DEVELOPMENT AND CHARACTERISATION OF INHALED ANDROGRAPHOLIDE AS AN ANTI-CANCER AGENT

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DEVELOPMENT AND CHARACTERISATION OF INHALED ANDROGRAPHOLIDE AS AN ANTI-CANCER AGENT

by

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LIST OF SYMBOLS

~	approximately
%	percent
ºC	degree Celsius
=	equal
±	plus minus
<	less than
≤	less than or equal to
>	more than
≥	more than or equal to
µg/mL	microgram
µL	microliter
L	littre

LIST OF ABBREVIATIONS

API	Active pharmaceutical ingredients
CO2	Carbon Dioxide
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate
DSC	Dynamic Scanning Calorimetric
ED	Emitted Dose
FPF	Fine Particle Fraction
FT-IR	Fourier Transform Infrared Spectroscopy
GSD	Geometric Standard Deviation
IC50	Inhibitory Concentration At Half Of Maximal Response
IC25	Inhibitory Concentration 25%
ISO	International Standard Of Organization
IU	International Unit
MMAD	Median Mass Aerodynamic Diameter
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
NaCI	Sodium Chloride
NGI	Next Generation Impactor
NMR	Nuclear Magnetic Resonance
NSCLC	Non small cell lung carcinoma
p	Probability
PBS	Phosphate Buffered Saline Solution
R2	Correlation Coefficient
SD	Standard Deviation

SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analysis
USM	University Science Malaysia
v/v	Volume Per Volume
w/v	Weight Per Volume
WHO	World Health Organisation
XRD	X-ray Diffractometry

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PEMBANGUNAN DAN PENCIRIAN INHALASI ANDROGRAPHOLIDE SEBAGAI AGEN ANTI-KANSER

ABSTRAK

Andrographolide ialah sebatian berasaskan tumbuhan yang mampu merawat kanser paru-paru. Walau bagaimanapun, kelarutan air dan bioavailabiliti yang rendah menghadkan pemberian secara oral. Dalam kajian ini, kami membandingkan prestasi aerosol *in vitro*, kestabilan penyimpanan, profil pelarutan, dan aktiviti antikanser dua formulasi andrographolide. Formulasi 1 disediakan menggunakan teknik presipitasi dan pengeringan secara semburan, manakala Formulasi 2 dihasilkan melalui pengeringan semburan larutan andrographolide. Morfologi dan sifat kristal formulasi disahkan menggunakan mikroskop elektron pengimbasan (SEM) dan analisis hamburan sinar-X (XRD). Profil penyebaran aerosol *in vitro* telah dinilai menggunakan impaktor generasi terbaru (NGI). Formulasi 1 terdiri daripada kristal yang memanjang dengan diameter purata 1.65 ± 0.34 µm manakala Formulasi 2 terdiri daripada zarah sfera amorfus dengan diameter purata 1.43 ± 0.19 µm. Kedua-dua formulasi mempunyai peratus yang boleh dihembus (<5 µm) melebihi 44%, menjadikannya sesuai untuk menghantar ubat ke paru-paru. Kepekatan perencat 25% (IC_{25}) bagi Formulasi 1 ialah 31.91 µg/ml manakala Formulasi 2 menunjukkan IC_{25} sebanyak 6.57 µg/ml terhadap sel karsinoma paru-paru (A549). Kedua-dua formulasi adalah stabil dalam vakum pada suhu 30°C sehingga 3 bulan. Kesimpulannya, kami telah menghasilkan dua serbuk kering tersedut andrographolide yang mempunyai profil aerosol yang baik, aktiviti antikanser mujarab, dan kestabilan dalam simpanan yang mencukupi, yang layak untuk diuji secara *in vivo*.

DEVELOPMENT AND CHARACTERISATION OF INHALED ANDROGRAPHOLIDE AS AN ANTI-CANCER AGENT

ABSTRACT

Despite the promising anti-cancer activity of andrographolide against lung cancer, its limited water solubility and low bioavailability pose significant challenges for effective oral administration. To address this issue, the current study focused on the development and comparison of two inhalable preparations of andrographolide for pulmonary drug delivery. Precipitation and spray drying , prepared using precipitation and spray drying techniques, and Direct spray drying, produced via direct spray drying of andrographolide solution, exhibited favorable in vitro aerosol performance, storage stability, and dissolution profiles. Morphology and crystalline properties of the preparations were confirmed using scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis, respectively. *In vitro* aerosol dispersion profile was evaluated using a next-generation impactor (NGI). Precipitation and spray drying consisted of elongated crystals with an average diameter of 1.65 ± 0.34 µm while direct spray drying was made of amorphous spherical particles with an average diameter of 1.43 ± 0.19 µm. Both preparations showed inhalable fractions (particle size < 5 µm) of more than 44% making them suitable for pulmonary drug delivery. The 25% inhibitory concentration (IC_{25}) for Precipitation and spray drying was 31.91 µg/ml while direct spray drying demonstrated IC_{25} of 6.57 µg/ml against lung carcinoma cells (A549). Both preparations were relatively stable in a vacuum condition at 30°C up to 3 months. In conclusion, two novel inhalable andrographolide dry powders successfully produced with good aerosol profiles, potent anti-cancer activity and sufficient storage stability.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Although lung cancer was considered a reportable disease a century ago, it is still the most common cause of cancer mortality in both men and women in developed counties (American Cancer Society, 2022). According to a World Health Organization study (2020), lung cancer killed 19 people per 100,000 in Malaysia, or 4,088 people a year, making it the nation's second most frequent cause of cancer death after breast cancer and the eighth most common cause of death overall. Lung cancer has also often been diagnosed at an average age of 60 among Malaysians while relatively uncommon among individuals under 40 (Siang & John, 2016).

Lung cancer is generally classified into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). These types are distinguished by their appearance under a microscope, their behavior and treatment options. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 85% of all lung cancer cases. NSCLC develops in the tissues of the lungs and is characterised by the uncontrolled growth of abnormal cells in the lung tissues (Travis et al., 2015). Small cell lung cancer, also called oat cell carcinoma, accounts for about 10-15% of lung cancers. It is strongly associated with smoking and tends to grow rapidly. Small cell lung cancer is more likely to spread to other parts of the body early on, which often limits treatment options (American Cancer Society, 2021).

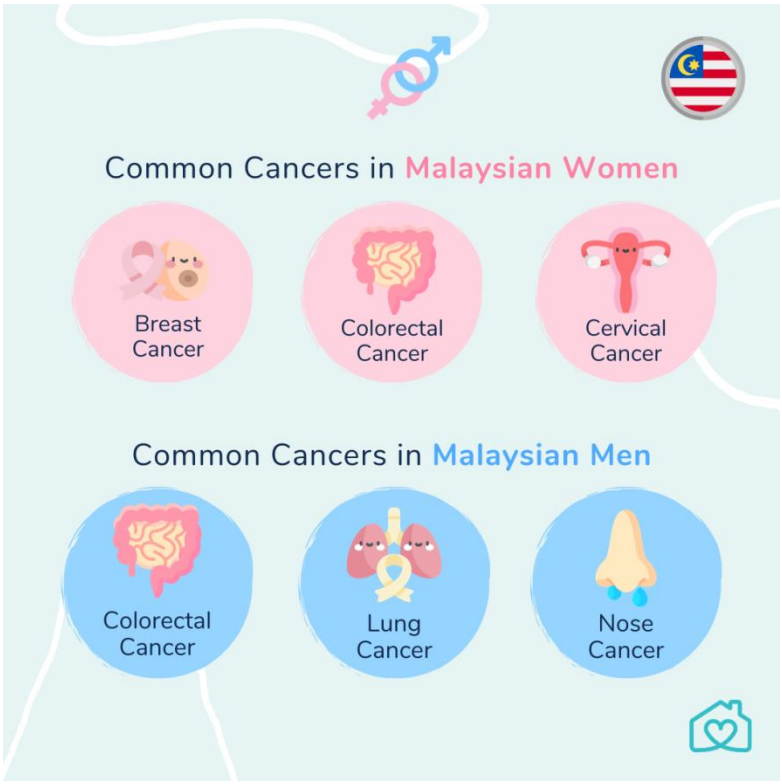

Figure 1.1: Cancer Statistics in Malaysia (National Cancer Registry, 2022)

Chemotherapy, radiation, surgery, or a combination of one or all of these has been the mainstay of treatment for the past 50 years (American Cancer Society, 2022). Management for lung carcinoma varies depending on the severity of the disease and the existence of metastases; however, surgical resection, molecularly targeted medicines and radiation therapy are all options. Combining chemotherapy and targeted radiotherapy are used to treat cancer patients as the primary therapy option (Kwon et al., 2020). Surgical procedures, particularly preoperative staging, have improved significantly over the past 50 years, and surgical procedure remains the primary modality for offering a possibility for lung cancer cure. Radiotherapy administration has also advanced, with larger dosage treatments becoming more practical, improved field design, and more targeting. Chemotherapy has also improved, with nearly none of the drugs used in the 1950s–1970s being used nowadays. Unfortunately, these advancements have only resulted in minor increases in the survival rate. The potency

of therapy is largely determined by the methods by which the medicine is administered and the drug's optimal concentration. The effectiveness of a treatment largely depends on how the medication is administered and achieving the optimal concentration of the drug. The limited progress in improving the effectiveness of therapies for severe illnesses underscores a growing demand for a multidisciplinary approach in distributing therapeutic agents to lung cancer tissues.

Interestingly pulmonary drug delivery provides prospective advantages over "traditional" drug administration via the oral or intravenous routes as seen by the growing interest in and number of marketed drugs for inhalation therapy. One of the essential features of aerosol drug delivery is targeting the medication administration directly into the affected lung cells. Some major benefits of pulmonary entity delivery are: (1) Rapid onset of action: inhalation delivers medication directly to the lungs allowing it to rapidly reach the cancer cells. This can result in a faster onset of action compared to oral medications, which must be absorbed through the gastrointestinal tract. (2) Targeted Delivery: Pulmonary drug delivery allows for targeted delivery of medication to the lungs, making it an ideal route of administration for lung cancers. (3) Lower Dose Requirements: Inhalation delivers medication directly to the site of action, which means that lower doses of medication may be needed compared to other routes of administration. This can reduce the risk of side effects and improve patient compliance. (4) Non-Invasive: Pulmonary drug delivery is a non-invasive route of administration, which means that it does not require needles or other invasive procedures. This makes it more comfortable for patients and reduces the risk of blood-borne infections. (5) Portable: Inhalers used for pulmonary drug delivery are portable and can be carried with the patient, making it easier for them to take their medication

as prescribed. This can improve patient compliance and reduce the risk of missed doses.

The uses of herbal medicines are well-established and widely acknowledged for their efficacy and safety, as well as accepted by national health agencies, including WHO [WHO, 2002]. Since the start of civilisation, medicinal plants have been vital to human life in battling various illnesses (Hossain et al., 2014). WHO indeed estimated that 86% of people worldwide depend on herbal medicines for some degree of their primary health care program. It is also well-documented that complementary medicines, as well as traditional medicines, have well-established usage in our healthcare system (Duraz et al., 2011).

About 65% of anticancer drugs introduced over the last 25 years have been derived from natural sources. Chemical synthesis (either partial or total) has played an important role in supplying these nature-derived compounds in large quantities and in preparing their novel analogs. Many of these compounds suffer from low solubility and poor bioavailability. Pulmonary delivery offers the advantages of increased bioavailability, prolongation of drug circulation time, and multiple drug loading, all contributing to improved efficacy and decreased toxicity (David J. Newman and Gordon M. Cragg., 2020).

There is immense scope for the nature-derived molecules to be formulated into inhalation based drug delivery systems targeting the tumor microenvironment to combat cancer. There is an existing gap and research initiatives to synthesise more tumor targeted micro therapeutic delivery systems with high quality and yield of cytotoxic agents obtained from natural resources could prove effective in the overall management of cancer (G. S. Katiyar., 2018)

Novel preparations containing plant based drugs and by pulmonary delivery could prolong drug circulation duration, provide coordinated drug release, a better efficacy-to-toxicity ratio that could lead them into clinical trials and eventually to the bedside. Efficient preparations targeting strategies and evaluation of the targeting efficiency of drug, and conforming to international standards for their toxicology and biocompatibility could pave the way for clinically viable phytochemical-based anticancer therapies.

Andrographolide is a natural compound found in the *Andrographis paniculata* plant, which is commonly used in traditional medicine for its anti-inflammatory and anti-infective properties. There is also evidence to suggest that andrographolide may have anti-cancer properties (Pramanik et al., 2011). Several studies have shown that andrographolide can inhibit the growth and proliferation of cancer cells, including breast cancer, prostate cancer, colon cancer, leukemia and lung cancer (Chen et al., 2015). It also has been shown to induce cell cycle arrest, apoptosis (programmed cell death), and inhibit angiogenesis (the formation of new blood vessels that tumors need to grow and spread) in cancer cells. In addition to its direct anti-cancer effects, andrographolide may also enhance the efficacy of chemotherapy and radiation therapy (Hsieh et al., 2018). While the exact mechanisms underlying andrographolide's anti-cancer effects are not exactly known , it is thought to act through multiple pathways, including modulation of the immune system and inhibition of several key signaling pathways involved in cancer cell growth and survival (Sheeja et al., 2007).

Many researchers have investigated the effects of andrographolide on NSCLC. They found that andrographolide inhibited the proliferation and growth of NSCLC cells in a dose-dependent mechanism and induced regular apoptosis (programmed cell death) by activating caspase-3 and caspase-9, which are key proteins involved in the

apoptotic process (Chen et al., 2021). Andrographolide also inhibited the growth and invasion of NSCLC cells by suppressing the expression of matrix metalloproteinase-9 (MMP-9), which is an enzyme that promotes cancer cell invasion and metastasis (Yang et al., 2017). The compound also found to sensitise NSCLC cells to the effects of radiation therapy by inhibiting the DNA repair machinery and inducing apoptosis, leading to enhanced anti-tumor effects. In a nutshell, andrographolide is a promising candidate for further development as a potential anti-cancer agent. However, its poor solubility in water limits its therapeutic efficacy (Yen et al., 2020).

1.2 Problem statement

The poor solubility of andrographolide in water can present a challenge for its absorption in the lungs, especially since the lungs primarily interact with substances in their dissolved form. This characteristic can pose challenges in formulating drug delivery systems or developing pharmaceutical products that require higher solubility for effective administration. To overcome this limitation, various strategies have been explored, including preparations techniques, co-solvents, and particle size reduction (Loureiro Damasceno JP et al., 2021,).

In a nutshell, research into the performance, stability, and treatment of lung cancer using andrographolide shows great prospects for expanding our knowledge of its therapeutic potential. It provides chances for developing new lung cancer therapy methods, more effective drug stability, and tailored drug delivery. Exploring and utilising the potential advantages of andrographolide via research in these areas can help us create new and effective treatments for people with lung cancer.

1.3 Objectives of the study

1.3.1 General objective

To produce a stable inhalable andrographolide dry powder for the treatment of NSCLC.

1.3.2 Specific objectives

i. To produce crystalline and amorphous andrographolide dry powders for aerosol delivery to the lungs
ii. To perform physicochemical and aerosol characterisation of the andrographolide dry powders
iii. To evaluate the storage stability of the andrographolide dry powders
iv. To examine in vitro anti-cancer properties of the andrographolide dry powders against adenocarcinoma human alveolar basal epithelial cells

CHAPTER 2

LITERATURE REVIEW

2.1 *Andrographis paniculata*

Andrographis paniculata (Table 2.1) has a long history of use in traditional medicine, particularly in Ayurveda, Chinese medicine, and other traditional healing systems. It has been traditionally used for its various therapeutic properties, including its potent anti-inflammatory, antiviral, antibacterial, and immunomodulatory effects. The key bioactive compound found in *A. paniculata* is andrographolide. This phytochemical has been extensively studied and is considered the major contributor to the plant's medicinal properties. Andrographolide has shown promising potential in various areas of health, such as supporting immune function, alleviating respiratory conditions, and exhibiting anticancer properties. In addition to andrographolide, *A. paniculata* contains other bioactive compounds, including diterpenoids, flavonoids, and polyphenols, which contribute to its overall therapeutic effects (Akbar, S., 2011).

Due to its medicinal properties, *A. paniculata* has gained attention in modern scientific research and is being studied for its potential applications in various health conditions, including colds, flu, respiratory infections, inflammatory disorders, and certain types of cancer (Vimalanathan et al., 2014). The extract's main ingredient, andrographolide, has been often linked to its pharmacological effects (Rajagopal et al., 2003, Okhuarobo et al., 2014)

Table 2.1: Taxonomical profiles

Kingdom	**Plantae, Plants**
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Angiosperma
Class	Dicotyledonae
Sub class	Gamopetalae
Series	Bicarpellatae
Order	Personales
Tribe	Justicieae
Family	Acanthaceae
Genus	*Andrographis*
Species	*paniculata*

2.1.1 The characteristics of *Andrographis paniculata*

A. paniculata is an annual blooming plant with upright and branching stems. Hedgerows, hill slopes, waste grounds, farms, damp environment, seashores, and roadsides are all good places to find this herb (Hossain MS, 2014). It is also possible to cultivate it in the garden. Forests, wastelands and wet and gloomy regions are preferred for their good growth (Shahid Akbar., 2011). It is a salt-sensitive plant; hence, its development is restricted under stress, particularly saline stress, which significantly impacts plant growth and crop output. Under ideal conditions, this plant may grow to a height of 30 to 110 cm. It has a dark green stem that is 30–110 cm long, 2–6 mm in diameter, and quadrangular with longitudinal grooves (Hossain et al., 2021). It is widely grown throughout southeastern Asia, including India, Pakistan,

Indonesia, and China. In its native locations, *A. paniculata* is commonly cultivated from seeds in pine, oak, and dry forest environments along highways and settlements. It is grown in India during the summer monsoon. This crop may be grown commercially in any soil with a reasonable level of organic content. For one hectare, 400 g of seed is adequate. The spacing is kept at 15 to 30cm (Anil Kumar et al., 2012). There have been no reports of serious insect or disease manifestation. (Mishra, et al., 2007)

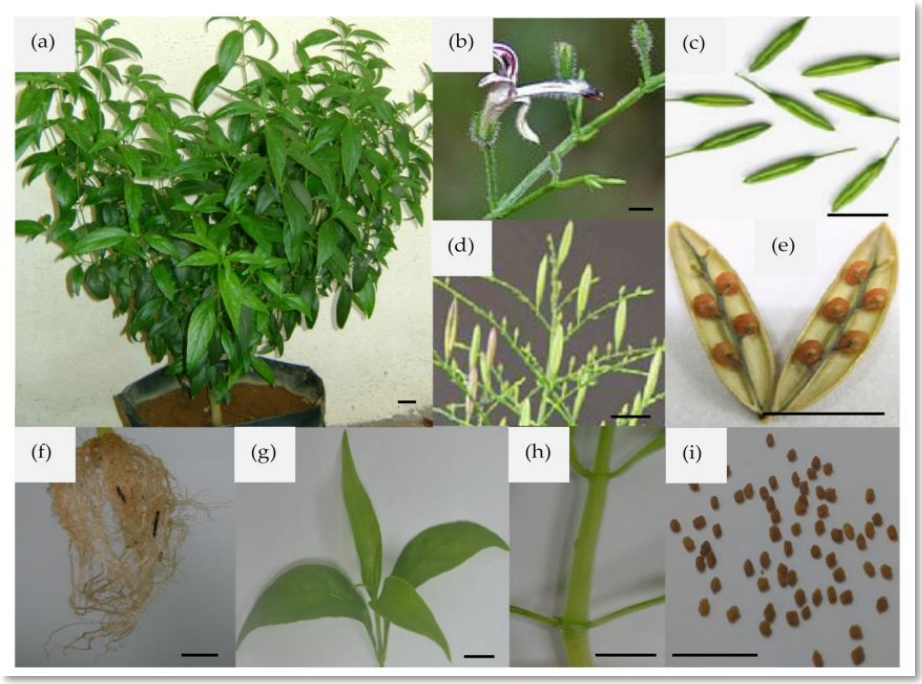

Figure 2.1: *Andrographis paniculata* and its different parts. (a) Aerial part, (b) flower, (c) pod stage with panicles: mature capsule, (d) fruit, (e) opened capsule, (f) roots, (g) leaves: opposite arrangement, (h) stem, and (i) seed. (Hossain, M.S. 2016)

2.1.2 Common benefits of *Andrographis paniculata*

A. paniculata's most prevalent traditional application is to enhance and improve the immune system. The andrographolide component of this plant is thought to be primarily responsible for its naturally resistant properties, according to research (Adiguna et al., 2021). Apart from that, *A. paniculata's* has the ability to support the

immune system. It has been extensively studied for its immunomodulatory effects, stimulating the production and activity of immune cells. By enhancing immune function, *A. paniculata* helps the body defend against pathogens and maintain overall wellness (Akbar, 2011).

Another significant benefit is its potent anti-inflammatory properties. *A. paniculata* contains bioactive compounds that inhibit the release of pro-inflammatory molecules, thereby reducing inflammation in the body (Intharuksa, 2022) This makes it potentially useful in managing conditions characterised by chronic inflammation, such as arthritis and inflammatory bowel disease. *A. paniculata* also exhibits broad-spectrum antimicrobial effects. Research has shown its efficacy against bacteria, viruses, and fungi, making it a potential natural remedy for various infections, including respiratory tract infections and skin infections (Kalra et al., 2013). The herb is rich in antioxidants, which play a crucial role in protecting cells from oxidative damage caused by free radicals. This antioxidant activity contributes to its potential in preventing chronic diseases such as cardiovascular disorders and neurodegenerative conditions (Mishra et al., 2008). *A. paniculata* has traditionally been used to support digestive health. It can help alleviate symptoms of indigestion, bloating, and diarrhea. Additionally, the herb possesses hepatoprotective properties, aiding in the detoxification and protection of the liver (Akbar, 2011)

2.1.3 The compounds isolated from *Andrographis paniculata* extract

Several bioactive compounds have been isolated from *A. paniculata* extract, contributing to its therapeutic properties (Figure 2.2). Among the major compounds identified is andrographolide, which exhibits diverse pharmacological activities such as antioxidant, immunomodulatory, anti-inflammatory and anti-cancer activity

(Kumar et al., 2010). Deoxyandrographolide, yet new significant compound found in the extract, also possesses anti-inflammatory, antioxidant, and anticancer properties (Dai et al., 2019). Apart from that, neoandrographolide, another major compound in *A. paniculata*, has demonstrated anti-inflammatory and hepatoprotective effects (Kumar et al., 2010).

Other minor compounds include 14-Deoxy-11,12-didehydroandrographolide, which stops the release of pro-inflammatory cytokines and contributes to the anti-inflammatory activity of the extract (Kumar et al., 2010), and a group of diterpenoid lactones known as andrographanoids A-G, which have shown various pharmacological properties, including anti-inflammatory, anticancer, and antimicrobial activities (Dai et al., 2019).

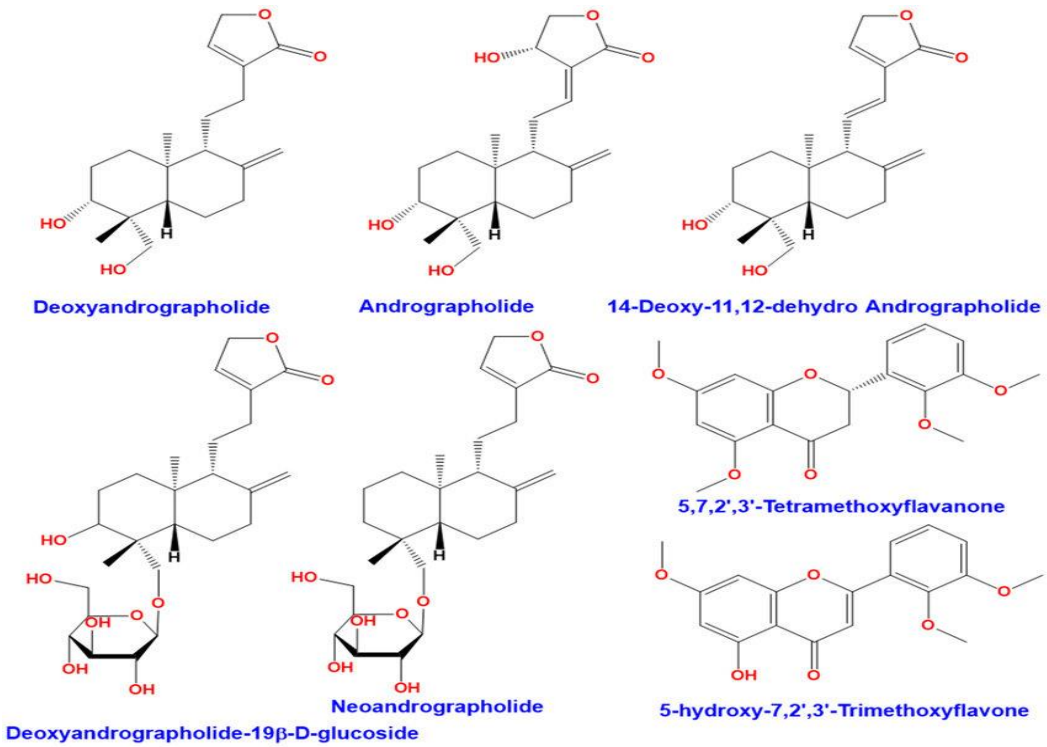

Figure 2.2: Chemical structures of active components in *A. paniculata* (Mussard et al., 2019)

2.1.4 The properties of andrographolide

Andrographolide is a diterpenoid lactone derived from *A. paniculata* only. The crystals are solid, white, and odourless powders that are obtained by the purification of andrographolide. The crystals are insoluble in water but soluble in organic solvents such as ethanol and acetone. They have a melting point of around 230°C and can be easily stored at room temperature. Andrographolide crystals are often used in the industry related to pharmaceutical for the development of various medicines. One of the most significant advantages of using andrographolide crystals is their high purity. The purification process readily removes impurities from the compound, resulting in a more potent product. This high purity is crucial for pharmaceutical applications, as it ensures that the drug is safe and effective for human use. Andrographolide crystals also possess good stability and can be easily stored for extended periods without any significant loss of potency. This stability is important for drug manufacturers, as it ensures that the drug will retain its potency and effectiveness (Chen et al., 2015; Sun et al., 2019).

Inflammation is a critical component of many diseases, including respiratory diseases such as asthma and cancer. Studies have shown that andrographolide can reduce inflammation in the airways, making them a promising treatment option for these diseases (Xia et al., 2017). Andrographolide has also been shown to possess potent anti-viral activity against several viruses, including influenza, herpes simplex virus, and human immunodeficiency virus (Sheeja et al., 2006).

Andrographolide have also been studied for their potential use in the treatment of cancer. The most explored cancers were colon cancer, breast cancer, lung cancer and head and neck carcinomas, followed by prostate cancer and glioblastoma.

Andrographolide has been shown to possess anti-cancer properties by inducing apoptosis in cancer cells (Li et al., 2018). Lee et al reported andrographolide inhibited dose-dependently the invasion and migration of adenocarcinomic human alveolar basal epithelial cells, under non-cytotoxic concentrations (Lee, Yi-Chieh, et al 2010). In a separate study by Xiangyu Luo, the activity of Na^+-K^+-ATPase in lung cancer cells was decreased by andrographolide, indicating malfunctioning of the α-subunit and/or impairment of mitochondrial membrane, and also suggesting that the mitochondrial dysfunction resulted from andrographolide might induce apoptosis of lung cancer cells. Andrographolide treatment has also shown to downregulate PI3K/AKT signaling pathway, consequently decreasing the expression of hypoxia-inducible factor-1α (HIF-1α) by the ubiquitin-dependent degradation (Lee et al., 2010). Further studies showed andrographolide regulates amino acid and arachidonic acid metabolism which have great potential as target pathways against lung cancer (Wen Luo et al., 2021). This mechanism of action makes andrographolide a promising candidate for the development of anti-cancer treatments.

2.1.5 Andrographolide crystal and amorphous form

Exploring andrographolide's crystal and amorphous form and its implications for medication delivery and preparations is one area of focus. Understanding andrographolide's crystal and amorphous forms aids in optimizing medication preparations, improving solubility, stability, and controlled release, crucial for effective drug delivery and therapeutic outcomes. Amorphous andrographolide is a highly promising drug delivery system due to its increased solubility and bioavailability compared to its crystalline form. Hongzhi Qiaoet al., (2017) compared the bioavailability of amorphous and crystalline andrographolide in rats. The results

showed that amorphous andrographolide had a significantly higher bioavailability than the crystalline form. This study highlights the potential of amorphous andrographolide as a drug delivery system for improving the therapeutic efficacy of andrographolide. They also noted that amorphous andrographolide had a more sustained release profile, allowing for prolonged drug exposure to cancer cells. Despite its potential benefits, there are also some challenges associated with the use of amorphous andrographolide. One major challenge is its physical stability, as amorphous materials have a tendency to recrystallise over time. This can result in a loss of drug efficacy and reduced bioavailability. However, several strategies such as stabilising excipients and developing amorphous solid dispersions, have been proposed to improve the stability of amorphous andrographolide (Lomlin et al., 2003).

On the other hand, the crystalline form of andrographolide is physically more stable than the amorphous form, but its solubility and bioavailability are limited. However, the crystal structure of andrographolide can be modified to improve its solubility and bioavailability. One such modification is the preparation of smaller size microcrystals of andrographolide, which have a high surface area and enhanced dissolution rate. Another approach is the preparation of co-crystals of andrographolide with other molecules, which can improve its solubility and bioavailability (Yan Y et al., 2018).

2.2 The Pulmonary drug delivery system

Recently, there has been a surge in interest in pulmonary medication delivery for the treatment of lung cancer. This is due to the fact that the ability of the lungs to absorb pharmacologically active substances for both systemic and local treatment. Some local respiratory ailments and number of systemic condition can be effectively

treated by administering medications via the pulmonary route. Topical treatment of pulmonary hypertension, local infectious illnesses, asthma, and the systemic administration of oxytocin , human growth hormones, and insulin are some examples (Klepser et al., 2004). Because of the high surface area and high air permeable barriers, drug distribution to the lungs via the respiratory system is very responsive. Comparatively low enzymatic activity, fast drug absorption, and ability to overcome first-pass metabolism are other advantages of pulmonary drug delivery over perioral uses.

The amount of droplet formation in the respiratory system is determined by both the patient's physiological and pathological conditions, such as breathing styles and overall lung health, and the physicochemical characteristics of the inhaled particles, such as form, bulk density, size, solubility in water, and humidity levels. The main approaches for particle deposition after inhalation includes impaction owing to inertial forces, gravitational deposition, and Brownian diffusion (Hofmann et al, 2011). Interception and electrostatic precipitation are two further processes that account for small rates of deposition.

The inhalation particles are subjected to a centrifugal force during inhalation, resulting in deposition in different bronchial areas subject to particle size. Different size ranges of particles tend to deposit in different regions of the respiratory tract due to their aerodynamic properties (Figure 2.3). The respiratory tract is divided into three main regions: the nasopharyngeal region (nasal cavity and pharynx), the tracheobronchial region (trachea and bronchi), and the alveolar region (respiratory bronchioles, alveolar ducts, and alveoli. Particles larger than (>5 μm) are deposited in the upper respiratory tract, while smaller particles (1–5 μm) get deposited in the bronchiolar region via sedimentation. Furthermore, particles with size < 1 μm are

deposited in deeper alveolar regions through Brownian diffusion, while particles smaller than 0.5µm are exhaled out during exhalation (Dolovich MB and Dhand R., 2011). Usmani et al. have thoroughly investigated this size-dependent particle sedimentation behavior in the respiratory tract.. In general, larger particles tend to deposit in the upper respiratory tract, while smaller particles tend to deposit deeper in the lungs. However, the exact deposition pattern is also influenced by factors such as the shape of the particles, the velocity of the inhaled air, and the breathing pattern.

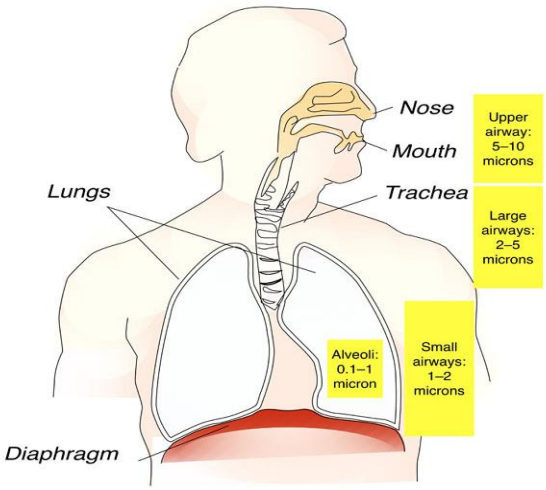

Figure 2.3: Lung deposition pattern corresponding to particle size (Adapted from A Guide to Aerosol Delivery Devices for Respiratory Therapists, 2017)

The shape of the particles can influence their ability to flow and disperse, affecting the aerosolisation performance of the DPI as well. Particles with irregular shapes may agglomerate, reducing the effective surface area and leading to poor dispersibility (Kumar et al., 2018). On the other hand, particle density affects the settling velocity and the gravitational forces that act on the particles, affecting their ability to remain airborne and to be delivered deep into the lung. Higher-density particles tend to deposit more rapidly and in the upper airways, while lower-density particles tend to deposit deeper in the lung. The surface chemistry of the particles can influence their adhesion to the inhaler device and the lung surfaces, affecting the

delivery efficiency of the drug. Surface charges and other surface properties can also influence the ability of the particles to agglomerate and form stable aerosols (Bhavane et al., 2003). Lastly, moisture content of the powder can impact its cohesive properties, leading to changes in the flowability and dispersibility of the powder. It also can affect the electrostatic charges on the particles, which can influence their aerosolisation (Heng et al., 2008).

2.3 Delivery devices

There are various types of inhalers available, each with their own unique design and method of drug delivery. Choosing the right inhaler device generally depends on various factors, including the type and severity of the respiratory condition, the patient's age and ability to use the device, and the specific medication being prescribed. Healthcare providers can work with patients to select the best inhaler device for their individual needs (Usmani OS., 2019)

Nebulisers

Nebulizers are appliances that disperse a liquid or a solid into tiny droplets or aerosols that may be breathed. Nebulizers typically aren't portable. The distribution of medications with a large therapeutic dosage, such antibiotics, benefits from their ability to administer a bigger volume of drug solution over a longer period of time (Lass et al., 2006). Additionally, no particular inhalation technique or coordination is required during delivery because the medication is breathed through a mouthpiece or facemask in conjunction with regular tidal breathing. Nebulization may therefore be practical for young patients, the elderly, acutely dyspneic patients, or refusing patients. However, employing them has a number of drawbacks, including being time-consuming, heavy, and less portable (Alharbi AS et al., 2021).

Pressurised metered dose inhalers (pMDIs)

A pressurized canister keeping medications that are dissolved or suspended in a liquid propellant or a combination of liquid propellants and various excipients is a component of pMDIs, which are multi-dose devices. A metering valve is installed in the canister, and when it is activated, it delivers a predefined amount in spray form. According to Dolovich and Dhand (2011), pMDIs are the most often utilized inhalation devices for treating asthma and chronic obstructive pulmonary disease because they are more portable and need less time to administer than nebulizers. The original pMDIs were not environmentally friendly as they employed chlorofluorocarbon (CFC) as a propellant, which can damage the ozone. However, alternative propellants, namely hydrofluoroalkanes are now used widely. A disadvantage of pMDIs is the need for actuation and inhalation coordination by patients, which can cause variation in the fraction of drug reaching the lungs (Pilcer and Amighi, 2010). The problem has been overcome by the introduction of breath-actuated pMDI devices (e.g. Autohaler®, Easi-breathe®), which diminish the need for coordination as the device can sense the inhalation through the actuator and fire the dose in synchrony. Alternatively, spacer devices or valved holding chambers may be used to minimise the need for coordination in children (Leach, C. L. 2009).

Dry powder inhalers (DPIs)

Dry powder inhalers (DPIs) are widely used for the delivery of dry powder to the lungs, particularly for the treatment of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). DPIs offer several advantages over other inhaler devices, including ease of use, portability, and the absence of propellants. DPIs work by delivering micronized drug particles directly to the lungs, where they

are deposited and absorbed into the respiratory system. The key advantage of DPIs is their ability to deliver medication without the need for coordination between inhalation and device actuation, as is required with pressurized metered-dose inhalers (pMDIs). This makes DPIs particularly suitable for patients with compromised lung function or coordination difficulties (Newman et al., 2008). One of the crucial aspects of DPIs is the preparations of the dry powder. The drug particles need to have the appropriate size, shape, and surface characteristics to ensure effective lung deposition. Fine particle fractions (FPFs) are critical for efficient drug delivery to the lungs, as larger particles tend to deposit in the oropharynx and are swallowed rather than reaching the desired site of action (Fink et al., 2012). Preparations techniques such as micronisation, spray drying, or particle engineering can be employed to achieve optimal particle size distribution (Vehring, 2016).

The inhalation technique and patient cooperation are also crucial for successful drug delivery with DPIs. Proper inhalation technique involves a deep and forceful inhalation to generate sufficient airflow to disperse and carry the drug particles into the lungs. Patients should be educated on the correct use of DPIs to maximise their therapeutic benefits (Lavorini et al., 2008). Furthermore, DPIs require careful device design to ensure consistent and reliable drug delivery. The design should consider factors such as airflow resistance, ease of loading the medication, dose counters, and mouthpiece design for optimal patient comfort (Lennernas, 2014). Device design improvements and advancements, including multi-dose DPIs and breath-activated devices, have enhanced the performance and ease of use of DPIs (Vehring, 2016). In conclusion, DPIs are valuable devices for delivering medication to the lungs. Their ease of use, portability, and lack of propellants make them a preferred choice for respiratory drug delivery. Proper preparations, particle engineering, inhalation

technique, and device design are critical factors in ensuring the efficacy and safety of DPIs in clinical practice.

2.4 Production of inhalable dry powder

Selecting appropriate procedures for particle preparation is equally critical. There are several preparation techniques used to produce particles for inhalation including:

1. Spray drying: Spray drying involves atomising a solution or suspension of the drug with or without carrier materials into a stream of hot gas, which dries the droplets and forms a powder.
2. Jet milling: Jet milling involves milling the drug and carrier material using a stream of high-pressure air or nitrogen to produce micron-sized particles.
3. Fluid bed coating: Fluid bed coating involves suspending particles in a fluidised bed and applying a coating material to the surface of the particles. This technique is used to improve the flow and dispersibility of particles in DPI preparations.

2.5 The spray drying

Spray drying is an advanced pharmaceutical manufacturing process used to produce respirable colloidal particles in the solid state efficiently. Spray drying was explored in the 1980s to produce fine particles for pulmonary delivery. Spray drying is a well-known method of particle production that transforms a fluid material into dried particles using a hot gas as a drying medium. In this process, the feed solution is supplied at room temperature and pumped to the nozzle where it is atomised by the nozzle gas into the hot gas stream (Ziaee et al,. 2018).

Bothiraja et al. (2009) and Chen et al. (2017) have studied the productions of andrographolide solid dispersion and amorphous form using spray drying technique. This method is reported to have better control on particle formation and can be easily translated to large-scale production. This process is also suitable for thermolabile materials, such as proteins and peptides, because mechanical high-energy input is avoided in this process (Mulle, & Keck, (2012). More importantly, spray drying can result in uniform particles. It is also a relatively inexpensive, short-time period process and allows continuous large-capacity production. Compared to freeze drying method, spray drying six (6) times less expensive for every kg of water removed (Chen et al., 2021).

Figure 2.4: The Buchi Mini Spray Dryer B-290, a typical laboratory-size model. (Reproduced from Buchi Labortechnik AG, Switzerland.)

The particle size and morphology of particles from spray drying are influenced by the feed rate. The transfer of feed solution into the nozzle per unit of time is represented by the feed rate. It has been demonstrated that bigger particle size occurs when the pump speed is increased in turn increased the solution feeding rate (Focaroli et al,. 2019).

The physicochemical properties of spray-dried powders also depends on other process variables such as characteristics of the liquid feed (solvent/solution, viscosity), drying air (pressure), and the type of atomiser (Tonon et al., 2008). Harjunen et al. found that lactose dried with 100% water and 100% ethanol was 100% amorphous and 100% crystalline, respectively. Likewise as mentioned above, slow feed rates result in smaller particle sizes with less moisture content and enhanced flow properties. In contrast, a high feed rate usually results in particles with bigger sizes, higher moisture content, and poor dissolution rates. The inlet temperature also has critical importance in the spray drying procedure by affecting the surface morphology, density, and water content of particles, as well as the overall product yield. Particles drying at low inlet temperatures simultaneously have higher water contents and poorer flow rates but have smoother surface morphologies (Tay et al., 2021).

The effect of temperature has been well demonstrated in a study by Littringer et al. (2012). In this study, the authors dried mannitol at three different temperatures (60 ◦C, 90 ◦C, and 120 ◦C) and compared the smoothness of the particles. Particles obtained at 60 ◦C and 90 ◦C were found to have smooth surfaces, whereas particles obtained at 120 ◦C were found to have rough surfaces. They also found that the particles obtained at higher temperatures were hollow in structure. The effect of inlet temperature was also studied by Coppi et al., in 2009 who prepared alginate microparticles loaded with lactate dehydrogenase and found that higher inlet temperatures decreased the water content in the dried powder while maintaining excellent storage stability. This can be explained by the fact that more fluid is available; hence there is less energy per droplet to dry them.

2.6 Lung cancer

Lung cancer remains a significant global health concern, representing a leading cause of cancer-related morbidity and mortality. It is characterised by uncontrolled cell growth in the tissues of the lungs, typically arising from the epithelial cells lining the bronchi and lungs. There are two primary types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), each with distinct characteristics and treatment approaches. In recent years, there has been growing interest in exploring the potential of natural substances in the management of lung cancer (Dela Cruz CS et al., 2011). Natural substances, often derived from plants, fruits, and other botanical sources, contain a diverse array of bioactive compounds known as phytochemicals. These compounds have been studied for their potential anti-cancer properties, including their ability to inhibit the growth of cancer cells, induce apoptosis, and exert anti-inflammatory effects.

One notable example is curcumin, a polyphenol found in turmeric. Curcumin has demonstrated anti-cancer activities in various preclinical studies, showing potential inhibitory effects on the proliferation and metastasis of lung cancer cells. Similarly, resveratrol, commonly found in grapes and red wine, has been investigated for its anti-cancer properties, including its ability to modulate signaling pathways involved in lung cancer development (H. Bar-Sela et al., 2014).

Green tea polyphenols, particularly epigallocatechin gallate (EGCG), have also attracted attention for their potential in lung cancer prevention and treatment. EGCG has been shown to possess anti-inflammatory and antioxidant properties, contributing to its potential anti-cancer effects (Singh BN et al., 2011).