ANTI-TUMOR POTENTIAL OF SEED KERNELS OF *CAESALPINIA BONDUCELLA* EXTRACTS AND ISOLATED PHYTOCONSTITUENTS: *IN VITRO* AND *IN SILICO* ANALYSIS

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI – 600032

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

MASTER OF PHARMACY

IN

Branch- II (PHARMACEUTICAL CHEMISTRY)

SUBMITTED BY

C.R. PUNITH KUMAR (261915009)

Under the Guidance of

Dr. N. RAMALAKSHMI, M.Pharm., Ph.D.

Professor and Head

Department of Pharmaceutical Chemistry

Department of Pharmaceutical Chemistry
C.L. BAID METHA COLLEGE OF PHARMACY.
(An ISO 9001-2008 certified Institute)
THORAIPAKKAM, CHENNAI-600097
October 2021

Phone : 24960151, 24960425, 24962592
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
Owned and Managed by Tamilnadu Chemists and Druggists Educational Trust
2/305, Rajiv Gandhi Salai, OMR, Thoraipakkam, Chennai-600097.
Affiliated to The Tamilnadu Dr.M.G.R Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi.

Srinivasan.R
Chairman

K.K. Selvan
Executive Trustee

Dr. Grace Rathnam
Principal

Prof. Dr. N. RAMALAKSHMI, M.Pharm., Ph.D,

Professor and Head,

Department of Pharmaceutical Chemistry.

CERTIFICATE

This is to certify that **C.R. PUNITH KUMAR (261915009)**, carried out the dissertation work on "**ANTI-TUMOR POTENTIAL OF SEED KERNELS OF *Caesalpinia bonducella* EXTRACTS AND ISOLATED PHYTOCONSTITUENTS: *IN VITRO* AND *IN SILICO* ANALYSIS"** for the award of degree of MASTER OF PHARMACY under THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, **CHENNAI- 600097** in the **academic year 2019-21** under my Supervision and Guidance in the Department of Pharmaceutical Chemistry, **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI-600 097** during the academic year 2019-2021.

PLACE: CHENNAI-97.
DATE:

Dr. N. RAMALAKSHMI, M.Pharm,Ph.D,
Professor andHead
Department of Pharmaceutical Chemistry,
C.L. BAID METHA COLLEGE OF PHARMACY
CHENNAI – 600 097.

Phone : 24960151, 24960425, 24962592
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
Owned and Managed by Tamilnadu Chemists and Druggists Educational Trust
2/305, Rajiv Gandhi Salai, OMR, Thoraipakkam, Chennai-600097.
Affiliated to The Tamilnadu Dr.M.G.R Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi.

Srinivasan.R
Chairman

K.K. Selvan
Executive Trustee

Dr. Grace Rathnam
Principal

Prof. Dr. Grace Rathnam, M. Pharm, Ph.D.,

Principal & HOD,

Department of Pharmaceutics.

CERTIFICATE

This is to certify that the project entitled **"ANTI-TUMOR POTENTIAL OF SEED KERNELS OF *Caesalpinia bonducella* EXTRACTS AND ISOLATED PHYTOCONSTITUENTS: *IN VITRO* AND *IN SILICO* ANALYSIS**" as submitted by **C.R. PUNITH KUMAR (261915009),** in partial fulfillment for the award of the degree of **Master of Pharmacy** in the **academic year 2019-21**. It was carried out at **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI** – **600097** under the guidance and supervision of **Dr. N. RAMALAKSHMI, M.Pharm, Ph.D.,** Professor and Head in the Department of Pharmaceutical Chemistry during the academic year 2019 –2021.

PLACE: CHENNAI-97.
DATE:

Dr. Grace Rathnam, M. Pharm,Ph.D.,
Principal &HOD,
Department of Pharmaceutics,
C.L. BAID METHA COLLEGE OF PHARMACY
CHENNAI – 600 097.

DECLARATION

I do hereby declare that the thesis entitled **"ANTI-TUMOR POTENTIAL OF SEED KERNELS OF *Caesalpinia bonducella* EXTRACTS AND ISOLATED PHYTOCONSTITUENTS: *IN VITRO* AND *IN SILICO* ANALYSIS**'' by **C.R. PUNITH KUMAR (261915009),** in partial fulfillment of the degree of **MASTER OF PHARMACY** was carried out at **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI-600 097** under the guidance and Supervision of **Dr. N. RAMALAKSHMI, M.Pharm, Ph.D.,** during the academic year 2019-2021. The work embodied in this thesis is original & is not submitted in part or full for any other degree of this or any other University.

PLACE: CHENNAI

DATE:

C.R. PUNITH KUMAR

(261915009)

ACKNOWLEDGEMENT

I humbly present this work to the **ALMIGHTY GOD** with whose blessings I was able to complete my project work**.** Indeed, my project is a small work done with the help of primitive persons at heart. So, it is my bounded duty to promulgate them individually.

At the outset, I express my sincere love and deepest sense of gratitude to my beloved Family for their excellent co-operation and support throughout my project.

It is with the great pleasure, I record my thanks and gratitude to our Principal **Dr. Grace Rathnam M.Pharm., Ph.D.,** C.L. Baid Metha College of Pharmacy, Chennai-600097 for the successful completion of research work.

I am very much privileged and happy in expressing my deep sense of gratitude to my respectable guide **Dr. N. Ramalakshmi, M.Pharm., Ph.D.,** for her gracious guidance, constant inspiration, endless consideration, encouragement, infinite help and efforts and memorable guidance for the successful completion of my research work. I heartily thank her for valuable suggestions and moral support throughout my research work. Her vision and perception of the uncommon will always continue to strive me towards perfection with lot of confidence.

I take great pleasure in acknowledging my sincere thanks to all the faculties **Dr. S. Amuthalakshmi M.Pharm., Ph.D, Mr. R. Vijayakumar M.Pharm., Mrs. K. Dhunmati M.Pharm, Mrs. R.S. Remya M.Pharm.**, of the Department of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy, Chennai-97 for their constant help and suggestions.

I sincerely thank our Chief Librarian, **Mrs. Rajalakshmi,** for providing necessary reference material for my project work.

I wish to thank **Dr. T. Purushoth Prabhu M.Pharm., Ph.D.,** HOD of Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy, Chennai-97 for his continuous support in the research work.

I extend my sincere gratitude to **Dr. Kathiresan** sir, Head R & D, Sattva Vaid Nature's Global Private Limited, Chennai for his continuous support and knowledge to help me in this research work.

I sincerely thank **Dr. Ganesh Jeevan** sir, Head QC, Kawman Pharma for his moral support throughout the completion of my project.

I also thank **Mr. Karthick**, **Mr. Jeyaraj** and **Mr. Chakravarthy** from Sattva Vaid Nature's Global Private Limited, Chennai for their constant support throughout the completion of the research work.

I acknowledge my sincere thanks to **Mrs. Muthulakshmi,** Lab Technician of the Department of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy, Chennai-97 for her help during my research work.

I owe special thanks to Mr. Srinivasan and Mrs. Shanthi, Stores in- charge, C.L. Baid Metha College of Pharmacy, Chennai, for their timely supply of all necessary chemicals and reagents required for the completion of my project work.

I express my thanks to all my class mates and batch mates **K. Yuvarani, A. Subhashini, K. Padma, V. Bala Aakash, V. Manimegalai, N. Helina, P. Pavithra, S. Bhuvaneswari, P. Buvaneswari, P. Kiruthika and T.R. Rajadevi** for their support from the beginning until the completion of my project work.

I thank my senior **P. Manimegalai M.Pharm.,** for her warm encouragement and thoughtful guidance.

I also wish to thank all my juniors for their support and help.

I take this wonderful opportunity to thank my friends **S. Swarna Kumar, N. Naresh, N. Jagan Karthick** for their support in my project.

Finally, I thank all those who helped me directly or indirectly in the successful completion of my thesis.

C.R. Punith Kumar

INDEX

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

WHO	**-**	World Health Organization
HIV	**-**	Human Immunodeficiency Virus
HPV	**-**	Human Papilloma Virus
LEEP	**-**	Loop electrosurgical excision procedure
IV	**-**	Intravenous
USFDA	**-**	Unites States Food and Drug Administration
DNA	**-**	Deoxyribo Nucleic Acid
HDL	**-**	High Density Lipoprotein
LDL	**-**	Low Density Lipoprotein
PTZ	**-**	Pentylene Tetrazole
MES	**-**	Maximal Electro Shock
Cm	**-**	Centimeter
IU/g	**-**	International Units/gram
PCOS	**-**	Poly Cystic Ovarian Syndrome
FT-IR	**-**	Fourier Transformed- Infrared Spectroscopy
MS	**-**	Mass Spectroscopy
HCL	**-**	Hydrochloric Acid
TLC	**-**	Thin Layer Chromatography
HPTLC	**-**	High Performance Thin Layer Chromatography
NaOH	**-**	Sodium Hydroxide
KOH	**-**	Potassium Hydroxide
H2SO4	**-**	Sulphuric Acid
EtOAc	**-**	Ethyl Acetate
AlCl3	**-**	Aluminium Chloride
DMSO	**-**	Dimethyl Sulfoxide
NCCS	**-**	National Centre for Cell Science

Nm	**-**	Nanometer
PDB	**-**	Protein Data Bank
Mg/g	**-**	milligram**/**gram
LYS	**-**	Lysine
VAL	**-**	Valine
HIS	**-**	Histidine
ILE	**-**	Isoleucine
LEU	**-**	Leucine
PHE	**-**	Phenylalanine

INTRODUCTION

INTRODUCTION

"The art of healing comes from nature and not from physician. Therefore, the physician must start from nature with open mind." Paracelsus.

Traditional medicine is now popular, with more than 60% of the population and about 80% of people in developing countries depending on traditional methods of treating their health needs. This is due to the low cost, affordability, and affordability of the traditional medicine system [1]. Different parts of plants, such as root, stem, bark, leaves, seeds, fruits, etc. they are used to treat certain diseases and to prevent the symptoms of certain diseases. Modern medicine uses only active compounds isolated from different parts of the plant, and about 80 percent of these active components show good performance in treating those specific problems, and in the traditional medicine system, the whole medicine is given [2].

Remedy is considered a chemical industry as it contains a large number of chemical compounds such as alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and fats (essential and organized). Other rare compounds such as furanocoumarins, hydroxycoumarins, naphthoquinones, acylphloroglucinols and sterones are all distributed among plants.

Herbal Medicines:

Herbal medicines are also called herbal medicines, herbal products, herbal medicines products, phytomedicines, Phyto therapeutic agents and phytopharmaceuticals. The use of herbal medicine in evidence- or a science-based approach to the treatment and prevention of diseases is known as (rational) phytotherapy. This approach to traditional medicine is in contrast to traditional medicine that uses traditional medicine in its entirety and especially based on its emotional and traditional uses. Although the two approaches - traditional / complete and logical / evidence-based - are completely different, in some cases they use the same terms [3 & 4].

According to the WHO, approximately 21,000 plant species have the potential to be used as medicinal plants. Remedies have become an accepted form of health care, although there are many differences between alternative therapies and conventional medicine. Generally, they have been found to be safe but there is little evidence of the effectiveness of herbal remedies. Although extracts from certain drugs have been shown to be effective in some cases, concerns about the absorption and digestion of herbal remedies are a major factor in judging their health effects [5 & 6].

Herbal Medicine in India:

The largest producer of medicinal plants is India. Currently there are around 250,000 Ayurvedic practitioners registered, as compared with about 700,000 practicing modern medicine. There are around 20,000 medicinal plants in India. Although traditional practitioners use 7,000 to 7,500 plants for treating different diseases, the proportion of plants used in the different Indian systems of medicine is Ayurveda 2000, Siddha 1300, Unani 1000, Homeopathy 800, Tibetan 500, Modern 200, and Folk 4500.More than 1.5 million Indians use the traditional medicinal system for health care. Around 25,000 effective plant-based formulations are used in Indian traditional and folk medicine.

India has more than 7800 manufacturing units involved in the production of natural health products and traditional plant-based formulations, which require more than 2000 tons of medicinal plant raw materials each year. There are more than 1500 herbal supplements and ethnic traditional medicines available as dietary supplements [7].

Advantages of Herbal Medicine:

- The cultivation and processing of medicinal herbs and herbal products is environmentally friendly.
- There is an undisputed clinical efficacy of several natural anticancer drugs. Vincristine/ vinblastine from *Catharanthus roseus,* etoposide from Podophyllum and the taxols from *Taxus brevifolia.*

- Herbal medicine has a very long history: it is a collection of processes based on the ideas, beliefs and experiences of various cultures and times, often obscure, used for health maintenance, such as prevention, diagnosis, development and health improvement. treatment of diseases.
- By using a well-studied biological phenomenon, even at the cellular level, bacteria and parasites can develop resistance to chemotherapeutics; that is, selectively leading to resistance to certain chemical compounds, this process has occurred, in part, with Plasmodium species, the causative agent of malaria, so that synthetic anti-malarial drugs lost an important part of its effectiveness so often it has been necessary to return to quinine.
- A diverse species of exotic species such as India have a variety of unique and rare plants with a variety of medicinal properties.
- Natural products can offer an alternative to established therapy because they act at a different stage in disease and be useful in combination therapy. The search for synthetic molecules active against human immunodeficiency virus (HIV) has resulted largely in reverse transcriptase inhibitors, but investigations into plant extracts have produced wide range of chemical compounds with various modes of action that result in viral non-proliferation [8].

Cancer

Cancer is a generic term for a large group of diseases that can affect any part of the body. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. Cancer begins when genetic changes interfere with this orderly process. Cells start to grow uncontrollably. These cells may form a mass called a tumor. A tumor can be cancerous or benign. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body. A benign tumor means the tumor can grow but will not spread.

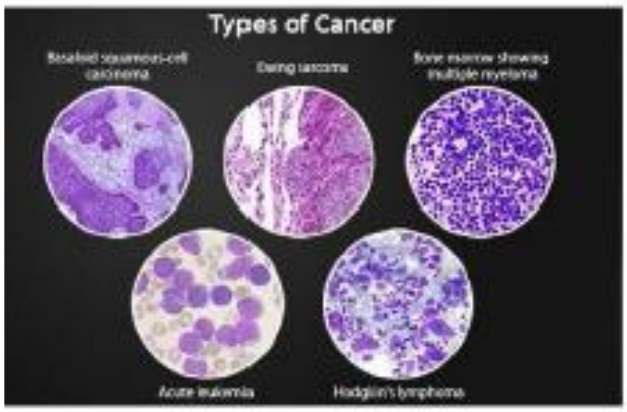


Figure 1: Types of Cancer

Types:

1. **Carcinoma** is a cancer that starts in the skin or the tissues that line other organs.
2. **Sarcoma** is a cancer of connective tissues such as bones, muscles, cartilage, and blood vessels.
3. **Leukemia** is a cancer of bone marrow, which creates blood cells.
4. **Lymphoma** and **myeloma** are cancers of the immune system [9].

Risk Factors:

Risk factors for cancer include exposure to chemicals or other substances, as well as certain behaviors. They include things that people cannot control, such as age and family history. Family history of certain types of cancer can be a sign of inherited cancer. Many cancer risk factors (as well as immunosuppression) are identified early in epidemiology studies. The list below includes the most-studied known or suspected risk factors for cancer.

- Age
- Alcohol
- Cancer-Causing Substances
- Chronic Inflammation
- Diet
- Hormones
- Immunosuppression
- Infectious Agents
- Obesity
- Radiation
- Sunlight
- Tobacco [10].

Cervical Cancer:

Cervical cancer is the fourth most common disease in women worldwide and represents a major health challenge worldwide [11].

About 90% of the 270,000 people who died of cervical cancer in 2015 occur in low-income and middle-income countries where death is 18 times higher than in developed countries. cancer and HPV screening programs and vaccinations are effective strategies for preventing infections [12]. Squamous cell carcinoma and adenocarcinoma are minor histological types accounting for about 70% and 25% of all cervical cancers,respectively [13].

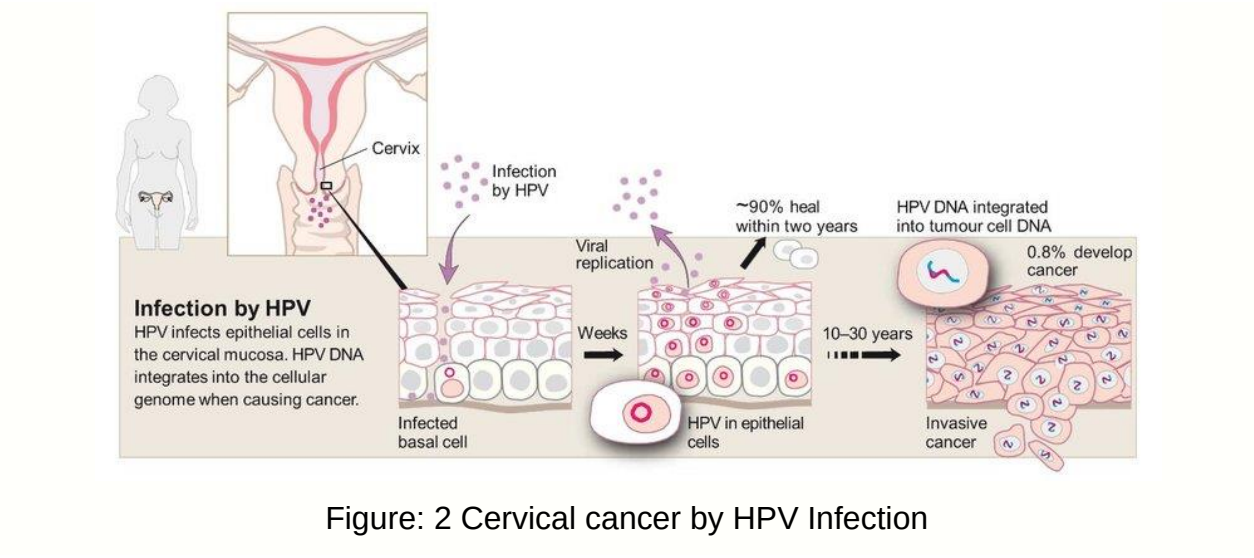


Figure: 2 Cervical cancer by HPV Infection

Risk factors for cervical cancer:

Chronic infection by high-risk oncogenic subtypes of human papilloma virus (HPV) causes almost all cases of cervical cancer [14] and, therefore, risk factors are those associated with acquiring HPV infection, severely impaired immune response to HPV infection, or both.[15 & 16] These risk factors include:

- early age of sexual debut
- multiple sexual partners or a high-risk sexual partner
- immunosuppression (eg, after organ transplantation or
- immunodeficiency disorders such as HIV)
- history of sexually-transmitted infection
- history of HPV-related vulvar or vaginal dysplasia
- non-attendance for screening and underscreening in countries with established cervical screening programmes (resulting in an estimated two-thirds of cervical cancers in such countries)[17 & 18].

Stages of cervical cancer:

Stage I: Cancer is found only in the cervix.

Stage II: Cancer has spread beyond the cervix but has not yet spread to the pelvic wall (the tissues that line the part of the body between the hips).

Stage III: Cancer has spread to the lower third of the vagina and may have spread to the pelvic wall and nearby lymph nodes.

Stage IV: Cancer has spread to the bladder, rectum, or other parts of the body

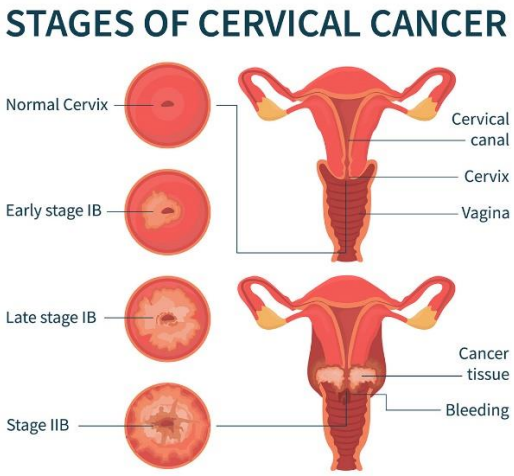


Figure: 3 Stages of Cervical cancer

Signs and Symptoms:

Symptoms often do not begin until the cancer becomes larger and grows into nearby tissue. When this happens, the most common symptoms are:

- Abnormal vaginal bleeding, such as bleeding after vaginal sex, bleeding after menopause, bleeding and spotting between periods, or having (menstrual) periods that are longer or heavier than usual. Bleeding after douching may also occur.
- An unusual discharge from the vagina − the discharge may contain some blood and may occur between your periods or after menopause.
- Pain during sex
- Pain in the pelvic region

- Fatigue, Loss of weight and appetite
- Swelling of the legs
- Problems urinating or having a bowel movement
- Blood in the urine [19].

Diagnosis:

Diagnosis is based on histopathological examination of cervical biopsy. Women with symptoms of cervical cancer need pelvic examination, examination of the cervix and vaginal mucosa, as well as cervical cytology. The cervix and lining of the vagina should be identified by speculum examination. The cervix may appear normal when the infection is slow or in the endocervical canal. Cervical cancer can metastase through lymphatic vessels to the pelvic, para-aortic, mediastinal, supraclavicular, and inguinal lymph nodes. Enlargement of the inguinal lymph nodes, enlarged and supraclavicular can be felt in advanced diseases.

Colposcopy and biopsy should be performed on patients with symptoms or women with a cytology that promotes invasion without visible lesions. Cone biopsy is mandatory if the toxicity is suspected to be harmful to the clinic or cervical cytology but is not guaranteed in the review of histopathological biopsies of the cervix. The cone should be type III extraction (depth> 1 · 5 cm) in a single piece [20].

Management of cervical cancer

Treatment of cervical cancer depends on a number of factors, including the type and stage of the cancer, the possible side effects, and the patient's choices and overall health.

1. Surgery

Surgical removal of a tumor and healthy surrounding tissue during surgery. A gynecologic oncologist is a physician who specializes in treating female cancer with surgery. With cervical cancer that does not spread beyond the cervix, these procedures are often used.

- **Conization**
- **LEEP**
- **Hysterectomy**
- **Exenteration**

2. Radiation Therapy

Radiation therapy is the use of high-powered x-rays or other particles to destroy cancer cells. Radiation therapy may be given alone, before surgery, or instead of surgery to reduce the tumor.

The most common type of radiation therapy is called external-beam radiation therapy, which is radiation emitted from an external body. When radiation therapy is given using herbs, it is called internal radiation therapy or brachytherapy. Radiation therapy, or schedule, usually consists of a set number of treatments given over a period of time that include external and internal radiation therapy. This combined approach is the most effective in reducing the risk of cancer recurrence, called recurrence

3. **Chemotherapy**

Chemotherapy is the use of drugs to destroy cancer cells, usually by keeping the cancer cells from growing, dividing, and making more cells. All medicines used to treat cervical cancer are given intravenously (IV). IV chemotherapy is injected directly into a vein or is given in a small tube called a catheter, which is a temporary tube inserted into a large vein to make it easier to inject.

4. Targeted Therapy

Targeted therapy is a treatment that targets the cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival. This type of treatment blocks the growth and spread of cancer cells and limits damage to healthy cells.

Drugs approved in treating cervical cancer:

- Avastin, Mvasi&Zirabev (Bevacizumab)
- Bevacizumab
- Bleomycin Sulfate
- Hycamtin (Topotecan Hydrochloride)
- Keytruda (Pembrolizumab)
- Pembrolizumab
- TisotumabVedotin-tftv
- Tivdak (TisotumabVedotin-tftv)
- Topotecan Hydrochloride

Drug Combinations:

- CARBOPLATIN-TAXOL
- GEMCITABINE-CISPLATIN.

Adverse effects:

- Nausea and vomiting
- Loss of appetite
- Hair loss
- Mouth sores
- Fatigue (tiredness)

Chemotherapy can damage the blood-producing cells of the bone marrow, the blood cell counts might become low. This can result in:

- An increased chance of infection from a shortage of white blood cells (called neutropenia)
- Bleeding or bruising after minor cuts or injuries because of a shortage of blood platelets (called thrombocytopenia)
- Shortness of breath or fatigue due to low red blood cell counts (called anemia) [21].

FLAVONOIDS

Flavonoids are second-line metabolites that are abundant in plants, fruits, and seeds, which are responsible for color, aroma, and taste. In plants, flavonoids perform many functions, such as controlling cell growth, attracting pollinators, and protecting against biotic and abiotic stress. In humans, these compounds are associated with a wide range of health benefits from their bioactive properties, such as anti-inflammatory, anticancer, anti-aging, cardio-protective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral [22, 23].

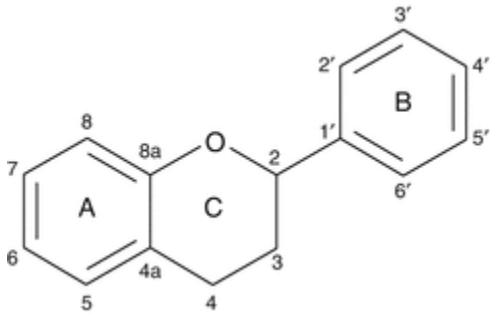


Figure: 4 Flavonoid basic skeleton

Flavonoids has a basic 15-carbon flavone skeleton, C6-C3-C6, with two benzene rings (A and B) attached to a three carbon Pyran ring (C). Position of catechol B-ring on the Pyran C-ring and the number and location of hydroxy groups in the catechol group of the B-ring influences the antioxidant capacity of flavonoids [24].

However, in the chemical structure of flavonoids, especially the presence of hydroxy groups, influences human bioavailability and biological activity. Based on the structure of flavonoids, they can be divided into six main classes, flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins [25].

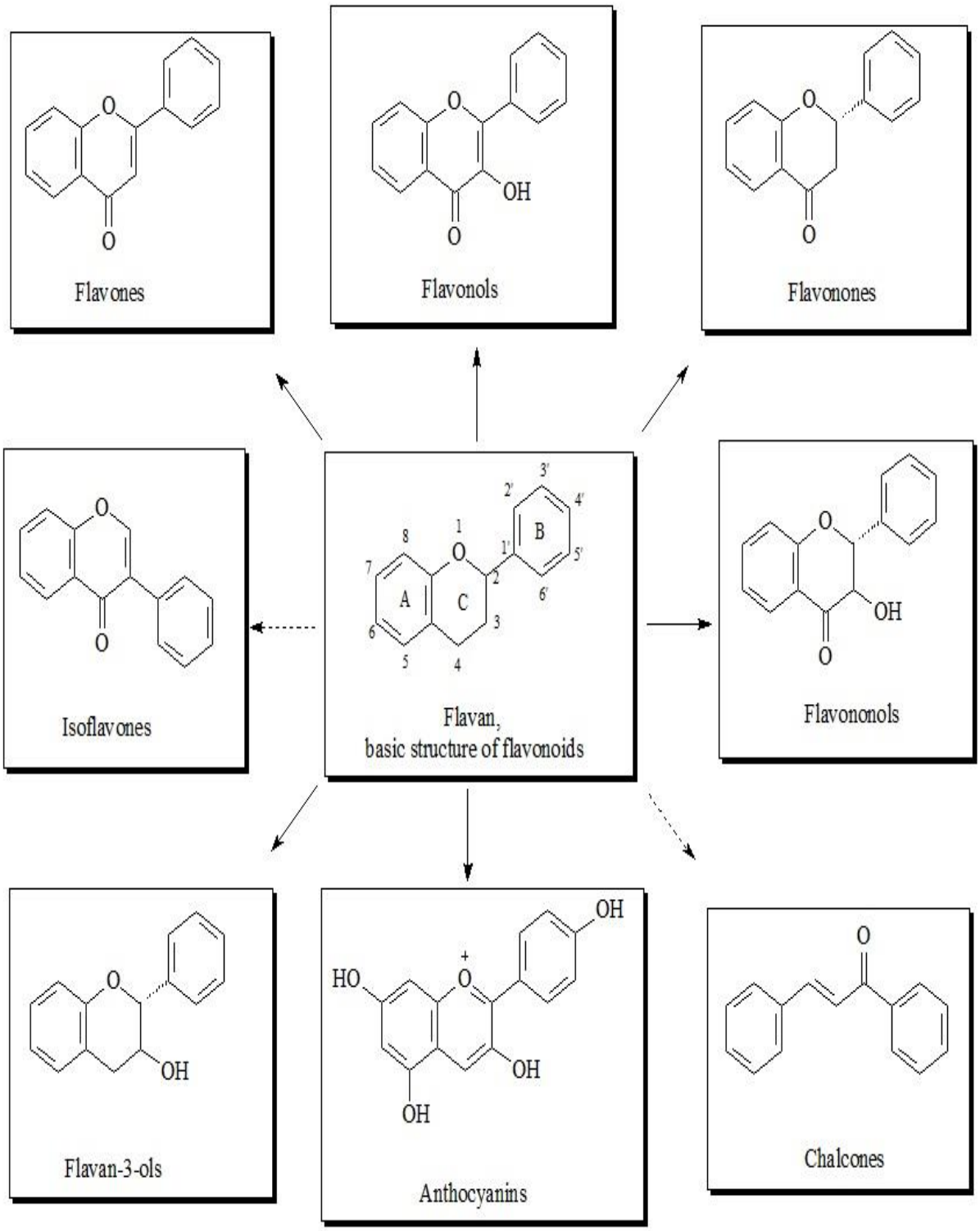


Figure: 5 Classification of flavonoids

Flavonols:

Flavonols have a ketone group. They are the building blocks of proanthocyanins. Flavonols are found in abundance in variety of fruits and vegetables. Flavonols are widely studied there are kaempferol, quercetin, myricetin and fisetin. Onions, kale, lettuce, tomatoes, apples, grapes and berries they are rich sources of flavonols. Apart from fruits and vegetables, red tea and wine are also sources of flavonols. Flavonol supplementation has been found to be associated with a variety of health benefits including antioxidant power and reduced risk of vascular disease compared to flavones, flavonols have a hydroxyl group in the middle position 3 of the C ring, which may also be glycosylated. Like flavones, flavonols are very different from methylation and hydroxylation patterns and, considering different glycosylation patterns, are probably the most common subgroup of flavonoids for fruits and vegetables. For example, Quercetin is present in many plant foods [26].

Flavonoids have a wide variety of anti-cancer effects, they modulate the enzymatic activities of ROS-scavenging, participate in stopping the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness.

Flavonoids have a proven ability to trap free radicals, regulate cell metabolism and prevent stress-related oxidative diseases.
While many flavonoids have been shown to have anti-cancer activity, the molecular mechanisms responsible for this effect have not been elucidated yet [27].

Cyclin Dependent Kinase 6

Cell division protein kinase 6 (CDK6) is an enzyme encoded by the CDK6 gene. It is regulated by cyclins, more specifically by Cyclin D proteins and Cyclin-dependent kinase inhibitor proteins. The protein encoded by this gene is a member of the cyclin-dependent kinase, (CDK) family, which includes CDK4.

In mammalian cells, cell cycle is activated by CDK6 in the early G1 phase through interactions with cyclins D1, D2 and D3. There are many changes in gene expression that are regulated through this enzyme. After the complex is formed, the C-CDK6 enzymatic complex phosphorylates the protein pRb. After its phosphorylation, pRb releases its binding partner E2F, a transcriptional activator, which in turn activates DNA replication. The CDK6 complex ensures a point of switch to commit to division responding to external signals, like mitogens and growth factors. CDK6 is important for the control of G1 to S phase transition [28].

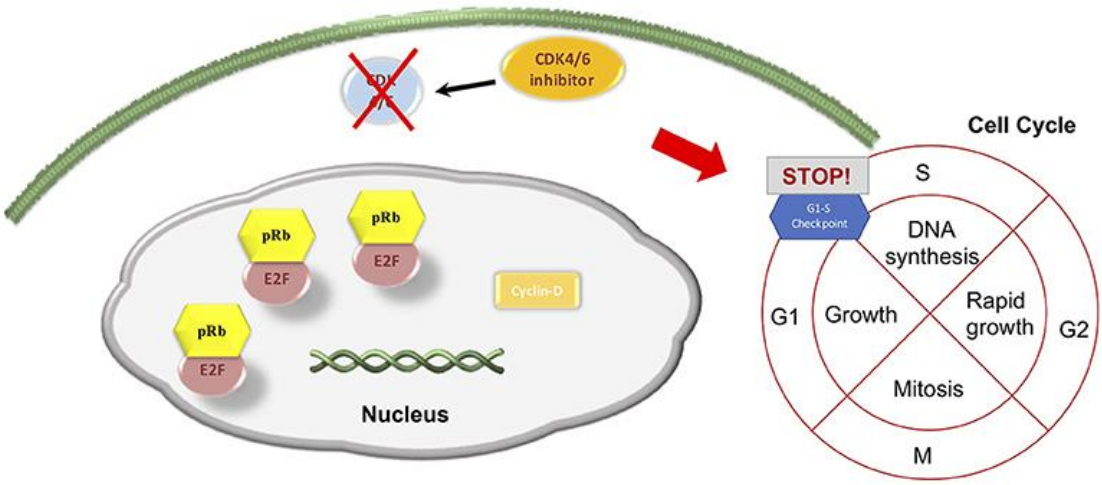


Figure: 6 Mechanism of CDK6

CDK inhibitors have therapeutic potential for a variety of diseases, including cancer, diabetes, kidney, neurodegenerative and infectious diseases. CDKs are unique in the protein kinase family because their activity depends on the association with their partner cyclins, and this could be a potential advantage for inhibitor design.

CDKs regulate critical checkpoints in the cell cycle and are considered highly validated targets for several proliferative diseases. CDK4 and CDK6 selective inhibitors either approved or currently in advanced registration trials for several cancers.

There are currently three CDK4/6 inhibitors that have been approved by the US Food and Drug Administration for treatment of Breast cancer, they are palbociclib, ribociclib, and abemaciclib [29].

Docking

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex.

Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed.

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.

The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy.

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are Lead optimization, Hit identifications, Drug- DNA interaction.

REVIEW
OF
LITERATURE

PHARMACOLOGICAL ACTIVITY

Anti-malarial activity:

The roots of *C.bonducella* were extracted with dichloromethane by the cold maceration method. The extract was subjected to column chromatography to isolate the compound Norcaesalpin-D and its structure was confirmed by spectral analysis and tested against Chloroquine- sensitive chloroquine-resistant *P.falciparum* and artemisinin-resistant *P.falciparum*. The extract was active against all above, but against the artemisinin-resistant *P.falciparum* it showed much better activity [30].

Anti-bacterial activity:

The seeds were extracted with methanol, and the extract was fractionated with ethyl acetate and water. The methanol extract, ethyl acetate, water fraction, and a pure compound bondenolide were tested for antibacterial activity against 10 different organisms by the agar diffusion method. All four samples showed good activity against *Pseudomonas aeruginosa*. The methanol, ethyl acetate extract, and bondenolide showed activity against *Klebsiellapneumoniae, Escherichia coli,* and *Staphylococcus aureus*. The water-soluble extract was found to be active against *Corynebacteriumdiphtheriae, Streptococcus pyogenes,* and *Shigella flexneri* [31].

Anti-fungal activity:

The seeds were extracted with ethanol, and they were fractionated with n-butanol, ethyl acetate, and chloroform. The antifungal activity was studied against organisms such as *Candida glabrata* and *Aspergillus flavus*. The crude extract and its fractions were tested against the organisms. There was mild activity in lower concentrations, but the activity was excellent in higher concentrations. The maximum antifungal activity was shown by the n-butanol and chloroform fractions against *Candida glabrata* and *Aspergillus flavus* [32].

Anthelmintic activity:

The leaves were extracted with petroleum ether, chloroform, and methanol. Anthelmintic activity was studied using adult earthworms such as *Pheretimaposthuma* and *Ascardiagalli.* The methanolic extract showed Anthelmintic activity in a dose-dependent manner by giving the shortest time of Paralysis (P) and Death (D). Piperazine citrate is used as the standard drug. The Anthelmintic activity observed was found to be produced by the tannins present in the extract [33].

Anti-viral activity:

The extraction was made with water. The plants were also extracted with ethanol, methanol, and chloroform. Standard viral isolates of *Orthomyxovirus* and *Paramyxovirus* were tested on the hen's eggs. Different dilutions of the extracts were incubated with the standard concentration of the virus. Aqueous and ethanolic extracts showed complete inhibitory effects on the growth of *paramyxovirus*. All extracts except the chloroform extract showed significant inhibitory activity against the *Orthomyxovirus* [34].

Wound healing activity:

The seeds were defatted with petroleum ether and extracted with alcohol. This alcoholic extract was fractionated with ether, ethyl acetate, butanone, and butanol. All extract and its fractions were tested for wound healing activity in excision, incision and dead space wound models in albino rats. Ethyl acetate fraction of the extract showed the best wound healing activity among all extracts [35].

Anti-oxidant activity:

The seeds were extracted with ethanol. The total phenolic content was determined by the Folin–Ciocalteau method using gallic acid as the standard. The free radical scavenging activity of the DPPH (2,2-Diphenyl-1-picrylhydrazyl) was determined by the inhibition percentage of the free radical. The radical scavenging ability of hydroxyl, nitric oxide and superoxide was determined. The ethanol extract showed strong antioxidant

activity by inhibiting DPPH, hydroxyl radical, nitric oxide, superoxide anion scavenging, and hydrogen peroxide scavenging activities when compared with standard ascorbic acid [36].

Anti-tumor activity:

The seeds were extracted with methanol and the methanolic extract was tested for antitumor activity on swiss albino mice having Ehrlich ascites carcinoma (EAC). The extract was administered in different doses and after the last dose, the animal was sacrificed and various parameters such as hematological profile and certain biochemical parameters were studied. The results showed a decrease in the tumor volume, packed cell volume, and viable cell count which in turn prolonged the lifetime of the mice having EAC [37].

Anti-diabetic activity:

The seed kernels were extracted with petroleum ether (a). This was again extracted with ethanol (b) and the seed coat was also extracted with ethanol (c). The hyperglycemic effect was induced by intraperitoneal injection of Alloxan to the Wistar rats. All three extracts were found to be acting well in the glucose tolerance test. The extract markedly reduced the blood glucose levels of the animals, but the extract (b) was found to be more potent and it also reduced the LDL levels and increased the HDL levels. From this, it shows that the extract was found to be antidiabetic against alloxan-induced hyperglycemia and anti-hyperlipidemic [38].

Antipyretic & Analgesic:

The seeds were extracted with petroleum ether and then extracted again with hot ethanol. Fever was induced in the rats by injecting brewer’s yeast in normal saline. The temperature of the animals was tested at regular intervals. The temperature of the drug-treated animals was found to be decreased and more potent than the standard ASA. The analgesic activity was confirmed by Eddy’s hot plate method and the tail-flick method [39].

Immunomodulatory activity:

The seeds were extracted with ethanol, dissolved in water, and administered orally to rats to determine the LD50 value. Three major tests were performed, Neutrophil adhesion test for immunostimulant action, Haemagglutinating antibody test to assess the humoral immune response, Delayed hypersensitivity test showed stimulation effect on the T cells. Cyclophosphamide induced myelosuppression in animals. The ethanolic extract along with cyclophosphamide normalized the bone marrow activity better than cyclophosphamide alone, which proved it a potent immunostimulant drug [40].

Anti-estrogenic activity:

The seeds were extracted with alcohol. Adult female Wistar albino rats were used. The control rats showed normal 4 days of the estrous cycle, whereas in the drug-treated rats the cycle was reduced to 1.5 - 2 days. There was a decrease in the ovary weight recorded and various histological changes such as distortion in the shape and size of the follicular antrum, dislocation of primary oocytes, degeneration of cumulous oophorus, detachment of primary oocytes from zona granulosa cells were observed in the drug-treated rats [41].

Anticonvulsant activity:

The seeds were extracted with petroleum ether. Male albino mice and Wistar rats were grouped separately. The convulsions were induced by PTZ, MES, Strychnine, and Picrotoxin. Diazepam was administered as a standard drug in all except in MES where phenytoin is used. The lowest dose of the extract did not show much activity, but the medium and higher doses showed potent anticonvulsant activity as that of the standard drug [42].

Anti-psoriatic activity:

The leaves were extracted with hydro alcohol. This extract was further fractionated with n-butanol(CBHAB) and water(CBHAW). The *in vivo* test was performed by mouse tail test with retinoic acid as the standard drug. Both extracts showed orthokeratosis and

changes in epidermal thickness. The *in vitro* test was performed in human keratinocyte cell lines. All extracts showed some antiproliferative activity in the keratinocyte cells. CBHAW had potent anti-psoriatic, anti-proliferative activity [43].

Anti-ulcer activity:

The leaves were extracted with normal distilled water. Female Wistar rats were divided into five groups where omeprazole was given as the standard drug. The extract was administered to the animals at three different doses. The ulcer was induced in the animals by pylorus ligation. The gastric contents of the animals were collected to determine the ulcer index. There was a dose-dependent increase in the ulcer index, pH of the gastric fluid, and decrease in the gastric volume, free acidity of the drug-treated rats [44].

Anti-filarial activity:

The seed kernels were extracted with water. Anti-filarial activity was determined by testing the extract against Litomosoides sigmodontis and Brugia malayi. The microfilaricidal, macrofilaricidal, and female worm sterilizing efficacy were determined. There was a decrease in the microfilariae count in L.sigmodontis and a good activity on the macrofilariae and female worm sterilizing effect. In B.malayi, there was a reduction in the microfilariae count and female worm sterilizing ability was good [45].

Anti-inflammatory activity:

The seeds were extracted with ethanol. Wistar albino rats were divided into four groups with the first group as control, the second and third groups were administered with the extract in different doses, and the other group was administered with the standard drug indomethacin. In carrageenan-induced paw edema, both groups, which received the extract, showed potent anti-inflammatory activity, and the highest dose was potent. Similarly, egg albumin induced edema, which was very effective in countering the acute inflammation [46].

Anti-cataract activity:

The seeds were extracted with ethanol. The lenses of the goat eyeballs were removed and incubated in artificial aqueous humor. Cataract was generated by incubating the lenses in high glucose culture. Lenses were divided into six groups with two groups being positive and negative control and one group taking the standard drug enalapril. Three groups were incubated with the extracted drug at three different concentrations. The extract possesses anti-cataract as well as antioxidant activity and slowed down the progression of the cataract [47].

Anxiolytic activity:

The seeds were extracted and tested for anxiolytic activity by various models. In the stair-case model, the extract was treated to the animals and the anxiolytic activity was increased in a dose-dependent manner by increasing the number of steps climbed. In the hole-board model, the high dose extract was found to be increasing the number and latency of head dipping of the animals but not the rearing. In the LDT model, there was an increase in the time spent and crossing in the light compartment and decreased time spent in the dark compartment. From this, it is evident that the seed extract of *C.bonducella* has potent anxiolytic activity [48].

Anti-fertility activity:

The seeds were extracted with ethanol. Female Wistar albino rats were administered with control and ethanolic extract. There was a decrease in implantation index and an increase in resorption index, pre-implantation, and post-implantation loss recorded in the seed extract treated rats. There was a decrease in progesterone levels in the extract-treated animals and craniofacial deformities observed in the embryos due to the anti-progesterone hormonal property [49].

Anti-diarrheal activity:

The leaves were extracted with methanol and fractioned with chloroform, petroleum ether, and ethyl acetate. Diarrhea was induced on the rats by injecting castor oil into them. Animals were administered with the methanolic extract and its fractions in different doses with loperamide as the standard drug. All extracts showed better activity against castor oil-induced diarrhea. Ethyl acetate fraction showed the best Anti-diarrheal activity [50].

Hepatoprotective activity:

The leaves of Caesalpinia crista were extracted with methanol. The iron overload was carried out in mice by injecting iron dextran intraperitoneally. The results showed that DPPH (2,2 Diphenyl-1-picrylhydrazyl) assay exhibited some antioxidant activity. Histopathological study showed some hepatocellular necrosis in iron dextran treated mice, and there was a reduction in hepatic injury in drug-treated mice. From this, it was found to have some hepatoprotective effect against Iron-overload-induced liver toxicity, by increasing antioxidant enzymes and chelating iron to excrete from the body [51].

Anti-spermatogenic activity:

The seeds were extracted with normal distilled water. Male albino rats were divided into four groups with one as control and the other three groups were administered with the aqueous extract in three different doses. The left and right epididymis was isolated and the sperm suspension were made by cutting off the caudal portion to determine the sperm count. There was a decrease in sperm density and an average increase in anti-spermatogenic activity and can be considered as a better drug for birth control programmes [52].

Aphrodisiac activity:

The roots were extracted with water and ethanol. Wistar rats of both sexes were administered with the aqueous and ethanolic extract with sildenafil as the standard drug. The sexual behaviors of the animals were studied by frequency of sniff, frequency

of going up, latent time of going up. The ethanolic extract showed a good effect by decreasing the latent time of going up while the aqueous extract increased the frequency of going up. The results showed the sexual behavior in the rats increased in a dose-dependent manner [53].

PHARMACOGNOSTIC CHARACTERS:

Table: 1 Review of Literature of Pharmacognostic characters

S.NO	PHARAMCOGNOSTIC CHARACTERS STUDIED	PART USED	REFERENCE
1	Macroscopy Microscopy Physico chemical constants Heavy metals	Seeds	Shan Sasidharan et al., 2021[54].
2	Macroscopy Microscopy Physico chemical constants	Seeds	SunayanVikhe et al., 2017 [55].
3	Macroscopy Microscopy Physico chemical constants	Seeds Seed coat	Prakash et al., 2018 [56].
4	Macroscopy Microscopy Fluorescence Analysis	Roots	Ganesh H Wadkar et al., 2017 [57].

ETHNOBOTANICAL SURVEY

ETHNOBOTANICAL SURVEY

PLANT PROFILE

Plant Name : *Caesalpinia bonducella; Caesalpinia crista*

Common Name : Fever Nut, Bonduc nut

Family : Caesalpiniaceae / Fabaceae

VERNACULAR NAMES

Hindi : Katkaranj, Gataran, Karanju, Katkaliji

Kannada : Gajaga, Gajjuga, Heggejjuga

Telugu : Gatchakai, Gachakaya

Sanskrit : Lata Karanja

Malayalam : Kazhanchikkuru

Tamil : Kalichikai, Kazharchikkaai

English : Fever nut, bonduc nut [58].

TAXONOMICAL CLASSIFICATION

Table: 2 Taxonomical classification of *Caesalpinia bonducella*[59 & 60].

Kingdom	**Plantae (Plants)**
Phylum	Magnoliophyta
Division	Magnoliopsida
Class	Angiospermae
Subclass	Rosidae
Order	Fabales
Family	Fabaceae/ Caesalpiniaceae
Genus	Caesalpinia
Species	Bonducella

Figure: 7 Aerial parts of *Caesalpinia bonducella*

Figure: 8 Seeds and seed kernels of *Caesalpinia bonducella*

PLANT DESCRIPTION

Table: 3 Morphological characters of *Caesalpinia bonducella* [61 & 62].

Parts of the plant	Diagram	Morphological Character
Stem		Stem is 5cm in diameter which grows as vine but flowers and fruits as a shrub. It has number of spines and the pith is white in colour and large diameter.
Leaf		Leaves are long and branch out to a length of up to 60cm. It has a pair of spiretules and a mucronate point on its base. There are 8 pairs of pinnate with spines that are 5 to 7.5cm long. The main leaf axis has a stout, recurved spine.
Leaflet		Leaflets are elliptic-oblong, marginated, mucuronate, glabrous above and puberulous at the bottom. Petioloules are very short with hooked spines. Pale golden hairs are present in the upper and lower leaf blades.

Flowers		Flowers are dense at the top, and they loosen up at the bottom. Erectiles are short in the lobes. Calyx is fulvous, hairy,6-8mm long and has an obovate-oblong shape. It has yellow petals and white hairs on its body. Ovary is 1mm long with 2 ovules.
Fruit		Pods are inflated with rigid spines and have short stalks. These fruits have a shape that is round and wiry.
Seed		The seed coat is hard, glossy with greenish to grey colour. It has circular and vertical crack markings on the surface which is 1-2cm long and oblong in shape. In dried ones, seed kernels are separated from the testa which is about 1-11.25mm thickness and is made of three layers. The outermost layer is thin and brittle, middle layer is broad and the innermost layer is white and pappery. The kernels are furrowed, ridged and hard with pale-yellowish

		white in color, circular to oval in shape. Plumule is cylindrical and straight with a thick radical axis and bitter taste and unpleasant odour.

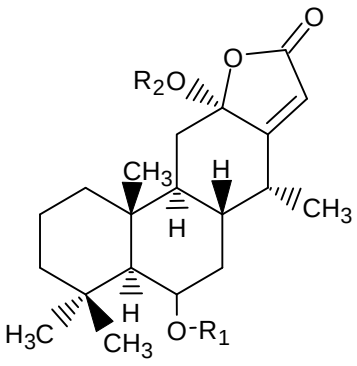
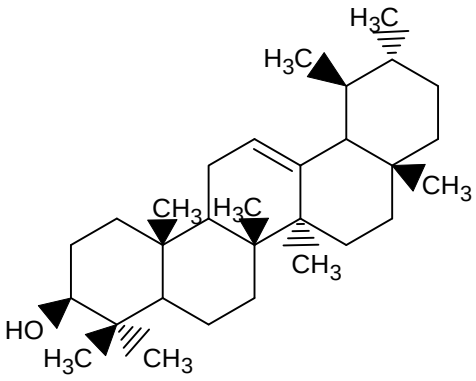
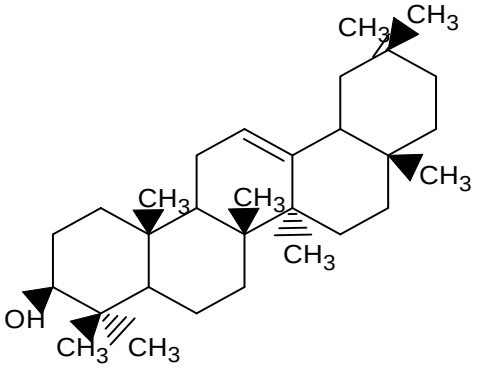
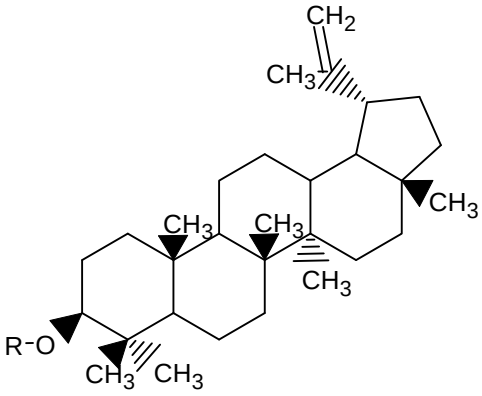
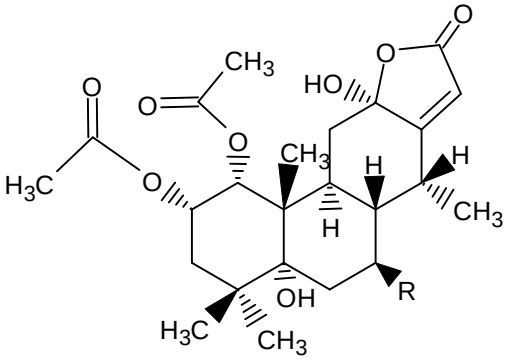
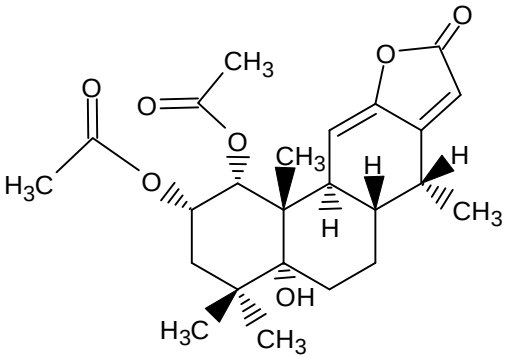
CHEMICAL CONSTITUENTS

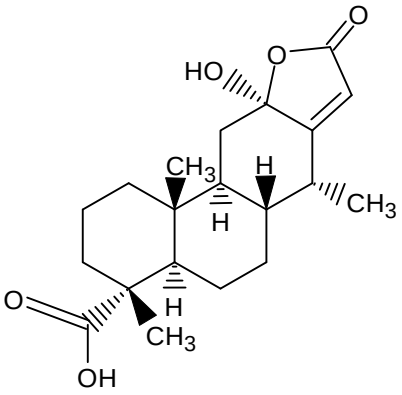
The various plant parts, such as the stem, root, flowers, leaves, and seeds of *Caesalpinia bonducella*, contain important chemical constituents including Steroids, Saponins, Terpenes, Terpenoids, Fatty Acids, Amino Acids, Phytosterols, Tannins, Flavonoids, Hydrocarbons, and Phenolics [63].

The plant contains cassane diterpene hemiketals such as Caesalpinolide C and Caesalpinolide E and also triterpenoids such as α-amyrin, β-amyrin, Lupeol and Lupeol acetate.There were some cassane furanoditerpenes isolated, including Bonducellpin D, Bonducellpin A, Bonducellpin B, Bonducellpin C, Bonducellpin E, Bonducellpin F, and Bonducellpin G.It was found that a new compound 1α-acetoxy-5α,7β-dihydroxycassa-11,13(15)-diene-16,12-lactone had potent anti-tumor activity. Caesalpin derivatives such as caesalpinin, caesalpin, caesalpin G and caesalpin H have also been isolated. Some of the important Phytochemicals present in *Caesalpinia bonducella* has been tabulated in Table.

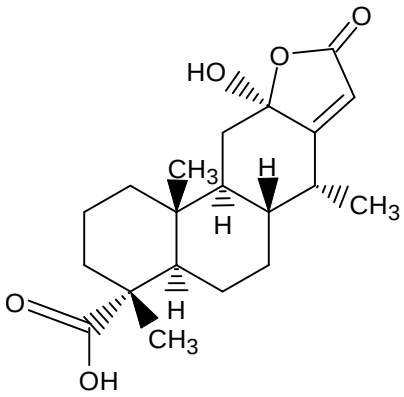
Caesalpinia bonducella has been reported to contain the nutrients such as crude fiber 12.79–14.07%, Protein 18.65-20.32%, Fat 6.54-7.23%, Carbohydrate 16.91-18.56%, Food energy (Kcal/100g)376.27–402.12, Calcium 0.150-0.184%, Phosphorus 0.17-0.22%, Sodium 0.07-0.08%, Iron 0.22-0.5%, Vitamin C 0.016-0.043(IU/g) and Vitamin A 416.75 –700.14 (IU/g) [64].

Table: 4 Structures of some important Phytochemicals in *Caesalpinia bonducella*

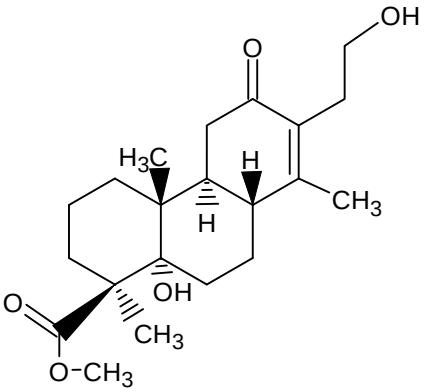
 **R_1= R_2=H** Caesalpinolide C **R_1= $COCH_3$, R_2=H**Caesalpinolide E	 α-Amyrin
 β-Amyrin	 **R=H** Lupeol **R=CH_3CO** Lupeol acetate
 **R=H** Neocaesalpin B	 Neocaesalpin D



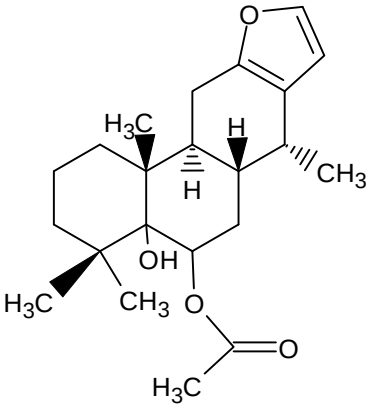
Neocaesalpin H



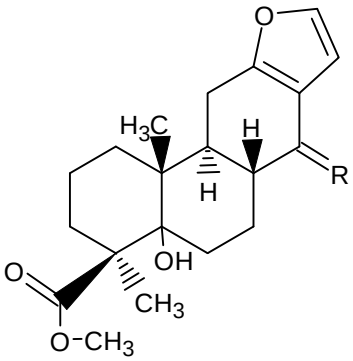
Neocaesalpin I



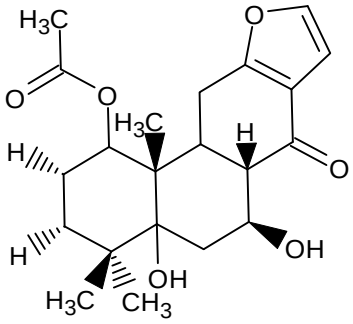
Ceasaldekarin G



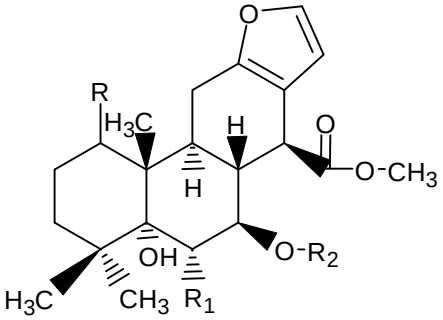
Caesaldekarin A



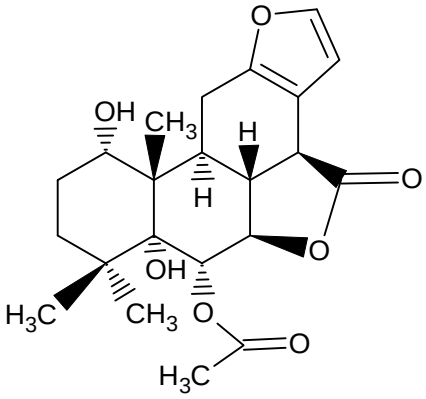
R=H, α-CH_3 Caesaldekarin C
R=CH_2Caesaldekarin F



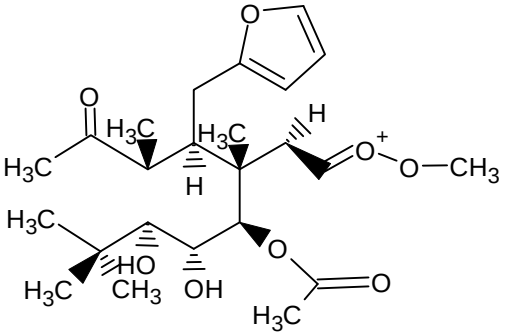
Norcaesalpinin E



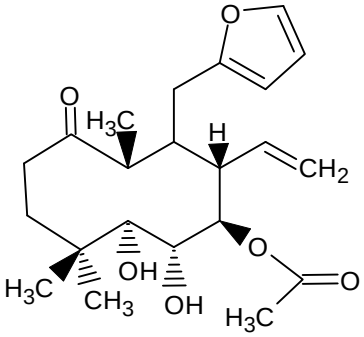
R=H, α-OAc, R_1= OAc, R_2=H Bonducellpin A
R=O, R_1= OAc, R_2=H Bonducellpin B
R=H, α-OAc, R_1=H_2, R_2=H Bonducellpin C



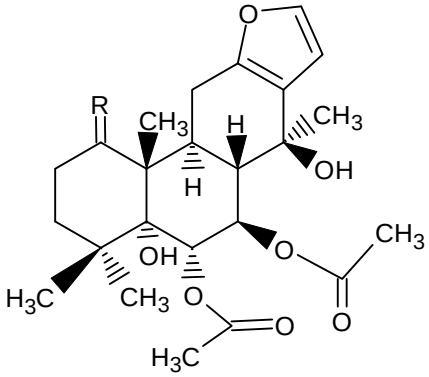
Bonducellpin D



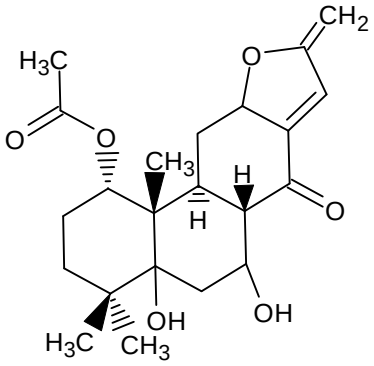
Bonducellpin E



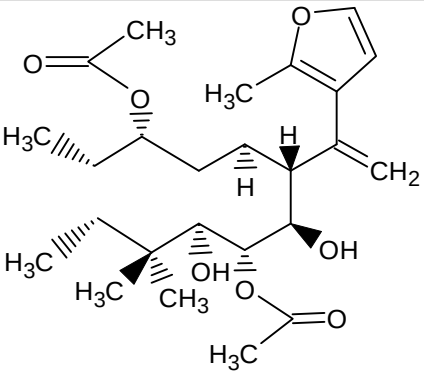
Bonducellpin F



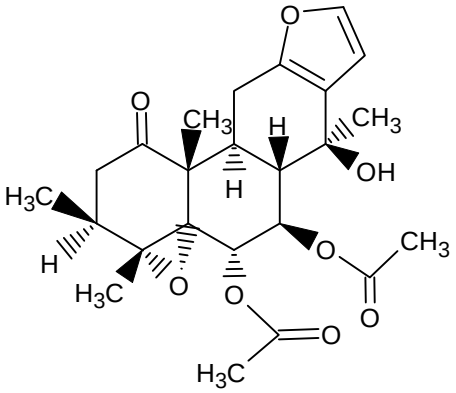
R=O, Caesalpin
R=H,α-OH Caesalpin G
R=H,α-OAc Caesalpin H



1α-acetoxy-5α,7β-dihydroxycassa-11,13(15)-diene-16,12-lactone



Bonducellpin G



Caesalpinin

ETHNOBOTANICAL USES:

- In Ayurveda, *Caesalpinia bonduc* is used in various diseases, such as tumors, diabetes, fever, cough, skin diseases, nausea, gout, etc., in Ayurveda. Kuberaksadi vati, Visama Jvaraghni Vati are among the major formulations of the plant in Ayurveda. Topically it is used as a local applicant to reduce inflammation and pain. Seed oil provides treatment for rheumatoid arthritis and osteoarthritis. Orally, it is used as anti-pyretic, diuretic, nerve tonic, anti-inflammatory agent and to counter ascites, haemorrhoids [65].
- In Siddha, Caesalpinia bonduc is used for treating various conditions and diseases. Kazharchikkai choornam (seed kernel powder) is the major formulation available which treats fever, asthma, malaria, constipation, PCOS, etc. It is used by traditional Siddha physicians in Malabar region for psoriasis treatment [66].
- In Homoeopathy, it is used for the treatment of different ailments of the head, eyes, nose, mouth, tongue, abdomen, liver, spleen and for control of mind in the homoeopathic system of medicine. It is also approved for the treatment of intermittent fever.
- In Unani, Fever nut is used in treating Ascites and Hydrocele when administered in the form of powder or paste.The seeds are thrown into fire and until the outershell is burntcompletely and use the kernel which provides immediate relief

in bronchial spasm in unani system.The oil prepared from burning seed is used to treat septic wounds and itching.Karanjwa,Habb-e-Mubarish, Jawarish-Gajga are some unani preparations of the plant [67].

RATIONALE
FOR
SELECTION

RATIONALE FOR SELECTION

The plant *Caesalpinia bonducella* belonging to the family Fabaceae/Caesalpiniaceae was selected for the work.

The seed of the plant possesses traditional claim for use in PCOS.

The plant exhibits various ethno medicinal uses hence used in various traditional systems of medicine such as Siddha, Ayurveda, Unani and Homoeopathy.

The plant is rich in Flavonoids and Polyphenolic compounds which are vital for the anticancer activity.

The anticancer activity of the plant against cervical cancer has not been scientifically validated so far for seed kernel extract.

So, the seed kernels of the plant *Caesalpinia bonducella*, was selected for evaluation against cervical cancer.

AIM

AND

OBJECTIVE

AIM AND OBJECTIVE:

Cervical cancer is one of the major types of cancer among women all around the world. It is the fourth most common type of cancer among women.

A majority of around 40% of women suffering from cervical cancer receive the standard treatment of surgical removal cancer tissues and several rounds of chemotherapy.

This standard treatment has proved to be effective among some patients whereas it also has a variety of side effects that may lead to be fatal.

This directs a vision towards completely naturally isolated compounds from *Caesalpinia bonducella* which has been already proved to be a good antitumor agent against breast cancer.

Hence, I've decided to work on isolating certain bioflavonoid compounds from *Caesalpinia bonducella* which might be having good antitumor activity against cervical cancer.

The isolated compounds are to be characterized by FT-IR and MS.

It is also to be tested for *invitro* Anti-cancer activity against cervical cancer cell lines and *in silico* docking analysis.

PLAN

OF

WORK

PLAN OF WORK

I PHARMACOGNOSTICAL STUDIES

- Macroscopical studies
- Microscopical studies
- Powder Microscopy
- Determination of Physicochemical constants
    - Ash Values
        - ❖ Total Ash
        - ❖ Acid Insoluble Ash
        - ❖ Water Soluble Ash
        - ❖ Sulphated Ash
    - Extractive Values
        - ❖ Alcohol soluble extractive value
        - ❖ Water soluble extractive value
- Determination of Loss on Drying

II PHYTOCHEMICAL STUDIES

- Preparation of Extracts
- Preliminary Phytochemical Screening
- Quantitative estimation of Phytochemical constituents
- Enrichment of extract
- Isolation of phytoconstituents
- Thin Layer Chromatography

III PHARMACOLOGICAL STUDIES

- *Invitro* Anticancer activity on cervical cancer cell lines.
- *Invitro* Anti-bacterial activity.
- *Invitro* Anti-fungal activity.
- *Insilico* docking analysis for Anticancer activity

IV CHARACTERIZATION

- Characterization of isolated compounds by FT-IR
- Characterization of isolated compounds by MS

MATERIALS
AND
METHODS

PHARMACOGNOSTIC STUDIES:

COLLECTION:

The seeds of *Caesalpinia bonducella* Linn., were collected from Chennai district in Tamilnadu during the month of October-2020.

AUTHENTICATION:

The collected plant material was botanically identified and authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-45. as the seeds of *Caesalpinia bonducella*. The seed were dried under shade, powdered and was stored in air tight container and then a portion of its used for the pharmacognostical and phytochemical studies.

PHARMACOGNOSTIC STUDIES:

Pharmacognosy is the systemic study of natural source, it is preliminary step in the standardization of crude drugs, which consist of identification, authentication and standardization of herbal medicinal plants through organoleptic character, histological character, powder microscopy, quantitative microscopy, histochemical analysis and physicochemical observations as the prescribed by an authoritative source as World Health Organization.

Evaluation of crude drugs which involves determination of authenticity, quality, purity, potency, safety, efficacy, reliability and reproducibility of the results for varying batch of crude drug. It is also used in the detection of nature of adulteration. Systemic identification of crude drugs and their quality assurance gives an integral part of drug description [68].

MACROSCOPY:

Fresh seeds of *Caesalpinia bonducella* were collected and different organoleptic features viz shape, size, colour, type, odour, taste were observed. These parameters

are considered useful in the quality control of the crude drug and evaluated as per standard WHO guidelines.

MICROSCOPY

This method is used to identify drugs on cellular level. It is to determine structure of organized drugs based on their histological characters. It includes examination of whole parts and also powdered drugs.

Histological characters studies:

- Size, shape and relative position of cells and tissues
- Chemical nature of cell wall.
- Fragments of plant cells or tissues.

Procedure:

In this study, transverse sections of seeds were examined under the microscope (10X and 40X). Staining reagents Phloroglucinol- Hydrochloric) were employed according to standard techniques. The different distinguishing characters were observed with or without staining and images of the obtained microscopy results were recorded by a camera.

POWDER MICROSCOPY:

Shade dried seeds of *Caesalpinia bonducella* were powdered well, then the powder was passed through sieve no.60 and used or powder analysis and organoleptic characters such as nature, colour, odour and taste were studied. Powder analysis using various staining reagents viz 1% phloroglucinol in 90% ethanol, concentrated HCL and N/50 iodine. Slide were observed under the microscope [69].

PHYSICO CHEMICAL CONSTANTS [70, 71 & 72]

Shade dried powdered plant materials of the seeds of *Caesalpinia bonducella* Linn., was used for the determination of the physio chemical constants in accordance with the WHO guidelines.

1. **ASH VALUE:**

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which complies of inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination of the quality and purity of the crude drug in the powdered form.

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.

Procedure:

Silica crucible was heated to redhot for 30 minutes and cooled in the desiccators. Incinerate about 2 to 3g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0c until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

The percentage of ash value = (weight of total ash/ weight of drug taken X 100)

WATER SOLUBLE ASH:

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

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter was collected in a ashless filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 4500. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

Percentage of water-soluble ash = (Weight of residue obtained/ weight of sample taken) X 100

ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air-dried drug is calculated.

Percentage of acid insoluble ash = (Weight of residue obtained/ weight of sample taken) X 100

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These tests are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible are heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly charred. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25o until all black particles have disappeared. Crucible are allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. This process is repeated until two successive weighing differ by more than 0.5 mg.

Percentage of acid sulphated ash = (Weight of residue obtained/ weight of sample taken) X 100

1.DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of Phyto constituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of Alcohol soluble extractive

5gm of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air-dried drug was calculated.

Percentage of Alcohol-soluble extractive value = (Weight of the dried extract/ weight of the sample taken) X 100

Determination of water-soluble extractive

5gm of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for rest eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Percentage of water-soluble extractive value = (Weight of the dried extract/ weight of the sample taken) X 100

DETERMINATION OF MOISTURE CONTENT:

Loss on drying

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Use of high-speed mill in preparing the samples are avoided. The sample in the tarred evaporating dish were placed in the drying chamber (105°C) for 5 hours and weigh. Drying and weighing is continued every one-hour interval until the difference between the two successive weight is not more than 0.25 percent. Constant weight is reached when the two-consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air-dried sample.

Percentage of moisture content = (Final weight of the sample / Initial weight of the sample) X 100

PHYTOCHEMICAL STUDIES:

The purpose of the phytochemical assessment is to determine the nature of the phytoconstituents present in the plant through appropriate chemical tests. It is critical to investigate the pharmacological activities of the plant. It may be done by confirmation with various chromatography techniques like TLC and HPTLC. Therefore, a comprehensive study is required for qualitative and quantitative characterization of Phyto constituents.

EXTRACTION

Extraction is the preliminary stage of the phytochemical evaluation. It highlights the metabolites in the extraction solvent depending on its polarity. The dried coarsely powdered sample (sieve no. 10) of *Caesalpinia bonducella*(1500gm) was extracted with methanol (4500ml) at a temperature of 65^0C. The extraction was performed by means

of Continuous Hot extraction method (Soxhlet extraction). The extraction was repeated for three times to get the complete extraction of the crude drug. The extract is then dried by Rotary vaccum evaporator (Buchi Rotavapor). The percentage of yield, colour and consistency of the extract were recorded and preceded by a more detailed phytochemical and pharmacologic examination.

PRELIMINARY PHYTOCHEMICAL SCREENING

The chemical tests for various phytoconstituents in the dried powder and extracts of seeds of *Caesalpinia bonducella* Linn., were carried out as described below and the results were recorded.

Test for alkaloids:

***Mayer's* Test**: To the extract, 2 ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for saponins:

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for tannins:

To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for cardial glycosides:

***Keller-Killani* test:** To 1ml of the extracts, 2 ml of glacial acetic acid containing a drop of FeCl3. Equal volume of conc. H2SO4 was added from the sides of the tube. A brown colour ring indicates the presence of cardial glycosides.

Test for flavonoids:

***Alkaline reagent* test**: Extract was treated with 10% NaOH solution; formation of intense yellow colour indicates presence of flavonoid.

Test for phenols:

Lead acetate **test:** The extract was taken; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

1 ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 was added from the side of test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence. This indicates the presence of steroid.

Test for terpenoids:

Salkowski **test**: 5 ml of extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Quinones:

The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of quinones.

Test for Proteins:

Ninhydrin **test:** The extract was taken and few drops of freshly prepared Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids [73].

FLAVONOID ENRICHMENT OF EXTRACT

The crude methanolic extract was further fractionated according to solvent polarity. The crude methanolic extract was dissolved in distilled water and stirred for 3 h followed by fractionation three times sequentially with chloroform, ethyl acetate and n-Hexane. These three fractions were centrifuged, filtered and concentrated. The ethyl acetate fraction was then separated and considered to be the flavonoid enriched extract [74].

ISOLATION

The methanol extract of seed kernel was dispersed in distilled water and partitioned with n-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (n-BuOH) consecutively based on increasing the polarity of solvents to gain n-hexane, $CHCl_3$, EtOAc, n-BuOH, and water-soluble fractions. The EtOAc soluble fraction was selected for more fractionations and purification.

In the final step, EtOAc soluble fraction was fractionated by the Sephadex LH-20 column chromatography and MeOH as eluent. Three sub-fraction (1-3) were gained. Sub-fraction 2 (240 mg) and 3 (110 mg) were purified by silica gel plate (20 × 20 cm) with chloroform-methanol (90:10) to get compound **1** and compound **2** [75].

QUANTITATIVE ESTIMATION OF TOTAL FLAVONOID CONTENT:

TOTAL FLAVONOID CONTENT

Estimation of the total flavonoids in the test sample was carried out using the method of (Ali *et al.*, 2018). To 1000 µg/ml of test sample a volume of 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. A calibration curve was constructed, using Quercetin (10 – 320 µg/mL) as standard. Total flavonoid contents were expressed as Quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.002x + 0.127$, where y was the absorbance [76].

CHROMATOGRAPHY:

Chromatography methods are important analytical tool in the separation, identification and estimation of components present in the plant.

THIN LAYER CHROMATOGRAPHY

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flow along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used [77].

TLC Plate Preparation

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase:

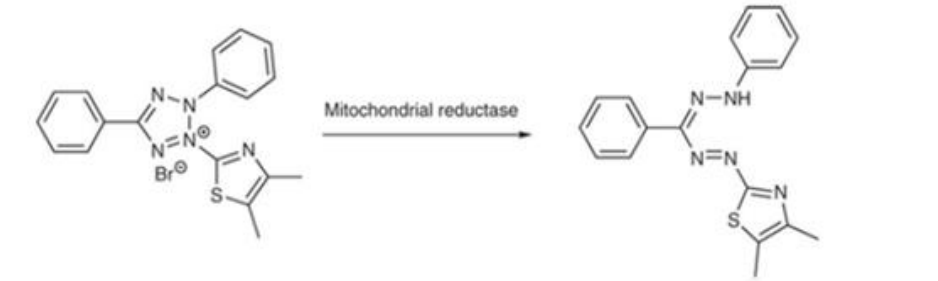
Solvent mixture was selected on the basis of the Phyto constituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents. From the vast analysis, best solvent was selected which showed good separation with maximum number of components [78].

$$Rf = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

PHARMACOLOGICAL STUDIES

EVALUATION OF *IN VITRO* ANTI-CANCER ACTIVITY AGAINST CERVICAL CANCER CELL LINES

The *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.



(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))

MTT

(2E,4Z)-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan

(Formazan)

Materials and Methods

1. MTT reagent (the solution is filtered through a 0.2 µm filter and stored at 2–8 °C for frequent use or frozen for extended periods)

2. DMSO

3. CO_2 incubator

4. Micro Plate reader

5. Inverted microscope

6. Refrigerated centrifuge

Preparation of test solutions

For MTT assay, serial two fold dilutions (3.125 – 100 µg/mL) were prepared from this assay.

Cell lines and culture medium

HeLa cell line was procured from NCCS, stock cell was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in an humidified atmosphere of 5% CO_2 at 37^oC until confluent.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1 x 10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37^oC for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37^oC in 5% CO_2 atmosphere.

The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm [79].

The percentage of viability was calculated using the following formula,

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

EVALUATION OF *IN VITRO* ANTI-BACTERIAL ACTIVITY

Name of the organisms used for the study:

Salmonella Typhi - Gram Negative

Escherichia coli - Gram Negative

Bacillus *subtilis* - Gram Positive

Staphytococcus aureus - Gram Positive

Disc-diffusion method:

The antibacterial activity of the test samples were carried out by disc diffusion method. The target microorganism were cultured in Mueller Hinton broth and incubated for 24 h. The petri dishes containing Mueller Hinton agar (MHA) medium were cultured with diluted bacterial strain. The prepared discs were placed on the culture medium. Test samples (500, 1000 and 2000µg) were injected to the sterile disc. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antibacterial activity [80].

EVALUATION OF *IN VITRO* ANTI-FUNGAL ACTIVITY

PRINCIPLE

The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

Potato dextrose agar medium, Amphotericin B antimycotic solution, test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

AGAR- WELL DIFFUSION METHOD

Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato influsion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

PROCEDURE

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain *(Candida albicans)* wells were cut and different concentration of sample 3u, 3q, CBE, 3o, 3m and 3r (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin

B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA) [81 & 82].

EVALUATION OF *IN SILICO* ANTI-CANCER ACTIVITY

Docking

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

In this study, PyRx is used to perform docking studies. Docking studies were performed with the active site of CDK 6(PDB ID: 1XO2). Protein was retrieved from RCSB and used for docking analysis.

CHARACTERIZATION

All the isolated compounds were characterized by using FT-IR and MASS Spectroscopy.

Infrared Spectroscopy

The infrared spectroscopy is one of the most powerful analytical techniques, this offers the possibility of chemical identification. The most important advantages of infrared spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly. The technique is based upon the simple fact that a chemical substance shows marked selectable absorption in the infrared region. After absorbing IR radiations the molecules of a chemical compound exhibit small vibrations, giving rise to closely packed absorption bands calledas IR absorption spectrum which may extend over a wide wavelength range.Various bands will be present in IR spectrum which corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical compound is a fingerprint for its identification.

Mass Spectroscopy

Mass spectrometer performs three essential functions. First, it subjects molecules to bombardment by a stream of more amounts of energy electrons, converting some of the molecules to ions, which are then accelerated in a field of electric. Second, the ions which are accelerated are divided according to their ratios of mass to charge in an electric or magnetic field. Finally the ions that have particular mass-to-charge ratio are detected by a device which can count the number of ions striking it. The detector's output is amplified and fed to a recorder. The trace from the recorder is a mass spectrum a graph of particles detected as a function of mass-to- charge ratio. The Mass spectra of the synthesized compounds were taken using Agilent spectrometer.

RESULTS
AND
DISCUSSION

RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

MACROSCOPY:

Organoleptic characters

Colour	: Green
Taste	: Bitter
Odour	: Characteristic
Shape	: Globular
Size	: 2.2-4cm long and 1-2cm in diameter

Caesalpinia bonducella seeds

MICROSCOPY

Microscopical characters

Detailed microscopic examination of a drug helps to identify the organized cellular structure of drugs material by their known histological characters. Use of various reagents or stains help to distinguish cellular structures depending on their chemical nature.

T.S of Seeds

The transverse section of the seed reveals an outermost compact and single-row arrangement of very narrow, translucent, radially elongated cells, which form the palisade. The cotyledons usually display a single outer layer of the epidermis containing small, isodiametric cells. The inner parenchymatous ground tissue cells are rich with fixed oil and are seen with uniformly distributed empty cavities. Cotyledons are also rich in starch grains.

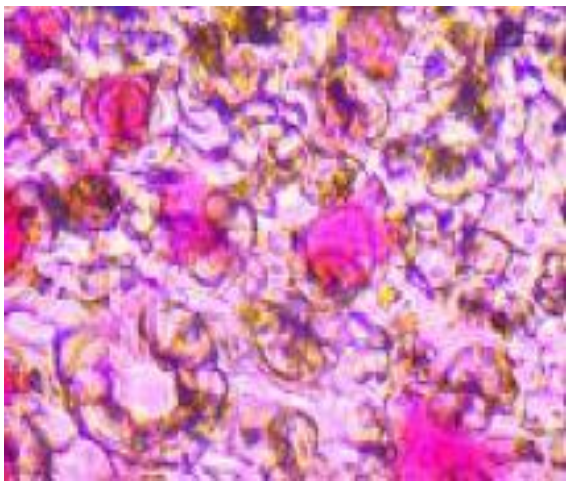
The transverse section of the seeds shows the presence of Cotyledons, Palisade cells, endosperm, collapsed parenchyma tissue.

(a) Sclereids

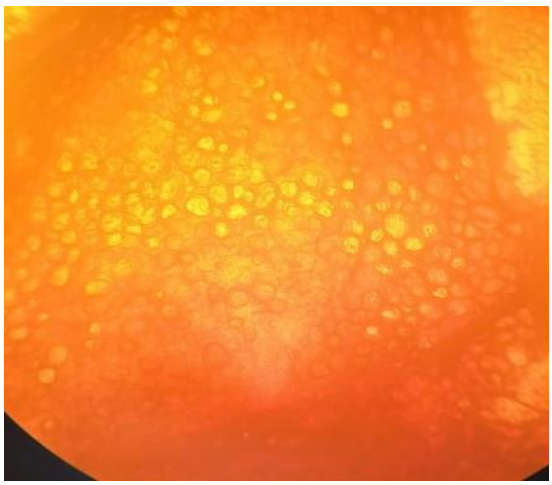
(b) Cotyledons

(c) Collapsed parenchyma

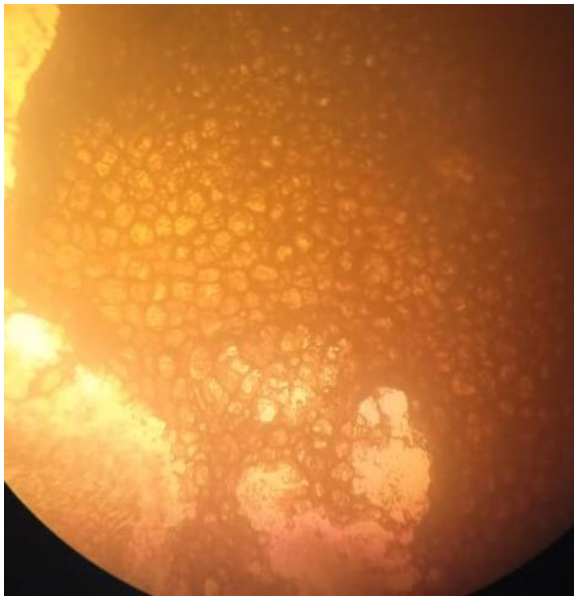
(d) Palisade cells and cotyledons



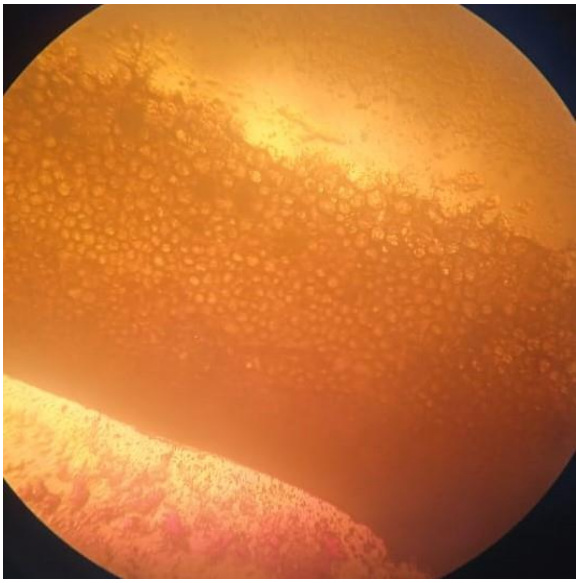
(a) Sclereids (Enlarged view)



(b) Cotyledons



(c)Collapsed parenchyma



(d) Palisade cells and cotyledons

Figure:9 Transverse section of seed kernels of *Caesalpinia bonducella*

POWDER MICROSCOPY:

The powder microscopy of *Caesalpinia bonducella* seeds shows the following characters.

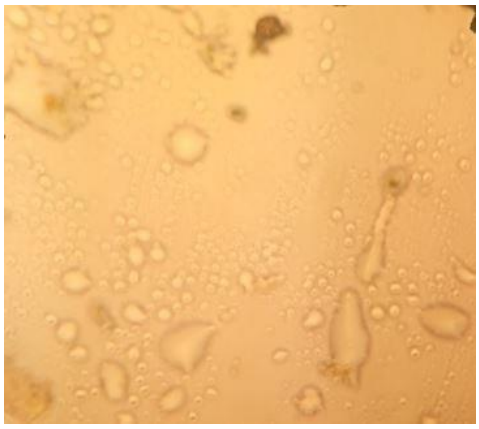
Organoleptic characters:

Nature	: Coarse powder
Colour	: Off-white
Odour	: Characteristic
Taste	: Bitter

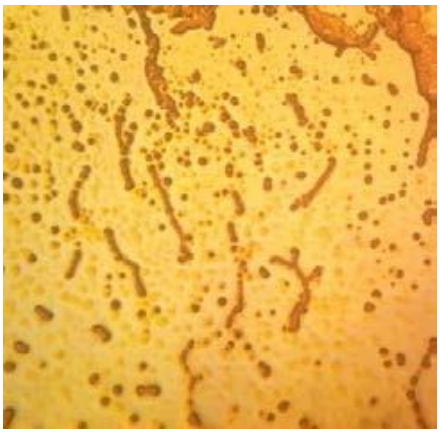
Microscopical characters:

The powder of the seeds shows the following inclusions when examined under the microscope which are of diagnostic values.

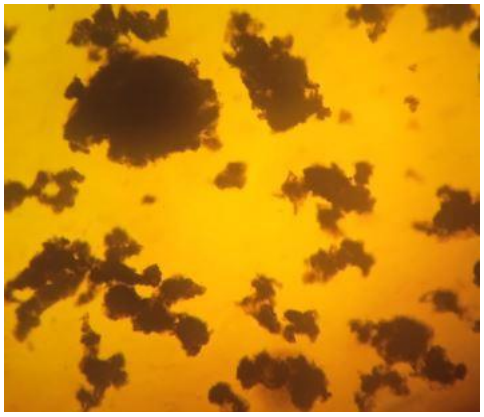
(a) Oil globules
(b) Schlerenchymatous Fibers
(c) Starch grains
(d) Parenchyma cells
(e) Stone cells
(f) Calcium Oxalate crystals



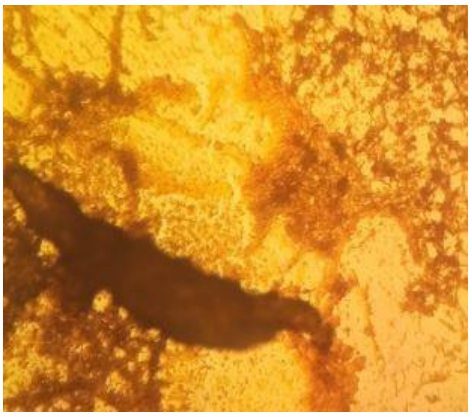
(a)



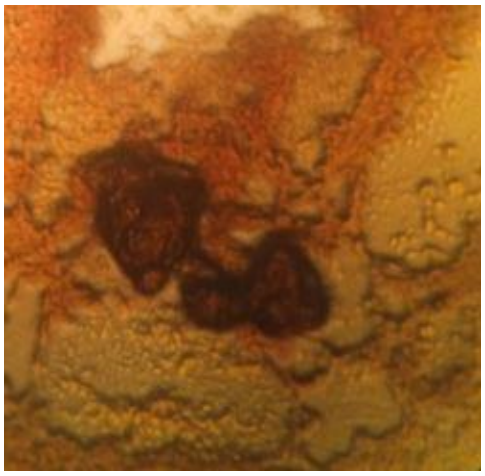
(b)



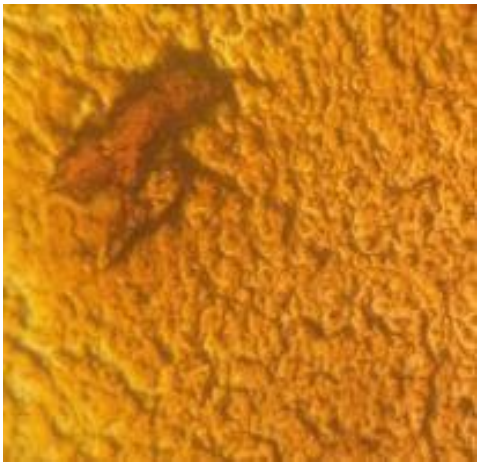
(c



(d)



(e)



(f)

Figure: 10 Powder Microscopy of *Caesalpinia bonducella* seed kernels

PHYSICOCHEMICAL CONSTANTS:

Table: 5 Physicochemical constants of seeds of *Caesalpinia bonducella*

S.NO	PARAMETERS	PERCENTAGE (%W/W)
I	**Ash Values**	
1	Total Ash	4.5
2	Acid Insoluble Ash	0.5
3	Water soluble Ash	1.8
4	Sulphated Ash	1.2
II	**Extractive Values**	
1	Water Soluble Extractive	10.3
2	Alcohol Soluble Extractive	11.5
III	Loss on Drying	6.6

PHYTOCHEMICAL ANALYSIS

PERCENTAGE YIELD OF EXTRACT

Table: 6 Results of Extraction

S.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD %w/w
1	Methanol	Hot Extraction	Solid Powder	Off-White	8.3

QUALITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Table: 7 Preliminary phytochemical analysis of extract and enriched medium of seeds of *Caesalpinia bonducella*

S.NO	PHYTOCHEMICAL TESTS	CRUDE METHANOL EXTRACT	ENRICHED EXTRACT
1	Alkaloids	+	-
2	Saponins	+	-
2	Tannins	+	-
4	CardiacGlycosides	+	+
5	Flavonoids	+	+
6	Phenols	+	+
7	Steroids	-	-
8	Terpenoids	+	-
9	Quinones	-	-
10	Proteins	-	-

+ indicates presence, -indicates absence

Figure: 11 Phytochemical analysis of Crude methanol extract

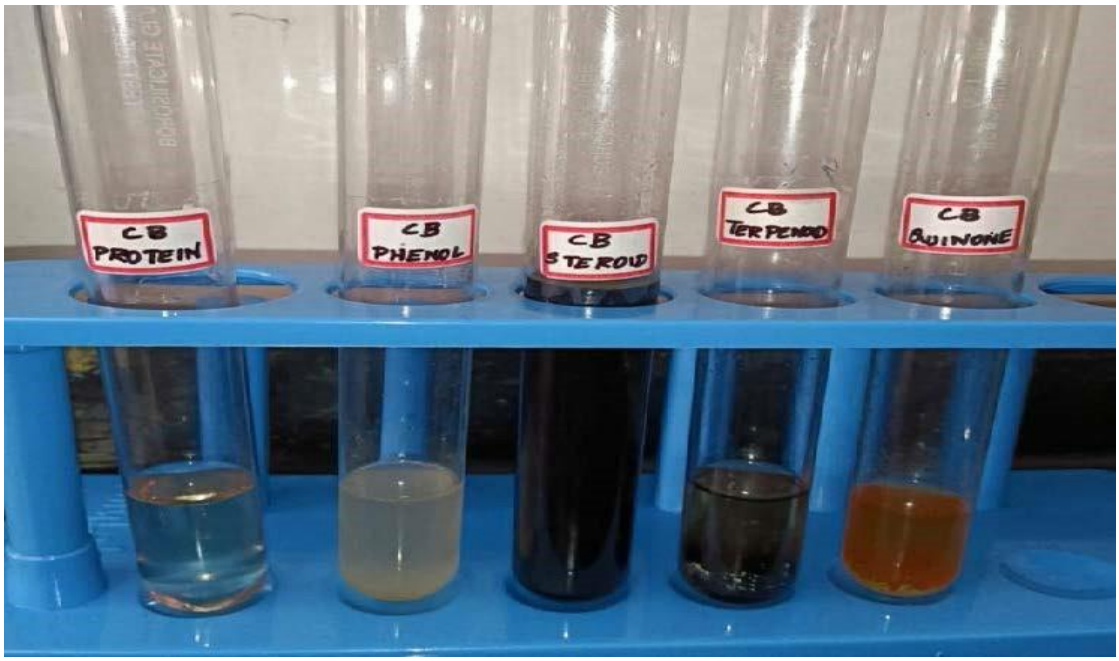
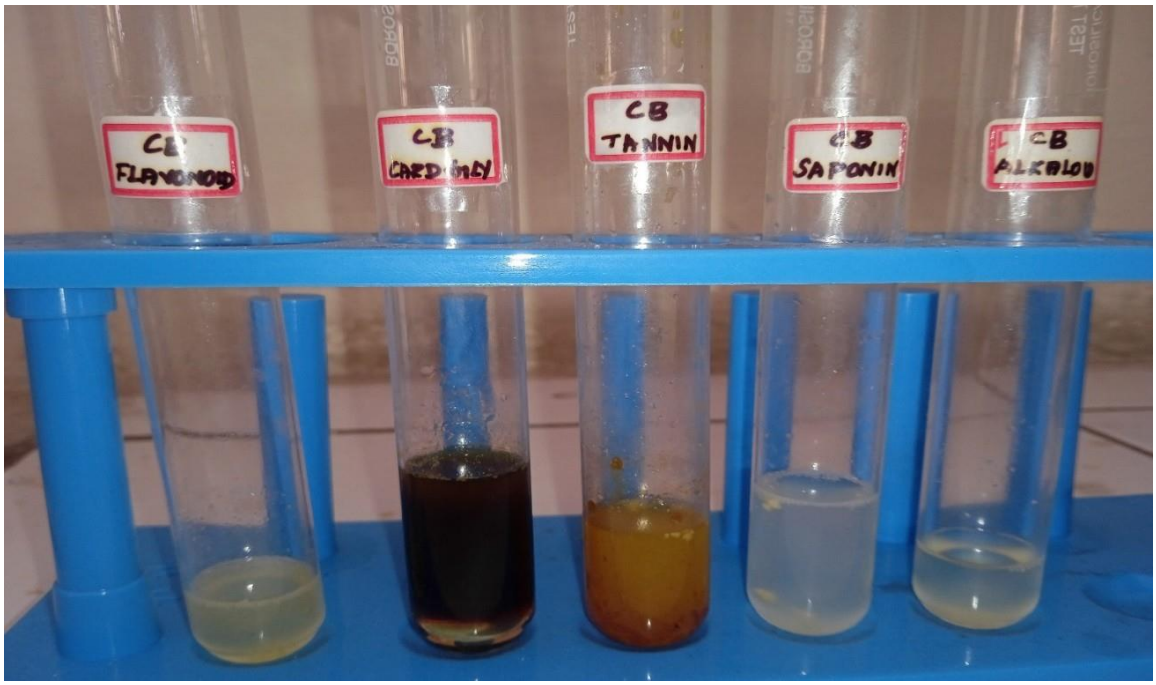
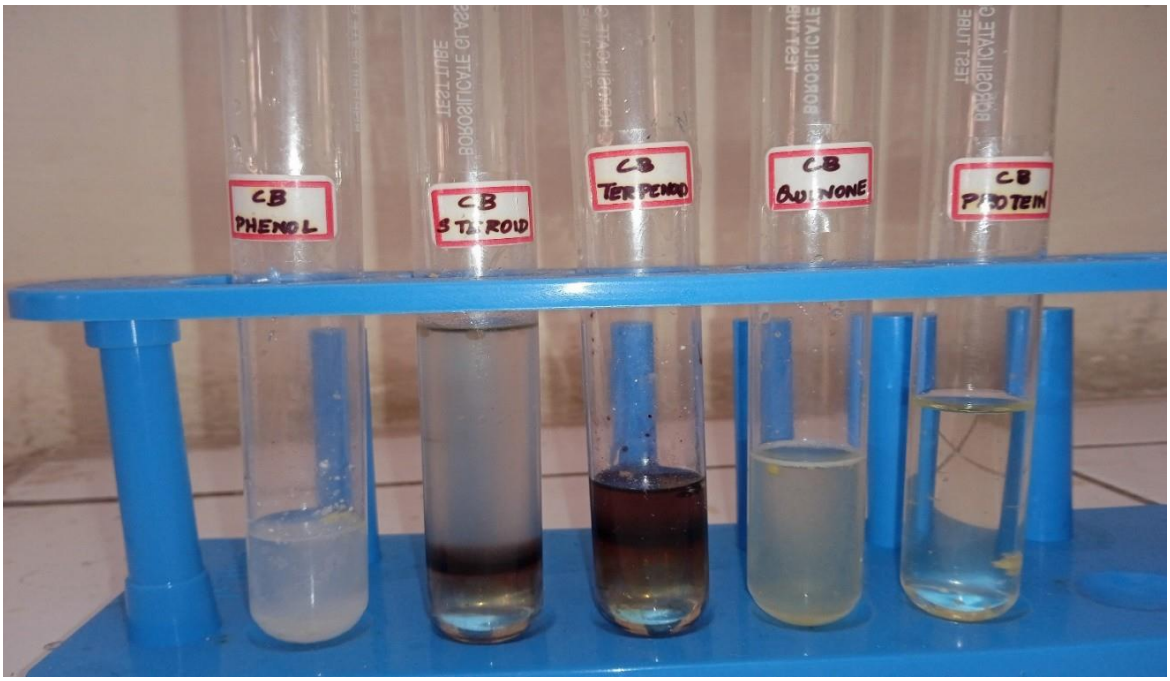


Figure: 12 Phytochemical analysis of Enriched extract





ENRICHMENT OF EXTRACT:

Table: 8 Results of Enrichment of flavonoids

S.NO	EXTRACT	METHOD OF ENRICHMENT	PHYSICAL NATURE	COLOUR	YIELD %w/w
1	Methanolic extract	Partition	Solid Powder	Off-White	4.7

ISOLATION

Table: 9 Results of Isolation of Phytoconstituents

COMPOUND	FRACTION USED	COLUMN	ELUENT	COLOUR	YIELD %w/w
Compound 1	Ethyl acetate soluble fraction	Silica gel	Chloroform: Methanol (90:10)	Yellow	101.3mg
Compound 2	Ethyl acetate soluble fraction	Silica gel	Chloroform: Methanol (90:10)	Yellow	90.7mg

CHARACTERIZATION

All the isolated compounds were characterized by using FT-IR and MASS Spectroscopy.

Infrared Spectroscopy

The infrared spectroscopy is one of the most powerful analytical techniques, this offers the possibility of chemical identification. The most important advantages of infrared

spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly.

The technique is based upon the simple fact that a chemical substance shows marked selectable absorption in the infrared region. After absorbing IR radiations the molecules of a chemical compound exhibit small vibrations, giving rise to closely packed absorption bands called as IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in IR spectrum which corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical compound is a fingerprint for its identification.

Mass Spectroscopy

Mass spectrometer performs three essential functions. First, it subjects molecules to bombardment by a stream of more amounts of energy electrons, converting some of the molecules to ions, which are then accelerated in a field of electric. Second, the ions which are accelerated are divided according to their ratios of mass to charge in an electric or magnetic field. Finally the ions that have particular mass-to-charge ratio are detected by a device which can count the number of ions striking it. The detector's output is amplified and fed to a recorder. The trace from the recorder is a mass spectrum a graph of particles detected as a function of mass-to- charge ratio. The Mass spectra of the synthesized compounds were taken using Agilent spectrometer.

Isolation of compound 1 (Quercetin)

The methanol extract of seed kernel was dispersed in distilled water and partitioned with n-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (n-BuOH) consecutively based on increasing the polarity of solvents to gain n-hexane, $CHCl_3$, EtOAc, n-BuOH, and water-soluble fractions. The EtOAc soluble fraction was selected for more fractionations and purification. In the final step, EtOAc soluble fraction was fractionated by the silica gel column chromatography and MeOH as eluent. Three sub-fraction (1-3) were gained. Sub-fraction 2 was purified by silica gel plate (20 × 20 cm) with chloroform-methanol (90:10) to get compound **1** (101.3mg).

Yellow solid; Yield: 101.3mg; MP: 310-313^{0}C; FT-IR (KBr cm^{-1}): 3268 (OH), 1665.41 (C=O), 1348.19 (C-O), 1506.36 (C=C), 1083.39 (C-O-C), Elemental analysis for C15H10O7, calculated C(59.61%), H(3.34%), O(37.05%) found: C(59.65%), H (3.31%), O(37.04%); M+ Calculated for C15H10O7 is 302.23, found: 302.16.

Isolation of compound 2 (Kaempferol)

The methanol extract of seed kernel was dispersed in distilled water and partitioned with n-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (n-BuOH) consecutively based on increasing the polarity of solvents to gain n-hexane, $CHCl_3$, EtOAc, n-BuOH, and water-soluble fractions. The EtOAc soluble fraction was selected for more fractionations and purification. In the final step, EtOAc soluble fraction was fractionated by the silica gel column chromatography and MeOH as eluent. Three sub-fraction (1-3) were gained. Sub-fraction 3 was purified by silica gel plate (20 × 20 cm) with chloroform-methanol (90:10) to get compound **2** (90.7mg).

Yellow solid; Yield: 90.7mg; MP: 268-273^{0}C; FT-IT (KBr cm^{-1}): 3290.63 (OH), 1650.31 (C=O), 1378.40 (C-O), 1604.98 (C=C), 1083.39 (C-O-C), Elemental analysis for C15H10O6, calculated C(62.94%), H(3.52%), O(33.54%) found: C(62.91%), H (3.56%), O(33.50%)M+ calculated for C15H10O6 is 286.23, found: 286.16.

IR Spectral Data:

Compound 1 (Quercetin)

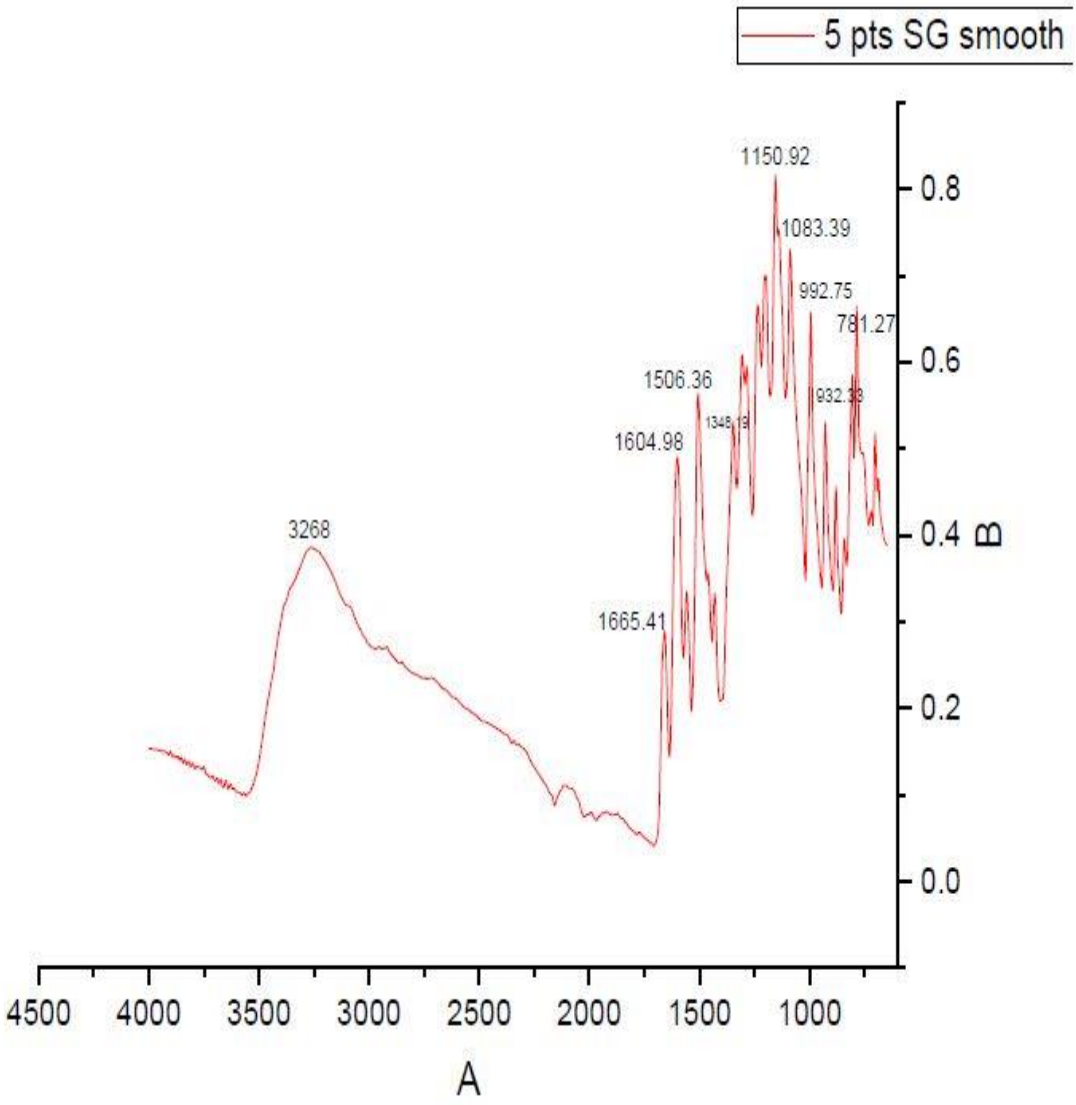


Figure: 13 FT-IR Spectra of compound 1

Compound 2 (Kaempferol)

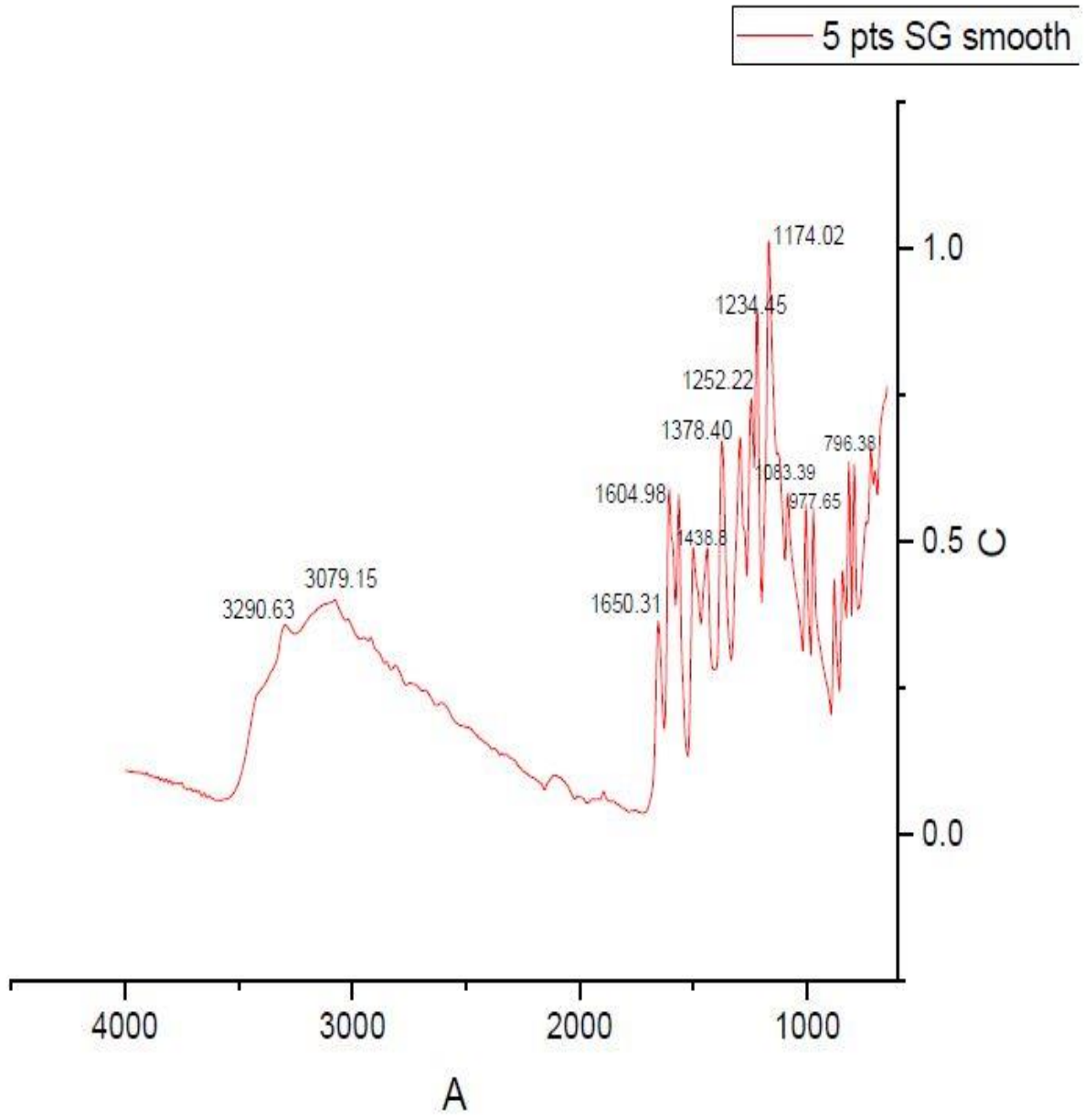


Figure: 14 FT-IR Spectra of compound 2

Mass Spectral Data:

Compound 1

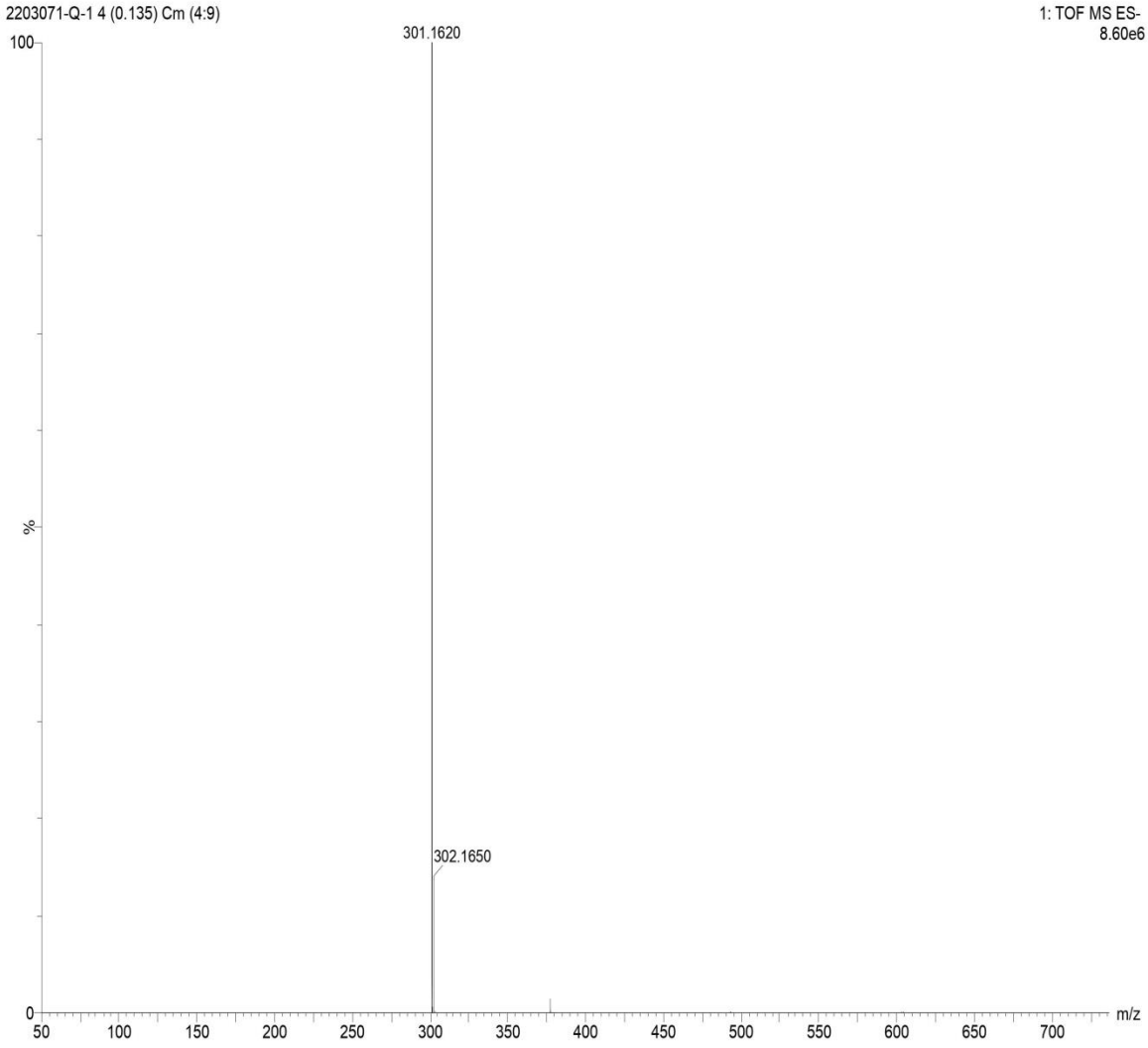


Figure: 15 Mass Spectra of compound 1

Compound 2

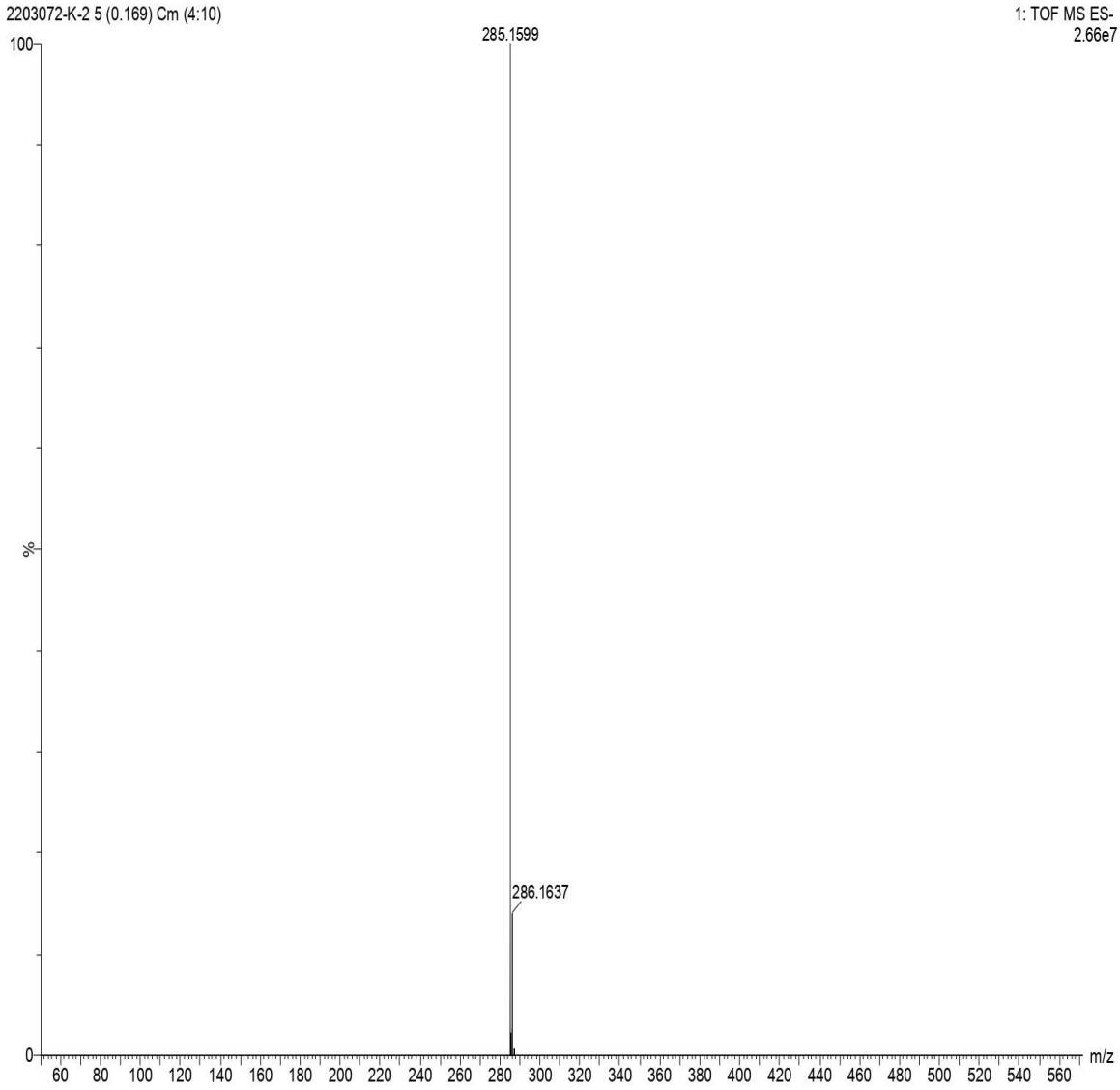


Figure: 16 Mass Spectra of compound 2

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The phytoconstituents such as flavonoid compounds were quantitatively estimated and tabulated for crude methanolic extract and enriched extract.

Table: 10 Quantitative estimation of Phytoconstituents

S.NO	PARAMETERS	VALUES
1	Total Flavonoid in crude extract	1.9mg/g
2	Total Flavonoid in enriched extract	7.5mg/g

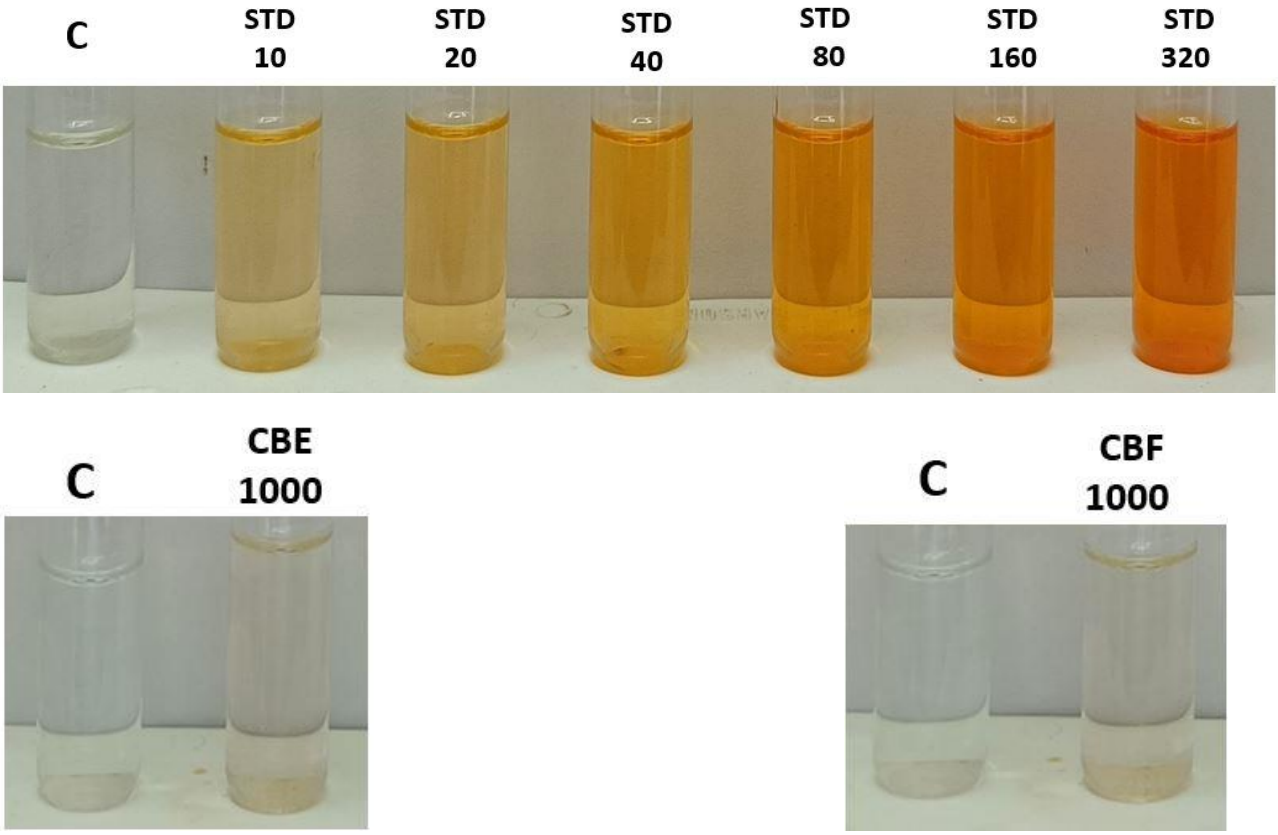


Figure: 17 Results of Flavonoid estimation

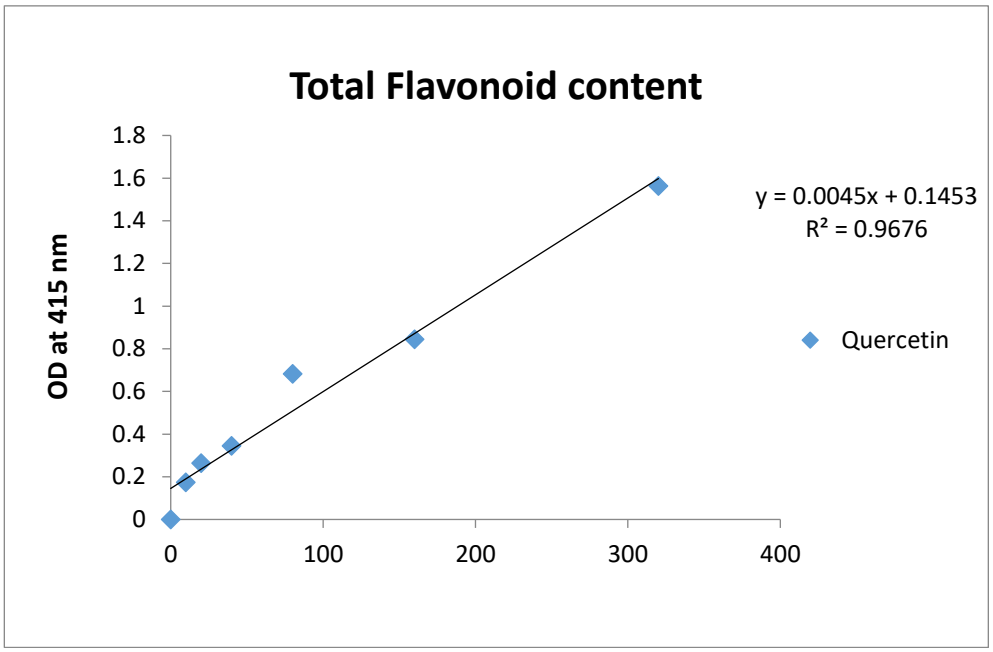


Figure: 18 Calibration curve for total flavonoid content

THIN LAYER CHROMATOGRAPHY:

Table: 11 TLC of methanolic extract of seeds of *Caesalpinia bonducella*

S.NO	Chemical Constituents	Solvent system	Spray Reagent	No of Spots	Rf Value
1	Enriched Extract	Ethylacetate:Methanol:Aceticacid(6:4:1)	Anisaldehyde-Sulphuric acid	4	0.89 0.44 0.32 0.12
2	Isolated compound 1	Ethylacetate: Methanol: Aceticacid (6:4:1)	Ethanolic Aluminium chloride solution	2	0.93 0.31
3	Isolated compound 2	Ethylacetate: Methanol: Aceticacid (6:4:1)	Ethanolic Aluminium chloride solution	1	0.92

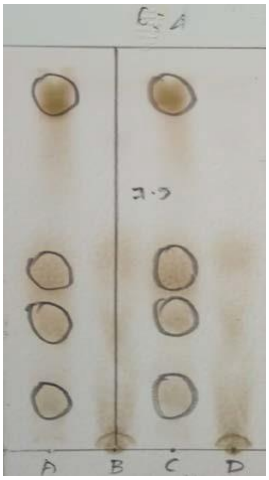


Figure: 19 TLC of enriched extracts

Figure: 20 TLC of isolated compound 1

Figure: 21 TLC of isolated compound 2

PHARMACOLOGICALSTUDIES

EVALUATION OF ANTI-CANCER ACTIVITY

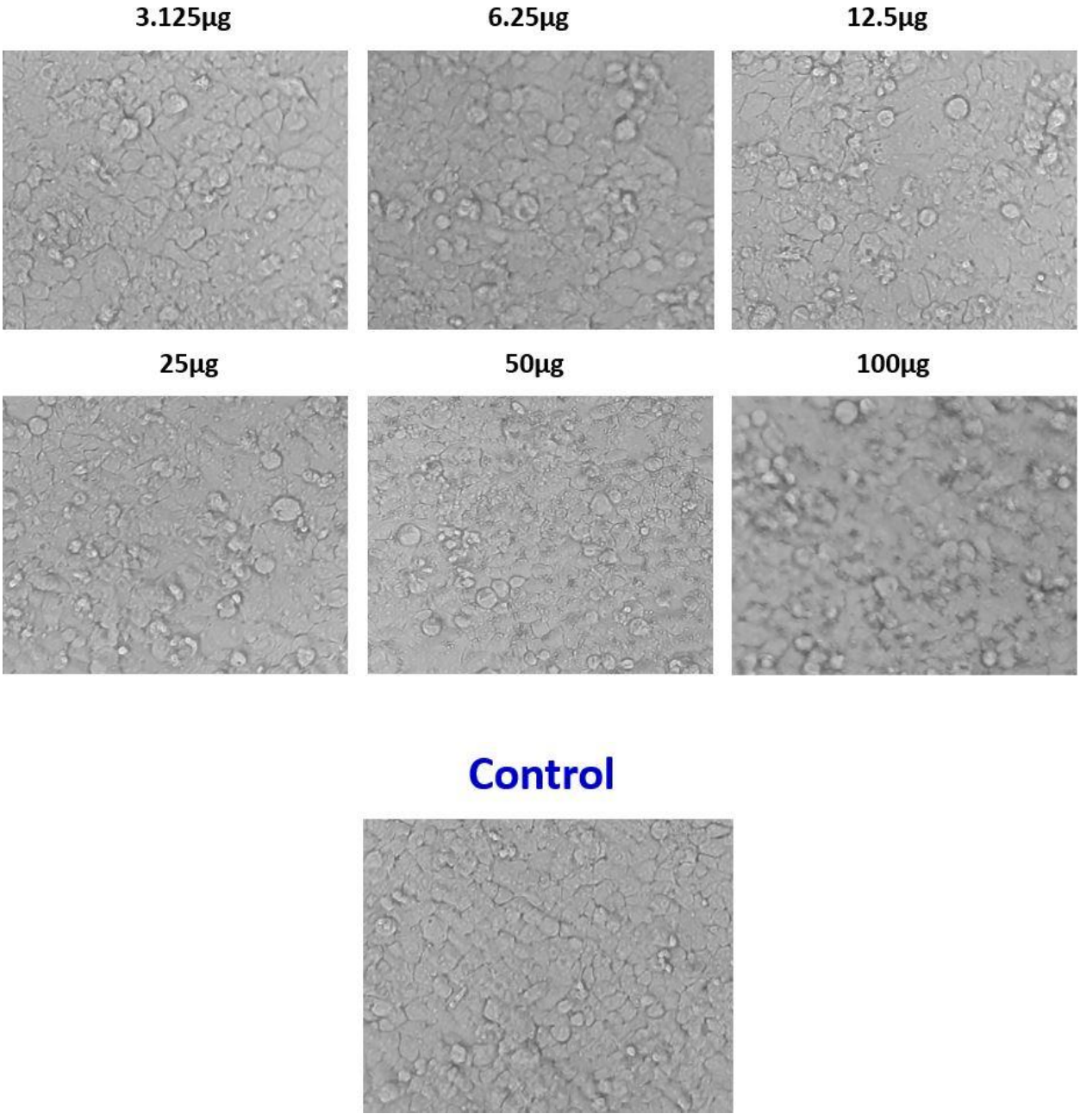


Figure: 22 Cell viability of enriched extract on HeLa cells.

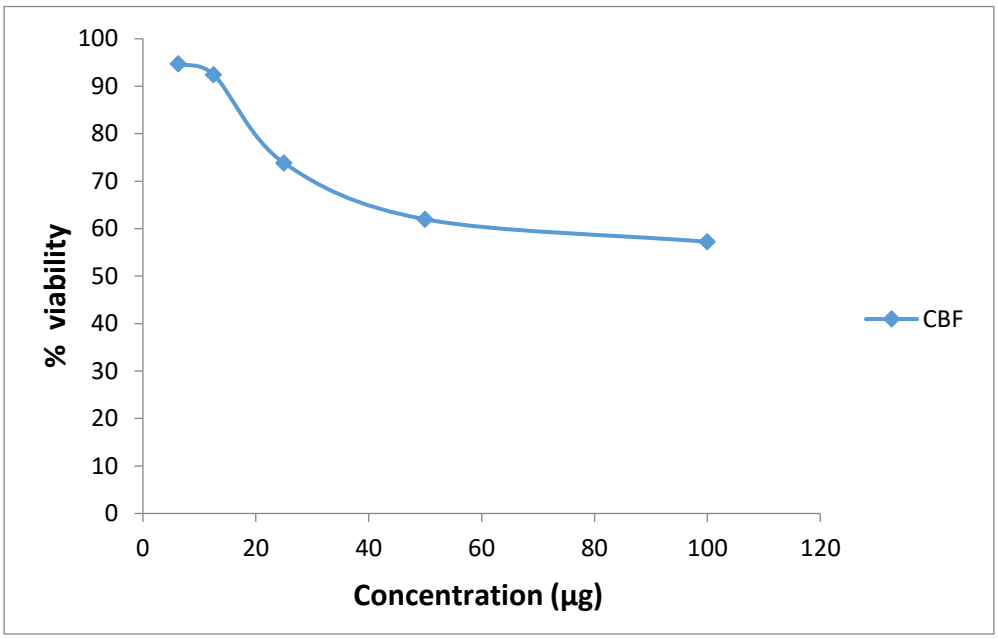
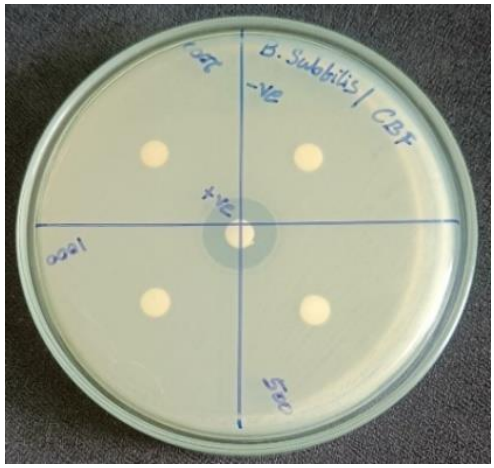


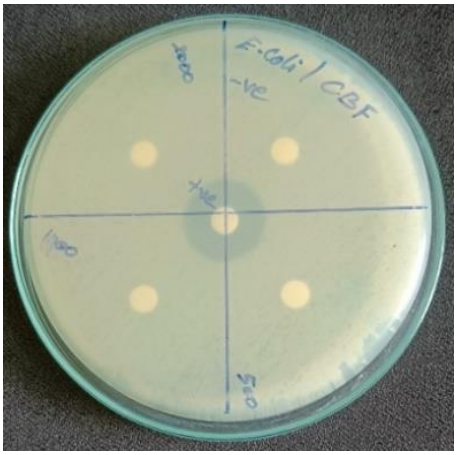
Figure: 23 Graph of percentage viability

The IC_{50} value of the flavonoid enriched extract was found to be **138.42 µg**.

EVALUATION OF ANTI-BACTERIAL ACTIVITY

Bacillus subtilis ***Escherichia coli***





Staphylococcus aureus ***Salmonella typhi***

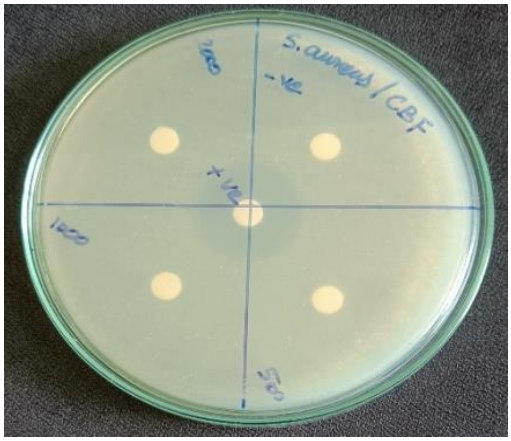


Figure: 24 Zone of inhibitions of microorganisms against enriched extract

Table: 12 Zone of Inhibition of microorganisms against enriched extract

Samples	Zone of Inhibition (mm)											
	S.aureus			***B.subtilis***			***E.coli***			***S.typhi***		
Concentration	500	1000	2000	500	1000	2000	500	1000	2000	500	1000	2000
Enriched Extract	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Streptomycin (20 µg)	**22**			**18**			**20**			**18**		

The above table shows that, the flavonoid enriched extract had no activity against the two gram positive and two gram negative organisms.

EVALUATION OF ANTI-FUNGAL ACTIVITY

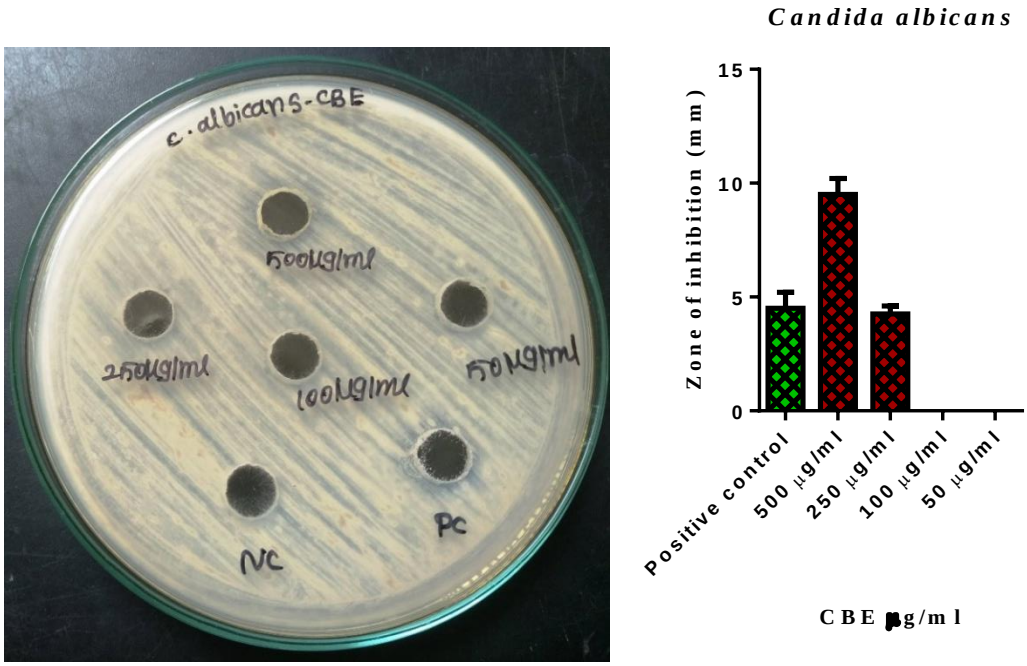


Figure: 25 Zone of Inhibition of *Candida albicans* against enriched extract

Table: 13 SD± Means of zone of inhibition obtained by enriched extract against *Candida albicans.*

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Candida albicans*	Flavonoid enriched extract	9.5±0.7	4.25±0.35	0	0	4.5±0.7

SD – Standard Deviation, *Significance - p< 0.05

DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

In this study, AutoDockVina using Pyrx toolwas used to perform docking studies. Docking studies were performed with the active site of human cyclin-dependent kinase 6 complex (PDB ID: 1XO2). Protein was retrieved from RCSB and used for docking analysis.

This molecular docking study was performed using the selected protein from the protein data bank (PDB ID: 1XO2).The cocrystal structure of the human cyclin-dependent kinase 6 complex protein complexed with ligand Fisetin in the active site is taken as protein.The docking for Fisetin was performed with its complex cocrystallized protein to validate the binding energy of ligand−protein interactions. The validation results showed a binding energy of −10.4 kcal/mol for Fisetin with two hydrogen bond interactions with VAL 101, HIS 100 and five hydrophobic interactions with ILE 19, LEU 152, PHE 98.

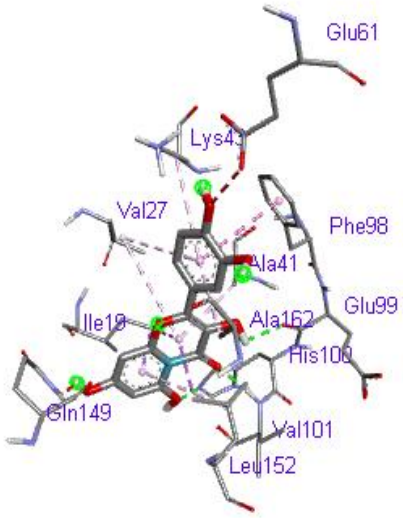
The most active compound, compound 2 showed hydrogen bond and hydrophobic interactions with binding affinity energy of -10.2 kcal/mol. Compound 1 exhibited binding energy of -9.9 kcal/mol.

Compound 2 showed interaction in a different mode, by forming strong hydrogen-bond interactions with three amino acids LYS 43, VAL 101 and HIS 100.The ligand showed interaction by forming Hydrophobic interactions with three amino acids ILE 19, ILE 19, LEU 152. The phenyl group of compound 2 showed hydrogen bond interaction with LYS 43. The hydroxyl group of compound 2 showed hydrogen bond interaction with VAL 101. Hydroxyl group showed hydrogen bond interaction with HIS 100. Naphthyl group of compound 2 showed hydrophobic interactions with ILE 19 two times. Also, phenyl group showed hydrophobic interaction with LEU 152.

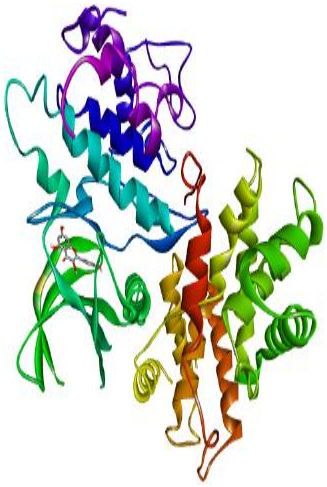
Compound 1 exhibited binding energy of -9.9 kcal/mol. The carbonyl group of compound 1 showed hydrogen bond interaction with VAL 101. The hydroxyl group of compound 1 showed hydrogen bond interaction with HIS 100. The Napthyl group showed hydrophobic interactions two times with ILE 19 and one time with LEU 152. Phenyl group of compound 1 showed hydrophobic interaction with PHE 98.

In comparison to validation results, there is, a hydrogen-bond interaction is common in both isolated compounds as well as Fisetin, which is His 100 and two hydrophobic interactions with ILE 19 in the essential active site in both the isolated compounds. These results indicate that ligands can act as anti-proliferative agents against CDK 6.

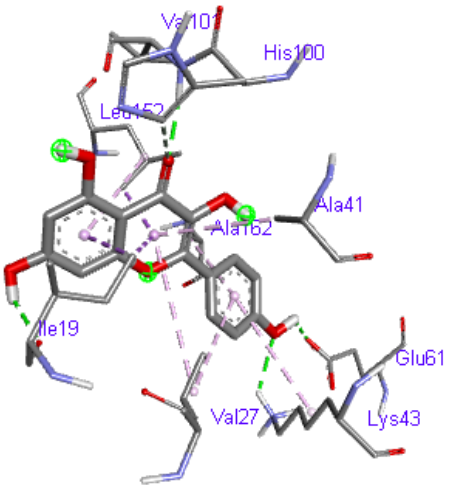
Compound 1- 3D



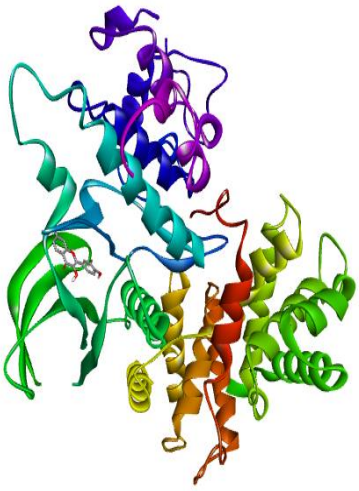
Compound 1- Ligand interaction



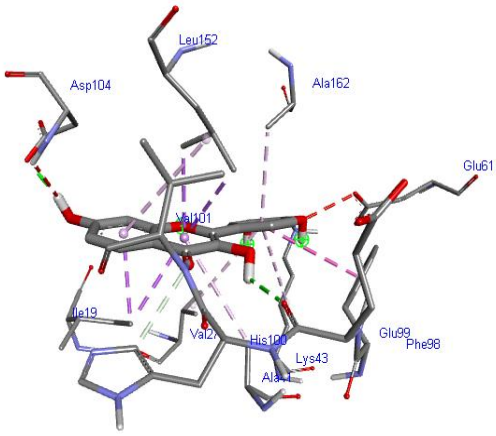
Compound 2 -3D Interaction



Compound 2 – Ligand Interaction



Standard compound-3D (Fisetin)



Standard Compound Ligand Interaction (Fisetin)

Figure: 26 Docking results with 3D and Ligand interaction

Table: 14 Docking results

S.No.	Compound No.	Docking score Kcal/mol	Amino acid interaction with Distance
1.	Isolated compound 1	-9.9	VAL 101 – 2.02 HIS 100 – 3.39 ILE 19 – 3.83 ILE 19 – 3.46 LEU 152 – 3.51 PHE 98 – 4.43
2.	Isolated compound 2	-10.2	LYS 43 - 2.45 VAL 101 - 2.01 HIS 100 – 2.45 ILE 19 – 3.81 ILE 19 – 3.47 LEU 152 – 3.51
3.	Validation	-10.4	VAL 101 – 2.00 HIS 100 – 3.34 ILE 19 – 3.89 ILE 19 – 3.41 LEU 152 – 3.89 LEU 152 – 3.43 PHE 98 – 4.70

SUMMARY
AND
CONCLUSION

SUMMARY AND CONCLUSION

Although there are several allopathic drugs available, many people still rely on nature that has blessed the human species with abundant herbs.

The occurrence of cervical cancer is still increasing worldwide. In India, cervical cancer contributes to approximately 6–29% of all cancers in women. It is estimated that cervical cancer will occur in approximately 1 in 53 Indian women during their lifetime. Apart from HPV Vaccine (*human papillomavirus vaccine)* the standard drugs used for treatment are cisplatin, carboplatin, etc. Although with the advent of new synthetic drugs, the side effects (tolerable or severe) on chronic use have led us to look for effective drugs with less or no side effects.

Therefore, there is an attempt to evaluate the traditional claims of *Caesalpinia bonducella* seed kernels for the treatment of cervical cancer.

The review on literature showed other works have been done on this plant but still activity against cervical cancer has not been reported so far.

The project entitled Anti-tumor potential of seed kernels of *Caesalpinia bonducella*(Fabaceae)extracts and isolated phytoconstituents: *in vitro* and *in silico* analysis has been achieved with the following results.

Macroscopical studies states the characteristic features of *Caesalpinia bonducella* seeds such as globular shape, characteristic odour, green in colour and other morphological characters has been well studied and established.

Microscopic investigations are the diagnostic features and its powder analysis helps to distinguish this particular plant (leaves) in whole form and in crude powder form. Microscopical analysis in *Caesalpinia bonducella* showed the presence of schlerenchymatous fibres, Starch grains, Parenchyma cells, Oil Globules, Stone cells and calcium oxalate crystals.

The above findings help to bring the identity determined the leaves by both morphology and anatomy.

Various physicochemical constants were determined for the seeds of *Caesalpinia bonducella* such as Ash values, Extractive values and loss on drying. This helps in confirming the identity and purity of this plant and to detect adulterants and its nature.

The phytochemical studies on the seeds of *Caesalpinia bonducella* were carried out to bring its importance as a valuable medicinal plant.

The total methanolic extract was carried out using Hot continuous extraction apparatus. The percentage yield of methanolic extract was 8.3% w/w.

The extract was subjected to preliminary phytochemical screening which revealed the presence of Flavonoids, Phenols, Saponins, Terpenoids, Alkaloids, Cardiac Glycosides and Tannins.

Quantification of phytoconstituents was done for flavonoids and phenolic compounds by UV spectroscopy method.

The obtained extract was enriched in flavonoid content by removing all other phytochemicals present in it by partition chromatography. Phytochemical screening of the enriched extract revealed the presence of Flavonoids, Phenols and Cardiac glycosides.

Also, the methanolic extract was subjected to column chromatography which resulted in the isolation of compounds Quercetin and Kaempferol which was confirmed in the later stage by FT-IR and Mass spectroscopy.

Thin Layer Chromatography was performed for the enriched extract and both the isolated compounds.

In vitro pharmacological studies, MTT assay on HeLa cells against cervical cancer cell lines were performed for the enriched extract to find out the anti-cancer activity. Also it has been tested for Anti-bacterial and Anti-fungal activity.

In silico docking analysis were performed on CDK 6 enzyme (1XO2) by the isolated constituents of *Caesalpinia bonducella* to evaluate the anti-cancer activity using molecular docking analysis.

The *Insilico* studies revealed that both the compounds, Quercetin and Kaempferol, possessed significant binding energy and inhibition constant values.

Hence from the studies it can be concluded that the seed kernels of *Caesalpinia bonducella* possess significant activity against cervical cancer. This investigation has a potential to be developed further into a natural Anti-cancer drug.

Further detailed studies on more flavonoids and especially Homo isoflavonoids (HIFs) which is a part not much explored by many has to be studied.

REFERENCES

REFERENCES

1. Shrestha PM, Dhillion SS. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of ethnopharmacology. 2003 May 1;86(1):81-96.
2. Sarkar S, Zaidi S, Chaturvedi AK, Srivastava R, Dwivedi PK, Shukla R. Search for a herbal medicine: anti-asthmatic activity of methanolic extract of *curcuma longa*. J PharmacognPhytochem. 2015;3:59-72.
3. Saroya AS. Herbalism, Phytochemistry&Ethnopharmacology. USA: Science publishers; 2011. p.1-3.
4. Barnes J, Anderson LA, Phillipson JD. Herbal Medicines. 3rd ed. London: Pharmaceutical press; 2007. p.4.
5. Cardini F, Wade C, Regalia AL, Gui S, Li W, Raschetti R et al. Clinical research in traditional medicine, priorities and methods. Com Ther Med.2006; 4: 282-87.
6. Firenzuoli F, Gori L. Herbal Medicine Today: Clinical and Research Issues. Evid Based Complement Alternat Med. 2007; 37(40).
7. Pandey MM, Rastogi S, Rawat AK. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine. 2013 Jun;2013.
8. Evans M. A guide to herbal remedies. Orient paperbacks: 1994
9. Cancer.Net. ASCO., https://www.cancer.net/navigating-cancer-care/cancer-basics/what-cancer (Accessed January 05, 2022)
10. National Cancer Institute., https://www.cancer.gov/about-cancer/causes-prevention/risk (Accessed January 05, 2022)
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424.
12. WHO. Cervical cancer. World Health Organization: Geneva, 2018. http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/ (accessed April 25, 2021).

13. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet 2013; 382: 889–99.

14. Small W Jr, Bacon MA, Bajaj A, et al. Cervical cancer: a global health crisis. Cancer 2017; 123: 2404–12.

15. International Collaboration of Epidemiological Studies of Cervical Cancer. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. Int J Cancer 2007; 120: 885–91.

16. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet 2007; 370: 59–67.

17. Bos AB, Rebolj M, Habbema JD, van Ballegooijen M Nonattendance is still the main limitation for the effectiveness of screening for cervical cancer in the Netherlands. Int J Cancer 2006; 119: 2372–75.

18. Sultana F, English DR, Simpson JA, et al. Rationale and design of the iPap trial: a randomized controlled trial of home-based HPV self-sampling for improving participation in cervical screening by never- and under-screened women in Australia. BMC Cancer 2014;14: 207.

19. Live long Lyndhurst., https://www.livelonglyndhurst.com/cervical-cancer.html (Accessed January 05, 2022)

20. American cancer society., https://www.cancer.org/cancer/cervical-cancer/detection-diagnosis-staging/signs-symptoms.html (Accessed January 05, 2022)

21. ASCO, Cancer.Net., https://www.cancer.net/cancer-types/cervical-cancer/types-treatment (Accessed January 05, 2022)

22. Saini, N.; Gahlawat, S.K.; Lather, V. Flavonoids: A nutraceutical and its role as anti-inflammatory and anticancer agent. In Plant Biotechnology: Recent Advancements and Developments; Gahlawat, S., Salar, R., Siwach, P., Duhan, J., Kumar, S., Kaur, P., Eds.;Springer: Singapore, 2017.

23. Fraga, C.G.; Croft, K.D.; Kennedy, D.O.; Tomás-Barberán, F.A. The effects of polyphenols and other bioactives on human health.FoodFunct. 2019, 10, 514–
24. Maleki, S.J.; Crespo, J.F.; Cabanillas, B. Anti-inflammatory effects of flavonoids. Food Chem. **2019**, 299, 125124.
25. D'Amelia, V.; Aversano, R.; Chiaiese, P.; Carputo, D. The antioxidant properties of plant flavonoids: Their exploitation by molecular plant breeding. Phytochem. Rev. 2018, 17, 611–625.
26. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of nutritional science. 2016;5.
27. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients. 2020 Feb;12(2):457.
28. Wikipedia., https://en.wikipedia.org/wiki/Cyclin-dependent_kinase_6#:~:text=Cell%20division%20protein%20kinase%206,)%20family%2C%20which%20includes%20CDK4 (Accessed January 05, 2022).
29. Li Z, Zou W, Zhang J, Zhang Y, Xu Q, Li S, Chen C. Mechanisms of CDK4/6 inhibitor resistance in luminal breast cancer. Frontiers in Pharmacology. 2020:1723.
30. Nondo RS, Moshi MJ, Erasto P, Masimba PJ, Machumi F, Kidukuli AW, Heydenreich M, Zofou D. Anti-plasmodial activity of Norcaesalpin D and extracts of four medicinal plants used traditionally for treatment of malaria. BMC complementary and alternative medicine. 2017 Dec;17(1):167.
31. Simin K, Khaliq-uz-Zaman SM, Ahmad VU. Antimicrobial activity of seed extracts and bondenolide from Caesalpiniabonduc (L.) Roxb. Phytotherapy Research. 2001 Aug;15(5):437-40.
32. Khan HU, Ali I, Khan AU, Naz R, Gilani AH. Antibacterial, antifungal, antispasmodic and Ca++ antagonist effects of Caesalpiniabonducella. Natural Product Research. 2011 Feb 1;25(4):444-9.

33. Wadkar GH, Kane SR, Matapati SS, Hogade MG. In-vitro anthelmintic activity of Caesalpiniabonducella (Linn). Flem. leaves. Journal of pharmacy research. 2010 May;3(5):926-7.
34. Usha P, Sharma MC. ANTIVIRAL ACTIVITY OF LATHAKARANJA (CAESALPINIA CRISTA L.) CRUDE EXTRACTS ON SELECTED ANIMAL VIRUSES. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2012 Sep 1;1(9):440.
35. Patil KS. Wound healing activity of the seed kernels of Caesalpinia crista Linn. J Nat Remed, 2005; 5: 26-30.
36. Shukla S, Mehta A, John J, Singh S, Mehta P, Vyas SP. Antioxidant activity and total phenolic content of ethanolic extract of Caesalpiniabonducella seeds. Food ChemToxicol. 2009 Aug;47(8):1848-51. doi: 10.1016/j.fct.2009.04.040. Epub 2009 May 5. PMID: 19422871.
37. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi ML. Antitumor activity and antioxidant status of Caesalpiniabonducella against Ehrlich ascites carcinoma in Swiss albino mice. Journal of Pharmacological Sciences. 2004;94(2):177-84.
38. Kannur DM, Hukkeri VI, Akki KS. Antidiabetic activity of Caesalpiniabonducella seed extracts in rats. Fitoterapia. 2006 Dec;77(7-8):546-9. doi: 10.1016/j.fitote.2006.06.013. Epub 2006 Jul 6. PMID: 16905279.
39. Archana P, Tandan SK, Chandra S, Lal J. Antipyretic and analgesic activities of Caesalpiniabonducella seed kernel extract. Phytother Res. 2005 May;19(5):376-81. doi: 10.1002/ptr.1339. PMID: 16106383.
40. Shukla S, Mehta A, John J, Mehta P, Vyas SP, Shukla S. Immunomodulatory activities of the ethanolic extract of Caesalpiniabonducella seeds. J Ethnopharmacol. 2009 Sep 7;125(2):252-6. doi: 10.1016/j.jep.2009.07.002. Epub 2009 Jul 14. PMID: 19607900.
41. Salunke KR, Ahmed RN, Marigoudar SR; Lilaram. Effect of graded doses of Caesalpiniabonducella seed extract on ovary and uterus in albino rats. J Basic ClinPhysiolPharmacol. 2011 Jun 17;22(1-2):49-53. doi: 10.1515/jbcpp.2011.006. PMID: 22865364.

42. Ali, A., MD SHALAM, MD ASHFAQ, NV RAO, SH GOUDA, and SM SHANTAKUMAR. "ANTICONVULSANT EFFECT OF SEED EXTRACT OF CAESALPINIA BONDUCELLA (ROXB.)." (2009): 51-55.
43. Muruganantham N, Basavaraj KH, Dhanabal SP, Praveen TK, Shamasundar NM, Rao KS. Screening of Caesalpiniabonduc leaves for antipsoriatic activity. J Ethnopharmacol. 2011 Jan 27;133(2):897-901. doi: 10.1016/j.jep.2010.09.026. PMID: 20920562.
44. Ansari JA, Ahmad S, Jameel M. Effect of Caesalpiniabonducellal.on ulcer and gastric secretions in pylorus ligated rat model. Journal of Drug Deliver &Therapeutics. 2012;2(5):102-4.
45. Gaur RL, Sahoo MK, Dixit S, Fatma N, Rastogi S, Kulshreshtha DK, Chatterjee RK, Murthy PK. Antifilarial activity of Caesalpinia bonducella against experimental filarial infections. Indian J Med Res. 2008 Jul;128(1):65-70. PMID: 18820361.
46. Kannur DM, Paranjpe MP, Sonavane LV, Dongre PP, Khandelwal KR. Evaluation of Caesalpiniabonduc seed coat extract for anti-inflammatory and analgesic activity. J Adv Pharm Technol Res. 2012 Jul;3(3):171-5. doi: 10.4103/2231-4040.101010. PMID: 23057003; PMCID: PMC3459446.
47. Kurmi P, Konwar M, Das S. In-vitro anticataract activity of ethanolic extract of seed kernel of Caesalpiniabonducella (L.) Fleming on goat lens. An International Journal of Pharmaceutical Sciences. 2015 Jan 1;6(1):244-53.
48. Rao NV, Shalam M, Shantakumar SM, Ali A, Gouda TS, Babu J. Anxiolytic Activity of Seed Extract of Caesalpinia bonducella (Roxb.) in Laboratory. Internet Journal of Pharmacology. 2008;5(2).
49. Ahmed RN. Effect of ethanolic seed extract of Caesalpinia bonducella pregnant female albino rats. Asian Pacific Journal of Reproduction. 2013 Jun 1;2(2):85-9.
50. Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA, Mia AA. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpiniabonducella (L.)Roxb leaves. BMC complementary and alternative medicine. 2013 Dec;13(1):1-7

51. Gbankoto A, Anago E, Houndjo PA, Adjahouinou DC, Gbaguidi F. Effect of aqueous and Ethanolic extracts of Caesalpiniabonduc root on sexual behaviour of male Wistar rats. Intl J of Multidiscipl and Current Res. 2015 Nov;3:1137-41.
52. Sarkar R, Hazra B, Mandal N. Hepatoprotective Potential of Caesalpinia crista against Iron-Overload-Induced Liver Toxicity in Mice. Evid Based Complement Alternat Med. 2012;2012:896341. doi: 10.1155/2012/896341. Epub 2012 Jul 17. PMID: 22919421; PMCID: PMC3418686.
53. Kanerkar UR, Bhogaonkar PY, Indurwade NH. Antispermatogenic effect of Caesalpinia bonduc (L.) Roxb. Seeds. Int Res J Sci Eng. 2015;3(4):173-8.
54. Sasidharan S, Srinivasakumar KP, Bhaumik A, Kanti S. Botanical and pharmacognostic evaluation of Caesalpinia bonduc seed, a prevalent Indian traditional drug having vivid therapeutic prospects. Journal of Pharmacognosy and Phytochemistry. 2021;10(6):297-304.
55. Vikhe S, Nirmal S. Evaluation Isolation and Characterization of Chemical constituents from C. bonducella L. seed. Pravara J Sci Technol. 2017;1.
56. Khandagale PD, Puri AV, Ansari YN, Patil RY. PHARMACOGNOSTIC, PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF CAESALPINIA BONDUCELLA [L.] Roxb. SEED.
57. Wadkar GH, Sayyad FJ. Pharmacognostic, physicochemical and phytochemical investigation of root bark of Caesalpinia bonducella. International Journal of Pharmacognosy and Phytochemical Research. 2017;9(1):26-30.
58. Sankara Rao, K., Raja K Swamy, Deepak Kumar, Arun Singh R. and K. Gopalakrishna Bhat (2019). Flora of Peninsular India.
59. Pournaghi N, Khalighi-Sigaroodi F, Safari E, Hajiaghaee R. A review of the genus Caesalpinia L.: emphasis on the cassane and norcassane compounds and cytotoxicity effects. J. Med. Plants. 2020 Dec 2;19:1-20.
60. Centre for Agriculture and Bioscience international., *Caesalpinia bonduc*https://www.cabi.org/isc/datasheet/10699 (Accessed December 21, **2020**)

61. Handa S.S, Kaul M.K. 1996. Supplement to Cultivation and Utilization of Medicinal Plants, RRL, JammuTawi,727-737

62. Sharma B.M, Singh P. 1972. Pharmacognostic study of seed *caesalpinia crista* Jour. Res. Ind. Med, 7(1): 8.
63. Kakade NR, Pingale SS, Chaskar MG. Phytochemical and pharmacological review of Caesalpiniabonducella. International Research Journal of Pharmacy. 2016;12:12-7.
64. Ravikanth K; Kanaujia A; Thakur D; Sharma A. Int J Adv Pharm Biol Chem., 2014; 3(3):698-702.

65. Bimbima. Ayurvedic herb Latakaranja- https://www.bimbima.com/ayurveda/ayurvedic-herb-latakaranja-caesalpinia-bonducella/348/ (Accessed January 3, **2021**)
66. Medicinal Plants. Caesalpiniabonduc (Latakaranjah) medicinal uses Adverse effects, Research & Pharmacology. https://www.indianmedicinalplants.info/herbs/index.php/c/1360-caesalpinia-bonduc-latakaranjah-medicinal-uses-adverse-effects-research-pharmacology (Accessed December 20, **2020**)
67. Ghose, S.C., Drugs of Hindoosthan, 8th edition, Hahnamann Pub. Co. Pvt, Ltd., Calcutta; p, 102-110, 1980
68. Esau, K. Anatomy of seed Plants. New York: John Wiley and sons; 1979. p. 550.
69. Sass JE. Elements of Botanical Micro technique. New York: McGraw Hill Book Co; 1940. pp.222.
70. World Health Organisation. Quality Control Methods for Medicinal Plant Materials, WHO Geneva, Switzerland. Materials. 1998; 128.
71. The *Ayurvedic Pharmacopoeia* of India. New Delhi: The controller of publications; 2001; 143.
72. *Indian Pharmacopoeia.* New Delhi. The controller of publications. 1996; 47-60

73. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.

74. Mahesh AR, Ranganath MK, Harish Kumar DR. Enrichment of flavonoids from the methanolic extract of Boerhaavia diffusa roots by partitioning technique. Research Journal of Chemical Sciences ISSN. 2013;2231:606X.

75. Pournaghi N, Khalighi-Sigaroodi F, Safari E, Hajiaghaee R. Bioassay-guided Isolation of Flavonoids from Caesalpinia bonduc (L.) Roxb. and Evaluation of Their Cytotoxicity. Iranian Journal of Pharmaceutical Research: IJPR. 2021;20(1):274.

76. Ammar Mohammed Ahmed Ali, MawahibElAmin Mohamed El-Nour, Sakina Mohamed Yagi. Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiberofficinale*Rosc.) rhizome, callus and callus treated with some elicitors. *Journal of Genetic Engineering and Biotechnology*, 16 (2018); 677–682.

77. Kasture AV, Mahadik KR, Wadokar SG, More HN. Chromatography, Pharmaceutical analysis. 15th ed. Pune: Nirali Publication. 2006; 51-67.

78. Gurdeep R Chatwal, Anand SK. Instrumental methods of chemical analysis. Mumbai: Himalaya publishing house; 2007; 2.272- 2.302.

79. Sodde, V. K., Lobo, R., Kumar, N., Maheshwari, R., &Shreedhara, C. S. (2015). Cytotoxic activity of Macrosolenparasiticus (L.) Danser on the growth of breast cancer cell line (MCF-7). *Pharmacognosy magazine*, 11(Suppl 1), S156.

80. EK Elumalai, M Ramachandran, T Thirumalai, P Vinothkumar. Antibacterial activity of various leaf extracts of MerremiaemarginataAsian Pac J of Trop Biomed. 2011; 1(5): 406-408.

81. De Magaldi, Silvia W., and Teresa Camero. "Suceptibilidad de Candida albicans" In vitro" mediante los posos de difusión." Bol. venez.infectol (1997): 5-8.

82. Clinical and Laboratory Standards Institute. "Reference method for broth dilution antifungal susceptibility testing of yeasts." Approved standard M27-A3 (2008).

INSTITUTE OF HERBAL SCIENCE
PLANT ANATOMY RESEARCH CENTRE

Prof.P Jayaraman, Ph.D
Director
Retd, Professor, Presidency College Chennai-5

AUTHENTICATION CERTIFICATE

Based upon the Organoleptic ✓ / Macroscopic ✓ / ~~Microscopic~~ Examination of Fresh ✓ / ~~Market~~ Sample, it is certified that the specimen given by C.R. Punith Kumar, M.Pharm., Dept. of Pharmaceutical Chemistry, C.L. Baid Metha College of Pharmacy. is identified as below :

Binomial : Caesalpinia crista L.

Family : Caesalpiniaceae

Synonym(s) : C. bonducella (L.) Fleming

Regional Names : Tamil :- Kalichikai

Reg. No of the Certificate : PARC/2020/4361

References : Nair, N.C & Henry, A.N. Flora of Tamil Nadu, India I : Pg : 128 .1983. ✓

Henry, A.N. *et al.* Ibid. — II : — .1987.

Ibid. — III : — .1989.

Ed : S.P.Ambasta,
The Useful Plants of India,
CSIR – Publication, 1986.

Date : 11/11/2020

(Prof. P.JAYARAMAN)

#4,2nd Street, Sakthi Nagar,
West Tambaram, Chennai-600 045
Ph:044-22263236,+919444385098
Email- herbalparc@yahoo.com

Prof. P. Jayaraman, M.Sc., Ph.D.,
Director
PLANT ANATOMY RESEARCH CENTER
(PARC)
West Tambaram, Chennai - 45.
Ph: 044-22263236, Cell: 9677237739
E-mail: herbalparc@yahoo.com

School of Pharmaceutical Sciences
Chettinad Academy
of Research and Education
(Deemed to be University)

Allied Health Sciences
Chettinad Academy
of Research and Education
(Deemed to be University)

This is to certify that C.R. PUNITH KUMAR, C.L. Baid Metha College of pharmacy has participated in the **One Day Technical Conclave on Pharma Quality Systems,** jointly organised by Chettinad School of Pharmaceutical Sciences (CSPS) & Faculty of Allied Health Sciences (FAHS), held on 11th February, 2020.

Prof. K. Lakshmi
Dean i/c - CSPS

Prof. Sripathi Kandula
Principal - FAHS

Prof. R. Murugesan
Director - Research, CARE

INTERNATIONAL PHARMA WEBINAR

MNR College of Pharmacy

MNR Higher Education and Research Academy
Sangareddy - 502 294, Gr. Hyderabad, Telangana.

Certificate

This is to certify that Dr./ Mr. / Ms. **C.R. Punith Kumar**
of **C.L.Baid Metha College Of Pharmacy, Chennai.**
actively participated in **"International Pharma Webinar on Molecular Docking with Autodock - An Easier Approach for Beginners "** organised by MNR College of Pharmacy on 10th June, 2020.

Shri. M. N. Raju
Chairman
MNR Educational Trust

Shri. Ravi Varma Manthena
Vice-Chairman
MNR Educational Trust

Dr. V. Alagarsamy
Convenor & Principal
MNR College of Pharmacy