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Natural Product of Nepal for Biomedical Application

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Preface

Madan Bhandari University of Science and Technology Development Board (MBUSTDB) is undertaking preparations for the establishment of a research-oriented world-class university. In this context, MBUSTB is engaging experts for identification of potential areas for research and teaching, which has the potential of directly contributing to economic development of the country.

This publication presents the outcome of a study related to the identification of research areas related to the utilization of natural products for biomedical application, the outcomes of which have the potential of directly contributing to country's economic development. This study is a part of wider studies aimed at exploring the potential of natural products for biomedical, technological and agricultural applications.

MBUSTDB highly appreciates the remarkable hard work and dedication of the author – Dr. Sagar Regmi – for preparing this publication. MBUSTB appreciates and thanks all other individuals and institutions who contributed to bringing out this publication.

Prof. Rajendra Dhoj Joshi
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Executive Summary

This report in biomedical engineering, could help in promotion of natural product of medicinal value. The work was done in close cooperation with a team consultant working in exploration and utilization of indigenous natural resources of Nepal for biomedical, technological, and agricultural application. The main content of the report is as following:

1. Review of literature on natural products of Nepal enriched with antioxidant and anticancer properties.
2. Identify the production of antioxidants supplements and its market scope and commercialization in Nepal and abroad.
3. Identify a novel model for drug screening from large database of natural products of Nepal.
4. Identify natural product available in Nepal to develop the relevant dyes for the biomedical application.
5. Assessment of prevalence and risk factors and pattern for cancer in Nepal.
6. Preliminary assessment of the use of Yarsagumba (cordyceps sinensis) for the perspectives of discovery of anti -cancer drugs.
7. Review of Himalayan herbs for other diseases such as cardiovascular, neurodegenerative, aging and diabetes.
8. Communication and networking with national and international R & D institutions of biomedical research.

9. Support design of master's and PhD level course in Madan Bhandari University of Science and Technology (MBUST) for research on interdisciplinary science. Formulation of topics for PhD research and master's degree research.
10. Estimation of cost for lab establishment in MBUST for research on natural product with biomedical application.

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Methodology

The methodology used in the report mainly consists of literature review to identify natural product of Nepal for various biomedical application. The google scholar was used to download the paper and End note was used to keep a systematic record and referencing. The information used in the report were collected from the review papers, research articles published in different journals. The certain ideas were extended from my PhD thesis of Nanyang Technological University Singapore. The experimental designs were developed from existing protocols as well as my own experience in the field. The figures in the report are taken from scientific journal which are properly cited. Furthermore, certain concepts developed in the report are from the discussion among the experts working in natural product extraction and their application in biomedical sectors.

List of abbreviations

AD	Alzheimer's Disease
ATP	Adenosine Triphosphate
AAR	Age-Adjusted Rate
BAX	BCL2 Associated X, Apoptosis Regulator
BCL-2	B-cell lymphoma 2
BMS	Bristol-Myers Squibb
CM	Confocal microscopy
CT	Computed Tomography
CVD	Cardiovascular Disease
DOX	Doxorubicin
DNA	Deoxyribonucleic Acid
DPPH	Diphenyl 1- Picrylhydrazyl
EMT	Epithelial Mesenchymal Transition
ERK	Extracellular Signal Regulated Protein Kinase
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas Chromatography Mass Spectrometry
GEM	Genetically Engineered Mouse

HPLC	High Performance Liquid Chromatography
HUVECs	Human Umbilical Vein Endothelial Cells
IL	Interleukin
LC-MS	Liquid Chromatography Mass Spectrometry
MBUST	Madan Bhandari University of Science and Technology
MBUSTB	Madan Bhandari University of Science and Technology Board
MDM2	Mouse Double Minute 2
MRI	Magnetic Resonance Imaging
MTT	3-4,5-Dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
NTFP	Non-Timber Forest Product
PD	Parkinson's Disease
PhD	Doctor of Philosophy
PI	Propidium Iodide
R and D	Research and Development
ROS	Reactive Oxygen Species
SEM	Scanning electron microscope
SRB	Sulforhodamine B

TGA	Thermo Gravimetric Analysis
TLC	Thin Layer Chromatographic Separation
TNF-Alpha	Tumor Necrosis Factor-Alpha
UV	Ultraviolet
WHO	World Health Organization
XRD	X-ray Powder Diffraction

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Chapter 1. Review of literature on natural products of Nepal enriched with antioxidant and anticancer properties.

1.1 Introduction

The small molecules produced in a natural level by any organisms including primary and secondary metabolites are called natural products [1]. In short, natural products can be anything that is produced by life or in other ways, natural products can be defined as any kind of organic compound which is synthesized by living organism. It is the field of study of natural products that gives rise to the development of synthetic organic chemistry where scientists modify the actual natural products to advance their properties [2]. It constitutes the small molecules like urea [3] to complex structure like Taxol [4]. It includes the biological derived material or biotic materials like wood, silk, bio-based materials like bioplastics, corn-starch, engineered wood, cellulose fibres, casein, or bodily fluids like milk, plant exudates or other types of natural materials which were once found in living organism like coal or soil. They possess a diversity in the structure and interesting biological activities [5]. The synthesis of natural products has interesting aspects of organic chemistry which plays an essential application in the discovery of drugs [6].

The research on the natural products is very important. We should care about natural products because of:

- Natural products have a diversity in the structure as well as unique pharmacological properties. The structural diversity of natural products is such that its capacity is more than that of compounds produce via synthetic organic chemistry in scientific laboratory [7].

- Both the *in vivo* and *in vitro* studies of natural products have confirmed for the antioxidants, antibacterial, antimicrobial, antifungal as well as anticancer properties [8].
- Natural products is widely used in the traditional Chinese medicine, Ayurveda as well as modern medicine for the treatment of diseases [9].
- Natural products are the source of the development of drugs particularly as an anti-cancer agents as well as anti-infectives [10].
- Almost one third of FDA-approved new molecular entities is from natural products [11].
- Majority of the natural products were from mammalian source, microbes, plants etc [12].
- The natural products research and development was highest in 1990s. The research in this area was reduced after that time [11].

Natural products have the potential to develop into anti-cancer drugs. In this review, we present the significance of natural products of Nepal for finding the therapeutics with focus on cancer. Out of 174 compounds for the approved for the treatment of cancer, 93 were natural products or derivatives of them [13]. Most of the anti-cancer drugs were discovered from the plants. The discovery of paclitaxel from the bark of Taxus brevifolia is the most success clinically approved anti-cancer drugs developed from the plants [13]. It is essential to figure out whether the natural products of Nepal have bioactive compounds of anti-cancer properties. It is necessary to use the latest technology to innovate and discover antioxidants supplements for the fight against cancer.

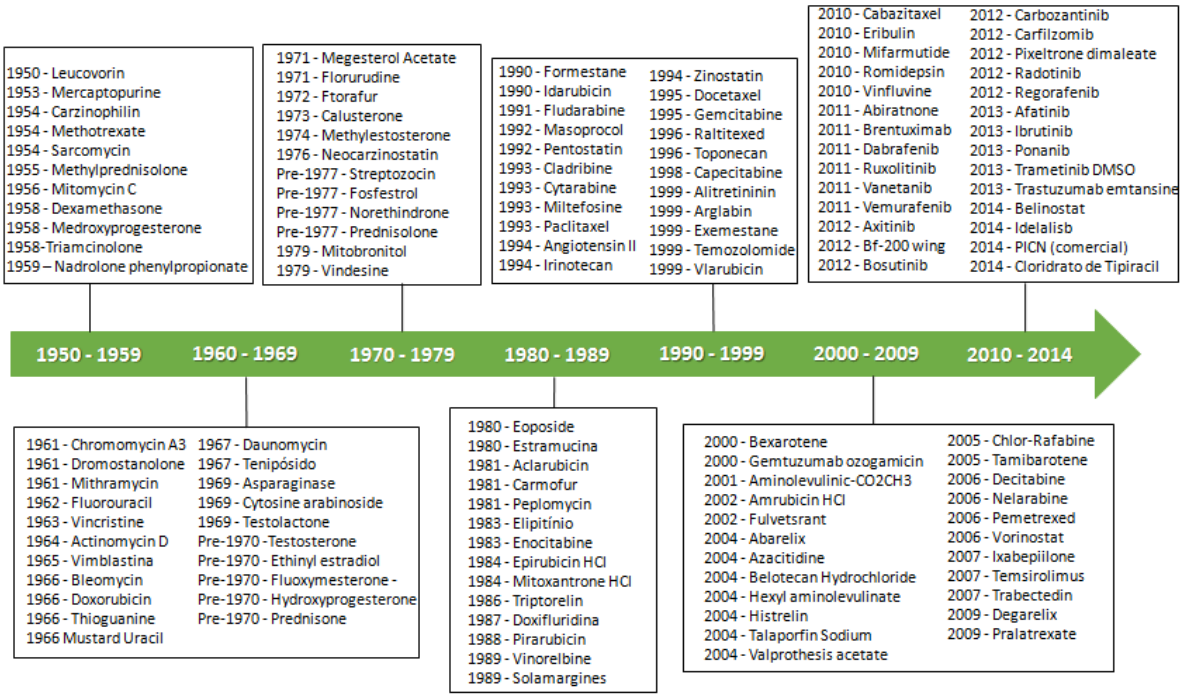

Figure 1.1. Anticancer drugs developed from natural products from 1950 to 2014. Reprinted from reference [13] with permission from Clinics in Oncology.

From the historic point of view, natural products of plants and animals are useful for the development of pharmaceutical drugs. However, recently natural products research is focused on finding biologically active molecules for target of anti-cancer and anti-microbial agents [14]. In between 1981-2014, almost 136 drugs were discovered against cancer with 17% were synthetic origin whereas 83% of these drugs were isolated from natural products [15]. The most successful discovery was anti-cancer drugs named paclitaxel against breast cancer, originated from bark of Taxus Brevifolia [16]. Regardless of such success of discovery of medicinal value drugs from natural products, many plant species are not investigated with a mission of drug discovery. Many plants are available in high-altitude range, mid-hilly region as well as in southern plane of Terai which needs further investigation. Nepal is rich in species of plant diversity around the globe [17]. Unfortunately, very little of these plant species for medicinal value were studied. There is a need

to explore the effect of biologically active compounds of these plant species on the various cancer cell lines [18]. In this review, we aim to investigate the significance of natural products of Nepal for discovery of anti-cancer drugs and antioxidants supplements.

Based on the information of world health organization, around 80% of people depends upon plant-based medicine. The compounds of natural plants and their derivatives has a success in finding the potential drugs [19].

Table-1.1 Medicine developed from natural products.

S. No.	Medications	Medicine
1.	Antibiotics	Penicillin, Tetracycline, Erythromycin
2.	Antiparasitic	Avermectin
3.	Antimalarials	Quinine, Artemisinin
4.	Lipid control agents	Lovastatin and Analogs
5.	Immuno suppressants for organ transplants	Cyclosporin, Rapamycin's
6.	Anticancer drugs	Paclitaxel, Doxorubicin

National Cancer Institute (NCI) recognized the importance to use natural products as anticancer agents in 1950s. Many investigations were done to find the natural products as anticancer agents using the advanced screening technologies. The most successful drugs discovered using the natural resources is paclitaxel (Taxol). In 1950, NCI has promoted an extensive research program to investigate anticancer properties in natural plant products. Almost after 13 years later, it was discovered antitumor activity in extracts of Taxus brevifolia from the pacific. In 1971, Monroo E.

Wall and Mansukh C. Wani identify the active principle (paclitaxel) of Taxus brevifolia. Then, in 1979 Susan B. Horwitz discover that paclitaxel blocks cell division by altering microtubular dynamics. Then NCI initiates phase I clinical trials in 1993 followed by phase II clinical trials in 1985. The major problem is that three mature trees of a century old can produce just 1 gm of anticancer agent, but 2 grams of drugs is required for a course of treatment. Therefore in 1990 Bristol-Myers Squibb (BMS) performs semi-synthesis of paclitaxel making the availability of product in 1993. The Taxol is one of the most popular drugs prescribed for 50,000 breast cancer treatment with the need of around 100-200 kg per year.

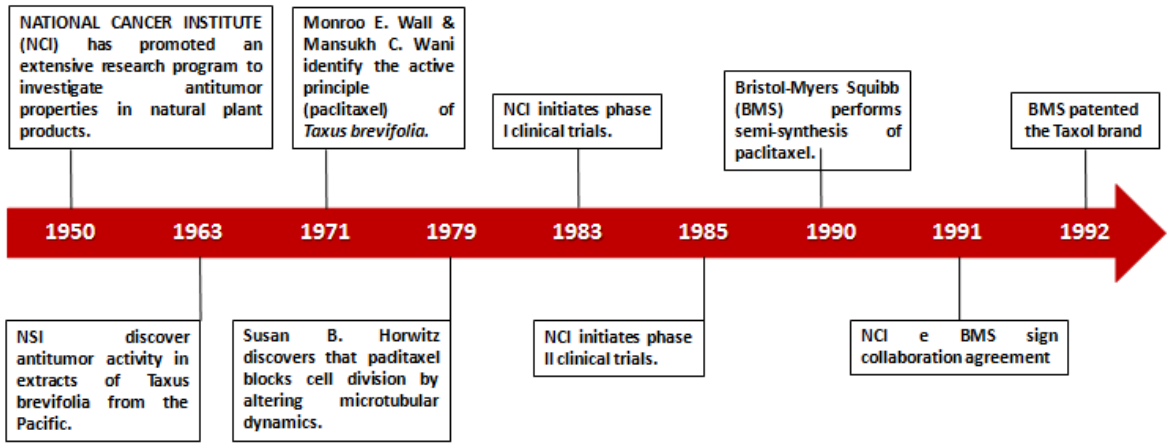

Figure 1.2. Trend of MAP and other non-timber forest product (NTFP) export from Nepal between 2005-2014

1.2 Mushroom of Nepal enriched with antioxidant and anticancer properties.

Mushroom, which is a popular cuisine around the world, has various novel constituent with miraculous biological properties. There are a great variety of mushroom which have been used as natural products, with the medicinal benefits as antioxidants [20], antidiabetic [21], hypocholesterolemic [22], antitumor [23], immunomodulatory [24], antiallergic [25], nephroprotective [26], antimicrobial agents [27] for the treatment of many diseases like cancer [28]. However, there is little information on the chemical structure of the extracts of these mushroom as well as their mechanism of action to understand the anti-cancer properties [29].

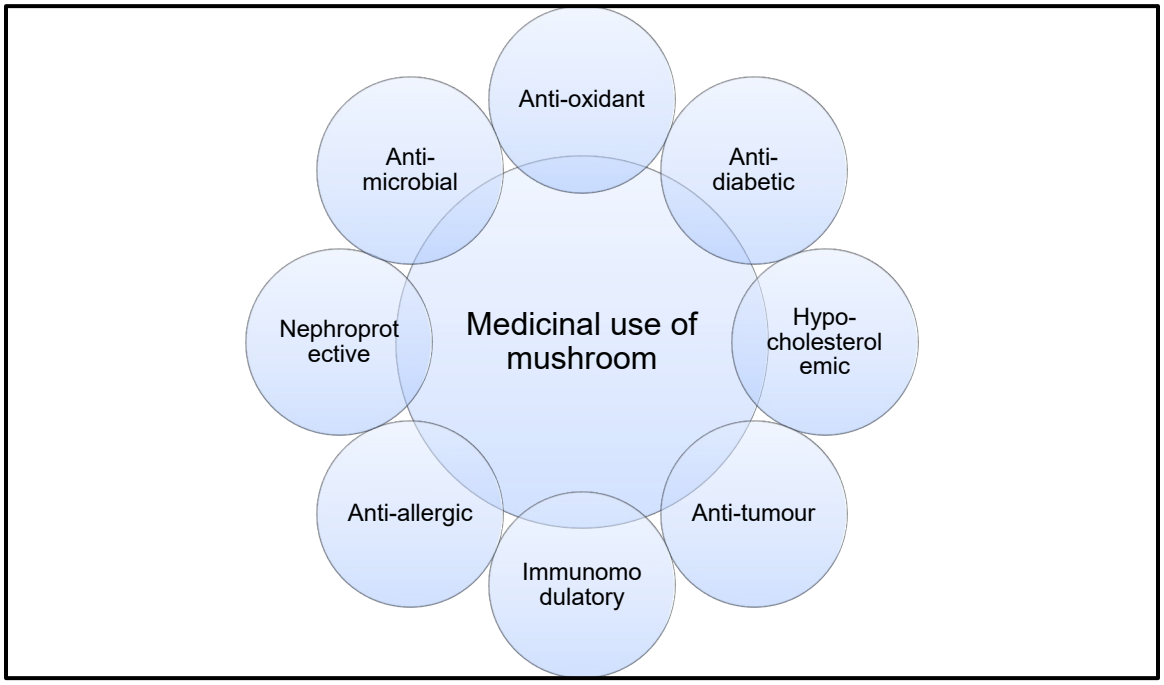

Figure 1.3. Medical use of mushroom

1.3 Objectives

Cancer metastasis is the biggest challenge in the cancer treatment, which mainly involves the spread of cancer cells from the primary sites to different region of the body [30]. Most of the research involves finding the treatment strategies for primary tumors. But it is the cancer metastasis which is responsible for almost 90% of cancer related deaths [31]. The process of cancer metastasis is complicated as it involves 1) departure of cells from primary tumors 2) entry of these cells in the blood vessels 3) circulation of these cells in bloodstream 4) extravasation of these cells from blood vessels to form secondary tumor in the other region of the body [32, 33]. This makes the cancer difficult to control treat which ultimately becomes a life-threatening.

Breast cancer is the very frequent cancer related with the woman [34]. There are around 2.1 million case of breast cancer each year around the globe and according to the report of World Health Organization (WHO), it is estimated that almost 6,27,000 women passed away due to breast cancer in 2018 that corresponds to around 15% of cancer deaths in women [35]. Around 90% of such breast cancer death is due to metastasis via lymphatic system which leads to the formation of bone and lung tumor. There is basically an epithelial- mesenchymal transition (EMT) associated with the metastasis where the tumor cells develops the potential for invasion as well as migrate around the blood vessels to form deadly secondary tumor with the alteration of genomic signaling [36].

Mushroom must undergo various stage of extraction, fractionation as well as purification of different active compounds. The different extracts and compounds need to be tested in different cancer cell lines *in vitro* study, *in vivo* modal as well as clinical trials. In addition to this, another approach of research is to understand the mechanism of actions of these active biomolecules of the mushroom. All these developments will lead to the formulation of natural drugs of cancer treatment [37].

There is a great development in medicinal mushroom research with the intention of finding anticancer drugs, but there is a lot more work to do to understand the synergistic role of various compounds of these medicinal mushrooms [38]. There are various studies with validation in clinical trials which shows that the extracts of compounds and their derivatives are effective in modern anticancer chemotherapy. However, the exact structure of these biomolecules of mushrooms, mechanism of action of these extracts as well as molecular targets needs to be further explored in detail.

The objectives of the research can be outlined as:

1.3.1 Primary objectives:

1. Developing a platform to engineer the desire breast cancer cells as well as other different kind of cancer cells.
2. Determine the compounds from mushrooms grown in Nepal with antioxidants as well as anti-cancer properties. (Based on literature review and past research work).
3. Screening of different compounds extracts from the mushrooms of Nepal with antioxidants as well as anti-cancer properties.
4. Exploring the cell signaling pathways in cancer by mushrooms and their bioactive molecules.
5. Comparison of nutritional value of edible mushroom of Nepal (In terms of content of amino acids, proteins, vitamins, dietary fibers, fats, minerals, antioxidants).

1.3.2 Broad objectives:

1. Establishment of a cancer research platform to identify the compounds extracts from mushroom with anti-cancer properties.

2. Promotion of mushrooms as new generation biotherapeutics.
3. Development of antioxidants supplements products from mushroom extracts.
4. Building a platform for commercialization of mushroom with the high antioxidant, vitamins, mineral etc. rich contents.
5. Enlargement of these mushroom research center in the areas of antidiabetic, anti-microbial as well as hypocholesterolemic based pharmaceutical's industries.

1.4 Literature review

Mushroom is a popular cuisine around the world which constitute various active biomolecules of medicinal importance [39]. It has been well studied that mushroom is rich in nutritional value and has the wonderful properties of antioxidants, anti-cancer, anti-inflammatory as well as antimicrobial. It is considered as a natural product for long time and used in treatment of various diseases [29]. The medicinal properties of mushroom are widely studied in India, Korea, China, Japan [29].

Cancer is the biggest challenge of modern medicine. It is the cancer metastasis that is responsible for almost 90% of cancer related death [40]. The effective treatment of cancer is still not available as well as have adverse side effects of these chemotherapeutic drug [41]. As we know that mushroom has some compounds with anti-cancer properties, there are some studies of clinical trials of developing these extracts as anti-cancer drugs [42]. It is also found that these extracts of medicinal mushroom lower the side effect of chemotherapy like anaemia, nausea etc. The polysaccharides found in mushroom has anti-cancer properties. In addition to this, certain scientific studies suggest that extracts of mushroom work effectively in synergetic way with anti-cancer drugs for the treatment of drug-resistant cancer. The bioactive compounds and active compounds responsible for anti-cancer properties of mushroom are presented in the table below:

Table-1.2. Bioactive compounds of mushroom.

Bioactive compounds of mushroom						
Polysaccharides	Proteins	Fats	Ash	Glycosides	Alkaloids	Folates
Volatile Oil	Tocopherols	Flavonoids	Phenolics	Carotenoids	Ascorbic acid	Organic acid

Table-1.3. Active compounds in mushroom responsible for anti-cancer properties

Active compounds in mushroom responsible for anti-cancer properties				
Lentinan	Krestin	Hispolon	Lectin	Calcaelin
Illudin S,	psilocybin	Hericium polysaccharide A & B	Ganoderic acid	Schizophyllan

Mushrooms *Phellinus linteus* has a phenolic compound named Hispolon which has a capacity to induce cell death of cancer of breast and bladder [43]. Hispolon induces the cell death of epidermoid and gastric cells. The downregulation of MDM2 is effective way of treating cancer as this proto-oncogene inhibits the tumour suppression activity of p53. Treatment with Hispolon increased the concentration of p21 and decreased the level of MDM2. The Hispolon plays a significant role in the regulation of cell signalling with the regulation of ERK1/2 activity [43].

There is a report of induction of apoptosis in human colon cancer cells (SW480) by the protein bound polysaccharide of Mushrooms Phellinus linteus via G2/M phase arrest [44]. The extracts of

this mushroom have the anti-angiogenesis effects which inhibits the growth of human umbilical vein endothelial cells (HUVECs) which was further validated *in vivo* modal. When the extract of this mushroom is given on a daily basis for 8 weeks to human hepatoma cell transplanted mice, there is an increase in the number of T cell as well as reduction of size of tumour [45]. These experimental results show that the mushroom can emerge as a new generation of biotherapeutics.

Figure 1.3: Mushrooms with anti-cancer potential. Reprinted from reference [29] with permission from Springer.

The extract of mushroom *Pleurotus ostreatus* inhibit the growth and induces apoptosis of colon cancer cells (HT-29). Moreover, the lectin extract of mushroom *Pleurotus citrinopileatus* has a potential anti-cancer activity as it reduces the growth of the tumour by 80% when the dose of 5 mg/kg is given daily for at least 20 days in mice with Sarcoma 180 [46]. Similarly, the polysaccharides extract from mushroom *Pleurotus tuberregium* inhibits the growth and induces apoptosis of leukemic cells via Bax/Bcl-2 mechanism [47]. The mushroom *Polyozellus multiplex* which is found widely in Korea increased the level of p53 tumour proteins and regulate the growth of stomach cancer [48]. The extract Polyozellin from this mushroom *Polyozellus multiplex* helps in differentiation in human myeloid leukemic cell lines [49].

The extracts of mushroom helped a lot in improving the treatment strategies of various types of cancer. Many companies are involved to identify the mushroom extract with anti-cancer behaviour and formulate them in a development of a product. The China based Zhejiang Fangge Pharmaceutical Co., Ltd. is involved in the research and development of mushroom extract and sells the product like mushroom powder, mushroom supplement, mushroom extracts like polysaccharides in different region of the globe. The Fin Eco Ltd is a research-based biotech company which involves in developing the products from the natural products by using the modern advance technologies. The Fin Eco Ltd focus on the medicinal mushroom to develop the anti-cancer drugs. Another US based company Mushroom Wisdom previously known as Maitake Products, Inc works is working since 1991 in East Rutherford, New Jersey for the development, production, and research of mushroom supplements. The Aloha medicinal Inc. is US based company which formulate cancer inhibiting Ganoderma capsules. Many more companies will use the mushroom to develop the therapeutic for cancer in near future. It is very important to

understand the mechanism of action of extracts of mushroom for the development of products related to cancer therapy.

Medicinal mushroom has a long history with the need of scientific research to understand them. They have various biologically active compounds of pharmaceutical importance whose mechanism of action is unknown. The understanding of these bioingredients of mushroom helps to makes something better for the benign as well as metastatic tumor. There is research to figure out the compounds of medicinal value along with the mechanism of action, however there is no clear understanding of signaling pathways for each biologically active extracts of mushroom. This research is very important as there is no effective anticancer agent for various types of cancer like acute myeloid leukemia, estrogen receptor negative human breast cancer, acute lymphocytic leukemia etc. The past research has hinted us for the possibility of discovery of anticancer drugs from mushroom. Therefore, a dedicated research team is required mainly to extract the compounds, purify it, identify the structure, and investigate the antioxidants and anticancer compounds from varieties of mushroom.

1.5 Research plan

The primary research objective is to screen the biological active compounds from mushroom with antioxidants and anticancer properties for the development of products related to cancer therapy. The research plan can thus be outlined as follows:

1.5.1 Engineer the breast cancer cells as well as other different kind of cancer cells

In the initial part of the project, various cancer cells like Human cervical carcinoma (HeLa), Human breast adenocarcinoma with high metastatic potential (MDA-MB-231) and low metastatic potential (MCF7), Human breast ductal carcinoma (BT-474), Human hepatocellular carcinoma

(HepG2), Human Lung carcinoma (A549), Human colorectal adenocarcinoma (HCT-15), Human chronic myeloid leukemia (K562), Mouse melanoma (B16-F10) and Human melanoma (MDA-MB-435) will be engineered for the research purpose.

1.5.2 Screening of compounds from mushroom extracts cultivated in Nepal with antioxidants and anticancer properties.

Different compounds from the extracts of mushroom varieties of Oyester, Bottom and Shiitake will be analyzed for the antioxidants and anticancer properties. The colorimetric assay will be used to determine the phenolic compounds in the methanolic extract of mushroom. The 1, 1-diphenyl; -2-picrylhydrazyl (DPPH) radical scavenging assay will be used to determine the activity of antioxidants. The effect of mushroom extracts on the morphology and response of mitochondria of cancer cells will be carried out using confocal microscopy. The apoptotic rate as well as necrotic status will be determined for a single compound as well as synergistic effect with other clinical approved anticancer drugs like Doxorubicin (DOX), Taxol, cisplatin, etoposide etc. These experiments will hint us to identify the extracts with antioxidants and anticancer agents from the mixture of compounds.

1.5.3 Exploring the cell signalling pathways in cancer by mushrooms and their bioactive molecules.

The ingredient of mushroom has a biological potential and usefulness in pharmaceutical industry. The recent progress in genomics, cancer genetics, translational research and proteomics helps to study the mechanism of cancer progression. The signaling pathway of Wnt, JAK-STAT regulates the growth of cancer. The regulation of Wnt, JAK-STAT pathways by ingredients of mushroom will be explored in detail connecting it with TGF/SMAD, Notch and TRAIL induced pathways.

The detail insights of multiple pathways by ingredients of mushroom will help to fill the gaps of knowledge and find the molecular target for effective therapeutics.

1.5.4 Building a platform for commercialization of mushroom with the high antioxidant, vitamins, mineral etc. rich contents.

Mushrooms are a powerhouse of nutrition. They are mostly low in calories and fat, free with cholesterol and contains fiber, mineral, vitamins, copper, potassium, zinc, folate etc. The comparison of nutritional value of edible mushroom of Nepal in terms of content of amino acids, proteins, vitamins, selenium, dietary fibers, fats, minerals, antioxidants will be carried out.

Chapter 2. Review on the production of antioxidants supplements and commercialization in Nepal and abroad

2.1 Research plan

The primary research objective is to find out the biologically active compounds with antioxidants properties for the development of products related to cancer therapy. The research plan can thus be outlined as follows:

2.1.1 Investigate the antioxidants compounds from plant extracts

We will investigate the absence or presence of active antioxidants using a qualitative phytochemical test. As we want to know the exact compounds present in the crude extract, we will gas chromatography - mass spectrometry (GC-MS). Some of the antioxidants can be detected by this method. Antioxidants are volatile in nature, and we will perform Liquid chromatography–mass spectrometry (LC–MS) to target specific antioxidant compound. The polyphenols or flavonoids, which are major constituents of antioxidants or vitamins can be detected using high performance liquid chromatography (HPLC) - mass spectrometry.

The thin layer chromatographic separation (TLC) is used to conform the antioxidant compounds. First, we apply the extract of plants on thin layer chromatographic on thin layer chromatographic plate as a rectangular spot followed by running it in solvent system upto 18-20 cm. Secondly, remove the plate followed by drying and spraying 0.2% solution of DPPH in methanol or ethanol. If there is a presence of antioxidant compounds, then plant extract become yellow against pinkish background on the thin layer chromatographic.

2.1.2 Research in various antioxidants supplements in collaboration with BP Koirala memorial cancer hospital

Based on the report obtained from medical oncology, Department at BP Koirala memorial cancer hospital in Chitwan, Doctors have recently stopped prescribing the supplements to cancer patients. Based on their report, these supplements to cancer patients neither current nor improve the treatment of the disease. It is necessary to investigate whether the normal food supply is enough for dietary for vitamins, minerals, and other fundamental requirements. There still need further research to know whether the antioxidants supplements should be given to cancer patients or not. This is a very controversial and debated topic.

2.1.3 To study the health benefits of antioxidants supplements

The supplements of antioxidants are sold in pharmacist regardless of warnings by Department of Drug Administration. Such kind of dietary supplements falls under food items, not drugs thus regulated by Department of food technology and quality control. It is important to regulate the quality of these food supplements and verify the health benefits as claimed by the company. Antioxidants like vitamin C and vitamin E reduces the side effect of some anti-cancer drugs. But the huge dose of vitamin B6 can cause damage to nerve if intake for long duration. Similarly, the vitamin E causes bleeding. Therefore, it is very important to understand the effects of antioxidants supplements on human health. On the other hand, it is important to study the effect of prescription of antioxidants supplements for cancer patients. There is a usual practice of prescribing the antioxidant supplements for cancer patients. There is a controversial in the research of antioxidants supplements. So, there is a need of more research in this area to identify whether these antioxidants supplements add additional complications in patient's health condition. There is a concept even in

patients to take such antioxidants supplements rather than taking natural foods, fruits, vegetables etc. Such intake of antioxidants even burdens the cancer patients economically.

2.1.4 Effects of antioxidants supplements in cancer cell lines

After identifying the antioxidant's supplements from plant extract, we will explore the effect of antioxidants supplements on various cancer cells like Human cervical carcinoma (HeLa), Human breast adenocarcinoma with high metastatic potential (MDA-MB-231) and low metastatic potential (MCF7), Human breast ductal carcinoma (BT-474), Human hepatocellular carcinoma (HepG2), Human Lung carcinoma (A549), Human colorectal adenocarcinoma (HCT-15), Human chronic myeloid leukemia (K562), Mouse melanoma (B16-F10) and Human melanoma (MDA-MB-435) together with the anticancer drugs.

2.1.5 Building a platform for commercialization of antioxidants supplements in Nepal and abroad

The composition of the nutritional value of each extract will be explored. The plant extracts with the high antioxidants, vitamins, minerals will be developed as a antioxidants supplement's products. Based on the scientific results, the market strategies of such product will be carried out in national and international level.

Chapter 3. Identify a novel model for drug screening from large database of natural products of Nepal.

3.1 Abstract

Cancer is a complicated diseases of unregulated cell growth killing millions of people worldwide. There are around 80% of compounds which has a good result in preclinical studies but could not get clear in Phase II clinical trials. This is due to the use of wrong model for drug screening from large database of compounds. The mostly used methods of screening are *in vitro* various human cancer cell line modal, *in vivo* mouse modal, circulating microfluidics modal, organ on chip model etc having their own certain advantages and disadvantages. The combined used of these preclinical screening modals 1) to discover anti-cancer drugs from a wide range of natural products 2) to understand the mechanism of action, will help to reduce the rate of failure of anticancer compound at the stage of clinical phase. In this review, we aim to provide the various modal used to screen the anti-cancer drugs from the large database of natural products with the merits and demerits.

Keywords

Microfluidics model, drug screening, natural product, cancer, cell line

3.2 Introduction

Cancer is a complex disease where the cells grows on its own way in a very unregulated manner having a potential to invade and migrate in different region of the body [50]. Cancer is a leading cause of death worldwide which occurs with the mutation of the gene. There is a report of around 9.6 million of cancer related death in 2018, worldwide [51, 52]. Out of 6 deaths around the globe, 1 death is due to cancer. Around 70% of death of cancer occurs in low- and middle-income countries. The number of cancer deaths is expected to be increased by 70% in next two decades i.e., it is predicted that 23.6 million new cases of cancer by 2030. The top three death causing

cancers are lung cancer (1.76 million deaths), colorectal (862 000 deaths) and stomach cancer (783 000 deaths) respectively [53]. In male, the lung cancer is the leading cause of death with 1,368,524 new cases diagnosed in 2018 [54]. In female, the breast cancer is the deadliest with 2,088,849 new cases diagnosed in 2018 [52].

Cancer begins when the normal cells lose its function and behaves in abnormal way. It can occur in the any region of human body. The cause of each cancer is different making the disease more complicated. In other words, the cause of cancer varies with the type of cancer as well as in every individual. Almost 33% of cancer occurs due to 1) high body mass index 2) low intake of fruits and green vegetables 3) lack of exercise 4) use of tobacco 5) use of alcohol. Tobacco use is associated for 22% cancer related death making it a significant risk factor. The higher death rate of 1) lung cancer is due to air pollution or smoking [55] 2) breast cancer is due to late diagnosis, lack of early detection, lack of health facilities, unhealthy lifestyle etc [56]. It has affected the people from all the countries of the globe. Cancer are mainly two types: 1) Benign tumour: Cancer that does not spread to different region of the body, 2) Malignant tumour: Cancer that spread to different region of the body [57]. Malignant tumour is responsible for almost 90% of cancer related deaths [58]. Malignant tumour is very dangerous and can be life-threatening [59]. In case of benign, only brain tumour can be very dangerous and can lead to a death. Sarcomas are malignant tumour of solid mesenchymal tissue whereas fibrosarcoma is cancer of fibrous tissue. Leukaemia's is cancer of mesenchymal cells of the blood [60].

As cancer is a disease of uncontrolled cell division the way to discover anticancer drugs is evaluated by 1) measuring the size of tumour 2) measuring the cytotoxic activity 3) measuring antiproliferative activity. Murine modal was developed to screen the new anti-cancer drugs. For example, Sarcoma 180, Carcinoma 755 and L1210 mouse leukaemia modal as well as P388 murine

leukaemia modal were used [61]. The clinically approved drugs like 5-flurouracil, 6-marcaptopurine, actinomycin D are discovered using such murine modal [61, 62]. In case of slow developing cancer like breast, lung, colorectal, the success is limited whereas in quickly growing cancer like lymphomas, childhood leukaemia, the modal is successful [63].

There are new targets developed for drug discovery with the development of proteomics and genomics. Such projects are carried out in different cancer cell line modal in screening potential anti-cancer drugs. The main objective of screening is to figure out compounds with anti-cancer properties which can proceed ahead in the drug development program. But such screening modal should be simple and cost effective.

The National Cancer Institute (NCI) is the largest centre to screen the compounds of anti-tumour properties since 1955 in collaboration with other organization like European Organization for Research and Treatment of Cancer and Cancer Research UK [64]. The NCI has modified the protocol for preclinical screening based on the experimental data of drug effects and growth of cancer cells [65].

3.3 Cancer Cell Line Modal

The cancer research is done mainly using human cancer cell line, the best modal for screening as they are immortal, easy handling as well as growth behaviour. There are collection of 60 different cancer cell lines from nine types of cancer namely leukaemia, colon cancer, brain cancer, non-small cell lung cancer, melanoma, ovarian cancer, prostate cancer, breast cancer and renal cancer for screening of anti-cancer compounds. Cancer cell line be grown in a 96 well plate. Then, the compounds from the natural products of interest will be added for the action for 12-hour, 24-hour, 48 hour and 72 hours to monitor the response [66].

The table shows the most cancer cell line used in the cancer research: -

Table 3. 1. Cancer cell lines for drug screening

S. No.	Cancer Cell Line	Origin
1	HeLa	Human cervical carcinoma
2	MDA-MB-231	Human breast adenocarcinoma
3	MCF-7	Human breast adenocarcinoma
4	BT-474	Human breast ductal carcinoma
5	HepG2	Human hepatocellular carcinoma
6	A549	Human Lung carcinoma
7	HCT-15	Human colorectal adenocarcinoma
8	K562	Human chronic myeloid leukaemia
9	B16-F10	Mouse melanoma
10	MDA-MB-435	Human melanoma

The cell growth can be determination by various assay such as 1) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay 2) propidium iodide (PI) assay, 3) luciferase assay and 4) sulforhodamine B (SRB) assay etc.

3.3.1 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay

MTT assay is the method developed by Mosmann in 1983. It can measure the level of reductase in mitochondria which reflects the level of cell metabolic activity. In this assay, the activity of NAD (P) H-dependent cellular oxidoreductase represents the total number of viable cells. This enzyme basically reduces the MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide into formazan (insoluble). After the treatment of cancer cells with compounds of interest in a 96-well plate,10 µl of sterilized MTT solution (5 mg/ml) need to be added to the cells. After 3 h of incubation, 100 µl of solubilization solution containing 10% SDS and 0.1% HCl should be added to each well. 12 h later, the optical density should be measured at 595 nm using a plate reader [67, 68].

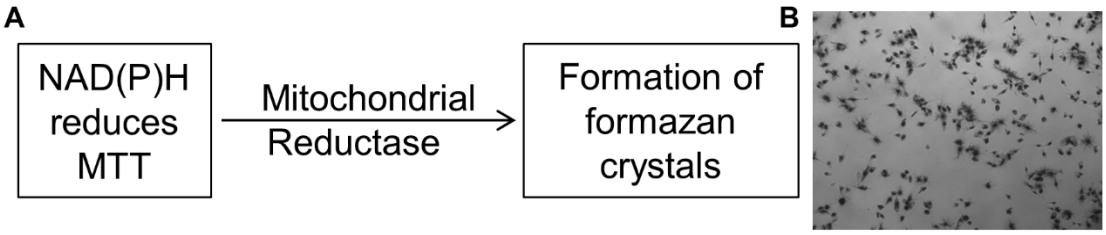

Figure 3.1 Mechanism of cell viability assay (B) Formation of formazan crystals

3.3.2 Propidium iodide (PI) assay

Propidium iodide (PI) can penetrate damaged cell membranes and stain the nucleus red; thus, PI staining has been used as the conventional assay for determining necrotic cell death [69]. PI is a cationic dye which can enter inside the nucleus of a dead cell and intercalate with the DNA. When these dyes bind with DNA, the fluorescent increases and can be detected. In this assay, after the treatment with various compound, the same volume of cell must be stained with 10 µM PI for 30 min in the dark. The images can be captured with an inverted fluorescence microscope at an

excitation wavelength of 590 nm and an emission wavelength of 630 nm. The assay is simple and quick which can be carried out without washing steps. But in this method, this PI can bind with double-stranded RNA in cytoplasm and give the wrong interpretation which can be solved using RNAase enzyme [70].

3.3.3 Luciferase assay

Adenosine triphosphate (ATP) is a principal donor of free energy and required by a cell in a living state to carry out the assigned function. However, the ATP reduces in the dead cells. So, if we can determine the amount of ATP, that is one of the ways to know the living status of a cell. After the cell is lysed, ATP reacts with luciferase to produce high quantum chemiluminescence. The intensity of emitted light is directly proportional to the concentration of ATP in cell. Luciferase assay is much better than MTT assay as it has good sensitivity and better reproducibility[71, 72].

3.3.4 Sulforhodamine B Assay

The Sulforhodamine B (SRB) assay is used to determine the density of cell based on the measurement of cellular protein content. It is popularly used to determine the toxicity screening of compounds using 96 well plate. The `10% wt./vol trichloroacetic acid was used to fix the monolayers of cell. It is stained for 30 min followed by washing by 1% vol/vol acetic acid. Then this protein is dissolved in 10 Mm Tris base solution. The OD is measured at 510 nm using a plate reader. This method is inexpensive and requires very simple equipment. This method can test many samples in a few days for screening [73, 74].

3.3.5 Screening modal by *In vivo* method

The cell lines are a cost, time effective and provide a quick result in screening the cytotoxic compounds. The *in vivo* is very important for screening the new anti-cancer drugs. The *in vitro* method is a factor in identifying the anti-cancer compound. The issues of cytotoxicity studies, physiochemical properties along with pharmacokinetics needs to evaluate for finding the anticancer drugs. The *in vitro* studies for screening are not suitable for developing anticancer drugs which needs further evaluation of compounds at clinical trials. The *in vivo* model is useful in finding the potential targets of anticancer drugs. The *in vivo* model is either of human cancer or mouse model and found to be effective in designing the anticancer drugs as it involves the various parameters like cytotoxicity studies, physiochemical properties, efficacy along with pharmacokinetics associated with the compounds [75, 76].

3.3.6 Mouse xenograft models vs Genetically engineered mouse (GEM) model

There are many animals developed to study the cancer related human. These models are used to explore the effect of intravasation, response of circulating tumour cells (CTCs), extravasation, malignant properties in response to compounds. The human tumour xenograft is commonly used modal to study the response of therapy [77]. The human cancer cells are taken and transplanted in the skin or in organ of tumour of origin in an immunosuppressed mouse which do not reject the cells of human. The tumour cells from human fit well in athymic nude mice as it is immunosuppressed. The tumour will develop in a period of 0.5-2 months depending upon the number of injected cells or size of the tumour implant, after which the response of various compounds can be examined *in vivo* model. Such transplant of human tumor in a mouse was first reported by Rygaard and Povelsen in 1969. Such kind of Human tumour xenografts in an immunosuppressed mouse can be done for all kind of tumours and it is a model for screening the

anticancer drugs *in vivo* system [78]. The efficacy of anticancer drugs in the xenograft model can be compared with the well-established parallel cell line which has more than 90% correct in predicting clinical activity of anticancer drugs.

Genetically engineered mouse (GEM) model is another animal model for studying the cancer associated with human [79]. The genetic profile of the mice is changed so that some of the genes that is associated with tumorigenesis is deleted, mutated, or overexpressed and the effect of such change of these genes is explored over time as well as the effects of compounds in response to tumour. This GEM models are used to study the cancer initiation process, intravasation, drug resistance [80].

3.4 Overview

There is no single method that can guide us to develop a compound to develop anticancer drugs in clinical trials. The invitro model are quick in screen but they are not useful in noncytotoxic compounds. The pharmacokinetics and pharmacodynamics of compounds is determined by Tumour xenograft model. The GEM model is very close with cancer associated with human and requires highly skilled expert and the method is expensive too. Thus, all the methods available need to be examined in preclinical stage to develop the natural products related compounds to drugs [66].

3.4.1 Proposed Microfluidics devices

The major problem of microfluidics device is the squeezing of the cells where the cells get trapped and killed through the mechanical force exerted by the rollers. Therefore, the best option to study the natural products on a target of circulating tumour cells is by ibidi Pump System [81] (Figure 2).

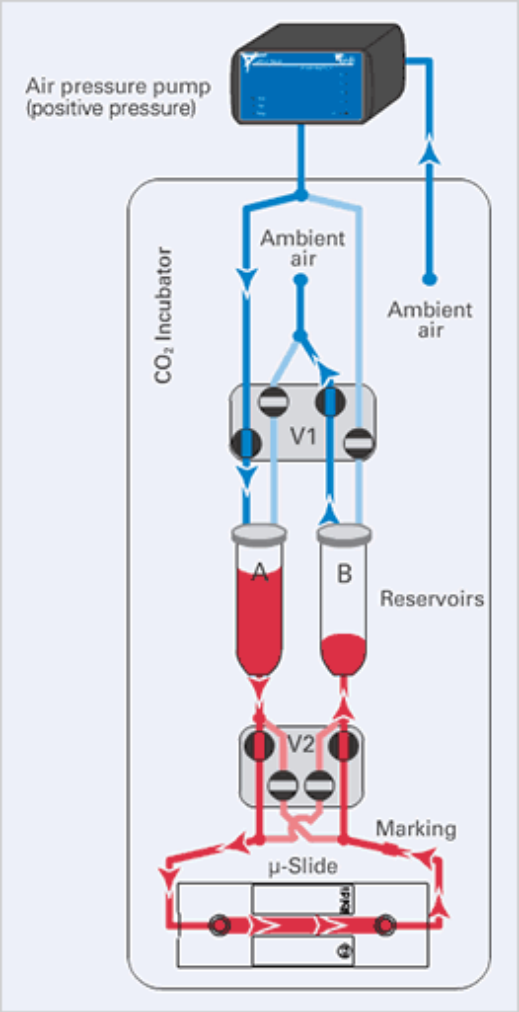

Figure 3.2. ibidi Pump System

The ibidi Pump System has more advantage than the typical microfluidics devices. This pump system can generate the shear stress that is on the scale of physiological relevant. The shear stress depends on the flow rate of the medium in the system and this pump system has a very precise control of the flow rate using the pump control software. The shear stress is well defined in this system for each ibidi channel slides. The flow in this system is unidirectional and the system can run for long duration even up to few weeks. This system can be set up to generate the oscillatory and pulsatile flow to generate turbulent as well as pulsatile blood flow to mimic the human circulatory system. The system is very easy to culture the cells, handle in the incubator and observe

it through microscope under sterile condition. The medium consume in this system is very limited under a flow condition. The major advantage of this system is the minimum destruction of the cells through the mechanical shear stress condition.

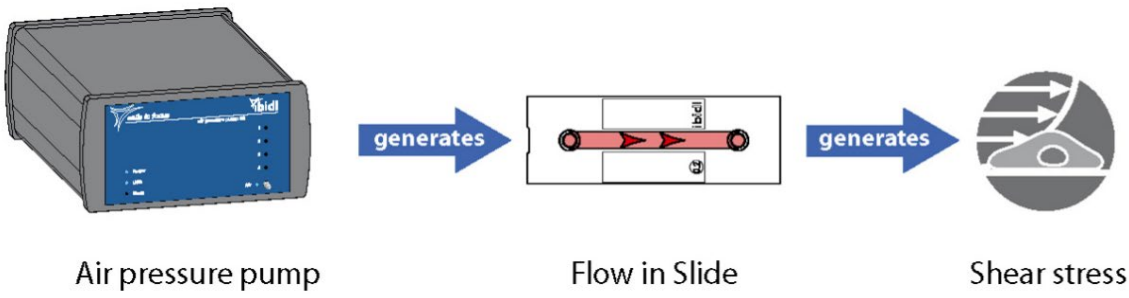

Figure 3.3 The principle of the ibidi Pump System

This pump system works together with fluidic unit which creates a unidirectional oscillatory flow of the medium in the channel where the shear stress can be calculated. The pump creates a constant pressure which make the medium to move from one chamber to another chamber in a uniform motion in a specific flow rate (ml/min). The flow rate is controlled by the input of the pressure. The shear stress is dependent on the 1) flow rate of the medium 2) Viscosity of the medium 3) Dimension of the flow in the system. This flow rate (ml/min) generate the shear stress on the wall in dynes/cm^2 where the cell will be exposed [82, 83].

Chapter 4. Identify natural product available in Nepal to develop the relevant dyes for the biomedical application.

4.1 Introduction

The need of natural dyes is increasing day by day around the globe due to huge application, low cost, and easy extraction from plants [84] . Dyes are colorants derived from plants or any other sources like invertebrates or minerals in which colouring matter is dissolved in liquid and absorbed by the material where it is applied [85]. Nepal has a wide variation of altitude from low land to highest peak of the world, making it rich in diversity of natural products [86]. Dyes are found in plants, animals, insects, minerals etc [87]. Natural dyes are widely used as it has no adverse effects and has a potential application in cosmetics, pharmaceutical, leather and food industry etc [88]. However, the dyes do not interact with the material and need the mordant to colour on the material of the interest. The mordant has the following advantages. 1) To prevent the colour from fading from light 2) To fix the fabric 3) To prevent the dye from washing out. The mordant that are commonly used are Alum, copper sulphate, potassium dichromate, tannic acid etc [89].

The natural dyes are environmentally friendly and harmless compared to the synthetic dyes [90]. The natural dyes are obtained from insects, animals, natural plants, minerals whereas the systematic dyes are made from various chemical reagents. The plant dyes are mainly extracted from the green methods and has a medicinal application in antifungal, antioxidants, antimicrobial as well as anticancer properties. We aim to explore the various plants that can yield many dyes as well the various techniques for the extraction of the dye. Moreover, we explore the application of natural dyes in medical, textile and food industry to replace the synthetic dyes.

In this review, we focus on the various dyes producing plants, methods of extraction and technologies associated with practices in Nepalese culture. It is a great research to explore the innovative ideas for the extraction of dyes and its testing for toxicity and application related to human health and environment. The review highlights the advancement of technology using natural dyes and replace the synthetic dyes in various sector of applications like fabric, drugs, textile, food, solar cells, and medical values.

4.2 Importance of natural dyes

The natural dyes have significant importance in the life of human being for many years. Indigo is the oldest and most popularly used dye [91]. The vegetables and fungi play a major role in the production of natural dyes along with the source of animals, plants, or minerals. The major advantages are: -

- Natural dyes are nontoxic, biodegradable, nonallergic and harmless to use by humans.
- Natural dyes colours are vibrant.
- Natural dyes are easy to extract from plants, animals, insects, flowers, fruits etc.
- Natural dyes have antifungal, antioxidants, antimicrobial as well as anticancer properties.
- Natural dyes do not have harmful reagents as well as carcinogens chemicals.
- The use of natural dyes will help in conserving the environment as well as reduce the human dependence on harmful products.
- Natural dyes can absorb the harmful UV light in the fabrics and prevent the damage of skin from harmful radiation of sunlight.

4.3 Types of Dyes Based on Origin

The dyes are divided into three categories based on the source from which they are extracted.

4.3.1 Plants

The plant is a rich in natural dye. Different part of the plants such as root, fruits, bark, stem, seeds etc can be used to extract the natural dyes. The same plants can be used to extract two or more than two different colours of the natural dyes [92].

4.3.2 Animals

The cochineal insect has a red whereas the cow urine has Indian yellow. Animals are also rich in the production of natural dyes. The Lac insects produce the red as well as violet dye. The Murex snail can be used to make purple dye whereas the Octopus has brown dye. Dyes from animals are extracted from the dried part of the body [93].

4.3.3 Minerals

Minerals are used to prepare the natural dyes like iron buff, manganese brown, nankin yellow, prussian blue etc.

4.4 Natural dyes available in Nepal

Plant-based coloring agents have been practiced since ancient times, such as Ayurveda, Uani, and Shidda, and have been part of human culture. History revealed that china had recorded dyes' use even before 2600 BC [94]. Natural dyes have been used in coloring clothes and other textiles since the beginning of the 18^{th} century. Afterward, the chemists began to synthesize synthetic dyes that were widely used because of their large amount of production and natural dyes. By the early 20^{th} century, only a small number of Natural dyes were being extracted. Since then, people have found

the harm synthetic dyes cause in the environment and living begins, which have increased the extraction of natural dyes. Lately, there has been increased interest in herbal dyes as consumers have been aware of synthetic dyes' ecological and environmental problems [95]. There are various herbal colorants which are currently attractive as they have 1) biodegradability, 2) no toxic effects, 3) green chemistry, and 4) potential application in the various industry and textile dyeing. The extracts from the nature shade of plant have usefulness in antimicrobial, UV protection as well as deodorizing. The intrinsic properties are then transferred to dyed substrate of the textile along with an herbal color. The dyeing fabrics along with the herbal colorats combine with dyeing of functionality finishes which is an eco-friendfly steps using less water and energy than other menthods of wet treatments. These days, there is a great interest in natural dyes in textile dying because of policy and regulations changes of many countries due to toxic effect of synthetic dyes. In this review, we present a table of commonly used dyes as well as methods available for dyeing textile materials to have other applications [96].

Table 4.1 List of commonly used dyes

Plant name	Parts used	Color	Medicinal uses	Reference
Acacia catechu (Catechu)	Wood	Reddish-brown	Sour Throat and cough	[97]
Azadirachta indica (Neem)	Bark	Brown	Skin disorder, antiseptic	[98]
Ocimum sanctum (Holy basil/ tulsi)	Leaves	Reddish Brown	Used as anti-cancer, hypoglycemic, anti-spasmodic, and as a hypotensive	[99]

Curcuma longa (Turmeric)	Rhizome	Yellow	Antioxidant, antiinflammatory, anticancer	[100]
*Aloe barbadensis (*Aloe Vera)	Whole plant	Red	Liver and spleen alignment, and eye infection	[101]
Ageratina Adenophora (Sticky snakeroot)	Leaves	Yellow	Regulating mensuration and activating blood	[102]
Allium cepa (Onion)	Fruits and Leaves	Yellow	Stimulate appetite, and digestion	[103]
Berberis aristana (Indian barberry)	Bark	Yellow	Antibacterial, anti-diarrheal, and anti-cancer	[104]
Bute utilis (Bhoj patra)	Tree gum	Brown	Antiseptic	[105]
Bute monosperma (Palas)	Flower	Yellow/Orange	Astringent, Anti-diarrheal	[106]
Calendula officinals (Marigold)	Flower	-	Antioxidant, skin infection	[107]
Camellia sinensis (Tea)	Leaves	Brown	Reduce inflammation, Anti-cancer	[108]

Capsicum annum. L, (Chilli)	Fruits	Red	Digestive, stimulant, anti-pyretic	[109]
Crocus sativusL. (Saffron)	Flower	Red, Yellow	Sores and rheumatic; Swelling	[110]
Embellia ribes (Embelia)	Fruits	Red	Antibacterial, lung diseases	[111, 112]
Gardenia jasminoides Ellis. (Cape jasmine)	Fruits	Yellow	Clearing away heat, purging fire, cooling blood	[113]
Gossypium herbaceum (Lavant cotton)	Flower	Yellow	Gastrointestinal issues, such as hemorrhages	(Sabane, 2018)
Hypericum cordifolium (Uraulo)	Leaves	Black	Used as anxiety, depression, insomnia, water retention, and gastritis.	-
Jatropa curcas L. (Physic nut)	Bark/Leaves	Blue	Used as scabies, ringworm, gonorrhea, dysentery, diarrhea	(Babel et al., 2013)
Juglans regia (Walnut)	Bark	Black/Brown	Antibacterial, Antiviral, Antidiabetics, Anticancer as well as cardiovascular benefits	[114]

Lawsonia inermis (Henna)	Leaves	Orange	Anti-diarrheal, anti- dysenteric, emmenagogue liver tonic, and anti- fungi	[115]
Mallotus philippiensis (Kamala tree)	Fruits	Red	Employed as an antioxidant and for cutaneous infection	(Kumar et al., 2016)
Michelia champakaL. (Champa)	Flower	Yellow	Used as a tonic for stomachache and carminative, used	[116]
Morus alba (White mulberry)	Bark/Leaves	-	Exhibit anti-bacterial activity against food poisoning microorganism	(Kumari et al., 2016)
Phyllanthus emblica (Amala)	Bark	Yellow/Green/Black	The bark of this plant rich in tannin so used for dysentery, diarrhea, liver disease, and cough	(Sakthivel, 2015)
Punica granatum (Pomegranate)	Bark	Pale Yellow	Antibacterial, Antiviral, Cardiac, Demulcent, Stomachic &Vermifuge	[117]
Solanum xanthocarpumL. (Tomato)	Fruits	Red	Antibacterial, anti-fungal, antimutagenic, Prostate cancer	[118]

Tagetes erecta L. (Marigold)	Flower	Yellow	They are used as antioxidants, analgesics, and anti-bacterial.	[119]
Zingiber officinale (Garden ginger)	Rhizome	Brown	They are used as cardioprotective, anti-inflammatory, antimicrobial, antioxidant, anti-cancer properties, immune stimulant, etc.	[120, 121]

Majorities of natural dyes are extracted from various parts of plants, like leaves, roots, flowers, fruit, and bark. The above table presents the other dyes yielding plants along with their medicinal values. There is a practice of using various traditional dye-generating plants for many medicinal purposes. For instance, extracts of plants like saaj, tulsi, turmeric, amala, khayer, neem, etc., are widely used in medical purpose. They can be used as an antioxidant, anti-inflammatory, anti-cancer, antiviral, anti-fungal, anti-bacterial, hyperglycemic, anti-diarrheal, anti-spasmodic, skin disorders, etc. [98, 122, 123]

Acacia catechu is widely found in sub-Himalayas. It is used to extract the brown dye. The major component available in the catechu is catechin which has the molecular formula of $C_{15}H_{14}O_6$. It has application in traditional medicines as well as it shows anti-inflammatory and anti-cancer activities.

Among the natural products, *Azadirachta indica* (Neem) is the most promising natural compound. Neem tree has the adaptability to a different range of climatic and geographic factors. It has many

properties such as antiviral, anti-fungal, anti-bacterial, and insecticidal biodegradable, least persistence, and least toxic to organisms, as well as economic and easy availability.

Ocimum Sanctum (Holy basil/tulsi) is often used for its medicinal properties and spiritual properties. There are mainly three different types of tulsi strains. Rama Tulsi has green leaves, whereas Krishna tulsi has purple leaves, and the third one which is wild is a vana tulsi. They are used in the treatment of common colds, stomach related problems, headaches etc. It is a different color of natural dyes with no toxic components.

Curcuma longa (Tumeric) Tumeric is a plant native to Nepal and other parts of Asia. Its rhizomes condiment is used as a colorant as well as an aromatic stimulant. The color component is isolated from the powder *Curcuma longa* which contain mainly alkaloids and had many flavonoids.

Camellia sinesis, also known as tea, is an evergreen plant. It contains many compounds like polyphenols (Catechins as well as flavonoids). Gallocatechin, Epicatechin is considered as a potential natural dye [108]. It is advantageous for natural industrial Dyeing which is helpful in saving energy and ecofriendly.

Capsicum annum. L. (chili) Red pigment is widely used as a food colorant and natural dye from this plant. The pigment is extracted from dry pepper's high-quality natural dye, which has anti-cancer and cosmetic properties. It is widely consumed as vegetables. They are a good source of red carotenoids, capsicum red pigments, and capsorubin [109].

Crocus sativus L. (Saffron) Saffron plants tiny, and its flower is the only part that is seen above the ground. Its smell is unique and sweet which is mostly used as a natural dye in Dyeing, the cosmetic industry, as well as cooking. Its microbial activity has been studied against different kinds of microbes like E. Coli [110].

Embelia ribes (black pepper) is a medicinal woody climber that belongs to the Myrsinaceae family. It is one of the 32 medicinal plants identified in Ayurveda. The fruits, leaves, and roots are used to cure various diseases, including constipation, indigestion, fungus infection, heart disease, and obesity. It shows properties like anti-bacterial and antifertility activities. The attractive color range of light brown to dark brown is acquired when dyed with false black pepper using different moderates.

Gardena jasminodes (cape jasmine) is an evergreen plant which is used commonly for landscape function. It is well known for its sweet, subtly fragrant flower. Widely found in Asia. Compounds like iridoids and triterpenoid ingredients have been found beneficial for bioactivities and could be potential for food additives and medicinally functional components. It is also used to extract yellow dye from its leave, flower, and fruits.

Globally, the need for natural dyes is increasing due to its low cost, various application and extraction done using the green methods from natural sources [124-126]. Dyes are corelated to the pigments, but they are different terminology. Dyes, which known by colorants where the coloring matter is dissolved in liquid and absorbed by the material where it is applied. Pigments consist of excellent particles of ground coloring matter which is suspended in a liquid and its forms a paint film that bonds to the surface where it is applied.

Nepal is rich in biodiversity with many natural products. Dyes are the widely extracted from plants, minerals, animals, insects, etc.[127, 128].

Natural dyes are widely used due to their nontoxic effect and wide application in pharmaceuticals companies, cosmetics, food industry, leather etc. [118]. Many of the dyes do not combine with

the materials of interest and they required a mordant to 1) stick to the fabric and 2) prevent the color from being degrade by washing as well as fade by the sunlight.

either washing out or fading with exposure to light. The widely used mordants in textile dyeing are Alum, potassium dichromate, tannin, tannic acid, ferrous sulfate, copper sulfate etc. [126].

On the other hand, synthetic dyes are harmful to environment and has adverse effect on health compared with natural dyes. The synthetic dyes is prepared from inorganic metal complex [106] whereas natural dyes are obtained from mineral, plant, and animal products which were the main sources of dyes as well as drugs which has application in medicinal values [129]. The synthetic dyes has a huge side effects as well as radiation hazards [130, 131]. For example, let us consider a 1) Turmeric which is a naturally occurring yellow dye that has application in skin revitalization as well as antiseptic 2) Indigo which gives a cooling sensation.

The popular methods known as green methods of extraction of plant dye is well studied and has a medicinal significance. It has antimicrobial, anti-fungal, antioxidant, as well as anti-cancer activities[132]. It has been reported that *Curcuma longa, Punica granatum L*, lawsone from *Lawsonia inermis L* possess anti-fungal, antimicrobial, anti-bacterial action [128].

4.5 Application

Natural dyes has uses in various sectors like textile, fabric dying, drugs, food dyeing as applications in solar cells [126, 130].

4.5.1 Textile/fabric dyeing

Dyes has wide applications in paper, fabric as well as leather factory. The synthetic dyes has pollution related to water due to discharge from textile dye manufacturing to non-biodegradable

colored effluents [133]. Natural dyes are more useful than synthetic dyes due to 1) non-allergic 2) non-toxic 3) friendly environment [131]. The mordants can be either inorganic or plant based which is widley used for fabric dyeing. The inorganic type mordant are chemicals like alum, potassium dichromate, ferrous sulfate, copper sulfate tannin, tannic acid, etc. From plant-based we have mango bark, pomegranate rinds, acorns, oak galls, *aloe vera*, etc. as mordant which has application in fabric dyeing.

Natural dyes is used in the protection of UV radiations in the various perspectives of textile industry [105]. There is a need of experimental design to optimize the pre-mordant dyeing for UV protection [134].

These days people are aware of sunburn and radiation allergy on skin by various chemical [135]. Natural dyes absorb the UV region and can prevent the harmful radiations[136]. The cloth dyed with natural dyes have UV prevention characteristics and they can absorb around 80% of the UV rays [135]. In the case of absence of mordants, the natural dyes have wide application in fabric dyeing.

4.5.2 Antimicrobial activity

The natural dyes have various properties like 1) antimicrobial 2) anti-fungal properties, however the detail information about these properties is largely unknown. The sample *Punica granatum* (Pomegranate), butterfly pea and marigold was examined for antimicrobial activity using four bacteria namely *E.coli, Klebsiella sp, S. aureus,* and *Bacillus sp* [137, 138]. The experimental result proved that *Punica granatum* (Pomegranate) has antimicrobial activities together with butterfly pea and marigold.

4.5.3 Therapeutic

Natural dyes has a many applications in medical field as they have anti-bacterial, antiviral as well as anti-fungal action[139]. There is various medicine available from the extract of natural dyes. In 1996, Escribano et al., studied the extract of saffron and its ingredients like crocin, safranal, and picrocrocin which was obtained from *Crocus sativus L.* and has an ability to inhibit the growth of cancer cells. The *Michelia champaka L.* has a dyes which is used to care the stomachache and carminative is used for the treatment of nausea, fever and diuretic in renal problem [140].

Furthermore, research shows the extracts from the plant has a wide application in circulation of blood [141], protection of ulcer [126], protection of liver [142], wound healing [143], etc. These results are enough to prove that natural dyes have a potential applications in the healthcare sectors.

Chapter 5. Assessment of Prevalence, Risk factors and Pattern for Cancer in Nepal

5.1 Abstract

Cancer is a non-communicable disease which exists widely around the globe. It has become a complex problem as the rate of incidence of cancer is increasing every day. Nepal is also facing this issue every day but being a developing country, this issue has become more critical. The incidence rate and mortality rate has increased in a huge amount over time. Lack of proper treatment and diagnosis equipment has been inducing factors for the increment. Different types of cancer are prevalent in Nepal which is explained in this article along with their potential risk factors and pattern of existence.

Keywords: Cancer, types of cancer, risk factors, mutation, smoking, alcohol consumption, awareness.

5.2 List of Abbreviation

AAR: Age-adjusted rate, TNF-alpha: Tumor necrosis factor-alpha, IL: Interleukin, MRI: Magnetic Resonance Imaging, CT: Computed Tomography

5.3 Introduction

In the globe, it is reported that 10 million deaths occurred in 2020 from cancer only. The death rate is more in developing countries like Nepal. According to World Health Organization, the mortality rate in Nepal due to different types of cancer is more in female than male. Cancer is the disease that results when cellular changes occur in an uncontrolled manner. In the human body, which is made of trillions of cells [144], it can start almost anywhere. Cancer can have any site of origin depending on which the names of cancers are given respectively. Generally, human cells grow and

divide to form new cells within the body to maintain the size and architecture appropriate for the body. Cells die as it grows old and new cells take their place. This orderly process breaks down as cancer arises [145]. The old or damaged cell which should have died, survives or the new unwanted cell formed are called additional cells. These additional cells can divide without stopping and may form growths called tumors. Tumors are of three types among which malignant tumors can grow and spread to other parts of the body. Over time, these malignant tumors grow more aggressive and become fatal as they disrupt the tissues and organs required for the entire organism to function.

Genes within the nucleus plays important role in the proliferation of the cancerous cell. Mostly, two classes of genes namely, proto-oncogenes and tumor suppressor genes play a vital role in triggering cancer. In normal condition, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation and stop cell death while tumor suppressor genes slow down cell division, repairs DNA mistakes, or help in apoptosis [146]. Proto-oncogenes, when mutated, can become carcinogenic oncogenes that can trigger excessive multiplication due to the high yield of encoded growth stimulatory protein. Similarly, tumor suppressor genes can be inactive when mutated. To develop cancerous tumors, mutations must occur in half a dozen or more of the cell growth controlling gene.

Mutation in the cell growth controlling genes increases the growth of the cell uncontrollably. This sudden change in the gene sequence occurs due to different actions like; smoking [147], alcohol consumption [148], air pollution [149], exposure to Ultra-violet (UV) radiations [150] and chemical, increase in the stress of tissues due to workloads etc. These actions when exists for a longer period becomes the potential risk factor for cancer.

5.4 Cancer in Nepal

Cancer is the uncontrolled growth of abnormal cells in the body. The growth of the abnormal cell does not look after the age, sex, and nationality. Cancer can occur in any age group, any sex and any caste or nationality. This implies for Nepal as well; it is found that approximately 8000-10000 new cases of cancer are identified each year in Nepal [151]. The most common cancer in Nepal is the lung cancer, which is followed by cervical cancer, breast, stomach, mouth, and colorectal cancer. The Kathmandu, capital city of Nepal, is the highest cancer affected district of Nepal, which is followed by Sunsari, Morang, Chitwan, Jhapa, Lalitpur, Rupandehi, Kaski, Nawalparasi and Bhaktapur [152]. In 2018, a total of 2156 new cases were reported out of 11,600 cases in Kathmandu valley, among which 999 were males and 1157 were females. While 670 deaths occurred due to cancer in 2018, among which 365 were male and 305 were female. The leading cause of death in male was mostly due to lung cancer, stomach cancer, urinary bladder cancer, gall bladder and non-Hodgkin's lymphoma. The rate of death and incidence in male was highest in the age group of 70-74. In the case of females, the incidence rate and death rate were more in case of breast, lungs, cervix, uterus, gallbladder, and ovarian cancer. The incidence and death rate were the highest in 75 years, followed by those aged 65 to 69. In the case of Rukum district, a total of 87 new cases were reported in 2018 among which 44 were males and 43 were females. The mortality rate was 38 among which 16 were males and 22 were females. Males aged 65 to 69 and females aged 55 to 59 had a higher incidence of cancer. Lungs are top leading cancer site in case of the male, which were followed by stomach, thyroid, prostate, gallbladder, and liver. Likewise, in females, the most common site is cervix/uterus followed by lung, ovary, and breast.

Similarly, in Siraha, Saptari, Dhanusha and Mahottari, a total of 1,106 new cancer cases were reported, including 155 men and 131 women. The higher cancer incidence was found among the

age group of 70-74 years in males and 65-69 years in females. The mouth was the top leading cancer site in males followed by lungs, gallbladder, liver, and stomach. Likewise, in females, the most common site was breast, followed by the cervix / uterus, gallbladder, lung, and stomach.

5.5 Types of cancer prevalent in Nepal

The most common type of cancer prevalent in females and males within Nepal along with potential risk factors are as follow:

5.5.1 Breast cancer

Breast cancer is the type of non-communicable disease in which the cells in the breast grow out of control. There are different types of breast cancer, depending on the site of origin. The most common are; ductal and lobular breast cancer [153]. Ductal breast cancer starts from the duct of the breast, which is further divided into in situ and invasive type. Lobular breast cancer begins from the milk-producing glands, it is also further divided into in-situ and invasive type. In-situ ductal carcinoma is the type of breast cancer, which has malignant cells contained within breast ducts, while invasive ductal carcinoma is the type of ductal breast cancer which has been spread to nearby tissue of the breast. On the other hand, in situ lobular carcinoma is the type of lobular breast cancer, which has malignant cell contained within the breast lobule while invasive lobular carcinoma has cells infiltrated into the surrounding breast tissue. In-situ ductal carcinoma is diagnosed through mammography while the invasive type of ductal carcinoma is diagnosed through mammography and is also palpable when large. The in situ lobular carcinoma is diagnosed through biopsy whereas the invasive lobular carcinoma is diagnosed microscopically.

Breast cancer is the most common type of cancer in the world, whereas in Nepal it is the second most common type of cancer among women after cervical and uterus cancer. Kathmandu valley

has the highest number of breast cancer patients in Nepal. In the three districts of Kathmandu valley (Kathmandu, Lalitpur and Bhaktapur) Kathmandu district had a total of 1351 new cancer cases out of which 721 were females with the age-adjusted rate (AAR) 92.6 per 100, 000 population. The mortality rate was 183 for females with AAR 24.9 per 100,000. The leading site for cancer in females was breast in Kathmandu district with AAR 21.5 per 100,000. Similarly, in Lalitpur and Bhaktapur district the new cases were 262 with AAR 109.2 and 174 with AAR 107.8 per 100,000 respectively in the female. While the mortality rate was 82 with AAR 34.7 per 100,000 and 40 with AAR 34.7 per 100,000 for Lalitpur and Bhaktapur district, respectively. With the total case of 262 in Lalitpur, the major site for cancer in females was breast with AAR 21.1 per 100,000 population. Similarly, in Bhaktapur with total new cases of 174, the major site for cancer was breast with AAR 24.9 per 100,000 population.

In Kathmandu valley, it is reported that in 2018, the total number of breast cancer cases was 259 which is 22.9% of total cancer cases in the Kathmandu valley in case of females. Out of 259 cases it was reported that 2 were from the age group of 20-24 while 6, 12, 28,34,37,38,33,27,19,11 and 12 from 25-29,30-34, 35-39,40-44,45-49,50-54,55-59,60-64,65-69,70-74 and above 75 years age group, respectively. The mortality case due to breast cancer was 35 in Kathmandu valley which is 11.5% of the total death rate in females due to cancer. Out of 35 cases, 2 were from the age group of 30-34 while 4,5,5,3,5,3,4,1,3 was from 35-39,40-44,45-49,50-54,55-59,60-64,65-69,70-74 and above 75 years age group respectively. Among 22.9% of total breast cancer cases, 4.6%were diagnosed with a non- microscopical investigation like radiography, clinical investigation, etc. While 93.8% were investigated by microscopy and 0.8 % were identified through death certificate record.

While in Rukum district, it is reported that among the new 110 cases of cancer, 43 were females with AAR 51.4 per 100,000 population and with total mortality case of 38, 22 were females. The most affected site was cervix followed by lungs, uterus, ovary, and breast. Among 43 cases of cancer, only 2 of them had breast cancer, which is 4.7% of total case of cancer in females of Rukum. Among 2 of them, 1 was from the age group of 55-54 years while the other was 65-69 years. The mortality rate is also 2 which is 9.1% of the total mortality rate of women in Rukum due to cancer. For the diagnosis, it is reported that microscopic and verbal investigation method was used.

In case of the Terai region of Nepal, district Dhanusha, Siraha, Mahottari and Saptari had total new cancer cases of 1106 out of which 542 were females. The total mortality rate of females due to cancer was 131 among 542 cases, 112 females had breast cancer, which is 20.9% of total cases of cancer in females of Dhanusha, Mahottari, Saptari and Siraha district. Siraha had the highest number of breast cancer patient with AAR 10.9 per 100,000 population, followed by Dhanusha with AAR 10.2 per 100,000 population, while Mahottari and Saptari had AAR 9 and 5.6 per 100,000 population.

Out of 112 cases in four districts 2 were from age group of 20-24 age group while 2,3,16,18,16,15,18,8,7,5 and 2 were from 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality rate due to breast cancer was 18 among which 4 were from age group of 60-64 while 1, 2, 3, 2, 2, 2 and 2 were from 30-34, 40-44, 45-49, 50-54, 55-59, 65-69- and 70-74-years age group, respectively. Among the diagnosed 20.7% breast cancer patient, 8.09% were diagnosed non- microscopically, 63.4% were diagnosed microscopically while others were either identified through death certificate records or through verbal information.

Males also get affected by breast cancer, but the incidence rate and mortality rate are very low as compared to females.

Risk factors

From the above data, it is seen that breast cancer is largely prevalent in women of Nepal. From Kathmandu to rural areas like Rukum and in Terai regions like Dhanusha, Siraha, Saptari and Mahottari, breast cancer has almost affected women of Nepal. The rate of incidence of breast cancer has been increasing at a huge rate. This might be due to various reasons like the lifestyle of women in different places within the country, environment, age of women, age of Menarche and Menopause, etc. the lifestyle or habit of women includes the workload, the diet, smoking and drinking habits. From the given data it is seen that women in Kathmandu valley are largely affected by breast cancer than the other reason, this might be due to the high amount of smoking and alcohol consumption rate in these areas as it is reported that smoking increases risk of breast cancer [154].

Hereditary factor is also one of the key risk causes of breast cancer. In several families, the dominant inclination to early onset breast cancer is reported to be the result of germ line mutation in a chromosome 17q gene known as BRCA1[155].The another breast cancer susceptibility gene for occurrence of hereditary breast cancer is BRCA2 which perform a vital role in repair of homologous Deoxyribonucleic acid (DNA) [156]. Hereditary mutation in BRCA1 and BRCA2 increases chromosomal instability and likely causes cancer by stimulating malignant transformation [157]. BRCA1 is estimated to confer a breast cancer risk of 54% by age of 65 years.

In a survey done in 2012 in Bhaktapur cancer hospital, within Kathmandu valley, it was seen that 48.2% and 55.4% of breast cancer patient were engaged in smoking and alcohol consumption.

Also from the same survey, it is seen that 18.1% had menarche before the age of 11 years while 53% had menopause at the age between 50 to 55years.

Age is also one of the potential risk factors for breast cancer. More the age of women more there is the chance of getting infected with breast cancer in women [158]. The risk of breast cancer increases exponentially until menopause, but the risk rate becomes steady after menopause [159]. Mostly the women of the age group of 40-65 years are at high risk of getting infected with this type of cancer. These age groups are at the age of getting the menopause which affects their health. The immune system is quite low during this age and women are prone to various diseases. In rural areas of Nepal, there is less hospital facility which plays a major role in the increase of mortality rate. Women from such areas do not have proper access to medicines, radiotherapy, or chemotherapies, also they are not benefitted from early diagnosis facilities.

5.5.2 Cervical and Uterine cancer

Cervical cancer is a type of cancer that occurs in the cells of the cervix which is the lower part of the uterus that connects the vagina [160]. Endometrial cancer or uterine cancer develops in the lining of the uterus called the endometrium.

In Kathmandu valley, the total number of the cervix and uterine cancer cases was 99 which is 8.7% of total cancer cases in Kathmandu valley in case of females. Out of 99 cases it was reported that 1 was from the age group of 20-24 while 2, 3, 5,10, 10, 16, 10, 15, 9, 7 and 11 from 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality case due to cervical and uterine cancer was 10 in Kathmandu valley which is 3.3% of total death rate in females due to cancer. Out of 10 cases, 1 was from the age group of 35-39 while 1, 3, 2, 1, 2 were from 45-49, 50-54, 60-64, 65-69 and above 75 years age

group, respectively. Among 8.56% of total cervix and uterine cancer cases, 2% were diagnosed with a non- microscopical investigation like radiography, clinical investigation, etc. while 94.9% were investigated by microscopy. In Kathmandu district, cervix and uterine cancer was the second most incidence site of cancer with AAR 8.9 per 100,000 population. In the case of Lalitpur and Bhaktapur district, these cancer does not take the second most place but still has a high number of incidences with AAR 10.9 and 4.5 per 100,000 population, respectively.

In Rukum district, the most prevalent cancer in the female is cervical and uterine cancer. Among the total new 43 cases of cancer in females, 11 of them had cervical and uterine cancer which is 25.6%. The mortality case due to this type of cancer was 2 which is 9.1% of total mortality case in female due to cancer. Among 11 of them, 2 were from the age group of 40-44 years while 3, 1, 3 and 2 were from 45-49, 50-54, 55-59 and 60-64 years. For the diagnosis, it is reported that microscopic and verbal investigation method was used.

In case of Terai region of Nepal [161], district Dhanusha, Siraha, Mahottari, Saptari had total new cancer cases of 1106 out of which 542 were females. The total mortality rate of females due to cancer was 131. Among 542 cases, 105 females had cervical and uterine cancer which is 19.6% of total cases of cancer in females of Dhanusha, Mahottari, Saptari and Siraha district. Mahottari had the highest number of the cervical and uterine cancer patient with AAR 12.4 per 100,000 population followed by Siraha with AAR 10.2 per 100,000 population while Dhanusha and Saptari had AAR 6.4 and 6.3 per 100,000 population. Out of 105 cases in four districts 1 was from age group of 20-24 age group while 2, 11, 12, 18, 9, 17, 14, 11, 5 and 5 were from 30-34, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality rate due to cervical and uterine cancer was 18 among which 4 were from age group of 60-64 while 1, 1, 2, 2, 1, 2, 2, 2 and 1 were from 25-29, 30-34, 40-44, 45-49, 50-54, 55-59, 65-69- and 70-74-

years age group, respectively. Among the diagnosed 19.6% cervical and uterine cancer patient, 4.8% were diagnosed non- microscopically, 72.4% were diagnosed microscopically while others were either identified through death certificate records or verbal information.

Risk factors

Cervical and uterine cancer is prevalent throughout Nepal. The rate of this type of cancer is found to be more prevalent in rural areas of the hilly region. This might be due to the workload of women in hilly areas. Also, due to lack of medical facilities, women are at the risk of losing their life either due to the absence of early detection facility [162] or due to lack of chemo and radiotherapies. Women of rural areas also have a lack of awareness about cancer, they are unaware of the symptoms, risk factors of cancer [163]. Age is also one of the risk factors for this type of cancer. Women with age group between 45 to 75 years are at higher risk of cervical and uterine cancer so women of this age group are supposed to get a regular health check-up [164]. Women who have given birth to one or more children or have become pregnant at early age are also at high risk of getting infected by cervical and uterine cancer [165]. Also, smoking and alcohol consumption increases the risk of getting infected.

5.5.3 Lung's cancer

Lung's cancer is the type of cancer that begins from the lungs and is one of the fatal diseases. It causes severe deaths due to damage in the lungs. Primarily, lung cancer starts at the lungs, but it can spread to other organs within the body fast. There are mainly two forms of primary lung cancer which are classified based on the type of cell in which cancer starts to grow [34]. They are:

- Non-small cell lung cancer- it is the most common form and can be any one of three types: squamous cell carcinoma, adenocarcinoma, or large cell carcinoma.

- Small-cell lung cancer- it is the least common form that usually spreads faster than non-small cell lung cancer.

Lung cancer is the most common type of cancer in the world [35]. In Nepal, the maximum number of the lung cancer patient are males. In the case of females, it is placed in the third position after breast cancer and cervical and uterine cancer [1].

In the three districts of Kathmandu valley, Kathmandu district had a total of 1351 new cancer cases out of which 630 were males with the age-adjusted rate (AAR) 90.4 per 100,000 population. The mortality rate was 210 for males with AAR 31.6 per 100,000. The leading site for cancer in males was lungs in Kathmandu district with AAR 15.3 per 100,000. Similarly, in Lalitpur and Bhaktapur district the new cases were 222 with AAR 101.3 and 147 with AAR 112.5 per 100,000 respectively in the female. While the mortality rate was 91 with AAR 43.3 per 100,000 and 64 with AAR 49.4 per 100,000 for Lalitpur and Bhaktapur district respectively.

With the total case of 222 in Lalitpur, the major site for cancer in males was lungs with AAR 23.7 per 100,000 population. Similarly, in Bhaktapur with total new cases of 147, the major site for cancer was breast with AAR 23.4 per 100,000 population.

In Kathmandu valley, the total number of lungs cancer cases was 177 which is 18.1% of total cancer cases in Kathmandu valley in case of males. Out of 177 cases it was reported that 1 was from the age group of 5-9 while 1, 2, 1, 2, 4, 6, 16, 19, 22, 29, 39 and 35 from 15-19, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality case due to lungs cancer was 89 in the Kathmandu valley which is 24.7% of total death rate in males due to cancer. Out of 89 cases 2 were from the age group of 40-44 while 8, 9, 11, 19, 20, 20 were from 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age

group, respectively. Among 17.7% of total breast cancer cases, 10.72%were diagnosed with a non-microscopical investigation like radiography, clinical investigation, etc. while 87% were investigated by microscopy and 2.3 % were identified through death certificate record.

In Rukum district, the most prevalent cancer in male was lungs cancer. Among the total new 44 cases of cancer in females, 10 of them had lungs cancer which is 22.7%. The mortality case due to this type of cancer was 3 which is 19% of total mortality case in male due to cancer. Among 10 of them,4 was from the age group of 60-64 years while 3, 2 and 1 were from 65-69, 70-44, 5 and above 75 years. For the diagnosis, it is reported that microscopic and verbal investigation method was used.

In case of Terai region of Nepal [29], district Dhanusha, Siraha, Mahottari, Saptari had total new cancer cases of 1106 out of which 564 were males. The total mortality rate of males due to cancer was 155.among 564 cases, 46 males had lungs cancer which is 8.3% of total cases of cancer in males of Dhanusha, Mahottari, Saptari and Siraha district. Siraha had the highest number of the lung's cancer patient with AAR 8 per 100,000 population followed by Mahottari with AAR 3.7 per 100,000 population while Saptari and Dhanusha had AAR 3.4 and 1.1 per 100,000 population. Out of 46 cases in four districts 1 was from age group of 20-24 age group while 2, 1, 1, 2, 1, 7, 7, 11, 6 and 7 were from 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality rate due to breast cancer was 19 among which 1 was from age group of 20-24 while 1, 1, 1, 3, 3, 3, 3 and 3 were from 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. Among the diagnosed 8.2% of the lung's cancer patient, 8.7% were diagnosed non- microscopically, 69.6% were diagnosed microscopically while others were either identified through verbal information.

In the case of females, Lalitpur district has more female lungs, cancer patient, among the three districts of Kathmandu valley with AAR17.9 per 100,000 population. While Kathmandu has AAR 8.5 per 100,000 population and Bhaktapur has AAR 8.2 per 100,000 population. The number of a female lung's cancer patient was 116 which is 10.2% of the total cancer female patient in the Kathmandu valley. In the case of Rukum district, cervix cancer was most incidence site of cancer followed by lungs cancer. The total cases in Rukum were 4 while mortality case is 3 for females. In Siraha, Saptari, Dhanusha and Mahottari district the total number of female lung cancer patients is 41while mortality cases is 17. In Kathmandu valley, it is seen that the incidence of lung cancer in the female has started from the age of 30 years old while the peak value of incidence is at the age of 75 years. In Rukum the incidence of lung cancer has started from 50 years and in the Siraha, Saptari, Dhanusha and Mahottari districts the incidence has been started from 40 years.

Risk factors

The major risk factor for lung cancer is smoking and air pollution. Smoking is a big issue among males throughout Nepal [165]. The person who smokes has the most risk of getting infected by lung cancer but the person sitting next to the one who smokes also becomes at the risk zone of getting infected by lung cancer [166]. In a survey done among the ethnic groups of Nepal about their smoking habits, it is reported [167], that people who smoked one type of product had highest lung cancer risk followed by bidi smokers, unfiltered cigarettes and filtered cigarettes. It is also reported [168] that the increase in the frequency of smoking increases the risk of lung cancer among all ethnic groups. Also, the air quality of Nepal is deteriorating day by day which causes airborne diseases and small children's health gets degraded. Even at the small age group of 5-9 years, children are suffering from lungs cancer. Household air pollution which results from the use of solid fuels or biomass fuels like wood, charcoal, dung, etc. also increases the risk of lungs cancer

especially in the case of women and children [169]. Another major risk factor for lungs cancer is tobacco. It is reported that, Tobacco exposure produces a heavy burden of genomic mutations in lung cancer, including mutation of the tumor suppressor TP53. TP53 alterations is found to be present in squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of the lung [170, 171]. Similarly, is reported that individuals with low education are more in the risk of getting infected by lungs cancer [172]. People from rural areas or western region are at higher risk of getting lung cancer. This might be since there is a comparatively low medical facility to diagnose cancer at an early stage. Lung cancer is difficult to be diagnosed at an early stage, but if there is no regular monitoring then people are at more risk of being infected.

5.5.4 Mouth cancer

Mouth cancer refers to cancer that develops from the part of the oral cavity like lips, gums, tongue, the roof of the mouth and floor of the mouth. Mouth cancer is one of the type of head and neck cancer. The oral cavity is the leading site of incidence for cancer for males in the terai region. In Saptari district the incident of mouth cancer was the highest with AAR 9.9 per 100,000 population followed by Dhanusha with AAR 6.2 per 100,000 population, Mahottari with 5.2 per 100,000 population and Siraha with 3.7 per 100,000 population. The total number of oral cavity cancer cases was 106 in case of males of Dhanusha, Saptari, Siraha and Mahottari which is 13.4% of the total cancer cases in these 4 districts. Among the 106 cases, 7 were lip cancer cases while 20 were tongue cancer cases, 74 were mouth cancer cases and 5 were salivary gland cases. From the data, it is seen that the incidence of oral cavity started at the age group of 15-19 years. The peak value of incidence cases is at the age group of 50 -54. The mortality cases due to oral cavity cancer were 24 among which 16 cases were due to mouth cancer only. In the case of Kathmandu valley, the total case of oral cavity cancer was 61 out of 999 cancer cases among which Bhaktapur had higher

cases with AAR 7 than Kathmandu and Lalitpur. The highest incident case was seen in the age group of 60-64 followed by 50-54 years. The mortality cases were 18 and the highest mortality case was seen in the age group of 40-69. In Rukum district, the cases of oral cavity cancer were found to be very less which only 4.5 % of total cancer cases.

In the case of females, the cases of oral cavity cancer are very low as compared to male. Saptari had a high number of oral cavity cancer cases with AAR 3.3 per 100,000 population. Kathmandu valley also had a smaller number of oral cavity cancer cases than other cancer cases.

Risk factors

The important risk factor of mouth cancer is the use of tobacco. Chewing tobacco, alcohol consumption, and smoking has shown to increase the risk of oral cavity cancer. It is reported that [173], using chewing products like surti, zarda and supari increases the risk of oral cavity cancer along with smoking of cigarette with a filter. It is also reported that [174], people using tobacco and cigarettes at low frequency also increased the risk of oral cavity cancer. Report suggests people who smoked less than 10 cigarettes per day had a high risk of developing oral cavity cancer. Similarly, people who take supari in high amount has increased risk of getting infected with oral cavity cancer as it contains carcinogenic compounds like arecoline and nitrosoamine [173]. The time of duration of habits also affects the risk. It is reported that person who smokes less than 3 cigarettes per day has a high chance of getting infected by oral cavity cancer after the continuous use of cigarettes for 30 years while people smoking more than 3 cigarettes per day has the risk of oral cavity cancer after 20 years of continuous use [173]. Along with cigarettes, alcohol consumption also increases the risk of oral cavity cancer.

5.5.5 Stomach cancer

Stomach cancer is an abnormal growth of cells that begins in the stomach. It is also known as gastric cancer that can affect any part of the stomach. It is most likely to affect the oesophagus in the gastroesophageal junction. Based on the microscopic examination, stomach cancer is divided into two types [175], and they are:

Well-differentiated/Intestinal type- this type of cancer occurs in the areas with a high incidence of gastric cancer and follows a predictable stepwise progression of cancer development from metaplasia. It is typically characterized by the presence of mitotically active columnar cells forming the gland with swollen, darkly stained nuclei (with hematoxylin), with mucin accumulation in the lumina of these malignant glands, and without much intracellular mucin accumulation.

Undifferentiated/diffused type- the diffuse carcinoma cells are smaller as compared to intestinal-type and are more uniform in overall shape and nuclear size. Diffuse gastric cancer is a specific type of stomach cancer, which is also known as “signet ring cell gastric cancer” or “linitis plastic.” The word "diffuse" is used because this cancer tends to affect much of the stomach, rather than staying within one area of the stomach.

Stomach cancer is one of the common types of cancer in the world. In Nepal, there are more male stomach cancer patients than female.

In the three districts of Kathmandu valley, Kathmandu district had a total of 1351 new cancer cases out of which 630 were males with the age-adjusted rate (AAR) 90.4 per 100,000 population. The mortality rate was 210 for males with AAR 31.6 per 100,000. The leading site for cancer in males was lungs followed by stomach cancer in Kathmandu district with AAR 7.8 per 100,000. Similarly,

in Lalitpur and Bhaktapur district the new cases were 222 with AAR 101.3 and 147 with AAR 112.5 per 100,000 respectively in male. While the mortality rate was 91 with AAR 43.3 per 100,000 and 64 with AAR 49.4 per 100,000 for Lalitpur and Bhaktapur district respectively.

With the total case of 222 in Lalitpur, the second major site for cancer in males was stomach with AAR 8.5 per 100,000 population. Similarly, in Bhaktapur with total new cases of 147, the second major site for cancer was stomach with AAR 11.6 per 100,000 population.

In Kathmandu valley, the total number of stomach cancer cases was 90 which is 9.2% of total cancer cases in Kathmandu valley in case of males. Out of 90 cases it was reported that 2 were from the age group of 25-29 while 2, 2, 1, 4, 8, 13, 11, 11, 12, 7 and 19 from 15-19, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. The mortality case due to stomach cancer was 38 in Kathmandu valley which is 10.5% of total death rate in males due to cancer. Out of 38 cases 1 was from the age group of 25-29 while 1, 3, 4, 1, 4, 5, 8, 3, 8 were from 30-34, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and above 75 years age group, respectively. Among 9.2% of total stomach cancer cases, 12.2%were diagnosed with a non- microscopical investigation like radiography, clinical investigation, etc. while 85.7% were investigated by microscopy and 2.2 % were identified through death certificate record.

In Rukum district, the most prevalent cancer in male was lungs cancer followed by stomach cancer. Among the total new 44 cases of cancer in females, 5 of them had lungs cancer which is 11.4%. The mortality case due to this type of cancer was 3 which is 19% of total mortality case in male due to cancer. Among 10 of them, 1 was from the age group of 45-49 years while 1, 1 and 1 were from 50-54, 55-59, 65-69 and above 75 years. For the diagnosis, it is reported that microscopic and verbal investigation method was used.

In case of Terai region of Nepal, districts dhanusha, siraha, mahottari, saptari had total new cancer cases of 1106 out of which 564 were males. The total mortality rate of males due to cancer was 155.among 564 cases, 24 males had lungs cancer which is 4.4% of total cases of cancer in males of dhanusha, mahottari, saptari and siraha district. Dhanusha had the highest number of the stomach cancer patient with AAR 3.5 per 100,000 population followed by mahottari with AAR 1.5 per 100,000 population. Out of 24 cases in four districts 1 was from age group of 30-34 age group while 2, 2, 6, 4, 1, 4, 3 and 1 were from 35-39, 40-44, 45-49, 50-54, 55-59, 65-69, 70-74 and above 75 years age group, respectively. The mortality rate due to stomach cancer was 6 among which 1 was from the age group of 0-4 while 2, 1, 1 and 1 were from 35-39, 50-54, 65-69- and 70-74-years age group, respectively. Among the diagnosed stomach cancer patient, 8.4% were diagnosed non- microscopically, 54.2% were diagnosed microscopically while others were either identified through verbal information.

In the case of females, the Bhaktapur district had more stomach cancer patient among the three districts of Kathmandu valley with AAR 4.6 per 100,000 population. While Kathmandu had AAR 4.2 per 100,000 population and Lalitpur had AAR 3.6 per 100,000 population. The number of female lungs cancer patient was 51 which is 4.5% of the total cancer female patient in the Kathmandu valley. In the case of Rukum district, the stomach cancer patient was only 2 while mortality case was 1 for female. In Siraha, Saptari, Dhanusha and Mahottari district the total number of female stomach cancer patients was 19 while mortality cases were 9. In Kathmandu valley, it is seen that the incidence of stomach cancer in the female has started from the age of 20 years old while the peak value of incidence is at the age of 75 years. In Rukum the incidence of stomach cancer has started from 45 years and in the Siraha, Saptari, Dhanusha and Mahottari districts the incidence has been started from the age 25 years.

Risk factors

The important risk factors that cause stomach cancer are Helicobacter pylori (*H.pylori*), obesity, red meat, alcohol and low socioeconomic status. In 1994, the international agency for research on cancer and the World Health Organization (WHO) classified *H.pylori* as a type I carcinogen. The impact of *H.pylori* infection on stomach cancer appears to be multifactorial, involving host and environmental factors as well as different strains of bacteria. *H.pylori* is closely related with intestinal gastric cancer which follows a stepwise pathway but towards malignancy similar to that in the colon [176]. Gastric inflammation leads to mucosal atrophy, metaplasia, dysplasia and, ultimately, carcinoma in the Correa gastric carcinogenesis model [176]. Reports [177, 178] indicate that *H. pylori* infection is an independent risk factor for distal gastric cancer, with an elevated risk of 3 to 6 times that of non-infected patients. It is reported that patients with *H.pylori*, due to the presence of unique gene polymorphisms there is an increased risk of gastric carcinoma development. Higher cancer rates have been associated with genes encoding tumour necrosis factor-alpha (TNF-alpha) and interleukins IL-1, IL-8, and IL-10 in the *H.pylori* environment [179]. Intestinal type of gastric cancer is strongly associated with *H.pylori* which is not same in case of diffuse gastric cancer. Diffuse stomach cancer is associated with other risk factors like obesity, socioeconomic factor.

It is reported that overweight increases the risk of esophageal and gastric cardia adenocarcinoma while tobacco and smoking increase the risk of esophageal, gastric cardia and non-cardia gastric adenocarcinoma. Similarly, there are a variety of genes that increases the risk of stomach cancer like MCC, APC and p53. These tumor suppressor genes have been identified in a large percentage of stomach cancers. Also, studies that reported that E-cadherin which is a calcium-dependent adhesion molecule responsible for cellular binding is an important component in the gastric

carcinogenesis cascade. Genetic susceptibility requires a single mutated CDH1 allele's inherited transmission. If there is an acquired mutation of the second allele in the E-cadherin gene, then the loss of intracellular adhesion which leads to the increased intracellular permeability. A wide variety of mutations in this domain have been identified in gastric cancer families.

5.5.6 Others

Cancers like urinary bladder cancer, gall bladder cancer, non-hodkings cancer, ovarian cancer skin cancer, kidney cancer, etc. are among the prevalent type of cancer in Nepal.

5.6 Conclusion

Cancer is one of the most prevalent non-communicable diseases in Nepal. The leading cause of deaths due to cancer in Nepal is highest for lung cancer followed by stomach cancer, mouth cancer and liver cancer in males. While breast cancer is the leading cause of death in females followed by cervix and uterine cancer and lungs cancer. With the highest population in the Kathmandu valley, the cases of cancer are also high in the valley. Lung's cancer prevails at a high rate in both males and females. This might be due to the increase in air pollution in the environment as well as within the house. Also, the increase in habit of smoking and alcohol consumption increases the risk in the cause of lungs cancer. Lung's cancer is mostly seen in the age group of people who are more than 45 years old. This shows that the increase in age also increases the risk of lung cancer. So, regular health checkup is necessary during old age. But due to the increase in pollution air-borne diseases are affecting the health of children so, they should be well known about air pollution and preventive measures. Children should be aware of the consequences of smoking and alcohol consumption on health. Well-equipped hospital with diagnosis machines like; Computed Tomography (CT) scan, X-ray, Magnetic Resonance Imaging (MRI), mammography, etc. should

be present in every village and cities throughout Nepal. This helps in early diagnosis of cancer ultimately reducing the mortality cases.

Breast, cervix, and uterus are the major cancer sites in case of females. Breast cancer is prevalent more in the Kathmandu valley while the cervix and uterine cancer are more prevalent in rural areas of Nepal. The increase in household work increases the risk of the cervix and uterine cancer. Women of rural areas mostly must face the problem of carrying heavy loads like water pots. Along with this, women give birth to many children which ultimately affects their health. The menarche and menopause age also influences the risk of having cervix, uterus, and breast cancer. This age cannot be controlled but women with less menarche and menopause age should frequently go to hospitals for checkups.

Intake of tobacco increases the chance of getting infected by oral cancer including mouth, lips, tongues, and salivary glands. More the use of tobaccos more will be the risk of getting oral cancer. People in terai areas of Nepal are likely to consume more tobacco products than another part of Nepal. So, the cases of oral cancer are also more in the Terai region than in other regions of Nepal. Similarly, smoking and alcohol consumption also increases the risk of oral cavity cancer.

Obesity is also one of the risk factors for cancer. Overweight people are likely in the risk of getting infected by stomach, liver intestinal cancers. Alcohol consumption and smoking also cause the risk of getting stomach cancer. Gall bladder, kidney, urinary bladder cancer is also among the most prevalent cancer in Nepal. Risk factors for these types of urinary system organ cancer are low water consumption, diet containing low fibers and other nutrients, unhealthy diets, and gender. Other risk factors for different types of cancers are the hereditary cause, exposure to UV rays,

diets, immunosuppression, exposure to chemical components and pollution, hormonal factors, chronic inflammations, etc.

Thus, prevention from smoking, alcohol consumption, air pollution can help people to prevent cancer. Regular checkups, controlled diet and less workload can also inhibit the risk of cancers. If individuals follow these acts, then they can maintain a large distance from cancer. But not only the individuals but the government should also focus on the preventive measures and awareness programs against cancer. Children should be kept far from alcohols and cigarettes. Also, they should maintain well-equipped facilities in every hospital throughout Nepal.

Chapter 6. Preliminary assessment of the use of Yarsagumba (Cordltceps sinensi's) for the perspectives of discovery of immune buster's anti-cancer drugs

6.1 Introduction

Yarsagumba/Cordyceps′ part insect; part mushroom' is a sporadic and exclusive herb found at around 3,000 meters high altitude [180]. It is a summer grass-winter insect, i.e., endo-parasitic living being [181]. It is a part of traditional medicine in different cultures. It is scientifically known as *Ophiocordyceps sinensis*/*Cordyceps sinensis* (caterpillar-fungus, Herbalists) and it highly prized. It is located in the south part of Asia (China, India, Bhutan, Bangladesh) and mainly in Nepal's different higher altitude districts [182, 183].

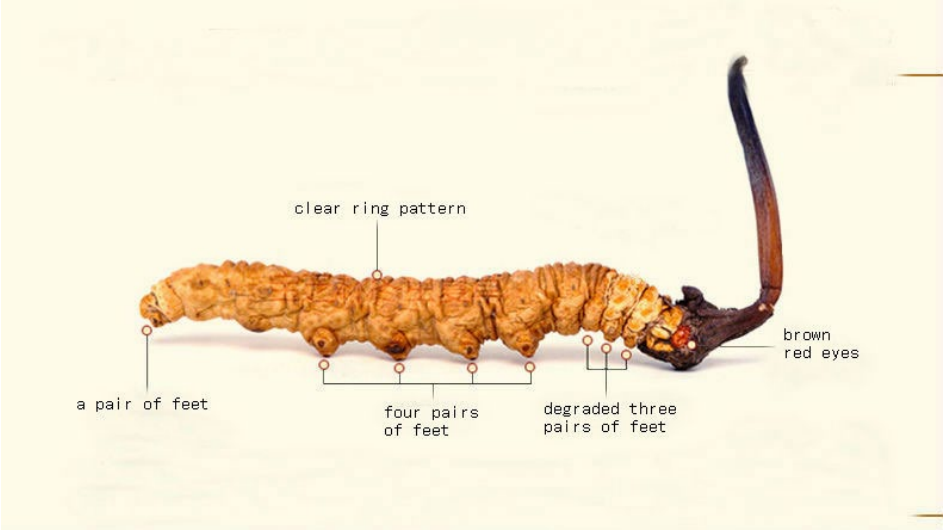

Figure 6. 1: Yarsagumba (Cordltceps sinensi's), taken from web source.

Yarsagumba is an expensive herb that has significant economic importance to many people in the rural himalayan district of Nepal. People believe it can cure sexual impotent, cancer, cough, and strength the immune system; many scientific articles support this belief. Therefore, they use its

medicinal tonic as an energy drink. However, many pharmacologically active components are still unknown, and the research is needed to explore more about it.

The chemical composition found in Yarsaghumbha (*Cordyceps Sinensis*) are: -

- Nucleosides are significant components found in Yarsagumbha. It measures/analyzes the quality of the caterpillar fungi (Cordyceps). Nucleosides show anticancer and antiviral behavior [184].
- Another significant component is Cordycepin, an active constituent present in *Cordyceps Sinensis* that also shows the similar property of Nucleosides with additional antimetastatic properties [185]. Metastasis is related to more than 90% of cancer related deaths.
- Cordyceps consist of three to eight percent polysaccharides by weight. They exhibit antitumor, antioxidant, antihyperglycemic, and anti-influenza properties [186].
- Cordyceps Sinensis contains vital nutrients – vitamins like vitamin B_1**,** B_2, B_{12}, and K, minerals [187]**.**
- The two major components Cordycepin (Deoxyadenosine) and cordycepic acid (Mannitol) are identified to have medicinal properties.

Cordyceps increases natural stamina, increases blood flow, sex drive naturally. Modern analysis of Cordyceps extract shows powerful antioxidant abilities and potential effects on bad cholesterol. Research done clinically has found that Deoxyadenosine named Nucleosides in Cordyceps helps the men significantly with their erectile dysfunction. These components help increase libido and desire in both genders. Many athletes and artists have also reported taking Yarsaghumba extract to boost their stamina and circulation routine [188]. Its copes against kidney and lung problems. It

stimulates the immune system [189]. Fatigue, sweating, respiratory problems, other heart diseases, and liver disease are cured using Cordyceps. Major uses of this unique species are for treatment of exhaustion, respiration, tuberculosis, cardiovascular diseases, back pain, lack of sex drive, and premature ejaculation [190-193].

6.2 Anticancer activity of Cordyceps.

Researchers have shown that nucleoside, key signing molecules in the body, helps fight cancer, nucleoside analogs as anticancer. By research in *Ophioocordyceps sinesis,* it has antitumor, anticancer, and antiviral activities. Immunomodulating reducing cholesterol, antioxidant process. [190]. We have collected different studies showing the antitumor activity in various cancers in different pathways. Both natural and cultivated cordyceps have antitumor effects [194, 195]. Research showed that cordyceps activate repressive effect on methA fibrosarcoma and Ehrlich ascites carcinoma [196], lymphoma (EL4) [197], melanoma (B16) [198], Lewis lung carcinoma [199], and tumors (H22) [200] in mice. Further, *C. sinensis* upturned the effect of leukopenia in mice, which directed the use of *C. sinensis* in other chemotherapy [201]. Cordyceps exhibited direct cytotoxic activity against different tumor cells, including lung carcinoma, melanoma, lymphocytic, prostate, breast, hepatocellular [198, 199, 202]. Although cordyceps shows a cytotoxic outcome on cancer cells, they don't have any toxic activity against normal cells [198]. Various mechanisms like cytotoxicity, apoptosis, selective inhibition of RNA, protein synthesis, antioxidant properties help *C.sinesis* to have an anticancer effect [194, 195, 203]. The critical measure of cordyceps is the apoptotic homeostasis mechanism [194, 204]. The apoptotic mechanism consists of Bax, caspase-3 (also -9, -8); inhibition of cyclooxygenase; and nuclear factor κB, protein appearance and down parameter of Bcl-2 level [194, 203]. Apoptosis of human breast carcinoma cells tempted by *C. militaris* aqueous extract reduced Akt activation and reversed

PI3K/Akt-pathway-improved apoptosis [205]. The apoptotic events encouraged by the section mediated by reduced telomerase activity [206].

6.3 Working mechanism

It shows that Yarshagumba/Cordyceps improve immunity by stimulating cells and immune system chemicals. It also has the terrific activity to cure tumor cells and shrinks its size, specifically lung cancers. Moreover, a medicinal mushroom decreases exhaustion, enhances the immune system, and sexual performance as it is an effective aphrodisiac. People also call it 'Himalayan Natural Viagra'.

6.4 Immune activation/booster property

Natural *C. Sinensis* has an ancient history in the use of respiratory problems and cancers. Its vital mechanism is associated with immune growth, mainly promoting specific immunity. Intake of *C. Sinensis* extracts enhanced mice fixed internally with syngeneic lymphoma (EL4) cells [197]. In addition, cultured *C. Sinensis* encouraged interleukin and tumor necrosis factor; raised phagocytosis of human peripheral blood mononuclear cells, and produced HO in mice [207].

Further research showed that an aqueous extract of mycelia of *C. Sinensis* boosted the TNF-α and nitric oxide (NO) issue. The extract also treated mice spleens showed declined bacterial burden. The results specify that *C. Sinensis* protect from the production of bacteria by stimulating immunity [208]. The *C. Sinensis* also improve natural T-cells' activity [196]. *C. militaris* extract encouraged T-cell responses against microbial pathogens and tumors [209]. Cordyceps encourages the adaptive immune system. Although natural and cultured yarsaghumba methanol extracts had no proliferation of primary mouse splenocytes *in vitro* [210] or mice *in vivo* [207]; the extracts improved stimulated proliferation of mouse splenocytes *in vitro* [210] or ovalbumin-induced proliferation and serum immunoglobulin in immunized mice [211]. Cultured *C. Sinensis* increased

CD25 appearance on lymphocytes *in vitro* [207], help patients with posthepatic cirrhosis [196]. Cordyceps had a positive consequence on bronchoalveolar fluids (BALF) cells. The *C. Sinensis* ethanol extract improves the immune response [212]. Fruiting bodies of *C. militaris* water extract meaningfully regulate gene expression [213]. Fruiting bodies of *C. cicadae* methanol extract improved proliferation [214].

6.5 Major benefits of Yarsagumba/Cordyceps Sinensis

Talking about the benefits of Yarsagumba, we can write a book if you investigate ancient medicinal textbook-like aayurved and other Chinese histories. Still, most of the services need to be put through scientific research to test. Taking these things just on people's beliefs can be dangerous to health and wealth in Yarsagumba. We have mentioned some points about the benefits of this among people. There is research that supports that the use of Cordyceps that reduces the tumor size. It also protects against free-radical damage in the human body. This research is first conducted in mice and put up to a human scale. Many people believe that Yarsagumba improves sexual function. There is also much scientific research to back up this fact.

Other benefits are:

1. Increase in T-cell production.
2. DNA Restoration
3. To maintains healthy cholesterol levels in the body.
4. Improves heartbeat, respiration, kidney disease, auto-immune system.
5. Increases oxygen absorption.
6. *C. Sinensis* upsurges ATP (adenosine triphosphate) in the body
7. Protects kidneys, purification of liver from contaminants.
8. Stables Antibiotic

9. Treats and prevents cirrhosis of the liver.
10. Inhibits tightening of smooth muscles, group a streptococcus bacteria.
11. Polysaccharide compounds trigger immune modulation.

Researchers in Japan showed that Cordyceps enhances the immune system against melanoma. Yarsagumba also treats coughs, chronic respiratory illnesses, sexual problems, asymmetrical heartbeat, high cholesterol, dizziness, weakness, irregular weight loss. In addition, it helps to cope with the addiction to opium, drugs, alcohol.

Chapter 7. Review of Himalayan Herbs for diseases such as cardiovascular, neurodegenerative, aging and diabetes.

7.1 Introduction

Himalaya is the mountainous range located on the south-east Asian region. It mainly separates the Indian subcontinent from Tibetan plateau. It has a spread across the five countries namely Bhutan, Nepal, India, Pakistan, and China. This region is a home to more than 110 peaks including Mount Everest which is the highest peak of the globe having an elevation of 8848.86 m. Based on the theory of plate tectonics, the formation of Himalayan range occurred 55 million years ago because of continental collision between Indian and Eurasian Plate. It gave rise to parallel mountain ranges: the Shivalik Hills on the south; the Lower Himalayan Range; the Great Himalayas, which is the highest and central range; and the Tibetan Himalayas on the north side.

Due to vast size, high altitude range and complex topography, Himalayan region experiences a wide range of climates from humid subtropical to dry desert conditions. This wide diversity of altitude, climate, rainfall, and soil conditions is favorable for a variety of distinct plant and animal species. The Himalaya's region consists of many lives sustaining herbs which are being exploited for long time. The plant compounds of these Himalayas region are vital part of western medicine, and they are very important source of novel compounds in the field of discovery of drugs. In Nepal, almost 1600 to 1900 species of plants are mostly used for practices of traditional medicinal. Furthermore, in Nepal, Traditional medicine is widely used by majority of the population in Ayurveda. This is the alternative medicine system with historical roots in the Indian subcontinent. For thousands of years, the people inhabitant to Himalayas has been using these medicinal herbs for treating common ailments. Due to recent developments of modern drugs, herbs are being over collected and unscientifically exploited the resources from Himalayas to suffice the global need

which is rendered threatening to the biodiversity of the region and many species are being endangered.

Table 7.1: Medicinal plants found in the Himalayas region which is used to treat different ailments along with its medicinal compounds.

Botanical Name	**Common Name**	**Parts used**	**Medicinal Compound**	**Usage**
Artenesia annua Linn.	Sweet wornwood	Plant	Artenisinin	Anti-malarial
Asparagus racemosus Willd.	Asparagus	Root	L-asparaginase, Rutin	Anti-cancer
Atropa belladonna Linn.	Belladona	Roots, inflorescence	Atropine	Anti-cholinergic
Berberis asiatica Roxb.	Tree turmeric	Root bark	Berberine	Anti-diarrheal
Camellia sinensis (L.) Kuntze	Tea	Leaf	Caffeine	CNS- stimulant
Cephaelis ipecacuanha (Brot.) A. Rich	Ipecac	Bark	Emetine	Emetic, expectorant
Cinchona officinalis Linn. F.	Cinchona	Bark	Quinine	Anti-malarial
Colchicum luteum Baker	Colchicine	Tuber	Colchicine	Anti gout
Digitalis purpurea Linn.	Foxglove	Leaf	Digoxin, getalin, digitoxin	Cardiotonic

Dioscorea deltoidea Wall.	Dioscorea	Tuber	Diosgenin	Contraceptive
Dioscorea prazerin Prain & Burkil.	Dioscorea	Stem, Roots	Diosgenin	Contraceptive
Ephedra gerardiana Wall.	Ephedra	Plant	Ephedrine	Bronchodilator
Gloriosa superb Linn.	Glory Lily	Tuber	Colchicine	Gout, cancer
Hyocyamus niger Linn.	Henbane	Leaves	Hyoscyamine	Anti-cholinergic, Anti spasmodic
Panax pseudoginseng Wall.	Indian ginseng	Root	Ginsenosides	Tonic, antioxidant
Picrorhiza kurroa Royle ex Benth.	Kutki	Root	Kutkin	Stomachic, cathartic
Papavar somniferum Linn.	Poppy	Latex	Morphine, codeine, papaverine	Analgesic, anti-tussive
Podophyllum hexandrum Royle	May Apple	Root	Podophyllotoxin	Anti-cancer
Silybium marianum (Linn.) Gaertn.	Holy Thistle	Leaf	Silymarine	Anti-hepatoxic
Taxus baccata Linn.	Himalayan Yew	Leaf	Taxol	Anti-cancer
Urginea indica Kunth.	Indian Squill	Bulb	Scillaren A, B	Cardiotonic

Valeriana wallichii DC.	Valerian	Roots	Valpotriates	Tranquilizer

Source: Dhani Raj Chhetri; Medicinal Plants of the Himalaya, Production, Technology and Utilization

7.2 Himalayan herbs for Treatment of cardiovascular diseases, neurodegenerative diseases, aging and diabetes.

For many years, local people in Himalayan region have been using these herbs to treat their common ailments. However, many have not been scientifically proven yet, they have been using it by their own experience. The Hindu religious scripture Atharvaveda contains 114 hymns which mentions the treatment of diseases by using medicinal herbs of the region which originated and developed Ayurveda, the system of traditional medicine native to Indian subcontinent.

Some Himalayan medicinal plants such as *Aconitum Ferox*, abundant in highest planes of Darjeeling Hills, are used as antidote of poison. Moreover, it is used for treating body pain, anxiety, and fever. Another herb *Clematis buchacaciana* is used for sinus inflammation, toothache, headache, and indigestion. *Drymaria cordata*'s leaves are used for treating clogged sinuses and inflammation. In China, it is considered as magical herb and is utilized for treating snakebites. Fruit of *Rhus semialata* which is found abundantly in northeastern part of India, is used for curing diarrhea and dysentery.

7.2 .1 cardiovascular disease (CVD)

It is a class of diseases that involve the heart or blood vessels. The Leaves of *Digitalis purpurea* (Foxglobe) are used in medicine to strengthen contraction of heart muscle. The drug obtained from the leaves, Digitalis, is commonly used to restore adequate circulation in patients with congestive

heart failure. It is also used to slow the ventricular contraction in patients with atrial filbrillation. *Hippophae rhamnoides* (Sea Buckthorn) is a yellow-orange berry rich in oil, vitamin C, tocopherols, carotenoids, and flavonoids. Flavonoids are polyphenolic compounds, which possess antioxidant properties, whose consumption has shown the inverse correlation with CVD risk [215]. The flowering shoots of *Arnebia benthami* are used in preparation of syrup used in treating cardiac disorder [216]. The Methanolic extract of *Moringa oleifera* leaves at 200 and 400 mg/kg bw plays an effective role in the treatment of obesity and reduction of cardiometabolic abnormalities [217].

7.2.2 Neurodegenerative diseases

They are the range of conditions that results in progressive degeneration or death of nerve cells, which are the building blocks of nervous system. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most common neurodegenerative diseases. Leaves of *Acacia catechu* (Cutch Tree) contain several antioxidant compounds with potential benefit in psychiatric disorders such as AD and PD [218]. *Azadirachta indica* is found effective in reversing the neurobehavioral changes, attenuating the cognitive deficits, and decreasing the oxidative stress in experimental AD models [219]. *Centella asiatica* extract is found to improve neurobehavioral activity and reduces tissue death due to lack of oxygen [220]. *Centella asiatica* extract contains the pharmacologically active compound asiatic acid and its derivatives that are neuroprotective, antinociceptive, and anti-inflammatory [221].

7.2.3 Diabetes

It is a metabolic disease caused when blood glucose is too high. *Berberis aristata* fruit helps in controlling blood glucose level. Moreover, Berberis fruit influences blood glucose regulation [222]. *Syzygium cumini* seed and its bark has proved effective in humans with diabetes due to rich

source of ellagitannins, 3,6-hexa hydroxyl diphenoyl glucose, isomer 4,6-hexahydroxy diphenoyl glucose, 1- galloylglucose, 3-galloylglucose, gallic acid, and ellagic acid [223]. From experimental studies, it is found that single or multiple doses of aqueous and alcoholic extracts of *Artemisia* species had anti-diabetic effect due to the active compounds of these plants and they all are effective in lessening the blood glucose level [223]. *Emblica officinalis* and some of its phytochemicals including gallic acid and ellagic acid possess anti-diabetic effects [224].

7.2.4 Aging

It is the process of being older. Many recent studies are being done for preventing the aging process. *Centella asiatica* is a common ingredient of cosmetics applied on photoaging skin, mainly due to enhancement of type I collagen, which amount in skin decreases with age [225]. *Bacopa monnieri* extracts can safely boost cognitive abilities during aging [226].

7.3 Conclusion

Himalayas have been home to thousands of medicinal plants which has been growing and thriving for centuries. They have been playing a crucial role in culture and economy of the region. Many researchers are working on finding the medicinal potential of these traditionally used herbs and are being used for development of modern drugs. So, there is a need of giving the emphasis on preserving these medicinal herbs and avoid the over collection of the endangered species.

Chapter 8. Communication and networking with national and international R & D institutions of biomedical research

8.1 Introduction

Biomedical Engineering is an interdisciplinary program which involves the chemistry, physics, and mathematics, biology to develop a useful product in an economic and safe way. It is about the application of science, engineering, and medicine to develop the advancement of medical technology. It has combinations with other branches of engineering say Chemical Engineering to manufacture Chemicals, food, pharmaceuticals, and many other useful products. It involves the engineering concept to solve the medical problems like robots of surgery, diagnostic equipment's as well as development of artificial organs. It is the best option for those who are interested in Engineering and human body.

Biomedical Engineering involves with: -

- Programming
- Mathematics for engineering
- Mechanical systems
- Medical biophysics
- Medical instrumentation
- Mechatronics
- Human anatomy and physiology

- Biological medical science

There is a need of manpower in developed as well as developing nations with the skills offer by Bachelor of Engineering degree in Biomedical Engineering (B.Eng.), Master of Engineering degree in Biomedical Engineering (M.Eng.), Doctor of Engineering degree in Biomedical Engineering (Ph.D.). As we are planning the world top ranked university, it is very important to develop an academic and practical approach of bioengineering system. We need to understand informative biology, structural biology, molecular biology as well human physiology. Thus, we need to have a dynamic faculty to run this program as well as have a collaboration with internationally renowned universities.

8.2 Communication and networking with national and international R & D institutions of biomedical research

S.No.	School	Address	Contact Information
1.	Department of Biomedical Engineering, City University of Hong Kong, SAR, China	Department of Biomedical Engineering (BME), Room Y6700, 6/F, Yellow Zone, Yeung Kin Man Academic Building, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong, SAR, China	Phone: (852) 3442-8420 Fax: (852) 3442-0172 Email: bmego@cityu.edu.hk
2.	School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore	Nanyang Technological University 50 Nanyang Avenue, Singapore 639798	Office: N1.3-B3-11 Phone: (65) 6316 8966 Email: slim@ntu.edu.sg (Prof. Sierin Lim)

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6.	Biomedical Engineering, University of Glasgow, UK	East Quadrangle, Glasgow, Lanarkshire, G12 8QQ, United Kingdom	Phone: + 44 (0) 141 330 2000 Email: Manuel.Salmeron-Sanchez@glasgow.ac.uk
7.	Biomedical Engineering Johns Hopkins University	3400 N. Charles Street, Wyman Park Building Suite 400 West Baltimore, MD 21218	Phone: 410-516-2039 Email: mim@jhu.edu
8.	MIT Department of Biological Engineering	21 Ames St #56-651, Cambridge, MA 02142, United States	Email: bedh@mit.edu Phone: (617) 324-2800
9.	School of Biomedical Engineering (SBME), University of British Columbia, Canada	2222 Health Sciences Mall Biomedical Research Centre (BRC)Vancouver, BC V6T 1Z3	Email: reception@sbme.ubc.ca Phone: 604-822-7810

10.	College of Engineering, University of Toledo, USA	5051 Nitschke Hall MS 303 2801 W. Bancroft St.Toledo, OH 43606-3390	Phone: (419) 530-8030 Fax: (419) 530-8076 Email:EngGradStudies@utoledo.edu

Chapter 9. Support design of master's and PhD level course in Madan Bhandari University of Science and Technology (MBUST) for research on interdisciplinary science. Formulation of topics for PhD research and master's degree research.

9.1 *Natural products of Nepal enriched with antioxidant and anticancer properties.*

- This requires the establishment of a cancer research platform to identify the compounds extracts from natural products with anti-cancer properties.
- We need to have cancer cell lines of different origin based on the engineered platform.
- There are wide varieties of medicinal plants in Nepal, and we need to prepare a list of medicinal plant with anticancer and antioxidant properties.

9.2 Production of antioxidants supplements and commercialization in Nepal and abroad

- We will investigate the antioxidants supplements products from various plants extracts.
- We aim to promote mushrooms as new generation biotherapeutics and focus mushroom extracts for antioxidants supplements.
- We will explore the health benefits of antioxidants supplements.
- We aim to develop a product based on antioxidant supplements.

9.3 Determine the compounds from mushrooms grown in Nepal with antioxidants as well as anti-cancer properties.

Table 9.1. Bioactive compounds of mushroom

Polysaccharides	*Proteins*	*Fats*	*Ash*	*Glycosides*	*Alkaloids*	*Folates*
Volatile Oil	*Tocopherols*	*Flavonoids*	*Phenolics*	*Carotenoids*	*Ascorbic acid*	*Organic acid*

Table 9.2. Active compounds in mushroom responsible for anti-cancer properties

Lentinan	Krestin	Hispolon	Lectin	Calcaelin
Illudin S,	psilocybin	Hericium polysaccharide A & B	Ganoderic acid	Schizophyllan

- This project aims to explore the bioactive compounds of mushroom.
- We will investigate in the active compounds in mushroom responsible for the anti-cancer properties.

9.4 Screening of different compounds extracts from the mushrooms of Nepal with antioxidants as well as anti-cancer properties.

- This project aims to explore the antioxidants and anti-cancer properties of mushroom.
- We will find the compounds with antioxidants in mushroom.
- We will find the compounds with anti-cancer in mushroom.
- We aim to develop a product based on anticancer properties and antioxidant supplements.

9.5 Exploring the cell signaling pathways in cancer by mushrooms and their bioactive molecules.

In this project, we aim to investigate the role of bioactive compounds of mushroom:

1. In Angiogenesis inhibitor
2. Topoisomerase inhibitor
3. ROS inducer
4. Amitotic kinase inhibitor
5. Antimitotic
6. Antioxidants (ROS)
7. DNA repair (Mutation)
8. Apoptosis (Transformation)
9. Immune system (Proliferation)

Some mushrooms in combination with commercial anti-cancer drugs work in synergy as an effective tool for treating drug-resistant cancers.

9.6 Comparison of nutritional value of edible mushroom of Nepal (In terms of content of amino acids, proteins, vitamins, dietary fibers, fats, minerals, antioxidants).

In this project, we aim to investigate the nutritional value of edible mushroom of Nepal in terms of content of amino acids, proteins, vitamins, dietary fibers, fats, minerals, antioxidants etc.

9.7 Development of a novel microfluidics devices for the screening of natural products extracts with anti-cancer properties.

In this project, we aim to design the microfluidics device which can mimic the human physiological system.

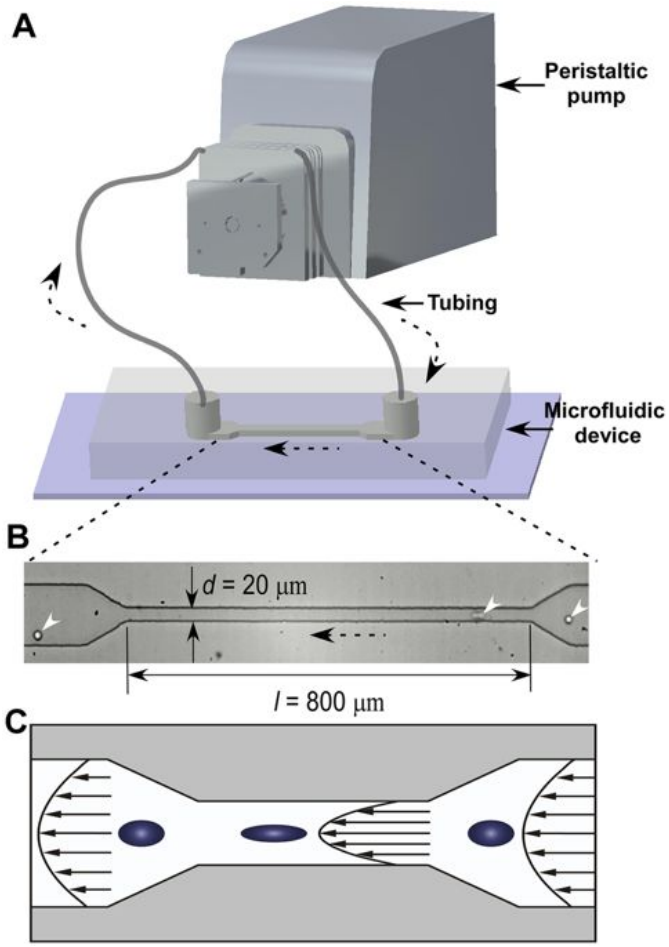

Figure 9. 1: Peristalatic pump design by Rong Fan et.al [227].

9.8 Academic Program of Cancer Research

This programs aims to establish the cancer research enviroment in MBUST and we can offer the graduate programe leading to the MSc and PhD degrees in the area of molecular and cell biology of cancer. It can provide the graduate students the opportunity to explore the reseearch skills, presentation and experience. The local of herbs and resource of Nepal can be used in developmental models, programmed cell death and drug resistance. We can offer the clinical trials, pediatric oncology, epidemiology and cancer imaging/screening and diagnosis courses in MBUST. This will be the first program in Nepal which can be offered by the Cancer research group. The following course can be developed in academic program of MBUST.

1. Molecular Biology of Cancer.
2. Etiology of Cancer.
3. Chemical and Radiation Carcinogenesis.
4. Cancer Biology.
5. Molecular Principles of Radiotherapay.
6. Chemotherapy.
7. Immunotherapy and gene therapy.

Chapter 10. Estimation of cost for lab establishment in MBUST for research on natural product with biomedical application

10.1 Introduction

It is very important to design the laboratory facilities in a safe and effective way. I have design and estimation of cost for the following in a teamwork: -

1. Cell culture faculty
2. Plate reader machine
3. Incubator
4. Refrigerators for laboratory
5. Liquid Nitrogen Tank
6. Fourier-transform infrared spectroscopy (FTIR)
7. Scanning electron microscope (SEM)
8. Thermo Gravimetric Analysis (TGA)
9. X-ray Powder Diffraction (XRD)
10. Confocal microscopy (CM)

10.2 Description of the equipment's

S.No.	Equipment	Specification	Remarks
1.	**Cell culture faculty**	It is used for cell culture media, serum, cell lines, cryopreservation of cell lines, mycoplasma screening. It consists of: 1. Laminar flow hoods 2. Cell culture hoods 3. Incubator 4. Water bath 5. Centrifuge 6. Refrigerator and freezer (–20°C) 7. Cell counter 8. Inverted microscope 9. Liquid nitrogen (N2) freezer 10. pH meter 11. Confocal microscope 12. Flow cytometer 13. Cell culture vessels like flasks, petri dishes etc. 14. Pipettes	It is recommended to have BSL2/Category 2.
2.	**Plate Reader Machine (VICTOR Nivo Multimode Microplate Reader)**	1. It is used for reading of all standard technologies like absorbance, luminescence, fluorescence intensity, time-resolved fluorescence, and fluorescence polarization etc.	
3.	**Incubator**	• It creates an environment for the cell growth	
4.	**Refrigerators for laboratory**	• It is used to store the reagents and media at the appropriate temperature.	
5.	**Liquid Nitrogen Tank**	• Liquid nitrogen is used for the storage of cells for the long durations	
6.	Fourier transform infrared spectroscopy, Scanning electron microscope, Thermo Gravimetric Analysis, X-ray Powder Diffraction, Confocal microscopy	• This is a group work and is completed by Natural Product Team of MBUST which is already submitted in an excel sheet.	

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