An *in vitro* study on the effects of homeopathic *Radium bromatum* (9CH, 12CH, and 30CH potencies) on cancer cells

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I hereby declare that this mini dissertation is representative of my own work unless explicitly acknowledged. The work has not previously been submitted in any form to the Durban University of Technology or to any other institution for assessment or for any other purpose

Signature of Student — **Date** 07/03/2025

APPROVED FOR FINAL SUBMISSION

Signature of Supervisor
Prof J.J. Mellem — **Date** 11 March 2025

DEDICATION

This work is dedicated to Science and Humanity for the pursuit of knowledge and healthy curiosity.

"The most beautiful thing we can experience is the mysterious. It is the source of all true art and science." – Albert Einstein.

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ABSTRACT

Background:

Cancer is an abnormal growth of cells that can replicate uncontrollably and, in its later stages, spread throughout the body, potentially leading to death. In recent years, cancer has become one of the leading causes of death globally. It is usually characterized by the dysregulation of cell growth and the body's resistance to the normal process of cell death (apoptosis). While conventional cancer treatments can be beneficial, they can also produce harmful side effects, such as nausea, vomiting, hair loss, blood clots, and infertility. The side effects experienced by cancer patients may vary depending on which healthy cells are affected by the treatment for the individual.

Rostock *et al.* (2011); Frass *et al.* (2020); Bagot, Theunissen and Serral (2021) have highlighted the potential benefits of complementary treatments using homeopathic medicines as supportive care for cancer patients to improve their quality of life by alleviating side effects from conventional treatments. Radiation therapy, a commonly used cancer therapy that uses radiation, can result in adverse effects such as fatigue, nausea, vomiting, skin complications, injury or pain at the radiation site, and secondary cancers may develop. Homeopathic *Radium bromatum* has been used to help relieve these side effects in cancer patients who receive conventional treatments. *Radium bromatum* is a homeopathic remedy made from the toxic and radioactive radium bromide, and it is important to study the effects of the highly diluted radium bromide in its homeopathic form on cancer cells.

Aim:

This research study aimed to investigate the anti-cancer activity of homeopathic *Radium bromatum* of different potencies (9CH, 12CH, and 30CH) on cancerous human lung carcinoma (A549) and human hepatocellular carcinoma cell lines (HEP-G2) *in vitro,* with non-cancerous human embryonic kidney cell lines (HEK293) used as a control.

Methods:

The MTT assay method was used to determine cell viability, and cell morphology was observed under an inverted microscope. Triplicate experiments were performed. The results

are presented as mean values with standard deviations (SD). Data were statistically analyzed using a one-way ANOVA test followed by Tukey's test for evaluating the statistical significance of variations observed between the groups. Mean values were considered statistically significant when $p<0.05$.

Results:

The results on morphological changes were observed after 48 hours post-treatment, showing preliminary evidence of the ability of *Radium bromatum's* anti-cancer potential against lung and liver cancers, while sparing normal kidney cells. The decrease in malignant cells compared to non-malignant cells was significant as demonstrated by the MTT assay, and assessed under the inverted microscope. Rad-br 9CH demonstrated the most significant anti-cancer effects, including reduced cell proliferation and viability, thus demonstrating cytotoxicity and cell termination of the cancer cells through possible apoptotic pathways.

Further assessments demonstrated anti-proliferation activities of the various *Radium bromatum* potencies, with Rad-br 9CH showing the most significant effect towards cancerous cells A549 and HEP-G2. While the overall cell cytotoxicity was minimal, there was an increase in cell growth inhibition associated with increased concentration of the different samples. The samples also exhibited low cytotoxicity towards healthy cells (HEK293). The data showed that the *Radium bromatum* potencies had higher inhibition effects compared to the placebo, indicating that all the *Radium bromatum* samples were more effective than the placebo. Comparison between the cancer cells, A549 responded more effectively than HEP-G2 when exposed to *Radium bromatum* overall, showing higher cytotoxicity. Thereby, indicating that *Radium bromatum* in different potencies of 9CH, 12CH, and 30CH were able to induce cytotoxicity in the cancerous cells, causing reduced cell viability and cell death.

Additionally, results observed in this study also showed that treatment with the placebo failed to elicit similar responses to the highly diluted *Radium bromatum* samples, highlighting that the *Radium bromatum* samples were superior to the placebo, and proving that the homeopathic medicines contain molecules or particles of the original source for it to cause a reaction beyond the placebo as demonstrated in this study. This study also exemplifies that homeopathic medicines cannot be interpreted as placebo. However, more research is needed in the form of an *in vivo* rat model to examine the systemic effects of this homeopathic preparation in order to facilitate its therapeutic application in cancer management.

Conclusion:

This research offers preliminary *in vitro* evidence of the homeopathic *Radium bromatum's* potential as an anti-cancer agent owing to its anti-proliferative properties.

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DEFINITION OF TERMS

Avogadro's constant: Refers to the number of chemical units – atoms, molecules or ions – presented in one mole of any substance. It is also called Avogadro's number and is named after an Italian chemist, Amadeo Avogadro in the early 1800's. The Avogadro's constant is therefore defined as the number of carbon-12 (^{12}C) atoms in exactly 12 grams of carbon-12, i.e. a value of $6.02214154 \times 10^{23}$ mol^{-1} (Schmit and Pollard 2016).

Dilution: In the context of manufacturing homeopathic medicines, the initial dilution ratio is 1 part starting material to 9 parts diluent (1/10, decimal potencies) or 1 part starting material to 99 parts diluent (1/100, centesimal potencies) to produce the 1st potency. The whole process involves trituration and/or serial dilution with succussion applied at each step. Starting materials are commonly sourced from nature, i.e. plant, mineral or animal substances (Bell and Koithan 2012).

Doctrine of Signatures: One of the chief methods practiced since ancient times for acquiring knowledge regarding medicinal plants. A doctrine which draws associations with the appearance, shape, color, taste, etc., of a plant or substance to that of a disease as an indicator for its medicinal practical application (Wood 2005: 19-20).

Homeopathy: Its basic principle is with respect to 'like cures like' conveying that what a medicinal substance is able to cause, conversely it is also able to cure. In other words, a disease can be cured by a medicinal substance of which it yields similar symptoms in the healthy person, the principle of similarity (Cukaci *et al*. 2020).

***In vitro*:** Latin meaning 'within the glass'. An experimental process performed outside of a living organism, typically in a test tube or culture dish (Martin 2015: 399).

***In vivo*:** Latin meaning 'within the living'. Experiments or procedures that are conducted in or on a living organism such as animals, plants or persons (Martin and Law 2017: 387).

Pharmacopoeia: A book which contains a listing of drug substances used in medicine describing specifications of their source, composition, properties, manufacturing and quality control standards, dosages as well as dispensing of drug substances (Martin 2015: 580).

Placebo: A non-medicated inactive drug or substance, given as a control in research, which is used for comparison with a medicated substance to be tested in controlled experiments; or given to placate the patient for the psychological benefit (Martin and Law 2017: 574).

Potency: In the context of homeopathy, potency translates to the strength of the remedy in a stage of altered therapeutic activity subjected to a measured process of potentization of the source material; the strength of the medicine is measured through ascending degrees of dilution which is expressed as the potency of the medicine (Ernst 2016: 127).

Potentization: Alternatively known as dynamization. It is a process of manufacturing homeopathic medicine by trituration, succussion and dilution. The founder of Homeopathy claimed this course of action transformed the original substance's properties and generated dynamical energies, which in turn provided healing influences in its minute dosages (Nandy and Bhar 2021).

Proving: Deriving from the Latin word '*probare*' which means 'to try' and from the German word '*pruefung*' meaning 'the test'. A proving is an experiment to ascertain what a substance can cause by dispensing it to healthy volunteers – so as to take note of the objective and subjective symptoms being produced (Jayasuriya 2010: 713).

Succussion: A process entailing vigorous vertical shaking (i.e. agitation) of the liquid solution. Otherwise known as 'dynamization'. Succussion can occur by means of mechanical or by hand (Elia *et al*. 2014).

Trituration: A method of dilution of insoluble solid source material prepared by grinding with lactose. Trituration is the first step in preparing homeopathic medicines in a ratio of 1:99. Hence, 1 mg of solid substance mixed and triturated with 99 mg of lactose to prepare the 1st potency. The 2nd potency is obtained by mixing and triturating 1 mg of the 1st potency with 99 mg of lactose. Up to the 3rd potency it can be reached through the trituration method, whilst subsequent potency levels are prepared through the liquid dilution process (Basu *et al*. 2017).

Vital force: A term also known as life force or vital energy described by Hahnemann who recognized an enlivening element within the body, which governs functions and feelings, for the purpose of one's existence. This vital force maintains health, balance and harmony within

the organism, and without this force the body is merely an inert corpse. According to Hahnemann, falling ill is an indication of a disturbed life force, and through homeopathic treatment it stimulates the life force to promote the body's natural abilities to self-heal and restore homeostasis in the organism (Kunzli, Naude and Pendleton 1982: Aphorisms 9-11; De Schepper 2001: 12; Ernst 2016: 115).

LIST OF ABBREVIATIONS

%	Percent
°C	Degrees Celsius
α	Alpha
µL	Microliter
10M	10 000C
50M	50 000C
A549	Lung adenocarcinoma cell line
ATP	Adenosine triphosphate
BCE	Before the common (or current) era
Br	Bromine
C	Centesimal dilution process (1:100).
CH	Centesimal Hahnemannian dilution process (1:100).
CK	Centesimal Korsakovian dilution process
cm	Centimeter
cm^2	Centimeters squared
CO_2	Carbon dioxide
D	Decimal dilution process (1:10).
DMEM	Dulbecco's Modified Eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribo Nucleic Acid
DUT	The Durban University of Technology
EtOH	Ethanol
FBS	Fetal bovine serum
GHP	German Homeopathic Pharmacopoeia
h	Hour
HEK293	Human embryonic kidney cell line
HeLa	Henrietta Lacks cervical cancer cell line
HEP-G2	Human hepatocellular carcinoma cell line
K	Korsakovian dilution process
KZN	KwaZulu-Natal
LM	Quinquagenimillesimal dilution process (1:50 000) i.e. 50 000C
M or 1M	1000C

mg	Milligram
Min.	Minutes
mL	Milliliter
MT	Mother Tincture
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
nm	Nanometer
NMR	Nuclear magnetic resonance
PBS	Phosphate-buffered saline
Q	Quinquagenimillesimal dilution process (1:50 000)
Ra	Radium
$RaBr_2$	Radium bromide
$RaCl_2$	Radium chloride
Rpm	Revolutions per minute
Rv	Required volume
UV	Ultraviolet
X	Decimal dilution process (1:10).

CHAPTER 1: INTRODUCTION

1.1 Overview

Cancer is a global health problem and the second-leading cause of death following after cardiovascular diseases (Global Burden of Disease 2019 Cancer Collaboration 2022). The World Health Organization (WHO) reported an estimated 20 million new cases of cancer and approximately 9.74 million cancer-related deaths in 2022. The WHO predicts a further increase in cancer incidence by 2040, with an estimated 29.9 million new cases and 15.3 million deaths worldwide. In South Africa, there were around 111,000 new cases and over 64,500 cancer-related deaths in 2022. The incidence of cancer in South Africa is on the rise, with projections of approximately 175,000 new cases and 110,000 cancer deaths by 2040 (International Agency for Research on Cancer 2023).

The exact cause of cancer is challenging to determine, however several factors have been identified as contributing risk factors for developing cancer, including smoking, alcohol consumption, social lifestyles, occupational and environmental exposures or carcinogens (Cassidy *et al.* 2015: 8-15). Preventive measures can help lower the risk of cancer by maintaining a healthy weight, avoiding tobacco, reducing intake of alcohol and processed foods, regular health check-ups and cancer screenings (Ayenigbara 2023). Conventional cancer therapies usually involve surgery, radiotherapy, chemotherapy and pharmaceutical drugs (Debela *et al.* 2021). However, these treatments face challenges due to the complex nature of cancer, such as cancer's ability to develop resistance to conventional therapies and the toxicity of these treatments to normal cells (Chakraborty and Rahman 2012; Rezayatmand, Razmkhah and Razeghian-Jahromi 2022). Cancer treatment is also extraordinarily costly, making it inaccessible to many patients in South Africa who rely on public healthcare, which strains the healthcare budget and limits treatment options (Sartorius *et al.* 2016; Mattila, Babar and Suleman 2021). Although conventional cancer treatments offer life-saving prospects, they often cause various unpleasant side effects (Vickers 2018: 25), in which radiotherapy and chemotherapy can be stressful for patients, therefore further impacting their health and quality of life (O'Reilly *et al.* 2020).

Radium and radiation therapy are closely connected due to their radioactive properties, with radium serving as a radiation source (Gianfaldoni *et al.* 2017). Radiation therapy, or radiotherapy, is routinely used for cancer patients to assist in diagnosis and treatment, but it

was discovered in the early 20th century that radiation exposure can also cause cancer (American Cancer Society 2014).

The health effects of radiation exposure depend on the dose and duration of exposure. Higher doses and longer durations increase the risk of developing adverse side effects such as anemia, dental and bone issues, and an increased risk of cancer due to DNA damage and cell mutations. (Blows 2005: 334; Massachusetts Department of Public Health 2017; Adler, Carlton and Stewart 2023: 122-123).

Chemotherapy is a drug-based approach aimed at destroying rapidly growing cancer cells in the body, but it also negatively affects normal cells. Drug resistance poses a major challenge in this form of cancer therapy (Chu and DeVita Jr. 2023: 1-2). Patients undergoing chemotherapy may experience common adverse effects such as nausea, vomiting, fatigue, impaired functioning, hair loss, anemia, decreased appetite, diarrhea, and cognitive changes. These side effects can have a significant impact on their quality of life (Gibbons and Groarke 2018).

The adverse effects of cancer can be devastating and life-altering for many individuals worldwide. The rising prevalence of drug-resistant cancers underscores the importance of continued research and treatment development. Therefore, in scientific experiments, using human cell lines as practical and valuable models to advance new cancer therapies is essential (Mirabelli, Coppola and Salvatore 2019).

Since 1952, cell culturing has been a common technique used by researchers globally for *in vitro* studies, particularly in cancer research. Cancer cell lines mirror their parent cells and can replicate and maintain certain genetic functions similar to the original cells, highlighting their importance in model systems for medical research (Mirabelli, Coppola and Salvatore 2019). Cell lines are widely used in cancer research to test hypotheses and conduct *in vitro* drug testing for the identification of anti-cancer activities. In this study, a homeopathic preparation was selected for testing its efficacy in cancer treatment.

Homeopathy was founded in the early 1800s by German physician Dr. Samuel Hahnemann. It is based on the principle of 'like cures like', where natural substances that mimic disease symptoms are used to stimulate the body's self-healing processes (Schmukler 2006: 7).

This concept proposes that a substance that induces symptoms in a healthy person can alleviate those same symptoms in a sick individual, known as the principle of similarity (Schmukler 2006: 7-12). Although homeopathy is practiced globally, it is also associated with limitations and controversies, highlighting the lack of scientific evidence to support homeopathy's safety and effectiveness including studies arguing that it is equivalent to the placebo effect (Mukerji and Ernst 2022; Moriarty 2024). In this quantitative experiment, the homeopathic medicine selected to test against cancer cells is *Radium bromatum*.

This substance is sourced from radium bromide, originating from pure radium, which is a radioactive alkaline earth metal obtained from a material called pitchblende containing uranium (Vermeulen 2004: 1116; Ropp 2013: 69). In modern times, refining uranium yields radium and it is commercially sold as radium bromide ($RaBr_2$) or radium chloride ($RaCl_2$) instead of in its pure form (National Center for Biotechnology Information 2022). With reference to Ropp (2013: 69) both radium chloride and radium bromide are used in medicine for cancer treatments.

The choice to explore the application of *Radium bromatum* is based on its clinical uses compared to some of the side effects associated with radiation therapy. Sourati, Ameri and Malekzadeh (2017) confirmed that radiation exhibits anti-cancer abilities but also leads to adverse effects. Depending on the targeted area of the body, some of the adverse effects observed include radiation-induced dermatitis, hair loss, oral mucositis, esophagitis, gastritis, pneumonitis, pericarditis, cystitis, fatigue, nausea, and vomiting. Taking this into account, Boericke (2021: 543-544) highlights the uses of radium in a highly diluted form categorized as a homeopathic medicine known as *Radium bromatum*. Clinical applications of *Radium bromatum* include treatment of skin disorders such as moles, ulcers, cancers, itching, and burning skin. Other indications encompass nausea, abdominal pains, throat pains, dermatitis, male and female disorders, as well as profound weakness and severe pain throughout the body, including the joints.

No research has been conducted on *Radium bromatum* in various potencies to assess its anti-cancer activities *in vitro*, so its impact on cancer cells is still unknown. However, limited *in vivo* studies suggest that *Radium bromatum* may be a helpful supportive therapy in integrative oncology to help manage the side effects of conventional cancer treatments, with no reported adverse effects linked to homeopathic treatment (Rossi *et al*. 2018; Samuels *et al*. 2018; Shukla *et al*. 2020; Veyrier *et al*. 2024). Several *in vitro* research on similar homeopathic

preparations for cancer treatment have shown anti-cancer effects, including cytotoxicity, anti-proliferation, DNA fragmentation, gene modifications, cell cycle arrest, and apoptosis in cancer cells, thereby demonstrating homeopathy as a potential anti-cancer therapy in cancer treatment (Arora and Tandon 2015; Mondal, Samadder and Khuda-Bukhsh 2016; Loonat *et al*. 2022; Gunes *et al*. 2024).

This *in vitro* research study aimed to investigate the effects of homeopathic *Radium bromatum* (9CH, 12CH, and 30CH potencies) as well as a placebo on cancer cell lines. The same techniques were applied to non-cancerous cell lines for comparison. The potencies chosen are standard concentrations commonly used in everyday practice by homeopathic practitioners, with the CH potency designated as centesimal, the original scale practiced by Hahnemann (Kayne 2006). A549, HEP-G2, and HEK293 cell lines were selected for this experiment. The A549 cell line is a model for human lung cancer, a prevalent form of cancer. HEP-G2 cells represent human liver cancer, another significant cancer. HEK293, derived from human embryonic kidney cells, are non-cancerous and frequently used in cancer research as a control. All these cell lines are widely used to study cancer development, progression, and treatment response (ATCC 2024).

This study addresses a critical gap in knowledge regarding *Radium bromatum*'s efficacy, particularly on specific potencies, where the expected outcome uncovering its potential anti-cancer effects will provide valuable insights previously unavailable to the scientific community.

1.2 Rationale for the Study

As cancer is a global issue with a significant increase in incidence, more research needs to be conducted on this disease. Conventional cancer therapies are beneficial, but they come with many challenges. Therefore, the exploration of new or complementary treatment options for cancer is crucial both nationally and internationally for the benefit of patients.

Research on the effects of *Radium bromatum* on cancer cells is limited. Due to anti-proliferative *in vitro* evidence of various homeopathic medicines, it is hypothesized that *Radium bromatum* may possess anti-cancer properties against cancer cells *in vitro*. While crude forms of radium or radium bromide have shown effects on cancers, there is a lack of research on radium bromide in high dilution (i.e., *Radium bromatum*) and its impact on cancer cells, particularly in terms of potencies. Since *Radium bromatum* is a homeopathic preparation derived from the

toxic and radioactive radium bromide, it is essential to investigate the effects of highly diluted radium bromide in its homeopathic form on cancer cells themselves.

In this study, the purpose was to evaluate whether the homeopathically-potentized high dilutions of *Radium bromatum* (9CH, 12CH and 30CH) have any anti-cancer effects on cancer cells *in vitro*. Normal cells were used as a control and the research variables were the growth of the cancer cells.

1.3 Aim and Objectives

1.3.1 Aim:

To investigate the effects of homeopathic *Radium bromatum* in different potencies and a placebo on both cancerous and non-cancerous cell lines.

1.3.2 Objectives:

- To prepare *Radium bromatum* (9CH, 12CH, and 30CH) and the placebo in deionized water from stock *Radium bromatum* (8CH, 11CH, and 29CH) and placebo all in 96% ethanol, respectively, using the homeopathic potentization method.
- To assess the cell growth inhibition effects of *Radium bromatum* (9CH, 12CH, and 30CH) on cancerous human lung carcinoma cell lines (A549), human hepatocellular carcinoma cell lines (HEP-G2), and non-cancerous human embryonic kidney cell lines (HEK293) using the MTT assay.
- To assess the cell morphological change using an inverted microscope.
- To compare the effects of *Radium bromatum* and the placebo (negative control).
- To compare the effects of *Radium bromatum* at various potencies on healthy (positive control) and cancer cell lines.

1.4 Importance of the study

It is crucial for the ongoing search for new or supportive cancer treatment options to enhance outcomes for cancer patients. Chemotherapy encounters significant challenges, including drug resistance, reduced efficacy, and undesirable side effects. Therefore, there is a continued need for new cancer treatment approaches that are more effective and less toxic.

This study aims to examine the effects of homeopathy at a cellular level to assess if the selected medicine exhibits potential anti-cancer activities. *In vitro* findings provide valuable

evidence but are limiting, thus potentially informing future research aimed at validating the findings in animal models, and ultimately in clinical trials to assess its anti-cancer safety and effectiveness in cancer patients.

This research could serve as a vital initial stage in broadening studies, influence integrative oncology or patient-care strategies, and expedite research on the use of homeopathy as an adjunctive treatment for cancer patients *in vivo*.

CHAPTER 2: LITERATURE REVIEW

2.1 Cancer

2.1.1 Introduction

Non-communicable diseases are rapidly increasing and contributing to higher morbidity and mortality rates in the 21st century (Khamis 2019). These diseases are chronic conditions that include cardiovascular diseases, stroke, cancers, diabetes and kidney diseases (World Health Organisation 2023b). Cancer results from uncontrolled cell divisions in the body, which can invade and destroy normal cells, causing an imbalance within the body (Almalki 2023). It is a disorder that arises from acquired mutations triggered by damage to the genome, leading to genetic modifications, genomic instabilities, diminished cell death capabilities, angiogenesis formation, and metastatic growth (Meyerson and Pellman 2011). DNA damage can result from exposure to UV radiation, ionizing radiation, and chemical carcinogens (Cassidy *et al.* 2015: 19). Therefore, cancerous cells are abnormal cell growths replicating uncontrollably and can metastasize throughout the body in later stages, potentially leading to the death of the patient (World Health Organisation 2023a).

2.1.2 Epidemiology of Cancer

In recent years, cancer has been identified as the second-leading cause of death globally, following cardiovascular diseases (Global Burden of Disease 2019 Cancer Collaboration 2022; American Cancer Society 2024). According to the International Agency for Research on Cancer (2023), there were approximately 20 million new cancer cases and an estimated 9.74 million cancer-related deaths reported worldwide in 2022. The WHO predicts that by 2040, the cancer trajectory is expected to reach to an estimated 29.9 million new cases, with approximately 15.3 million cancer-related deaths globally (International Agency for Research on Cancer 2023).

In South Africa, over 111,000 individuals were diagnosed with cancer in 2022, resulting in more than 64,500 cancer-related deaths. By 2040, the incidence and mortality figures are projected to increase to approximately 175,000 new cases and 110,000 deaths (International Agency for Research on Cancer 2023). In 2022, Asian countries accounted for 49.2% of all cancer cases and 56.1% of cancer-related deaths, followed by Europe (22.4% incidence, 20.4% mortality), America (21.1% incidence, 14.9% mortality), Africa (5.9% incidence, 7.8% mortality), and Oceania (1.4% incidence, 0.8% mortality) (Bray *et al.* 2024).

Globally, it is estimated around one in five men or women are diagnosed with cancer, with mortality rates averaging at one in nine for men and one in twelve for women (Bray *et al.* 2024). The most common cancer diagnoses worldwide include lung cancer (incidence at 12.4% of all cases), female breast cancer (11.6%), colorectal cancer (9.6%), prostate cancer (7.3%), stomach cancer (4.9%), liver cancer (4.3%), thyroid cancer (4.1%), cervix uteri cancer (3.3%), bladder cancer (3.1%), and non-Hodgkin lymphoma (2.8%) as the tenth most frequently diagnosed type of cancer. The top three causes of cancer-related deaths are lung cancer (18.7% of all deaths), colorectal cancer (9.3%) and liver cancer (7.8%). Breast cancer is the most frequent diagnosis for women, while lung cancer is the most common type for men (Bray *et al.* 2024).

Cancer continues to be a significant health challenge in both developing and developed countries, with complex causes that include tobacco use, alcohol consumption, environmental contaminants, infectious agents, custom habits and lifestyle influences (Cassidy *et al.* 2015: 8-15).

2.1.3 Cancer Risk Factors

Environmental and occupational factors that play a part in cancer deaths include tobacco use, obesity, diet, radiation exposure, infections, heredity, stress, environmental pollutants, and lack of physical activity (lifestyle, economic and behavioral factors) (Lewandowska *et al.* 2019). Smoking contributes to 90% of lung cancer cases with passive smoking also identified as a potential carcinogen. Additionally, smoking is linked to kidney, stomach, pancreas, larynx, and bladder cancers (Cassidy *et al.* 2015: 206). Globally, tobacco is responsible for approximately one in four cancer-related deaths (Jassem 2019).

Physical inactivity, obesity, and poor diet contribute to about 15-20% of cancers worldwide (American Cancer Society 2024). Research revealed that physical inactivity increases cancer risk, and data showed that a sedentary lifestyle is associated with chronic diseases (Marino *et al.* 2024). There is growing evidence of the link between obesity and cancer risk attributable to excessive nutrient intake or imbalanced nutrition (Zitvogel, Pietrocola and Kroemer 2017: 846). Specific foods are also linked to certain types of cancers such as a high-salt diet causing gastric cancer (American Cancer Society 2024), aflatoxin B1 causing liver cancer and chewing betel nut causing oral and esophageal cancers (Park *et al.* 2008; Asghar, Ahmed and Asghar 2020).

Exposure to ionizing and non-ionizing ultraviolet radiation contributes to about 2-3% of cancer cases (Lewandowska *et al*. 2019). Radon gas and medical imaging are sources of ionizing radiation which are relatively weak mutagens and carcinogens. When combined with other cancer-causing agents like tobacco smoke, radiation can have a more damaging effect. Prolonged exposure to ultraviolet rays from sunlight can result in skin cancer (Ayenigbara 2023).

Hereditary factors are also a contributory cause in the development of cancer in some cases, with up to 10% of the population carrying genetic mutations which makes them genetically predisposed to the disease (Wang 2016). Examples of cancer owing to hereditary causes include inherited mutations in the BRCA1 and BRCA2 genes for ovarian or breast cancers, and familial adenomatous polyposis for colorectal cancer (Wang 2016). Some hormones also play an important role in the cancer development by promoting cell proliferation activity (Lappano *et al*. 2022). Understanding the specifics of cancer development is relevant to comprehend, as cancer is a major health concern. The process of carcinogenesis is complex, multistage and multifactorial (Compton 2020: 26).

2.1.4 Carcinogenesis

Carcinogenesis is a multistep process involving biomolecular and cellular events that lead to cell transformation and tumor development undergoing complex phases including initiation, promotion, progression, and metastasis. During the initiation phase, normal cells undergo gene mutations either spontaneously or as a result of carcinogenic exposures. These gene modifications lead to dysregulation of molecular signaling pathways, causing genome instability and affecting cell proliferation, differentiation, and survival. Chemo-preventive agents can help prevent neoplastic formation during the initiation process (Cassidy *et al*. 2015).

The promotion phase follows, where pre-malignant cells begin to proliferate in a relatively long but reversible process of event. Chemo-preventive agents can influence the rate of cell growth during this stage. The progression phase occurs between pre-malignant and malignant cells, where neoplastic cells undergo phenotypic and genetic changes. This results in the rapid increase of the size of the tumor and further mutations that can induce invasive and metastatic properties (Compton 2020: 30).

Metastasis is the final phase involving malignant cells disseminating from the primary site to other areas of the body through the blood or lymphatic systems. Chemo-preventive interventions can be used to prevent the evolution of cancer metastases. **Figure 1** provides an overview of the carcinogenesis process (Cassidy *et al*. 2015: 212).

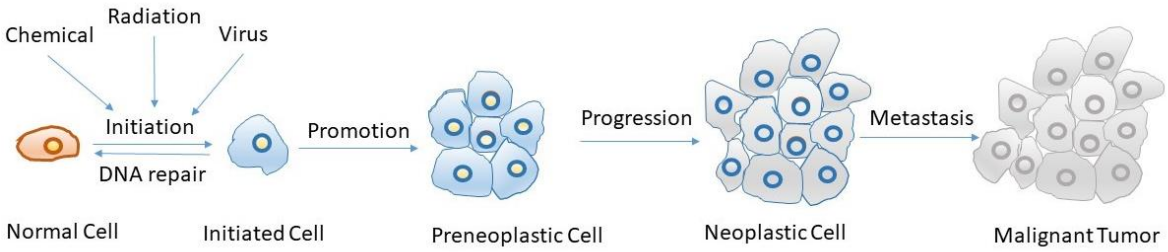

Figure 1: The multistep process of carcinogenesis illustrating the transformation from a normal cell into malignant tumor. Initiated cells will progress to cancer triggered by various cancer risk factors such as radiation, environmental pollutants, and infections. The carcinogenesis process undergoes four main complex phases including initiation, promotion, progression, and metastasis (Libretexts Medicine 2024).

2.1.5 Characteristics of Cancer Cell

A cancerous cell is the resultant manifestation of the carcinogenesis development. The morphology of cancer cells differs from normal cells; the cancerous cell presents with a large nucleus, pronounced or multiple nucleoli, small cytoplasm, it is irregular in is shape and size, and are poorly differentiated from normal cells (Bidram *et al*. 2019). The features of cancer cells exhibit several biological hallmarks characteristic of malignancy, including autonomy in proliferation due to the ability to activate oncogenes, evasion of growth-inhibitory signals by means of deactivation of tumor suppressor genes, resistance to apoptosis by suppressing and inactivating apoptotic genes and pathways, uncontrolled replication through the activation of specific genes or pathways, sustained angiogenesis where cancer cells are able to chemically signal for the formation of new blood supply to allow delivery of oxygen and nutrients (tumor angiogenesis), invasion of tissue and metastases in which cancer cells spread to other parts of the body causing further damages to normal tissues and its surrounding environment, the capacity to reprogram metabolic pathways, and lastly the ability to avoid immunological destruction by natural killer cells, T and B lymphocytes and macrophages (Hanahan and Weinberg 2011; Chakraborty and Rahman 2012; Singh *et al*. 2016).

2.1.6 Oncogenes and Tumor Suppressor Genes

Two specific types of genes, oncogenes and tumor suppressor genes, play crucial roles in regulating the cell cycle of normal cells. Initially, proto-oncogenes control normal cell growth and differentiation, however when a proto-oncogene undergoes DNA mutation, it transforms into an oncogene, leading to overexpression or the formation of cancer (Kontomanolis *et al.* 2020). Examples of commonly activated oncogenes include *RAS* and *MYC* that can upregulate angiogenesis signals and other cancer-related characteristics (Almalki 2023).

Proto-oncogenes promote cell proliferation, while tumor suppressor genes are responsible for downregulating cell growth (anti-proliferation). The tumor suppressor genes are transcription factors that are also involved in DNA repair by halting cell division in response to DNA damage or cellular stress. During the DNA repair process, these genes assist in preventing mutations from transferring on to daughter cells. The *P53* and *RB1* genes are typical examples of tumor suppressor genes participating in modulating the normal cell cycle, cell division, and apoptosis. Mutations in tumor suppressor genes have damaging effects leading to disabled or inhibited DNA repair functions, ultimately resulting in the development of cancer (Kontomanolis *et al.* 2020).

Specific genes, such as *MYC, BAX*, *P53*, and *E2F*, assist in the activation of apoptotic pathways. While in contrast, genes like *RAS*, *BCL2* and *ABL* prevent programmed cell death. Dysregulation or dysfunction of apoptosis is accountable for the formation of cancer (Kontomanolis *et al.* 2020). In addition to genetic factors influencing cell growth, various other factors also play a role in the development of tumors.

2.1.7 Factors Controlling Tumor Growth

2.1.7.1 Genetic Instability

Tumor growth is influenced by several factors that stimulate or promote tumor activity, these include genomic instability, cell cycle dysregulation, and apoptotic impairment. Genetic instability is a driving feature in tumorigenesis, leading to the accumulation of extra chromosomes or DNA (aneuploidy), chromosomal inversions or deletions, telomere damage, epigenetic changes, and other genetic alterations. This instability inevitably results in defective

gene regulation of the normal cell cycle, malfunctioning of DNA repair and the progression of malignancy (Ferguson *et al.* 2015).

Research has shown that certain dietary components rich in antioxidants such as vitamin B3 (niacin), vitamin B9 (folate), and vitamin B12, vitamin C, vitamin D, carotenoids, selenium, and glutathione, as well as nutraceuticals like resveratrol, are supportive in the prevention and stabilization of the genetic integrity, suggesting potential anti-tumor effects. However, some antioxidants like β-carotene may exhibit pro-tumorigenic activities in certain cases (Ferguson *et al.* 2015). Subsequently, each normal cell goes through a regulated cell cycle, but disruptions of this process can lead to the development of malignant tumors and eventual cancer (Almalki 2023).

2.1.7.2 Cell Cycle Dysregulation

The cell cycle is a controlled mechanism that coordinates a series of events within a cell to activate cellular division, resulting in two genetically identical cells. It also activates DNA repair of damaged cells by initiating cell cycle arrest to maintain genomic integrity. Simultaneously, cell cycle checkpoints ensure a regulated process to prevent genetic errors that could lead to uncontrolled proliferation or cell immortality. However, cell cycle dysregulation occurs when cancer-related mutations disrupt the cycle causing a network of malfunctioning pathways that include faulty cell cycle checkpoints, atypical cyclin expression and activity, as well as alterations or overexpression of cyclins and cyclin-dependent kinases (CDKs) that are involved in the control of the cell cycle. When the integrity of the cell cycle is disturbed it can result in tumorigenesis or carcinogenic processes such as continuous cell growth, immortal replication, resistance to apoptosis and the potential for metastasis (Almalki 2023).

2.1.7.3 Apoptosis

Apoptosis, also known as programmed cell death, is a crucial and tightly regulated process in cell biology that is also responsible for preventing uncontrolled malignancy. It can be triggered through two main pathways: the intrinsic and extrinsic pathways. Within the intrinsic pathway, internal signals prompt cells to self-destruct in response to cellular stressors like reduced growth factors and deprivation of oxygen. Whereas the extrinsic pathway involves external signals from neighboring cells, such as ligands binding to death receptors on the cell surface to activate the cell's self-destruction process (Chaudhry *et al.* 2022).

The sequence of events in both pathways induce cell death by activating the initiator caspases, which are protease enzymes that then trigger effector caspases, in order to cause proteolytic cleavage of regulatory proteins and ultimately cell termination (Chaudhry *et al.* 2022).

Specific proteins such as Caspase-9, SMAC and Bcl-2 are key regulators in the intrinsic pathway, while extrinsic pathway mediators such as TRAIL (TNF-related apoptosis-inducing ligand receptors), TNF (tumor necrosis factor), and Fas (CD95/APO1) receptors are involved in promoting apoptotic cell death (Chaudhry *et al.* 2022).

In addition to these pathways, there are caspase-independent apoptotic pathways that involve mitochondrial pro-apoptosis proteins, such as AIF (apoptosis-inducing factor) and endonuclease-G. These pathways suggest that apoptosis activation can occur without the involvement of caspases (Negara *et al.* 2020).

Cancer is an intrinsically complex and multifactorial disease that arises from the dysregulation of genomic stability, leading to uncontrolled cell growth which ultimately impacts on the patient's overall health and well-being. Therefore, it is imperative to take preventive measures against cancer development.

2.2 Prevention and Conventional Treatment for Cancer

Cancer prevention helps reduce one's risk of developing cancer by making healthy choices. The important preventive methods for most cancers include making wholesome dietary changes, stopping the use of tobacco products, regular exercise, maintaining a healthy body weight, getting adequate sleep, undergoing cancer screenings, avoiding unnecessary radiation exposure, effectively treating inflammatory diseases, and taking nutritional supplements that aid immune function. These practices can contribute to a healthier and longer life (Ayenigbara 2023).

Conventional treatment strategies for cancer usually involve radiation therapy, chemotherapy and surgery, and often these modalities may be applied synergistically to help patients overcome the disease. Recent advancements in oncology have introduced other treatment modalities that include targeted therapy, immunotherapy, nanoparticles, stem cell therapy, natural antioxidants, radionics, sonodynamic therapy, and ablation therapy (Debela *et al.*

2021). Although conventional cancer therapies have led to a decline in cancer incidences, they come with many challenges and limitations (Debela *et al*. 2021: 2).

The high cost of oncology drugs and cancer treatments is a well-known issue, making cancer a costly disease to manage both globally and locally (Sartorius *et al*. 2016). In 2018, the Medical Brief reported a severe shortage of radiation oncologists in South Africa's public healthcare sector, highlighting the escalating demand for cancer specialists and equipment to meet the rising cancer rates in the country (Medical Brief 2018; van Dyk 2018).

Drug resistance to chemotherapy is a major problem resulting in cancer relapses, which limits its therapeutic effectiveness, and many of the chemotherapeutic agents are toxic to healthy cells (Rezayatmand, Razmkhah and Razeghian-Jahromi 2022). In general, conventional cancer treatments often result in various unwanted side effects (Vickers 2018: 25). Additionally, radiotherapy and chemotherapy can create a great deal of strain on patients, potentially causing further health complications and reducing their quality of life (O'Reilly *et al*. 2020).

2.2.1 Radiation Therapy

Radium and radiation therapy are closely associated due to their radioactive properties, with radium serving as a source of radiation (Gianfaldoni *et al*. 2017). Radiation therapy, also known as radiotherapy, is routinely practiced in the treatment against cancer to eliminate localized cancerous cells and reduce the risk of metastasis (Rangel 2013: 74). The types of radiation effective in radiotherapy consist of electromagnetic radiation, such as X-rays and Gamma-rays, as well as particulate radiation represented by neutrons, electrons, and protons. Radiation therapy can be administered as an internal or external application, using high-energy radiation or radioactive substances to control or kill malignant cells, in addition to inhibiting their proliferation and replicating inclinations. However, radiation not only affects cancer cells but also normal cells, leading to potential side effects (Gianfaldoni *et al*. 2017).

Emerging research on the application of local radiation therapy not only demonstrates selective eradication of tumor cells within a localized area, but also triggers the host's immune system to attack residual tumor cells at specifically irradiated sites as well as distant sites (abscopal effects) (Formenti and Demaria 2009; Golden and Apetoh 2015). Radiotherapy activates ICD (immunogenic cell death), a new form of cell termination, to initiate molecular signals involving the translocation of CRT (calreticulin), and the release of ATP (adenosine

triphosphate) and HMGB1 (high-mobility group box 1) for the pivotal stimulation of $CD8^+$ T-cells which targets and kills infected and cancerous cells. **Figure 2** shows anti-tumor immunity in response to radiation therapy (Golden and Apetoh 2015). Kerr *et al.* (2016: 587); Veness *et al.* (2019) highlights the significant role of radiotherapy in modern cancer treatment as a non-surgical option that can assist in reducing the high risk of cancer-related morbidity and mortality. Patients undergoing radiotherapy may experience a range of side effects, with some experiencing few or none, while others may suffer from multiple side effects. Fatigue, nausea, and vomiting are the most common negative effects experienced following treatment. Other adverse effects may include injury or pain at the radiation site, secondary cancers may develop due to radiotherapy, skin complications may develop, as well as specific symptoms may occur related to the radiation site such as alopecia, dental caries, incontinence, fertility issues, loss of appetite, temporomandibular disorders, and dysphagia if the radiation was directed at the head or neck (Mercadante 2020: 421; Adler, Carlton and Stewart 2023: 123).

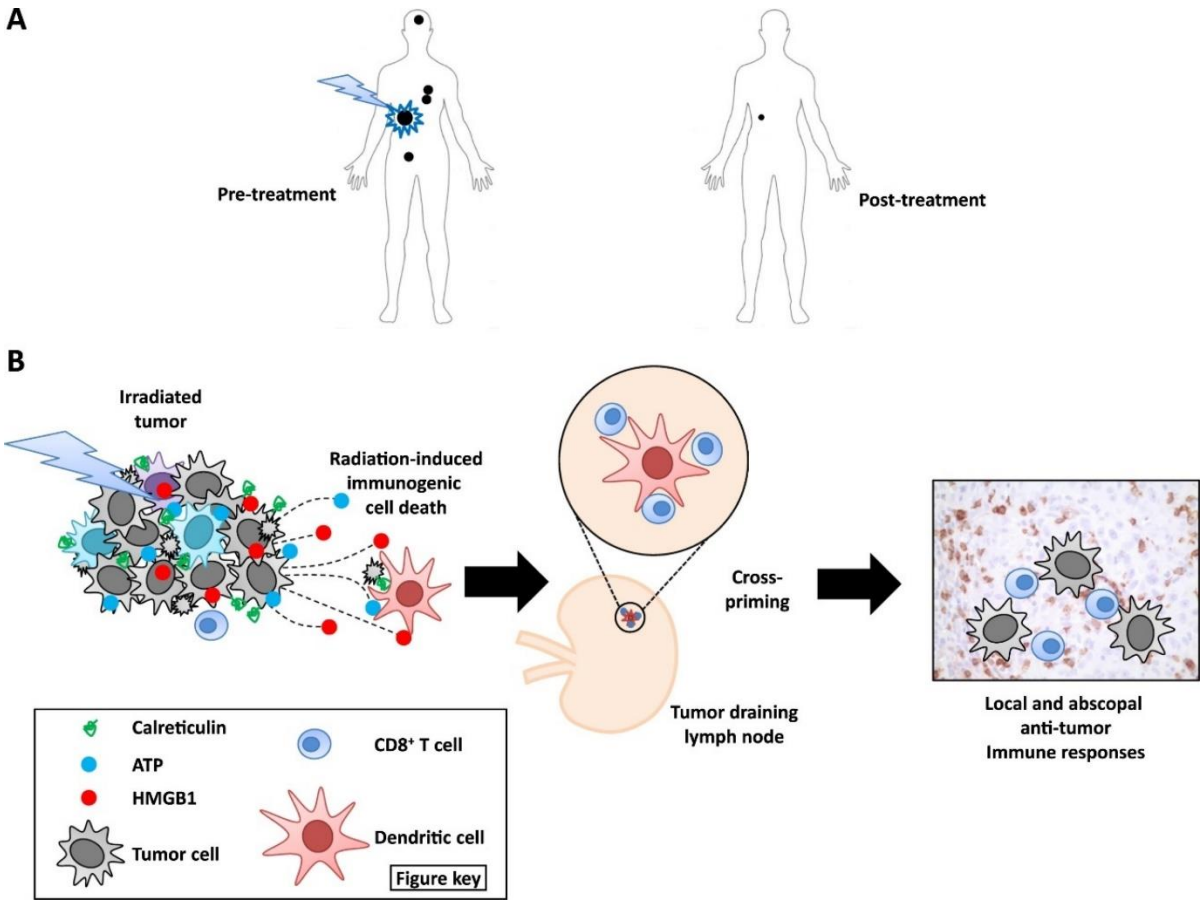

Figure 2: Anti-tumor immunity in response to radiation therapy. (A) Emerging evidence suggests that radiotherapeutic treatments affect irradiated sites and can trigger immune responses to kill

remaining tumor cells locally and remotely. (B) Radiation therapy activates ICD involving the release of CRT, ATP, and HMGB1 signals to promote $CD8^+$ T-cells, resulting in local and abscopal anti-cancer effects (Golden and Apetoh 2015).

2.2.2 Chemotherapy

Chemotherapy plays a key role in cancer treatment by using drug therapy to target rapidly growing tumor cells in the body. However, it simultaneously affects healthy cells, leading to drug resistance, mutations and DNA replication problems (Chu and DeVita Jr. 2023: 1-2). Unlike radiation therapy, which targets specific parts of the body, chemotherapy is a more generalized treatment suitable to treat tumors that cannot be surgically removed or cancers that have spread throughout the body (Cancerquest 2023). Common types of cancer chemotherapy comprise of anti-metabolites, alkylating agents, platinum analogs, and anti-tumor antibiotics (Amjad, Chidharla and Kasi 2023).

Chemotherapy-induced apoptosis is a potential approach in combating cancer through the use of drugs inhibiting proliferation and activating cell death, including apoptosis, of tumor cells. However, the apoptotic cells not only release molecules signaling its removal by phagocytic cells (macrophages) but can also stimulate tumor-promoting factors, such as PS (phosphatidylserine) and S1P (sphingosine-1 phosphate) resulting in the production of TGF-β (transforming growth factor-β), anti-inflammatory cytokines, PGE2 (prostaglandin-G E2 type), VEGF (vascular endothelial growth factor), and EGF (epidermal growth factor). These factors aid in immune suppression and cancer progression (Dehne *et al.* 2017; Franzese *et al.* 2024). Additionally, chemotherapeutic drugs that trigger the release of ATP (adenosine triphosphate), CRT (calreticulin) and HMGB1 (high-mobility group box 1) in order to activate M1 macrophages and ICD (immunogenic cell death) can lead to a more favorable tumor microenvironment. Conversely, some chemotherapy drugs fail to activate ICD and may lead to drug-resistance. **Figure 3** shows the outcome of cancer cells after chemotherapy treatment (Franzese *et al.* 2024). Unfortunately, chemotherapy can have psychological impacts, reduce quality of life and cause various complications (Anand *et al.* 2022). Common side effects that a chemotherapy patient may experience include fatigue, reduced functioning, bruising, hair loss, nausea, vomiting, bowel issues, and mood disturbances. The repercussions of chemotherapy vary from person to person, with some experiencing mild symptoms while others may face recurrent and severe complications (Gibbons and Groarke 2018; Anand *et al.* 2022).

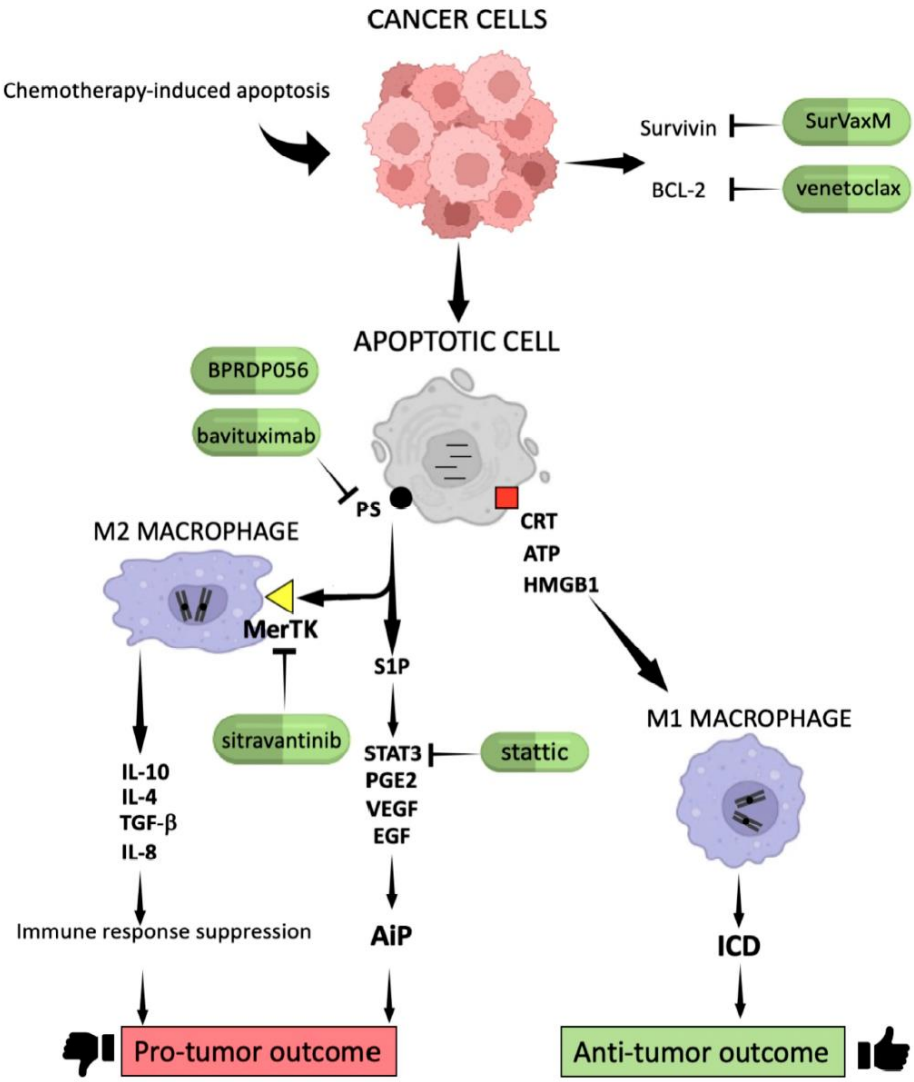

Figure 3: The outcome of cancer cells after chemotherapy treatment. The pointed arrows refer to activation, and flat-tipped arrows refer to inhibition. Diagram illustrates the effects of chemotherapeutic drugs activating apoptosis of cancer cells, leading to two potential effects: the fostering of cell survival of the tumor and suppression of immunologic responses, or the induction of M1 macrophage and immunogenic cell death resulting in anti-tumor development. Abbreviations: AiP (apoptosis-induced proliferation), CRT (calreticulin), HMGB1 (high-mobility group box 1), ICD (immunogenic cell death), MerTK (mer tyrosine kinase), PS (phosphatidylserine), S1P (sphingosine-1 phosphate) (Franzese *et al.* 2024).

2.2.3 Surgery

Surgical intervention is a critical component of cancer treatment, alongside radiation therapy and chemotherapy. It is most effective when the cancer is in its early stages, as it involves physically removing the cancerous tissue. However, surgery can also pose risks, such as an

increased chance of metastasis during the perioperative period (Tohme, Simmons and Tsung 2017).

Research indicates that during surgical resection, damage to surrounding tissues of the excised tumor can lead to the diffusion of tumor cells into the bloodstream. Factors like blood transfusions, anesthesia, and postoperative complications, such as infections, can impact cancer recurrence (Tohme, Simmons and Tsung 2017). Patients may also experience hospitalization, additional surgeries, severe complications, reduced functionality, and substandard cosmetic concerns occurring post-surgery in some cases (Veness *et al.* 2019).

2.2.4 Biological Therapy

Other new cancer therapeutics, such as biological therapy, also known as immunotherapy, is a treatment form involved in the activation or suppression of the immune system to combat tumors using immunomodulators like interleukins and interferons. Examples of anti-cancer immune drugs include Atezolizumab and Tisagenlecleucel. Vaccine therapy, a type of immunotherapy, has been developed to train the immune system to recognize and attack tumors (Anand *et al.* 2022). Other forms of immunotherapy, such as gene therapy, are also a relatively new modality, designed to genetically manipulate and correct mutated genes to prevent cancer. While these new therapies show great potential, they are still in the early stages of development (Bidram *et al.* 2019).

Despite the promising effects of biological therapy, there is emerging evidence of adverse effects, including rashes, fatigue, diarrhea, and, in rare cases, hematological toxicity and immune-related myocarditis. Additionally, there is reduced overall efficacy as cancer patients develop drug resistance due to T-cells' reduced ability to recognize antigens (Zhang, Bai and Shan 2020).

It is evident that numerous populations will be affected by cancer in one form or another, whether physically, emotionally or mentally. More interventions and supportive measures are needed to alleviate the burden pertaining to the blight of cancer (Stein, Syrjala and Andrykowski 2008; American Cancer Society 2024). The increasing prevalence of drug-resistant cancers highlights the need for further research and treatment development. In addition to conventional treatment options, alternative and complementary systems of medicine, such as Eastern or Western herbal medicines, ayurvedic medicine, and homeopathic medicine, also demonstrate anti-cancer properties.

2.3 Use of Complementary and Alternative Medicines by Cancer Patients

Natural forms of medicine are increasingly sought after globally owing to their beneficial effects on adverse reactions from conventional cancer treatments, as well as their low costs and minimal toxicity to normal cells (Singh *et al.* 2016). Rossi *et al.* (2017) compiled various studies on integrative oncology revealing a growing demand for complementary and alternative systems of medicines in cancer care worldwide. In Europe, one in three cancer patients make use of complementary medicines alongside conventional cancer treatment, with homeopathy, herbal medicines, and spiritual therapies being the most commonly used therapies. A 2007 French study showed that 60% of cancer patients in two public hospitals used homeopathy as a supplementary therapy. Other studies from Tuscany found that patients receiving chemotherapy also used complementary medicines, with herbal medicine (52%), homeopathy (30%), and acupuncture (13%) being popular choices. Complementary therapies were also observed in pediatric cancer cases with 12.4% of hospitalized children receiving complementary medicines to minimize the adverse effects of conventional treatments. Several studies reported that a high percentage of cancer patients (66.3%) informed their physicians about their use of complementary therapies and experienced benefits from them (89.6%) (Rossi *et al.* 2017). The majority of French oncologists and general practitioners view homeopathy favorably as a supportive care option integrated with conventional therapies (Bagot, Theunissen and Serral 2021).

In India, complementary and alternative medicine is a popular choice among cancer patients, with homeopathy being highly valued and integrated into public health systems, similar to other countries like the UK, Mexico and Brazil (Chavan 2021). A 2005 study surveyed nearly 1000 cancer patients from fourteen European countries and found that approximately 36% were using complementary therapies, especially homeopathy and herbal medicines. However, 4.4% of these patients experienced transient side effects (Molassiotis *et al.* 2005).

Most patients chose complementary medicines to enhance the body's ability to fight cancer (50.7%), boost physical health (40.6%), and improve emotional well-being (35.2%) (Molassiotis *et al.* 2005). Another European survey conducted in 2015 reported that 236 oncology centers in Europe provided integrative therapies, including homeopathy, in the public healthcare sector (Rossi *et al.* 2015).

A small group of breast cancer patients undergoing chemotherapy in South Africa were prescribed individualized homeopathic medicine. This study revealed a statistically significant

reduction in side effects, with patients experiencing shorter durations and better coping mechanisms after taking homeopathy (Moodley 2006: 96). In a recent study also conducted in South Africa, 47 out of 65 cancer patients opted for adjunctive approaches for the management of side effects from conventional cancer treatments, including homeopathy, herbal products, and health supplements (especially probiotics, minerals and vitamins). The majority (81.5%) of these patients disclosed their use of complementary medicines to their oncologists as supportive therapy, and over 50% of these cases were supported by the oncologists. While some patients reported adverse reactions, most patients had positive outcomes after using complementary medicines (Kalla 2021: 89).

2.4 Herbal Drugs and Herbal Products Useful in Cancer

Numerous studies have shown that herbal medicines exhibit anti-cancer properties such as cytotoxicity in tumor microenvironments, cancer immunity, and anti-inflammatory effects. Research indicates that certain phytoconstituents in herbal drugs, such as curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, and berberine, modulate autophagy and apoptosis pathways in various types of cancers. Traditional herbal medicines containing alkaloids, flavonoids, and polyphenols, such as artesunate, coumarins, terpenoids, and quinone, are considered anti-cancer agents, however their mechanisms of action require further investigation (Ali *et al*. 2023).

Studies on green tea and its active polyphenolic compounds, especially EGCG, have demonstrated anti-cancer effects against prostate cancer cells. However adverse effects like nausea, fatigue, and diarrhea have been reported, suggesting that green tea may have limited anti-cancer benefits. Recent studies report low toxicity of herbal medicines and their phytochemical compounds. In cases of lung cancer, herbal plants like *Prunus armeniaca, Rhus verniciflua, Stemona japonica,* and *Tussilago farfara* have been utilized as anti-cancer treatments (Yin *et al*. 2013).

In a long-term randomized controlled study, a Japanese traditional medicine, Sho saiko-to (TJ-9), appeared to prevent the development of hepatocellular carcinoma. However, limited data is available on the efficacy of TJ-9, thus indicating the need for further research (Yin *et al*. 2013).

Several phytochemical studies have reported various mechanisms of action of anti-cancer compounds. For example, apigenin, a flavone found in celery and chamomile, affects the

leptin/leptin receptor pathway to induce apoptosis in lung cancer cells. Gingerol induces caspase-dependent apoptosis in colon cancer cells by targeting the Erk1/2/JNK/AP-1 signaling pathways. Similarly, lycopene prevents the development of gastric cancer cells by disrupting Bcl-2 and Erk signaling, as well as the PI3K/Akt pathway in pancreatic malignant cells (Singh *et al*. 2016).

Several challenges in the use of herbal drugs for cancer treatment include the lack of reliable and consistent sources of medicinal plants, inconsistent preparations of biochemical ingredients and phytocompounds, limited evidence on the efficacy of herbal drugs against cancers, personalized prescriptions in some traditional medicine practices brings about confusion in drug formulation, and general safety considerations of herbal drugs. Despite the long history of using herbal plants globally for various diseases, including cancer, more evidence-based studies are needed in the field of medicinal plant research (Yin *et al*. 2013). Another form of natural medicine includes homeopathy, and it presents its own unique set of challenges.

2.5 Homeopathic Medicines in Cancer Treatment

Despite its enduring popularity, Cukaci *et al*. (2020) have contended that homeopathy is not effective, asserting that homeopathic remedies do not have any effects beyond the placebo effect. *The Lancet* has also highlighted the lack of proven benefits associated with homeopathy, attributing it to a lack of sufficient evidence (Anon 2005). A systematic review by Milazzo and colleagues found that homeopathy lacks a plausible mechanism of action due to highly diluted substances and limited evidence to support its efficacy in cancer care (Milazzo, Russell and Ernst 2006).

Despite the challenges and controversies that homeopathy encounters surrounding its efficacy, it is practiced in over 80 countries worldwide (Arora and Tandon 2015). Research by Frenkel (2015) suggested cellular changes in cancer cells after treatment with homeopathic remedies *in vitro*. In animal experiments, inhibitory effects of several homeopathic medicines were seen on certain tumors.

Human clinical studies indicated that homeopathy enhanced quality of life, reduced side effects of conventional therapies, and improved survival in cancer patients. These findings suggest potential positive effects of homeopathic medicines in cancer care (Frenkel 2015).

Several homeopathic medicines have been used to alleviate side effects resulting from conventional cancer therapies, such as *Cadmium sulphuratum* 30CH, *Carcinosin* 30CH, *Phosphoricum acidum* 30CH, and *Radium bromatum* 30CH (Samuels *et al*. 2018). Homeopathic remedies like *Ruta graveolens* 200CH, *Hydrastis canadensis* 200CH, *Thuja occidentalis* 200CH, *Lycopodium clavatum* 200CH, *Phosphorus* 1M, and *Arsenicum album* 200CH have shown anti-malignant activities in various animal models (Preethi *et al*. 2012).

In vitro studies have investigated the effects of highly diluted homeopathic medicines. A study on homeopathic *Ruta graveolens* mother tincture (MT) and *Ruta graveolens* 30CH against human colon cells demonstrated anti-cancer effects through *in vitro* assays. Evidence of cytotoxicity, anti-proliferation, gene modifications, DNA fragmentation, cell cycle arrest, and the activation of apoptosis were identified, such as upregulations of caspase-9, caspase-3, *P21*, *P27* and *Bax* expressions as well as downregulations of the *Bcl-2* expression. These findings are indicative of the hallmarks of anti-cancer development (Arora and Tandon 2015). Certain homeopathic remedies were reported to induce apoptosis in cancerous cells. *Carcinosinum* 200CH increased expressions of tumor suppressor gene *P53,* inducing apoptosis (Preethi *et al*. 2012). *Lycopodium clavatum* (5CH and 15CH) caused apoptosis in cervical cancer cells, through the evidence of DNA fragmentation, increase of caspase-3 and *Bax*, decrease in *Bcl-2* and *Apaf* gene expressions as well as the release of cytochrome-c. It also demonstrated no cytotoxic effects in normal cells. This study illustrated the ability of a potentized homeopathic medicine causing anti-cancer activities, highlighting its potential as an anti-cancer therapy (Samadder *et al*. 2013).

Another study investigated the anti-cancer properties and nanoparticulate nature of *Terminalia chebula*. It was found that certain potencies of *Terminalia chebula* (3X, 6CH and 30CH) reduced cell viability of breast cancer cells without affecting healthy cells, showing decreased cell growth, and the presence of nanoclusters and nanoparticles (Wani *et al*. 2016). Many other homeopathic medicines have demonstrated anti-cancer effects against various cancer cells *in vitro,* such as Phytolacca decandra, *Calcarea carbonica* (Fuselier *et al*. 2023), *Conium maculatum*, *Thuja occidentalis* and *Sabal serrulata* (Frenkel 2015).

Homeopathy has its own set of distinct principles and values which need to be understood in order to appreciate its holistic approach to healing and it is crucial for the comprehending of how its treatment differs from conventional medicine.

2.6 Homeopathy

2.6.1 History

An alternative and complementary medical system known as Homeopathy was discovered by a German physician, Dr. Christian Friedrich Samuel Hahnemann (1755-1843) in the early 19th century. The term "homeopathy" originates from the Greek words "*homoios,*" meaning 'similar,' and "*pathos,*" meaning 'suffering' (Bellavite and Signorini 2002: 12; Castro 2016: 4-7). Homeopathy is based on the fundamental principle known as the 'Law of Similars,' expressed in the Latin phrase "Similia similibus curentur," which translates to "let likes be cured by likes" (Grams 2018). The law of similars is not a new concept and has historical roots dating back to figures like Hippocrates and Paracelsus. In the 4th century BCE, Hippocrates stated, "Through the like, disease is produced, and through the application of the like it is cured." Paracelsus, a renowned physician and alchemist in the 15th century, practiced the law of similars to a great extent and introduced the concept of the "Doctrine of Signatures" (Ullman 1991: 7; Castro 2016: 3).

Homeopathy has been practiced for over 200 years and is widely used by hundreds of thousands of physicians and millions of individuals worldwide. It offers a natural and gentle form of medicine that can be safely used in conjunction with conventional treatments (Prasad 2007; Frenkel 2015: 5; Tinney and Rice 2023). Despite controversial studies questioning its efficacy, homeopathy remains popular (Anon 2005; Frenkel 2015: 2; Cukaci *et al*. 2020). In the year 2012, it was reported that 100 countries around the world uses homeopathy, following acupuncture, herbal medicines and indigenous traditional medicine practiced in 113, 110 and 109 countries, respectively (World Health Organization 2019: 47). Research by Relton *et al*. (2017) indicated a consistent use of homeopathy across various countries from 1986 to 2012, with a growing demand for its services globally.

Annually there is a continuous rise in the demand for homeopathy around the world, reflecting a significant trend in healthcare preferences towards individualized treatments and access to traditional and complementary health professionals and medicines worldwide (World Health Organization 2013: 25-26). Homeopathy is commonly used to treat acute and chronic disorders and is considered non-toxic due to its systematic potentization method. It is safe for individuals of all ages, including the elderly, infants, children, and pregnant or breastfeeding women (Coyle-Demetriou and Demetriou 2018: 69).

Homeopathic medicines stimulate the body's natural self-healing abilities and treatment is personalized with a holistic perspective by considering the patient's physical, mental, and emotional symptoms or behaviors (Matzo and Sherman 2015: 247).

2.6.2 Homeopathic Principles

Dr. Hahnemann was dissatisfied with the conventional treatment methods of the 18th century, prompting his determination to find alternative healing approaches. At that time, the cinchona tree's bark, containing quinine, was valuable in treating malaria. Through self-experimentation, Hahnemann ingested the cinchona bark whilst in a healthy state and observed the development of symptoms similar to malaria. As a result, it led to his realization that a substance causing symptoms in a healthy individual can cure similar symptoms in a sick person, known as 'like cures like' (Vithoulkas 2000: 8-10; Sankaran 2014: 1), thus marking the inception of homeopathy. In 1810, Hahnemann published the first edition of his seminal work, "Organon of the Rational Art of Healing," commonly referred to as "The Organon." This book provided a ground plan outlining the theoretical and practical elements of homeopathic practice. Hahnemann also established fundamental homeopathic principles including the concept of the Law of Similars, the Law of Provings, the Law of the Minimum Dose, the Theory of Drug Dynamization (potency), the Law of the Single Remedy (individualization), and the Doctrine of Miasms (Kayne and Kayne 2007: 5; Jayasuriya 2010: 15-35).

2.6.3 Homeopathic Potentization and Provings

Potentization is a process that is essential and distinctive to homeopathy. It involves a mathematically precise method that converts drug substances into an altered state in order to be physically soluble, physiologically assimilable, and therapeutically active (Jayasuriya 2010: 62; Basu *et al*. 2017). This process entails a quantitative reduction of a raw substance which subsequently results in therapeutic effects based on remedy provings. Remedy provings follow the Law of Similars, where substances are systematically tested on healthy individuals to reveal the symptoms that indicate the medicine's use, known as the 'proving' of the drug. The proving of a substance encompasses all the symptoms it produces in healthy individuals (Castro 2016: 21). The potentization process consists of serial dilution and succussion or trituration at each stage. Succussion is used for liquid and soluble constituents, while trituration is implemented in the case of dry and insoluble constituents in the preparation of homeopathic remedies (Bell and Koithan 2012; Basu *et al*. 2017).

2.6.3.1 Succussion Rationale

According to Torres (2002); Basu *et al*. (2017), succussion, a method involving rhythmic and vigorous vertical shaking with impact, was rationalized as having a high turbulent arrangement with vortices constantly forming, leading to the circulation of energy. Various pharmacopoeias emphasized that the purpose of succussion is to separate the very fine particles of a substance by creating an intermixture within the inert liquid vehicle (Jahr 1842: 40; British Homeopathic Society 1876). Through the systematic process of serial dilution, succussion or trituration, Hahnemann discovered a peculiar phenomenon: in every substance found in nature, there lies hidden an inner life, a force that can demonstrate healing properties and neutralize any toxic effects of the crude materials through progressive dilution. Additionally, there is immeasurable energy dormant within substances that can be activated and utilized for the benefit of living beings if extracted correctly (Kunzli, Naude and Pendleton 1982: Aphorism 269; Shah 2016).

It is further explained that the vigorous shaking with impact provides kinetic energy to the liquid solution, and mere dilution alone does not produce the same effect. Therefore, both actions of dilution and succussion are necessary (Nandy and Bhar 2021). Kayne (2006: 94); Mordeniz (2017) suggested that the role of succussion may induce electrochemical changes in the structuring of solvent molecules, thereby enabling the 'memorizing' ability to imprint the original substance. Other studies found that sequential dilution and succussion of the homeopathic substance could modify the water structure in an ethanol-water solution (Singh *et al*. 2022), as well as further hypotheses suggesting that the potentization process enhances α-amylase activity *in vitro*, resulting in the structure of the starting material imprinting on water polymers. Additionally, the stabilization of water structure by ethanol molecules has shown preservation activities over extended periods (Sukul *et al*. 2002), and the potential to carry the information of the original drug molecules (Chakraborty *et al*. 2014). Recent studies also demonstrated the existence of nano-sized solvent super-structures in dynamized solutions (Demangeat 2013; Elia *et al*. 2014). Based on quantum electrodynamics or quantum mechanics, both Mordeniz (2017); Manzalini and Galeazzi (2019) confirmed that succussed serial dilutions in aqueous solution contain information associated with the starting material or coding of the original substance, leading to the transfer of its information.

The number of succussions used depends on the individual's health status as well as on the pharmacopoeia being utilized. However, the number of succussions executed during the manufacturing process remains consistent (Shah 2016).

Through Hahnemann's experiments, by 1839, he recommended '10, 20, 50 or more powerful strokes', eventually up to 100 strokes of succussion at each potency level, modifying the degree of dynamization (Schmidt 1994: 47). Whereas prior to administering a liquid form to a patient, depending on whether the condition is acute or chronic, approximately 10 powerful strokes of vigorous shaking are applied to the bottle using the full force of the arm (Kunzli, Naude and Pendleton 1982: Aphorism 248; Schmidt 1994; Shah 2016: 127). In this study, 100 strokes of succussion were applied at each potency step during the manufacturing process.

2.6.4 Homeopathic Potencies

Different scales of remedy potencies are commonly used in homeopathy, including the decimal scale (D or X), the centesimal scale (C or CH), the fifty millesimal or quinquagintamillesimal scale (50M or LM or Q), and the Korsakovian scale (K or CK). The D potency involves a 1:10 ratio, with one part of mother tincture (MT) or previous potency added to nine parts of the diluent (e.g. 1X). The CH potency consists of a 1:100 ratio, with one part of mother tincture (MT) or previous potency in 99 parts of dilution (e.g. 1CH), while the LM potency refers to a dilution of 1:50 000 ratio (e.g. LM1 or Q1). The letter 'M' denotes a 1000 dilution and 'L' represents 50 in Roman numerals. The LM potency was introduced by Hahnemann in the 6th edition of *The Organon of the Medical Art*. In the Korsakovian potentization method (e.g. 1K or 1CK), one vial is used throughout the entire process. For example, to prepare a Hahnemannian 30CH, thirty vials are used, whereas for a Korsakovian 30CK, the same vial is utilized (Kayne 2006: 95-97; Jayasuriya 2010: 64). **Figure 4** shows the potentization process using the Hahnemannian centesimal and decimal scales.

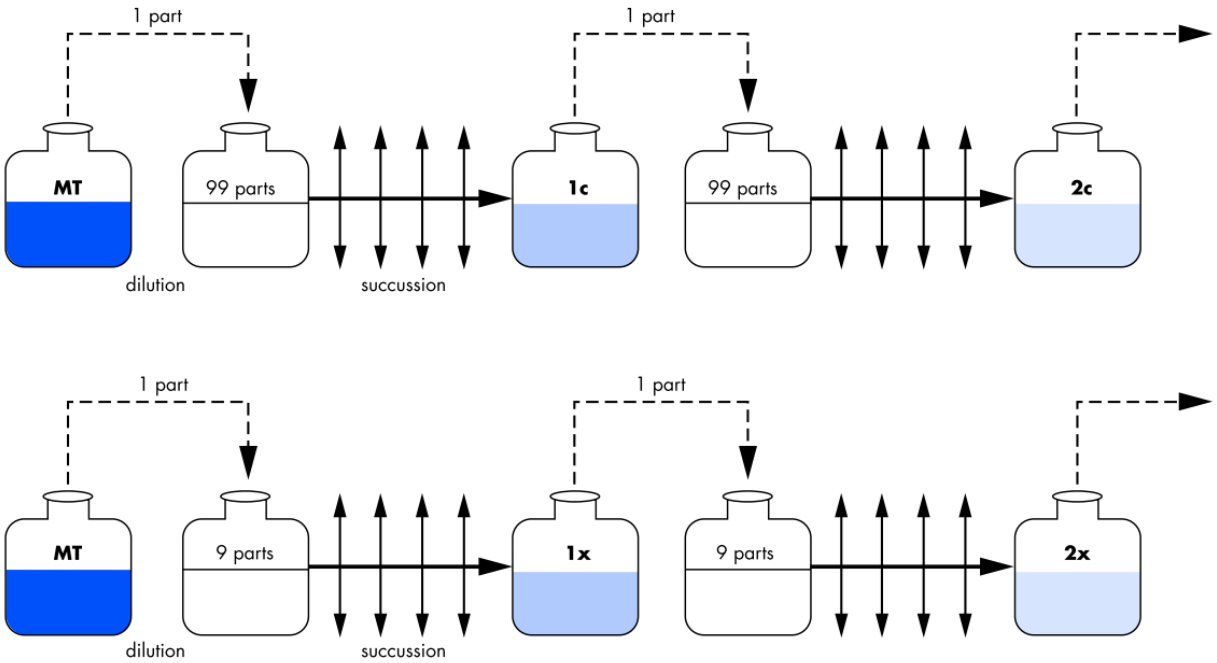

Figure 4: The potentization process using centesimal and decimal scales (Hahnemannian method). The C or CH potency consists of a 1:100 ratio, with one part of mother tincture (MT) or previous potency in 99 parts of dilution (e.g. 1C), whereas the X or D potency involves a 1:10 ratio, with one part of mother tincture (MT) or previous potency added to nine parts of the diluent (e.g. 1X) (Kayne and Kayne 2007).

Low centesimal potencies were used in this research as this scale of potency was extensively practiced by Hahnemann himself, while the decimal scale was credited to Constantine Hering, an American homeopath, who introduced the decimal dilution process later on (Sukul and Sukul 2004: 5; Kayne 2006: 96).

Several *in vitro* studies have shown the anti-cancer effects of low homeopathic potencies on different cancer cells (da Silva *et al*. 2011; Samadder *et al*. 2013; Arora and Tandon 2015; Wani *et al*. 2016; Fuselier *et al*. 2023). See Appendix A illustrating a comparison between dilution, concentration and centesimal potency (Kayne 2006: 96).

The highly diluted source material in potentized remedies is a contentious topic as it is claimed that the potency increases with each dilution step. The potentization process is believed to enhance the medicinal influence of the remedy (Frank 2002: 799; Nandy and Bhar 2021). Consequently, ongoing research plays a critical role in providing scientific evidence for the mysteries of homeopathy. This study adopted a quantitative approach due to the lack of research on testing radium bromide in its potency form on cells. Research at a cellular level provides factual and reliable data, ensures quality-controlled parameters, eliminates bias in

data collection, and focuses on numerical data and statistical analysis (Matanda and Mawere 2022: 79).

2.6.5 Possible Mechanisms of Action of Homeopathic Medicines

According to the founder of homeopathy Dr. Samuel Hahnemann, the action of homeopathic remedies is to stimulate one's own *vital force* to overcome the state of disease, and thereby return to a healthier state (Ernst 2016: 10). However, the exact mechanisms of action of homeopathic remedies are still under investigation in medical communities worldwide. The discussions center around the rationale for using ultra-high dilutions, often diluted beyond Avogadro's constant, suggesting that no single molecule of the original substance is present in the final product due to the method of repeated dilutions (Ernst 2016: 50; Basu *et al*. 2017: 241). Bellavite and Signorini (2002: 5-6) proposed that the understanding of homeopathy involves nanopharmacology, quantum physics, electromagnetic phenomena, and the theory of water memory, among other fields.

Several studies have explored the unusual behavior of ultra-high dilutions. Chikramane *et al*. (2010); Bell and Koithan (2012); Wani *et al*. (2016); Nandy and Bhar (2021) demonstrated the existence of nanoparticles of the original materials even in ultra-high dilutions which consequently challenges the traditional view of the role of dilutions in homeopathic medicines. Other studies revealed that apoptosis is a mechanism of action of homeopathic medicine by means of tumor reduction or shrinkage. Additional research has confirmed similar biological activities found in ultra-dilutions compared to their mother tincture or original substance (Preethi *et al*. 2012: 172). Saha, Roy and Khuda-Bukhsh (2013: 164) provided evidence that homeopathic remedies are more than just placebos and that their mechanism of action is linked to the alteration of gene expression. Recent investigations by Tournier *et al*. (2019: 896) identified various techniques used to explain the potential physicochemical properties of homeopathic medicines, including optical spectroscopy, nuclear magnetic resonance (NMR) relaxation, and electrical impedance measurements.

Despite ongoing research, homeopathy is becoming increasingly popular and it is widely used in many countries around the world (Bodeker *et al*. 2005; Frenkel 2015: 2). Concurrently, homeopathy is a subject of ongoing discussion, with questions surrounding its efficacy and limitations. Some critics argue against homeopathy's lack of strong scientific evidence to support its efficacy beyond the placebo effect. Another point of contention is the debate around the high dilutions which cannot cause any biological effects (Grams 2019). Further

scientific research is warranted to validate the phenomena of homeopathy and understand its molecular mechanisms.

2.6.6 Radium Bromatum

Vermeulen (2004: 1116) explained the origins of *Radium bromatum,* sourced from the crude substance called radium bromide. Radium was discovered in 1898 by French physicists Marie and Pierre Curie.

It is a radioactive alkaline earth metal, the sixth element in group 2 on the periodic table, and known to easily combine with halogens, namely chlorine and bromine. The metal is silver-white in color, oxidizes and blackens rapidly when exposed to air, decomposes water violently, and is volatile in nature (National Center for Biotechnology Information 2022).

In the 1900s, radium was used to make glow-in-the-dark clocks or watches and added to products like toothpaste and hair creams. Its other uses gained popularity for medical disorders like infections, arthritis, and cancer. However, it was later discovered that radium had high toxicological effects on health. The metal element is now used and managed in the form of radium chloride and radium bromide, which are used to treat some types of cancers. (Ropp 2013: 21-23). Radiation from radium causes adverse consequences on living cells and overexposure results in radium burns. Numerous radiation injuries have been reported, and repeated or prolonged exposures to radiation could cause various effects such as amenorrhea, male or female disorders, anemia, leukopenia, thrombocytopenia, cataracts, alopecia, ulcerations, skin atrophy, and ultimately leading to squamous cell carcinomas (Vermeulen 2004: 1118; Iddins *et al.* 2022).

The provings of radium bromide were conducted by Clarke and Dieffenbach in 1904 and 1910, respectively. The homeopathic remedy became known as *Radium bromatum,* which is based on the preparation of serial dilution with succussion (Vermeulen 2004: 1119). *Radium bromatum* has been documented as effective in treating various conditions, including gout and rheumatic conditions, skin diseases such as acne rosacea, moles, naevi, ulcers, and cancers. It has also been used to lower blood pressure, alleviate severe aching pains deep in joints, restlessness, and is better if the individual is moving about. Other symptoms indicated for this remedy include ulcers due to radium/x-rays or burns with slow healing and great fatigue. A person who may benefit from *Radium bromatum* is someone who is anxious, depressed, fearful of being alone in the dark, has a strong desire to be around others, and tends to be tired

and irritable. Other physical or general symptoms would also accompany the mental picture of the remedy in order to achieve an overall match between the patient and the remedy picture (Vermeulen 2001: 808-809; Boericke 2021: 543).

Given the threatening and growing phenomenon of cancer affecting millions of people, investigating any potential cancer treatment on a cellular level is crucial to combat this global health burden. It is hypothesized that *Radium bromatum* may inhibit the growth of cancer cell lines. As *Radium bromatum* is a homeopathic preparation made from toxic and radioactive radium bromide, it is important to observe its effects on cancer cells.

Consequently, if *Radium bromatum* demonstrates anti-cancer activities on a cellular level, it could expedite further research into its adjunctive treatment for cancer patients *in vivo*.

No research has been conducted on *Radium bromatum* in different potencies to assess any anti-cancer activities *in vitro*, and its effect on cancer cells remains unknown. However, there are a few cases of *in vivo* studies that show promising results. One study reported the use of *Radium bromatum* 30CH as a supportive therapy in an integrative oncology setting, with no adverse effects associated with the homeopathic treatment, but the study offered insufficient evaluation of patients' response to homeopathy (Samuels *et al*. 2018). Another study showed that patients prescribed *Radium bromatum* experienced significant decreases in skin damage caused by radiotherapy compared to those without the homeopathic protocol. However, the study lacked a control group to serve as a baseline for comparison(Rossi *et al*. 2018). In a randomized controlled trial, pre-defined homeopathic medicines, including *Radium bromatum* 30CH, were found to be beneficial in the management of adverse radiotherapy-induced dermatological effects. However, limitations included analysis using only centesimal potencies, and only assessing the effects on dermatological side effects post radiation and vomiting experienced after chemotherapy, without exploring other possible adverse events. Although the pre-selected medicines were superior to placebo, further independent replications and research evaluations were recommended (Shukla *et al*. 2020). In a recent study on early breast cancer, homeopathic medicines like *Radium bromatum* were integrated alongside conventional cancer treatments, which provided advantageous supplementary care to manage the side effects of conventional therapies. However, the study had limitations in its sampling, and the use of homeopathy was offered as supportive care but other uses were not included (Veyrier *et al*. 2024). There is a definite need to explore *Radium bromatum* starting at the cellular level.

CHAPTER 3: MATERIALS AND METHOD

3.1 Research Introduction and Design

This quantitative study investigated a homeopathic medicine tested against human cancer cell lines at a cellular level. The *in vitro* study utilized human pulmonary carcinoma cell lines (A549) and human hepatocellular carcinoma cell lines (HEP-G2), as well as non-cancerous human embryonic kidney cell lines (HEK293) as a control. Cells were treated with homeopathic *Radium bromatum* (9CH, 12CH and 30CH) and a placebo. Refer to Appendix B for the flow diagram illustrating a summary outline. The experiment was conducted in the cell culture laboratory in the Department of Biotechnology and Food Science, at Durban University of Technology (DUT) in Durban, South Africa.

The specific potencies were selected based on standard concentrations commonly used by homeopathic practitioners, with the CH referred as the centesimal potency, the original scale practiced by Hahnemann (Kayne 2006). Cancer cell lines, A549 and HEP-G2, were chosen for this study as both cell lines are models for human lung and liver cancers, respectively, and are also prevalent forms of cancer. The non-cancerous cells, HEK293, represent human embryonic kidney cells commonly used in cancer experiments as a control. All these cell lines are widely used to study cancer development, progression, and treatment response (ATCC 2024).

3.2 Consumables and Equipment

Refer to Appendix C for the list of the media, reagents and equipment used in this research.

3.3 Aseptic Technique

Aseptic techniques were practiced under controlled conditions in order to prevent contamination by pathogens; all work were conducted under laminar flow units, in a closed sterile room for cell culture; work surfaces were sterilized using ultraviolet germicidal irradiation; materials and techniques were prepared under sterile conditions; latex rubber gloves were used and work surfaces, laminar flow units and gloves were disinfected with 70% ethanol to maintain a clean environment.

3.4 Source and Preparation of the Medicines

CoMED Health (Pty) Ltd. company supplied the *Radium bromatum* in 8CH, 11CH, 29CH and the placebo. According to CoMED Health (Pty) Ltd. company, the source material, radium bromide, was manufactured according to the German Homeopathic Pharmacopoeia, GHP 6 method, i.e. trituration of insoluble substances in lactose monohydrate as the vehicle, followed by the GHP 8a method, i.e. liquid preparations made from trituration obtained as per GHP 6. The liquid dilution ratio used was 1 part substance to 99 parts of 96% ethanol (1:100 centesimal dilution), with succussion by hand 100 times between potencies. After the manufacturing process, the remedy potencies were contained in previously sterilized 30 mL amber glass dropper bottles, then stored in a temperature-controlled room with no direct sunlight.

From the supplier, the remedy bottles, including the control, all contained 96% ethanol at a quantity of 25 mL each. However, a final step before use was to potentize by hand to 9CH, 12CH, 30CH respectively, including the placebo bottle with 100 succussions each in deionized water solution, in a sterile environment. This was to remove the physiological effect of the 96% ethanol on cell lines. The remedy in serial potencies are standard concentrations and are used in everyday practice by homeopathic practitioners in South Africa and internationally. Refer to Appendix D for the manufacturing process and mathematical calculations.

3.5 Cytotoxicity of *Radium Bromatum* against Cancer Cells

3.5.1 Cell Lines

The A549, HEP-G2 and HEK293 cell lines were acquired from Dr. Rene Khan at the School of Medical Biochemistry, University of KwaZulu-Natal, South Africa. Cells were cultured in 25 cm^2 tissue culture flasks and maintained at 37°C in a humidified incubator with 5% CO_2 (SnjidersHepa by United Scientific, Cape Town, South Africa). Upon arrival, the cells were transferred to two separate 75 cm^2 flasks (by Greiner, Germany) with subculturing performed after three days or when the cells reached approximately 80% confluence in each flask.

3.5.2 Cell Maintenance

All cell culture maintenance experiments were conducted in a sterile environment within a laminar flow hood to prevent contamination. Prior to use, the hood was disinfected with UV

irradiation and wiped down with 70% ethanol. The cells were cultured in separate layers in flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with glucose, L-glutamine, sodium pyruvate, heat-inactivated fetal bovine serum, and antibiotics. When the cell layers reached 80% confluence, they were sub-cultured to maintain exponential growth. For subculture of cell, the medium was aspirated, and cells washed with phosphate-buffered saline. Trypsin was then added to detach the cells from the flask surface. After incubation at 37°C with 5% CO_2, the flask was gently tapped to dislodge the cells. The cells were resuspended in fresh medium and transferred to new flasks. The flasks were then placed in a humidified 5% CO_2 incubator at 37°C for further incubation with cultures monitored daily for contamination by observing changes in medium color and turbidity. Cell growth was assessed visually using an inverted microscope.

3.5.3 Storage of Cells

The cell culture flasks with 80% confluence were washed with 5 mL of phosphate-buffered saline (PBS). The cells were then treated with trypsin following the subculture protocol. Subsequently, 10 mL of Dulbecco's Modified Eagle Medium (DMEM) was added to each flask, and the cells were transferred to 50 mL tubes. The tubes were centrifuged at 1500 rpm for 10 minutes to form cell pellets. To reconstitute the cell pellets, 2 mL of cryoprotective medium (comprising 10% dimethyl sulfoxide (DMSO), 20% fetal bovine serum (FBS), and 70% DMEM) was added. Next, 1 mL of the cryoprotective cell suspension was aliquoted into cryotubes (Corning, South Africa). The cryotubes were initially cooled on ice before adding the cryoprotective solution. They were then placed in thermos flasks and stored overnight at -20°C. Finally, the cells were preserved in a -80°C freezer until required for experiments, at which point they were thawed.

3.5.4 Cell Regeneration

When freshly incubated cells were not available for immediate use, cells were thawed from -80°C storage and transferred to 20 mL of pre-warmed supplemented DMEM in 75 cm^2 tissue culture flasks. The flasks were then placed in a 37°C humidified incubator with 5% CO_2.

3.5.5 Cell Enumeration

The viability of the cells was determined using trypan blue dye exclusion. Trypan blue is a dye that helps differentiate between viable and non-viable cells. Viable cells with intact membranes do not absorb the dye, while non-viable cells with damaged membranes take up

the dye and turn blue. This property makes trypan blue useful for counting viable cells in a cell suspension. In this experiment, equal amounts of the cell suspension and trypan blue were mixed and incubated for about a minute. A small amount of the mixture was then loaded onto a hemocytometer for cell counting. The total cell count was calculated using a specific formula. This method not only allowed for the enumeration of viable cells but also provided information on cell morphology, as viable cells remained clear while non-viable cells were stained blue.

Total cell count = 16 squares x 4

= Cell counts in 4 sets of 16 squares

16 squares = 2×10^4/mL

Therefore, cells per mL = $\frac{\text{total cell count}}{4}$ x 2×10^4 per mL

= cells per mL

3.5.6 Morphological Analysis Using an Inverted Microscope

A standard inverted microscope was used for morphological studies to observe the changes in cell death morphology in malignant (A549 and HEP-G2) and non-malignant (HEK293) cell lines induced by homeopathic samples, Rad-br 9CH and Rad-br 12CH. Cell morphological changes were observed under the inverted microscope 48 hours post-treatment. All treated cell lines were compared to untreated control cells used as the negative control.

3.5.7 Cytotoxicity Assessment

The cytotoxicity of the prepared samples, *Radium bromatum* (9CH, 12CH, 30CH) and the placebo, against A549, HEP-G2, and HEK293 cells were assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. A cell volume of 90 µL of $1x10^5$ cells was placed in each well of a 96-well microtiter plate, with the surrounding wells filled with phosphate-buffered saline to prevent medium evaporation during incubation. The plates were then incubated at 37°C for 24 hours to allow the cells to attach to the plate wells. The cells were tested at the concentration of $1x10^{-18}$ (9CH), $1x10^{-24}$ (12CH), and $1x10^{-60}$ (30CH) of *Radium bromatum*. Each well of the plate was treated with 10 µL of varying concentrations of the *Radium bromatum* solution, including the placebo. The cell-containing microtiter plates were then incubated for two days at 37°C in a humidified incubator with a 5% CO_2 atmosphere. After incubation, the MTT reagent (5 mg/mL) was added to each well and the plate was further incubated at 37°C for four hours. Subsequently, the medium was removed, and 100 µL of DMSO (Dimethyl Sulfoxide) was added to each well to dissolve the formazan crystals generated in metabolically active cells. Absorbance was measured at 570 nm with a

reference wavelength of 650 nm using a microplate reader (BioTek Instruments, Inc. USA) (Kasumbwe *et al*. 2017).

$$\%\text{GI} = \left(\frac{\text{Abs of negative Control} - \text{Abs of drug}}{\text{Abs of negative control}}\right) \times 100$$

%GI: Percentage growth inhibition

3.6 Experimental Controls

A few control groups were conducted in this study to provide a standard benchmark; treating non-cancerous human embryonic kidney cells (HEK293), no treatment, and a vehicle control (placebo). The control groups were used to ensure that the observed effects were caused by the *Radium bromatum* samples and not due to other factors.

The treatment of normal cells (HEK293) established a baseline showing how healthy cells behave, relevant for comparison to observe if the treatment caused any effect, positive or negative, compared to the normal state of the cells and cancer cells (A549 and HEP-G2).

The untreated control contained cells that were free from any treatment which also provided a basis for comparison against the treated cells. It is essential to see the effects of the cells without any intervention, in order to understand the natural behavior of the cells within a controlled environment.

The vehicle control (placebo) contained the same solution as the *Radium bromatum* samples but without the *Radium bromatum* solute. This control ruled out any effects caused by the solvent, therefore any differences between the treatment group and the vehicle control group can be attributed to the samples tested.

Three trials were performed to verify that the results remained consistent. These control groups and the validation process in this study helped ensure that the results observed were valid, reliable and unbiased. However, general limitations of the control groups include imperfections of an artificial environment replicating the complex entity of a living organism, the influence of unforeseen factors affecting experimental results, and the control itself might produce subtle effects without clear reasoning.

3.7 Statistical Analysis

The experiments were repeated three times. The results are presented as mean values with corresponding standard deviations (SD). Data were statistically analyzed using a one-way ANOVA test followed by Tukey's test for evaluating the statistical significance of variations observed across the groups. A probability value of $p<0.05$ indicated statistical significance.

CHAPTER 4: RESULTS

In this study, the aim was to evaluate whether the homeopathically-potentized high dilutions of *Radium bromatum* (9CH, 12CH, and 30CH) have any anti-cancer effects on cancer cells *in vitro*. A placebo-vehicle and normal cells were used as controls. Morphological studies were analyzed, after 48 hours post-treatment, using a standard inverted microscope to observe the changes in cell death morphology in malignant (A549 and HEP-G2) and non-malignant (HEK293) cell lines induced by Rad-br 9CH and Rad-br 12CH. Cell cytotoxicity was conducted using the MTT method to evaluate anti-proliferation and reduced cell viability. To ensure reproducibility, experiments were performed three independent times. Results are expressed as mean values with standard deviations (SD). Statistical differences between groups were evaluated using one-way ANOVA and Tukey's tests. Mean values with a probability value of $p<0.05$ were considered statistically significant.

4.1 Solvent Exposure

Since the stock *Radium bromatum* (8CH, 11CH, and 29CH) and the placebo were all in 96% ethanol from the supplier, therefore it was important to remove the physiological effect of the 96% ethanol on cell lines. Consequently, *Radium bromatum* (9CH, 12CH, and 30CH) and the placebo were homeopathically potentized in deionized water from the stock. The solvent used in this study was deionized water, devoid of the high concentration of ethanol, which pose no harm to the cancer cells for the purpose of this experiment.

4.2 Morphological Evaluation by an Inverted Microscope

Morphological changes were observed in the cancer cells A549 and HEP-G2, as well as in the non-cancer cells HEK293 after 48 hours of incubation with Rad-br 9CH and Rad-br 12CH (**Figures 5**, **6**, and **7**). There was a remarkable reduction in the number of living cells and volume. The decrease in malignant cells compared to non-malignant cells was highly significant. The decrease in cell number was significant in Rad-br 9CH at higher concentrations against A549 and HEP-G2 (**Figure 5** and **6**, respectively). Additionally, a slight decrease in cell number was also observed in Rad-br 12CH against A549 and HEP-G2. No morphological changes were observed in the Rad-br 9CH and Rad-br 12CH-treated HEK293 cells. After 48 hours, no clear morphological changes were detected in the HEK293 cells following treatment with Rad-br 9CH and Rad-br 12CH (**Figure 7**).Rad-br 30CH had the lowest concentration compared to Rad-br 9CH and Rad-br 12CH, as a result the cells observed

showed that Rad-br 30CH had no statistically significant changes compared to untreated controls and was therefore not included in this analysis. All treated cell lines were compared to untreated control cells. Untreated cells displayed a high density of cells, maintained their normal shape, and remained attached. However, treated cells of A549 and HEP-G2 with Rad-br 9CH showed morphological alterations and a decline in living cells, possibly due to cell death mechanisms. A slight decrease in cell density was also observed in A549 and HEP-G2 cells treated with Rad-br 12CH. It is evident that treated malignant A549 and HEP-G2 cells exposed to Rad-br 9CH and Rad-br 12CH showed a decrease in the density of attached cells after 48 hours. In contrast, non-malignant HEK293 cells exposed to Rad-br 9CH and Rad-br 12CH showed no significant reduction in cell density compared to the untreated control, indicating no harm to healthy cells.

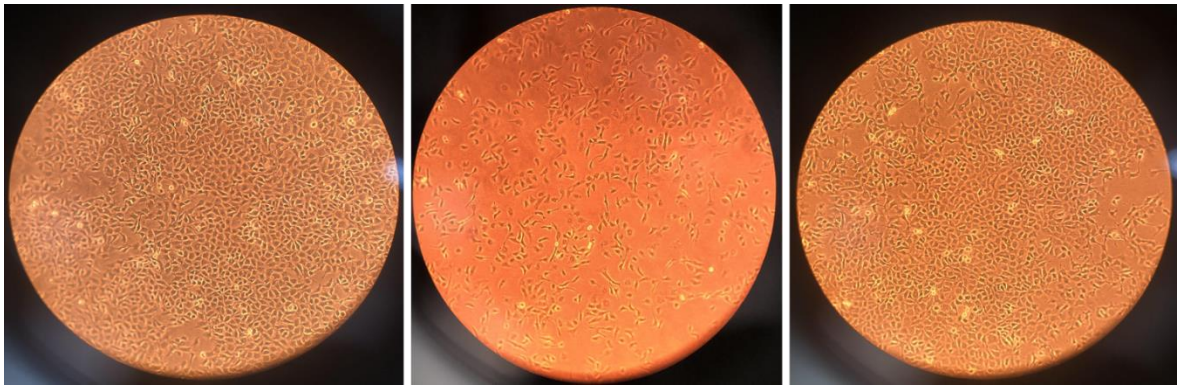

Figure 5: Cells morphology of malignant A549 (10X magnification) untreated representing the negative control (A), treated with Rad-br 9CH after 48 hours showing damage in the cells and reduction in the number of living cancer cells (B), treated with Rad-br 12CH after 48 hours showing damage in the cells and reduction in the number of living cancer cells (C). Cytotoxicity increased at higher concentrations.

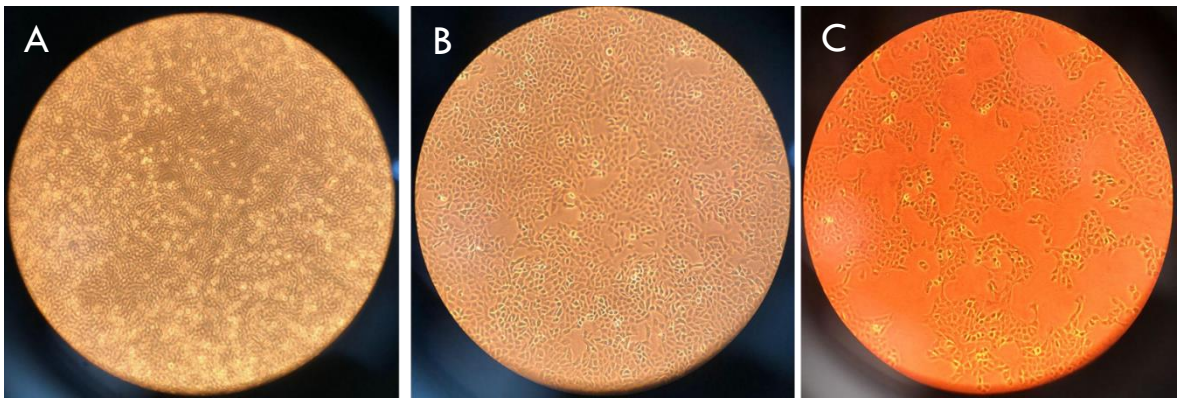

Figure 6: Cells morphology of malignant HEP-G2 (10X magnification) untreated representing the negative control (A), treated with Rad-br 12CH after 48 hours showing damage in the cells and reduction in the number of living cancer cells (B). treated with Rad-br 9CH after 48 hours showing damage in the cells and reduction in the number of living cancer cells (C).

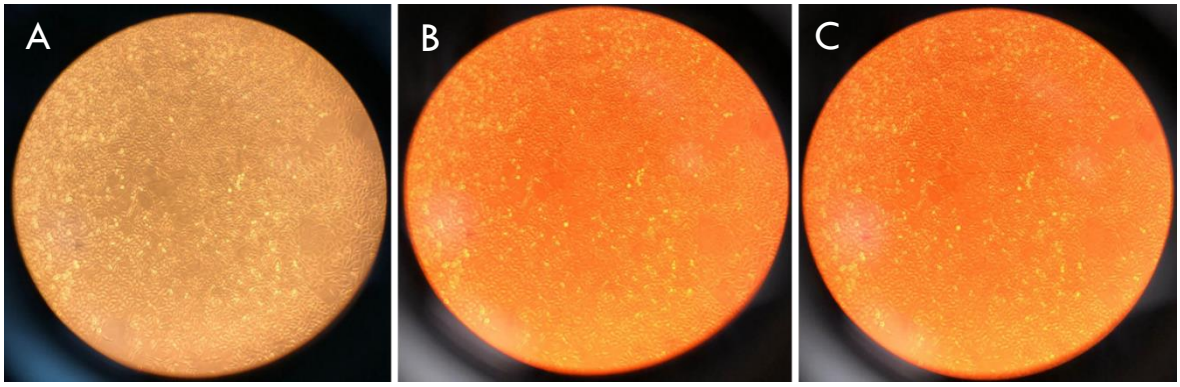

Figure 7: Cells morphology of non-malignant HEK293 (10X magnification) untreated representing the negative control (A), treated with Rad-br 9CH after 48 hours showing no morphological changes nor reduction in the number of living cells (B), treated with Rad-br 12CH after 48 hours showing no morphological changes nor reduction in the number of living cells (C).

4.3 Cytotoxicity Assessment by MTT Assay

Homeopathic treatments are prepared by diluting homeopathic stock (MT, mother tincture), sourced from a raw material, undergoing a series of sequential steps. After numerous dilutions, the remedies are diluted to levels exceeding Avogadro's number, thereby indicating that their therapeutic effects are not based on molecules. Despite skepticism regarding the scientific basis for the efficacy of homeopathic treatments, researchers are studying the *in vitro* effects of potentized remedies on molecular and cellular systems (Witt *et al.* 2007).

In this study, the 12CH and 30CH potencies are considered to have been diluted beyond Avogadro's number. The cytotoxic effects of various *Radium bromatum* (Rad-br) samples on A549, HEP-G2, and HEK293 cells were evaluated using the MTT assay. Assessing the impact of these samples on cancer and normal cell lines was crucial for understanding their effects on cell survival and growth. **Table 1** summarizes the percentage of cell growth inhibition for each sample. Mean absorbance values were calculated based on the negative control in three trials for each experiment. Overall, the test samples exhibited low cell growth inhibition on A549, HEP-G2, and HEK293 cells, with the most significant effect seen in Rad-br 9CH at 37.80±0.33% and 31.50±2.10% against A549 and HEP-G2 cells, respectively. Rad-br

12CH showed cell growth inhibition of 26.50±0.32% on A549 cells and 12.30±1.50% on HEP-G2 cells. Rad-br 30CH had a cell growth inhibition effect of 14.4±0.43% on A549 cells and 10.60±0.80% on HEP-G2 cells. Rad-br 9CH was found to be the most effective among the samples tested. Although the overall cell growth inhibition was minimal, there was an increase in activity as the concentration of the samples increased.

The samples also exhibited low cytotoxicity towards healthy cells (HEK293 cells). Furthermore, all *Radium bromatum* samples showed higher cell growth inhibition effects compared to the negative control (placebo). Refer to Appendix E for an overview represented on a diagrammatic graph.

Table 1: Percentage growth inhibition of *Radium bromatum* in 9CH, 12CH, 30CH, and the placebo against A549, HEP-G2, and HEK293 cells

	Percentage inhibition (%)		
Samples	**A549**	**HEP-G2**	**HEK293**
Rad-br 9CH	37.80 ± 0.33^{dC}	31.50 ± 2.10^{cB}	5.60 ± 0.70^{bA}
Rad-br 12CH	26.50 ± 0.32^{cC}	12.30 ± 1.50^{bB}	4.90 ± 1.20^{bA}
Rad-br 30CH	14.40 ± 0.43^{bC}	10.60 ± 0.80^{bB}	2.70 ± 0.50^{aA}
Placebo	4.30 ± 0.35^{aB}	3.40 ± 0.60^{aB}	1.80 ± 0.90^{aA}

Values are mean ±SD, n=3, $^{a-d}$ Different lowercase superscripts in the same column indicate significant differences ($p<0.05$), $^{A-C}$Different uppercase in the same row indicate significant differences ($p<0.05$).

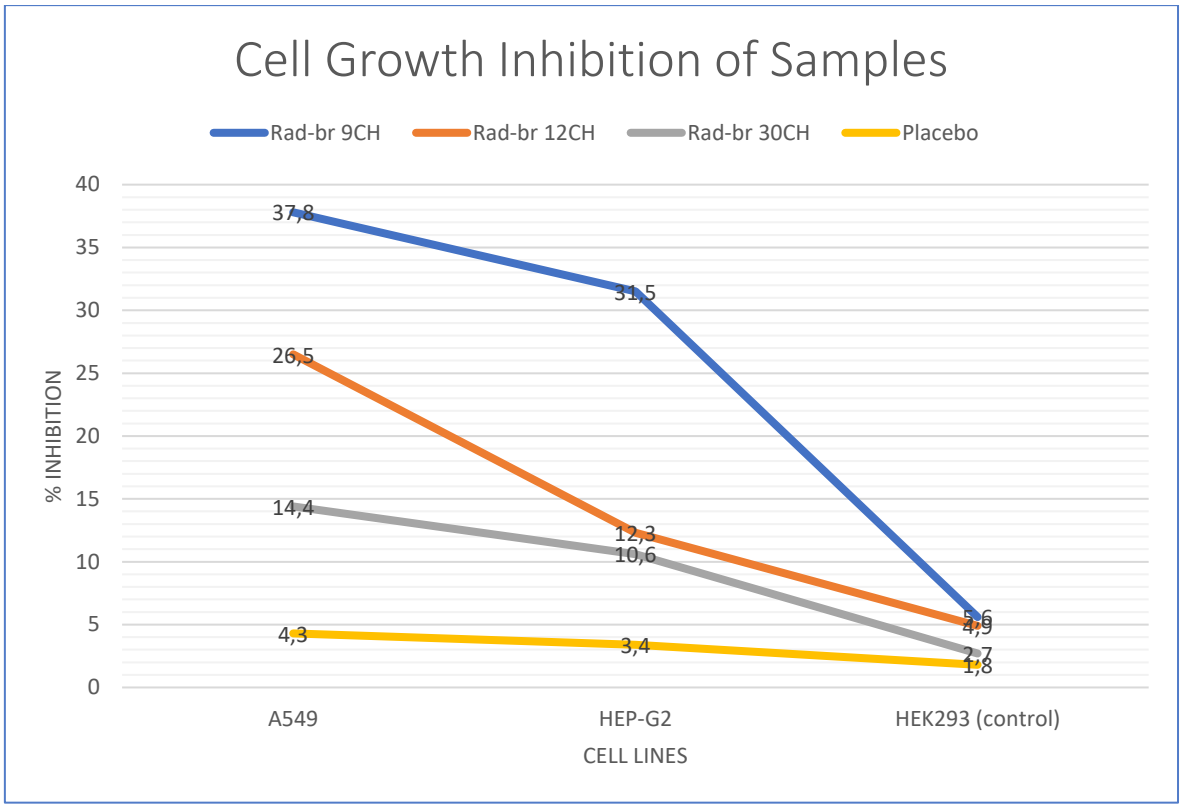

Figure 8: Graph showing an overview of the percentage of cell growth inhibition for each sample tested (*Radium bromatum* 9CH, 12CH, 30CH, and the vehicle control/placebo) against cancer cells, A549 and HEP-G2, as well as non-cancer cells, HEK293, used as a control.

CHAPTER 5: DISCUSSION

Cancer has affected populations worldwide, leading to a continuous increase in its incidence and mortality rates, impacting millions globally (International Agency for Research on Cancer 2023). Despite significant research efforts to combat cancer, conventional treatments often result in unpleasant adverse effects for patients (Vickers 2018: 25). The complex nature of cancer can also lead to drug resistance, cancer recurrence, and toxicity to normal cells, affecting patients' health and quality of life (Rezayatmand, Razmkhah and Razeghian-Jahromi 2022). This has prompted ongoing investigations into alternative, less toxic, and more effective cancer treatment options. This study aims to explore the potential anti-cancer properties of *Radium bromatum*, a non-toxic and non-radioactive form of radium bromide, against cancer cells.

Homeopathic *Radium bromatum* presents promising opportunities as a complementary or alternative therapy to conventional cancer treatments. Previous *in vivo* studies have demonstrated the potential of *Radium bromatum* in managing cancer and alleviating adverse effects of conventional therapies. Samuels *et al*. (2018) reported the use of *Radium bromatum* 30CH alongside conventional cancer treatments without any adverse effects. Another study showed the positive impact of *Radium bromatum* in reducing dermatological damage caused by radiation therapy (Rossi *et al*. 2018). A randomized controlled trial also found homeopathic medicines, including *Radium bromatum* 30CH, to be effective against radiation-induced dermatitis (Shukla *et al*. 2020). Integrating complementary medicines like *Radium bromatum* with conventional cancer treatments has been shown to improve the quality of life for patients with early breast cancer (Veyrier *et al*. 2024).

This study aims to build on previous research by investigating the anti-cancer activity of different potencies (9CH, 12CH, and 30CH) of *Radium bromatum* using the MTT assay on human lung carcinoma cell lines (A549), human hepatocellular carcinoma cell lines (HEP-G2), and non-cancerous human embryonic kidney cell lines (HEK293). Morphological changes in the cells will also be observed under an inverted microscope 48 hours post-treatment, with all treated cell lines compared to untreated control cells.

5.1 Solvent Exposure

The solvent used in this study was deionized water, aimed to remove the physiological effect of the 96% ethanol on cell lines. The solutes, *Radium bromatum* (9CH, 12CH, and 30CH) and the placebo were homeopathically potentized in deionized water from stock *Radium bromatum* (8CH, 11CH, and 29CH) and placebo all in 96% ethanol, respectively. Introducing solvents like ethanol on the growth medium of cell cultures can be toxic to cells, potentially changing the cellular environment, including damages in cell membranes leading to the potential amplification of the effects of the treatment drugs, therefore influencing the outcomes of the experiment where the results become non-reproducible, and the effect of the drug incorrectly evaluated (Timm *et al*. 2013; Nguyen, Nguyen and Truong 2020).

Therefore, ethanol in high concentrations can cause retardation of cell proliferation, while low concentrations were not significantly impacting on cells indicating non-toxic effects (Nguyen, Nguyen and Truong 2020). Research has confirmed the advantages of using purified forms of water as a vehicle containing homeopathic substances in testing cancer cells (Fuselier *et al*. 2023; Gunes *et al*. 2024). Therefore, in this study, deionized water was the preferred vehicle used, devoid of the high concentration of ethanol, thus posing no harm to the cancer cells for the purpose of this experiment.

5.2 Morphological Evaluation by an Inverted Microscope

Cancer cells are characterized by a large nucleus, irregular shape and size, prominent nucleoli, small cytoplasm, and are poorly differentiated from normal cells (Baba and Catoi 2007). Direct observation, also known as an observational study, of the morphology of cells *in vitro* provides a preliminary determination to evaluate cell death, with morphological features playing a primary role in the interpretation of cell death (apoptosis, necrosis and necroptosis) (Wu, Dong and Sheng 2020). There are many hallmarks of cell death including cell shrinkage, nuclear fragmentation, chromatin condensation, membrane blebbing, and reduction in cell density (Chaudhry *et al*. 2022).

Cellular morphology of cell death in terms of decreases in cell volume, cell density, cell viability, and cell proliferation were considered in the assessment.

In this study, the cell morphology results provided preliminary evidence of the ability of *Radium bromatum's* anti-cancer potential against lung (A549) and liver (HEP-G2) cancers, while sparing healthy kidney cells (HEK293). Rad-br 30CH had the lowest concentration compared to lower dilutions of Rad-br 9CH and Rad-br 12CH. The lower the dilutions are, the more concentrated it is. In this study, Rad-br 9CH was the lowest dilution and had a higher concentration.

Rad-br 9CH was found to be the most effective among the samples tested on malignant cells, inducing morphological changes as analyzed under the inverted microscope. These included signs of decreased proliferation, cell viability, cell density, and volume, thus demonstrating cytotoxicity and cell termination of the cancer cells through possible apoptotic pathways. Definitive morphological features of cell death were not present in the non-cancerous cells (HEK293) in both treated and untreated culture plates. This suggests the harmless effect of the homeopathic preparations against normal cells.

It is difficult to clearly justify why Rad-br 30CH was less effective compared to Rad-br 9CH and Rad-br 12CH as they are all highly diluted substances. From the viewpoint of traditional science that high dilutions, often diluted beyond Avogadro's constant, contain no single molecule of the original substance, and they do not display any effects beyond the placebo effect (Mukerji and Ernst 2022). Current available evidence of the mechanism of action is limited, ongoing research is necessary. However, the observations seen in this study are not without precedent.

Literature data provides similar evidence demonstrating anti-cancer effects of different homeopathic preparations against various cancer cells. A study conducted by Loonat *et al.* (2022) made use of *Thuja occidentalis* in mother tincture, investigated its morphological effects on A549 lung cancer cells by an inverted light microscopy. The treated cells revealed significant cellular morphological changes compared to the control. The cells of the control group remained attached to their culture plates in high density and maintained their normal shape. Whereas the treated cells with *Thuja occidentalis* showed rounded up dead cells, cell debris, cell detachment from culture plates, cell shrinkage, and reduction of cell density which are indicative of cell death (Loonat *et al.* 2022).

An *in vitro* study using homeopathic samples such as *Hydrastis* 200C, *Thuja occidentalis* 200C and *Thuja occidentalis* 1M did not demonstrate any cell morphological changes related to cell

death of cancer cells, however *Ruta graveolens* 200C and C*arcinosinum* 200C produced morphological changes inducing classical features of apoptosis in DLA tumor cells. Additionally, these features were not observed in vehicle-treated cells (Preethi *et al*. 2012).

Other studies also analyzed cell morphological changes of different homeopathically treated cancer cells under an inverted microscope. These findings resulted in reduction of cell viability, inhibition of cell proliferation, DNA damages, and decreases in cell volume which are suggestive of cell death, therefore potential anti-cancer inclinations (Arora and Tandon 2015; Mondal, Samadder and Khuda-Bukhsh 2016).

The findings of this research is therefore supported by other studies of a similar nature using high dilutions of homeopathic medicines against select cancer cells to elicit anti-proliferation, reduced number of cells and decrease in cell volume, attributed to possible cell death mechanisms.

5.3 Cytotoxicity Assessment by MTT Assay

The cytotoxicity results demonstrated toxicity towards cancer cells A549 and HEP-G2, while unaffecting healthy cells, HEK293. Rad-br 9CH was the lowest dilution and contained a higher concentration compared to Rad-br 12CH and Rad-br 30CH. In this study, Rad-br 30CH had the lowest concentration, with results showing it was less effective compared to Rad-br 9CH and Rad-br 12CH. Rad-br 9CH was found to be the most effective among the samples tested. Although the overall cell growth inhibition was minimal, there was an increase in activity as the concentration of the samples increased.

The observations of this study have also been confirmed by other precedent research assessing cytotoxicity. Previous studies have demonstrated similar *in vitro* results when using highly diluted homeopathic medicines to treat various cancer cells. Arora *et al*. (2013) conducted an *in vitro* study using several potencies of different homeopathic samples to evaluate cell cytotoxicity through the MTT method. The results of the three malignant COLO-205 colon, MCF-7 breast, and ACHN kidney cell lines exposed to their respective homeopathic treatment for 48 h revealed significant cytotoxicity of all the potencies compared to the untreated cells, with mother tinctures (MT) showing the greatest effect of toxicity towards cancer cells. Extremely high potencies of 10M revealed low cytotoxicity ranging from 21.8±4.6 to 31.7±4.1% with respect to controls. The percentage cytotoxicity of the 1M preparations ranged from 21.5±3.1 to 36.5±3.9%, and the 200CH potencies

showed 35.6±6.7 to 42.1±7.0%. Potencies of 30CH preparations exhibited cytotoxicity ranging from 42.2±2.8% to 48.6±3.6% compared to the control, while MT showed a range of 66.5±5.0 to 82.3±9.8%. Generally, higher concentrations of the samples were associated with increased cytotoxic activity. Treatment against normal renal cells (MDCK) showed low toxicity (Arora *et al*. 2013).

Other findings conducted by Samadder *et al*. (2013) evaluated potential anti-cancer properties of the high dilutions of *Lycopodium clavatum* 5C and *Lycopodium clavatum* 15C tested against HeLa cells by method of the MTT assay. The results demonstrated reduced proliferation of HeLa cells as the concentration of the samples increased by evidence of decreased cell viability. The study also tested the effect of the homeopathic samples against normal peripheral blood mononuclear cells which showed minimal or no cytotoxicity. The overall findings concluded that the potentized *Lycopodium clavatum* 5C and *Lycopodium clavatum* 15C samples exhibited signs of anti-cancer properties in cancerous HeLa cells (Samadder *et al*. 2013).

A Brazilian study using various potencies of homeopathic *Euphorbia tirucalli* and ultra-diluted latex (5CH, 15CH and 30CH) were tested *in vitro* on human melanoma (MV3) cells to analyze the proliferation of the cancer cells by the MTT method for 24 to 72 hours. For control measures, 0.5 and 5% of 70°GL EtOH solutions were used. The results observed minimal effects on the cancer cells when treated with 0.5% 70°GL EtOH solution (control), whereas exposure with 0.5% solution of *Euphorbia tirucalli* 30CH showed increased inhibition of the proliferation of melanoma (MV3) cells (da Silva *et al*. 2011).

In another study, Wani *et al*. (2016) investigated the potential anti-cancer effects of commercially available *Terminalia chebula* (TC) homeopathic medicines, examining their nanoparticle properties. The study tested various homeopathic preparations (MT, 3X, 6C, and 30C) of TC on breast cancer (MDAMB231 and MCF7) and non-cancerous (HEK 293) cell lines using the MTT assay. The results revealed that MT reduced the cell viability of both breast cancer and non-cancerous cells, while the other potencies (3X, 6C, and 30C) only affected the survival of breast cancer cells without harming non-cancerous cells (Wani *et al*. 2016).

In a recent study where human multiple myeloma (MM) cell lines were exposed to four homeopathic samples, all at the 200CH potency in distilled water, to assess viability of the cancer cells by method of the MTT assay. Decreased cell viability occurred over time

particularly at 72 and 96 hours when treated with all the homeopathic samples, with 50% of cells viable as demonstrated by the MTT method (Gunes *et al*. 2024).

Examining this evidence suggests that not all low potencies will demonstrate anti-cancer activities nor prove to be effective in all types of cancers.

An experiment testing homeopathic *Ruta graveolens* against human colon cancer cells used other means of assessing cell cytotoxicity using trypan blue exclusion assay. The findings revealed that extremely high dilutions of *Ruta graveolens* (10M, 1M, and 200CH) exhibited low cytotoxicity ranging from 30.3±1.9% to 42.1±2.4%, while *Ruta graveolens* in 30CH and MT demonstrated maximal toxicity with a cell growth inhibition effect of 72.2±2.6% and 78.7±1.3%, respectively. This study showed a wide range of varying cytotoxicity of the various potencies, highlighting that low potencies like 30CH, can also show significant cytotoxic activities against cancer cells (Arora and Tandon 2015). Therefore, homeopathic research on anti-cancer properties should be based on scientific evidence.

Further investigations into the molecular mechanisms on the activities of homeopathic potencies are necessary to help explain the reasoning behind Rad-br 30CH's less effectiveness compared to Rad-br 9CH and Rad-br 12CH.

5.4 Beyond the Placebo Effect

The cytotoxic responses of highly diluted substances are perplexing and contrasting to the findings posed by Cukaci *et al*. (2020), arguing against homeopathy's scientific efficacy that they do not display any effects beyond the placebo effect, and other studies deeming its implausibility due to its high dilution (Anon 2005; Milazzo, Russell and Ernst 2006; Mukerji and Ernst 2022). In this study, the results demonstrated anti-proliferation activities of the various *Radium bromatum* potencies, particularly *Radium bromatum* 9CH, towards cancerous cells A549 and HEP-G2. Although the cell growth inhibition percentages were minimal overall, it revealed higher inhibition effects compared to the placebo, indicating that all the *Radium bromatum* samples were more effective than the placebo.

While comparison between the cancer cells, A549 responded more effectively than HEP-G2 when treated with *Radium bromatum* in general, revealing higher cell growth inhibition. The variation in their responses may have various possibilities, including their different genetic profiles influencing potential drug metabolism and cellular signaling pathways, as well as

influencing drug delivery responses whereby the A549 and HEP-G2 cells have different cell membrane transporters which can affect drug delivery to cells, thus affecting the efficacy of the drug being tested (Nagai *et al*. 2021).

The data also showed similar percentage inhibition with regards to each potency against the different cancer cells, indicating parallel consistencies of the testing samples. *Radium bromatum* 9CH showed the most significant effect due to a higher concentration compared with *Radium bromatum* in 12CH and 30CH. Therefore, indicating that cell proliferation decreases with an increase in concentration of the therapeutic medicines. This indicates that *Radium bromatum* in different potencies of 9CH, 12CH, and 30CH were able to induce cytotoxicity on the cancerous cells, causing reduced cell viability and cell death. Finally, no significant cytotoxic effects were observed on the healthy cell (HEK293 cells) when exposed to the various potencies, thus highlighting the value of the positive control in validating and comparing with the samples used in this experiment.

The effect of the potentization process of homeopathic medicines have been claimed to be enormously important, with substances diluted to 12CH or above is known to surpass Avogadro's number, suggesting that no molecules of the original source exist (Witt *et al*. 2007; Ernst 2016: 50), which has become a controversial subject for the scientific communities. Research have also confirmed the existence of nanoparticles of the original substances found even in extremely high dilutions, challenging the traditional understanding of the Avogadro's constant and the role of dilution practiced in homeopathy (Chikramane *et al*. 2010; Bell and Koithan 2012; Demangeat 2013; Elia *et al*. 2014; Wani *et al*. 2016; Nandy and Bhar 2021). Based on quantum mechanics or quantum electrodynamics, the homeopathically potentized dilutions contain information linked to their original material or coding of the original substance, resulting in the transfer of its information (Mordeniz 2017; Manzalini and Galeazzi 2019).

Other studies showed that homeopathic medicines goes beyond the placebo effect due to its association with the modification of gene expressions and evidence of similar biological activities to their mother tincture or starting materials (Preethi *et al*. 2012; Saha, Roy and Khuda-Bukhsh 2013). Recent investigations aimed to assist in the explanation of the potential physicochemical properties of homeopathic medicines include different techniques such as optical spectroscopy, nuclear magnetic resonance (NMR) relaxation, and electrical impedance measurements (Tournier *et al*. 2019). While the use of homeopathy is increasingly

in demand and extensively practiced in many countries worldwide, continuous research are ongoing to validate the homeopathic phenomenon and further understand its molecular mechanisms (Bodeker *et al.* 2005; Frenkel 2015).

Consequently, quantitative experiments with scientifically approved parameters are paramount in the investigation and assessment of homeopathically potentized and highly diluted medicines, in order to scientifically discern outcomes without prejudice. In this study, the placebo, which contained no initial material of the source substance, underwent the same homeopathic potentization process as the *Radium bromatum* samples. In the final step before use, all samples including the placebo were homeopathically potentized in deionized water. The results observed in this study showed that treatment with the placebo failed to elicit similar responses to the highly diluted *Radium bromatum* samples, highlighting that the *Radium bromatum* samples were superior to the placebo, and proving that the homeopathic medicines contain molecules or particles of the original source in order for it to cause a reaction beyond the placebo as demonstrated in this study.

Several studies proved similar outcomes of the various homeopathic medicines used, demonstrating better results of anti-cancer activities compared to the placebo, used as the control in their research (da Silva *et al.* 2011; Arora *et al.* 2013; Samadder *et al.* 2013; Arora and Tandon 2015; Fuselier *et al.* 2023; Gunes *et al.* 2024).

In biological research, the effects of cell growth inhibition and cell death (apoptosis) are important markers to analyze, particularly in the development of cancer treatment. As an innovative and promising strategy for cancer prevention, considerable interest has been focused on the manipulation of apoptosis in cancer cells. The molecular mechanisms of apoptosis are essential for comprehensive understanding in anti-cancer research.

Therefore, the findings of this study warrant further *in vitro* studies to elucidate the apoptotic pathways involved and to determine whether the apoptotic effects of these samples contribute to their overall chemotherapeutic abilities in combating human lung and liver cancers, thus potentially leading to future therapeutic applications.

5.5 Gaps and Limitations of this study

This study explored a crucial gap in our understanding of *Radium bromatum*'s effectiveness, especially at specific potencies. It further addressed the lack of scientific evidence for

homeopathy by employing a controlled experimental design, the limited research available on the efficacy of different potencies of *Radium bromatum* in cancer treatment, and the lack of pre-clinical data on this medicine that limits its exploration in clinical settings.

There were other limitations identified in this study which will be necessary to overcome in order to improve therapeutic outcomes, thus enabling more productive and effective treatment strategies for improving patient survival. The limitations found include:

- The study only considered the effects of low centesimal potencies; the effects of low decimal potencies may have been useful for comparison and may prove to be more optimal.
- Studies on the *in vitro* mechanisms of *Radium bromatum* were not carried out.
- Since homeopathy is known to encompass the physical, mental, and emotional state of an individual during treatment, therefore, the effectiveness of *Radium bromatum in vivo* was not ascertained in this *in vitro* study which limits the full understanding of the medicine.

By investigating *Radium bromatum's* potential anti-cancer properties, the *in vitro* research provides valuable new insights previously unavailable to the scientific community. This early-stage study can serve as a foundation for further research to evaluate *Radium bromatum's* safety and efficacy in animal models and human cancer patients, potentially leading to the development of novel cancer therapies incorporating *Radium bromatum* or similar homeopathic substances, such as targeted cancer therapy, combination therapy, and drug development.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Cancer is a significant global health concern affecting millions of individuals physically, mentally, and emotionally (Stein, Syrjala and Andrykowski 2008; American Cancer Society 2024). Conventional cancer therapies face challenges such as drug resistance, reduced efficacy, and adverse effects that impact patients' quality of life and survival (Rezayatmand, Razmkhah and Razeghian-Jahromi 2022). Homeopathy is gaining international and national attention, with research showing promising outcomes from previous *in vitro* studies on the potential anti-cancer properties of highly diluted substances, capable of inducing cell cytotoxicity, anti-proliferation, gene alterations, DNA fragmentation, cell cycle arrest, and cell death.

This study aimed to investigate the effects of homeopathy at a cellular level to determine if *Radium bromatum* of different potencies (9CH, 12CH, and 30CH) exhibits potential anti-cancer activities in cancerous human lung carcinoma cell lines (A549) and human hepatocellular carcinoma cell lines (HEP-G2) in vitro, using non-cancerous human embryonic kidney cell lines (HEK293) as a control. The findings of this investigation support the hypothesis that highly diluted *Radium bromatum* can inhibit cell proliferation in cancer cell lines, particularly A549 and HEP-G2. Morphological changes in cancer cells, including decreased proliferation, cell viability, density, and volume, were observed under an inverted microscope, indicating cytotoxicity and cell death through possible apoptotic pathways. The MTT assay also demonstrated anti-proliferative activities of the various *Radium bromatum* potencies, with Rad-br 9CH showing the most significant effect on A549 and HEP-G2 cells, leading to reduced cell viability and cell death. The data from this study showed that the placebo treatment did not produce similar responses to the highly diluted *Radium bromatum* samples, underscoring the superiority of *Radium bromatum*.

Thus, this research provides preliminary *in vitro* evidence of *Radium bromatum* as a potential anti-cancer agent due to its anti-proliferative properties against cancerous cells (A549 and HEP-G2) while sparing non-cancerous cells (HEK293). It also emphasizes that homeopathic medicines should not be dismissed as placebos. This study addresses a significant gap in scientific literature with respect to *Radium bromatum* and its efficacy, specifically on the effects of different potencies. A controlled experimental design was conducted to investigate

the effects which directly addresses the need for more rigorous scientific evaluation, also addressing the scarcity of studies on *Radium bromatum* in cancer research and the absence of pre-clinical data.

Ethical considerations or challenges in the use of homeopathy in cancer research may include the lack of scientific evidence, the welfare of animals, the potential harm, and the need for informed consent. A careful evaluation regarding these ethical implications is important prior to conducting any research in this area.

The feasibility of transitioning these *in vitro* findings into pre-clinical (animal) and clinical models is complex and subjected to several factors, including key considerations on reproducibility of these *in vitro* results, selection of appropriate animal models that can closely mimics human cancers, and comprehensive safety assessments in pre-clinical models before considering human trials.

This study opens the door for further investigations into the therapeutic applications of *Radium bromatum* for cancer treatment. Further research is warranted.

6.2 Recommendations

Based on the findings of this study, future research can be considered on the following:

- The effects of *Radium bromatum* on other cancer cell lines as different types of cancers have different metabolic characteristics, gene expressions, and signaling pathways.
- An exploration to address the need for advanced analytical techniques, such as genetic analyses or molecular assays, for further validation.
- Investigation into the mechanism of action of *Radium bromatum* that may enrich the understanding of the homeopathic remedy.
- Investigation into the apoptotic pathways involved in the anti-cancer effects of *Radium bromatum*.
- The effects of *Radium bromatum* on cancer cell lines previously exposed to radiation.
- Using other approaches to test the cell viability or cytotoxicity in response to treatment.
- An *in vivo* rat model to test *Radium bromatum*.

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LIST OF APPENDICES

Appendix A

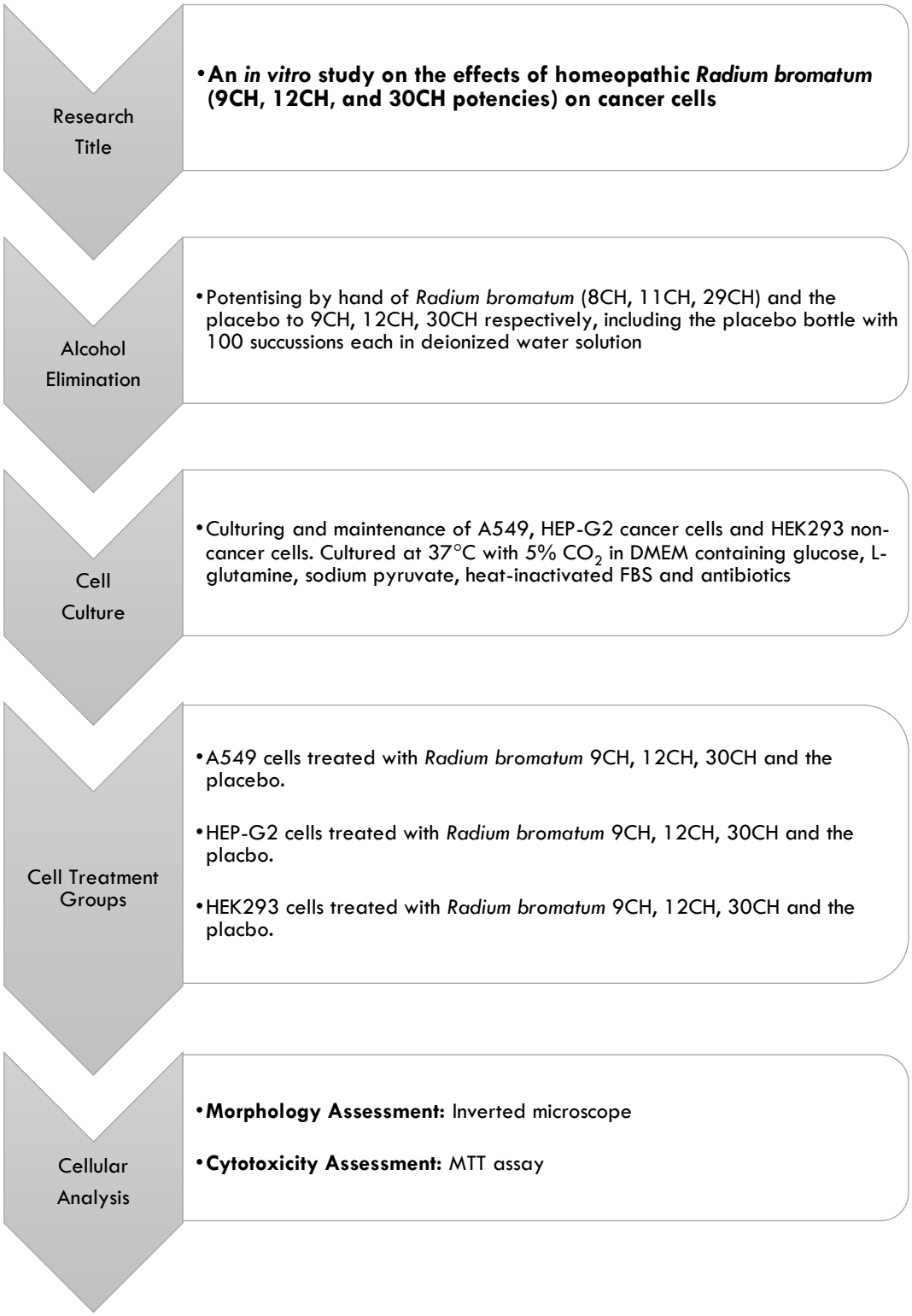
Comparison between dilution, concentration and centesimal potency

Dilution	Concentration	Centesimal potency
1:100	10^{-2}	1C or 1CH
1:10 000	10^{-4}	2C or 2CH
1:1 000 000 or $1:10^{6}$	10^{-6}	3C or 3CH
$1:10^{8}$	10^{-8}	4C or 4CH
$1:10^{10}$	10^{-10}	5C or 5CH
$1:10^{12}$	10^{-12}	6C or 6CH
$1:10^{14}$	10^{-14}	7C or 7CH
$1:10^{16}$	10^{-16}	8C or 8CH
$1:10^{18}$	10^{-18}	9C or 9CH
$1:10^{20}$	10^{-20}	10C or 10CH
$1:10^{22}$	10^{-22}	11C or 11CH
$1:10^{24}$	10^{-24}	12C or 12CH
$1:10^{26}$	10^{-26}	13C or 13CH
$1:10^{28}$	10^{-28}	14C or 14CH
$1:10^{30}$	10^{-30}	15C or 15CH
$1:10^{60}$	10^{-60}	30C or 30CH
$1:10^{400}$	10^{-400}	200C or 200CH
$1:10^{2000}$	10^{-2000}	1000C or M
$1:10^{20\,000}$	$10^{-20\,000}$	10 000C or 10M

Homeopathic centesimal potency (Kayne 2006: 96).

Appendix B

Flow diagram of the research methodology – a summary outline



Appendix C

List of media, reagents and equipment used for cell culture

Product	Supplier/Manufacturer
96-well microtiter plate	The Scientific Group South Africa
A549 cell lines	Dr. Rene Khan at the School of Medical Biochemistry, UKZN
Cell culture flasks (25cm^2)	The Scientific Group South Africa
Cell culture flasks (75 cm^2)	The Scientific Group South Africa
Cryotubes	Corning, South Africa
Dimethyl sulfoxide (DMSO)	ThermoFisher Scientific
Dulbecco's Modified Eagle Medium (DMEM)	ThermoFisher Scientific
Fetal bovine serum (FBS) and antibiotics	ThermoFisher Scientific
HEK293 cell lines	Dr. Rene Khan at the School of Medical Biochemistry, UKZN
HEP-G2 cell lines	Dr. Rene Khan at the School of Medical Biochemistry, UKZN
Humidified incubator	SnjidersHepa by United Scientific, Cape Town, South Africa
Microplate reader	BioTek Instruments, Inc. USA
MTT reagent	Sigma-Aldrich
Phosphate-buffered saline (PBS)	ThermoFisher Scientific
Trypan blue dye	ThermoFisher Scientific
Trypsin	ThermoFisher Scientific

Appendix D

Manufacturing process and mathematical calculations of Radium bromatum

Part 1: Manufacturing of *Radium bromatum* from source material

The medicinal substance was supplied by a reputable homeopathic manufacturer and supplier, CoMED Health (Pty) Ltd.

According to CoMED Health (Pty) Ltd., the source material, radium bromide, was manufactured according to the German Homeopathic Pharmacopoeia, GHP 6 method, i.e. trituration of insoluble substances in lactose monohydrate as the vehicle, followed by the GHP 8a method i.e. liquid preparations made from trituration obtained as per GHP 6.

For this experiment, CoMED Health (Pty) Ltd. manufactured and supplied *Radium bromatum* 8CH, 11CH and 29CH including a placebo, as per standard serial dilutions under the rules stipulated in the Pharmacopoeia. The liquid dilution ratio used was 1 part substance to 99 parts of 96% ethanol (1:100 centesimal dilution), with succussion by hand 100 times between potencies. The placebo bottle contained the same 96% ethanol used for potentized *Radium bromatum* bottles. Additionally, the supplier certified that the excipients and the packaging materials used were tested and released for quality.

Part 2: Manufacturing of final potency solutions from *Radium bromatum* (8CH, 11CH, and 29CH) and placebo

Aim:

To produce final potencies of *Radium bromatum* (9CH, 12CH, and 30CH) and the placebo into deionized water from *Radium bromatum* (8CH, 11CH, and 29CH) and placebo all in 96% EtOH, respectively.

Apparatus:

- *Radium bromatum* (8CH, 11CH, and 29CH) and placebo all in 96% EtOH (batch *75857*, exp 06/2026).
- Deionized water

- 4 x 30 mL amber glass dropper bottles
- Micropipette
- Micropipette tips
- Pipettes (10 mL)
- Laminar flow.

Calculations (for centesimal potencies):

1/100 x required volume

1/100 x 20 mL = 0,2 mL of *Radium bromatum* (8CH or 11CH or 29CH) or placebo

Hence 20 mL - 0,2 mL = 19,8 mL of deionized water.

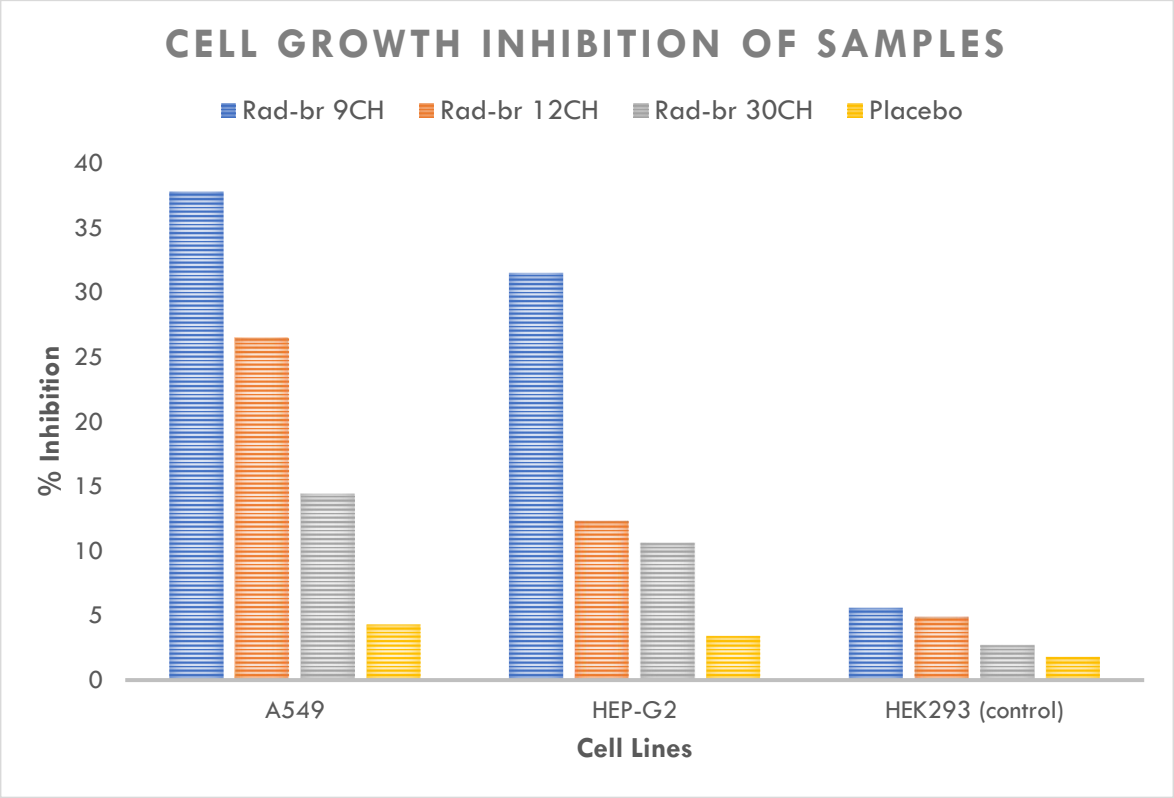
Method:

(Cleaned all equipments and worktops. Materials and techniques were prepared under sterile conditions. Laboratory rules and safety regulations were strictly adhered to).

1. Measured out 19,8 mL of deionized water by method of a 10 mL pipette & micropipette.
2. Added into a 30 mL amber glass dropper bottle.
3. Measured out 0,2 mL of previous potency (e.g. *Radium bromatum* 8CH) by method of micropipette.
4. Added into same 30 mL amber glass dropper bottle.
5. Securely closed remedy bottle lid.
6. Succussed remedy bottle by hand 100 times.
7. Labelled bottle accordingly: "Rad-br 9CH in deionized water", batch 049K23, exp 06/2024.
8. Repeated steps for *Radium bromatum* (12CH and 30CH), and placebo bottles.

Appendix E

Diagrammatic graph showing an overview of the percentage of cell growth inhibition for each sample tested



Appendix F

Gatekeeper's permission (Biotechnology and Food Science Department)

10/11/2022

To:

Professor Feroz Mahomed Swalaha

Head of Department of Biotechnology and Food Science.

Durban University of Technology

Request for Permission to Conduct Research

Dear Prof F. Swalaha,

My name is Ting-Yen Yang, a MHSc Homeopathic student at the Durban University of Technology. The research I wish to conduct for my Master's dissertation involves an *in-vitro* study on the effects of homeopathic *Radium bromatum* in different potencies on cancer cells.

I am hereby seeking your consent to please have access to utilise the Biotechnology laboratory facilities to assist in conducting my research.

I have provided you with a copy of my proposal which has been accepted by the Faculty Research Committee (FRC) with an approval letter. If you require any further information, please do not hesitate to contact me on gjackie.y111@gmail.com. Thank you for your time and consideration in this matter.

Yours sincerely,

T Yang

Durban University of Technology

Appendix G

Gatekeeper's permission (Homeopathy Department)

10/11/2022

To:

Professor Ashley Ross

Acting-Head of Department of Homeopathy

Durban University of Technology

Request for Permission to Conduct Research

Dear Prof A. Ross,

My name is Ting-Yen Yang, a MHSc Homeopathic student at the Durban University of Technology. The research I wish to conduct for my Master's dissertation involves an *in-vitro* study on the effects of homeopathic *Radium bromatum* in different potencies on cancer cells.

I am hereby seeking your consent to please have access to utilise the homeopathic laboratory facilities to assist in conducting my research.

I have provided you with a copy of my proposal which has been accepted by the Faculty Research Committee (FRC) with an approval letter. If you require any further information, please do not hesitate to contact me on gjackie.y111@gmail.com. Thank you for your time and consideration in this matter.

Yours sincerely,

T Yang

Durban University of Technology

Appendix H

Supplier's release certificate of samples

CoMED Health (Pty) Ltd
313 Kuit St, Waltloo, Pretoria, 0184
P O Box 659, Silverton, 0127
T: 012 813 9400 • F: 012 813 9698
www.comedhealth.co.za
SA Pharmacy Council No. 0095
[Vat. No. 4010253583] [Reg. No. 2008/005773/07]
Directors: C. Dillon (B.Sc; MBA); G. Shayne (B.Com; CA)*; M. Levien (HD.; ND.; DO.);
W. Jonker (B.Com (Hons); B.Compt.; MBA); P. Kreft (B.SC. (Hons))**
*British **Managing
Bank: Nedbank Business Banking
Branch No: 198765
Acc.No: 1497218365

Release certificate

Name of Product: **Radium bromatum 8CH (96%)**
Starting substance batch number: **20R02044**
Potency batch number: **75857**
Reference date: **15/06/2023**
Expiry date: **06/2026**
Order number: SO223999

The Head of the Dispensing Laboratory certifies:

- that the homoeopathic starting substances have been manufactured according to standards of German Pharmacopoeia,
- that the excipients used have been tested and released for quality,
- that manufacturing process for the potency was carried out according to the rules as stipulated in the Pharmacopoeia,
- that the packaging materials have been tested and released for quality,
- that the labelling on the finished product has been controlled,

In view of the inspection performed, the Head of the Dispensing Laboratory authorises release for shipment.

28/07/2023

Dr. R. Mazibuko
Dispensing Laboratory Manager

CoMED Health (Pty) Ltd
313 Kuit St, Waltloo, Pretoria, 0184
P O Box 659, Silverton, 0127
T: 012 813 9400 • F: 012 813 9698
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SA Pharmacy Council No. 0095
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Directors: C. Dillon (B.Sc; MBA); G. Shayne (B.Com; CA)*; M. Levien (HD.; ND.; DO.);
W. Jonker (B.Com (Hons); B.Compt.; MBA); P. Kreft (B.SC. (Hons))**
*British **Managing
Bank: Nedbank Business Banking
Branch No: 198765
Acc.No: 1497218365

Release certificate

Name of Product: **Radium bromatum 11CH (96%)**
Starting substance batch number: **20R02044**
Potency batch number: **75857**
Reference date: **15/06/2023**
Expiry date: **06/2026**
Order number: SO223999

The Head of the Dispensing Laboratory certifies:

- that the homoeopathic starting substances have been manufactured according to standards of German Pharmacopoeia,
- that the excipients used have been tested and released for quality,
- that manufacturing process for the potency was carried out according to the rules as stipulated in the Pharmacopoeia,
- that the packaging materials have been tested and released for quality,
- that the labelling on the finished product has been controlled,

In view of the inspection performed, the Head of the Dispensing Laboratory authorises release for shipment.

28/07/2023

Dr. R. Mazibuko
Dispensing Laboratory Manager

CoMED Health (Pty) Ltd
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W. Jonker (B.Com (Hons); B.Compt.; MBA); P. Kreft (B.SC. (Hons))**
*British **Managing
Bank: Nedbank Business Banking
Branch No: 198765
Acc.No: 1497218365

Release certificate

Name of Product: **Radium bromatum 29CH (96%)**
Starting substance batch number: **20R02044**
Potency batch number: **75857**
Reference date: **15/06/2023**
Expiry date: **06/2026**
Order number: SO223999

The Head of the Dispensing Laboratory certifies:

- that the homoeopathic starting substances have been manufactured according to standards of German Pharmacopoeia,
- that the excipients used have been tested and released for quality,
- that manufacturing process for the potency was carried out according to the rules as stipulated in the Pharmacopoeia,
- that the packaging materials have been tested and released for quality,
- that the labelling on the finished product has been controlled,

In view of the inspection performed, the Head of the Dispensing Laboratory authorises release for shipment.

28/07/2023

Dr. R. Mazibuko
Dispensing Laboratory Manager

Appendix I

Turnitin plagiarism report

Thesis

ORIGINALITY REPORT

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7	Shagun Arora, Ayushi Aggarwal, Priyanka Singla, Saras Jyoti, Simran Tandon. "Anti-proliferative effects of homeopathic medicines on human kidney, colon and breast cancer cells", Homeopathy, 2013 Publication	<1%