ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF FRANKINCENSE ESSENTIAL OIL

SAN ASEANA KOH

BACHELOR OF SCIENCE (HONOURS)

BIOMEDICAL SCIENCE

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

MAY 2023

ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF FRANKINCENSE ESSENTIAL OIL

By

SAN ASEANA KOH

A project report submitted to the Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Honours) Biomedical Science

May 2023

ABSTRACT

ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF FRANKINCENSE ESSENTIAL OIL

San Aseana Koh

One of the most rapidly expanding fields of research is the investigation of natural products particularly medicinal plants as a potential source of anticancer drugs. Frankincense essential oil, from the Burseraceae family has been used during religious ceremonies as a ritual incense. The oil is also used to cure wounds, treatment of allergies, snake and bug bites, colds, coughs, diarrhoea, headaches, syphilis, stomach aches and backache. In this study, purchased frankincense essential oil was used and evaluated for its polarity using thin layer chromatography (TLC), detection of classes of secondary metabolites via phytochemical analysis, antioxidant activity using DPPH assay and cytotoxicity on Vero and A549 cell lines via MTT assay. Numerous spots and a broader range of retention factor values were observed in hexane : ethyl acetate (10:3) indicating the presence of polar and non-polar bioactive compounds. The results correlate with the presence of phenols, tannin, coumarin, flavonoids, saponin, alkaloids and glycosides. In DPPH assay, both frankincense essential oil (v/v) and ascorbic acid presented an EC_{50} value of of 4.73 ± 0.01% and 4.60 ± 0.02 mg/mL, respectively. In MTT assay, the frankincense essential oil did not exhibit cytotoxicity on Vero cells. The essential oil and doxorubicin showed higher cytotoxicity towards the A549 cells with IC_{50} values of 76.04 ± 0.09 μg/mL and 0.19 ± 0.10 μg/mL for 24- and 72-hours, respectively. This result

indicates Frankincense essential oil could be a potential antioxidant agent with low toxicity to normal cells. Thus, further investigations should be conducted using pure isolates from the resin to further elucidate its capability as a pharmaceutical drug.

ACKNOWLEDGEMENTS

Without the help of numerous people around me, I could not have completed this endeavour. First and foremost, I must say that words cannot adequately express my gratitude to my distinguished supervisor, Dr. Sangeetha Arullappan. Her unwavering encouragement and excellent direction throughout the research have been a big factor in the success of our project. It is truly a privilege to learn from her tremendous guidance. Thank you very much!

Sincere appreciation to UTAR's Faculty of Science for providing the necessary research facilities and a pleasant working atmosphere for the completion of this study. In addition, I would like to express my gratitude to Mr. Saravanan and the other lab officers for their continuous assistance in providing the necessary consumables, chemicals, and laboratory glassware throughout the project.

Finally, I want to express my gratitude to my family and partner for their unwavering support and confidence in me throughout my academic career. My sincere gratitude also goes out to my bench mates, friends, and everyone else who helped me with the project by giving me advice or by supporting me in some other way.

Finally, a big thank you to all the opportunities that made this possible.

DECLARATION

I hereby declare that the project report is based on my original work except for the quotations and citations which have been dully acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

(SAN ASEANA KOH)

APPROVAL SHEET

This project report entitled "**ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF FRANKINCENSE ESSENTIAL OIL**" was prepared by SAN ASEANA KOH and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Dr. SANGEETHA A/P ARULLAPPAN) Date: 06/06/2023

Supervisor

Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 6th June 2023

PERMISSION SHEET

It is hereby certified that **SAN ASEANA KOH** (ID No: **19ADB04163**) has completed this final year project thesis entitled "ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT OF FRANKINCENSE ESSENTIAL OIL" under the supervision of Dr. SANGEETHA A/P ARULLAPPAN (Supervisor) from the Department of Allied Health Sciences, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project thesis in PDF format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(SAN ASEANA KOH)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
CDK	Cyclin-dependent kinase
CO_2	Carbon dioxide
EC_{50}	Half-maximal effective concentration
EDTA	Ethylenediaminetetracetic acid
FBS	Foetal bovine serum
FEO	Frankincense essential oil
FRAP	Ferric reducing ability of plasma
HPLC	High performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
NMR	Nuclear magnetic resonance
ORAC	Oxygen Radical Absorbance Capacity
TRAP	Telomerase Repeated Amplification Protocol
USDA	United States Department of Agriculture
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Natural products are substances obtained from biologically active natural sources, such as plants, animals, and microorganisms (Bernardini, et al., 2018). In particular, the development of novel medications for the treatment of infectious and malignant diseases as well as other therapeutic disorders including multiple sclerosis and cardiovascular disease has depended heavily on natural components (Atanasov, et al., 2021). It is the phytochemical components of the natural products which provides the basis of therapeutic effects. The phytochemical components of plants can generally be divided into two groups, namely primary and secondary metabolites. However, the definition of a natural product in the fields of medical and pharmaceutical chemistry has only ever included secondary metabolites. These secondary metabolites produce primary chemicals for the manufacture of drugs used in modern medicine to treat conditions ranging from migraine to cancer (Teoh, 2016). Secondary metabolites are the foundation for the therapeutic effects of many modern drugs and medicinal plants as the small primary chemicals produced have a variety of effects on both the plant and other living things (Hussein and El-Anssary, 2019). Secondary metabolites are categorised into four main groups based on their chemical structures, namely terpenoids, phenolic compounds, alkaloids, and sulfur-containing chemicals (Teoh, 2016).

The aromatic and flammable liquids of secondary metabolites is known as essential oil which is a natural product obtained from a single plant species, specifically from a variety of plant parts including flowers, roots, bark, leaves, seeds, and whole plants (Halcon, 2016). Steam distillation, solvent extraction, maceration, cold press extraction, water distillation, or carbon dioxide extraction are the techniques used to extract essential oils (Soares, et al., 2021). Due to their potential as therapeutic agents, essential oils are used in pharmaceutics. Most typically, diluted essential oils are used topically as part of massage therapy, usually when combined with a carrier oil. They can also be inhaled by using a humidifier by adding a few drops of essential oil in steaming water. Furthermore, they can be used as compresses, ointments, and creams or even, taken as soft capsules or as tea with sugar, which allows for a more precise dose and prevents several negative effects (Ríos, 2016).

Since ancient times, the use of natural products as a source for treatments has been acknowledged. Natural products were used ever since 2600 BC where cuneiform clay tablets from Mesopotamia showed the pioneer illustrations of natural products. These clay tablets display oils from *Commiphora* species (myrrh) and *Cupressus sempervirens* (cypress), which are still employed today to treat inflammation, coughs, and colds. The monks in England, France, and Germany conserved this Western knowledge during the Dark and Middle Ages, whilst the Arabs kept the Greco-Roman knowledge and developed the utilisation of their own natural resources as well as herbs from China and India that weren't common in the Greco-Roman era. The foxglove, *Digitalis purpurea*, was first detected in Europe in the 10th century, but it wasn't until the

1700s that researchers discovered its main ingredient, digitoxin, a cardiotonic glycosides that increases cardiac conduction and strengthens cardiac contractibility (Dias, et al., 2012; Rocha, et al., 2001).

Essential oils are widely used and identified as medicinal agents from ancient times due to their pharmaceutical and psychological components as they are believed to possess spiritual, physical, and mental relieving agents (Soares, et al., 2021). Essential oils have been utilised since at least 4500 BC when the Egyptians first used them to make tinctures, powders, salves, and ointments for use in healing practices and religious rituals (Leburmuth, 2019). Going forward in time to 2597 BC, Huang Ti, the Yellow Emperor of China, is known to have incorporated essential oils while writing the classic book ‘The Yellow Emperor's Book of Internal Medicine’, which still has applications for a variety of aromatics today (Leah, 2015; Leburmuth, 2019). Over three thousand years ago, the 'Ayur Veda', a centuries-old Indian system of medicine, began using essential oils in its medicinal concoctions. About 700 oils, including those from cinnamon, ginger, myrrh, and sandalwood, are stated in the Vedic literature as being useful for healing (Leah, 2015).

Natural product research has recently gained more attention because of the inability of alternative drug discovery techniques to produce many leading compounds in important therapeutic areas like immunosuppression, anti-infectives, and metabolic illnesses. Only as last-resort treatments for terminal diseases like cancer are many non-natural, synthetic medications' negative effects, acceptable. Since these non-natural and synthetic medications must

accumulate within living cells, the metabolites found in natural therapeutic plants may escape the side effects of the synthetic medications. One of the key features of natural product medicines are the vast structural and chemical variation of natural goods. Secondary metabolites derived from natural sources are regarded to have more ‘drug-likeness’ and ‘biological friendliness’ than entirely synthetic compounds since they have evolved within living systems. As a result, they are desirable options for medication development in the future (Atanasov, et al., 2021; Lahlou, 2013).

According to Soares, et al. (2021), essential oils can have a range of pharmacological actions that target the central nervous system (CNS), such as neuroprotection, anxiolytic effects, anti-depressant effects, anti-convulsant effects, analgesic effects, and sedative effects. In order to prevent and treat the symptoms of CNS-based diseases like insomnia, depression, dementia, and Alzheimer's disease, essential oils can be utilised as an adjuvant therapy. There are also studies that utilise essential oils to treat respiratory tract infections, according to Horvath and Acs (2015). Due to their volatility, essential oils can quickly enter the upper and lower respiratory tracts through inhalation. Furthermore, they provide a successful treatment for respiratory tract infections because of their effectiveness against microbes and inflammation. In the study, it was shown that oxygenated terpenoids, such as alcohols, aldehydes, esters, ketones, peroxides, and phenols, are what give essential oils their potent antibacterial properties and have an impact on bacterial development.

Even though there are many diseases that can be cured through natural remedies, the subject of this research focus is on cancer because it is a major concern for worldwide public health. The prevalence of cancer and the rate at which it results in fatalities continue to be worldwide health issues (Mondal, et al., 2014). Cancer is a disorder in which a small number of the body's cells grow out of control and spread to other bodily spaces (National Cancer Institute, 2021). Human cells normally grow and divide as needed by the body, die when they become damaged or getting old, and being repaired with new cells. Cancerous cells, however, disturbs this biological process, causing damaged or aberrant cells to proliferate indefinitely.

The World Health Organization (WHO) (2020) estimated 48,639 new cases of cancer were reported in Malaysia in 2020, the country's cancer incidence would double by 2040, and that one in ten Malaysians will receive a cancer diagnosis throughout their lifetime. From the 2007 to 2011 to the 2012 to 2016 reports, there was also an 11% increase in new cancer cases and over 30% more cancer-related deaths reported. As the cancer burden continues to increase and place a significant financial, mental, and physical strain on communities, people with cancer, and the nation's health care system, the increase in cancer cases will become a significant public health concern. Breast cancer, colorectal (colon) cancer, lung cancer, nasopharyngeal cancer, and liver cancer are the top five most prevalent malignancies in Malaysia (Masturah and Ishak, 2022; Ministry of Health, 2019; WHO, 2020).

Even though certain traditional cancer treatment techniques such as chemotherapy, radiation therapy, and surgery are successful, they can have significant negative side effects and toxicities (Sharma, et al., 2021). According to Mondal, et al. (2014), the greatest negative effects from chemotherapy on patients are caused by the overdosing of drugs. Most of the adverse effects are caused by the chemotherapy harming healthy cells that keep the body functioning. Anaemia and thrombocytopenia can result from immune suppression during chemotherapy because it reduces formation of blood cells in the bone marrow. Other examples of side effects are neutropenia, lymphedema, deep vein thrombosis, and the commonly heard side effects of hair loss, nausea, and vomiting (Centers for Disease Control and Prevention, 2022). Due to these severe side effects, the creation of new, all-natural chemotherapeutic agents are urgently needed given the paucity of safe and efficient methods.

Radionics, nanoparticles, natural antioxidants, targeted therapy, ablation therapy, targeted therapy involving stem cells, chemo-dynamic therapy, sonodynamic therapy, and ferroptosis-based therapy are only a few of the recent noteworthy advancements. Natural antioxidants have shown to be capable of detecting free radicals and blocking their damaging impacts, possibly curing or preventing cancer (Debela, et al., 2021).

Despite many advancements in cancer research, the focus of this study is particularly on essential oils as the desirable ‘first choice’ for cancer suppression therapy due to their appealing pharmacological qualities (Sharma, et al., 2021). Essential oils also offer the benefit of being naturally occurring, non-toxic, and

safe when used properly and at right doses (Soares, et al., 2021). When used topically or by inhalation, aromatherapy has a comparatively low toxicity profile (National Health Institute, 2023). According to research by Sharma, et al. (2021), essential oils may cause cancer cells to die in a controlled manner by apoptosis, necrosis, cell cycle arrest, and dysfunction of the major organelles.

Therefore, this research is conducted to assess the antioxidant activity and cytotoxic effect of the purchased Frankincense essential oil. It has been claimed that this essential oil from the *Boswellia* species is abundant in secondary metabolites that are associated in a variation of biological functions. Nevertheless, biochemical and phytochemical investigations are still limited. Thus, preliminary screening for the Frankincense essential oil was executed in this investigation.

The objectives for this study are

1. To determine the polarity of the purchased Frankincense essential oil via thin layer chromatography.
2. To determine the presence of secondary metabolites using phytochemical analysis in the purchased Frankincense essential oil,
3. To evaluate the antioxidant activity of Frankincense essential oil via DPPH assay,
4. To analyse cytotoxicity of Frankincense essential oil against Vero and A549 cell lines using MTT assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Frankincense

2.1.1 Botanical Description

The French word ‘Frankincense’ (*Boswellia* species) implies ‘pure incense’. It is a fragrant resin that is formed from released gums that were secreted by tropical *Boswellia* trees, which generally have a limited natural growing range and grow as little trees or bushes (Davison, Bongers and Philips, 2022). The resin from numerous *Boswellia* species are obtained from scraping the trunk and collecting the dried gums later, while the essential oil is derived by steam distillation of the resin-gum. The freshly abraded resin is white in colour and turns light yellow after it dries to become resin-gum. The essential oil produced after the extraction method will be colourless. The trees produce resin as an immunological reaction to mend and close the wounds in their bark caused by environment or human activity and this serves as natural protection against rotting, fungus, and insects (Soliman, et al., 2020). According to Hamidpour, et al. (2013), only three years of high-quality resin production are necessary before the quality of the resin that is collected dramatically deteriorates. As a result, the tree should be given some time to rest following the harvest. The tree of *Boswellia* species is shown in **Figure 2.1.**

Figure 2.1: The Frankincense tree of the genus *Boswellia* (Adapted from The Editors of Encyclopaedia Britannica, 2022).

The three major components of Frankincense are gum, resin, and essential oils. *Boswellia sacra* is the primary *Boswellia* tree from which Frankincense, a fragrant dried sap is obtained (Encyclopaedia of Living Forms, 2005). The *Boswellia* species from Burseraceae family is a woody deciduous plant with one or more trunks that grows to a height of 1.5 to 8.0 m. Its bark is easily peeled and has a paper-like texture. It has an odd number of leaflets that grow in opposition to one another along its branches, as well as compound leaves that are grouped at the tips of tangled branches. The leaves have tiny, yellowish-white flowers, which have five petals and ten stamens, are grouped in axillary clusters (Encyclopaedia of Living Forms, 2005).

The stem which has a strong trunk is frequently slightly swollen at the base or branched nearly from the ground up. Individual trees on steep slopes frequently develop some buttressing that rises from the roots up into the stem. This creates a cushion-like structure that sticks to the rock and provides support. The bark is

pale brown and has a papery outer covering that flakes off over a thick layer of reddish-brown resin layer (Encyclopaedia of Living Forms, 2005). **Figure 2.2** presents the tree trunk of the *Boswellia* species following abrasion producing the *Boswellia* resin.

Figure 2.2: White *Boswellia* resin due to natural abrasion from the tree trunk *(*Adapted from Davison, Bongers and Philips, 2022).

The leaves are 10 to 25 cm long, oblanceolate in shape with a petiole that is less than 10 mm long, as shown in **Figure 2.3**. Up to 5 cm long and 2 cm wide, the leaflets are widely ovate, oblong, or elliptic in shape. At the apex, the leaves are broadly cuneate or truncate, and frequently asymmetric at the base. The bottom part of the leaves is much whiter, densely tomentose with a distinct network, while the top surface is densely hirsute to subglabrous. The seed is a four-pointed structure with wider lateral points and narrower apical and basal horns, as it is frequently encircled by a persistent wing (Encyclopaedia of Living Forms, 2005).

Figure 2.3: Leaves of the *Boswellia* tree (Adapted from Top Tropicals, 2023).

The flowers of the *Boswellia* tree are small, sparsely pubescent racemes or panicles that measure 6 to 26 cm in length. The peduncle is about 0.5 to 4.0 cm and contains bracts that are 1.0 to 2.5 mm long, pedicels that measure 2 to 8 mm, and calyxes that measure 2.0 to 2.5 mm in length. The white petals have lengths of 4 to 5 mm and widths of 2.0 to 2.5 mm. The disc is short tubular, yellowish orange in colour, and is 1.0 to 1.5 mm deep. The filaments are glabrous, linear, and 2.0 to 2.5 mm long (Encyclopaedia of Living Forms, 2005).

2.1.2 Taxonomical Classification

The taxonomical hierarchy of Frankincense is shown in **Table 2.1** as it has the Sapindales order and Burseraceae family.

Table 2.1: Taxonomical classification of Frankincense (Integrated Taxonomic Information System, 2011).

Rank	Taxonomical classification
Kingdom	Plantae
Subkingdom	Viridiplantae
Super division	Embrophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Sapindales
Family	Burseraceae
Genus	Boswellia Roxb. ex Colebr
Species	*Boswellia*
Sub-species	*Boswellia serrata, Boswellia carterii, Boswellia frereana* and *Boswellia sacra*

2.1.3 Habitat

The *Boswellia* trees are widespread in arid woodlands, on steep, precariously eroding slopes and gullies. The trees can withstand the most challenging conditions as it frequently grows in calcareous soil as they can also be found in the ‘fog oasis’ forests of the Arabian Peninsula's southern coastal mountains (Fobar, 2019). The trees use a cushion-like swelling at the base of the trunk to hold onto rocks or rock walls. The range of elevation is 5 to 1230 m above sea level. The older trees that are still standing in Oman appear to be dying since animals frequently nibble on the tree's foliage, blossoms, and saplings (Health Jade, 2017). According to Encyclopaedia of Living Forms (2005), overexploitation is causing the *Boswellia* tree populations to decline.

2.1.4 Geographical Distribution

The Frankincense tree that comes from about four species namely *Boswellia serrata*, *Boswellia carterii, Boswellia frereana*, and *Boswellia sacra* can be found in India, East Africa and China, Northern Africa, Middle East, respectively (Integrated Taxonomic Information System, 2011).

2.1.5 Traditional and Medicinal Uses

The Frankincense gums are typically sold as chewing gum and post-meal mouth fresheners in Saudi Arabia and Yemen. While in Sudan and Eritrea, Frankincense is burned during religious ceremonies as a ritual incense. Additionally, the Frankincense essential oil (FEO) is utilised in Somalia and Ethiopia to cure wounds on people and animals topically, as well as for treatment of allergies, snake and bug bites, colds, coughs, diarrhoea, headaches, syphilis, stomach aches and backaches (Davison, Bongers and Philips, 2022; Lemenih and Teketay, 2003).

In terms of its medicinal uses, FEO serves as anti-arthritic, anti-proliferative, and anti-inflammatory agents for the treatment of diseases in the Indian medical system. Frankincense is used in traditional Chinese medicine for the relief of discomfort associated with leprosy, gonorrhoea, and cancer as well as to improve blood circulation, detoxification, moving qi (a vital life force) and eliminating body humidity. In China, Frankincense is also used as an astringent and to heal wounds and bleeding diseases (Davison, Bongers and Philips, 2022; Lemenih and Teketay, 2003).

Today, Frankincense is frequently employed industrially in a variety of fields, including pharmacology, pesticides, food, beverages, perfumes. Frankincense is prized for its aromatic scents and is frequently used in incense, cleansers, detergents, creams, and balms. It is also used in meditation combinations since it helps to deepen the meditative experience and strengthens the mind. In general, inhaling and diffusing essential oils may have a calming and mood-enhancing impact. In the food sector, beverages, sweets, chewing gum, gelatines, nut products, puddings, and canned vegetables employ Frankincense products (Davison, Bongers and Philips, 2022; Lemenih and Teketay, 2003).

2.1.6 Chemical Constituents

Pentacyclic triterpenoids, tetracyclic triterpenoids, macrocyclic diterpenoids, and a few essential oils, including α-pinene, β-pinene, camphene, α-thujene, sabinene, myrcene, limonene, linalool, and octyl acetate, are the primary chemical components isolated from Frankincense. The most distinctive and well researched constituents of Frankincense are pentacyclic triterpenoids. They can be classified as ursolidine, oleanolic, and lupinane based on their structural differences. β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, 3-acetyl-11-keto-β-boswellic acid, α-boswellic acid and acetyl-α-boswellic acid (shown in **Figure 2.4**) are the representative compounds that have been recognised as the primary constituents and are thought to be the biomarkers of Frankincense (Al-Yasiry and Kiczorowska, 2016; Cao, et al., 2019). Morikawa, Matsuda and Yoshikawa (2017) revealed that Frankincense exhibits a range of biological activities in association with their chemical components as stated in **Table 2.2.**

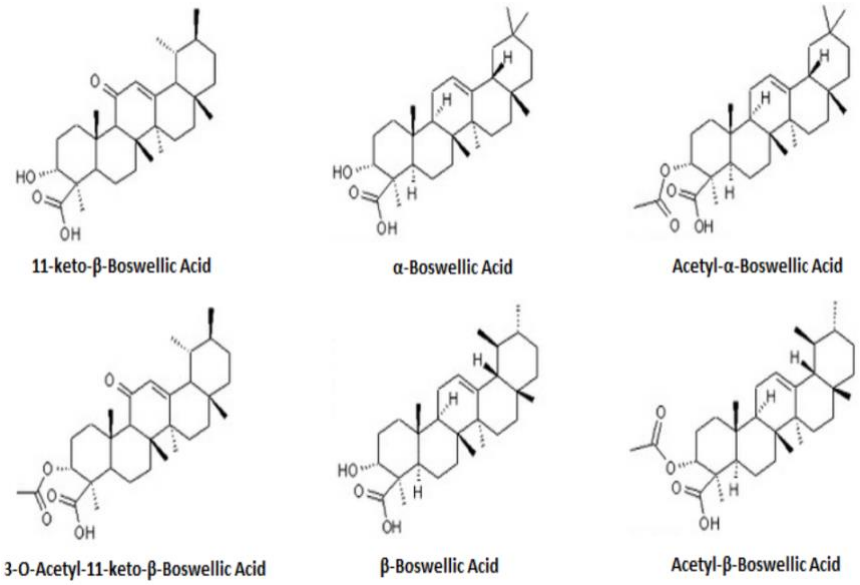

Figure 2.4: *Boswellia* species' main pentacyclic triterpenic acids *(*Adapted from Al-Yasiry and Kiczorowska, 2016).

Table 2.2: Pharmacological activities of Frankincense.

Compounds	Activity	References
Pentacyclic triterpens: 11-keto-β-boswellic acid	Anti-inflammatory	Catanzaro, et al., 2015; Liang, et al., 2010
Essential oil extract: α-pinene, linalool and 1-octanol		
Macrocyclic diterpenoids: Incensole and incensole acetate	Cytotoxic effect	Forcellese, Nicoletti and Petrossi, 2017; Wang, et al., 2011
Pentacyclic triterpens: 11-keto-β-boswellic acid and 3-acetyl-11-keto-β-boswellic acid	Anti-cancer	Bairwa and Jachak, 2016; Siddiqui, 2011
Pentacyclic triterpens: 3-acetyl-11-keto-β-boswellic acid	Anti-ulcer and anti-oxidant	Efferth and Oesch, 2022; Liang, et al., 2010

The main types of phytochemicals such as phenols, steroids, saponin, proteins and amino acids, alkaloids, carbohydrates, terpenoids, tannins, and alkaloids are present in Frankincense extracts, according to a qualitative phytochemical screening by Soliman, et al. (2020). According to a different study by Efferth and Oesch (2022), these components imply that Frankincense has the potential to be biologically active. A few of the ways that these bioactive compounds function to combat cancer, either alone or in combination with other substances, include the regulation of metabolic and signalling pathways, suppression of enzymes necessary for blood vessel development, microtubule assembly, and stimulating apoptosis are some of the strategies used to treat cancer (Ramakrishna, et al., 2021). **Table 2.3** shows the mode of action of secondary metabolites and their target cancer cells.

Table 2.3: Mode of action and targets of respective secondary metabolites.

Metabolite	Mode of action and their target cells	Reference
Phenols	Prevents the glucose transporter-4 from taking up glucose in MCF-7 breast cancer cells by blocking the phosphoinositide 3-kinase pathway.	Harmon and Patel, 2004; Kundu, et al., 2014
Alkaloids	Inhibits hypoxia-inducing factor-1 in cervical cancer by binding to the vascular endothelial growth factor ligand. Thus, reducing vasodilators mediators and reducing oxygen flow to cancer cells.	Lee, et al., 2014; Robati, et al., 2008
Terpenoids	Activates AMP-activated protein kinase in cancer cells, which has been shown to inhibit the growth of prostate (PC-3) and breast cancer (MCF-7) cancer cell lines.	Liu, et al., 2014a; Pan, Chai and Kinghorn, 2012
Flavonoids	Inhibit cyclin- dependent kinases by inhibiting cancer proliferation by blocking progression through the cell cycle by targeting P53, P21, cyclin D and cyclin A.	Engeland, 2018
Phenols, terpenoids, flavonoids, tannins, quinones	Enhancing tumour necrosis factor-related apoptosis-inducing ligand; Frankincense can increase TNF-α which causes ovarian cancer cells to undergo apoptosis.	Ham, et al., 2014; Lee, et al., 2014

2.2 Pharmacological Studies

As shown in **Table 2.4**, many components of the *Boswellia* species, including its extracts, resin, bark, and essential oil, exert biological activities and effects.

Table 2.4: Various pharmacological activities of *Boswellia* species.

Parts	Country	Activity / Effect	Mechanism of action	Reference
Resin	Poland	Analgesic effect	Act on CNS opioid receptors which can be blocked by morphine antagonist naloxone	Al-Yasiry and Kiczorowska, 2016
Extract	China	Anti-inflammatory	Inhibits inflammatory infiltration induced by nociceptive stimulation and overexpression of cyclooxygenase 2.	Cao, et al., 2019
Oil	Korea	Anti-oxidant	The AKBA component impairs nuclear factor-kappa B activation and the expression of the NF-kB regulatory gene, which inhibits the production of osteoclasts. It also enhances apoptosis produced by tumour necrosis factor.	Yang, et al., 2010
Bark	India	Anti-ulcer	To treat recurrent skin ulcers, AKBA directly suppresses MMP activity and secretion by regulating the activity of human collagenase.	Singh, et al., 2012
Oil	United Kingdom	Hepato-protection	Increases the expression ratio of bax/bcl-2 in mitochondria to cause apoptosis in the human liver carcinoma cell line SMMC-7721 as this apoptosis is cell cycle-dependent.	Hakkim, et al., 2019

2.2.1 Antioxidant Activity

The ethanol and chloroform extracts of Frankincense resin from *B. sacra* both shown antioxidant activity in a study by Soliman, et al. (2020), with increasing mean percentage inhibition from 14.26 to 47.69% and 13.00 to 44.75%, respectively when the concentration increases from 2 to 8 mg/mL. In another study by Singh, et al. (2012), the aqueous and ethanol extracts from the bark of *B. serrata* also shown antioxidant activity with mean percentage inhibition increasing from 7.25 to 96.71% and 4.22 to 72.45%, respectively as the concentration increased from 2 to 1024 μg/mL. The IC_{50} values were discovered to be at 23.53 and 91.97 μg/mL for the aqueous and ethanol extracts, respectively.

Furthermore, the nitric oxide scavenging method also shows antioxidant action in Frankincense extracts from the bark of *B. serrata*. As the concentration increased from 70 to 100 μg/mL, the mean percentage inhibition increased from 47.29 to 70.11%. Nitric oxide inhibition has an IC_{50} of 69.67 μg/mL (Singh, et al., 2012).

2.2.2 Cytotoxic Effect

Human melanoma (FM94) and healthy human epithelial melanocytes (HNEM) cell lines were treated with Frankincense essential oil (FEO) at concentrations of 3 to 10 μg/ml over 24 hours in a study by Hakkim, et al. (2019). As the concentration of FEO rose, the percentage of viable FM94 cells decreased from 60 to 15%. In contrast, the viability percentage of HNEM cells are still largely viable with only a slight decrease from 90 to 80%. When compared to the

HNEM and the untreated control, the FEO significantly reduced the vitality of FM94 cells. The HNEM cells retained their morphology, however the FM94 cells lost their adhesion and had entirely disrupted morphology. This shows that the FEO had selective cytotoxicity towards the FM94 cells, which is in accordance with studies by Yang, et al. (2019) and Singh, et al. (2012).

In another study by Pistelli, et al. (2019), *in vitro* cytotoxicity screening of FEO from *B. serrata* and *B. sacra* were tested against breast cancer cell lines (MCF7, T47D and MDA-MB-231), human myelogenous erythroleukemia (K562) and malignant tumour cell lines (SH-SY5Y), using MTT reduction assay. The IC_{50} values in parts-per million (ppm) obtained after treatment with the FEO from *B. serrata* were 71.6 ppm (MCF7), 89.4 ppm (MDA-MB-231), 75.4 ppm (K562) and 112.9 ppm (Sh-SY5Y). Meanwhile, the IC_{50} values after treatment with FEO from *B. sacra* were 231.0 ppm (MCF7) and 13.7 ppm (K562).

2.3 Cancer

2.3.1 Overview of Cancer

Uncontrolled growth and spread of abnormal cells describe the group of disorders known as cancer (Mathur, et al., 2015). Human cells frequently divide, multiply, and proliferate to create new cells when the body requires them. New cells replace old ones when they are destroyed by damage or ageing. Occasionally, this systematic process fails, allowing defective or damaged cells to proliferate when they should not. These cells possess the potential to develop into tumours, that are clumps of tissue. Malignant or benign (non-cancerous) tumours are both possible. Malignant or cancerous tumours can metastasise or

proliferate to other parts of the body, where they can infiltrate nearby tissues and grow new tumours. After removal, benign tumours frequently do not return, in contrast to malignant tumours, which can occasionally do so. Benign tumours do not invade or spread to surrounding tissues (National Cancer Institute, 2021).

Since the process of developing a tumour involves several stages, normal cells gradually transition to the neoplastic stage and acquire properties that make them tumorigenic as they do so. There are seven primary hallmarks of cancers: maintaining proliferative signalling, avoiding growth inhibitory signals, avoiding apoptosis, unlimited potential for replication, activating angiogenesis, activating invasion and metastasis, transforming energy metabolism, and avoiding immune response (Gutschner and Diederichs, 2012). Due to genetic defects that alter how our cells function, particularly how they grow and divide, cancer is a hereditary disease (National Cancer Institute, 2021).

A group of compounds known as carcinogens are directly responsible for causing DNA damage and either causing or promoting cancer. Carcinogens include substances like tobacco, asbestos, arsenic, radiation from the sun, gamma and x-rays, and exhaust gases from moving vehicles. Free radicals are created when carcinogens are exposed to our bodies, and they attempt to deprive other molecules in the body of their electrons. These free radicals harm cells and impair their ability for regular functions (Mathur, et al., 2015).

2.3.2 Prevalence of Cancer

According to estimates from the World Cancer Research Fund International (2022), there were 18.1 million new cases of cancer in the world in 2020, 9.3 million of which involved males and 8.8 million included women. The cancers that were most prevalent worldwide for both sexes were lung and breast cancer, which accounted for 12.5% and 12.2% of all newly diagnosed cases, respectively, in 2020. About 1.9 million new cases of colorectal cancer were reported in 2020, making up 10.7% of all cancer cases. The most common form of the disease, lung cancer, will account for 15.4% of the total new cases of cancer in men worldwide in 2020. The leading three cancers were colorectal, prostate, and lung cancers. On the other hand, breast cancer, which accounted for 25.8% of all new cases that were identified in 2020, is the cancer affecting women most commonly worldwide.

Meanwhile in Malaysia, there are an estimated 48,639 new cancer cases in 2020 and 29,530 deaths. From that, 23,052 cases were in males and 25,587 cases were in females. The top three cancer incidences for both genders are breast cancer (17.3%) followed by colorectal cancer (13.6%) and lung cancer (10.6%). Meanwhile, the top three leading cancers in males are lung cancer (17%), colorectal cancer (15.4%) and prostate cancer (9.3%). For females, breast cancer is the top cancer prevalence with 32.9% followed by colorectal cancer at 11.9% and ovary cancer with 7.2% (Global Cancer Observatory, 2021).

2.4 Vero Cell Line

The Vero cell line was developed on March 27, 1962, by Y. Yasumura and Y. Kawakoita at Chiba University in Chiba, Japan, from kidney tissue taken from an adult, healthy African green monkey (*Cercopithecus aethiops*) (American Type Culture Collection, 2020). B. Simizu transported the cell line from Chiba University to the Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health on June 15, 1964. In 1966, ATCC received the cell line (Ammerman, et al., 2009).

The Vero cell line exhibits epithelial shape and adherent growth as shown in **Figure 2.5**. This lineage's cells are elongated, resembling fibroblast cells in shape, develop in monolayers, and have minimal cytoplasmic granulation. Sub-culture needs to be done when the population reaches cellular confluence; otherwise, the cells would die or lift off the surface of the culture flask (Adams, et al., 2015).

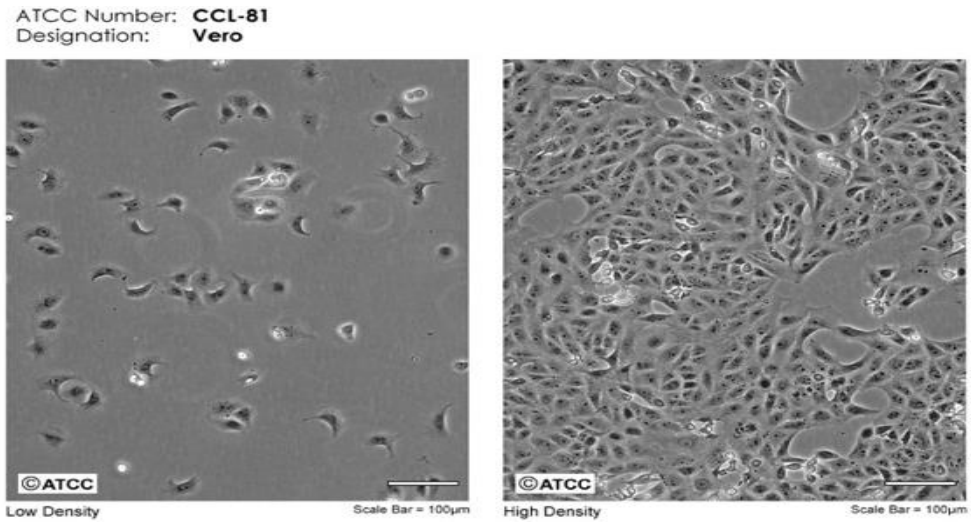

Figure 2.5: The morphology of Vero cells at low density (left) and high density (right) *(*Adapted from American Type Culture Collection, 2020).

This cell line has a hypodiploid number of chromosomes. In most cells, structurally changed marker chromosomes account for more than 50% of each cell complement's chromosomes. When a cell passage is not prolonged, Vero cells are non-tumorigenic and contain pseudo-diploid karyotypes (American Type Culture Collection, 2020). Approximately 25,877 potential protein-coding genes were found in the 2.97 Gb genome sequence of the Vero cell line, which represents the genome landscape (Osada, et al., 2014).

2.5 Lung Cancer (A549) Cell Line

Giard and colleagues created the A549 cell line in 1972 by extracting and cultivating lung carcinoma tissue from the explanted tumour of a 58-year-old Caucasian male (American Type Culture Collection, 2021). This adenocarcinoma cell line falls under the category of non-small-cell lung carcinoma which accounts for 85 to 88% of all cases of lung cancer (Altogen Biosystems, 2023). **Figure 2.6** shows the cells' morphology which is squamous in nature and epithelial-like structure. When cultivated *in vitro*, these cells attach to the culture vessel and form a monolayer. These cells have a high quantity of unsaturated fatty acids and the ability to synthesise lecithin, both of which are needed for maintaining membrane phospholipids (Foster, et al., 2018; Synthego, 2023). The A549 cells normally double in 22 hours, but this can occasionally increase to 40 hours (American Type Culture Collection, 2021; Synthego, 2023).

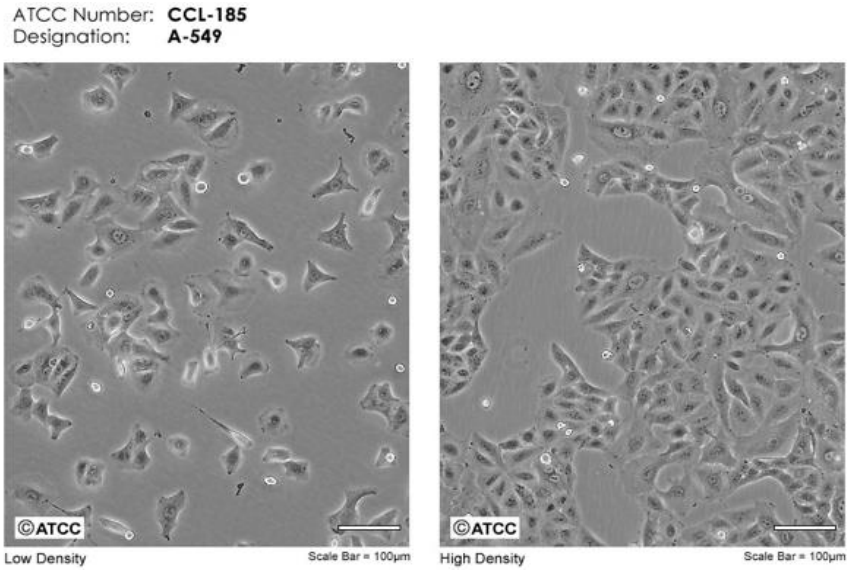

Figure 2.6: The morphology of A549 cells at low density (left) and high density (right) *(*Adapted from American Type Culture Collection, 2021).

2.6 Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to determine the polarity of mixtures. A sheet of glass, plastic, or aluminium foil that has been lightly coated with an adsorbent substance typically cellulose, silica gel, or aluminium oxide can be used in TLC. This adsorbent layer is referred to as the stationary phase. The sample is placed on the plate and a solvent or solvent mixture (mobile phase) is drawn up the plate by capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved (Sherma and Fried, 2003). The basis for compound separation is the competition among the solute and the mobile phase for binding sites on the stationary phase. Silica gel is one example of a material that can be categorised as polar. When two compounds with differing polarity are present, the more polar molecule has a stronger interaction with the silica and is hence more capable to remove the mobile phase from the binding sites (Bele and Khale, 2011).

The retention factor (R_f) value is larger for the less polar molecule as a result of its increased upward mobility on the plate. If the mobile phase is shifted to a more polar solvent or mixture of solvents, which is better suited for releasing solutes from the silica binding sites, all compounds on the TLC plate will move higher up the plate. The components of the mixture appear as spots at appropriate levels on the plates after the separation process is completed. R_f value is a number that describes a compound's behaviour in TLC (Bele and Khale, 2011).

2.7 Antioxidant Assay

Natural antioxidant research has gained popularity recently across many areas. Selecting an appropriate assay based on the chemical(s) of interest is essential to evaluate their antioxidant activity (Moon and Shibamoto, 2009). Because natural chemicals are used in food, medicine, and cosmetics, evaluations of their antioxidant effects are crucial. Reactive species are produced in living systems by a variety of metabolic processes and environmental stressors. These are free radicals and mainly reactive oxygen species (ROS). A rise in ROS over time can lead to oxidative stress at systemic levels, which manifests a number of health issues including cancer, age-related disorders, and cardiovascular diseases (Mishra, et al., 2011). The EC_{50} (concentration necessary to produce a 50% antioxidant effect) is a commonly used measure to express the antioxidant capacity (Chen, Bertin and Froldi, 2013). The antioxidant assay used in this study was DPPH assay however, there are other assays that can be utilised as tabulated in **Table 2.5**.

Table 2.5: Examples of other antioxidant assays (Mishra, et al., 2011).

Assay	Principle of Method	End product determination
ABTS	Antioxidant reaction with an organic radical.	Colorimetry
FRAP	Antioxidant reaction with a Fe (III) complex.	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals induced by AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride)	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals generated from AAPH decomposition.	Photo chemiluminescence quenching

DPPH assay is one of the common and simple colorimetric techniques for assessing antioxidant properties of pure compounds and determining the free radical scavenging capability of an antioxidant molecule (Mishra, et al., 2011). The assay is based on the evaluation of the scavenging ability of antioxidants against a stable free radical called DPPH (α, α-diphenyl-β-picrylhydrazy). By obtaining a hydrogen atom from antioxidants and converting it to the equivalent hydrazine, the odd electron of the nitrogen atom in DPPH is lowered (Sagar and Singh, 2011). The DPPH (•) radical scavenging activity is normally evaluated in terms of inhibition percentage of the pre-formed free radical by antioxidants. (Chen, Bertin and Froldi, 2013).

DPPH is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule (**Figure 2.7**), so that the molecules do not

dimerise. The delocalisation also causes the deep violet hue which is absorbed in methanol solution at a wavelength of about 517 nm. When DPPH solution is combined with something that can donate a hydrogen atom, it transforms into the reduced form and loses its violet colour (Sagar and Singh, 2011). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is as below.

$$Z\bullet + \mathbf{AH} = \mathbf{ZH} + A\bullet$$

Where ZH is the reduced form and A• is free radical produced in the first step. The DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH (Sagar and Singh, 2011).

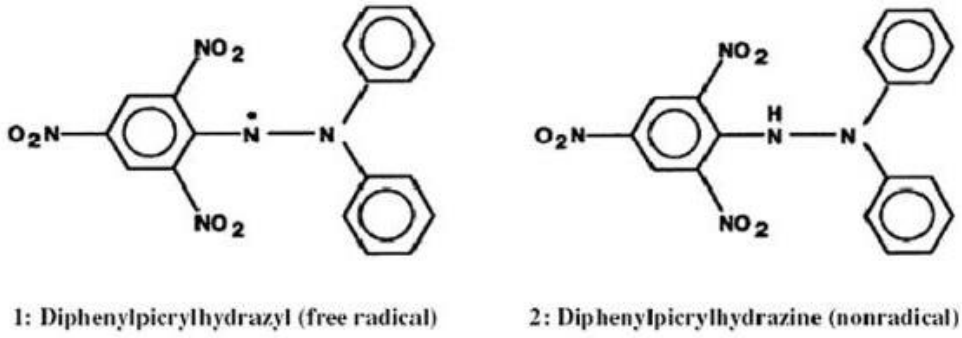

Figure 2.7: DPPH radical (left) and its stable form (right) (Adapted from Sagar and Singh, 2011).

2.8 Cytotoxic Assay

Based on the conversion of pale yellow tetrazolium, the MTT assay makes it easy, accurate, and reliable to count metabolically active cells (Riss, et al., 2016). The MTT reagent is a mono-tetrazolium salt that has three aromatic rings, comprising two phenyl moieties and one thiazolyl ring, around a positively charged quaternary tetrazole ring core with four nitrogen atoms.

When MTT is reduced, the central tetrazole ring is broken, creating the violet-blue, water-insoluble chemical known as formazan (Ghasemi, et al., 2021). Tetrazolium compounds are reduced by nicotinamide adenine dinucleotide in metabolically active live cells to produce the vivid formazan products (Riss, et al., 2016). Due to its positive charge and lipophilic nature, the MTT reagent can pass through the cell membrane and the inner membrane of mitochondria in living cells and is then converted to formazan by metabolically active cells (Ghasemi, et al., 2021). The amount of formazan is determined by measuring variations in absorbance at 550 nm is directly proportional to the number of live cells.

MTT is converted into a purple formazan product in live cells with an active metabolism as shown in **Figure 2.8** (Ghasemi, et al., 2021). Although the precise biological process by which MTT is converted into formazan is not fully understood, it most likely involves an interaction with NADH or other reducing molecules that can donate electrons to MTT. The MTT tetrazolium's formazan product accumulates near the cell surface, inside cells, and in the culture medium as an insoluble precipitate (Harshitha, et al., 2019). There are other cytotoxicity assays that can be utilised to determine cell viability as stated in **Table 2.6**.

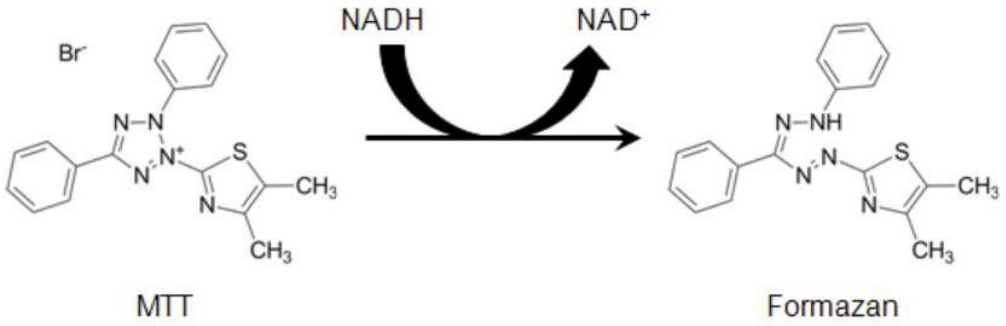

Figure 2.8: Structures of MTT and coloured formazan product (Adapted from Riss, et al., 2016).

Table 2.6: Examples of other cytotoxic assays.

Assay	Principle of Method	References
DNA fragmentation	DNA fragments resulting from apoptotic DNA fragmentation and are visualised by gel electrophoresis.	Harshitha, et al., 2019
Comet Assay	Measure DNA strand breaks in damaged cells induced by chemical and physical agents.	Collins, 2004
Necrosis Assay	Flow cytometric analysis of the cells stained positive for both FITC Annexin V and PI are undergoing the stage of necrosis as dead cells while cells stained negative are alive and not undergoing apoptosis or necrosis.	University of California, 2022
Enzyme Assay	Monitor passaging of lactate dehydrogenase, due to loss of cell membrane integrity when cells are exposed to cytotoxic compounds.	University of California, 2022

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Frankincense Essential Oil

The USDA certified Frankincense Serrata essential oil (product number: RM00121) was purchased from Plant Therapy Essential Oils Corporate in October 2022 via online purchase (Lazada Malaysia). The oil was obtained from the resin of the *Boswellia Serrata* from India and a maximum dilution of 3% for topical applications is recommended (Plant Therapy, 2023).

Figure 3.1: Frankincense essential oil from Plant Therapy Essential Oils Corporate (Adapted from Plant Therapy, 2023).

3.1.2 Cell Lines

The adult African green monkey kidney cell line (Vero cell) (CCL-81) and the lung carcinoma cell line (A549) (CCL-185) were used in this study.

3.1.3 Chemicals and Solvents

Table 3.1 lists all of the substances, chemicals and solvents that were used during the research.

Table 3.1: List of solvents and chemicals along with their corresponding manufacturer and brand.

Solvents/ Chemicals	Manufacturer / Brand
Basic DMEM powder	Sigma-Aldrich, United States
Chloroform	SYNERLAB, Malaysia
DPPH powder	Sigma-Aldrich, United States
DMSO	Nacalai Tesque INC, Japan
Doxorubicin hydrochloride	Sigma-Aldrich, United States
Ethyl acetate	Copens Scientific (M) Sdn. Bhd., Malaysia
Foetal bovine serum	Tico Europe, Netherlands
Hexane	Rank Synergy Sdn. Bhd., Malaysia
L-ascorbic acid powder	Fisher Scientific, United Kingdom
Methanol	GENE Chemicals, Malaysia
MTT powder	Chemical Solutions, Malaysia
Penicillin-streptomycin	Bio Basic Inc, Canada
Phosphate buffer saline tablet	Oxoid Limited, United Kingdom
Silica-coated aluminium sheet	Merck, Germany
Sodium hydrogen carbonate	SYSTERM, Malaysia
Hydrochloric acid	SYSTERM, Malaysia
0.25% Trypsin-EDTA	GE Healthcare, United States
0.4% Trypan blue dye	Sigma-Aldrich, United States
37% Hydrochloric acid	QReC Chemicals, Malaysia
95% Ethyl acetate (Industrial grade)	Chemical Solutions, Malaysia
98% Sulphuric acid	RCI Labsan Limited, Thailand
99.8% Acetic acid	HmbG Chemicals, Malaysia

3.1.4 Equipment

The list the equipment that were utilised during the study are presented in **Table 3.2.**

Table 3.2: List of equipment and their corresponding manufacturer and brand.

Equipment	**Manufacturer / Brand**
Autoclave	Hirayama, Japan
Biosafety Cabinet	Esco Micro (M) Sdn. Bhd., Malaysia
Centrifuge	Heraeus, Germany
Electronic balance	RGS Corporation Sdn. Bhd., Malaysia
Freezer (-20 °C)	Pensonic, Malaysia
Haemocytometer	Hecht-Assistant, Germany
Inverted phase contrast microscope	Nikon, Japan
Laminar hood	Edamix, Malaysia
Microplate reader	BMG Labtech, Germany
Refrigerator (4 °C)	Samemax, Malaysia
Ultraviolet lamp	Spectronics Corporation, United States
Ultra-low freezer (-80 °C)	Eppendorf Asia Pacific, Malaysia
Vortex	PLT Scientific, Malaysia
Water bath	PMI Labortechnik GmBH, Switzerland
5% carbon dioxide (CO_2) incubator	BINDER, Germany

3.2 Methodology

A simplified workflow of the study is shown in **Figure 3.2**.

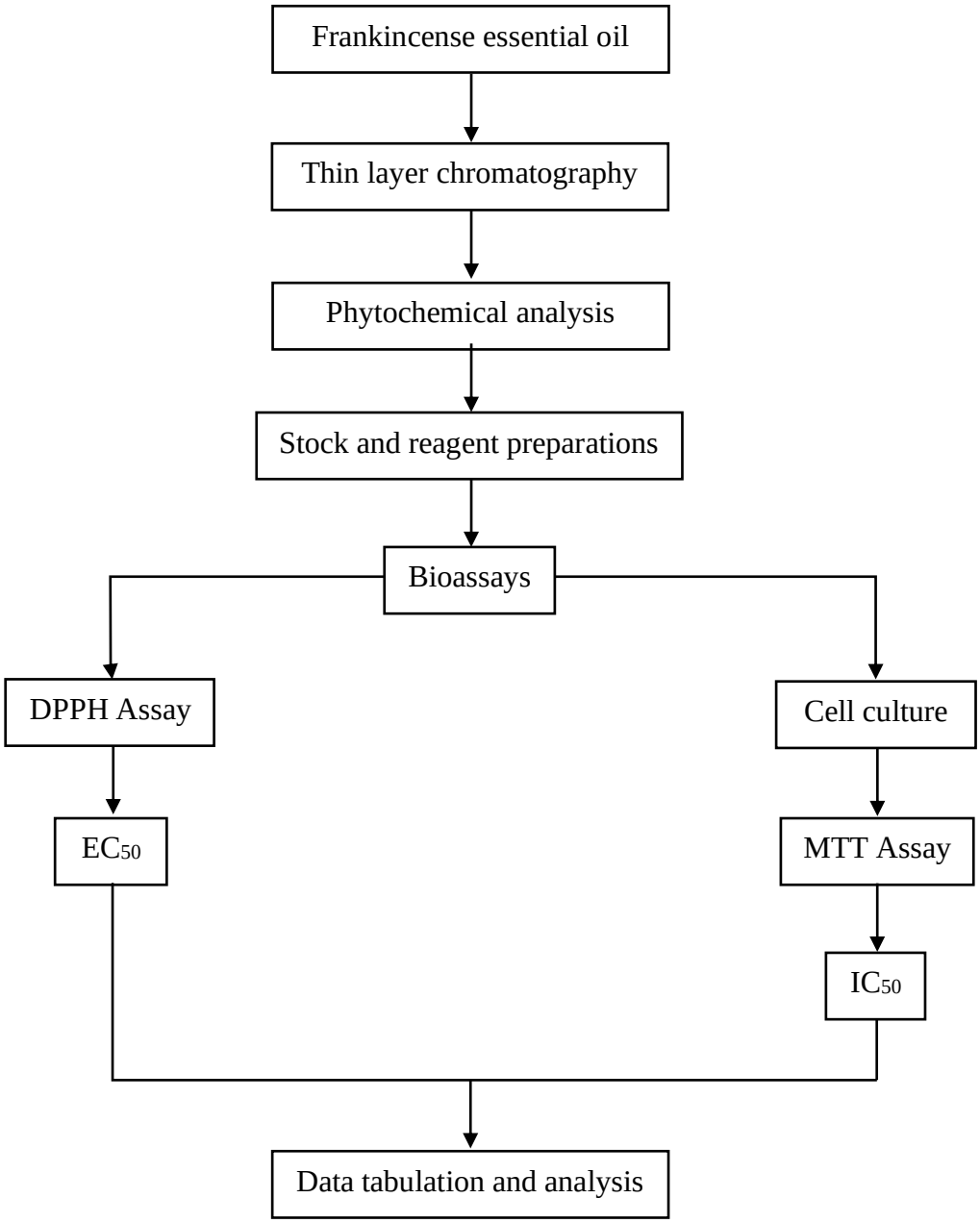

Figure 3.2: A brief summary of workflow for the study of Frankincense essential oil.

3.3 Phytochemical Screening

3.3.1 Negative Control

In a test tube, 200 µL of Frankincense essential oil (FEO) was added into 800 µL of ethanol and vortexed together. This negative control was used in all the tests.

3.3.2 Phenols

Phenols was tested using a ferric chloride test as Aloitaibi (2019) with slight modifications in terms of the volume. In a test tube, 200 µL of FEO was added with a drop of 5% ferric chloride solution. Gallic acid act as positive control and was prepared by adding 10 mg of gallic acid powder into 1 mL of methanol, which will form a deep blue or black solution in the presence of phenols.

3.3.3 Tannins

Braymer's test was used to evaluate tannins as per the study by Efiong, et al. (2019). About 600 µL of distilled water and 200 µL of FEO were added into a test tube along with a drop of 10% ferric chloride. Tannic acid was used as the positive control, where 1 mL of distilled water was used to dissolve 10 mg of tannic acid powder to produce a solution that would indicate the presence of tannins by turning to blue or green colour solution.

3.3.4 Coumarin

Coumarin's test was utilised to test the presence of coumarin as per Ismail, et al. (2017). In a test tube, 200 µL of FEO and about 300 µL of 10% NaOH were added. Methanol was mixed with hydroxycoumarin (positive control) to create a clear yellow colour solution that served as a coumarin presence indicator.

3.3.5 Flavonoids

Pew's test was used to test for the presence of flavonoids as per Efiong, et al. (2019). About 200 µL of FEO was added into 0.01 g of metallic zinc and dropwise addition of 1M HCl, into a test tube. Presence of flavonoids will cause the formation of white precipitates. Due to the lack of availability, no positive control was used for comparison.

3.3.6 Terpenoids

Salkowski's test was used to evaluate terpenoids in accordance with Aloitaibi (2019). Into a test tube, 200 µL of FEO and 200 µL of chloroform were mixed as one drop of 1M H_2SO_4 was added. The formation of a red colour solution indicates that terpenoids is present. No positive control was used to compare.

3.3.7 Saponin

Foam test was conducted to test for the presence of saponin as per Ismail, et al. (2017). Approximately 200 µL of FEO and 1 mL of distilled water were added into a test tube and shaken vigorously with a stopper. When saponin is present, a cloudy white solution with foamy bubbles forms. Because of the lack of positive control, none was used to compare.

3.3.8 Alkaloids

To test for the presence of alkaloids, Wagner's test was done according to Efiong, et al. (2019). Wagner's reagent is made up of iodine and potassium iodide in distilled water, was added along the sides of a test tube which contained 200 µL of FEO. Alkaloids will be present if a reddish-brown precipitate is present. Due to the lack of availability, no positive control was used.

3.3.9 Quinones

According to Efiong, et al. (2019), a sulphuric acid test is conducted to determine the presence of quinones. About 200 µL of FEO was added to 200 µL of 1M H_2SO_4 in a test tube. For quinones, a yellow precipitate is expected. No positive control was utilised due to its unavailability.

3.3.10 Glycosides

Keller-Killani test was used to test for the presence of glycosides as in a study by Yadav and Agarwala (2011). In a test tube, 200 µL of FEO was added into 120 µL of 1M of glacial acetic acid and one drop of 5% ferric chloride. Then, five drops of concentrated H_2SO_4 were added along the sides of the tube. A brown ring at the interphase indicates the presence of glycosides. The lack of positive control led to it not being used.

3.4 Thin Layer Chromatography

Thin layer chromatography (TLC) was conducted using a silica-coated aluminium plate that was cut into 2.0 cm width and 10.0 cm length. On the plate, a pencil was used to draw a baseline and frontline of 1.0 cm from the bottom and the top, respectively as seen in **Figure 3.3**. The essential oil was dissolved with chloroform. A small, concentrated dot of essential oil was spotted with a capillary tube on the baseline. The plate was then placed in an airtight compartment that was filled with several organic solvent mixtures. The organic solvent systems used were hexane alone as well as hexane and ethyl acetate mixture of ratios 1:1, 10:1 and 10:3. The plate was taken out and examined using an ultraviolet lamp under short (254 nm) and long (365 nm), respectively (SpectroUV, 2023). By dividing the compound's travel distance by the solvent front's distance, the retention factor (R_f) value of each spot formed on the plate was calculated (Row and Lee, 2000).

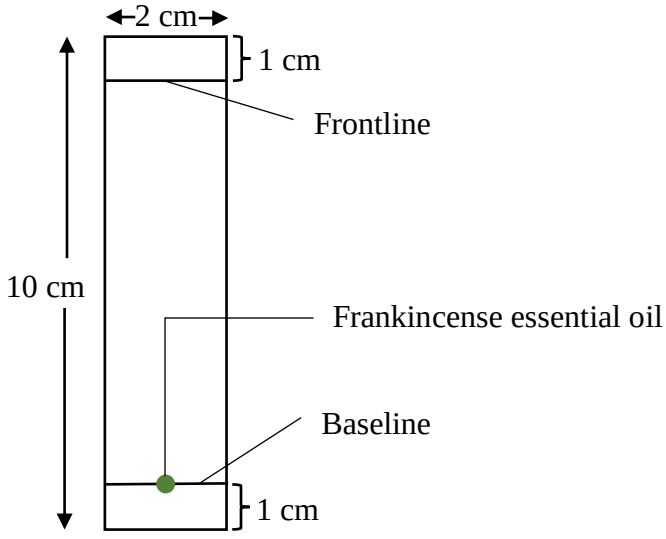

Figure 3.3: Design of the thin layer chromatography plate.

3.5 Preparation of Stock Solutions for Bioassays

In DPPH assay, two stock solutions namely weight per volume (w/v) and volume per volume (v/v) of FEO were prepared. The w/v concentration was produced by adding 100 mg of FEO into 1 mL of methanol into a sample vial. Meanwhile, 100% FEO directly from the bottle was used as the v/v concentration. The stock solutions were kept in a 4 °C refrigerator.

In MTT assay, the FEO stock solution was prepared by mixing 100 mg of the oil into 1 mL of 100% dimethyl sulfoxide (DMSO) which was then vortexed for solubilization. Addition of 50 μL stock solution to 4950 μL of basic DMEM allowed the stock solution to be diluted, hence producing a working solution of 1 mg/mL concentration. The stock solution was stored in a -20 °C freezer.

3.6 Preparation of Positive and Negative Controls

Ascorbic acid (100 mg/mL) was prepared in a sample vial for DPPH assay as a positive control. The stock solution was made by thoroughly vortexing 1 mL of distilled water with 100 mg of ascorbic acid powder. Methanol alone was used as the negative control in DPPH assay. Meanwhile, doxorubicin hydrochloride was the positive control utilised in the MTT assay. By mixing 20 μL of doxorubicin hydrochloride with 9.8 mL of basic DMEM, the stock solution was diluted to a concentration of 100 g/mL. The solution was kept at -20 °C freezer. DMSO (1%) was used as the negative control and was made by mixing 0.5 mL of 100% DMSO with 50 mL of basic DMEM in a falcon tube.

3.7 Preparation of Reagents and Medium

3.7.1 DPPH Reagent

The 0.2 mM concentration of DPPH reagent was produced by diluting 4 mg of DPPH powder with 50 mL of methanol, in a Schott bottle. The reagent was incubated in the dark for at least 30 minutes to allow complete solubilization and reactivity. Due to the fact that DPPH is a chemical that is sensitive to light, the bottle was covered in aluminium foil and prepared in the dark. The reagent was used freshly for the assay.

3.7.2 MTT Reagent

In order to achieve a concentration of 5 mg/mL for the MTT reagent, 150 mg of MTT powder was mixed in 30 mL of sterile, autoclaved PBS. The mixture was then filtered into a test tube using a cellulose filter membrane with a diameter of 0.22 μm. Since the MTT reagent is sensitive to light, the tube was covered with aluminium foil and produced in the absence of light. The reagent was stored in a -20 °C freezer.

3.7.3 Complete Growth Medium

About 500 mL of sterile distilled water was added with 6.7 g of DMEM powder and 1.85 g of sodium bicarbonate to produce basic DMEM. A sterile disposable filter unit and vacuum pump were used to filter the mixture. A quality check was performed to the prepared medium by adding 3 mL of the solution into a small petri dish and further incubated for 24 hours. The following day, the medium was checked for any trace of contamination by observing under an inverted microscope.

Complete growth medium (CGM) was then prepared by adding 10 mL of 10% heat-inactivated FBS into 90 mL of basic DMEM into a sterile Schott bottle. The bottle was parafilm-sealed and kept in a 4 °C refrigerator for later use. A quality check was performed by adding 3 mL of CGM into a small petri dish and incubated for 24 hours. The medium was examined under an inverted microscope the next day to look for any signs of contamination.

3.7.4 Phosphate Buffer Saline

Four phosphate buffer saline **(**PBS) tablets were dissolved in 400 mL of distilled water in a sterile Schott bottle. The PBS solution was autoclaved and allowed to cool down to room temperature. In a laminar hood, the PBS solution was filtered using a filter unit and vacuum pump. A small petri dish was filled with 3 mL of PBS, incubated for 24 hours, and was checked the following day for contamination as part of a quality control procedure.

3.8 Culture and Subculture of Cells

Vero and A549 cell lines were quickly thawed in a water bath for one minute at 37 °C. A new 25 cm^3 tissue culture flask containing 5 mL of pre-warmed CGM was added with the cells using a pipette. The cells were subsequently examined using an inverted microscope and cultured at 37 °C in a 5% CO_2 incubator. To make sure there was no contamination, the cultured cells were regularly observed.

Subculture was carried out once the cells had reached confluency of 70 to 90%. The old media was removed, and 5 mL of PBS was used to rinse the cells twice.

The flask was filled with 3 mL of 0.25% trypsin-EDTA solution and incubated for 15 minutes at 37 °C in a 5% CO_2 incubator. After checking the cells under an inverted microscope to make sure they had detached, 5 mL of CGM was added to the flask right away to cease trypsinization. The cells were placed in a 15 mL centrifuge tube and centrifuged for 10 minutes at 25 °C in 1000 rpm. The pellet was resuspended after the supernatant was discarded. A new 25 cm^3 flask was first added with 5 ml of CGM and subsequently 1 mL of cells. The flasks were then labelled accordingly and incubated in a humidified incubator with 5% CO_2 at 37 °C.

3.9 DPPH Assay

DPPH assay was performed on a 96-well plate (round- bottom). All of the wells, aside from columns 1 and 2 were filled with 100 µL of methanol. As illustrated in **Figure 3.4**, 100 µL each of the stock solutions of ascorbic acid (w/v) and FEO (w/v; v/v) were added to columns 3 through 12. With the pre-added methanol, serial dilution of the samples was done to produce concentrations that ranged from 0.39 to 50.00 mg/mL (w/v) and % (v/v). Columns 1 and 2 then received 100 µL of methanol. All of the wells except columns 11 and 12, which serve as a background absorbance for the FEO, received about 20 µL of DPPH reagent. After being covered in aluminium foil, the plate was incubated in the dark for 20 minutes. Absorbance was read at 517 nm using a microplate reader. The percentage of radical scavenging activity versus sample concentration (w/v or v/v) graphs was plotted and EC_{50} values were determined. The following formula was used to calculate the percentage of radical scavenging activity (Sagar and Singh, 2011).

$$\text{Radical scavenging activity (\%)} = \frac{A0-A1}{A0} \times 100\%$$

Where,

A0 = Absorbance of negative control

A1 = Absorbance of sample

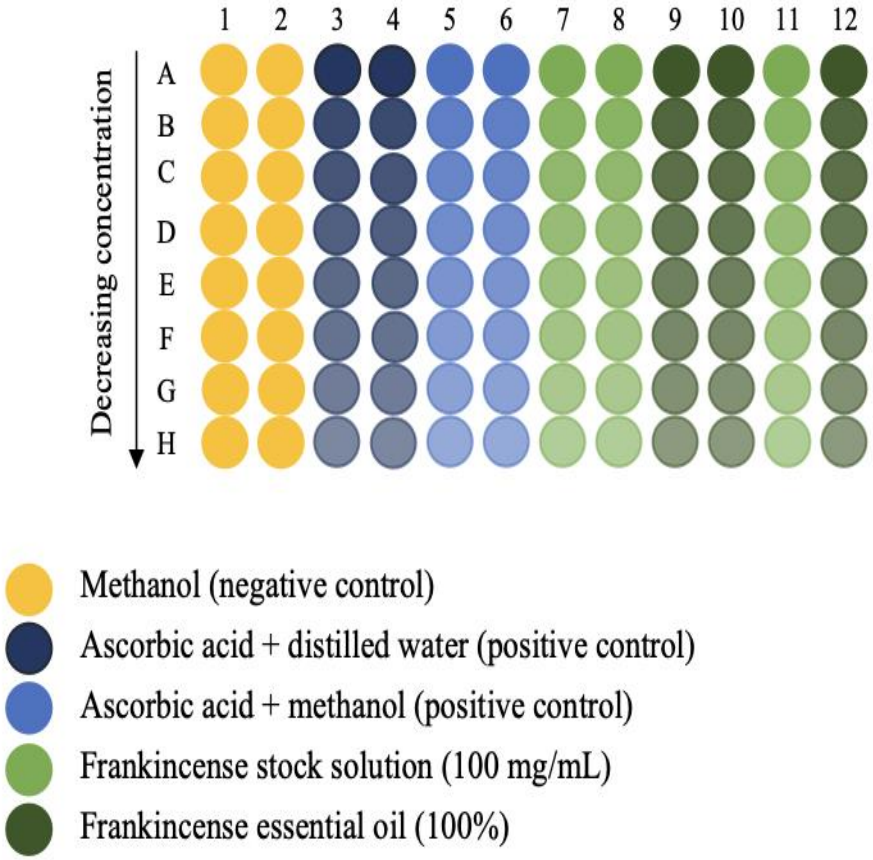

Figure 3.4: 96-well plate design utilised in DPPH assay.

3.10 MTT Assay

Approximately 50 µL of cell suspension and 50 µL of 0.4% trypan blue was mixed at a 1:1 dilution, into a microcentrifuge tube. The mixture was incubated at room temperature for five minutes. The mixture was then mixed again to prevent sedimentation before loading 20 µL of the mixture into one chamber of a clean haemocytometer. Microscope was used to view the cells under 100x

magnification. Dead cells were stained blue whereas viable cells were unstained and thus only the viable cells were counted using a hand tally counter (Fuentes, 2014). The counting process was done following the notion that only those cells touching the top and left boundary lines were counted while those touching the bottom and right boundary lines were excluded to prevent counting the same cell twice as shown in **Figure 3.5**. According to Katsares, et al. (2015), the average number of live cells in each of the four grids were calculated and the cell concentration was determined using the following formula.

Concentration of cells = average number of living cells in four grids x 10^4 x dilution factor x original volume of cell suspension.

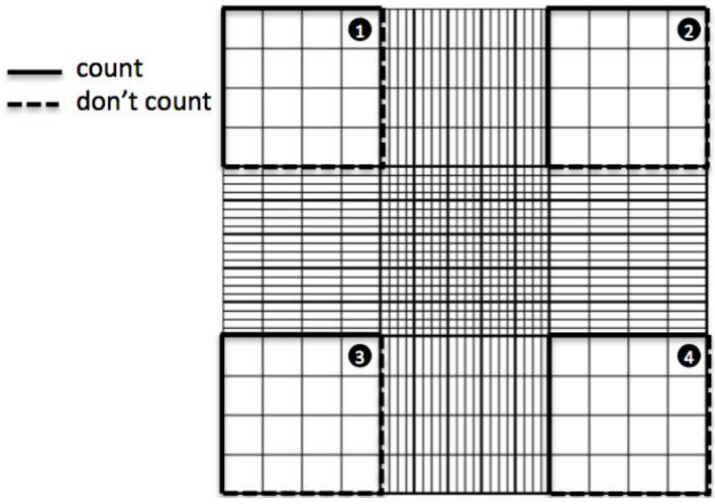

Figure 3.5: Grid lines present in one chamber of a haemocytometer and the squares labelled 1 to 4 where cells were counted (Adapted from Fuentes, 2014).

Cells at concentration of 1×10^4 cells/mL were seeded into the wells of columns 1 to 8 in a flat-bottom 96-well plate. The plate was then sealed with parafilm. The plate was observed under an inverted microscope for even distribution of cells into each well. The plate was then incubated in a 5% CO_2 incubator overnight at 37°C. In the following day, the plate was checked to confirm cell attachment before discarding the medium from the wells. In another 96-well plate, both FEO and doxorubicin hydrochloride were serially diluted (two-fold), respectively using basic medium to a final volume of 100 µL (**Figure 3.6)**. The plate was sealed with parafilm and incubated at 37 °C for 24-, 48- and 72 hours, respectively.

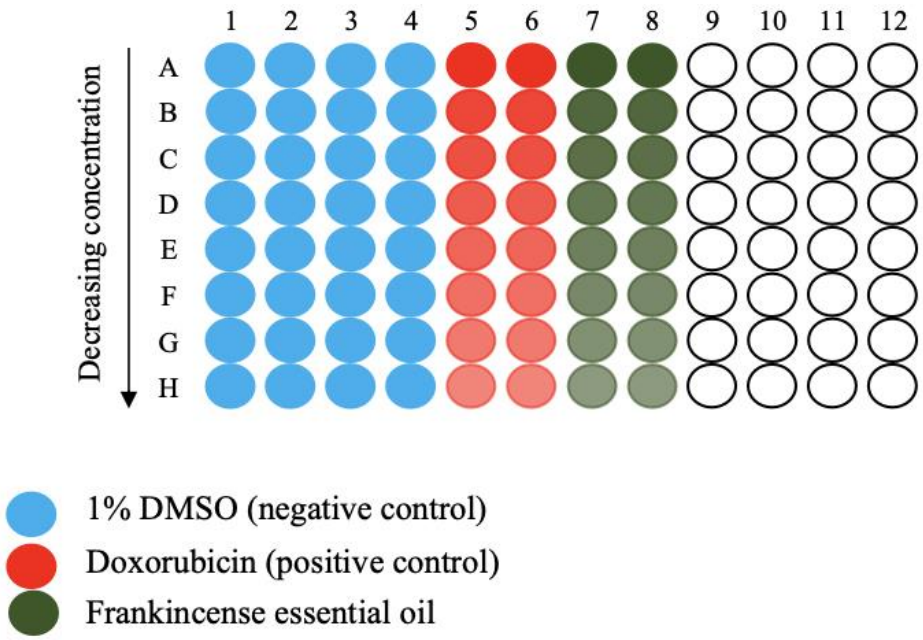

Figure 3.6 Design of 96-well plate used in MTT assay.

The mixtures from each well were completely discarded at the end of each period of incubation, and 20 µL of MTT reagent was then added in the absence of light. The plate was covered in aluminium foil and incubated for an additional 4 hours at 37 °C incubator. After incubation, 100 µL of 100% DMSO

was added to each well to break down the purple formazan crystals. For 10 to 15 minutes, the plate was incubated at room temperature. Finally, using a microplate reader, the absorbance was determined spectrophotometrically at a wavelength of 570 nm. The data obtained was tabulated to determine the percentage of viable cells using the following formula (Mahavorasirikul, et al., 2010). Graphs of cell viability (%) against various concentrations of different samples were produced and the IC_{50} values were determined from the graphs.

$$\text{Percentage of cell viability} = \frac{A1}{A0} \times 100\%$$

Where,

A0 = Absorbance of negative control

A1 = Absorbance of treated cells

3.11 Data Analysis

DPPH assay was repeated thrice, whereas MTT assay was done twice. The data obtained was presented as mean ± standard deviation (SD) which was then tabulated and analysed using GraphPad Prism 9. The data were either analysed using Student t-test or one-way ANOVA to determine the significance level of $P < 0.05$.

CHAPTER 4

RESULTS

4.1 Thin Layer Chromatography

The number of spots formed, and the range of retention factor (R_f) values were calculated and tabulated in **Table 4.1** according to their respective solvent systems. **Figure 4.1** shows the TLC profile of FEO in various solvent systems using hexane and ethyl acetate. The solvent system of hexane and ethyl acetate combination of ratio 10:3 showed the widest range of R_f values ranging from 0.48 to 0.90, followed by the 1:1 ratio with a range of 0.60 to 0.75 and the 10:1 ratio presented a range from 0.65 to 0.78. Meanwhile the solvent system containing hexane alone showed a single R_f value of 0.63.

In addition, the hexane-ethyl acetate mixture at a 10:3 ratio produced the highest total number of spots at four spots, followed by the ratio of 10:1 and 1:1 forming two spots each. Meanwhile, the solvent system containing only hexane generated only one spot. The spots produced also seem to have a pattern where those that moved higher towards the frontline are larger in size as compared to those nearer to the baseline.

Table 4.1: Number of spot(s) and range of retention factor (R_f) values formed from FEO in respective solvent systems, on thin layer chromatography.

Solvent System (ratio)	Number of spot(s)	Range of R_f value
Hexane	1	0.63
Hexane : Ethyl Acetate (1:1)	2	0.60 – 0.75
Hexane : Ethyl Acetate (10:1)	2	0.65 – 0.78
Hexane : Ethyl Acetate (10:3)	4	0.48 – 0.90

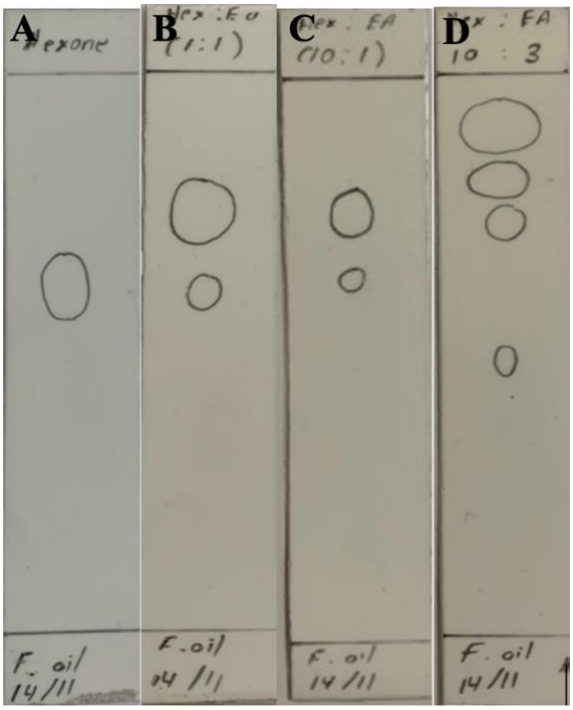

Figure 4.1: TLC profiles of FEO in different solvent systems. (A) Hexane alone; (B) Hexane: ethyl acetate (1:1); (C) Hexane : ethyl acetate (10:1); (D) Hexane : ethyl acetate (10:3).

4.2 Phytochemical Analysis

In **Table 4.2**, a summary of secondary metabolites in FEO is presented in which '+' and '-' symbols indicate the presence and absence, respectively. From the results, it can be inferred that the FEO contains seven of the nine secondary metabolites that were examined.

Table 4.2: The presence or absence of respective secondary metabolites in Frankincense essential oil via qualitative phytochemical analysis.

Secondary metabolites	Presence
Phenols	+
Tannins	+
Coumarin	+
Flavonoid	+
Terpenoids	-
Saponin	+
Alkaloids	+
Quinones	-
Glycosides	+

4.2.1 Phenols

A positive result of phenols in the FEO was confirmed via the ferric chloride test as presented in **Figure 4.2**. The positive control, gallic acid showed a clear, dark blue solution while the negative control (FEO and ethanol) was a clear, colourless solution. The FEO displayed a yellowish solution with some bubbles formed on the surface.

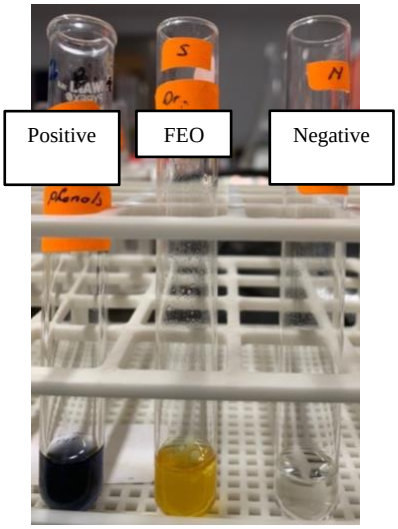

Figure 4.2: Results of ferric chloride test to detect the presence of phenols in FEO.

4.2.2 Tannins

Figure 4.3 shows how Braymer's test verified positive tannins result in the FEO. The positive control, tannic acid, displayed a clear, dark blue solution while the negative control (FEO and ethanol) was a clear, colourless solution. The FEO presented a clear, greenish yellow solution with a blue colour hue.

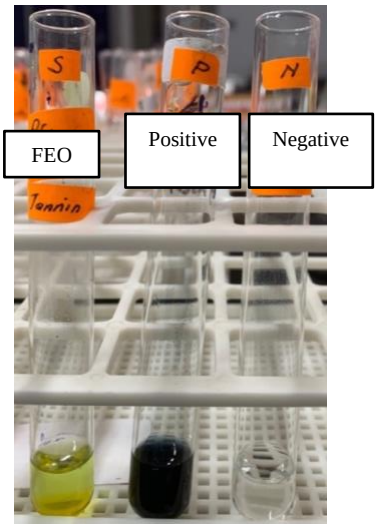

Figure 4.3: Results of Braymer's test to detect the presence of tannins in FEO.

4.2.3 Coumarin

Coumarin test shown in **Figure 4.4** confirmed a positive coumarin result in FEO. The negative control (FEO and ethanol) was a clear, colourless solution while the positive control (hydroxycoumarin) showed a clear, light orange solution. The FEO revealed a clear, yellow solution.

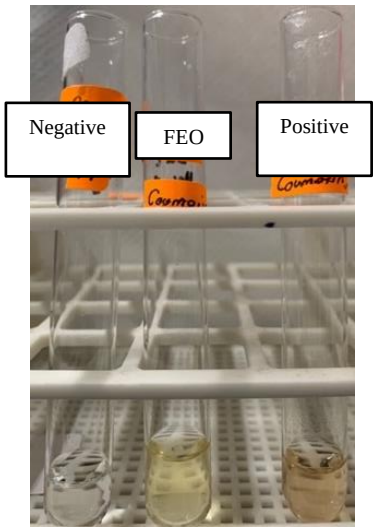

Figure 4.4: Results of coumarin test to detect the presence of coumarin in FEO.

4.2.4 Flavonoids

Pew's test was conducted as shown in **Figure 4.5** and it presented a flavonoids-positive result in the FEO. The negative control (FEO and ethanol) was a clear colourless solution while the FEO revealed a cloudy, white solution with white precipitates floating around.

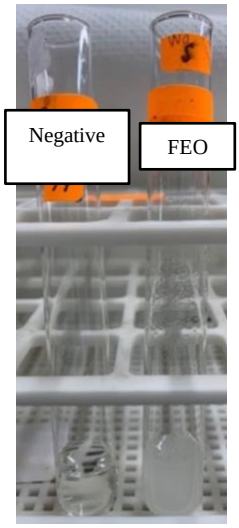

Figure 4.5: Results of Pew's test to detect the presence of flavonoids in FEO.

4.2.5 Terpenoids

Salkowski's test shown in **Figure 4.6** confirmed a negative terpenoids result in the FEO. Both negative control (FEO and ethanol) and FEO presented a clear, colourless solution which indicated the absence of terpenoids in FEO.

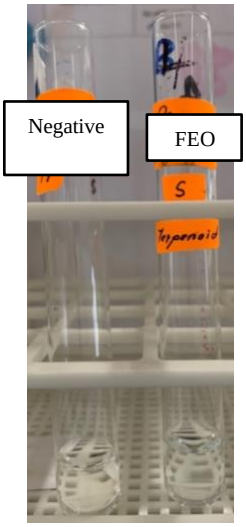

Figure 4.6: Results of Salkowski's test to detect the presence of terpenoids in FEO.

4.2.6 Saponin

Figure 4.7 shows how the Foam test verified a positive saponin result in the FEO. The negative control (FEO and ethanol) was a clear, colourless solution while the FEO revealed a cloudy, white solution with foamy bubbles formed on the surface.

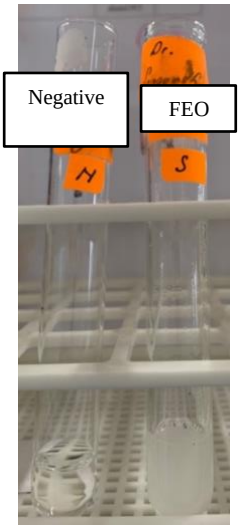

Figure 4.7: Results of Foam test to detect the presence of saponin in FEO.

4.2.7 Alkaloids

Wagner's test shown in **Figure 4.8** confirmed positive alkaloids result in the FEO. The negative control (FEO and ethanol) was a clear, colourless solution meanwhile the FEO had a clear, yellow solution with some bubbles and an orange-red cloudy precipitate with a thin layer of brownish-orange suspension on the surface.

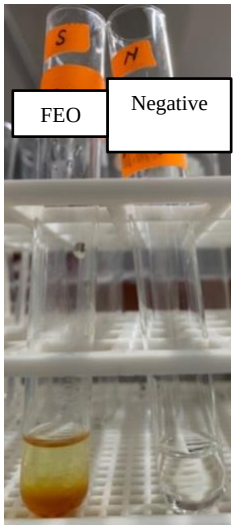

Figure 4.8: Results of Wagner's test to detect the presence of alkaloids in FEO.

4.2.8 Quinones

Sulphuric acid test shown in **Figure 4.9**, confirmed a negative quinones result in the FEO. The negative control (FEO and ethanol) is a clear colourless solution meanwhile the FEO presented a cloudy white solution.

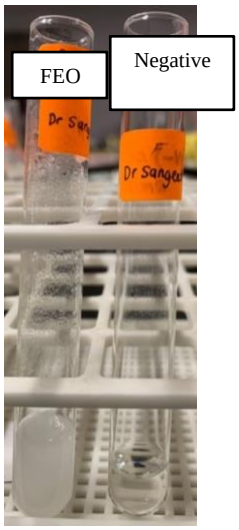

Figure 4.9: Results of sulphuric acid test to detect the presence of quinones in FEO.

4.2.9 Glycosides

Keller-Killani test shown in **Figure 4.10** confirmed positive glycosides result in the FEO. The negative control (FEO and ethanol) is clear and colourless meanwhile the sample presented a cloudy, white solution with a yellow precipitate and a thin yellow suspension on the surface.

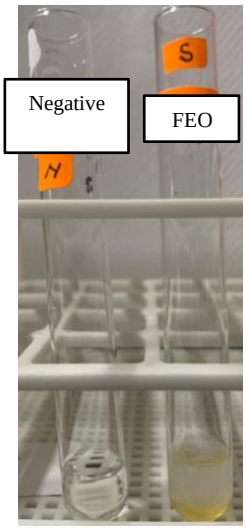

Figure 4.10: Results of Keller-Killani test to detect the presence of glycosides in FEO.

4.3 DPPH Assay

The radical scavenging activity (%) of FEO and ascorbic acid at varying concentrations (v/v or w/v) were tabulated and graphed in **Table 4.3** and **Figure 4.11,** respectively. The FEO of v/v concentration demonstrates a greater percentage of radical scavenging activity as compared to the w/v as shown in **Table 4.3**. Therefore, it is concluded that the v/v concentration of FEO has higher radical scavenging activity. However, the FEO's percentage of radical scavenging activity is still considerably lower than that of ascorbic acid, which acts as the positive control.

There is a general pattern of enhanced percentage of radical scavenging activity as the v/v concentration of FEO also increased as indicated in **Table 4.3**. The percentage of radical scavenging activity continued to increase from 19.79 ± 0.05 % to 78.47 ± 0.08 % in response to its increasing concentration from 0.39 to 50.00%. The FEO at 50% (v/v) exhibited the highest percentage of radical scavenging activity of 78.47 ± 0.08 %, while the lowest percentage of radical scavenging activity of 19.79 ± 0.05 % was associated with the lowest concentration of 0.39%.

Meanwhile, the percentage of radical scavenging activity in response to the increasing w/v concentration of FEO demonstrates a varied pattern as shown by **Table 4.3**. As the concentration of FEO of w/v increases, there is a fluctuation in the percentage of radical scavenging activity. The percentage of radical scavenging activity varies with increasing concentration from 0.39 to 50.00 mg/mL. The greatest percentage of radical scavenging activity at 43.58 ± 0.08 % corresponds to the highest FEO concentration (50.00 mg/mL) while the lowest radical scavenging activity percentage of 18.16 ± 0.06 % corresponds to the concentration of 6.25%.

The percentage of radical scavenging activity in ascorbic acid was rather constant as the concentration increased. Although the percentage of radical scavenging activity does not have a consistent increase from each value, however, still shows higher values. Their corresponding radical scavenging activities are 84.49 ± 0.01 %, 79.27 ± 0.04 %, 81.60 ± 0.01 %, 83.70 ± 0.01 %,

80.87 ± 0.02 %, 79.04 ± 0.03 %, 79.50 ± 0.01 %, and 80.55 ± 0.01 %, in order of increasing ascorbic acid concentration from 0.39 to 50.00 mg/mL.

Figure 4.11 demonstrates the rising trends in the radical scavenging activity for both concentrations of FEO (v/v and w/v), however they are still below the ascorbic acid's percentage radical scavenging activity, which is the positive control. As the concentration of FEO increased, the percentage of radical scavenging activity increased as well, revealing a dose-dependent relationship. Ascorbic acid, on the other hand, demonstrated a rather constant percentage of radical scavenging activity throughout the rising concentrations.

Table 4.3: Percentages of radical scavenging activity in various concentrations of FEO and ascorbic acid.

Concentration (%v/v); (w/v, mg/mL)	**Radical Scavenging Activity (%)**		
	Frankincense Essential Oil (v/v)	**Frankincense Essential Oil (w/v)**	**Ascorbic Acid (w/v)**
0.39	19.79 ± 0.05	19.20 ± 0.06	84.49 ± 0.01
0.78	20.10 ± 0.11	20.45 ± 0.09	79.27 ± 0.04
1.56	20.62 ± 0.08	18.61 ± 0.05	81.60 ± 0.01
3.13	36.48 ± 0.05	18.44 ± 0.05	83.70 ± 0.01
6.25	50.42 ± 0.05	18.16 ± 0.06	80.87 ± 0.02
12.50	63.34 ± 0.09	26.25 ± 0.06	79.04 ± 0.03
25.00	68.64 ± 0.05	30.66 ± 0.12	79.50 ± 0.01
50.00	78.47 ± 0.08	43.58 ± 0.08	80.55 ± 0.01

Data is represented as mean ± standard deviation (SD), n=3 ($P < 0.05$).

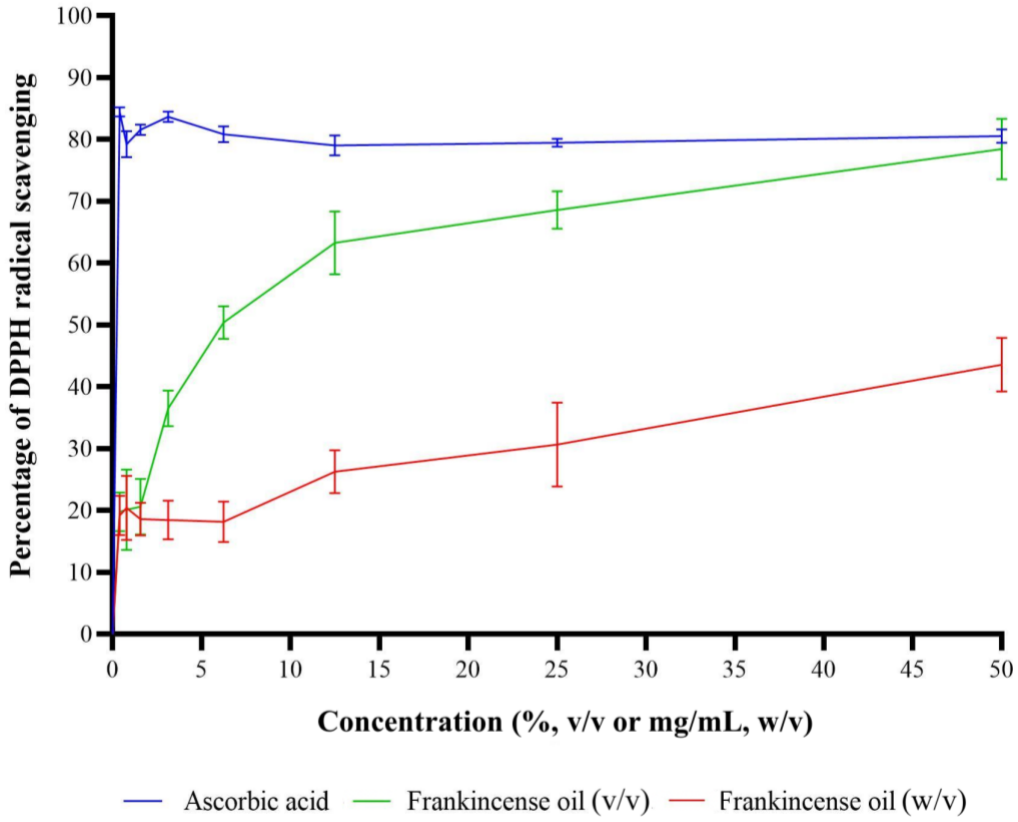

Figure 4.11: The increasing trend in the percentage of radical scavenging activity of ascorbic acid and FEO (v/v; w/v) at various concentrations.

The FEO (v/v and w/v) and ascorbic acid in all incubation periods showed significant difference ($P < 0.05$) based on Student t test. The EC_{50} values in **Table 4.4** were determined by graphical interpolation via the statistical graphing software, GraphPad Prism 9. In descending order of EC_{50} values, FEO of w/v displayed the highest EC_{50} value at 28.95 ± 0.37 mg/mL, followed by FEO (v/v) of 4.73 ± 0.01 % and ascorbic acid at 4.60 ± 0.02 mg/mL. The EC_{50} value is inversely proportional to the radical scavenging activity of the sample.

Table 4.4: EC_{50} values for FEO and ascorbic acid.

Sample	EC_{50} value (%; mg/mL)
Frankincense essential oil (v/v)	4.73 ± 0.01
Frankincense essential oil (w/v)	28.95 ± 0.37
Ascorbic Acid	4.60 ± 0.02

Data is represented as mean ± standard deviation (SD), n=3 ($P < 0.05$).

4.4 MTT Assay

4.4.1 Vero Cells

Figure 4.12 illustrates the characteristics of Vero cells, which are adherent cells with a fibroblast-like appearance and an elongated form.

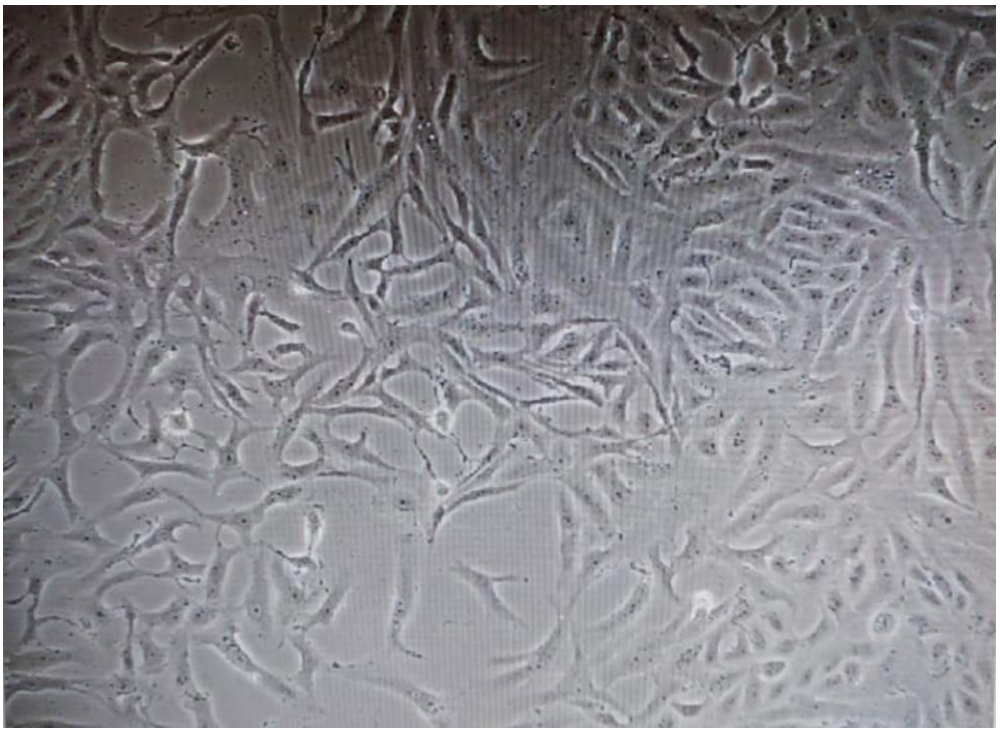

Figure 4.12: Morphology of Vero cells cultured in DMEM, at 100x magnification.

Table 4.5 lists the percentage cell viability of Vero cells treated with FEO and doxorubicin hydrochloride after 24 hours and 48 hours of incubation. After 24 hours incubation, the highest and lowest percentage cell viability of Vero cells treated with the FEO was 74.3 ± 0.02 % at 125.00 mg/mL and 45.6 ± 0.01 % at 31.25 mg/mL, respectively. The percentage of cell viability relatively increased at 48 hours where the highest percentage cell viability was 114.4 ± 0.04 % at 7.81 mg/mL and the lowest was 98.2 ± 0.00 % at 500.00 mg/mL.

After incubating the doxorubicin hydrochloride-treated Vero cells for 24 hours, it was deduced that the highest and lowest percentage cell viability was 113 ± 0.05 % at 25.00 mg/mL and 51.1 ± 0.02 % at 0.78 mg/mL, respectively. Meanwhile, after 48 hours of incubation, 131.2 ± 0.15 % at 100.00 mg/mL was the highest percentage cell viability while the lowest was 95.0 ± 0.00 % at 3.13 mg/mL. FEO showed significant difference however, doxorubicin hydrochloride was not significantly different ($P < 0.05$) based on student t test.

Meanwhile, **Figures 4.13** and **4.14** compares the percentage of Vero cells that remain viable after being treated with different concentrations of FEO and doxorubicin hydrochloride, respectively, after 24 hours and 48 hours of incubation. In both figures, the percentage cell viability shows a general increase from all the incubation periods from 24 hours to 48 hours, except for percentage cell viability of Vero cells treated with doxorubicin hydrochloride at concentration 6.25 μg/mL.

Table 4.5: The percentage of cell viability of Vero cells (24- and 48 hours incubation) and A549 cells (24-, 48-, and 72 hours incubation) treated with various concentrations of FEO and doxorubicin hydrochloride.

Sample	Concentration (µg/mL)	Cell viability (%) of Vero cells		Cell viability (%) of A549 cells		
		24 hours	48 hours	24 hours	48 hours	72 hours
Frankincense Essential Oil	7.81	48.5 ± 0.02	114.4 ± 0.04	78.5 ± 0.03	49.1 ± 0.26	67.2 ± 0.04
	15.63	52.2 ± 0.01	113.7 ± 0.01	92.7 ± 0.02	68.5 ± 0.17	69.5 ± 0.10
	31.25	45.6 ± 0.01	103.8 ± 0.00	66.4 ± 0.11	61.4 ± 0.26	80.7 ± 0.08
	62.50	56.9 ± 0.02	100.7 ± 0.01	95.0 ± 0.06	80.2 ± 0.06	86.8 ± 0.01
	125.00	74.3 ± 0.02	105.0 ± 0.01	79.3 ± 0.15	68.6 ± 0.08	71.6 ± 0.20
	250.00	50.7 ± 0.01	104.3 ± 0.01	75.5 ± 0.14	72.0 ± 0.03	78.3 ± 0.15
	500.00	45.7 ± 0.02	98.2 ± 0.00	69.9 ± 0.13	53.9 ± 0.25	57.0 ± 0.06
	1000.00	46.5 ± 0.02	98.3 ± 0.01	59.6 ± 0.11	51.8 ± 0.05	46.9 ± 0.17
Doxorubicin hydrochloride	0.78	51.1 ± 0.02	107.3 ± 0.01	97.7 ± 0.15	48.5 ± 0.11	54.1 ± 0.39
	1.56	55.2 ± 0.10	100.3 ± 0.01	89.5 ± 0.14	47.6 ± 0.00	39.9 ± 0.19
	3.13	62.1 ± 0.02	95.0 ± 0.00	58.7 ± 0.04	36.6 ± 0.06	26.3 ± 0.05
	6.25	111.4 ± 0.29	103.9 ± 0.01	63.3 ± 0.00	47.6 ± 0.14	26.2 ± 0.04
	12.50	83.7 ± 0.04	107.3 ± 0.12	55.2 ± 0.01	51.5 ± 0.05	32.6 ± 0.04
	25.00	113.0 ± 0.05	114.6 ± 0.04	74.0 ± 0.02	47.3 ± 0.04	31.1 ± 0.01
	50.00	96.8 ± 0.11	127.7 ± 0.10	97.2 ± 0.27	61.1 ± 0.19	40.6 ± 0.03
	100.00	107.8 ± 0.12	131.2 ± 0.15	104.1 ± 0.00	73.4 ± 0.09	51.5 ± 0.03

Data is represented as mean ± standard deviation (SD), n=2 ($P < 0.05$).

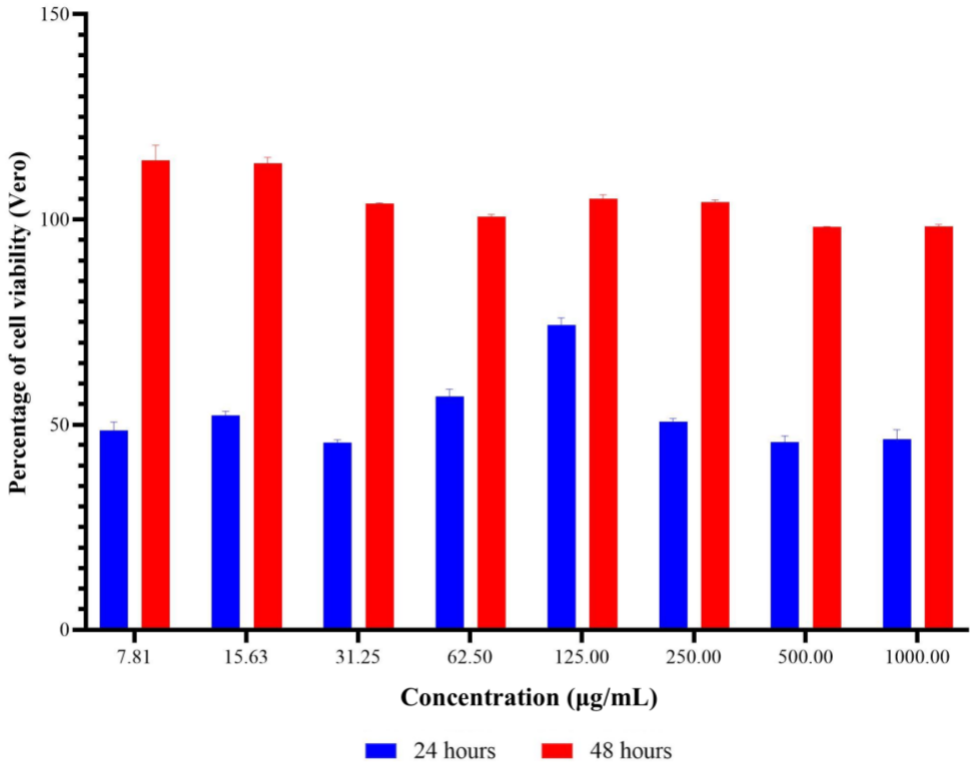

Figure 4.13: Bar graph representing the comparison of the percentage cell viability of Vero cells upon treatment with various concentrations of FEO at 24 hours and 48 hours.

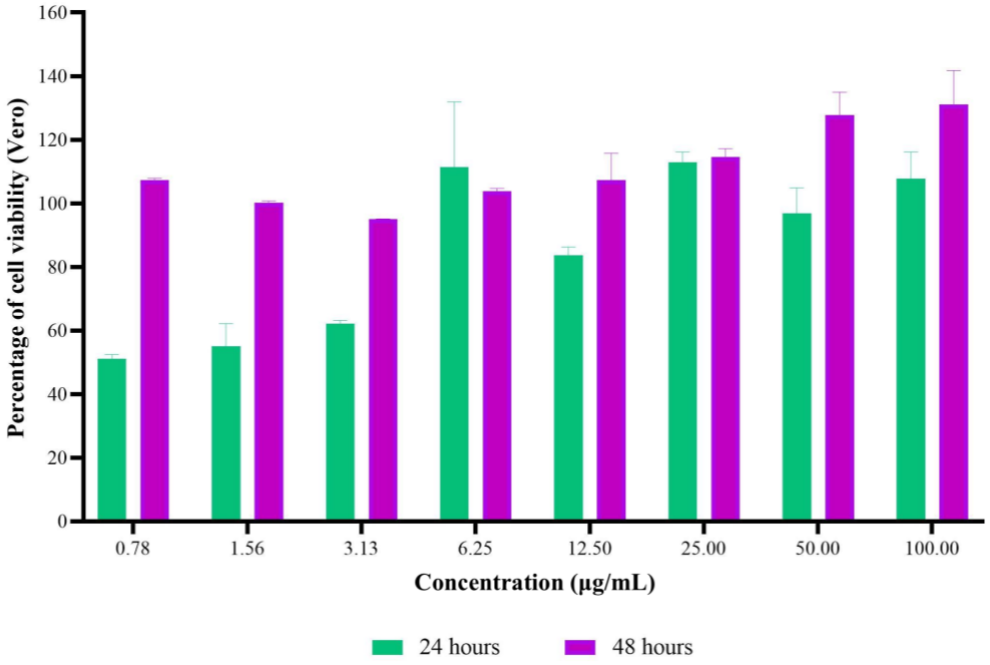

Figure 4.14: Bar graph representing the comparison of the percentage cell viability of Vero cells upon treatment with various concentrations of doxorubicin hydrochloride at 24 hours and 48 hours.

4.4.2 A549 Cells

A549 cells has the characteristics of adherent cells with the morphology of fibroblast-like appearance and an elongated shape as shown in **Figure 4.15**.

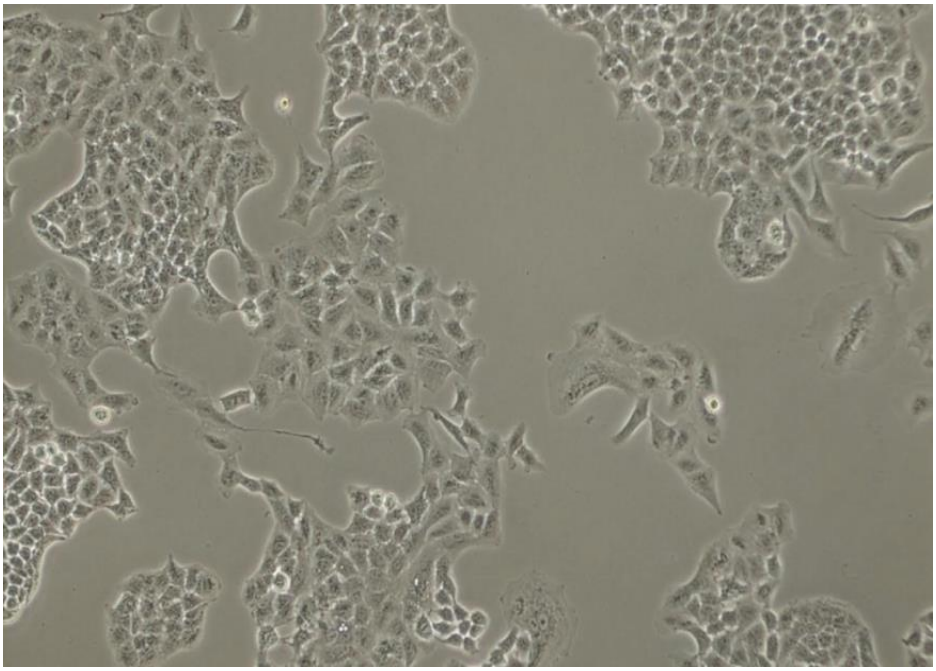

Figure 4.15: Morphology of A549 cells cultured in DMEM, at 100x magnification.

The percentage cell viability of A549 cancer cells after treatment with FEO and doxorubicin hydrochloride after 24-, 48- and 72-hours incubation are tabulated in **Table 4.5**. Referring to the treatment of FEO in A549 cancer cells after 24 hours incubation, the highest and lowest percentage cell viability were 95.0 ± 0.06 % at 62.50 mg/mL and 59.6 ± 0.11 % at 1000 mg/mL, respectively. Upon incubating for 48 hours, the highest percentage cell viability is 80.2 ± 0.06 % at 62.50 mg/mL and the lowest is 49.2 ± 0.26 % at 7.81 mg/mL. Meanwhile, after 72 hours of incubation, 86.8 ± 0.01 % at 62.50 mg/mL is the highest percentage of cell viability and 46.9 ± 0.17 % at 1000.00 mg/mL is the lowest.

After incubating the A549 cancer cells with doxorubicin hydrochloride for 24 hours, it was deduced that the highest percentage cell viability is 104.07 ± 0.00 % at 100.00 mg/mL while the lowest is 55.2 ± 0.01 % at 12.50 mg/mL. Upon incubating for 48 hours, cell viability ranges from 36.6 ± 0.06 % at 3.13 mg/mL to 73.4 ± 0.09 % at 100 mg/mL, with it being the lowest and highest percentage cell viability. The greatest and lowest percentages of cell viability after 72 hours are 54.1 ± 0.39 % at 0.78 mg/mL and 26.2 ± 0.05 % corresponding to 3.13 mg/mL, respectively.

After analysis of **Figures 4.16** and **4.17**, it may be deduced that after all the incubation periods (24-, 48- and 72-hours), FEO-treated A549 cells demonstrated a decreasing trend in cell viability as the incubation prolonged. In terms of their relation to the concentration, FEO-treated A549 cell viability decreased with increasing concentration. Meanwhile, A549 cells treated with doxorubicin hydrochloride (positive control) showed an increasing trend in cell viability in response to an increasing concentration.

The FEO showed no significant difference, while doxorubicin hydrochloride showed significant difference among the incubation periods ($P < 0.05$) based on one-way ANOVA. The IC_{50} values were determined by graphical interpolation via GraphPad Prism 9 and are tabulated in **Table 4.6.** FEO demonstrated increasing IC_{50} values upon increasing incubation with 76.04 ± 0.09 μg/mL to 191.10 ± 0.15 μg/mL and 358.50 ± 0.10 μg/mL. Meanwhile, doxorubicin hydrochloride exhibited fluctuating IC_{50} values from 0.45 ± 0.08 μg/mL to 1.01

± 0.08 µg/mL and 0.19 ± 0.10 µg/mL as the incubation period prolonged. The IC_{50} value is inversely proportional to the percentage cell viability of the sample.

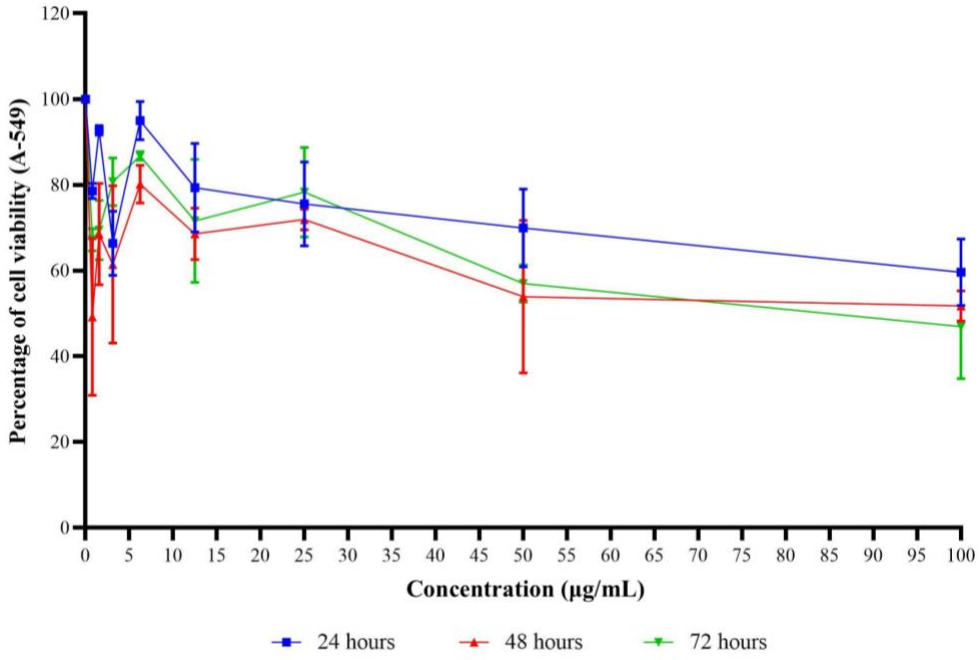

Figure 4.16: The percentage of cell viability of A549 cells versus concentration (µg/mL) of FEO at 24-, 48-and 72 hours incubation.

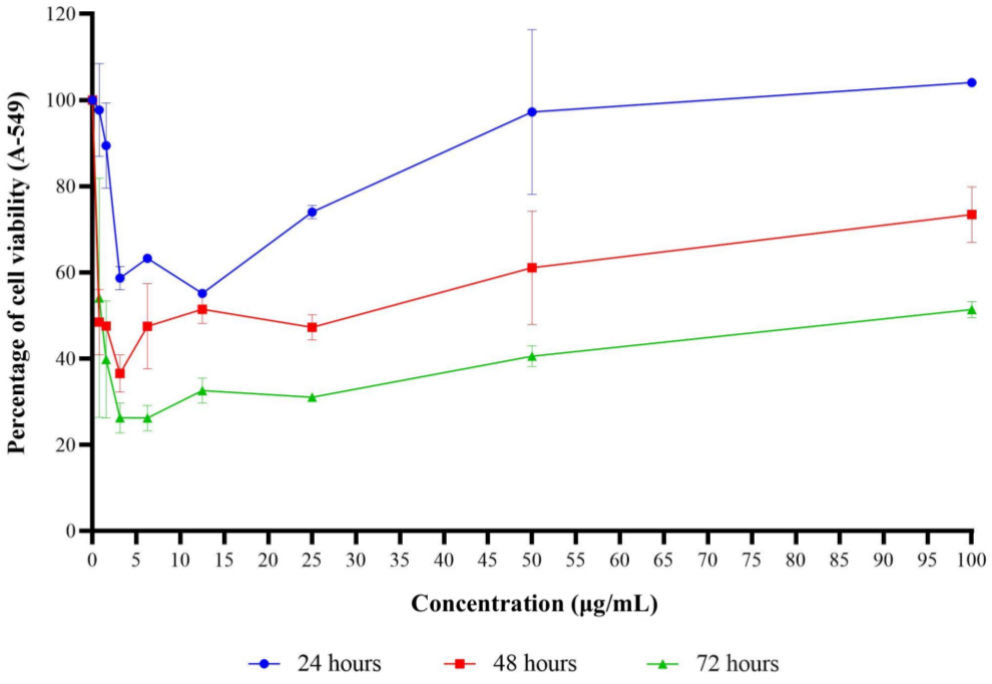

Figure 4.17: The percentage of cell viability of A549 cells versus concentration (µg/mL) of doxorubicin hydrochloride at 24-, 48-and 72 hours incubation.

Table 4.6: IC_{50} values of Frankincense essential oil and doxorubicin hydrochloride treatment after 24-, 48- and 72 hours against A549 cells.

Sample	IC_{50} (µg/mL)		
	24 hours	**48 hours**	**72 hours**
Frankincense Essential Oil	76.04 ± 0.09	191.10 ± 0.15	358.50 ± 0.10
Doxorubicin hydrochloride	0.45 ± 0.08	1.01 ± 0.08	0.19 ± 0.10

Data is represented as mean ± standard deviation (SD), n=2 ($P < 0.05$).

CHAPTER 5

DISCUSSION

5.1 Thin Layer Chromatography

Solvents with various degrees of polarity were utilised in the mobile phase of thin layer chromatography (TLC), notably hexane and ethyl acetate. According to Al-Harrasi, et al. (2013), Frankincense essential oil (FEO) comprises both polar and non-polar molecules. As a result, both polar and non-polar solvents that can separate different bioactive compounds must be utilised in the selection for mobile phase. The non-polar solvent is hexane, and the intermediate polar solvent is ethyl acetate. An aluminium plate covered in silica gel served as the stationary phase.

According to Vedantu (2023), the first solvent system in a chromatography should start with a non-polar solvent and increase the solvents' polarity slowly. In this study, only one spot was formed when using hexane alone to develop the chromatogram. This is because the non-polar molecule in the FEO has little attraction to the gel-coated aluminium plate hence the oil spends most of its time dissolved in the moving solvent. This is supported by the relatively high R_f values produced. The results obtained coincide with a study by Paul, et al. (2011), where they obtained a higher R_f value of the β-Carophyllene oxide, a non-polar compound found in the FEO.

After increasing the polarity of the solvent with the addition of ethyl acetate, varying spots were also separated because R_f values are affected by the varying ratios. R_f values ranges from 0 to 1, with 0 indicating that the solvent polarity is very low while 1 indicating a very high solvent (LibreTexts, 2023). A good separation occurs when one component of the FEO is insoluble in the hexane and ethyl acetate solvent mixture, while another component is freely soluble in it. Hence, the solvent mixtures that formed more spots are said to have a better separation than those that formed only one spot in the solvent containing hexane only. These results are supported by a study by Paul, et al., (2011) where the Frankincense resin compounds were found to have better separation as they increased the polarity of the solvents.

FEO can be inferred to have secondary metabolites that are non-polar in nature because the hexane-ethyl acetate mixture at 10:3 ratio obtained the greatest number of spots formed and widest range of R_f values. Because the solvent mixture contains a higher volume of hexane (non-polar) than ethyl acetate (intermediate polar), the compounds have less attraction towards the stationary phase. This inference is supported when no spots were formed when only ethyl acetate was used as the solvent (data not shown), hence it can be inferred that the FEO contains more non-polar molecules than polar.

The almost similar range of R_f values at ratios 1:1 and 10:1 correspond to their somewhat identical respective TLC profiles in terms of the location and number of spots. This can be explained by the polarity of the compounds and the solvent polarity. The FEO may consist of more non-polar compounds thus the

increasing polarity in the solvent had no effect. This can be supported by the fact that hexane: ethyl acetate of ratios 1:1 and 10:1 only had a small difference in their R_f values which indicates a poor separation. According to Columbia University (2007), the difference in R_f values between two spots is used as a measure of the performance of the separation. The bigger the difference, the better the separation.

The solvent system with the broadest range of R_f values was at ratio 10:3. Ethyl acetate isolates intermediate polar molecules such as phenols, tannins, flavonoid, saponin, alkaloids and glycosides, while hexane isolates non-polar compounds like coumarin, terpenoids and quinones (Kusuma, et al., 2022; Moreira, et al., 2014). Since FEO comprises chemicals with varying polarities, thus the hexane and ethyl acetate mixture at ratio 10:3 results in the FEO's highest attraction for the silica gel. The hexane only solvent system, on the other hand, produced the lowest R_f value, indicating that it had the least affinity for silica gel.

The ability of compounds to form hydrogen bonds and the polarity of the sample and solvent play a big role in the separation of compounds. The University of Wisconsin (2023) asserts that the polarity of solvents increases their ability to elute. Polar substances move up slowly or not at all with lower R_f value, whereas non-polar compounds move up faster with greater R_f value. This is because polar molecules move more slowly as they have a larger affinity for the stationary phase. Conversely, non-polar substances will move more quickly than polar substances, resulting in broader R_f values.

One of the spots formed in the 10:3 ratio is said to have the strongest hydrogen bond formation with the silica gel because it travelled the shortest distance with an R_f value of only 0.48, while the compounds with the least or no hydrogen-donor capabilities are also present in the same solvent system because they travelled the furthest, with an R_f value of 0.90. Compounds that are capable of donating a hydrogen bond will adsorb to the stationary phase more strongly than similar compounds with no hydrogen-donor capabilities (University of California, 2018). A substance that can form a hydrogen bond will adhere to the silica gel's surface more firmly than a substance that can only form weaker interactions. The compound briefly stops running while adhering to the silica gel, while the solvent continues to function without it. This means that the distance a compound can go up the plate depends on how firmly it is adsorbed (Clark, 2020).

Only the presence of polar and non-polar molecules may be determined from a TLC analysis while the precise chemicals present cannot be identified. To determine the presence of secondary metabolites in the Frankincense essential oil, a qualitative phytochemical study was then carried out.

5.2 Phytochemical Analysis

The phytochemical analysis of FEO was conducted to identify the major chemical constituent groups present using colour reactions, hence it can only be used to determine a substance's presence or absence on a qualitative level. **Table 4.2** shows that seven out of nine tests for secondary metabolites were successful in detecting those substances. Phenols, tannins, coumarin, flavonoids, saponin,

alkaloids, and glycosides are reported to be in the FEO, however terpenoids and quinones were not found.

Harmon and Patel (2014) stated that the flavonoids in FEO can prevent MCF-7 breast cancer cells from acquiring glucose by blocking the phosphoinositide-3-kinase (PI3K) pathway, which controls the glucose transporter-4 (GLUT-4). In fact, phenols are also said to be an inhibitor of GLUT4 which in turn reduces tumour progression. In another study by Mouhid, et al. (2017), flavonoids have been reported to target the AKT1 (protein kinase B), which is a downstream target of the PI3K pathway, thereby hindering uncontrolled cancer cell proliferation.

Robati, et al. (2008) stated that the alkaloids present in Frankincense can inhibit the hypoxia-inducible factor 1 (HIF1) in cervical cancer, which is a key transcription factor for controlling the growth of cancer. According to Lee, et al. (2014), Frankincense works by inhibiting HIF1-induced genes by decreasing the expression of HIF1. Additionally, tannins can activate the cancer-causing enzyme AMP-activated protein kinase (AMPK), which inhibits the growth of the PC-3 prostate cancer cell line and the MCF-7 breast cancer cell line. Tannins have also been shown to interfere with cyclin-dependent kinases (CDKs), which stop the progression of the cell cycle by targeting P53, P21, cyclin D, and cyclin A. This stops the growth of cancer (Liu, et al., 2014b). Frankincense resins cause cancer cells to express more death reporters which has an anticancer effect. As an example, phenols, saponin, flavonoids, tannins, quinones and glycosides were found to induce apoptosis via upregulation of TNF-α and has

been reported to induce apoptosis in ovarian cancer cells by increasing tumour necrosis factor related apoptosis-inducing ligands (TRAIL) (Liu, et al., 2014). In HeLa cells, these metabolites can increase the levels of death receptors and death receptor downstream elements for example fas ligand (FASL), TRAIL and fas-associated death domain (FADD) proteins (Ham, et al., 2014).

Several of these metabolites present in Frankincense have also been reported to initiate apoptosis in cancer cells by mitochondrial perturbations (Cincin, et al., 2015). For instance, the saponin in the gum resins of the *B. sacra* tree triggers apoptosis by lowering mitochondrial potential and raising permeability while having no impact on proliferating healthy cells. Through a reduction in the potential of the mitochondrial membrane, coumarin can also cause apoptosis in the DLD-1 colon cancer cell line. According to Wang, et al. (2011), the secondary metabolites present in Frankincense inhibits angiogenesis in cancer cells. They are reported to suppress the aminopeptidase-N enzyme, which controls angiogenesis and is overexpressed in a few malignancies (Fulda, 2008).

Flavonoids are also claimed to combat lung cancer by regulating redox homeostasis, upregulating apoptosis, arresting cell cycle progression, autophagy, reducing cell proliferation and invasiveness, maintaining inflammation response, downregulating anti-apoptotic factors, and targeting lung cancer signalling pathways, according to a study by Nath, et al. (2023). When used to treat lung cancer, flavonoids can work independently or synergistically with other therapies.

5.3 DPPH Assay

Reactive oxygen species (ROS) are a type of oxygen-containing unstable molecules that readily interact with other molecules in a cell. DNA, RNA, and proteins may become damaged in cells when there is an accumulation of ROS. Free radicals can harm cells, particularly DNA, which may contribute to the growth of cancer. Free radicals and reactive oxygen species are similar terms (National Cancer Institute, 2010; National Cancer Institute, 2019). Oxidative stress results when the body's defences against free radicals are overpowered. Free radicals and antioxidants must coexist in balance for appropriate physiological function (Lobo, et al., 2010). In order to stop free radicals from harming cells, antioxidants can interact and neutralise them. 'Free radical scavengers' is another name for antioxidants (National Cancer Institute, 2010). According to research by Al-Yasiry and Kiczorowska (2016) and Yang, et al. (2010), FEO exhibits antioxidant activities as it has the ability to scavenge free radicals. These properties make Frankincense important as a treatment for a variety of diseases brought on by oxidative stress. The antioxidant activity of the FEO is correlated with the presence of phytochemical substances.

Two concentrations of FEO were prepared which are the v/v (%) and w/v (mg/mL) concentrations. Both concentrations were made to compare the concentrations at which the minimum radical scavenging activity is shown by the FEO. This is because radical scavenging activity corresponds to a dose-dependent relationship. Theoretically, free radical scavenging activity also increased with increasing concentration.

According to Holford (2017), the concentration effective in producing 50% of the maximal response is said to be a good pharmacodynamic property for a drug. Hence, after analysing **Table 4.3**, only some of the v/v concentrations of FEO is said to have a good radical scavenging activity while the w/v concentrations were not exhibiting the desired percentage of radical scavenging activity This is evidenced by the fact that none of the radical scavenging activities (%) displayed in the w/v concentration of FEO were greater than 50%, indicating that it did not meet the minimum criteria for exhibiting good pharmacodynamic properties. However, v/v concentrations of 6.25 to 50.00% presented a radical scavenging activity of more than 50% thus, it can be deduced that the FEO (v/v) possesses its antioxidant activity at a minimum concentration of 6.25%. This is supported by a study from Yang, et al. (2010), in which they also utilised a purchased FEO that was extracted by steam distillation. The study stated that the FEO exhibited a relatively low scavenging activity of 26.6% maximum at various concentrations.

Therefore, the findings for the low percentage of radical scavenging activity from this study may be due to the fact that this FEO was purchased from Plant Therapy Essential Oils Corporate. Thus, the method of how the FEO was extracted from the *B. serrata* tree and the distillation method used to process them into essential oil cannot be confirmed. The total antioxidant substances that can successfully be extracted out of their source is highly influenced by the composition of the extracting solvent, various chemical properties, and polarity of various antioxidant substances which might or might not be able to dissolve in a specific solvent (Sultana, Anwar and Ashraf, 2009). Another reason for the

low radical scavenging activity in FEO (w/v) may be because the w/v concentration was measured and diluted hence the secondary metabolites were diluted. Meanwhile, the v/v concentration of FEO was taken directly from the stock solution.

According to **Figure 4.11**, the concentration of FEO is proportional to the percentage of radical scavenging activity, indicating that the FEO demonstrates antioxidant activity in a dose-dependent manner. This is because at low concentration, the bioactive compounds exhibiting antioxidant activity were present in minimal amounts and were diluted. The scavenging level of the essential oil is indicated by the degree of discoloration of the DPPH solution.

Because phenolic substances like flavonoids and tannins have an abundance of hydroxyl groups, which are efficient hydrogen donors and can rapidly oxidise ROS, they are always thought to have significant antioxidant properties. In a termination reaction, flavonoids and tannins play a key role as significant free radical scavengers, preventing the generation of new ROS (Aminudin, et al., 2015; Brewer, 2011). Furthermore, it has been proposed that phenolic chemicals that block ROS and scavenges free radicals, contribute to antioxidant activity (Shoeb, Madkour and Refahy, 2014). From the phytochemical analysis, FEO confirmed the presence of phenols and tannins.

The determination of the FEO concentration required to produce 50% of the activity of free radical scavengers (EC_{50}) is another crucial component of this test. According to Sowndhararajan and Kang (2013), a lower EC_{50} value

suggests a stronger antioxidant activity. Ascorbic acid had the lowest EC_{50} value of 4.60 ± 0.02 mg/mL, followed by FEO (v/v) at 4.73 ± 0.01 %, and FEO (w/v) at 28.95 ± 0.37 mg/mL, according to the results summarised in **Table 4.4**. The samples' reactive metabolites played a role in the antioxidant activity.

In this investigation, ascorbic acid was used as the positive control. From **Table 4.3**, ascorbic acid presented an excellent antioxidant activity which is supported by its high radical scavenging activity percentage, ranging from 79.27 ± 0.04 to 84.49 ± 0.01 %. It scavenges free radicals directly in the aqueous phases of cells and the circulatory system, which is one of their crucial responsibilities in acting as an antioxidant to preserve cellular components from damage (Chambial, et al., 2013). By providing electrons to the α-tocopherol radical, ascorbic acid interacts with the plasma membrane and becomes the ascorbate radical. Ascorbate's recycling of α-tocopherol aids in preventing peroxidation of membrane lipids (Kurutas, 2016).

FEO have been found to have a significant ROS-scavenging activity, which is associated with cytotoxicity toward cancer cells, and thus could be used as therapeutic and preventive agent (Sammar, et al., 2019). According to Liou and Storz (2010), high levels of ROS in the body of an individual encourage the growth and spread of tumours. As a result, substances with strong antioxidant qualities can inhibit the proliferation of cancer cells. Therefore, the FEO that showed higher antioxidant activity with lower EC_{50} values may have a greater cytotoxic effect in this study.

5.4 MTT Assay

Cells can be affected by toxic substances whether it be through cytotoxicity or cytostatic action. Drugs known as cytotoxic agents causes cell death and eventually cause tumours to shrink, whereas cytostatic medications only limit tumour growth indirectly (Kummar, et al., 2006). Therefore, it is difficult to clearly distinguish between cytotoxic and cytostatic drugs. This is because protracted cell cycle arrests in stages other than G_0 generated by cytostatic substances would ultimately result in cellular death, while cytotoxic drugs can cause stasis at low dosages or in apoptosis-resistant cells (Anttila, et al., 2019).

In this assay, two types of cells were used namely the Vero cells (CCL-81) and A549 cells (CCL-185) which are the normal and lung cancer cells, respectively. The goal of this assay is to observe whether the FEO can selectively kill cancer cells but not normal cells. This is in line with the goal of targeted therapy in which the genes and proteins that help cancer cells to survive and grow are targeted (Patel, 2022). It is also the foundation of precision medicine and is thought to be the ideal cancer drug (National Cancer Institute, 2022). Because of their targeted action, these drugs have an effect on the cancer cells and mostly leave normal, healthy cells alone. Targeted drugs often work by blocking cancer cells from copying themselves. This means they can help stop a cancer cell from dividing and making new cancer cells (American Cancer Society, 2021). In accordance with Wilson (2023), there are evidence that suggests Frankincense can target cancer cells without harming healthy cells. FEO have been shown to possess cancer cell's targeting activity and can increase the efficacy of commonly used chemotherapy drugs including paclitaxel and docetaxel, having

also shown pro-immune functions when administered to the cancer patient (Blowman, et al., 2018).

Three incubation durations of treatment were applied to the cell lines. According to Katzung, Masters and Trevor (2012), these incubation times were chosen from a pharmacological perspective since membrane permeability has a significant impact on a drug's effectiveness in terms of absorption, metabolism, distribution, and elimination. The size and degree of hydrophobicity in these compounds affect how quickly they diffuse. As a result, polar molecules are less permeable across the phospholipid bilayer while hydrophobic or nonpolar chemicals can easily pass through it (Fulton, 2019). According to the TLC analysis, FEO does indeed include polar and non-polar molecules. The three most significant routes for medications to cross cell membranes are (1) through channels or pores, (2) via a transport mechanism, and (3) through direct membrane penetration (Kell and Oliver, 2014). Each secondary metabolite found in FEO acts in a unique manner and at a different pace. A broad variety of incubation times may therefore provide the metabolites enough time to manifest their effects. This shows that the FEO acts in a time-dependent manner.

Figure 4.13 depicts the rise in Vero cell viability from 24 to 48 hours incubation. Since cell viability increased with longer incubation period, it was clear that cell proliferation continued while the FEO was being fed to the cells. The addition of complete growth medium, which supplied the cells with nutrients and promoted their proliferation, allowed the cells to multiply. This shows that the normal Vero cells were not harmed by the FEO. This is in line

with the objective of targeted therapy, where the drug does not destroy any of the normal, healthy cells, according to the concept of an ideal cancer drug. Frank, et al. (2009) claims that FEO may distinguish between malignant and healthy cells and can reduce cancer cells viability. The Vero cells showed no dose-dependent suppression of cell viability. This is consistent with research by Dozmorov, et al. (2014) and Frank, et al. (2009), which found that when FEO was used to treat normal bladder human urothelial cells, it had no cytotoxic effects.

The IC_{50} value is the concentration that causes 50% growth inhibition. The cytotoxicity of a compound can be classified as high cytotoxic activity if IC_{50} < 20 μg/mL, moderate cytotoxic activity when IC_{50} ranged between 21 to 200 μg/mL, weak cytotoxic activity if IC_{50} ranged between 201 to 500 μg/mL, and no cytotoxic activity when IC_{50} > 500 μg/mL (National Cancer Institute, 2015). Based on these criteria, the FEO with IC_{50} value of 76.04 ± 0.04 μg/mL (24 hours), 191.10 ± 0.15 μg/mL (48 hours) and 358.50 ± 0.10 μg/mL (72 hours) shows moderate cytotoxicity at 24- and 48-hours but weak cytotoxicity as it reached 72 hours. Meanwhile, IC_{50} values of doxorubicin at 0.45 ± 0.08 μg/mL (24 hours), 1.01 ± 0.08 μg/mL (48 hours) and 0.19 ± 0.10 μg/mL (72 hours) was considered to have high cytotoxic activity. The lower the IC_{50} value, the more cytotoxic is a substance.

Based on **Figure 4.16,** it is observed that the A549 cell viability decreases with FEO. At the higher range of concentrations and longer incubation period, the cell viability has reduced at least half which corresponds to the criteria by

National Cancer Institute which stated at least 50% of cell viability must be inhibited to be considered a good cytotoxic agent.

The results of the presence of cytotoxic effect in FEO is in accordance with the previous evaluation by Dozmorov, et al. (2014). However, their study inferred that the FEO showed high cytotoxic effects. From their study, at high concentrations, no viable cells were detected as the FEO significantly suppressed the viability of cancer cell. The cancer cells were more sensitive than the normal healthy cells to FEO-suppressed cell viability. The IC_{50} obtained by the study were 1:1250 and 1:600 dilutions (v/v) for cancer cells and normal healthy cells, respectively; and this corresponds to the criteria by National Cancer Institute to be classified as having a high cytotoxic activity.

There is another research by Frank, et al. (2009) which is in line to the inference obtained in this study in which FEO showed cytotoxic effect. They derived that the human bladder cancer J82 cells underwent significant morphological changes such as detaching from the tissue culture plates and cell shrinking as early as 3 hours following exposure to FEO. At 24 hours, the cancer cells had already completely detached from the tissue culture plates. This observation signifies that the FEO indeed has cytotoxic activity. The cancer cells responded to FEO treatment in a dose-dependent manner where the cancer cells viability decreased when treated with increasing concentrations of FEO which agrees to the study by Al-Yasiry and Kiczorowska (2016) and Abd-Rabou and Edris (2022).

One reason that the FEO had moderate and weak cytotoxic effect instead of a high cytotoxic effect as per the previous studies may be because the FEO was first diluted in DMSO to create the stock solution. Therefore, the moderate and weak cytotoxicity inferred from the IC_{50} values may be due to the FEO being highly diluted that it was unable to display the metabolic activity that is thought to have led to its high cytotoxic effect.

In addition, the antagonistic interactions of various secondary metabolites in FEO, which caused them to operate differently, may be another explanation for the moderate and low cytotoxic effect. According to Milugo, et al. (2013), the phytochemical interactions in the FEO led to a reduction in biological effects instead of elevating them. The FEO did not have any effects in some cases, but their pure constituents, for example resins, were found to be effective. This may be because the FEO contains certain compounds that may operate to hinder the activity of other substances. As a result, these antagonistic interactions will lessen the extracts' cytotoxic effect (Mazza, Shi and Le Maguer, 2002; Schinor, et al., 2007).

Doxorubicin hydrochloride served as the study's positive control. As presented in **Figures 4.14** and **4.17** in Vero and A549 cells, respectively. The cell viability after doxorubicin treatment were found to be increasing in Vero cells and decreasing in A549 cancer cells. According to Sadeghi-Aliabadi, Minaiyan and Dabestan (2010), this indicates that the doxorubicin was specific in killing cancer cells cancer and not normal cells as it has the ability to differentiate the cancer and healthy cells apart. Doxorubicin hydrochloride is often utilised in

cancer therapy with a broad spectrum intercalating anticancer drug produced from *Streptomyces peucetius*. Doxorubicin hydrochloride expresses toxicity on malignant cells by means of integrating into DNA, disrupting topoisomerase-II-mediated DNA repair, and producing free radicals. Cell death is the result of each of these pathways. Moreover, it raises the production of free radicals, which destroys cellular macromolecules by oxidation (Nikerel, et al., 2018).

5.5 Limitations of the study

In DPPH assay, improper pipetting and dilution technique in the 96-well plate may have given an inaccurate and inconsistent absorbance reading for the assay. Hence, to obtain a reliable and significant result, the assays were repeated for at least thrice and the average of the absorbance was calculated.

In MTT assay, Vero cells were used, which are kidney cell line isolated from African Green Monkey. Therefore, it may not represent a good model of human cell lines for toxicity testing.

5.6 Future Studies

Further research is required to examine the bioactive components found in Frankincense essential oil in greater depth. Using methods like high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), certain elements of Frankincense essential oil can be separated and purified from mixtures of other substances. Moreover, the isolated pure bioactive chemicals' structures can be elucidated using nuclear magnetic resonance (NMR) (Sasidharan, et al., 2011).

In addition, positive controls for the respective phytochemical analysis tests can be done as a qualitative reference in comparison with the sample. This helps in a good decision and comparison of colour and observations with the expected result.

Single tests make it exceedingly difficult to identify the potential activity demonstrated by Frankincense essential oil due to the different paths and activities associated with anticancer and antioxidant activities. As a result, other bioassays can be carried out to achieve more accurate results. Antioxidant assays like the Trolox equivalent antioxidant capacity, ferric reducing antioxidant power assay, total radical trapping antioxidant parameter approach and nitric oxide scavenging activity can be carried out to deeply assess the antioxidant activity of FEO (Prior, Wu and Schaich, 2005). To further evaluate the cytotoxicity of the essential oil against cancer cells, *in vitro* procedures such the neutral red assay, protease viability marker assay, DNA fragmentation assay, and ATP assay may be performed (Riss, et al., 2016).

Cytotoxicity of Frankincense essential oil were analysed with only one type of cancer cell line which is the A549 cell line. More anchorage-dependent and anchorage-independent cell lines should be investigated in order to get a better understanding of the anticancer activity. The stock solution and working solution were prepared with a w/v concentration of the Frankincense essential oil that was too low for the MTT assay. A greater w/v concentration can be prepared, or a v/v concentration can be employed to get better results.

CHAPTER 6

CONCLUSIONS

The mixture of hexane and ethyl acetate at ratio 10:3 showed the widest range of retention factor (R_f) values and formation of four spots which indicates the presence of polar and non-polar bioactive chemicals in the Frankincense essential oil (FEO). The phytochemical analysis of FEO revealed the presence of phenols, tannin, coumarin, flavonoids, saponin, alkaloids, and glycosides.

In DPPH assay, FEO (v/v) and ascorbic acid showed the higher radical scavenging activity of 78.47 ± 0.08% while ascorbic acid displayed a radical scavenging activity of 84.49 ± 0.01%. Meanwhile, the EC_{50} value of FEO (v/v) and ascorbic acid were 4.73 ± 0.01% and 4.60 ± 0.02 mg/mL, respectively.

The FEO did not display cytotoxicity against Vero cells. However, FEO presented reduction towards the A549 cancer cells as it showed a decrease in A549 cell viability for all incubation periods from 24 to 72 hours. At the end of the incubation period, FEO showed the highest IC_{50} value at 358.50 ± 0.10 μg/mL and doxorubicin displayed the lowest IC_{50} value of 0.19 ± 0.10 μg/mL.

In a nutshell, FEO has a potential as an antioxidant with low toxicity to normal cells hence, further analysis on the FEO should be carried out. Further investigations should be conducted using pure compounds isolated from the tree to further elucidate its capability as pharmaceutical drugs.

REFERENCES

Al-Harrasi, A., Ali, L., Ceniviva, E., Al-Rawahi, A., Hussain, J., Hussan, H., Rehman, N. U., Abbas, G. and Al-Harassi, R., 2013. Antiglycation and antioxidant activities and HPTLC analysis of *Boswellia sacra* oleogum resin: the sacred frankincense. *Tropical Journal of Pharmaceutical Research*, 12(4), pp. 597-602.

Abd-Rabou, A. A. and Edris, A. E., 2022. Frankincense essential oil nanoemulsion specifically induces lung cancer apoptosis and inhibits survival pathways. *Cancer Nanotechnology*, 13(22), pp. 1-24.

Adams, T., Anwar, R., Mfarej, M., Rundatz, T., Coyle, M. and McLaughlin, J. S., 2015. Nutritional stress of cultured Vero cells causes altered and morphology as seen in neoplastic transformation. *American Journal of Undergraduate Research*, 12(3), pp. 63-75.

Al-Yasiry, A. R. M. and Kiczorowska, B., 2016. Frankincense-therapeutic properties. *Biology and Animal Breeding*, 13(70), pp. 380-391.

Aloitaibi, S. H., 2019. Biophysical properties and dinger print of *Boswellia* sp. Burseraceae. *Saudi Journal of Biological Sciences*, 26(1), pp. 1450-1457.

Altogen Biosystems, 2023. *A549 cell line transfection protocol.* [online] Available at: <https://a549.com> [Accessed 25 January 2023].

American Cancer Society, 2021. *How targeted therapies are used to treat cancer*. [online] Available at: <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/targeted-therapy/what-is.html> [Accessed 27 March 2023].

Ammerman, N. C., Beier-Sexton, M. and Azad, A. F., 2009. Growth and maintenance of Vero cell lines. *Current Protocols in Microbiology*, 4(10), pp. 1-10.

American Type Culture Collection, 2020. *Vero*. [online] Available at: <https://www.atcc.org/products/ccl-81> [Accessed 17 January 2023].

American Type Culture Collection, 2021. *A549*. [online] Available at: <https://www.atcc.org/products/ccl-185> [Accessed 17 January 2023].

Aminudin, N. I., Ahmad, F., Taher, M. and Zulkifli, R. M., 2015. Cytotoxic and antibacterial evaluation of coumarins and chromanone. *Journal of Applied Pharmaceutical Science,* 6(1), pp. 23-27.

Anttila, J. V., Shubin, M., Cairns, J., Borse, F., Guo, Q., Mononen, T., Ignacio, V. G., Pulkkinen, O. and Mustonen, V., 2019. Contrasting the impact of cytotoxic and cytostatic drug therapies on tumour progression. *PLOS Computational Biology*, 15(11), pp. 1-18.

Atanasov, A., Zotchev, S., Dirsch, V. M. and Supuran, C. T., 2021. Natural products in drug discovery: advances and opportunities. *Journal of Drug Discovery*, 20(1), pp. 200-216.

Bairwa, K. and Jachak, S. M., 2016. Nanoparticle formulation of 11-keto-beta-boswellic acid (KBA): anti-inflammatory activity and *in vivo* pharmacokinetics. *Pharmacologic Biology,* 54(1), pp. 2909-2916.

Bele, A. A. and Khale, A., 2011. An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*, 2(2), pp. 256-267.

Bernardini, S., Tiezzi, A. Laghezza, M. and Ovidi, E., 2018. Natural products for human health: a historical overview of the drug discovery approaches. *Journal of Natural Product Research,* 32(16), pp. 1926-1950.

Blowman, K., Magalhaes, M., Lemos, M. F. L., Cabral, C. and Pires, I. M., 2018. Anticancer properties of essential oils and other natural products. *Evidence Based Complement Alternative Medicine*, 31(9), pp. 1-45.

Brewer, M., 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety,* 10(4), pp. 221-247.

Cao, B., Wei, X., Xu, X., Zhang, H., Luo, C., Feng, B., Xu, R., Zhao, S., Du, X., Han, L. and Zhang, D., 2019. Seeing the unseen of the combination of two natural resins, frankincense and myrrh: changes in chemical constituents and pharmacological activities. *Molecules*, 24(17), pp. 1-27.

Catanzaro, D., Rancan, S., Orso, G., Dall'Acqua, S., Brun, P., Giron, M. C. Carrara, M., Castagliuolo, I., Ragazzi, E. and Caparrotta, L. J. *Boswellia serrata* preserves intestinal epithelial barrier from oxidative and inflammatory damage. *Collection Personalise Medicine,* 10(5), pp. 1-27.

Centers for Disease Control and Prevention, 2022. *Side effects of cancer treatment.* [online] Available at: <https://www.cdc.gov/cancer/survivors /patients/side-effects-of treatment.htm> [Accessed 10 January 2023].

Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J. and Sharma, P., 2013. Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry,* 28(4), pp. 314-328.

Chen, Z., Bertin, R. and Froldi, G., 2013. EC_{50} estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chemistry*, 138(1), pp. 414-420.

Cincin, Z. B., Unlu, M., Kiran, B., Bireller, E. S., Baran, Y. and Cakmakoglu, B., 2015. Apoptosis effects of quercetin on DLD-1 colon cancer cell. *Pathology Oncolology*, 21(2), pp. 333-338.

Clark, J., 2020. *Thin-layer chromatography*. [online] Available at: <https://chem.libretexts.org/Courses/Providence_College/CHM_331_Advanced_Analytical_Chemistry_1/14%3A_Liquid_Chromatography/14.06%3A_Thin-Layer_Chromatography> [Accessed 14 March 2023].

Collins, A. R., 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. *Molecular Biotechnology*, 26(3), pp. 249-261.

Columbia University, 2007. *Chromatography*. [online] Available at: <http://www.columbia.edu/cu/chemistry/ugrad/hssp/EXP_8.html> [Accessed 14 March 2023].

Davison, S. C., Bongers, F. and Philips, D., 2022. The future of frankincense: understanding the plant's diversity is key to its conversation. *Herbalgram*, 133(1), pp. 40-59.

Debela, T. D., Muzazu, S. G. Y., Heraro, K. D., Maureen, T. N., Mesele, B. W., Haile, D. C., Kitui, S. K. and Manyazewal, T., 2021. New approaches and procedures for cancer treatments: current perspectives. *SAGE Open Medicine*, 9(1), pp. 1-10.

Dias, D. A., Urban, S. and Roessner, U., 2012. A historical overview of natural products in drug discovery. *Metabolites*, 2(1), pp. 303-336.

Dozmorov, M. G., Yang, Q., Wu, W., Wren, J., Suhail, M. M., Woolley, C. L., Young, D. G., Fung, K. M. and Lin, H. K., 2014. Differential effects of selective frankincense (Ru Xiang) essential oil versus non-selective sandalwood (Tan Xiang) essential oil on cultured bladder cancer cells: a microarray and bioinformatics study. *Chinese Medicine*, 9(18), pp. 1-19.

Efferth, T. and Oesch, F., 2020. Anti-inflammatory and anti-cancer activities of frankincense: targets, treatments, and toxicities. *Seminars in Cancer Biology*, 8(1), pp. 39-57.

Efiong, E. E., Akumba, L. P., Chukwu, E. C., Olusesan, A. I. and Obochi, G., 2019. Comparative qualitative phytochemical analysis of oil, juice and dry forms of garlic (*Allium sativum*) and different varieties of onions (*Allium cepa*) consumed in Makurdi metropolis. *International Journal of Plant Physiology and Biochemistry*, 12(1), pp. 9-16.

Encyclopaedia of Living Forms, 2005. *Boswellia sacra flueck.* [online] Available at: <http://www.llifle.com/Encyclopedia/TREES/Family/Burseraceae/12199/Boswellia_sacra> [Accessed 20 January 2023].

Engeland, K., 2018. Cell cycle arrest through indirect transcriptional repression by p53. *Cell Death Differentiation*, 25(1), pp. 114-132.

Fobar, R. 2019, *Frankincense trees of biblical lore are being tapped out for essential oils.* [online] Available at: <https://www.nationalgeographic .com/animals/article/frankincense-trees- declining-overtapping> [Accessed 20 January 2023].

Forcellese, M. L., Nicoletti, R. and Petrossi, U., 2017. The structure of isoincensole- oxide. *Tetrahedron*, 28(1), pp. 325-331.

Foster, K. A., Oster, C. G., Mayer, M. M., Avery, M. L. and Audus, K. L., 2018. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. *Experimental Cell Research*, 243(2), pp. 359-366.

Frank, M. B., Yang, Q., Osban, J., Azzarello, J. T., Saban, M. R., Saban, R., Ashley, R. A., Welter, J. C., Fung, K. M. and Lin, H. K., 2009. Frankincense oil derived from *Boswellia carteri* induces tumor cell specific cytotoxicity. *BMC Complementary and Alternative Medicine*, 9(6), pp. 1-39.

Fuentes, M., 2014. *Hemocytometer: squares to count.* [online] Available at: <https://www.hemocytometer.org/hemocytometer-protocol/hemocytometer-squares-to-count/> [Accessed 8 February 2023].

Fulda, S., 2008. Betulinic acid for cancer treatment and prevention. *International Journal of Molecular Science*, 9(6), pp. 1096-1107.

Fulton, T. B., 2019. *Diffusion and transport across cell membranes*. [online] Available at: <http://biochemistry2.ucsf.edu/programs/ptf/prologue %20links /Diff%20& % 20Trans%20Membranes.pdf> [Accessed 28 March 2023].

Ghasemi, M., Turnbull, T., Sebastain, S. and Kempson, I., 2021. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *International Journal of Molecular Sciences*, 22(12827), pp. 1-30.

Global Cancer Observatory, 2021. *Malaysia.* [online] Available at: <https://gco.iarc.fr/today/data/factsheets/populations/458-malaysia-fact-sheets.pdf> [Accessed 25 January 2023].

Gutschner, T. and Diederichs, S., 2012. The hallmarks of cancer. *RNA biology*, 9(6), pp. 703-719.

Hakkim, F. L., Bakshi, H. A., Khan, S., Nasef, M., Farzand, R., Sam, S., Rashan, L., Al-Baloshi, M. S., Hasson, S. S., Jabri, A. A., McCarron, P. A. and Tambuwala, M., 2019. Frankincense essential oil suppresses melanoma cancer through down regulation of bcl-2/bax cascade signaling and ameliorates heptotoxicity via phase I and II drug metabolizing enzymes. *Oncotarget*, 10(37), pp. 3472-3490.

Halcon, L., 2016. *What are essential oils?* [online] Available at: <https://www.takingcharge.csh.umn.edu/what-are-essential-oils> [Accessed 24 February 2023].

Ham, S., Kim, K. H., Kwon, T. H., Bak, Y., Lee, D. H., Song, Y. S., Park, S. H., Park, Y. S., Kim, M. S., Kang, J. W, Hong, J. T. and Yoon, D. Y., 2014. Luteolin induces intrinsic apoptosis via inhibition of E6/E7 oncogenes and activation of extrinsic and intrinsic signaling pathways in HPV-18-associated cells. *Oncology Reports*, 31(6), pp. 2683-2691.

Hamidpour, R., Hamidpour, S., Hamidpour, M. and Shahlari, M., 2013. Frankincense: from the selection of traditional applications to the novel phytotherapy for the prevention and treatment of serious diseases. *Journal of Traditional and Complementary Medicine*, 3(4), pp. 221-226.

Harmon, A. W. and Patel, Y. M., 2004. Naringenin inhibits glucose uptake in MCF-7 breast cancer cells: a mechanism for impaired cellular proliferation. *Breast Cancer Respiratory Treatment*, 85(2), pp. 103-110.

Harshitha, B., Subhada, B., Mustafa, M., Solanki, H., Safiya, N. A. M. and Tiwari, R. V. C., 2019. DNA laderring to evaluate cytogenic damage in patients with periodontitis. *Journal of International Society of Preventive and Community Dentistry*, 9(5), pp. 486-491.

Health Jade, 2017. *Boswellia*. [online] Available at: <https://healthjade.net/boswellia/> [Accessed 21 April 2023].

Holford, N., 2017. Pharmacodynamics principles and the time course of immediate drug effects. *Translational and Clinical Pharmacology*, 25(4), pp. 157-161.

Horvath, G. and Acs, K., 2015. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action. *Flavour and Fragrance Journal*, 30(1), pp. 331-341.

Hussein, R. A. and El-Anssary, A., 2019. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal Medicine*, 1(1), pp. 1-10.

Integrated Taxonomic Information System, 2011, *Boswellia Roxb. ex Colebr.* [online] Available at: <https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search value=50074 8#null> [Accessed 18 January 2023].

Ismail, M. Ul-Haq, N., Ali, S., Ali, I., Shaheen, R., Murtaza, G. and Razaq, A., 2017. Qualitative analysis for phytochemicals of selected medicinal plants from Gilgit-Baltistan, Pakistan. *Asian Journal of Chemistry*, 29(9), pp. 1929-1932.

Katsares, V., Petsa, A., Felesakis, A., Paparidis, Z., Nikolaidou, E., Gargani, S., Karvounidou, I., Ardelean, K. A., Grigoriadis, N. and Grigoriadis, J., 2015. A rapid and accurate method for the stem cell viability evaluation: the case of the thawed umbilical cord blood. *Laboratory Medicine,* 40(9), pp. 557-560.

Katzung, B. G., Masters, S. B. and Trevor, A. J., 2012. *Basic and clinical pharmacology*. [e-book] San Francisco: McGraw-Hill. Available at: Google Books <https://pharmacomedicale.org/images/cnpm/CNPM_2016/katzung-pharmacology.pdf> [Accessed 28 March 2023].

Kell, D. B. and Oliver, S. G., 2014. How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. *Frontiers Pharmacology*, 5(231), pp. 1-109.

Kummar, S., Gutierrez, M., Doroshow, J. H. and Murgo, A. J., 2006. Drug development in oncology: classical cytotoxics and molecularly targeted agents. *British Journal of Clinical Pharmacology*, 62(1), pp. 15-26.

Kundu, J., Chun, K. S., Chae, I. G. and Kundu, J. K., 2014. Phloretin: an apple polyphenol with cancer chemo preventive potential. *Archives of Basic and Applied Medicine*, 2(1), pp. 17-23.

Kurutas, E. B., 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, 15(71), pp. 1-23.

Kusuma, S. B., Wulandari, S., Nurfitriani, R. A. and Awaludin, A., 2022. The potential solvent for tannin extraction as a feed additive made of coffee husk (*Coffea canephora*) using soxhlet method. *Journal of Food and Agriculture*, 980(1), pp. 1-12.

Lahlou, M., 2013. The success of natural products in drug discovery. *Pharmacology and Pharmacy*, 4(1), pp. 17-31.

Lee, S. H., Jee, J. G., Bae, J. S., Liu, K. H. and Lee, Y. M., 2014. A group of novel HIF-1 inhibitors, glyceollins, blocks HIF-1 synthesis and decreases its stability via inhibition of the PI3K/AKT/mTOR pathway and Hsp90 binding. *Journal of Cell Physiology*, 230(4), pp. 853-862.

Leah, M., 2015. *History of essential oils*. [online] Available at: <https://healingscents.net/blogs/learn/18685859-history-of-essential-oils> [Accessed 24 February 2023].

Leburmuth, 2019. *Essential oils: a journey through history*. [online] Available at: <https://blog.lebermuth.com/essential-oils-a-journey-through-history> [Accessed 24 February 2023].

Lemenih, M. and Teketay, D., 2003. Frankincense and myrrh resources of Ethiopia: II medicinal and industrial uses. *Ethiopian Journal of Science*, 26(2), pp. 161-172.

Liang, Y. H., Li, P., Zhao, J. X., Liu, X. and Huang, Q. F., 2010. Acetyl-11-keto-beta-boswellic acid and arsenic trioxide regulate the productions and activities of matrix metalloproteinases in human skin fibroblasts and human leukaemia cell line THP-1. *Journal of Chinese Integrative Medicine,* 8(1), pp. 1060-1069.

LibreTexts, 2023. *Thin layer chromatography*. [online] Available at: <https://chem.libretexts.org/Ancillary_Materials/Demos_Techniques_and_Experiments/General_Lab_Techniques/Thin_Layer_Chromatography> [Accessed 13 April 2023].

Liou, G. Y. and Storz, P., 2014. Reactive oxygen species in cancer. *Free Radical Research,* 44(5), pp. 1-32.

Liu, J., Zheng, L. H., Wu, N., Ma, L. N., Zhong, J. T., Liu, G. and Lin, X. K., 2014a. Oleanolic acid induces metabolic adaptation in cancer cells by activating the AMP-activated protein kinase pathway. *Journal of Agriculture Food Chemistry*, 62(1), pp. 5528-5537.

Liu, Y., Yang, Z., Gong, C., Zhang, Y., Yu, G. L. and Gong, W., 2014b. Quercetin enhances apoptotic effect of tumour necrosis factor related apoptosis-inducing ligand (TRAIL) in ovarian cancer cells through reactive oxygen species (ROS) mediated CCAAT enhancer binding protein homologous protein (CHOP)-death receptor 5 pathway. *Cancer Science*, 105(5), pp. 520-527.

Lobo, V., Patil, A., Phatak, A. and Chandra, N., 2010. Free radicals, antioxidants, and functional foods: impact on human health. *Pharmacognosy Review*, 4(8), pp. 118-126.

Mahavorasirikul, W., Viyanant, V., Chaijaroenkul, W., Itharat, A. and Na-Bangchang, K., 2010. Cytotoxic activity of Thai medicinal plants against human cholangiocarcinom, laryngeal and hepatocarcinoma cells *in vitro*. *BMC Complementary and Alternative Medicine*, 55(10), pp. 1-8.

Mastura, Y. and Ishak, W. Z. W., 2022. *Cancer in my community: addressing increasing cancer cases in Malaysia.* [online] Available at: <https://www.cancer.net/blog/2022-02/cancer-my-community-addressing-increasing-cancer-cases-malaysia> [Accessed 25 February 2023].

Mathur, G., Nain, S. and Sharma, P. K., 2015. Cancer: an overview. *Academic Journal of Cancer Research*, 8(1), pp. 1-9.

Mazza, G., Shi, J. and Le Maguer, M., 2002. *Functional foods*. [e-book] Boca Raton: Taylor & Francis Group. Available at: Taylor & Francis e-book <https://www.taylorfrancis.com/books/mono/10.1201/9781420012873/functional-foods-giuseppe-mazza-marc-le-maguer-john-shi> [Accessed 29 March 2023].

Milugo, T. K., Omosa, L. K., Ochanda, J. O., Owuor, B. O., Wamunyokoli, F. A., Oyugi, J. O. and Ochieng, J. W., 2013. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra sond*.): further evidence to support biotechnology in traditional medicinal plants. *BMC Complementary and Alternative Medicine*, 13(1), pp. 285-291.

Ministry of Health, 2019. *Malaysia national cancer registry report (MNCR) 2012-2016.* [pdf] Malaysia: National Cancer Institute. Available at: <https://www.moh.gov.my/moh/resources/Penerbitan/Laporan/Umum/2012-2016%20(MNCRR)/MNCR_2012-2016_FINAL_(PUBLISHED_2019).pdf > [Accessed 25 February 2023].

Mishra, K., Ojha, H. and Chaudhury, N. K., 2011. Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. *Food Chemistry*, 130(12), pp. 1036-1043.

Moon, J. K. and Shibamoto, T., 2009. Antioxidant assays for plant and food components. *Journal of Agriculture and Food Chemistry*, 57(5), pp. 1655-1666.

Mondal, J., Panigrahi, A. K. and Khuda-Buksh, A. R., 2014. *Journal of Molecular and Cellular Biology*, 1(5), pp. 1-10.

Moreira, L. M., Melo, M. M., Martins, P. A., Lyon, J. P., Romani, A. P., Codognoto, L., Santos, S. C. and Oliveira, H. P., 2014. Photophysical properties of coumarin compounds in neat and binary solvent mixtures: evaluation and correlation between solvatochromism and solvent polarity parameters. *Journal of Brazilian Chemical Society*, 25(5), pp. 1-8.

Morikawa, T., Matsuda, H. and Yoshikawa, M., 2017. A review of anti-inflammatory terpenoids from the incense gum resins frankincense and myrrh. *Journal of Oleo Science*, 66(1), pp. 805-814.

Mouhid, L., Corzo, M., Torres, C., Vazquex. L. and Reglero, G., 2017. Improving *in vivo* efficacy of bioactive molecules: an overview of potentially antitumour phytochemicals and currently available lipid-based delivery systems. *Journal of Oncology*, 1(12), pp. 73-85.

National Cancer Institute, 2010. *Antioxidants and cancer prevention*. [online] Available at: <https://www.cancer.gov/about-cancer/causes-prevention/risk/diet/antioxidants-fact-sheet> [Accessed 20 March 2023].

National Cancer Institute, 2015. *Special concentration parameters GI50, TGI and LC50*. [online] Available at: <https://dtp.cancer.gov/databases_tools/docs/compare/compare_methodology.htm> [Accessed 29 March 2023].

National Cancer Institute, 2019. *Reactive oxygen species*. [online] Available at: <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/reactive-oxygen-species> [Accessed 20 March 2023].

National Cancer Institute, 2021. *What is cancer?* [online] Available at: <https://www.cancer.gov/about-cancer/understanding/what-is-cancer> [Accessed 15 January 2023].

National Cancer Institute, 2022. *Targeted therapy to treat cancer*. [online] Available at: <https://www.cancer.gov/about-cancer/treatment/types/targeted-therapies> [Accessed 27 March 2023].

National Health Institute, 2023. Aromatherapy with essential oils. [online] Available at: <https://www.ncbi.nlm.nih.gov/books/NBK65874/> [Accessed 6 June 2023].

Nath, R., Das, C., Kityania, S., Nath, D., Das, S., Choudhury, M. D., Patra, J. K. and Talukdar, A. D., 2023. Natural flavonoids in the prevention and treatment of lung cancer: a pharmacological aspect. *Combinatorial Chemistry and High Throughput Screening*, 26(5), pp. 863-879.

Nikerel, H. T., Karabekmez, M. E., Eraslan, S. and Kırdar, B., 2018. Doxorubicin induces an extensive transcriptional and metabolic rewiring in yeast cells. *Scientific Reports*, 8(1), pp. 1-14.

Osada, N., Kohara, A., Yamaji, T., Hirayama, N., Kasai, F., Sekizuka, T., Kuroda, M. and Hanada, K., 2014. The genome landscape of the African green monkey kidney-derived Vero cell line. *DNA Research*, 21(6), pp. 673-683.

Pan, L., Chai, H. B. and Kinghorn, A. D., 2012. Discovery of new anticancer agents from higher plants. *Frontiers of Bioscience*, 4(1), pp. 142-156.

Patel, J., 2022. *What is targeted therapy?*. [online] Available at: <https://www.cancer.net/navigating-cancer-care/how-cancertreated/ persona lized-and-targeted-therapies/what-targeted-therapy> [Accessed 27 March 2023].

Paul, M., Brüning, G., Bergmann, J. and Jauch, J., 2011. A thin-layer chromatography method for the identification of three different olibanum resins (*Boswellia serrata, Boswellia papyrifera* and *Boswellia carterii*, respectively, *Boswellia sacra*). *Phytochemical Analysis*, 20(23), pp. 184-189.

Pistelli, L., Shortrede, J. E., Najar, B. and Buhagiar, J. A., 2019. Chemical composition and *in vitro* cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. *Chemistry and Biodiversity*, 17(1), pp. 1-10.

Plant Therapy, 2023. *Frankincense serrata essential oil*. [online] Available at: <https://www.planttherapy.com/frankincense-serrata-essential-oil> [Accessed 22 April 2023].

Prior, L. R., Wu, X. and Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), pp. 4290-4302.

Ramakrishna, W., Kumari, A., Rahman, N. and Mandave, P., 2021. Anticancer activities of plant secondary metabolites. *Rice Science*, 28(1), pp. 13-30.

Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J. and Minor, L., 2016. Cell viability assays. *National Library of Medicine*, 32(4), pp. 1-25.

Ríos, J. L., 2016. Essential oils. *Essential Oils in Food Preservation, Flavour and Safety*, 1(1), pp. 3-10.

Rocha, D. A., Lopes, R. M. and Schwartsmann, G., 2001. Natural products in anticancer therapy. *Current Opinion in Pharmacology,* 1(4), pp. 364-369.

Robati, M., Holtz, D. and Dunton, C. J., 2008. A review of topotecan in combination therapy for advanced cervical cancer. *Therapeutic Clinical Risk Management,* 4(1), pp. 213-218.

Row, K. H. and Lee, J. W., 2000. Determination of retention factors of phospholipids in gradient HPLC. *Chromatographia*, 51(1), pp. 61-64.

Sadeghi-Aliabadi, H., Minaiyan, M. and Dabestan, A., 2010. Cytotoxic evaluation of doxorubicin in combination with simvastatin against human cancer cells. *Research in Pharmaceutical Sciences*, 5(2), pp. 127-133.

Sagar, B. K. and Singh, R. P., 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science Technology*, 48(4), pp. 412-422.

Sammar, M., Abu-Farich, B., Rayan, I., Falah, M. and Anwar R., 2019. Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: *in vitro* evaluation of 57 medicinal and edible plant extracts. *Oncology Letters*, 18(6), pp. 6563-6571.

Sasidharan, S., Chen, Y., Saravanan, D. Sundram, K. M and Latha, L. Y., 2011. Extraction, isolation and characterization of bioactive compounds from plant's extracts. *African Journal of Traditional, Complementary and Alternative Medicine,* 8(1), pp. 1-10.

Schinor, E., Salvador, M., Ito, I. and Dias, D., 2007. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. *Brazilian Journal of Microbiology*, 38(1), pp. 145-149.

Sharma, M., Grewal, K., Jandrotia, R., Batish, D. R., Singh, H. P. and Kohli, R. K., 2021. Essential oils as anticancer agents: potential role in malignancies, drug delivery mechanisms, and immune system enhancement. *Biomedicine and Pharmacotherapy*, 146(1), pp. 1-36.

Sherma, J. and Fried, B., 2003. *Handbook of thin layer chromatography*. [e book] CRC Press. Available at: Google Books <https://books.google.com.my/books?hl=en&lr=&id=Qc79DwAAQBAJ&oi=fnd&pg=PP1&dq=thin+layer+chromatography+principle&ots=67FO3GA0oF&sig=vKp3LzugBNxZJMbRi4gdbO0HnM&redir_esc=y#v=onepage&q=thin%20layer% 20chromatography%20principle&f=false> [Accessed 26 January 2023].

Shoeb, H. A., Madkour, H. M. F. and Refahy, L. A., 2014. Antioxidant and cytotoxic activities of *Gmelina arborea* leaves. *British Journal of Pharmaceutical Research*, 4(1), pp. 125-144.

Siddiqui, M. Z., 2011. *Boswellia serrata*, a potential anti-inflammatory agent: an overview. *Indian Journal of Pharmacology Science*, 73(1), pp. 255-261.

Singh, H. P., Yadav, I. K., Chandra, D. and Jain, D. A., 2012. *In vitro* antioxidant and free radical scavenging activity of different extracts of *Boerhavia diffusa* and *Boswellia serrata. International Journal of Pharmacology Sciences and Research,* 3(1), pp. 503-511.

Soares, G. A., Bhattacharya, T., Chakrabarti, T., Tadge, P. and Cavalu, S., 2021. Exploring pharmacological mechanisms of essential oils on the central nervous system. *Plants*, 11(21), pp. 1-25.

Soliman, A. M., Majeed, R. R., Himyari, H. F., Dhanalekshmi, U. M. and Khan, S. A., 2020. *In vitro* cytotoxic, antioxidant and antimicrobial activities of alcoholic and chloroform extracts of frankincense. *Dhaka University Journal of Pharmaceutical Science*, 19(2), pp. 105-110.

Sowndhararajan, K. and Kang, S.C., 2013. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii*. *Saudi Journal of Biological Sciences*, 20(4), pp. 319-325.

SpectroUV, 2023. *CM series: Fluorescence analysis cabinet.* [online] Available at:<https://cdn.shopify.com/s/files/1/0560/5919/9536/files/CM_Series_Flyer_A1092023.pdf?v=11747739222831778051> [Accessed 4 February 2023].

Sultana, B., Anwar, F. and Ashraf, M., 2009. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14(6), pp. 2167-2180.

Synthego, 2023. *Everything you need to know about A549 cells.* [online] Available at: <https://www.synthego.com/a549-cells#overview> [Accessed 25 January 2023].

Teoh, E. S., 2016. Secondary metabolites of plants. *Medicinal Orchids of Asia*, 1(1), pp. 59-73.

The Editors of Encyclopaedia Britannica, 2022. *Frankincense.* [online] Available at: <https://www.britannica.com/topic/frankincense> [Accessed 18 January 2023].

Top Tropicals, 2023. *Boswellia sacra, Boswellia carteri, Boswellia undulato crenata, frankincense, olibanum tree.* [online] Available at: <https://toptropicals.com/catalog/uid/boswellia_sacra.htm> [Accessed 20 January 2023].

University of California, 2018. *TLC analysis of spinach pigments*. [online] Available at: <https://cpb-us-e1.wpmucdn.com/sites.ucsc.edu/dist /9/291/files /2015/11/Exp-3-TLC-1.pdf> [Accessed 14 March 2023].

University of California, 2022. *Different cytotoxicity assays*. [online] Available at: <https://chem.libretexts.org/Bookshelves/Environmental_Chemistry/ Toxicology_MSDT/03%3A_Principles_of_Genetic_Toxicology/3.04%3A_New_Page> [Accessed 22 April 2023].

University of Wisconsin, 2023. *Thin layer chromatography*. [online] Available at: <https://www2.chem.wisc.edu/deptfiles/OrgLab/handouts/CHEM%20344%20TLC%20info.pdf> [Accessed 14 March 2023].

Vedantu, 2023. *How does the solvent affect chromatography?* [online] Available at: <https://www.vedantu.com/question-answer/does-the-solvent-affect-chromatography-class-9-chemistry-cbse-5feafee007b310683149b974> [Accessed 9 April 2023].

Wang, F., Li. Z. L., Cui. H. H., Hua, H. M., Jing, Y. K., Liang, S. W., 2011. Two new triterpenoids from the resin of *Boswellia carterii*. *Journal of Asian National Product Research*, 13(1), pp. 193-197.

Wilson, D. R., 2023. *Does frankincense have fighting properties?* [online] Available at: <https://www.medicalnewstoday.com/articles/314366> [Accessed 27 March 2023].

World Cancer Research Fund International, 2020. *Worldwide cancer data.* [online]. Available at: <https://www.wcrf.org/cancer-trends/worldwide-cancer-data/> [Accessed 26 January 2022].

World Health Organization, 2020. *Malaysia fact sheet.* [online] Available at: <https://gco.iarc.fr/today/data/factsheets/populations/458-malaysia-fact-sheets.pdf> [Accessed 15 January 2023].

Yadav, R. N. S. and Agarwala, M., 2011. Phytochmical analysis of some medicinal plants. *Journal of Phytology*, 3(12), pp. 10-14.

Yang, S., Jeon, S. Lee, E., Shim, C. and Lee, I., 2010. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Natural Product Research*, 24(2), pp. 140-151.

Document Viewer

Turnitin Originality Report

Checked and Verified by Dr. Sangeetha Arullappan

28/04/2023 ; 11 11am

Processed on: 25-Apr-2023 13:53 +08
ID: 2074857784
Word Count: 17771
Submitted: 1

FYP Thesis First Submission By San Aseana Koh

Similarity Index
12%

Similarity by Source
Internet Sources: 11%
Publications: 7%
Student Papers: N/A

include quoted | include bibliography | excluding matches < 8 words | mode: quickview (classic) report | print | refresh | download

<1% match (Internet from 21-Feb-2018)
https://link.springer.com/article/10.1007/s13197-011-0251-1

<1% match (Internet from 01-Mar-2022)
https://link.springer.com/article/10.1007/s13318-019-00599-z?code=7fc172e7-7045-446e-b953-8ee0f250e606&error=cookies_not_supported

<1% match (Internet from 29-Feb-2020)
https://link.springer.com/article/10.1007%2Fs13197-017-2807-1

<1% match (Internet from 20-Jan-2023)
https://www.science.gov/topicpages/c/cell+lines+derived

<1% match (Internet from 14-Dec-2022)
https://www.science.gov/topicpages/z/zc3h14+gene+generate

<1% match (Internet from 19-Feb-2023)
https://www.science.gov/topicpages/m/mammalian+cell+line

<1% match (Internet from 16-Mar-2023)
https://www.science.gov/topicpages/t/tea+catechins+reduce

<1% match (Internet from 18-Mar-2023)
https://www.science.gov/topicpages/b/breast+adenocarcinoma+mcf7

<1% match (Internet from 18-Oct-2022)
https://www.geeksforgeeks.org/what-is-cancer-introduction-types-stages-treatment/?ref=rp

<1% match (Internet from 20-Jan-2022)
https://chem.libretexts.org/Bookshelves/Environmental_Chemistry/Toxicology_MSDT/03%3A_Principles_of_Genetic_Toxicology/3.04%3A_New_

<1% match (Internet from 16-Dec-2022)
https://pdfcoffee.com/thin-layer-chromatography-7-pdf-free.html

<1% match (Internet from 22-Jan-2021)
https://amp.cancer.org/treatment/treatments-and-side-effects/treatment-types/targeted-therapy/what-is.html

<1% match (Kannupriya, Yeshika Bhatia, Seemha Rai, Nidhi Mahajan, Veena Puri. "Peanut Shell Carbon Nanocellulose Preparation, Analysis and Plausible Role in Regenerative Medicine Using Mesenchymal Stem Cell Line C3H10t1/2", Cold Spring Harbor Laboratory, 2023)
Kannupriya, Yeshika Bhatia, Seemha Rai, Nidhi Mahajan, Veena Puri. "Peanut Shell Carbon Nanocellulose Preparation, Analysis and Plausible Role in Regenerative Medicine Using Mesenchymal Stem Cell Line C3H10t1/2", Cold Spring Harbor Laboratory, 2023

<1% match (Internet from 04-Nov-2022)
https://ijpsr.com/bft-article/anti-microbial-ovicidal-larvicidal-anti-diabetic-and-cytoxicity-activity-of-silver-nanoparticles-from-brown-seaweed-stoechospermum-marginatum/?view=fulltext

<1% match (Internet from 15-Dec-2019)
http://ijpsr.com

<1% match (Fui Joo Bong, Nelson Jeng Yeou Chear, Surash Ramanathan, Nethia Mohana-Kumaran, Sreeramanan Subramaniam, Bee Lynn Chew. "The development of callus and cell suspension cultures of Sabah Snake Grass (Clinacanthus nutans) for the production of flavonoids and phenolics", Biocatalysis and Agricultural Biotechnology, 2021)
Fui Joo Bong, Nelson Jeng Yeou Chear, Surash Ramanathan, Nethia Mohana-Kumaran, Sreeramanan Subramaniam, Bee Lynn Chew. "The development of callus and cell suspension cultures of Sabah Snake Grass (Clinacanthus nutans) for the production of flavonoids and phenolics", Biocatalysis and Agricultural Biotechnology, 2021

<1% match (Internet from 05-Jan-2023)
http://prr.hec.gov.pk

<1% match (Internet from 16-Feb-2022)
https://noteshippo.com/separation-of-amino-acids-by-thin-layer-chromatography-tlc-principle-procedure-visualization/

<1% match ()
Henrietta, Ikwunne Helen. "In Vitro Antioxidant And Alpha-Glucosidase Inhibitory Activities Of Fruit Extracts From Seven Species Of Citrus", 2018

<1% match (Internet from 11-Apr-2023)

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	San Aseana Koh
ID Number(s)	19ADB04163
Programme / Course	Bachelor of Science (Honours) Biomedical Science
Title of Final Year Project	Antioxidant Activity and Cytotoxic Effect of Frankincense Essential Oil

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 12 % **Similarity by source** Internet Sources: 11 % Publications: 7 % Student Papers: — %	NIL
Number of individual sources listed of more than 3% similarity: —	NIL

Parameters of originality required and limits approved by UTAR are as follows:

(i) Overall similarity index is 20% and below, and
(ii) Matching of individual sources listed must be less than 3% each, and
(iii) Matching texts in continuous block must not exceed 8 words

Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor
Name: Dr. Sangeetha A/P Arullappan

Date: 28/04/2023

Signature of Co-Supervisor
Name: _____

Date: _____