site (96).

MATERIAL AND METHOD

CHAPTER 3

3.1 MATERIALS

3.1.1. Chemicals Used

Lantana camara leaves were collected from local region of Shimla (6,117 ft.) of Himachal Pradesh, India, and the berries *Rubus niveus* extract were purchased from the Nutri-Fruit™ (Division of Scenic fruit company of Gresham, Oregon)

S.No.	Ingredients	Source
1	Gelatin	Loba chemie
2	Albumin(BSA)	Loba chemie
3	Glutreldehyde 0.8%	Merck Chemicals
4	Acetone	Loba chemie
5	Methylene chloride	Spectrochem pvt. Ltd. Mumbai
6	Clove oil	sachee fragrance and chemicals ltd. India
7	Ethanol	Merck Chemicals
8	Methanol	Himedia Laboratories Pvt. Ltd
9	Potassium dihydrogen ortho phosphate	Nice Chemicals
10	Sodium chloride	Nice Chemicals
11	Dialysis membrane	Himedia Chemicals

Table 4. List of Chemicals

3.1.2. Glass wares used

The list of glass wares used is given in Table 3.

S.No.	Glass wares
1	Beakers 500 ml
2	Beakers 100 ml
3	Beakers 50 ml
4	Volumetric flasks 100 ml
5	Volumetric flasks 1000 ml
6	Volumetric flasks 10ml
7	Pestle Motor
8	Pipettes 10 ml
9	Pipettes 5 ml
10	Measuring cylinders 10 ml
11	Measuring cylinders 50 ml
12	Measuring cylinders 100 ml
13	Liquid Funnel
14	Glass Rod
15	Soxhlet Assembly
16	Test tube

Table 5. List of Glass wares

3.1.3. Instruments Used

S.No.	Instruments	Manufacturer
1	Double beam U.V Spectrophotometer	Elico(R)
2	PH meter digital	Harco & Co.
3	Digital Weighing Balance	Citizen Equipment service Pvt. Ltd.
5	Rotary flash evaporator	Superfit
6	Magnetic stirrer	Macro scientific
7	Hot plate	Thamson
8	Nanodrop Spectrophotometer	Thermo scientific
9	Hot air oven	Relitech
10	Surological water bath	Relitech
11	Incubator	Labnet
12	Ultrasonicator	Citizen
13	Lypholyzer	~NA~

Table 6. List of Instruments

3.2 Methods

3.2.1 Preparation of *Lantana camara* leaves extract

The hydroalcholic extraction was done as was mentioned in the shazam et.al. The fresh collected leaves of *Lantana camara* were dried till they become firm and then these dried leaves about 100g of them were grinded using a mixer grinder at high r.p.m. Grinding was done till the thin powder was obtained. Now the powder about 100g of it was then inserted into the soxhlet apparatus's (1000ml) siphoning tube with about 500 ml of the petroleum ether ,which was then placed over a round bottom flask (1000ml) and then was closed at the top by the condenser. The complete apparatus was then kept above the heating mantel held by the support from the side . The tube filled with the pure petroleum ether for the first cycle of 24 hrs for the process of oil extraction and after oil were removed The powdered extract was again dried in air so that ether evaporates away and the extract is used once again with the hydro alcoholic solution (250ml water and 250ml methanol) in the process to obtain hydro alcoholic extract.

3.2.2 Preparation of *Rubus niveus* berry extract

Whereas in case of *Rubus niveus* the process of macreation was used which was performed by dissolving about 20g of freeze dried extract in the 100ml of hydro alcoholic solution and was kept for rotation in a conical flask of 500 ml over a heated magnetic stirrer at 40 C^{o} for about 24 hrs. After each cycle of about 24 hrs the process was stopped and the solvent was filtered. The filtered liquid was rota evaporated and freeze died to obtain the extract that was in it whereas the rest of the filtrate was used with a fresh solvent. It was carried on for 7 days and done till the solution became from dark red to light pink.

3.2.3 Phytochemicals Screening of *Rubus niveus* berry extract and *Lantana camara* Leaves extract

Phytochemicals from *L.camara* & *Rubus niveus* contains numerous categories such as tannins, glycosides, alkaloids, and phenols etc, which have shown various pharmacological activities. Qualitative and quantitative analysis were performed using standard procedure as follows.

3.2.3.1 Determination of Total Phenols

Total phenolic content was quantified as for the method made by Alya *et al.* (2011) (84). For 0.2 ml of plant extract (1mg/ml), 0.4 ml of Folin-Ciocateu reagent was mixed and solution was allowed to stand at 25°C for 5-8 min before adding 0.2 ml of 7% sodium carbonate solution. Using deionized water, the final volume was made up to 10 ml and kept at room temperature for 2 h. After 2 h absorbance was measured at 765 nm. Thus, the calibration curve was plotted using Gallic acid as a standard for the determination of total phenolic.

3.2.3.2 Determination of Tannin contents

For the determination of total tannins content method of Tyler, (1994) and Harborne, (1973) was used. For 5ml of plant extract (1mg/ml) add 0.2 ml of 0.1M ferric chloride solution. The absorbance was measured at 395nm using UV spectrophotometer. Calibration curve of tannic acid was plotted to determine the total tannins [85,86].

3.2.3.3 Determination of total Flavonoids

For the assessment of flavonoids the method of Dewanto *et al.* (2002) was used [87]. In this method quercetin was used as a standard and flavonoids contents were measured as quercetin equivalent. For this purpose calibration curve of quercetin was plotted. To determine the flavonoids contents 1.50 ml of distilled water was added to 0.25 ml sample extract (1mg/ml), followed by addition of 90 µl of 5% sodium nitrite ($NaNO_2$). After addition of 180 µl of 10% $AlCl_3$, the mixture was allowed to stand for 5 min before adding 0.6 ml of 1M NaOH. At the end final volume was made with water up to 3 ml and absorbance was measured at λ_{max} 510 nm using UV spectrophotometer .

3.2.3.4 Determination of total Alkaloids

Total alkaloids were determined by the method as mentioned by [88]. Atropine was used as standard for the determination of total alkaloids. For this purpose calibration curve of Atropine was plotted. For determination of total alkaloids Bromocresol green (BCG) solution was prepared by heating 69.8mg BCG with 3 ml of 2N NaOH and 5 ml of distilled water and volume was made up to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by mixing 8.5g of sodium acetate in 31.5 ml of distilled water this mixture was mixed with 31.5 ml of dilute acetic acid solution. For quantitative estimation 1 ml of plant extract (1mg/ml) was mixed with 5

ml of phosphate buffer pH 4.7, followed by mixing of 5 ml BCG solution and 4 ml of chloroform in separating funnel. Finally, the chloroform layer containing plant extract was collected in the volumetric flask and absorbance was measured at λ_{max} 470 nm using UV spectrophotometer.

3.2.4 Antioxidant Assay (DPPH)

DPPH scavenging activity of the plant extracts was carried out according to the method of Koleva et al. and Mathiesen et al.[89,90] Ethanol solution of plant extracts (0.2 ml) at different concentrations (50–200 µg/ml) was mixed with 0.8 ml of tris HCl buffer (100 mM, pH 7.4). One milliliter DPPH (500 mM in 1.0 ml ethanol) solution was added to the above mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer. All the assays were carried out in triplicates.

$$\% \text{ DPPH radical scavenging} = \left(\frac{\text{Absorbance of control- Absorbance of test sample }}{\text{(Absorbance of control)}}\right) \times 100$$

Decreased absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity. In this study, petroleum ether, ethanolic, and aqueous extracts of *L.camra and hydroalcoholic* extract of *R.niveus* was used fruits were used.

3.2.5 Determination of Anthocyanin content using pH differential method

Anthocyanin content was determined using Ph differential method as mentioned by (Giusti and Wrolstad, 2005). This method is based on change of the color of monomeric anthocyanin pigment on changing the pH ascolored oxonium form exists at Ph 1.0 and colorless hemiketal form predominates at Ph 4.5. A dilution factor of 10 was used to ensure that when black raspberry was diluted using Ph 4.5 buffer of potassium chloride their absorbance lies within linear range of spectrophotometer (less than 1.2). Solutions were set in dark for 15min before taking reading in spectrophotometer. Spectral measurement was done at 570 nm (maximum absorption of anthocyanin) and 700 nm for haze correction. Monomeric anthocyanin content was determined using following equations: Absorbance (A) = (A520 – A700) pH 1.0 – (A520 – A700) pH 4.5 Monomeric anthocyanin (mg/L) = (A x MW x DF x 1000) / (e x 1) Where MW= molecular weight, DF= dilution factor, e= molar absorptivity coefficient, A= absorbance Pigment content was calculated as cyanidin-3-glucoside, where MW = 449.2 and e = 26,900 cm-1 mg-1.

3.2.6 Solubility study

The solubility of *R.niveus* and *L.camra* extract in a mixture of calendula oil, surfactant and co-surfactant was determined by mixing excess amount of extract in 0.5 ml of the mixture, separately in 2 ml of eppendrof and mixed using vortex mixer. The mixture vial then kept at 25±1.0 ℃ in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3,000 rpm for 15 min. The supernatant was filtered through 0.22 µm membrane filter and the concentration of extract in mixture was determined using UV spectrophotometer at λ_{max} 350 nm using linear equation of *B.aristata* extract (91,92).

3.2.7 Prepration of albumin based nanoparticle

The main aim of the formulation was to create a controlled realease of the drug ,increase efficacy, blood circulation time to increase target specificty by the use of albumin .Briefly, bovine serum albumin between 50 mg and 200 mg was dissolved in 2.0 ml of purified distilled water. Subsequently, drug was dissolved in 8 ml ethanol, which was added drop wise into the aqueous albumin solution under magnetic stirring (500 rpm). This resulted in the formation of an opalescent suspension spontaneously at room temperature. After this desolvation process, 0.11 ml of 8% glutaraldehyde in water (v/v) was added to cross-link the desolvated bovine serum albumin nanoparticles. The cross linking process was performed under stirring of the colloidal suspension over a time period of 24 hours. This method was utilized to produce the drug loaded part of the nanoformulation for the prepration of the blank formulation only 8m of ethanol is added without the dissolved drug in it.

3.2.8 Characterization of Nanoparticles

.

3.2.8.1 Dynamic Light Scattering

To determine the size and size distribution of albumin nanoparticle, DLS measurements were performed using a zeta sizer at *(IIT, mandi*). The intensity of the scattered light was detected at 90° to the incident beam. The light source was a 35 mW He-Ne laser emitting monochromatic light at a wavelength of 632.8 nm, which was focused onto the sample, and the scattered light was detected by a photo-multiplier tube.

3.2.8.2 Measurement of Zeta Potential and PDI

The zeta potential of the ANP's was measured by varying the pH in a Nanoparticle Size Analyzer. About 3 mL of the suspension (1 mg/mL) was added to a cuvette and adjusted to pH values in the range from 2–10 using 0.1 N HCl or 0.1 N NaOH. The suspension was equilibrated for 4 min at 25 °C. The measurement was performed with three runs, with each run consisting of 10 single measurements.

3.2.8.3 Encapsulation efficiency

Determination of drug entrapment, the amount of drug present in the supernatant (obtained at the end of preparation of nanoparticles) after centrifugation was determined (w) by UV-spectrophotometer. A standard calibration curve of concentration vs. absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the desolvation process (W). (W-w) will give the amount of drug entrapped in the pellet. Then exact percentage entrapment is given by *(W–w)/W * 100.*

3.2.8.4 In-vitro drug release profile

In-vitro release of *R.niveus* and *L.camara* from the nanoparticle was evaluated using a dialysis bag-diffusion technique[93]. Distilled water (100 ml) was used as the release medium at 37°C ± 0.5°C, under constant stirring on magnetic stirrer. An appropriate amount of extract NP`*s* was suspended in the dialysis bag and immersed into the release medium. At certain time interval, 3 ml aliquot of the medium was pipette out, and at each withdrawal the medium was replaced by the same volume of fresh medium. The amount of extract released in the medium was calculated using UV spectrophotometer at 330 nm and 540nm respectively from the calibration curve of both the extract. A control experiment to determine the release behavior of the free drug was also performed for both the extract. An appropriate amount of the drug equivalent to that of Np`s was dissolved in water, and same volume of this solution was enclosed in the dialysis bag and was immersed into the release medium, at 37°C ± 0.5°C. Then procedure, described above for the nano formulation was followed. Each experiment was performed in triplicate (n=3).

3.2.9 Evaluation of cytotoxicity using the MTT assay

The MTT assay (as described above) is used to assess the in vitro cytotoxicity of phytochemical (Sgouras & Duncan, 1990). In brief, HT-29 cells (100 µl; 1x105 cells/ml) were seeded into 96 well micro titre plates as before and left to adhere for 24 h. The next day, the medium was removed from the wells and replaced with filter sterilised complete medium containing extract dissolved in DMSO 100mg/ml . The plates were then incubated with extract solution for either 6 h or 24 h. In the case of the 6 h incubation; medium was removed after 6 h and replaced with culture medium only and further incubated to the total time of 24 h. In the case of the 24 h incubation media containing extract was removed at 24 h and replaced with complete media. MTT (20 µl of 5 mg/ml in PBS) was added to each well of the plates for both incubation times. Plates were incubated for a further 5 h. Then the medium was removed and DMSO (100 µl) added before a further incubation of 30 min at 37 ºC. Finally the absorbance at 570 nm of the plates was read with the Tecan plate reader. Absorbance values were blanked against DMSO and the absorbance of cells exposed to medium only were taken as 100 % cell viability (i.e. the control).

RESULTS

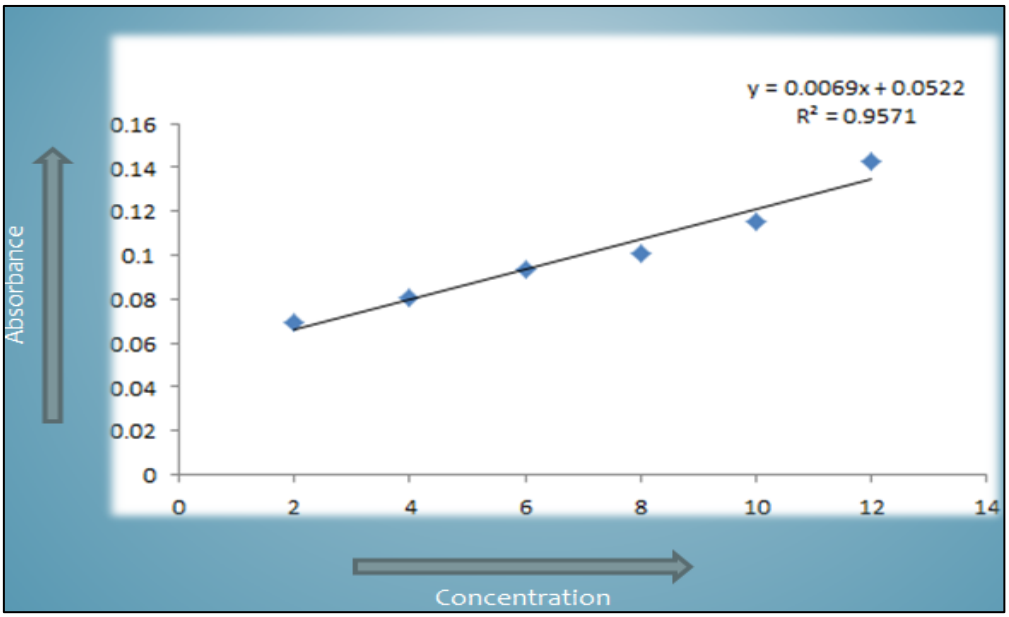
CHAPTER 4

4.1 Phytochemical Screening

Total Alkaloid

Atropine(µl)	Phosphate buffer(ml)	BCG(ml)	Chloroform (ml)	Abs at 470nm
200	5	5	4	**0.0692**
400	5	5	4	**0.0806**
600	5	5	4	**0.0932**
800	5	5	4	**0.1008**
1000	5	5	4	**0.1157**
1200	5	5	4	**0.1430**

Table 7. Reading for standard plot alkaloids



Graph 1. Standard plot for atropin

Lantana camara alkaloid content:

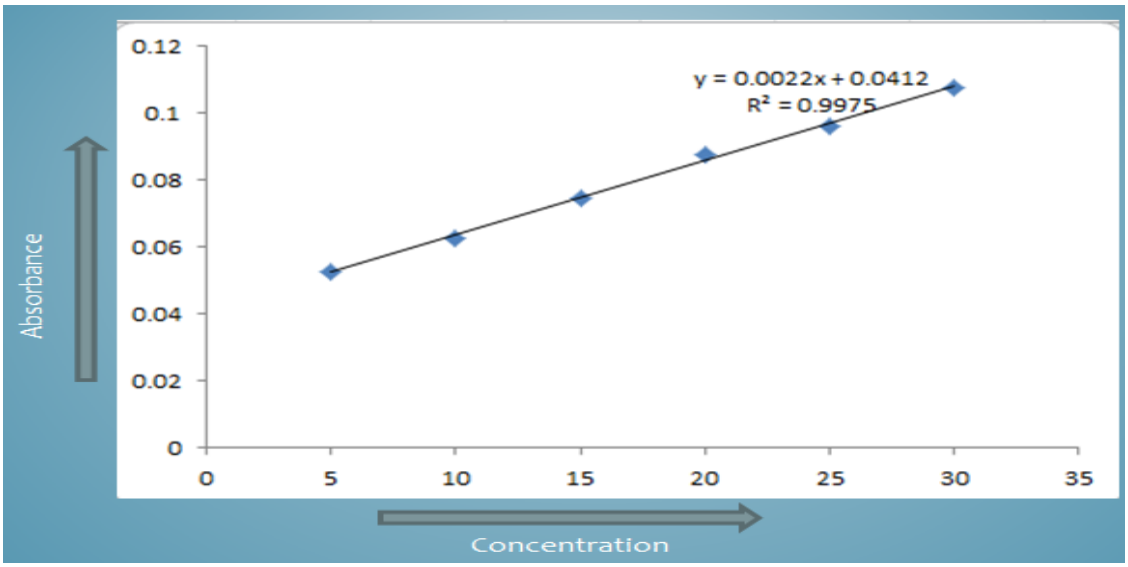
- OD of sample = 0.2532
- After equating the above values in equation of

- Y=mx + c
- We get **29.13µgAE/mg**

Amount of flavonoid

Quercetin (*µl*)	**Ethanol(*µl*)**	**Ab at 510 nm**
500	2500	**0.0525**
1000	2000	**0.0626**
1500	1500	**0.0745**
2000	1000	**0.0877**
2500	500	**0.0961**
3000	0	**0.1077**

Table 8. Reading for standard plot quercetine



Graph 2. Standard plot for quercetine

***Lantana camara* flavonoid content:**

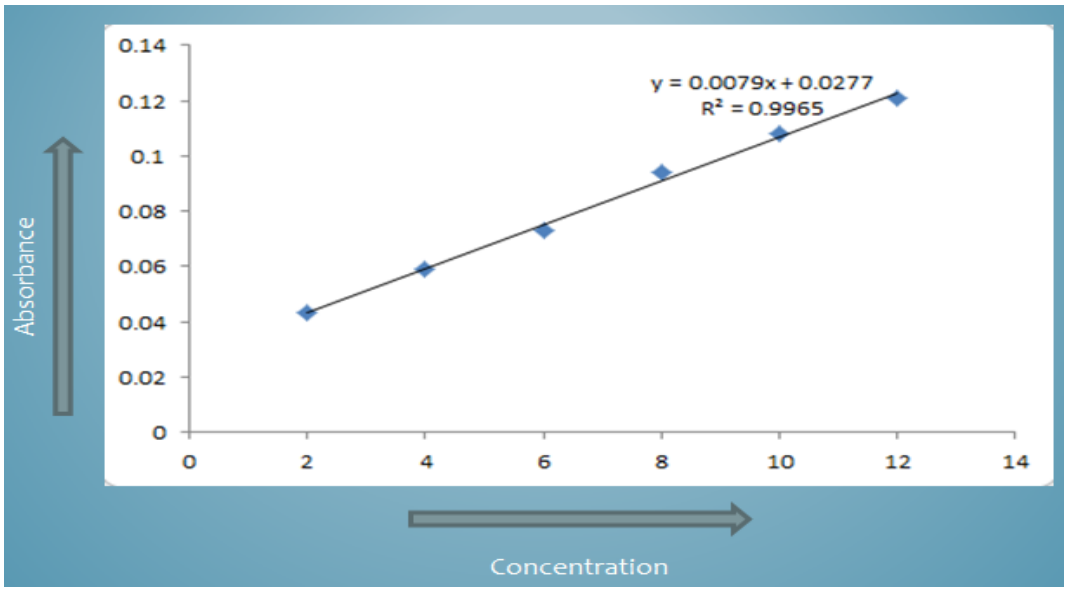
- OD of sample = 0.0767nm

- After equating the above values in equation of
- Y=mx + c
- We get x=**17.85 µg QE/mg of extract**

Amount of Tannins

Tannic Acid (µl)	Distilled water	Folin Colin reagent (ml)	Sodium Carbonate(ml)	Abs at 700nm
200	1000	5	4	**0.0435**
400	800	5	4	**0.0591**
600	600	5	4	**0.0733**
800	400	5	4	**0.0938**
1000	200	5	4	**0.1079**
1200	0	5	4	**0.121**

Table 9. Reading for standard plot Tannic acid



Graph 3. Standard plot for tannic acid

Lantana camara

- OD of extract =0.5748
- y = mx + c
- Putting the given data and equating we get
- **X=78.14 µgTAE/mg**

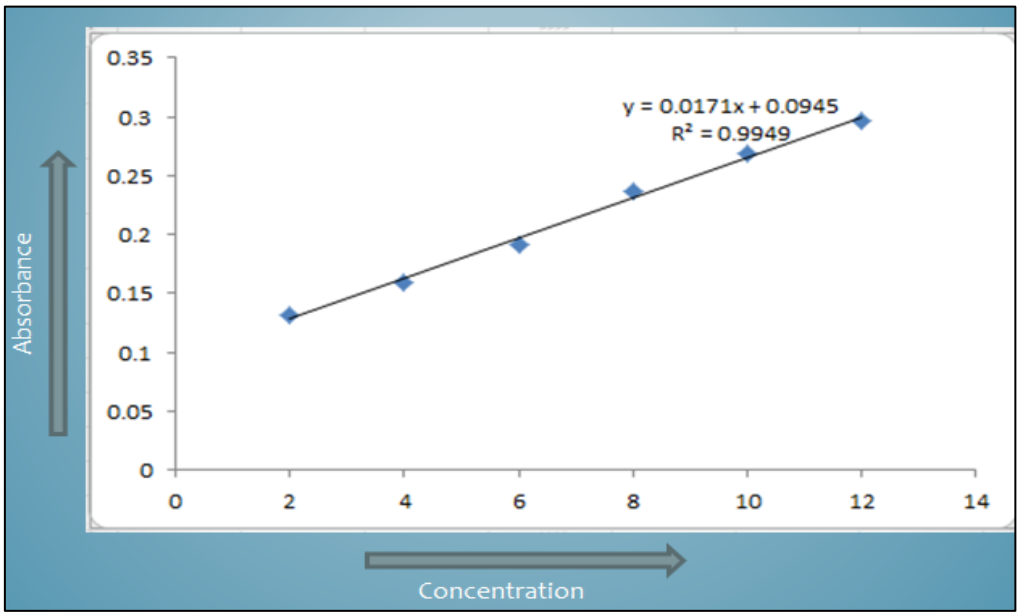
Rubus niveus

- OD of extract =0.4055
- y = mx + c
- Putting the given data and equating we get
- **X=54.05 µgTAE/mg**

Amount of Gallic acid

Gallic acid solution (µl)	Distilled water (*µ*l)	Folin colin reagent(ml)	Sodium Carbonate(ml)	Abs at 765nm
200	1000	5	4	**0.1318**
400	800	5	4	**0.1590**
600	600	5	4	**0.1917**
800	400	5	4	**0.2366**
1000	200	5	4	**0.2685**
1200	0	5	4	**0.296**

Table 10. Reading for standard plot of gallic acid



Graph 4. Standard plot for gallic acid

Lantana camara

- OD extract = 0.2864
- y = mx + c
- Putting the given data and equating we get
- **X= 14.87 µgGAE/mg**

Rubus niveus

- OD extract = 0.6753
- y = mx + c
- Putting the given data and equating we get
- **X= 34.25 µgGAE/mg**

Sum analysis of all the components

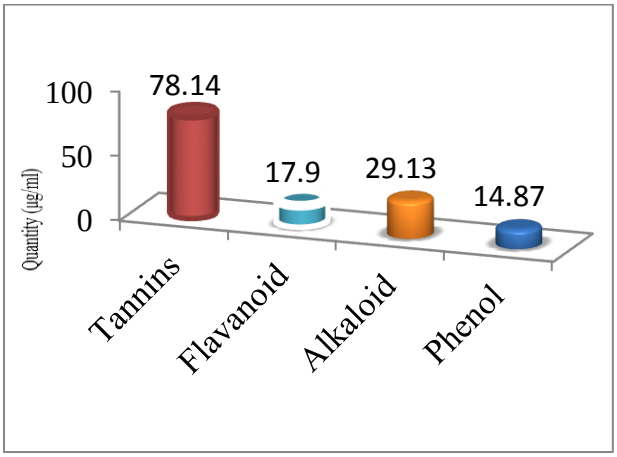


Fig 13. Phytochemical component in *Lantana camara*

Determination of anthocyanin content in Rubus niveus

Wavelength	pH=1	pH=4.5
540nm	**0.850**	**0.283**
700nm	**0**	**0**

Table 11. Anthocyanin content

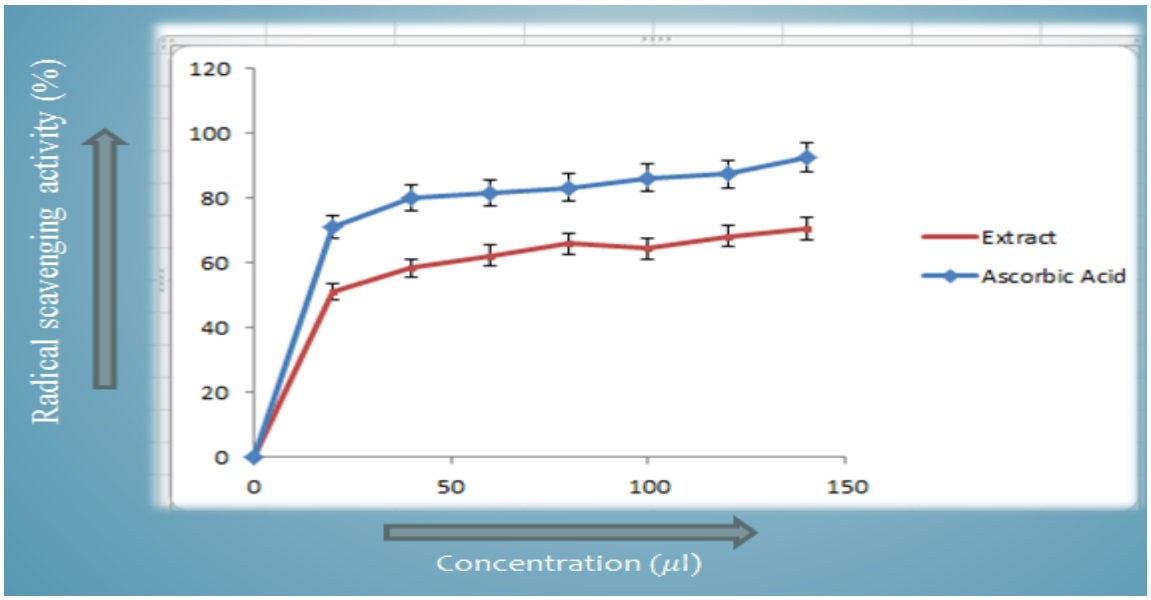
Concentration (mg/l) = 42.6 mg/l

4.2 Antioxidant activity

- (*lantana camara*) Extract vs. Control

Concentration (*µl*)	Positive control	Extract (*L.camra*)
200	71.20%	**50.9%**
400	80%	**58.4%**
600	81.6%	**62.3%**
800	83.3%	**64.5%**
1000	86.2%	**65.9%**
1200	87.5%	**68.3%**
1400	92.5%	**70.8%**

Table 12. Antioxidant Assay



Graph 5. Extract vs Positive control

4.3 Solubility and standard plot

Lantana camra

Solubility :- 6mg/ml

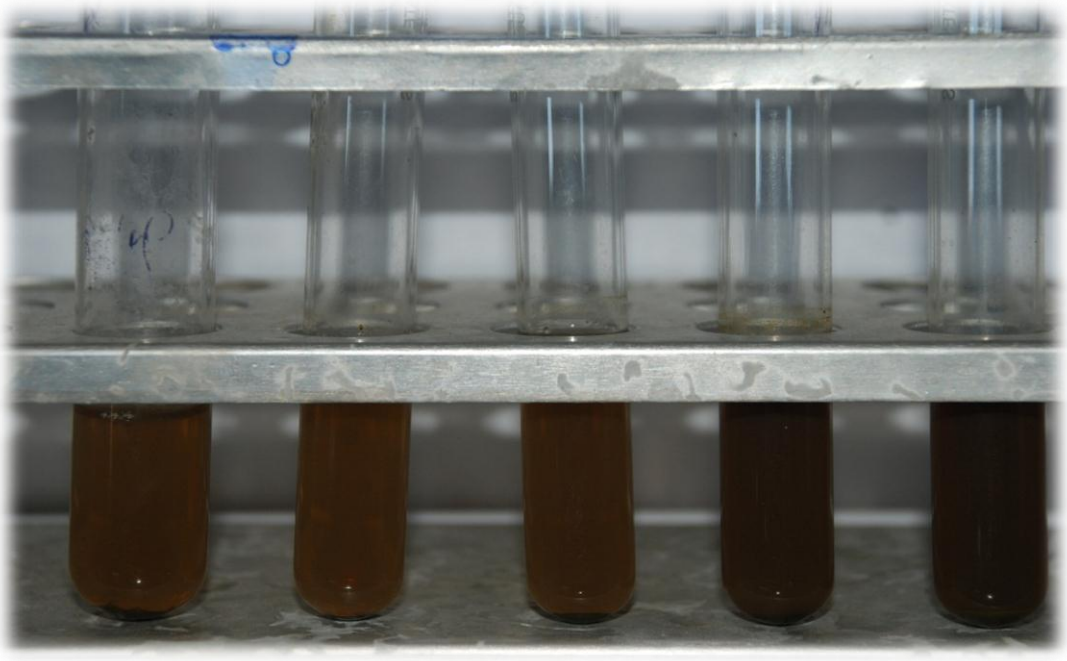


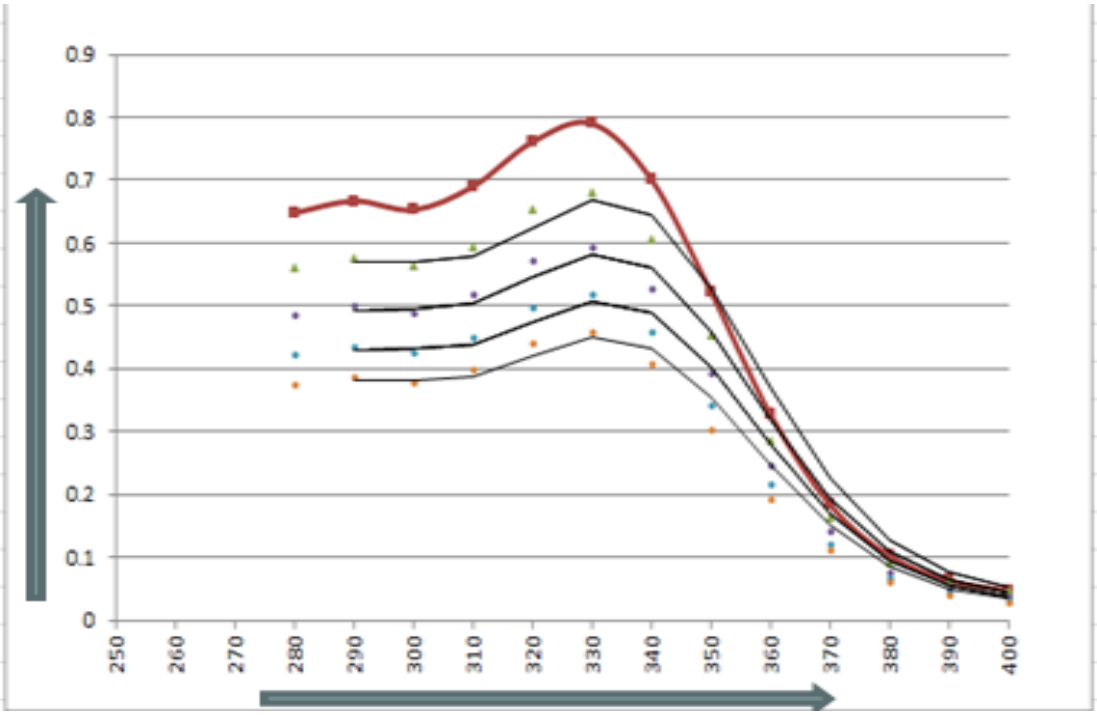
Fig 14. Different concentration of extract in 5ml of water

Standard Plot

Extract (μl)	Water	O.D. at 330nm
200	2800	**0.2649**
400	2600	**0.3509**
600	2400	**0.4252**
800	2200	**0.4865**
1000	2000	**0.5875**
1200	1800	**0.6858**

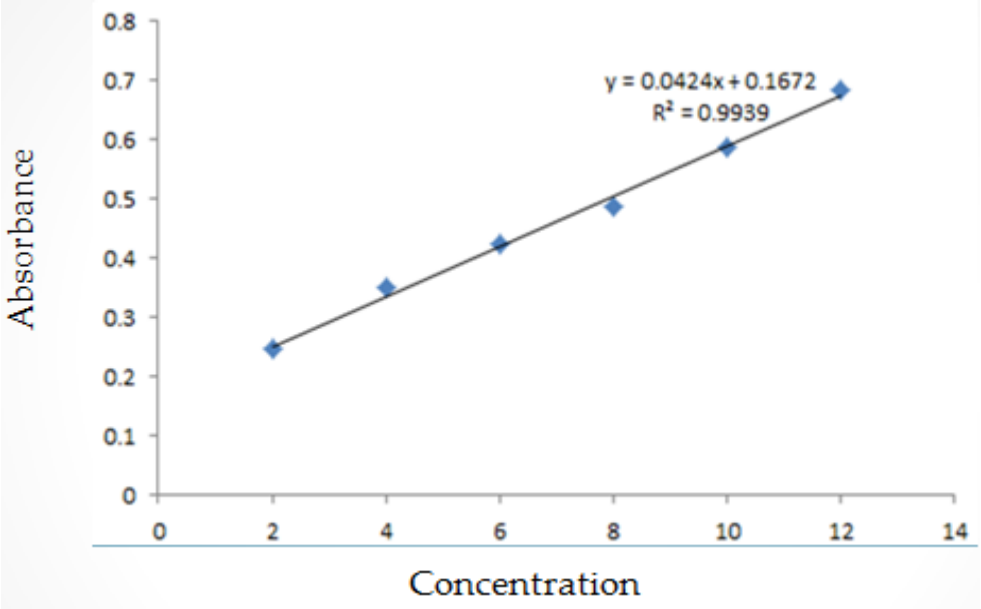
Table 13. Standard reading for *L.camara*

Lambda max :-



Graph 6. Lamda max*(L.camara)*

Lambda max :- 330nm



Graph 7. Standard plot of Lantana camara

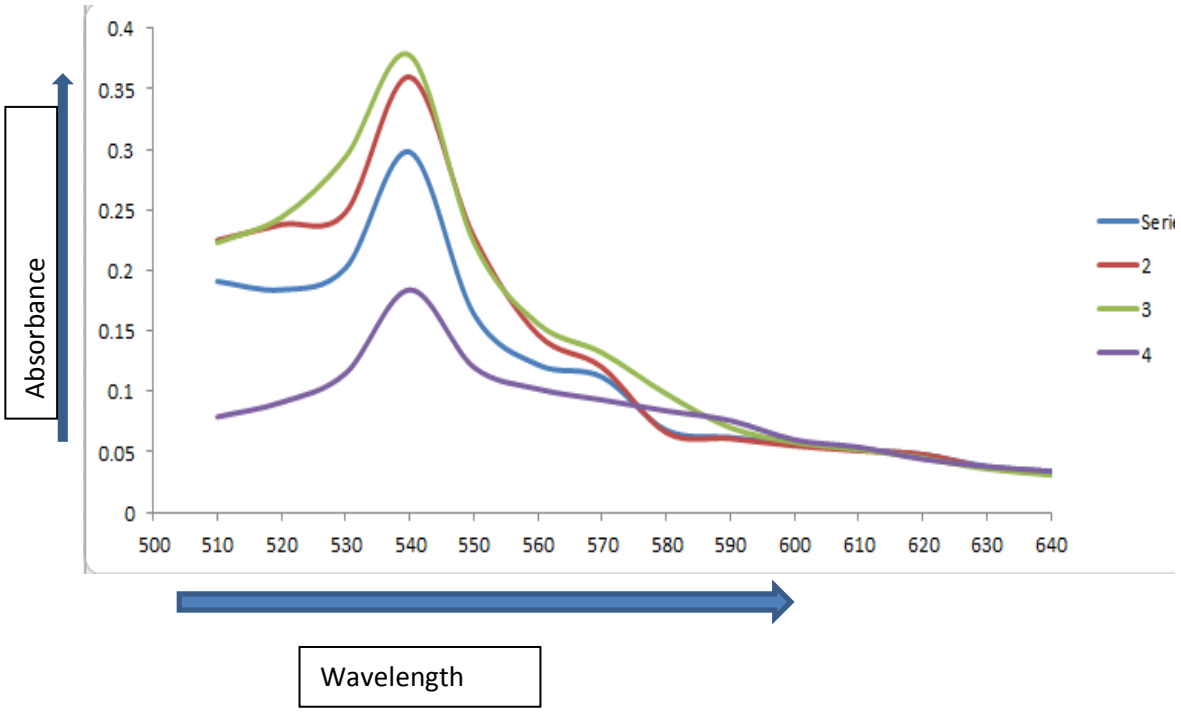
Rubus nivus

Solubility:- 8mg/ml in hydroalcoholic solution

Extract(*µ*l)	**Water**	**O.D. at 540nm**
200	2800	**0.1208**
400	2600	**0.1964**
600	2400	**0.2525**
800	2200	**0.3538**
1000	2000	**0.4420**
1200	1800	**0.6334**

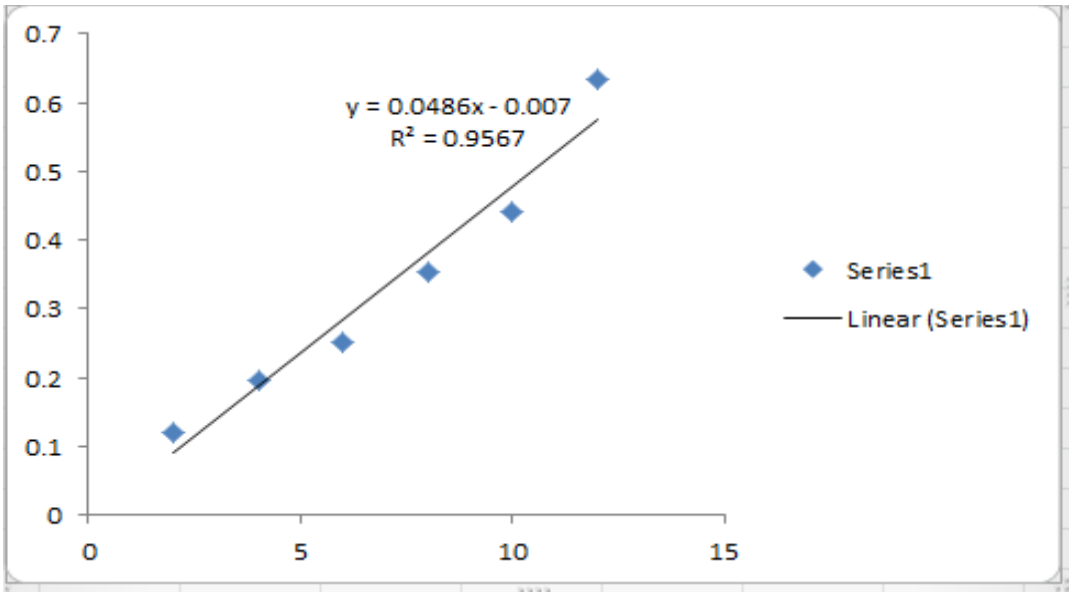
Table 14. Standard reading for *R.nivus*

Lamda max



Graph 8. Lambda max *(R.niveus)*

Standard plot



Graph 9. Standard plot *Rubus niveus*

Encapsulation efficency

Came Out to be

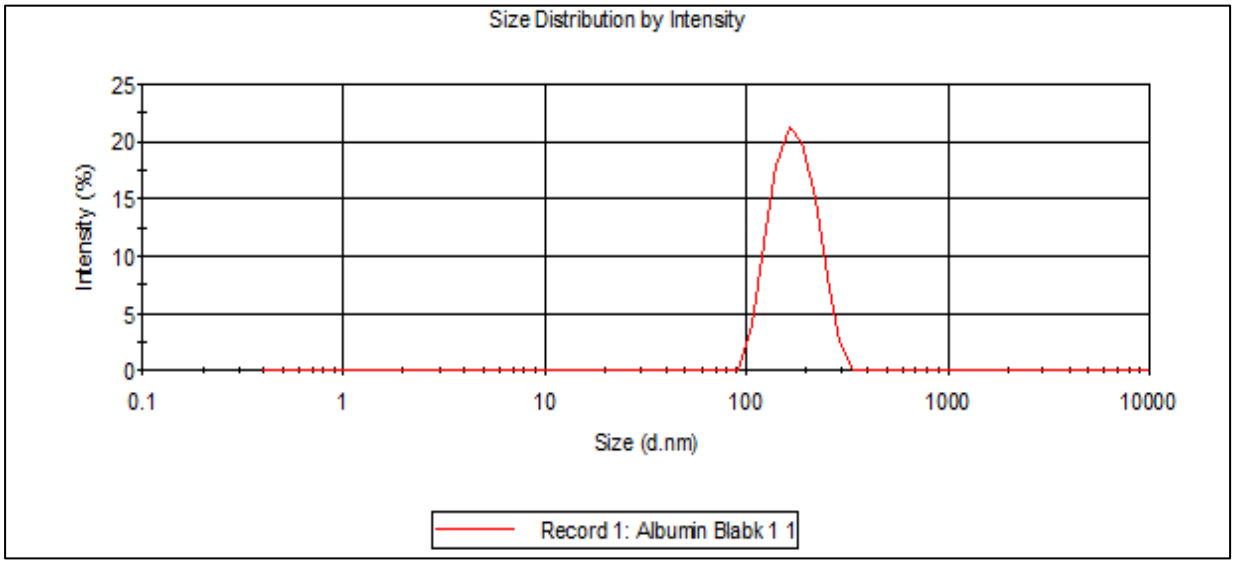
Lantana camara :-36.4%

Rubus niveus :- 42.6%

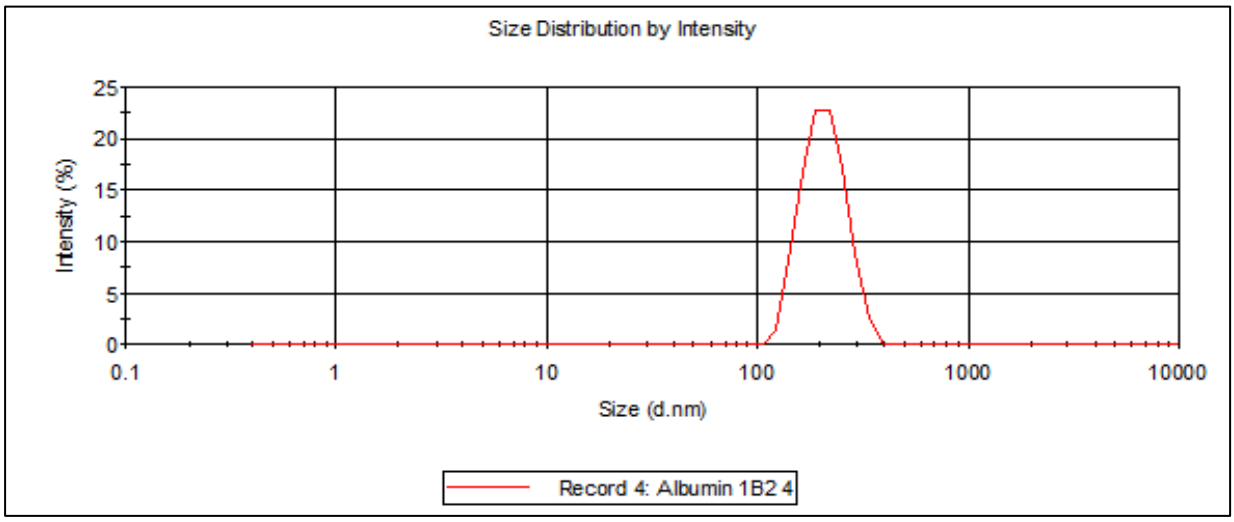
4.4 Characterization of nanoparticle

S.no	Code (At 100μl glutreladehyde)	Particle Size (nm)	Polydispersity Index (PDI)	Zeta potential mV
1	Albumin (blank) 1(a)	**167.4**	**0.050**	**-88.4**
2	Albumin(R.niveus) 1(b)	**235.4**	**0.092**	**-36.2**
3	Albumin(L.camara) 1(c)	**201.3**	**0.044**	**-74**

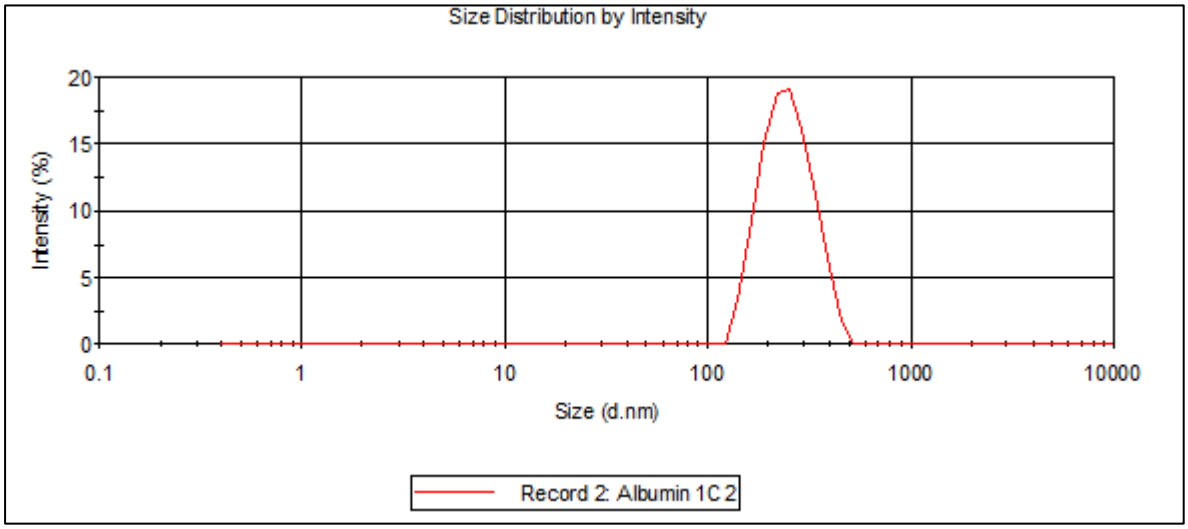
Table 15. Characterization (DLS, PDI and zeta potential) of albumin based nanoparticle



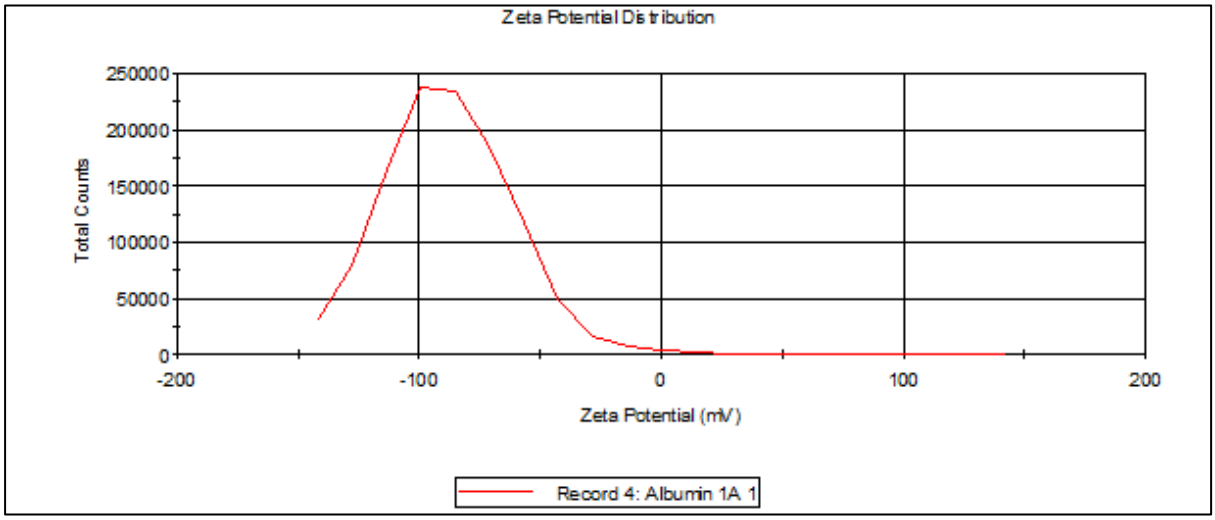
Graph 10. DLS of blank *albumin* NP`s



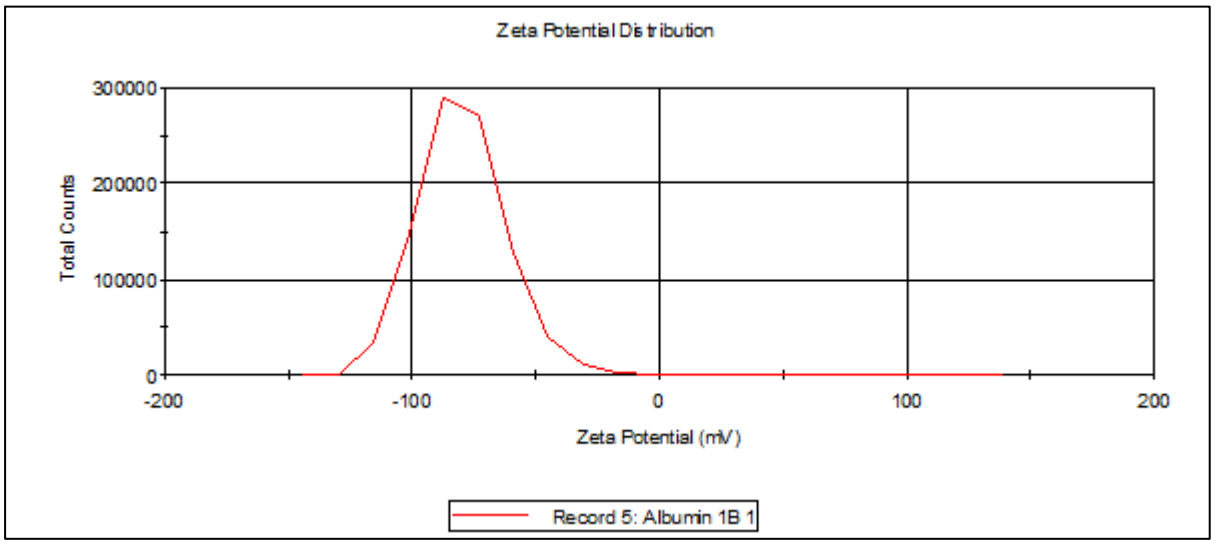
Graph 11. DLS of *Rubus niveus* NP`s



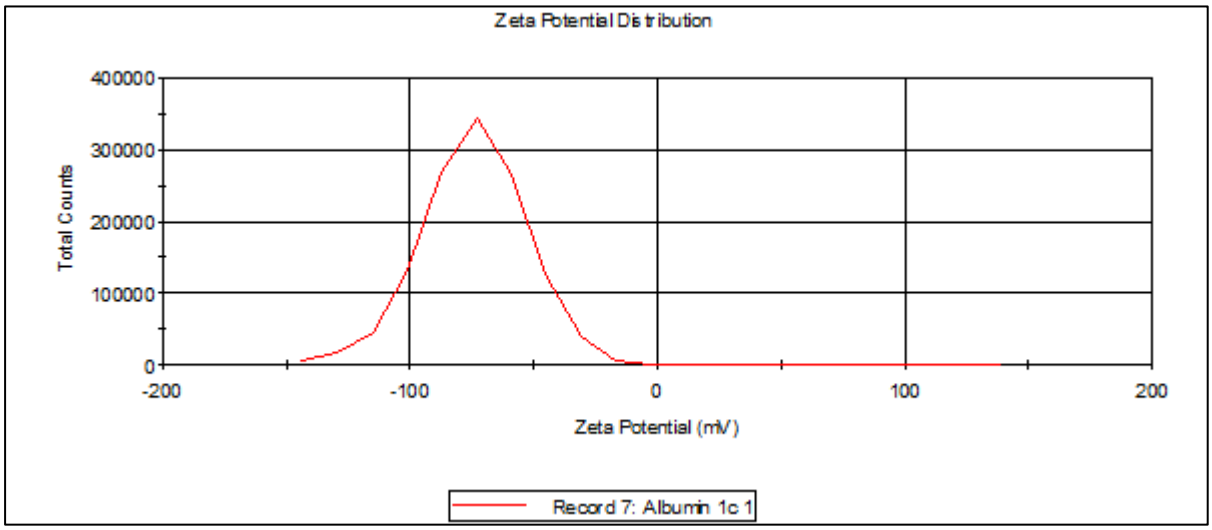
Graph 12. DLS of *L.camra* NP`s



Graph 13. Zeta potential of blank albumin NP`s



Graph 14. Zeta potential of *Rubus* NP`s



Graph 15. Zeta potential of *Lantana* NP`s

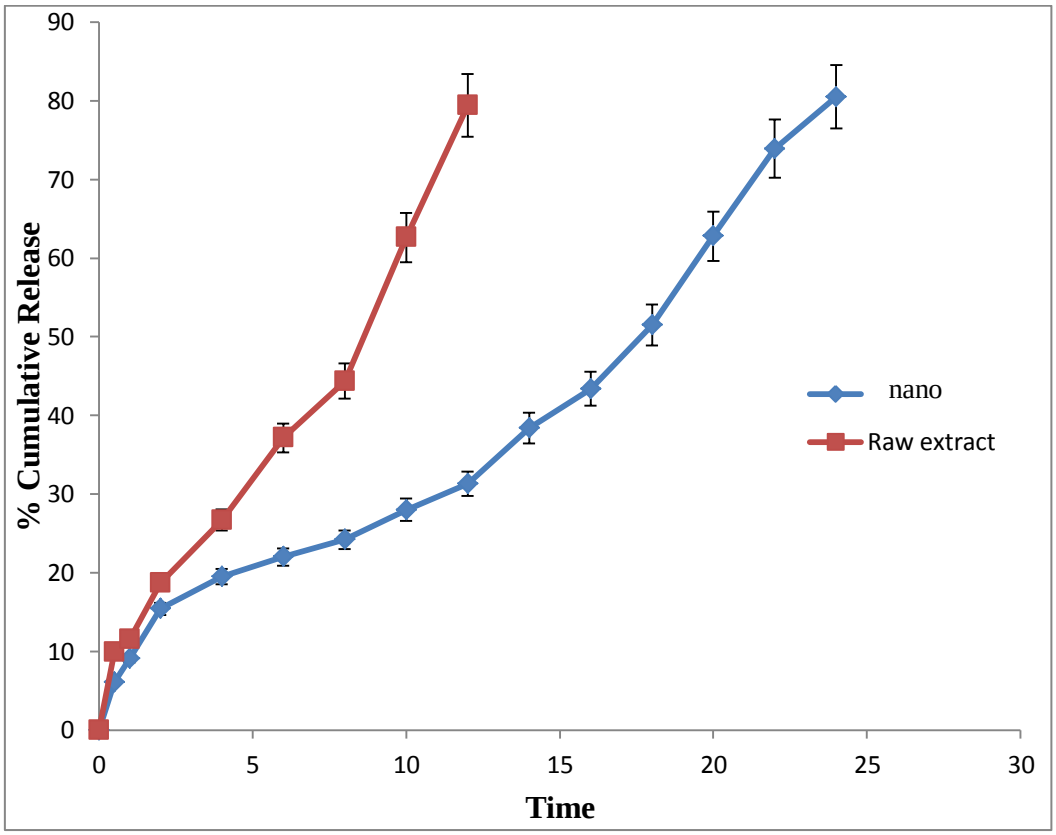
4.5 Invitro release study

Release studies of *Lantana camara* in (phosphate buffer 7.4)

(n=3, Mean ±SD)

Time	Crude extract	Albumin nanoparticle
0.50	9.94±0.2	6.05±1.2
1	11.55±0.6	9.09±1.6
2	18.73±1.2	15.41±2.3
4	26.70±2.2	19.51±0.5
6	37.15±1.0	22.3±0.7
8	44.37±1.4	24.20±1.6
10	62.62±0.6	28.02±1.9
12	79.4.±1.5	31.32±0.6
14		38.40±2.1
16		43.40±0.9
18		51.5±0.9
20		62.8±0.9
22		73.9±0.9
24		80.5±0.9

Table 16. Invitro release



Graph 16. % cumulative release vs time graph of raw and albumin nanoparticle loaded drug.

Discussion

Chapter 5

Today even with the presence of modern technology people still preferably choose natural medicine over other synthetic medicine because ,it is safer and with prolong use will not cause any side effects which are generally outcome of using modern synthetic medicines in excess. Hence all the researchers world-wide believe to have more of a natural approach for the preparation of medicine.

Keeping this thought in mind two of the well-known plants were taken *Lantana camra* and *Rubus niveus* which have been critically acclaimed for their anti-cancerous and anti-oxidant properties world-wide. From the above plants species leaves (*L.camara*) and berries (*R.niveus*) were taken and then hydro alcoholic were prepared for both of them through the process of maceration and use of soxhelet apparatus subsequently. After the extract was obtained for both of the species it was then analysed thoroughly for phytochemical screening which showed subsequent amount of tannins(78.14$\mu g/ml$),flavonoid(17.9 $\mu g/ml$), alkaloid(29.13 $\mu g/ml$) and phenols(14.87 $\mu g/ml$) in the extract of (*L.camra*) . These active compounds are the main components which play an important part in activity against cancerous cell as shown in many of the studies (*oyazio et.al 1986*). Similarly antioxidant studies were also performed which showed about 70% of the radical scavenging activity in the extract which again is the proof of high anti-cancerous activity which is shown by the plant. Similar assays were performed for the *Rubus berry* extract which showed higher antioxidant properties than the *L.camara* and a subsequent amount of the phenol was detected. In addition anthocyanin test was also performed on the extract of *Rubus niveus* one of the major component showing the anti-cancerous activity besides ellagatanin present on the berry surface. About 42.6 mg/ml of the anthocyanin content were noted in the extract. After the extract was analysed maximum wavelength was measured for both the extract which came out to be 330nm and 540nm subsequently for *L.camra* and *R.niveus* .From this standard plot for both the extract were plotted. Which are helpful in the further analysis of the given extracts.

Then the preparation of the albumin nanoparticle was done which constituted of three formulations including the blank, loaded 1 (R.niveus), loaded 2 (*L.camara*). These nanoparticle were then analysed under DLS to get the appropriate size of nanoparticle which greatly reflect the overall efficacy of the formulation. If the size is to big the circulation time and the absorbance of the formulation is greatly affected, if the size is too small the quantity of extract that gets loaded is too low and the hence effects get nullified so an appropriate amount of the size of the particle are required to make the drug fully efficient . On analysis an

average size of 167.4nm ,235.4nm ,201.3nm for blank, *R.niveus* & *L.camra* loaded albumin nanoparticle were obtained which are considered optimal as size less than 300 nm is highly efficient and gets absorbed very easily resulting in appropriate amount of the extract reaching the target organ. Also size is one of the factors that cause the formulation to stay in blood for a longer time period. Simultaneously Zeta potential at stable pH was done for checking the stability of the formulation. More negative is the result more stable the formulation is hence showing less coagulation on the other hand if the value of the zeta is less determines that the formulation is not stable and can coagulate. The reading showed highly stable formulations of the extract were made which had values as 88.4, -36.2, -74 for blank , *Rubus* and *Lantana* subsequently. After the positive characterisation of results the samples of the extract were given for the cytotoxic analysis which had some contradictory results with the concentration of the extract. Hence more of time needs to be devoted for the in-vitro analysis of these formulations to discover its true potential.

Blank Albumin Nanoparticle

Drug Loaded Albumin Nanoparticle

Conclusion

Chapter 6

The result obtained for the analysis of both the extract showed that both of the extract has a very high antioxidant activity as well as the amount of the phytochemical present inside them have a potential of generating tremendous result in cytotoxic studies. Simultaneously the formulation made from these extract were also highly stable and were of appropriate size which thereby show that further deep analysis into this topic can fetch valuable results.

The future direction for this study would call for cytotoxic studies both *in-vitro* as well as *in-vivo*.

Refrences

1. Varricchio, Claudette G. (2004). A cancer source book for nurses. Boston: Jones and Bartlett Publishers. p. 229. ISBN 0-7637-3276-1.
2. Cohen E et al. (1999) Induced differentiation in HT29, a human colon adenocarcinoma cell line. Journal of Cell Science 112: 2657-2666.
3. Fogh J et al. (1977) One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. Journal of the National Cancer Institute 59 : 221-226.
4. Halliwell B.et al.(1991): DNA damage by oxygen-derived species–Its mechanism and measurement in mammalian systems. FEBS Lett 281:9–19.
5. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H (2002): Free radicalinduced damage to DNA: Mechanisms and measurement. Free Rad Biol Med 32: 1102–1115.
6. Dreher D, Junod AF (1996): Role of oxygen free radicals in cancer development.Eur J Cancer 32A: 30–38.
7. Jackson JH (1994): Potential molecular mechanisms of oxidant-induced carcinogenesis.Environ Health Perspect 102: 155–158.
8. NR JENA et al.(2012). DNA damage by reactive species: Mechanisms, mutation and repair.J. Biosci. 37(3), 503–517, Indian Academy of Sciences.
9. Liu Y, Fiscum G and Schubert D (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain.J. Neurochem. 80 780–787
10. Loschen G.et al.(1974) Superoxide radicals as precursors of mitochondrial hydrogen peroxide.FEBS letters. 42(1):68-72.
11. Gungor N. et al. (2010).Genotoxic effects of neutrophils and hypochlorous acid. Mutagenesis 25 149–154.
12. Porter NA (1986): Mechanisms for the autooxidation of polyunsatrurated lipids. Acc Chem Res 19: 262–268.
13. Harrison LB.et al. (2002). "Impact of tumor hypoxia and anemia on radiation therapy outcomes". Oncologist 7 (6): 492–508. doi:10.1634/theoncologist.7-6-492
14. Srinivasan K (2005). "Spices as influencers of body metabolism: an overview of three decades of research". Food Research International 38 (1): 77–86.doi:10.1016/j.foodres.2004.09.001.
15. Arts, I.C. et al. (2005).Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr, 81(1 Suppl).
16. Halliwell, B.(2007) Dietary polyphenols: good, bad, or indifferent for your health? Cardiovasc Res.73(2): p. 341-7.

17. Erdman.et al.(2007). Flavonoids and Heart Health: proceedings of the ILSI North America Flavonoids .Washington, DC. J Nutr, 2007. 137(3): p. 718S-37S.
18. Dinkova-Kostova, A.T.(2008) . Phytochemicals as Protectors Against Ultraviolet Radiation: Versatility of Effects and Mechanisms. Planta Med.
19. Dressman J, Reppas C (2007). Drug solubility: How to measure it, how to improve it. Adv Drug Deliv Rev,59: 531–532.
20. Ehud Gazit.(2007). Plenty of room for biology at the bottom: An introduction to bionanotechnology. Imperial College Press,ISBN 978-1-86094-677-6
21. Yadav D. et al.(2011) Novel approach: Herbal remedies and natural products in pharmaceutical science as nano drug delivery systems. Int J Pharm Tech. 3:3092-116.
22. Jahanshahi M (2004). Re-design of downstream processing techniques for nanoparticulate bioproducts. Iranian J. Biotechnol. 2: 1-12.
23. Weber C, Coester C, Kreuter J, Langer K (2000). Desolvation process and surface characterisation of protein nanoparticles. Int. J. Pharm.194(1): 91-102.
24. Kratz F, Fichtner I, Beyer U, Schumacher P, Roth T, Fiebig HH, Unger C (1997). Antitumor activity of acid labile transferrin and albumin doxorubicin conjugates in vitro and in vivo human tumor xenograft models. Eur. J. Cancer. 33: S175
25. Takakura Y, Fujita T, Hashida M, Sezaki H (1990). Diposition characteristics of macromolecules in tumir-bearing mice. Pharm. Res.7: 339-346.
26. Bairwa NK. Sethiya NK, Mishra SH.(2010). Protective effect of stem bark of Ceiba pentandra Linn. against Paracetamol-induced hepatotoxicity in Rats. Pharmacognosy Res 2010;2:26-30.
27. Tulio Jr. AZ.et al.(2008).Cyanidin 3-Rutinoside and Cyanidin 3-Xylosylrutinoside as Primary PhenolicAntioxidants in Black Raspberry. Journal of Agricultural and Food Chemistry 56(6):1880-8.
28. Halvorsen BLet al.(2002).A systematic screening of total antioxidants in dietary plants.J Nutr. 132(3):461-71.
29. Duthie et al.(2005).DNA stability and lipid peroxidation in vitamin E-deficient rats in vivo and colon cells in vitro--modulation by the dietary anthocyanin, cyanidin-3-glycoside.Eur J Nutr. 44(4):195-203.
30. Olsson et al.(2004).Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels.J Agric Food Chem. 52(24):7264-71.
31. Lansky et al.(2005).Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions.Invest New Drugs. 23(1):11-20.
32. Stoner GD, Wang L, Zikri N, Chen T, Hecht SS, Huang C, Sardo C, Lechner JF. (2007).Cancer prevention with freeze-dried berries and berry components. Seminars in Cancer Biology 17:403-10.

33. Ozgen M.et al. (2008). Antioxidant Capacity and Phenolic Antioxidants of Midwestern Black Raspberries Grown for Direct Markets Are Influenced by Production Site.HortScience 43(7):2039-47.
34. Lodish H, Berk A, Kaiser CA, Krieger M, Scott MP, Bretscher A, Ploegh H, Matsudaira P. (2007). Cell Signaling II: Signaling Pathways that Control Gene Activity. In: Ahr K, editor. Molecular Cell Biology. 6th ed. New York, NY: W.H. Freeman and Company. P665-712.
35. Huang C, Huang Y, Li J, Hu W, Aziz R, Tang M, Sun N, Cassady J, Stoner GD. 2002.Inhibition of Benzo(a)pyrene Diol-Epoxide-induced Transactivation of Activated Protein 1 and Nuclear Factor kB by Black Raspberry Extracts. Cancer Research 62:6857-63.
36. Deepak Ganjewala, Silviya Sam, Kishwar Hayat Khan(2009). Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red and white flowers. Eur Asia J Bio Sci, 3, 69-77.
37. Ghisalberti EL.(2000) Review Lantana camara L. (Verbenaceae). Fitoterapia, 71, 467-486
38. Bhakta D, Ganjewala D (2009); Effect of leafpositions on total phenolics, flavonoids and proantho-cyanidins content and antioxidantactivities in Lantana camara (L). Journal ofScientific Research,1(2): 363-369.
39. Pan WD.et al.(1993). Studies on the chemical constituents of the leaves of Lantana camara. Yao Xue Xue Bao.28(1):35-9.
40. Safayhi H, Sailer E-R.(1997) Planta Med .63:487.
41. Sharma VS, Kaul KN. (1959)Indian 59418. Chem Abstr 53:652.
42. Funk,C.D. (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science, 294, 1871–1875.
43. Vivanco,I. et al. (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat. Rev. Cancer, 2, 489–501.
44. Castellone,M.D. et al. (2005) Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science, 310,1504–1510.
45. Bonni,A. et al. (1999) Cell survival promoted by the Ras-MAPK signalling pathway by transcription-dependent and -independent mechanisms. Science,286, 1358–1362.
46. Castellone,M.D. et al. (2005) Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science, 310,1504–1510.
47. Steinmetz,K.A. et al. (1991) Vegetables, fruit and cancer. II mechanisms. Cancer Causes Control, 2, 427–442.
48. Block,G. et al. (1992) Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer, 18, 1–29.

49. Willett,W.C. (1995) Diet, nutrition, and avoidable cancer. Environ. Health Perspect.103, 165–170.
50. Eberhardt,M.V. et al. (2000) Antioxidant activity of fresh apples. Nature, 405, 903–904.
51. Sun,J. et al. (2002) Antioxidant and antiproliferative activities of fruits. J. Agric. Food Chem., 50, 7449–7454
52. Stoner,G.D. et al. (2003) Foods and food components in the prevention of cancer. In Neeser,J.-R. and German,J.B. (eds.) Bioprocesses and Biotechnology for Functional Foods and Nutraceuticals. Marcel Dekker, Inc., New York, pp. 331–373 Chapter 17.
53. Rietveld. et al. (2003) Antioxidant effects of tea: evidence from human clinical trials. J. Nutr., 133, 3285S–3292S.
54. NHan . et al. (2005) SM.Inhibition of the growth of premalignant and malignant human oral cell lines by extracts and components of black raspberries.utr Cancer. 2005;51(2):207-17.
55. Duthie . et al.(2005) DNA stability and lipid peroxidation in vitamin E-deficient rats in vivo and colon cells in vitro--modulation by the dietary anthocyanin, cyanidin-3-glycoside.Eur J Nutr. 44(4):195-203.
56. Olsson. et al. (2004).Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels.J Agric Food Chem. 52(24):7264-71.
57. Glei. et al. (2003) in vitro toxicity testing of functional foods rich in catechins and anthocyanins in human cells.Toxicol In Vitro. 17(5-6):723-9.
58. Seeram. et al. (2004). Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines.J Agric Food 52(9):2512-7.
59. Lansky. et al. (2005). Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions.Invest New Drugs. 23(1):11-20.
60. Ozgen M. et al. (2008). Antioxidant Capacity and Phenolic Antioxidants of Midwestern Black Raspberries Grown for Direct Markets Are Influenced by Production Site. HortScience 43(7):2039-47.
61. .Maas JL, Galletta GJ, Stoner GD. (1991). Ellagic Acid, an Anticarcinogen in Fruits, Especially in Strawberries: A Review. HortScience 26(1):10-4.
62. Harris. et al. Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2'-deoxy-guanosine levels in the Fischer 344 rat.Nutri. Cncer, 40:125-133. doi: 10.127/S15327914NC402_8.PMID:11962247
63. Emma M Coates. et al (2007).Colon-available raspberry polyphenols exhibit anti-cancer effects on in vitro models of colon cancer.J Carcinog, 6:4

64. Rodrigo KA. et al. (2006).Suppression of the Tumorigenic Phenotype in Human Oral Squamous Cell Carcinoma Cells by an Ethanol Extract Derived From Freeze-Dried Black Raspberries. Nutrition and Cancer 54(1):58-68.
65. Kresty,L.A. et al. (2001) Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. Cancer Res, 61,6112–6119
66. Stoner,G.D. et al. (2007) Cancer prevention with freeze-dried berries and berry components. Semin. Cancer Biol., 17, 403–410.
67. Felgines,C. et al. (2002) Blackberry anthocyanins are slightly bioavailable in rats. J. Nutr., 132, 1249–1253.
68. Borges,G. et al. (2007) The bioavailability of raspberry anthocyanins and ellagitannins in rats. Mol. Nutr. Food Res., 6, 714–725.
69. Rodrigo KA. et al (2006).Suppression of the Tumorigenic Phenotype in Human Oral Squamous Cell Carcinoma Cells by an Ethanol Extract Derived From Freeze-Dried Black Raspberries. Nutrition and Cancer 54(1):58-68.
70. Huang,C. et al. (2002) Inhibition of BPDE-induced transactivation of AP-1 and NFjB by black raspberry extracts. Cancer Res., 62, 6857–6863.
71. Lin A, Karin M. (2003). NF-kB in cancer: a marked target. Seminars in Cancer Biology13:107-14.
72. Lu H. et al. (2006). Molecular Mechanisms Involved in Chemoprevention of Black Raspberry Extracts: From Transcription Factors to Their Target Genes. Nutrition and Cancer 54(1):69-78.
73. Hecht SS. Et al. (2006). Identification of cyanidin glycosides as constituents of freeze-dried black raspberries which inhibit anti-benzo[a]pyrene-7,8-diol-9,10-epoxide induced NFkB and AP-1 activity. Carcinogenesis 27(8):1617-26.
74. Keppler K. et al. (2005). Metabolism of anthocyanins and their phenolic degradation products by the intestinal micro flora. Bioorganic and Medicinal Chemistry 13:5195-205.
75. Wu X. et al. (2009). Phenolic acids in black raspberry and in the gastrointestinal tract of pigs following ingestion of black raspberry. Mol. Nutr. Food Res. 53:1-9.
76. McGhie. Et al (2007). The bioavailability and absorption of anthocyanins: Towards a better understanding. Molecular Nutrition and Food Research 51:702- 13.
77. Carlton PS.et al. (2000). Failure of dietary lyophilized strawberries to inhibit 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone-and benzo[a]pyreneinduced lung tumorigenesis in strain A/J mice. Cancer Lett 159:113-17.

78. Bhakta D, Ganjewala D (2009). Effect of leaf positions on total phenolics, flavonoids and proantho-cyanidins content and antioxidant activities in Lantana camara (L). Journal of Scientific Research, 1(2): 363-369.
79. Mayee R. et al (2011). Evaluation of Lantana camara Linn. (Verbenaceae) for antiurolithiatic and antioxidant activities in rats. International Journal of Pharmaceutical and Clinical Research 3 (1):10-14.
80. Pan WD. Et al (1993). Studies on the chemical constituents of the leaves of Lantana camara. Yao Xue Xue Bao .28(1):35-9.
81. Safayhi H, Sailer E-R. Planta Med 1997;63:487.
82. Sharma VS, Kaul KN. Indian 59418. Chem Abstr 1959;53:652.
83. Barua AK, Chakrabarti P, Dutta SP, Mukherjee DK, Das BC. Tetrahedron 1971;27:1141.
84. Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L. J Nat Prod 1998;61:1212.
85. Williams, C.S., Smalley, W., and DuBois, R.N. (1997). Aspirin use and potential mechanisms for colorectal cancer prevention. J. Clin. Invest. 100, 1–5
86. Castellone,M.D. et al. (2005) Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science, 310, 1504–1510.
87. Araki,Y. et al. (2003) Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res., 63, 728–734.
88. Bonni,A. et al. (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science, 286, 1358–1362.
89. McNeil, S.E (2009). Nanoparticle therapeutics: A personal perspective. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 1, 264-271.
90. Cuenca, A.G.; Jiang, H.; Hochwald, S.N.; Delano, M.; Cance, W.G.; Grobmyer, S.R. Emerging implications of nanotechnology on cancer diagnostics and therapeutics. Cancer 2006, 107, 459-466.
91. Ferrari, M. Cancer nanotechnology: Opportunities and challenges. Nat. Rev. Cancer 2005, 5, 161-171.
92. Weber C, Coester C, Kreuter J, Langer K (2000). Desolvation process and surface characterisation of protein nanoparticles. Int. J. Pharm.194(1): 91-102.
93. Kratz F, Fichtner I, Beyer U, Schumacher P, Roth T, Fiebig HH, Unger C (1997). Antitumor activity of acid labile transferrin and albumin doxorubicin conjugates in vitro and in vivo human tumor xenograft models. Eur. J. Cancer. 33: S175

94. Takakura Y, Fujita T, Hashida M, Sezaki H (1990). Diposition characteristics of macromolecules in tumir-bearing mice. Pharm. Res. 7: 339-346.
95. Desai, N.et al (2006) 'Enhanced efficacy and safety of nanoparticle albumin-bound nab-docetaxel versus taxotere', paper presented at American Association for Cancer Research (AACR), 96th Annual Meeting, Washington, DC, 1-5 ApriL.
96. Trieu, v., Hwang, J. and Desai, N., 2007, 'Nanoparticle albuminbound (nab) technology may enhance antitumour activity via targeting of SPARC protein', paper presented at conference 'New Targets and Delivery System for Cancer Diagnosis and Treatment', Sidney Kramer Cancer Center, San Diego, CA, 5-7 March.

Students bio data

[Pick the date]

Kunal Kapoor :- 4th year BTDD student

Enrolment No :- 101707

Batch:-Z-3

Field of interest :- Cancer biology, Stem cells, Nanotechnology & molecular biology

Namrata gautam :- 4th year BTDD student

Enrolment No:-101728

Batch:-Z-3

Field of interest:- Cancer biology, microbiology, nanotechnology