CHAPTER II

REVIEW OF LITERATURE

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Cancer is a collection of diseases that can cause death in vertebrates and are defined by abnormal cell proliferation. These cancer cells can penetrate and obliterate healthy cells (Madhuri *et al.,* 2009). A set of more than 200 diseases with similar symptoms are collectively referred to as cancer under the general word. Uncontrolled, limitless development and the spread of cells to other parts of the body are characteristics of cancers (Yarbro *et al.,* 2011).

2.1 Cancer

The second most prevalent cause of mortality worldwide, cancer is a serious issue for public health. Uncontrolled development and spread of aberrant cells characterize cancer, a group of illnesses that can be fatal if the spread is not stopped (Siegel *et al.,* 2019). Tumor suppressor genes undergo cumulative genetic and epigenetic alterations that lead to cancer (Wang *et al.,* 2018). Cancer treatment tries to eradicate cancer cells while minimizing damage to healthy cells (Fournier, 2013). Localized, systemic, or supportive medicines are used in the treatment of cancer to minimize side effects (Miller *et al.,* 2019). Twenty-three percent of cancer fatalities worldwide and 23.4% of cancer incidences occur in Europe.

In Bangladesh, there are 13 to 15 lakh cancer patients, with 2 lakh people receiving a cancer diagnosis for the first time every year (Uddin *et al.,* 2013). The two most common malignancies in men are lung cancer and mouth and oropharynx cancer. Esophageal cancer, stomach cancer, lymphomas, and multiple myeloma are some more cancer kinds that are frequently mentioned. The most common cancers in women are those of the cervix and breast. Lung cancer, esophageal cancer, and mouth and oropharynx cancer are other cancer kinds that afflict women (Noronha *et al.,* 2012). There are around 4000 compounds in tobacco smoke, at least 438 of which can lead to cancer. The single most significant modifiable risk factor for cancer (30%) is tobacco use. Unfortunately, since 1980, bidi manufacture in Bangladesh has increased even more quickly than cigarette production. According to a WHO research, 5 million women and 20 million men smoke tobacco in Bangladesh, and 57,000 people pass away each year from illnesses linked to tobacco use

(Islam *et al.,* 2013). In Bangladesh, 41% of men who are 15 years of age or older smoke. When looking at women over the age of 15, it was 1.8% (Islam *et al.,* 2013).

In cancer cells that utilize this metabolic route for ATP production, increased glycolysis serves as the primary source of energy (Gill *et al.,* 2016). Glycolysis breaks down glucose into two pyruvate molecules and two net molecules of ATP (Chaudhry *et al.,* 2019). In the presence of oxygen, normal cells use glycolysis and oxidative phosphorylation to produce energy (Dhup *et al.,* 2012). Even if there is a lot of oxygen available, cancer cells primarily generate energy by increasing the rate of glycolysis in comparison to normal cells (Alfarouk *et al.,* 2014). Cancer cells are far more proliferative than normal cells, hence they need more ATP to satisfy their metabolic demands (Cairns *et al.,* 2011). The Warburg effect encourages the growth of cancer cells. Cancer cells depend on the Warburg effect because they require a lot of energy to continue growing (Koppenol *et al.,* 2011). When essential glycolytic enzymes are inhibited, ATP is depleted and lactate production is reduced (Guo *et al.,* 2016). Reduced lactate synthesis disrupts cancer cells (Lu *et al.,* 2011). Reduced glucose absorption and lactate production result from starvation (Gonin-Giraud *et al.,* 2002). Once entering the cell, glucose undergoes a sequence of enzyme processes to produce pyruvate (Lu *et al.,* 2011). Even in the presence of oxygen, pyruvate is preferentially transformed into lactate in cancer cells. Apoptosis is potently induced by ATP depletion brought on by glycolytic inhibition. Breast cancer cells undergo apoptosis when they are starved (Fu *et al.,* 2007). Apoptosis is the focus of current cancer therapeutic techniques. Tumor elimination with oncolytic virotherapy alone is ineffective (Chu *et al.,* 2004). Combination medicines target tumor cells while halting the emergence of treatment resistance. Chemotherapy and virotherapy together provide promise for treating cancer (Shammari *et al.,* 2016).

2.2 Sarcoma type cancer

Bone, cartilage, muscle, endothelium, and blood cells are all produced by the mesoderm. Sarcomas are malignant non-hematological tumors that resemble these tissues. Except for a small number of neoplasms that are likely neuro ectodermal in origin, the bulk of sarcomas are assumed to develop in pluripotent mesenchyme stem cells (Ewing family of

tumors). Sarcomas are uncommon tumors that account for 1% of all malignancies on a steady basis (Ries *et al.,* 1999); nonetheless, they are somewhat more prevalent in childhood and adolescence, where they account for 6-7% (Ries *et al.,* 1999). Soft tissue sarcomas (STS), which originates from soft tissues, and bone sarcomas (BS), which originates from bone or cartilage, are the two primary types into which sarcomas are typically categorized.

The gi mesoderm is the four most prevalent adult STS subtypes, out of the more than 50 available, are pleomorphic undifferentiated sarcoma, which accounts for 20–70% of STS, liposarcoma (10–16%), fibrosarcoma (10–65%), and leiomyosarcoma (5–10%). The ranges mentioned show how sarcoma histotype definition and perception have changed significantly throughout time and among pathologists (Weissmueller *et al.,* 2014). The average age of STS is 65 years old 8. Rhabdomyo sarcoma and fibro sarcoma are the most prevalent STS in children. Osteosarcoma (OS), chondrosarcoma, and Ewing's sarcoma are the three types of bone sarcomas. OS and Ewing's sarcoma have peaks in frequency in late childhood and adolescence, whereas chondrosarcoma is largely a tumor of adulthood (Huvos *et al.,* 1991). Sarcomas' etiology is not well understood. Chemicals (phenoxy acetic acids and chlorophenols used as herbicides (Balarajan *et al.,* 1984); dioxin (Eriksson *et al.,* 1990); vinyl chloride (Ward *et al.,* 2001); previous radiotherapy (Murray *et al.,* 1999); viral infections (human herpes virus 8 is the causative agent of Kaposi's sarcoma (Jenner *et al.,* 2002); EBV is involved in the development of smooth muscle tumours; and acquired immunological defects are risk factors. Certain sarcomas are caused by some germline mutations that disrupt tumor suppressor genes in the Li Fraumeni syndrome (Malkin *et al.,* 1990), Rb in retinoblastoma (Friend *et al.,* 1986). Sarcomas can develop at any anatomic site, and their distribution varies widely within and among histological subtypes. Almost half of STS are detected in the extremities, and bone sarcomas as a whole have a similar distribution (Pisters *et al.,* 2008). 90% of OS cases are restricted to the extremities, according to (Bielack *et al.,* 2002).

2.3 Factors that can cause cancer

2.3.1 Extrinsic factors

2.3.1.1 Diet

According to human ecology research, a variety of dietary factors may contribute to the genesis of different types of cancer. Much eat animal fat and too little fresh fruits and vegetables in western countries, which raises the risk of colorectal cancer. Moreover, the risk of certain cancers, such as colorectal adenoma, is increased by meals that contain preservatives, flavors, and coloring agents (Sinha *et al.*, 2005).

2.3.1.2 Environment

There are numerous carcinogens in the environment. Some of those carcinogens are man made, such as radiation, pollution, while others are natural, such as prolonged exposure to sunshine (Berrington 2009). The most common exogenic risk factors for the development of cancer appear to be alcohol drinking, smoking, and exposure to X-ray and gamma radiation (Seitz *et al.*, 1998). Target cells are penetrated by carcinogenic chemicals, which also routinely reduce cell metabolism and result in a relative immunological deficit (Carvalho 1997).

2.3.1.3 Tobacco Smoking

Additionally, the risk increases with the amount of time spent smoking. Because of this, the yearly lung cancer death rate among 55–64-year-olds who smoked 21–39 cigarettes per day is almost three times greater for those who started smoking at age 15 than for those who started at age 25. The smoking technique (cigarette, cigar, pipe, hookah, etc.) and, for any one method, the "depth" of inhalation, both of which may be determined, determine the intensity of exposure to tobacco smoke. The majority of tobacco-related malignancies are more likely to be contracted from smoking black tobacco cigarettes than blond cigarettes. Much like filtered and low tar cigarettes, unfiltered and high tar cigarettes have a higher risk for most tobacco-related malignancies. There is no such thing as a "safe" cigarette; smoking tobacco products has a carcinogenic risk. Together, the epidemiological information shown above establishes "causation" due to the epidemiological data's

consistency, strength, specificity, temporal order of exposure and disease, and dosage response connection. Smoking and lung cancer are strongly correlated with socioeconomic status in many societies (Stellman *et al.,* 1997). Lung cancer incidence varies significantly between localities globally. Some regions of North America have high rates, whilst the lowest rates are seen in underdeveloped nations. 57-80% of lung cancer in women and 83-92% of lung cancer in males in the United States, Europe, and Japan, respectively, is a result of tobacco use. When smoking has reached its highest frequency and has persisted for the majority of smokers' lives, lung cancer has its greatest influence on the population. Lung cancer will certainly become more and more prevalent in the developing countries in the ensuing decades as smoking becomes more common there (Chen *et al.,* 1997). In addition to lung cancer, smoking causes cancers of the larynx, oral cavity, pharynx, oesophagus, pancreas, kidney and bladder (Law *et al.,* 1996).

2.3.1.4 Viruses

Viral infections are thought to be responsible for 15–20% of all human malignancies (Pagano *et al.,* 2004). As necessary intracellular parasites, viruses produce proteins that alter host cell signaling pathways that regulate cell proliferation, differentiation, cell death, genomic integrity, and immune system recognition (Johnston *et al.,* 2007).

2.3.1.5 Human Papilloma Virus (HPV)

More than 200 distinct members of the human papilloma virus family, or HPV types, infect human mucous membranes in the vaginal and oral regions in addition to the skin (McKaig *et al.,* 1998). The majority of HPV types are benign, and the infection either goes away on its own or, less frequently, the virus may cause the growth of warts or papillomas, which is how they acquired their name since these lesions are comparable to those that closely related papilloma viruses may cause in animals (Elson *et al.,* 2000). However, certain HPV strains are known as high-risk (HR) HPV types because they have been linked to cancer in people. Anogenital malignancies, including cervical cancer and certain cancers of the head and neck, can be brought on by HR-HPVs. It has been demonstrated during the past several decades that various cancers of the upper neck have become more common, and this is mostly due to tonsillar and base of tongue tumors that are HPV positive (Davis *et al.,* 2018).

2.3.2 Intrinsic factors

2.3.2.1 Age and Genetic instability

People of various ages are affected by cancer. Even without a family history of cancer, older persons have a higher risk of developing cancer. The genetic damage in the cells and protracted exposure to toxins may be to blame for this. The National Cancer Institute (NCI), United States of America (USA), reports that adults over 65 accounts for 60% of newly diagnosed malignancies (Jemal *et al.,* 2010).

According to research, tumor cells in some types of human cancer have an average of ten mutations per several hundred genes (Stites *et al.,* 2009). This is partly because cellular DNA damage can be a continuous process brought on by several mutagens (Loeb *et al.,* 2003). The structure of DNA is known to be altered by reactive metabolites and some environmental organisms, and these chemical changes to the genetic material can cause mutations and cancer. By producing hazardous reactive oxygen species, which are known to change DNA, carcinogens may directly damage DNA, leading to mutations or other undesirable chromosomal events. Moreover, carcinogens are linked to epigenetic alterations, which are changes in gene expression without altering the genetic code (Marsit *et al.,* 2006). Reactive oxidative species are also produced during tissue repair, which includes inflammation and reparative cellular proliferation to replace injured cells. Oxidative damage to DNA can change 10,000 nucleotide bases per cell every day (Loeb *et al.,* 2003). Mutagenesis is more likely since there are so many different ways that genetic harm might take place. Yet, the development of cancer typically cannot occur after a single mutagenesis event (Loeb *et al.,* 2003; Tran *et al.,* 2008).

Instead, the formation of cancer is a complex process that depends on numerous mutagen (Bild *et al.,* 2006). These tests demonstrate that at least two genetic alterations are required for the development of tumors, and research using human cells demonstrates that additional alterations in the genetic code are necessary for the development of human cancers (Loeb *et al.,* 2003).

This is demonstrated by the exponential rise in tumor incidence with age (Loeb *et al.,* 2003), which suggests that ongoing mutagenesis contributes to the buildup of DNA and cellular machinery damage. Experiments using cultured rodent cells to study cancer have

revealed additional evidence of rate-determining mutagenesis events in the development of cancer.

The mutation phenotype theory was put out by (Loeb *et al.,* 2003). It states that malignant phenotypes result from mutations in genes that, in their unaltered state, maintain genetic stability. The impacted genes generally control DNA replication and repair accuracy, preserve the original nucleotide sequence, control damage checkpoints, and control cellular responses like apoptosis. It is argued that the high number of mutations frequently discovered in tumor cells cannot be explained by a normal mutation rate. The mutation phenotype theory postulates that the significant number of genetic abnormalities found in malignant cells may be explained by an early mutation that subsequently boosts the mutation rate. According to the hypothesis, mutations in genes that code for proteins essential for maintaining genetic stability would make it difficult to stop or correct existing mutations, leading to a chain reaction of risky mutagenesis across the entire genome.

After developing enough mutations, cells become malignant, and as they multiply, rounds of cellular selection favor cells that have mutations that provide advantages for proliferation. (Anderson *et al.,* 2003). According to one theory, malignant cells may experience negative effects if they accumulate a lot of genetic changes because most mutations are bad in and of themselves (Loeb *et al.,* 2003).

2.3.2.2 Oncogenes

Oncogenes require dominant mutations of the gene's healthy version, known as a proto-oncogene, to become active. Because the activity of the non-mutated allele may not be adequate to maintain normal cellular function, they can be activated by changing a single allele (Moy *et al.,* 2017). Since the precise function of an oncogene can vary, oncogene activation or mutation can provide a cancer cell with a variety of skills (Jones *et al.,* 2009). Because oncogenes play such a significant role in tumor development and maintenance, some cancers require oncogenic activation to survive for an extended period (Tran *et al.,* 2008). Cancerous cells need oncogenes and their byproducts to fulfill their specific roles to function and survive, a condition described as "oncogene addiction" (Jones *et al.,* 2009).

For instance, oncogenic addiction could make cancer cells dependent on a certain metabolic route to proliferate (Thompson *et al.,* 2009). The discovery of numerous more oncogenes has revealed the variety of functions they play in the genesis of cancer as well as the wide range of cancer types that depend on oncogenes ever since the function of c-Src, the first proto-oncogene to be discovered, was clarified (Adjei *et al.,* 2009).

For instance, some oncogenes contribute to blocking apoptotic pathways, whereas others promote apoptosis (Jaishree *et al.,* 2008). By mimicking signals for healthy development, oncogenes can change specific mechanisms, and the identification of specific oncogenes has increased understanding of how cancer cells hijack the signaling pathways that growth factors employ to promote proliferation (Hanahan *et al.,* 2000).

2.4 Conventional cancer therapy methods

2.4.1 Surgery

It is the primary form of cancer treatment, and it seeks to totally or partially remove the tumor. Surgery, however, might be performed to explore a specific location and collect a sample for the identification of a suspicious growth (Eyre *et al.,* 2001).

2.4.2 Chemotherapy

The basic goal of chemotherapy, which is a key component of cancer treatment, is to either stop the growth and spread of tumor cells or to eradicate them. Chemotherapy has a greater impact on the patient's tumor cells than on healthy cells (Mycek *et al.,* 1998). In general, it is especially intended for the prevention of metabolic pathways involved in cell replication. Moreover, chemotherapy is done if a tumor metastasizes and surgery is not an option. In order to stop micro-metastases, it is also applied following surgical and radiation treatments. Poorly differentiated and quickly growing tumors are those that respond to chemotherapy the worst (Mycek *et al.,* 1998). Yet, many cancer chemotherapy drugs adversely harm patients' normal cells (Mascarenhas *et al.,* 1994). For instance, several chemotherapy drugs can be harmful to the heart, kidneys, liver, and other organs. This

necessitates the discovery of novel cancer therapeutic agents (Soini *et al.,* 1998). Many different tumor forms are treated primarily with chemotherapy, either with or without surgery. This covers a wide range of hematological and solid malignancies.

To guarantee the total elimination of any malignant cells still present in the body following surgery, it is also employed as adjuvant therapy. While treating cancers, some patients receive simultaneous chemotherapy and radiotherapy (chemo-radiotherapy), which may be more effective than receiving either treatment alone. It may not be feasible to cure cancer when it spreads to other organs, and in this situation, Even though chemotherapy is still one of the most effective methods for treating cancer, it can be harmful to healthy cells as well (Coffey *et al.,* 2009).

2.4.3 Radiotherapy

Radiotherapy, often known as radiation therapy (RT), is used to destroy cancer cells and/or shrink a main or secondary tumor. Ionizing radiation is a frequent cancer treatment that kills tumor cells. Due to the undesirable biological effects of irradiation, which are not limited to malignant tissues solely but extend to nearby healthy tissues, it is nevertheless associated with substantial side effects and potential risks. The production of free radicals and elevated levels of lipid peroxides in tissues, particularly cell membranes, are two of the main causes of cellular harm after radiation exposure (Hospers *et al.,* 1999). Several researchers have noted suppression of antioxidant systems in mice and rats' blood and tissues, along with both in irradiated animals caring Ehrlich ascites cancer and an increase in lipid peroxidation product following irradiation exposure (Oliiyk *et al.,* 2001). Humans receiving radiotherapy were also reported to experience the same effect (Muravskaya *et al.,* 1994).

2.4.4 Hormone therapy

This technique involves treating cancer patients by using specific hormones that have been linked to particular cancers. Hormone treatment doesn't always work well for treating cancer. To delay or stop the growth of hormone-dependent malignancies such as breast,

prostate, and ovarian cancer, hormonal therapy is typically utilized as a kind of treatment (Kent *et al.*, 2003).

2.4.5 Biological therapy

This largely consists of vaccinations, gene therapy, and monoclonal antibodies. This form of treatment makes use of the immune system to combat cancer. The immune system's cells, antibodies, and organs work together to safe guard and defend the body from outside threats. The immune system may also be able to distinguish between cancer cells and healthy cells in the body, as well as to get rid of the cancer cells (Restifo *et al.*, 1993). Cytokines are the primary immunotherapeutic medications employed (proteins that activate the immune system). The two cytokines that are most frequently employed are interleukin-2 (IL-2) and interferon-alpha (INF). In 10% to 20% of patients, cytokines induce the tumor to shrink to less than half its original size. Tumor vaccines and dendritic stem cells have recently been mentioned as cutting-edge immunotherapeutic techniques (Restifo *et al.*, 1993).

2.4.6 Photodynamic therapy

A well-known strategy for treating both neoplastic and non-neoplastic disorders, photodynamic therapy (PDT) is a form of light therapy. PDT uses photosensitizer-enhanced photochemical processes that destroy tumors with a high degree of selectivity (Dougherty *et al.*, 2002). When light activates the photosensitizers present in the targeted tissue, singlet oxygen can be produced. The produced singlet oxygen is a highly reactive oxidant that can interact with proteins and lipids to selectively destroy localized cells (Korbelik *et al.*, 2003).

2.4.7 Atomic Radiation

The energy that manifests as waves or particles is known as radiation. Every radiation's accompanying energy can be transferred to matter. The removal of electrons from atoms' orbits by this energy transfer can result in the production of ions. Ionizing radiation refers to all radiation types that can create ions in materials (Cooper *et al.,* 2003). Ionizing radiation has received a lot of interest recently because of both its potential benefits and risks to the human population. Ionizing radiation is becoming a crucial component of contemporary medicine. It serves both therapeutic and diagnostic objectives (Jagetia *et al.,* 2003). In this present study cannabis plant extract had been used for cancer prevention and treatment purpose of EAC induced cancer cells in albino mice.

2.5 Ehrlich Ascites Carcinoma (EAC)

Ehrlich Ascites Carcinoma Ehrlich ascites tumor cells is an undifferentiated tumour that originated spontaneously as a carcinoma of the mammary gland of a stock mouse (Stewart *et al.,* 1959). Ehrlich ascites carcinoma (EAC), an animal model of mammary tumor, is a rapidly growing undifferentiated malignancy with very aggressive behavior (Luksiene *et al.,* 2006), which is able to grow in almost all strains of mice (Dongre *et al.,* 2007) and is frequently employed in cancer research (Kim *et al.,* 1964; Nusse 1981; Bhattacharyya *et al.,* 2004; Bredov *et al.,* 2004; Izhevatkin 2004; Luksiene *et al.,* 2006; Prabhakar *et al.,* 2006; Dongre *et al.,* 2007). It has been reported that Ehrlich ascites tumor cells lack H-2 histocompatibility antigens (Chen *et al.,* 1970), which apparently is the reason for their rapid proliferation in almost any mouse host (Patt *et al.,* 1956). The kinetics of intact EAC cell proliferation in vivo has been studied in detail (Izhevatkin 2004). The EAC cell implantation induces per se a local inflammatory reaction, which results in a progressive ascitic fluid formation (Nascimento *et al.,* 2006). The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Gupta *et al.,* 2004). This ascites tumor was obtained from the Ehrlich mouse carcinoma, which originated as a tumor of the mammary gland. Intraperitoneal injection of the tumor emulsion produces ascites (Loewenthalh *et al.,* 1932). Solid tumors are obtained by subcutaneous injection of fresh ascitic fluid containing cancer cells, and ascites tumors are obtained by intraperitoneal

injection into mice. Upon intraperitoneal injection of fresh ascetic fluid containing about 1 million cancer cells, mice regularly develop large amounts of milky ascites (5-20 ml.) in 7-14 days and die in 2-6 weeks. The tumor generally has 100 per cent takes and regresses in about 5 per cent. The milky fluid contains about 5 to 100 million cancer cells/ml usually about 25 21 million cancer cells, and about 5-10 per cent of normal cells. Cancer cells in ascitic form are much larger than those in solid form. Hemorrhagic exudates were occasionally observed in 12-14 day old ascites tumors. Inoculation of the ascitic fluid containing a fairly large number of erythrocytes had no inhibitory effect on the normal course of ascites formation. The frequency of appearance of hemorrhagic ascites increased with time, so that at 21 days as many as 50 per cent of animals showed hemorrhagic ascites. At this time, the fluid had a tendency to clot (Sugiura 1953). It is well known that the microenvironment of the cells of in vivo ascites tumors is generally hypoxic. EAC cells were grown in the peritoneal cavity of mice in the low end of normal physiologic oxygen tension between 2.6 and 5.2% (Lin *et al.,* 2003). These cells are considered to be only transiently oxygenated when fluctuating into the vicinity of blood vessels of the peritoneum. The frequency of such transient oxygenations diminishes as the tumor volume increases, thereby causing an increasing proportion of quiescent cells registering in earlier investigations (Probst *et al.,* 1988). Formation of ascites and pleural effusion is a common problem for patients with advanced stage of cancer (Verheul *et al.,* 2000). These fluid accumulations cause severe symptoms such as abdominal distention, shortness of breath and fatigue. In this study cannabis extract had used to observe the prevention and treatment effects on albino mice.

2.6 Cannabis plant

Cannabis sativa L. is one of the earliest known cultivated plants since agricultural farming started around 10,000 years ago (Schultes *et al.,* 1974). It is a multi-purpose crop plant with diverse agricultural and industrial applications ranging from the production of paper, wood, and fiber, to potential use in the medicinal and pharmaceutical industries. The first-ever report to reveal the prospects of *Cannabis sativa* as a medicinal plant was already published in 1843 and described the use of plant extracts to treat patients suffering from tetanus,

hydrophobia, and cholera (Shaughnessy 1843). However, the first chemical constituent identified was oxy-cannabis (1869) (Bolas and Francis 1869), isolated cannabinoid, and fully identified in 1940 was cannabidiol (CBD) (Jacob *et al.,* 1940) followed by tetrahydrocannabinol (THC) (Gaoni *et al.,* 1964; Santavy 1964) and cannabigerol (CBG) in 1964, and cannabichromene (CBC) in 1966 (Gaoni *et al.,* 1966). Identification of THC later led to an understanding of the endocannabinoid system followed by the discovery of the first cannabinoid receptor (CB1) in 1988 (Devane *et al.,* 1988; Russo 2016). CB1 receptor acts as a homeostatic regulator of neurotransmitters for pain relief mechanisms, but the same mode of action was responsible for intoxicating effects from cannabinoids' excessive use. Thus, the understanding of mode of action of CB1 receptor raised concerns about the adverse effects of cannabis use. Consequently, the plant was removed from the medicinal category and recategorized exclusively to the category of drug-type plants. Cultivation and use of cannabis plants for recreational, medical, and industrial use were strictly banned and severely limited the scientific research in the field. Owing to strict legal regulations, the plant remained unexplored for its incredible potential in drug discovery for an extended period until it was legalized for medical use first in California and later in many countries around the globe. Extensive research followed legalization to explore the chemodiversity of cannabinoids for potential clinical value. In total, more than one thousand compounds—278 cannabinoids, 174 terpenes, 221 terpenoids, 19 flavonoids, 63 flavonoid glycosides, 46 polyphenols, 92 steroids—have been identified (ElSohly *et al.,* 2005; Gould, 2015; Radwan *et al.,* 2017). Nearly 278 of these compounds are cannabinoids and classified as phytocannabinoids (plant-based) to distinguish them from endocannabinoids (non-plant). Cannabimimetic drugs binding to CB1-receptors in the endocannabinoid system can also be found in algae, bryophytes, and monilophytes (Carvalho 2017; Kumar *et al.,* 2019). The major cannabinoids in cannabis include THC, CBD, and CBC, their precursor CBG and cannabinol (CBN) (Flores *et al.,* 2008). To date, 10 CBN-type, 17 CBG-type, 8 CBD-type, and 18 THC-type cannabinoids have been isolated (Gaoni and Mechoulam, 1964). Cannabigerolic acid (CBGA), a CBG-type cannabinoid, is the central precursor for the biosynthesis of psychoactive THC, non-psychoactive CBD, and CBC (ElSohly *et al.,* 2005; Gould 2015; Radwan *et al.,* 2017).

2.7 Cannabinoids identification by GC-MS

Gas chromatography (GC) is a chromatographic technique that is used for the separation and analysis of compounds, which can be vaporized without decomposition, from various matrices (Sarker *et al.,* 2012). In this chromatography, the mobile phase is an inert gas, e.g., helium, whilst the stationary phase is a microscopic layer of liquid or polymer on an inert support, often a glass or metal tubing called a GC column. The most commonly used detectors with a GC are flame ionization detector (FID) and thermal conductivity detector (TCD), but the use of a mass spectrometer (MS) with different ionization modes has recently become routine in most analytical labs (Verheul *et al.,* 2000). Other types of detectors used infrequently with a GC may include alkali flame detector (AFD), atomic emission detector (AED), dry electrolytic conductivity detector (DELCD), electron capture detector (ECD), flame photometric detector (FPD), helium ionization detector (HID), nitrogen-phosphorus detector (NPD), photoionization detector (PID), pulsed discharge ionization detector (PDD) and thermionic ionization detector (TID). The most recent developments in detection technology for GC have enabled multiple hyphenations with MS and NMR together to offer GC-MS-NMR, and a vacuum ultraviolet detector to give the new hyphenated technique GC-VUV; the use of an infrared detector with GC is also now available (GC-IRD). Because of the quality and extent of structural information that can be obtained from MS data, GC-MS, and GC-MS/MS, also known as tandem MS gas chromatography, have now been used routinely for the analysis of various types of natural products, including cannabinoids. In GC-MS, electron impact (EI) ionization mode is the most popular mode for the analysis of cannabinoids as this mode produces MS spectra that contain characteristic fragment ion peaks essential for the identification of compounds. However, in this interface, in most cases, the molecular ion is absent because of the extent of fragmentation of any molecule. Softer ionization techniques, e.g., chemical ionization (CI), that offers information on molecular ions, are also frequently used with GC nowadays for the analysis of cannabinoids. It can be mentioned that GC-MS analysis of cannabinoids is not necessarily restricted to the use of these two ionization techniques, rather, other ionization techniques, e.g., photoionization (PI) technique (Akutsu *et al.,* 2017), are also in use. One of the major components of *Cannabis sativa* is thermolabile tetrahydrocannabinol acid (THCA), which is the precursor of THC, formed through in situ heat-mediated

decarboxylation of THCA during smoking, cooking, or at the hot injection port of any GC. Therefore, in a GC operation, THCA cannot be detected, but its decarboxylated product, THC, can be. A GC method is typically simpler and faster than an HPLC method for the detection of cannabinoids, which makes a GC method preferable to an HPLC method. In most of the routine analyses of cannabinoids, a GC-FID is the method of choice because of its simplicity and reliability in routine identification and quantification of cannabinoids in plant materials and/or other matrices. However, for the correct identification of individual cannabinoids, a GC-MS is more appropriate. The use of a GC method for the determination of the potency of cannabis is based on the concentration of THC and CBD. In the following sections/subsections, various specific GC-based analytical methods for the analysis of cannabinoids in various matrices will be discussed.

2.7.1 GC Analysis of Natural Cannabinoids

Many studies on the usage of GC-based techniques for the analysis of cannabinoids that were published over the past ten years show that GC is still one of the most often used techniques. The published research demonstrates that numerous detection methods may now be employed for GC and that chemometric tools and other mathematical and computational modeling techniques can improve the usefulness and reliability of GC data processing. There are several GC column types now available, and selecting the right one and using the right detection technology is essential for the success of cannabis analysis by GC. Typically, thin-film nonpolar stationary phase columns with tiny diameters (such as 100% dimethyl silicone) are utilized in cannabis analysis. Cannabidiol (CBD) and cannabichromene (CBC), which are critically necessary for the precise assessment of the THC/CBD ratio, cannot be separated and distinguished by nonpolar columns such as 100% dimethyl silicone or 5% diphenyl in dimethyl silicone. In this case, a column with an intermediate polarity, such as a 35% diphenyl in dimethyl silicone column, can be used to separate CBD and CBC effectively. Two common GC starting conditions for the study of cannabis, one using an FID and the other using an MS detector.

Table 1: Two typical starting GC conditions for the analysis of cannabinoids.

GC method	Run time	Column	Carrier gas	Injector time	Detector
GC-FID	9 min	15m×0.53 mm ×0.5 μm	4 mL/min hydrogen	Capillary split injector, split liner with glass wool, 275°C	FID
GC-MS	30 min	30m×0.25 mm × 0.25 μm	1.3 mL helium	Capillary split injector, split liner with glass wool, 275°C	MS detector. Mass range 50-400 da

2.8 Cannabinoids induce cancer cell death

The capacity of these compounds to promote autophagy-mediated apoptosis in cancer cells serves as the basis for the majority of the mechanism behind cannabis anticancer activity (Velasco *et al.,* 2012). As a result, THC binds to cannabinoid receptors, stimulating sphingo lipid synthesis from scratch and activating a signaling pathway related to ER stress that involves up-regulating the transcriptional co-activator nuclear protein 1 (Nupr1, also known as p8) and its effector the pseudo-kinase tribbles homolog 3 (TRIB3) (Armstrong *et al.,* 2015; Blazquez *et al.,* 2004) Car Via TRIB3-mediated suppression of the AKT/mTORC1 axis, the activation of this pathway stimulates autophagy in turn (Salazar *et al.,* 2009).

Although its activation might result in cell death, autophagy is thought to be largely a cyt oprotective process (Eisenberg *et al.,* 2009; Galluzzi *et al.,* 2015; Mizushima *et al.,* 2008). Many studies showed that autophagy comes before apoptosis in the process of cannabinoi dinduced cell death (Armstrong *et al.,* 2015; Salazar *et al.,* 2009; Vara *et al.,* 2011). In several cancer cell types, the direct involvement of the autophagy pathway in the anticancer effect of cannabis has been well proven (Armstrong *et al.,* 2015; Carracedo *et al.,* 2006; Salazar *et al.,* 2009; Vara *et al.,* 2011). CB receptors may increase cancer cell death via this signaling pathway as a universal mechanism. In any event, additional mechanisms, some of which are cell type-specific, could work in conjunction with this route to cause the death of cancer cells (Vara *et al.,* 2011; Caffarel *et al.,* 2006, 2012; Guzman, 2003; Sarfaraz

et al., 2008; Vara *et al.,* 2013). It has also been demonstrated that cannabidiol and other cannabinoids produced from marijuana (Ligresti *et al.,* 2006) cause cancer cells to undergo apoptosis. CBD causes an increase in the formation of reactive oxygen species, which, at least in part, causes these anticancer effects (Massi *et al.,* 2008; Shrivastava *et al.,* 2011). Moreover, it has been suggested that CBD could cause cancer cells to die by activating TRPV2 receptors (Nabissi *et al.,* 2012).

2.8.1 Cannabinoids prevent metastasis, invasion, and angiogenesis

Treatment with cannabis has been demonstrated to normalize tumor vasculature in addition to the impact of these chemicals on inducing cancer cell death that was previously mentioned. The capacity of cannabis to prevent the activation of the vascular endothelial growth factor (VEGF) pathway appears to be the basis for these effects. As a result, different cancer types have been shown to exhibit down-regulation of various VEGF-activated pathway components, such as the active forms of its best-established receptors (VEGFR1 and VEGFR2), in response to cannabinoid treatment (Casanova *et al.,* 2003; Blazquez *et al.,* 2003, 2004; Portella *et al.,* 2003). In addition, cannabinoid receptor activation reduces migration and proliferation as well as triggers death in vascular endothelial cells, which may contribute to the antiangiogenic impact (Blazquez *et al.,* 2003; Pisanti *et al.,* 2007). Also, in animal models of naturally occurring and artificially generated metastasis, cannabinoids have been demonstrated to inhibit the growth of distant tumor masses. Moreover, these substances prevent several cancer cell types from migrating, adhering, and spreading (Blazquez *et al.,* 2008; Grimaldi *et al.,* 2006; Preet *et al.,* 2008; Qamri *et al.,* 2009; Ramer *et al.,* 2008). Cannabinoids' anti-metastatic effects are, at least in part, dependent on the control of extracellular proteases and their inhibitors (Blazquez *et al.,* 2008; Ramer *et al.,* 2008). Several findings suggest that cannabis activities may be regulated by the ER stress-related signaling pathway, which is also implicated in the activation of autophagy-mediated cancer cell death (Blazquez *et al.,* 2004, 2008). It is noteworthy that CBD, acting independently of cannabinoid receptors, has a sizable anticancer impact in several animal models of cancer, specifically through inhibiting invasiveness and metastasis. According to studies (McAllister *et al*., 2011; Murase *et al.,*

2014 and Soroceanu *et al.*, 2012), ID-1, a transcription factor inhibitor of DNA binding-1, is downregulated as a result of CBD use, at least in part, to produce this effect.

2.8.2 Antitumor potency of phytocannabinoid

Although being less 'translatable' to human therapy than animal studies, in vitro studies are commonly accepted as valuable first indicators for a possible effect also in vivo. Several in vitro experiments including various cannabinoids have been conducted in the past, not only to investigate their toxic effects on cancer cell lines but also to gain insight into the mechanisms of action (Grimaldi *et al.*, 2006). Twenty-three in vitro studies have been identified that investigated the anticancer potency of CBD in comparison with other cannabinoids (mainly THC, but occasionally cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), cannabidivarin (CBDV), and their extracts (CBDE, THC-E). Three other studies comparing THC and CBG with extracts are also included. To avoid confusion, the term extract (E) is used interchangeably with similar terms such as 'botanical drug substance' (BDS; CBD-BDS or THC-BDS), 'CBD-oil', 'CBD-tincture', 'CBD full spectrum extract' or 'cannabis drug preparation' (CDP). Overall, comparisons covered 86 cancer cell lines, the large majority of which were of human origin. Some had been included in more than one study, allowing a comparison of results (Blazquez *et al.*, 2008).

2.9 Cannabidiol (CBD)

The stereochemistry and structure of CBD, which was initially isolated in 1940 (Adams et al., 1940; Mechoulam *et al.*, 2002), were discovered in the 1960s. According to early research (Laprairie *et al.*, 2015; Mechoulam *et al.*, 2007; Petitet et al., 1998; Thomas *et al.*, 2007; Turkanis *et al.*, 1986), CBD acts as an allosteric negative modulator (antagonist) at CB1R and CB2R. It is perhaps premature to make definite assumptions regarding all of the compound's modes of action because more research has revealed the pharmacological promiscuity of CBD (Campos *et al.*, 2012; De Petrocellis *et al.*, 2010; Pertwee, 2009). There is growing evidence that CBD interacts with serotonin 1A (5-HT1A) receptors in

rodents to facilitate several of its effects in vivo (Gomes *et al.*, 2012; Magen *et al.*, 2010; Resstel *et al.*, 2009; Stern *et al.*, 2012). In male rats, CBD decreases immobility in a forced swim in a way that depends on 5-HT1A activity (Sartim *et al,* 2016). Both the anxiolytic effects of infralimbic administration of CBD and the anxiolytic effects of intra peri aqueductal gray CBD on elevated plus maze in male rats are blocked by a 5-HT1A antagonist in male rats (Fogaca *et al.*, 2014; Campos *et al.*, 2008). The latter is unaffected by the CB1R antagonist AM251. The subjective effects of CBD in male rhesus monkeys are similar to those seen for a 5-HT1A agonist, 8-hydroxy-2-dipropylaminotetralin, and do not significantly demonstrate CB1R agonist or antagonist-like activity, demonstrating that the evidence also includes nonhuman primate models (McMahon, 2016). Moreover, there is proof that CBD interacts with the opioid receptors as well as the transient receptor potential vanilloid type 1 (TRPV1) cation channels (Pertwee, 2008). Due to AEA reuptake inhibition and FAAH inhibition, CBD also raises levels of AEA (Bisogno *et al.*, 2001; Ligresti *et al.*, 2006). CBD may have antipsychotic effects rather than psychotomimetic ones, in contrast to THC. In addition to potential anti-inflammatory benefits, CBD may also have clinical effects on malignancies, epilepsy, neuropathic pain, anxiety disorders, and movement disorders (Izzo *et al.*, 2009).

2.9.1 Δ9 –Tetrahydrocannabinol (THC)

In the 1960s, the stereochemistry and structure of THC were established. (Gaoni *et al.*, 1964). Both CB1R and CB2R are partial agonists of THC. THC's euphoric effects are mostly mediated by CB1R agonist actions, and it may also have immunological or anti-inflammatory properties, which are presumably mediated by CB2Rs (Pertwee 2008). In healthy individuals, intravenous (i.v.) THC administration causes a variety of psychoactive effects, such as feeling "high," anxiety, paranoia, perceptual alterations, and cognitive deficits, particularly deficits in verbal recall (D'Souza *et al.*, 2004); in patients with schizophrenia (D'Souza *et al.*, 2005), it aggravates psychotic symptoms. Yet, stress is frequently cited by cannabis users as the main cause of chronic usage (Hyman *et al.*, 2009). Although the amount of dopamine released by THC is minimal in comparison to drugs like amphetamine and cocaine, which cause more striatal dopamine release, activation of CB1R

by THC results in the disruption of (GABA) glutamatergic neurotransmission (Bossong *et al.*, 2015). The psychotomimetic effects of THC may be influenced by the disturbance of inhibitory/excitatory balances (Farkas *et al.*, 2010; Hampson *et al.*, 2011; Li *et al.*, 2010). Cognitive impairment brought on by acute THC exposure is typically brief, temporary, and self-contained. Although other studies have not confirmed this finding, persistent cannabis use, particularly in adolescents, is linked to more severe chronic memory losses (Meier *et al.*, 2012; Ranganathan *et al.*, 2006).

2.9.2 Synthetic THC formulations

Nabilone (a synthetic derivative of THC) and dronabinol are the only two synthetic pharmaceutical versions of cannabinoids that have received approval for use in the United States (synthetic THC). Clinically, both have effects that are comparable to those of cannabis when taken orally (Badowski 2017).

Both synthetic versions of THC have received approval for the treatment of nausea and vomiting brought on by chemotherapy in individuals who did not react to antiemetic drugs of the traditional type (Marinol, 2017; Cesamet, 2013). Moreover, dronabinol is authorized to treat AIDS patients' anorexia which is accompanied by weight loss.

2.9.3 Δ9 -Tetrahydrocannabinol (THC) and cannabidiol combination drug

Nabiximols (Sativex), a class of botanical drugs available only by prescription, are combinations of THC and CBD that have been chosen cannabis strains in a 1: 1 ratio (Bisogno *et al.*, 2001). Tetranabinex, one strain, produces a lot of THC, whereas Nabidiolex, another, produces a lot of CBD. To create nabiximols, dried, extracted flowers are used.

Nabiximols contain additional phytocannabinoids obtained from plant material but are mostly made up of THC and CBD (70% w/w) and other cannabinoids (Russo and Guy, 2006). THC: in a 1 to 1 ratio. Because CBD counteracts some of the sedative and euphoric effects of THC without interfering with its intended effects, such as muscle relaxation and

pain reduction, it appears to allow for greater dosages of THC without raising the risk of negative side effects.

2.10 Cannabis uses in cancer

Cannabis is currently recognized as a strong cancer treatment agent. More than 40 years ago, Munson *et al.* made the first in vivo demonstration of THC's ability to prevent lung cancer cell proliferation (Munson *et al.,* 1975). Cannabinoids mimic endogenous compounds to modify the cell cycle and signaling pathways. In pre-clinical research, they have proven to have anti-cancer properties on a range of cancer cells and several animal models. There is some debate concerning the effects of cannabinoids on tumor cells because research conducted on lung, brain, and genitourinary carcinoma cell lines showed that while high cannabis concentrations have anti-proliferative effects, low amounts can promote the growth of cancer cells (Hart *et al.*, 2004).

However, these effects are highly cancer-specific and rely on the kind of tissues, the damaged pathways, and potentially other underlying alterations as well. Also, the effects of various cannabinoids on cancer cells in vitro could differ greatly from those in vivo. While there is still much to learn about the advantages and disadvantages of cannabinoids, cannabis has already demonstrated some promising features as an adjunct or single-agent therapy for cancer (Turgeman *et al.,* 2019).

2.10.1 Using cannabinoids in conjunction with other therapies

It is well-known that cannabinoids have pain-relieving, appetite-stimulating, and chemotherapy-induced nausea and vomiting-reducing effects on cancer patients (Goncalves *et al.,* 2019). The THC pill Dronabinol (Marinol; Solvay Pharmaceuticals) and its synthetic equivalent Nabilone (Cesamet; Meda Pharmaceuticals) are used to treat nausea and vomiting brought on by chemotherapy (Pertwee *et al.,* 2012). Moreover, dronabinol is employed to treat anorexia in AIDS patients (Pertwee *et al.,* 2012). Moreover, the THC and CBD combination drug nabiximols (Sativex; GW Pharmaceuticals) is approved for the treatment of neuropathic pain in adult patients with multiple sclerosis and the management

of pain in adult patients with advanced cancer (Barnes *et al.*, 2006). Cannabinoids enhance the effectiveness of each medicine when used in conjunction with traditional treatment techniques, which also reduces negative effects (Torres *et al.,* 2011). For instance, it has been shown that when paired with chemotherapy, cannabinoids and synthetic cannabinoids have synergistic inhibitory effects via increasing autophagy in pancreatic cancer, glioblastoma multiforme, and colorectal cancer tumors (Donadelli *et al.,* 2011).

For instance, combining THC with temozolomide consumption is an effective anticancer strategy in glioma xenograft models and in tumors that are temozolomide-resistant (Gustafsson *et al.,* 2009). A mouse model showed that THC and CBD further increase the anticancer effects against glioma when paired with radiation therapy (Scott *et al.,* 2014). Moreover, because they can change the expression of ABC efflux pumps, which cause chemotherapy resistance, cannabis may help make cells more sensitive to chemotherapy (Holland *et al., 2*008). For instance, leukemia cells are more susceptible to chemotherapy when THC is administered along with cytotoxic drugs (Liu *et al.,* 2008).

2.10.2 Cannabinoids' antiproliferative properties

Several studies document how cannabis inhibits the growth of cancer cell lines and some in vivo mice models. Although several pathways are modulated and various receptors are activated to achieve this result, the tumor cell frequently experiences the same outcome. However, the anticancer effects of cannabis rely on various factors, including their concentration and the kind of cancer (Calvaruso *et al.,* 2012). As mediators of cannabis' antiproliferative effect in melanoma, induction of cell cycle arrest, autophagy, and apoptosis have been discovered (Daris *et al.,* 2019). Few data are demonstrating that cannabis has tumor-promoting effects in melanoma cancer, although several experimental investigations have shown that they have antiproliferative effects (Carpi *et al.,* 2017).

As an illustration, Carpi et al. showed how the CB1 receptor promotes tumor growth and asserted that silencing the CB1 receptor greatly decreased the number of viable melanoma cells in comparison to control cells (Carpi *et al.,* 2017). Another illustration is the direct activation of cannabinoid receptors type 1 and 2 by UV irradiation, and the reduction of skin cancer in animals lacking these receptors (Cudaback *et al.,* 2010).

2.10.3 Cannabinoids' antiangiogenic properties

The proper flow of nutrients and oxygen is necessary for tumor growth. Angiogenesis is crucial for the progression of cancer for this reason, and it is known that inhibiting it can decrease the growth of the disease (Carmeliet *et al.,* 2000; Petrovic *et al.,* 2016). The use of cannabinoids may reduce tumor angiogenesis and decrease vascularization at malignant tissues' microenvironmental locations. Vascular endothelial growth factor (VEGF), VEGF receptors, placental growth factor (PlGF), and angiopoietin-2 expression levels are all decreased in conjunction with these effects (Ang-2). In vivo research by Casanova *et al.* on skin cancers has shown that activating CB receptors can change blood vessel architecture while also drastically reducing blood vessel size and affecting tumor vascularization (Abrams *et al.,* 2019, Casanova *et al.,* 2003). Moreover, studies using THC or CBD-treated animals with lung cancer xenografts or malignant glioma models have indicated that angiogenesis and pro-angiogenic factors are inhibited (Blázquez *et al.,* 2003; Picardi *et al.,*2014, Preet *et al.,* 2008, Ramer *et al.,* 2014).

2.10.4 Cannabinoids' anti-intrusive properties

The crucial and fatal stage in the development of cancer is the invasion and spread of aggressive tumor cells. Detachment of cancer cells from primary tumors, migration, invasion, intravasation, attachment at a distant place, extravasation, and the development of secondary lesions are all signs of metastasis (Ramer *et al.*, 2016). Evidence suggests that cannabinoids' effects on tumor cells' capacity for invasion and metastasis are mediated, among other things, by altering the activity of matrix metalloproteinases (MMPs), including by blocking matrix metalloproteinase-2 (MMP-2) and altering the expression of ld-1 and tissue inhibitor of matrix metalloproteinases (TIMP)-1. Similarly, administering cannabinoids may prevent breast cancer cells from migrating. Activating CB1 and CB2 receptors in melanoma has been proven to reduce the likelihood of metastasis. Activating CB2 receptors may also lessen the likelihood of brain metastases by preventing melanoma cancer cells from crossing the blood-brain barrier (Haskó *et al.,* 2014, Takeda *et al.,* 2014).

2.10.5 Effects of Terpenes on Cancer

Cannabinoids are mostly responsible for the effects of cannabis, although they are not responsible for all of its therapeutic benefits (Nuutinen *et al.*, 2018). Terpenes are believed to have biological qualities such as antioxidant, anticancer, antitumor, and anti-14 inflammatory, among others, according to numerous clinical and in vitro investigations. They frequently promote apoptosis to produce anti-cancer activity, but they do not affect healthy cells or tissues (Nuutinen *et al.*, 2018). Terpenes also have very low toxicity and no adverse effects, and they are well tolerated (Nuutinen *et al.*, 2018). In support of this hypothesis, studies in animal models have shown that limonene slows the growth of pancreatic, stomach, colon, skin, and liver cancer. By boosting the production of reactive oxygen species (ROS) and the activity of the caspase enzyme, limonene can suppress the growth of prostate cancer tumors more potently than standard medicines (Huang *et al.*, 2012).

Similar to this, there is growing data that suggests other terpenes may slow the growth of tumors. Myrcene is cytotoxic against human cervical carcinoma, lung carcinoma, and colon adenocarcinoma (Tomko *et al.*, 2020). Caryophyllene was found to hinder lung cancer cells' ability to advance through the G1 phase of the cell cycle (Chung *et al.*, 2019.). Further research demonstrated that linalool and elements might, respectively, cause lung cancer cells and oral cancer cells to undergo apoptosis. In colon and ovarian cell lines, it has been demonstrated that humulene, one of the primary cannabis terpenes, improves the antiproliferative effects of common medications (Tomko *et al.*, 2020.).

2.10.6 The surrounding effect

Inactive compounds boost the activity of the endogenous cannabinoids anandamide and 2-arachidonoyl glycerol in the endocannabinoid system (Ben-Shabat *et al.*, 1998.). They then utilized this to illustrate the rationale behind why botanical medications typically outperform their isolated constituents (Mechoulam *et al.*, 1999). The "entourage effect" refers to the cooperative interaction that develops when all of cannabis' secondary metabolites are present in a single cannabis extract. For instance, in breast cancer cells, limonene causes apoptosis synergistically with CBD and CBG, and the anti-inflammatory

properties of -pinene and -myrcene is enhanced when coupled with CBD (Russo, 2011.). Moreover, using CBD and THC together is more efficient than using THC or CBD alone at suppressing the proliferation of melanoma and glioblastoma cells (Marcu *et al.,* 2010). Generally speaking, cannabis secondary chemicals reduce the negative effects of THC, such as anxiety, psychoses, and motor incoordination, while enhancing its positive effects (Abrams *et al.,* 2015).

Moreover, they might lower the THC effective dose and improve the tolerability of cannabis-based medicines (Abrams *et al.,* 2015). High THC extracts have been shown to kill breast and melanoma cells more effectively than THC alone (Baram *et al.,* 2018; Blasco *et al.*, 2018). Furthermore, in a glioma cell line, a high CBD extract showed noticeably higher cytotoxicity than pure CBD (Ligresti *et al.,* 2006). Consequently, compared to THC or CBD alone, cannabis extract may be more effective at treating cancer.

2.11 Cell vaccines

One of the vaccine techniques being tested uses autologous tumor cells, which are cancer cells that were grown on a patient. Irradiated tumor cells are given out coupled with an adjuvant in this method. As the vaccine is made from tumor cells, it may be able to stimulate T cells that are specific to any antigen that the cells' antigens express. The drawback of this approach is that it can be challenging to find enough cells at times (Berger *et al.,* 2007; Hoover *et al.,* 2000; Maver *et al.,* 1979; Schulof *et al.,* 1988.).

Many malignancies, including lung cancer (Schulof *et al.,* 1988; Nemunaitis *et al.,* 2003), colorectal cancer (Weger *et al.,*2012; Hanna *et al.,* 2001; Ockert *et al.,* 1996) melanoma (Baars *et al.,* 2002; Berd *et al.,* 1990), renal cell carcinoma (Antonia *et al.,* 2002; Fishman *et al.,* 2008; Kinoshita *et al.,* 2001) and prostate cancer (Tani *et al.,* 2004) have been treated with this strategy. Tumor cells are frequently genetically altered to produce cytokines, such as IL-2 (Asada *et al.,* 2002) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Dranoff *et al.,* 1993; Mach *et al.,* 2000; Salgia *et al.,* 2000; Jaffee *et al.,* 2001), and to costimulate cells, such as B7-1 (Fishman *et al.,* 2008).

A cancer vaccine called GVAX uses tumor cells that have been genetically altered to release GM-CSF. It is applied following cancer radiotherapy to halt the unchecked spread of cancer cells. There are two different GVAX vaccine strategies: one uses allogeneic cells, which are not patient-specific, and the other uses autologous cells, which are patient-specific1 (Fishman *et al.,* 2008).

Good outcomes from GVAX phase 1/2 clinical studies have been observed in patients with non-small-cell lung cancer, correlating GM-CSF secretion and patient prognosis (Nemunaitis *et al.,* 2006). However, phase 3 clinical trials for prostate cancer have not revealed any effects (Arlen *et al.,* 2009). In conjunction with body radiation, the mesothelin-expressing Listeria monocytogenes vaccine, or cyclophosphamide (CY), and many phase 2 trials using GVAX therapy for advanced pancreatic cancer are now being done, with promising outcomes. The impossibility of customizing autologous tumor vaccines may be circumvented by an allogeneic tumor cell vaccine that contains tumor cell lines, such as Canvaxin (Morton *et al.,* 2006). Prostate (Simons *et al.,* 2007), breast (Emens *et al.,* 2009), and pancreatic malignancies have all been evaluated concerning these vaccinations (Lutz *et al.,* 2011).

A combination approach combining allogeneic GVAX against metastatic castration-resistant prostate cancer (CRPC) and an immune checkpoint inhibitor is being researched (Eertwegh *et al.,* 2012; Wang *et al.,* 2011), despite homologous GVAX against metastatic CRPC not meeting its phase 3 clinical trial objectives. DCs that deliver antigens to T cells and stimulate immune system activation are the focus of a novel treatment strategy. Since Dr. Ralph M. Steinman, who discovered DCs, realized their potential and the idea of employing DCs as a vaccine (Steinman *et al.,* 2007), DC therapy has been thoroughly researched (Nestle *et al.,* 1998). DCs may be loaded with a range of antigens, including tumor cells, tumor-derived proteins or peptides, and DNA/RNA viruses. There are further approaches, like fusing tumor cells and DCs. On the surface of DCs, many types of receptors are expressed. For instance, it has been observed that binding of an antigen to the scavenger receptor on DCs results in the induction of suppressive CD4(+) T lymphocytes that are specific for the antigen. Notably, not every antigen presented by DCs results in immunological activation (Romain *et al.,* 2012). Provenge, a prostate cancer vaccine that

was given FDA approval in 2010, has raised awareness of the use of autologous immune cells in immunotherapy. It is a crude leukocyte fraction that has been isolated from a patient's peripheral blood and cultivated with a prostate cancer antigen (prostatic acid phosphatase, or PAP) while being given GM-CSF. The PAP antigen is displayed by DCs, which make up the majority of Provence's active ingredients (Small *et al.,* 2000) and stimulate and generate antigen-specific T cells in patients. Provence is an excellent illustration of the difficulty of customized medicine because it successfully allows the development of personalized cancer vaccines. Nevertheless, because each patient's needs must be catered to throughout the whole process of creating a personalized vaccination, from sample collection to transport, processing, shipping, and delivery of the cells, more work and money are required.

2.11.1 Protein- and peptide-based vaccines.

Immunity against particular antigenic epitopes obtained from the vaccinated protein/peptides that are produced in cancer cells can be brought on by protein/peptide vaccinations (and preferably not expressed in normal tissues). An artificially created antigen protein or peptide is pre-scented in complex with the HLA molecules on the cell surface after being delivered by professional APCs. Immune responses specific to malignancy are triggered when T cells detect the antigens (Meena *et al.,* 2007). Many HLA-binding antigenic epitopes originating from tumor-associated antigens (TAAs) have been discovered. Moreover, neoantigens produced from cancer-specific gene alterations absent in normal tissue have gained interest recently. Algorithm based computer searches are being developed quickly to search for these neoantigens effectively (Tsoi *et al.,* 2020).

Phase 3 trials have been conducted to verify the results as many early protein/peptide vaccine clinical trials produced positive outcomes. The majority of these studies were unsuccessful, indicating that single-protein/peptide vaccines do not sufficiently inhibit tumor growth (Buonaguro *et al.,* 2011). Even if protein or peptide-derived epitopes triggered T-cell responses, anticancer effects are infrequently observed with response rates less than 10% (Melief, 2008). Many factors, including tumor immune escape pathways and

immunosuppressive TMEs, may account for these surprising findings (Marincola *et al.*, 1999).

The majority of peptide vaccines created to date include short-chain peptides (SPs) that are only allowed in MHC class I, suggesting potential issues with the vaccination formulations. In contrast to long-chain peptides (LPs), short-chain peptides (SPs) can bind to any cell without processing and, if given to CD8 T cells without the secondary cost stimulatory signal, may cause energy (Bijker *et al.,* 2008; Hailemichael *et al.,* 2013). Immune tolerance is produced in these circumstances, fostering a setting that is conducive to the development of cancer. Additionally, MHC class II-restricted helper T cells, which are necessary for effective cytotoxic T lymphocyte (CTL) induction, cannot be activated by MHC class I-restricted SPs. Yet, professional APCs can convert LPs into MHC class I- or class II-restricted antigens and present them, whereas other cell types cannot. Professional APCs that deliver LP-derived antigens can signal through both T cell receptors (TCRs) and costimulatory molecules to activate helper T cells without producing energy (Bijker *et al.*, 2007; Janssen *et al.,* 2008; Rosalia *et al.,* 2013.).The creation of LP vaccines with epitopes for both CTL and helper T cells is currently a high priority. Moreover, a cutting-edge technique utilizing an immune stimulatory adjuvant has been created to enhance the responsiveness to peptide vaccinations (Drake *et al.,* 2014; Liu *et al.*, 2018; Hos *et al.,* 2018).

2.11.2 Nucleic acid vaccines (DNA, RNA, or Viral Vector)

The ability of nucleic acid (DNA/RNA) vaccines to simultaneously elicit immunity against several epitopes is a benefit (Aurisicchio *et al.,* 2012). These vaccines can also be manufactured successfully and are affordable. Contrary to peptide vaccinations, adjuvants are less crucial when an immunogenic viral vector is utilized. However, when a viral vector is not utilized, creating a reliable delivery system becomes crucial, especially given that nucleic acid uptake into cells might not be very effective (Jorritsma *et al.,* 2016). In numerous exploratory investigations, DNA vaccines have shown promise (Ferraro *et al.,* 2011). For instance, phase 3 clinical studies for VGX3100, a DNA vaccine for cervical cancer, are ongoing (Trimble *et al.,* 2015). Unlike DNA vaccines, RNA vaccines do not

integrate into the genome, preventing carcinogenicity. Moreover, RNA vaccines can work in the cytoplasm as opposed to DNA vaccines, which must penetrate the nucleus. As a result, clearance happens quickly and there may be little chance that side effects may develop. Although RNA is more susceptible to degradation than DNA, it can nevertheless be made more stable through a variety of modifications, including formulations with liposomes or stabilizing adjuvants (Espuelas *et al.,* 2005). Moreover, methods for stabilizing the RNA molecule itself have been developed (Pascolo *et al.,* 2008) (5′ cap structure, untranslated regions, and codon use in translated regions). Melanoma and kidney cancer phase trials are ongoing (Weide *et al.,* 2008; Weide *et al.,* 2009; Oshiumi *et al.,* 2003).

There is also an ongoing phase I research of liposome-encapsulated mRNA for patients with metastatic melanoma (Kranz *et al.,* 2016). A breakthrough in nucleic acid vaccines will come from further advancement of nucleic acid delivery techniques. Nucleic acids for vaccines are efficiently transported via viral vectors. Viral vectors, which can stimulate innate immunity and can cause immunological reactions to viruses, do not require adjuvants. Frequently used viral vectors are attenuated or replication-defective for safety and derived from poxvirus, vaccinia virus, adenovirus, and alphavirus. To target the MUC1 and T4 antigens of renal cell cancer, certain modified vaccinia virus Ankara (MVA) vector-based vaccines are employed (Amato *et al.,* 2011).

Because they can transduce both dividing and nondividing cells and are simple to make, recombinant adenoviruses are frequently utilized in cancer gene therapy (Das *et al.,* 2012; Liu *et al.,* 2008; Raty *et al.,* 2008). An oncolytic virus is the encapsulated dsDNA virus known as herpes simplex virus type 1 (HSV-1). Melanoma can benefit from HSV-1 which expresses GM-CSF. The drawback of viral vectors is that because antiviral immune responses are induced, repeated delivery may be challenging. A heterologous prime-boost strategy is evolving as a result. For instance, Bavarian Nordic (Denmark) is developing PROSTVAC, a vaccine that combines two poxvirus vectors that express the tumor-associated antigen prostate-specific antigen (PSA) with three immune-enhancing cost stimulatory molecules collectively known as TRICOM (LFA-3, ICAM-1, and B7.1), to elicit an immune response in prostate cancer (Hodge *et al.,* 2010; Gulley *et al.,* 2010).

Bacteria and yeasts are now getting attention as potential novel vaccine carriers, in addition to viruses (Remondo *et al.*, 2009; Wansley *et al.*, 2008).

2.11.3 Combined Therapy with cancer vaccines

Except for a few particular cancer types, monotherapies utilizing cancer vaccines frequently had minimal therapeutic effects. The diverse immune evasion mechanisms of cancer, which are difficult to manage by any cancer vaccine alone, were blamed for the comparatively low efficiency of monotherapies. As was previously mentioned, any antitumor effects brought on by the cancer vaccination may be overridden by the immunosuppressive TME (Zou 2005). Combination medicines have received increased interest as different immunotherapy types have developed. Many strategies, such as conventional chemotherapy/radiation therapy or the most recent antibody therapies (Kumar *et al.*, 2018; Hwang *et al.*, 2018) have been used in conjunction with immunotherapies either concurrently or sequentially.