Introduction

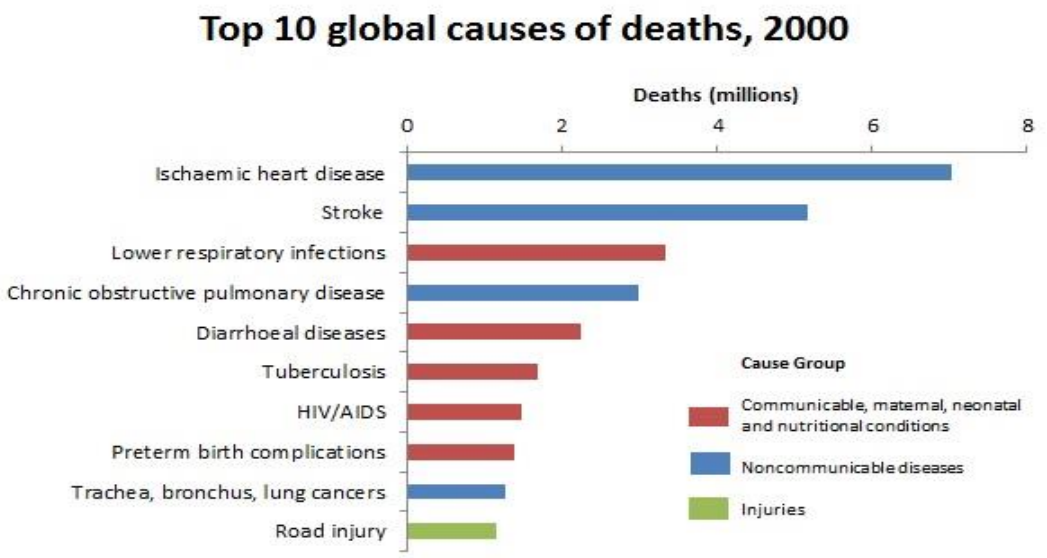
1.1. Cancer

"Cancer is an expansionist disease; it invades through tissues, sets up colonies in hostile landscapes, seeking "sanctuary" in one organ and then immigrating to another"- a definition of the disease 'Cancer' given by Siddhartha Mukherjee in his bestselling book 'The Emperor of All Maladies: A Biography of Cancer' [1]. Scientists from all over the world continue to spend time, energy and funds to understand this disease better and come up with viable solutions to tackle it. According to a report of the World Health Organisation, cancer has leapt to the sixth position in 2016 from the ninth position among the top ten causes of death globally in 2000 (**Fig 1.1. A & B**) [2]. It is a group of more than 100 diseases, also known as malignancies, characterized by aberrant cell proliferation, enabling a bodywide exodus and engendering tumor formation. In general, the latter are of two types, malignant or cancerous and benign or non-cancerous, classified on the basis of their capacity to spread or migrate. Benign tumors remain confined to the tissue of origin, and generally, stop proliferating after some point and therefore are curable and not fatal like malignant tumors [3]. The pandemic statistics for cancer occurrence and mortality are formidable with figures of 18.1 and 9.6 million in 2016 respectively, which is inclusive of data for nonmelanoma skin cancer. India accounts for over 50% of cancer incidence among the 24 countries under study; a statistics that is very initimidating [3]. If we analyze global cancer-related mortality, then lung, breast, prostate, colorectal and stomach cancers are responsible for 18.4%, 11.6%, 7.1%, 9.2% and 8.2% of the total mortality respectively [2]. The figures in Indian females are 9.8% and 7.3% for cancer incidence and mortality; whereas in males, the figures are 9.4% and 6.3% respectively [3].

There are a variety of causes of cancers or risk factors of cancers, among which ingestion of tobacco is considered as one of the prime cancer initiators [4]. Other avoidable causes of cancer are smoking, alcohol consumption, occupational exposure to carcinogens, overweight and obesity, high meat-based diet, inferior air quality, unhealthy lifestyles, unhygienic medical and sexual practices and infections like human papillomavirus infection [4-8]. The increase in breast cancer-related odds is augmented by BRCA1 and BRCA2 gene status; ancestral and personal reproductive history, endocrine profile, aging, socioeconomic position, alcohol intake and radiation vulnerability. Reproductive health parameters like mensuration initiation age, late first

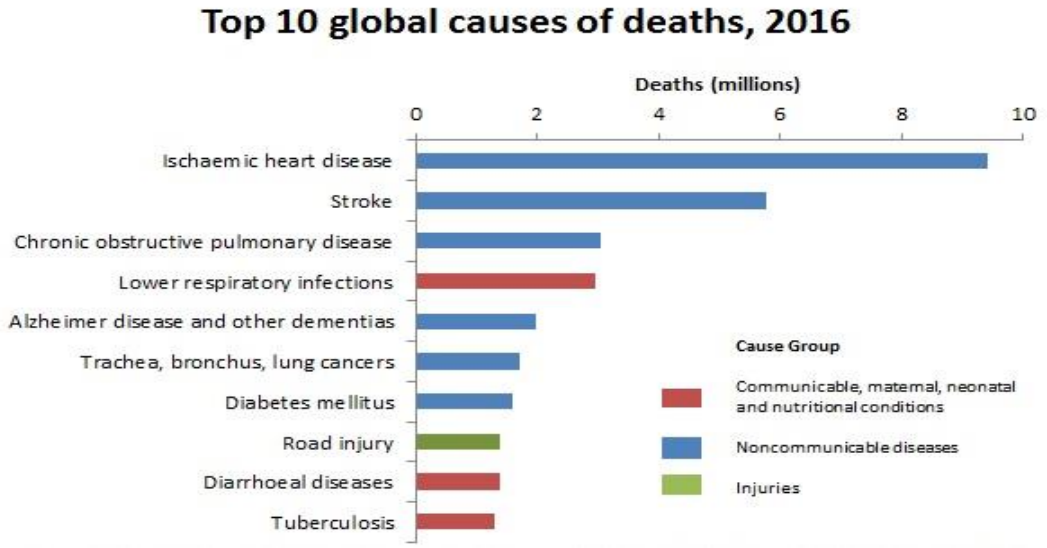
conception, minimal breastfeeding, delayed menopause, postmenopausal obesity, protracted oral contraceptives intake and estrogen replacement therapy play significant roles [9-12].

A



Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organisation; 2018

B



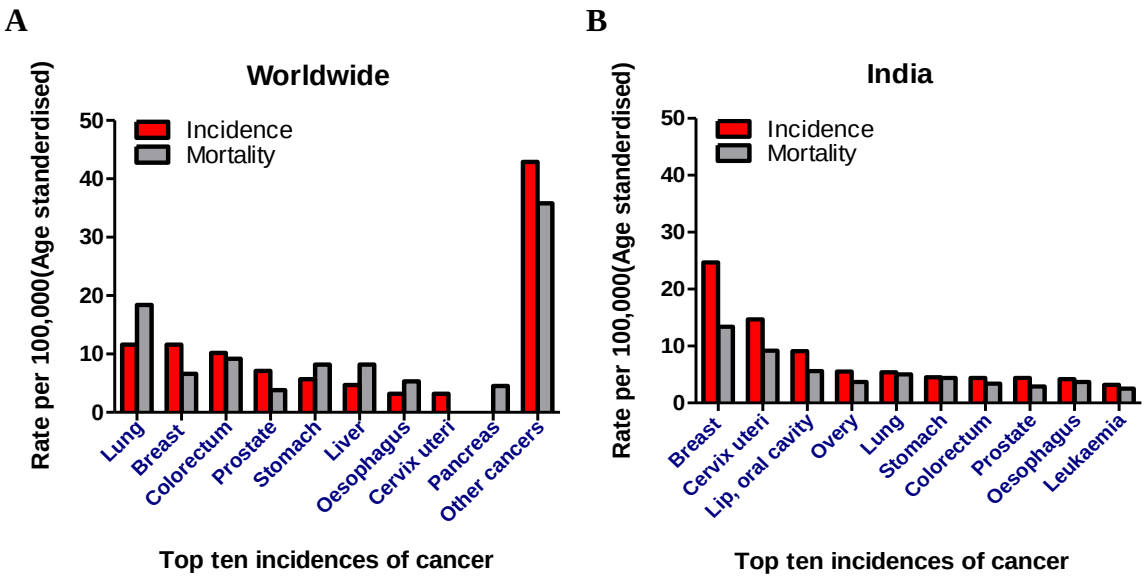
Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organisation; 2018

Figure 1.1.: A comparative global scenario of the top 10 causes of death in 2010 and 2016

1.2. Breast cancer

Breast cancer ranks second among all global cancer mortality and is the principal reason of cancer death among women in India (**Fig 1.2. A & B**) [3,13,14]. Though rare and

uncommon, breast cancer also occurs in men [15,16]. Breast cancer in men is similar to women; however, hormone receptor-positive breast cancer in men is more and might be responsive to hormonal therapy [16]. Tumors grow in the epithelial cells that line all organs and tissues and in the case of breast cancer, it specifically begins in the mammary lobules and duct. Most breast cancers belong to the adenocarcinoma group and therefore also named breast adenocarcinomas. The breast cancers are variously classified, based on their clinical features, expression of tumor markers, and histologic types. Based on the cell and position within the mammary tissue, histologically, they are classified as ductal or lobular and either situ or invasive [17-19]. The incidence rate of lobular cancer is more than that of ductal cancer and the former are more likely to be hormone positive [18]. On the basis of the hormone receptors present on the cells of breast cancer for the hormones estrogen and progesterone, they have been classified as hormone receptor-positive and hormone receptor-negative cancers. The status of these hormone receptors is critical for cancer treatment [20]. They may have one, both or none of the receptors and therefore vary in their characteristics from one type to another. The majority of the breast cancer mortality cases are primarily due to metastasis of primary cancer to different sites including organs like bones, brain, liver, lymph nodes and lungs [21].



Source: Globacon 2018: All cancers [22] *Source: Globacon 2018: India Factsheet* [23]

Figure 1.2.: A comparative scenario of cancer incidences in the world and India

1.3. Metastasis of cancer

Metastasis is the process by which cancer cells migrate to other regions or tissues of the body and form single or multiple tumors. This is also called 4th or last stage cancer [24]. Metastasis is considered as the end product of complex cell biological events (**Fig 1.3.**) [25,26]. This is the property that empowers certain cancer cells to spread into local or distant tissues, along with complex processes involving migration, adhesion, and invasion. These processes can be targeted by an anti-metastatic agent leading to attenuated aggression on cancer cells. The metastatic efficiency of different cancer cells is different. Migration of cancer cells to different tissues is an important initial step in metastasis. Initiation of metastasis also depends on adhesion property of the cells, which in turn, involves interaction with extracellular matrix following detachment from the primary sites. Collagen IV is an integral part of the basement membrane, along with other macromolecules like glycoproteins and proteoglycans, laminin, integrins, entactins and dystroglycan [27,28]. The process of invasion is critical for metastasis because the motile cells need to cross the extracellular matrix to enable migration into surrounding tissues [29].

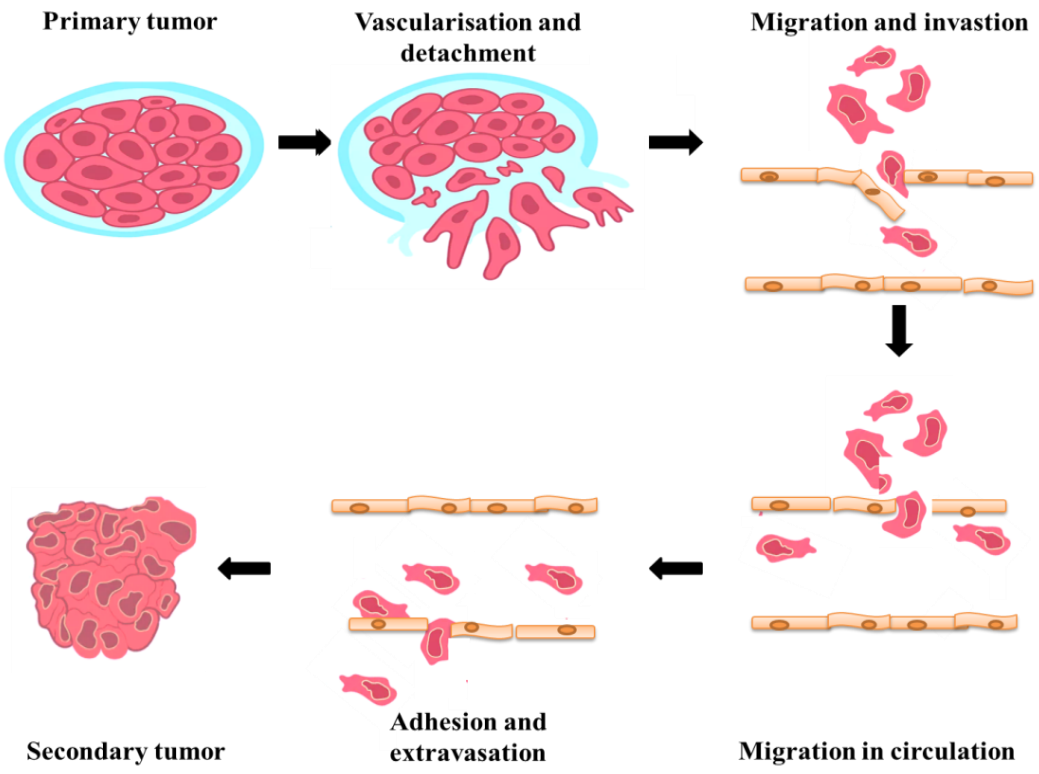


Figure 1.3.: A schematic representation of the process of metastasis

Invasion and metastasis are accompanied by degradation of connective tissues, and as a result expression of matrix-degrading enzymes e.g. matrix metalloproteinases (MMPs) increases. MMP-2 and 9 have been shown to overexpress and contribute to metastatic efficacy of MDA-MB-231 which is the most aggressive cancer cell line [30]. The main sites of metastasis of breast cancer are bone, brain, liver, lungs. Similarly, other cancers like lung cancer metastasize mainly to the adrenal gland, bone, brain, liver; bladder cancer to the bone, liver, lung and colon cancer to liver, lung, peritoneum [24]. Therapies that target primary, as well as metastatic cancer cells, are important for cancer treatment. Current research initiatives are also directed towards strengthening the immune system to combat cancer in conjunction with targeting steps of the metastatic process [24]. Metarrestin, a cancer drug still in an experimental stage with plans for human trials, targets metastatic tumors in the breast and prostate cancer models [31,32]. Zoledronic acid in less-frequent doses targets cancers of bone metastasis and reduces the symptoms [33].

1.4. Cancer treatment approaches and risks

There are various therapeutic options available for cancer that exploits the potential of surgery, radiation, chemicals, hormones, the immune system, stem cell transplant, in addition to targeted and personalized medicine [34]. The type and degree of cancers, decides the nature of treatment to be applicable to a particular patient. Sometimes multiple treatment approaches are used to treat cancer. All these treatment approaches are certainly expensive and have numerous individual side effects [35]. The most frequently employed approach is surgery, where the tumor is removed from the body. The entire tumor or a part of the tumor may be removed to alleviate the symptoms of cancer [36]. There are risks associated with surgery such as infection, pain, bleeding, damage to nearby tissues, and reactions of anesthesia; besides entailing a high monetary burden [36]. In radiotherapy, a high dose of radiation is used to kill the cancer cells, which unfortunately also kills neighboring normal cells resulting in numerous side effects; aggravated by the expenses for maintaining a specialized diet [37]. Chemotherapy may be used alone or in combination with other therapies to stop or slow down the growth of cancer cells and ease cancer-related problems [38]. Chemotherapy is the use of chemical agent *i.e*. drugs to treat cancer and most of the chemotherapeutic agents are expensive and have side effects as they kill or slow down the growth and

development of normal cells. It also frequently demonstrate other symptoms like fatigue, exhaustion and complete debilitation [39]. Immunotherapy is one of the current favorites among treatment measures against cancer. In this therapy, the immune system of the body is enhanced to fight against cancer with agents like cytokines and BCG [40,41]. This approach has a plethora of side effects ranging from flu-like symptoms, vertigo, change in blood pressure, edema, cardiac discomfort, respiratory problems, gastric issues and heightened susceptibility to infection [42]. Targeted therapy is another approach that targets the modifications in cancer cells at different stages [41,43]. These agents may be small molecules or antibodies which function by enhancing the immune system to prevent proliferation, induce apoptosis, inhibit angiogenesis, and starve cancer cells from hormones it needs to grow [43]. The important drawback of this therapy is that some cells become resistant and in most cases finding the right target is difficult. They have multiple side-effects associated with digestion, thrombosis, healing, hypertension, skin and hair [43]. Breast and prostate cancers are often targetted by hormone therapy that manipulates their endocrine growth inducers. A combination of hormone and adjuvant therapy is frequently used in tandem with other cancer treatments [44]. This approach has side effects as it interferes with the body's own mechanisms and reduces the ability to produce the hormones [44]. Hormone therapy is not without its symptomatic fallout in both men and women, especially reflected in reproductive and associated malfunctions, besides nausea, mood changes, and fatigue [44]. Stem cell transplants in cancer therapy are important to restore the blood-forming cells which are lost during the procedure of radiation and/or chemotherapy. In some leukemia and myeloma, this stem cell transplant approach efficiently works against cancer but these procedures are very expensive and have side effects like the risk of infection and bleeding [45]. Another treatment approach is precision medicine or personalized medicine, where doctor selects the most suitable treatment for a patient based on the genetic understanding of the particular patient. Many drugs of targeted therapy are part of this approach. This approach is in the developmental stage and new thresholds are crossed on a regular basis [46]. Doctors and scientists see a bright future in cancer treatment, as it has the potential to easily determine the genetic basis of the disease. Consequently, it allows the patient to get the most effective treatment by narrowing down the trial and error process of selecting best suitable medicines. This will also overcome the inconvenience of multiple combinations

therapies that are regularly required for patients. Though there are various drugs to treat primary cancer, as on date, there is no recognized and market ready anti-metastatic drug [47,48]. Metarrestin, an experimental anticancer drug successfully targets metastatic tumours without affecting others tissues or organs and the clinical trials are underway using the breast and prostate cancer models [31,32].

1.5. Medicinal plants in drug discovery

In search of the best cost-effective medicine against cancer without side effects, researchers worldwide are focusing on natural sources of medicine which could act as precision medicines [49-52]. Medicinal plants play a crucial role in drug discovery against a lot of diseases and as anticancer agents and are gaining increasing acknowledgment [51,53-56]. Medicinal plants are rich sources of bioactive molecules and can be exploited as anti-cancer agents [57,58]. The ethnobotanical approach to drug discovery has been recognized since the seventeen century [59-61]. Plant-derived compounds play an important role in many diseased conditions like Alzheimer's, cancer, diabetes, malaria, inflammation and pain [51]. Numerous leading compounds from plants are in the market or in a clinical trial from both edible as well as non-edible sources [50,51,62]. Studies are in progress to identify more efficacious and cost-effective drugs with minimal side effects [63-66]. Undiscovered compounds from medicinal plants may play a critical role in cancer therapy by causing alterations at the molecular level to treat different cancers. It is very important to address the problem of metastasis along with primary cancer as the 5-year survival rate of metastatic breast cancer patients is about 25%. This suggests the importance of targeted therapy as precision medicines for metastasis along with the tumor/s at the site of origin [67,68]. Despite this, no first-line anti-metastatic drugs are available in the market [47,48].

In the last few decades, numerous traditional knowledge-based commercial drugs have been isolated and commercialized [69-71]. Multiple molecules of medicinal plant origin are currently used as drugs to combat cancer (e.g. vincristine, vinblastine, taxol, paclitaxel, Podophyllotoxin) [72]. North-East India is a well-regarded reservoir of traditional medicinal plants, as it is one of the important biodiversity regions of the world [73]. So in search of the new anti-cancer molecule of natural origin, we did an extensive literature survey and selected a few medicinal plants which have traditional medicinal uses but were not exploited for molecular level elucidation.

1.6. Extraction of phytochemicals

It is crucial that extraction of the active molecules from the plant part at the appropriate time, temperature and solvent are non-toxic to living cells of the recipients [74-76]. Some of the organic solvents used for extraction have high toxicity to living cells like methanol, acetone, chloroform, dichloromethane. In addition to this laboratory safety, cost-effectiveness and working efficiency are important factors to be considered. Extractions with the solvents *e.g.* hexane, ethyl-acetate, ethyl alcohol, and water have almost no/minimal toxicity to living cells, animals, and human beings. Compounds and small molecules that are either hydrophilic or hydrophobic result from water or ethanol based extraction procedures. The latter comprises high hydrophilic composites which are very polar neutral, basic and acidic compounds. Likewise, ethyl acetate extract mainly contains medium hydrophobic compounds like steroids, wax, fatty acids, and alkaloids. Hexane extracts contain low or non-polar hydrophobic compounds with extremely high lipophilicity [77,78]. To allow maximum secondary plant compounds to be extracted into the solvent, use of lower boiling point solvent methanol or higher boiling point solvent ethanol, acetone or acetone: water mixtures for most of the polar and semi-polar constituents are reported. However, for lipophilic compounds, polar solvents like chloroform are used. Use of solvents like acetone, chloroform and diethyl ether are limited due to either their inherent toxicity to liver, inflammability or explosive tendency. If the plant constituent has thermolabile compounds, the use of ethanol and water combination or ethanol are optimal for extraction in cold [79]. A mixture of organic and inorganic solvents in the equal ratio (*i.e.* 1:1) is appropriate for the polar compounds including alkaloids. Therefore proper extraction methods with the most effective, non-toxic solvent system have to be employed to optimize the potential of the extract.

1.7. Objectives

The current situation of India in terms of incidences of breast cancer is very alarming and there is a critical need to combat the situation. Late detection due to ignorance and metastatic potential makes this cancer difficult to treat. There are quite a few generalized options to treat cancer and its metastasis irrespective of the origin or point of malfunction, that does not work often and leads to death within a very short span of time. It is now very exigent to develop new medicines which can target precisely the

point where it originates and the reason why it originated. For this, a safer and cost-effective approach like plant-derived compounds is the most viable option. However, to obtain such novel compounds from plant sources, several steps are to be followed before delivery of the product from "Bench-to-Bedside" such as proper extraction methods, evaluation of its activity in cancerous and normal cells, *in vivo* efficacy, its metabolic kinetics, etc. Based on the above current need and the knowledge available, the following few research questions were raised:

1. Can we select a few candidate plants with anti-cancer activity from the vast reservoir of traditional medicinal plants of North-East India?
2. What is their general mechanism of anticancer action?
3. Whether the selected plant extracts would show anti-metastatic activity?
4. Would these extracts work in *in-vivo* models?
5. Whats are the active compounds in the extracts that could be responsible for the anti-cancer effect?

To find the answer to these questions, the work in this thesis is designed on the following research objectives:

1. **Screening of extracts from selected medicinal plants based on their cytotoxic activity against breast cancer cell lines and phytochemical characterization of the selected extracts**
2. **To study the anticancer activity of two most potent extracts against breast cancer cell lines and identification of active compounds in the selected extracts**

    2.1. To study the anticancer activity of the fruit extract of *Ricinus communis* L. (RCFE) by *in vitro* and *in vivo* approaches and identification of its major compounds.

    a. Efficacy of RCFE for inhibition of migration, adhesion, and invasion in breast cancer cell lines.
    b. To study the efficacy of RCFE to induce apoptosis.
    c. Effect of RCFE on tumor suppression in a syngeneic mouse model.
    d. Identification of bioactive compounds in RCFE and their efficacy against breast cancer cell lines.

2.2. To study the mechanism of anticancer activity of edible tuber *Amorphophallus paeoniifolius* (APTE) in breast cancer cells and identification of major compounds.

a. Effect of APTE on migration, adhesion, and invasion *in vitro*.
b. To study the mechanism of cancer cell death by APTE.
c. Identification of major compound(s) in APTE by HR-LCMS

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