REVIEW ARTICLE

A Review on Supporting Roles of Phytochemicals and Advances in Their Nanoformulations for Lung Diseases

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ABSTRACT

Phytochemicals have shown potentials in preventing and treating various lung diseases, including asthma and lung cancer. However, their use has been hampered by limitations such as poor water solubility and instability. Nanoformulations, which include organic nanoparticles (e.g. liposomes) and inorganic nanoparticles (e.g. gold/silver nanoparticles) have potential to enhance drug solubility and subsequently increase their absorption and bioavailability. These drug formulations can be administered via various routes such as oral, intravenous, and pulmonary. This review highlights the potential of some phytochemicals in managing lung diseases and discusses the mechanism of actions of these phytochemicals and the impact of using the nanoformulations to deliver phytochemicals, particularly through pulmonary route. While pre-clinical studies have shown promising therapeutic activities of phytochemicals-loaded nanoformulations, further research is required. In conclusion, the application of phytochemicals and nanoformulations as drug delivery systems could be a promising approach to facilitate optimal therapy for lung diseases.

Keywords: Phytochemicals; Lung diseases; Drug delivery system; Drug formulation; Nanoparticles

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INTRODUCTION

Since ancient times, herbal medicines have been used for numerous medicinal applications, and the curative effects of plants have been described in detail by the Chinese and Egyptians since 3000 BC. The native communities included herbal medicines as part of their healing rituals, while more structured traditional systems such as Ayurvedic and Traditional Chinese Medicine developed guided application of the plants. Nonetheless, the use of herbal medicine has been diminishing in the late 19th and early 20th centuries as the use of glycoside and alkaloids isolated from the plants became more popular, though it gained back its reputation due to cultural acceptance since the mid-20th century [1].

Medicinal plant refers to a plant in which one or more of its parts consists of substances that have therapeutic benefits or can be used as precursors for the synthesis of pharmaceutical drugs [2]. These substances, also known as "phyto-" (plant) chemicals, are secondary metabolites which are the non-nutrient chemical components of the plants. The phytochemicals can be found in various parts of the plant, such as leaves, seeds, barks, roots, rhizomes, grains, stems, fruits, or flowers [3] and may function as part of the defence mechanism for the plant against microbial infections or infestations by pests [4]. There is a multitude of reports in the literature that describe the activity of these phytochemicals, ranging from the common cold to deadly diseases such as cancers. Their activities vary between diseases, depending mostly on the type of plant products, either crude extracts or pure isolated chemical compounds. Among the common pharmacological activities of phytochemicals being studied and reported include anticancer, anti-inflammatory, antioxidant, antimicrobial, and antidepressant, to name a few [5-7]. Phytochemicals are also known to have the potential to aid in the treatment or prevention of various lung disorders and diseases, which may be caused by several factors, including genetic and environmental or the interaction between the two [8].

Although phytochemicals have shown their preventive and therapeutic potential, their usage has been hampered by several limitations, including poor water solubility, instability, and poor bioavailability. To overcome these limitations, the formulation approach has to be innovative, with mild-processing conditions, to protect the phytochemicals that may be unstable under harsh conditions. The advances in drug delivery systems have given a new breath of opportunity to enhance the physicochemical properties of phytochemical-based formulations. Nanotechnological advances have produced different delivery systems such as liposomes, lipid-, polymeric- and metal-based nanocarriers, as well as cyclodextrin complexes which could be combined with other strategies, namely drug targeting and local application, to improve their delivery. While several reviews have discussed the usage of nanotechnology in the delivery of phytochemicals in general [9] and in anticancer drug delivery [5], this review will focus on the nanotechnology-based approaches in the formulation of phytochemicals and their overall impact in supporting the treatment of lung-related conditions. In addition, examples of phytochemicals for specific lung diseases will also be described as a future reference for scientists working in the area.

THE USE OF PHYTOCHEMICALS FOR LUNG DISEASES

In the past decades, the study of phytochemical compounds as an alternative medicine has gained popularity among scientists for disease prevention and treatment support [10]. With regard to lung-related disorders, studies have focused on common diseases such as asthma and lung injury, as well as more complicated diseases such as lung cancer. Numerous phytochemicals have been investigated for their ability to support the treatment of the aforementioned diseases, however only few of these phytochemicals will be discussed in this section as examples.

a) Phytochemicals used in asthma

Asthma with acute exacerbation is a chronic inflammatory disease of the respiratory tract, which has remained a globally serious disease with high morbidity and mortality [11, 12]. In asthma, histamine is the key trigger that increases airway resistance, leading to breathlessness, wheezing, coughing and chest tightness [13]. The disease is also associated with free radicals that cause the inflammatory response and gene expression of proinflammatory mediators through stimulation of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) [14, 15]. Thus, free radical scavengers or antioxidant molecules may be used to protect the lung against this problem.

Alkaloids, tannins, flavonoids, and phenolic compounds were reported as suitable candidates for the prevention of asthmatic attacks [14, 15]. These compounds are present in various parts of plants, such as barks, leaves, roots, and seeds. The majority of the activities are related to their ability to suppress cytokine, chemokine, or adhesion molecule synthesis or through instructing enzymatic breakdown via endogenous anti-oxidative enzymes, synthesis of different antioxidants and quenchers. Several antioxidant vitamins (i.e. carotenoids, vitamin C, vitamin E) and volatile compounds (i.e. phytoncides) [15] have demonstrated potential activity in asthma.

Carotenoids, vitamins C and E, can act as powerful antioxidants, with few studies recommending dietary consumption of antioxidants for asthmatic patients. A systematic review and meta-analysis done by Allen and co-workers revealed that lower dietary intake of vitamins C and A was associated with an increased risk of asthma [16]. Further, in another study in Thai children, low intake of vitamin C was associated with the severity of asthma and decreased pulmonary function in children [17]. A higher mean plasma of 8-iso-prostaglandin F2α (PGF2α), an oxidative stress product, in the vitamin C deficiency group suggested the effect of high oxidative stress in severe asthmatic patients.

Phytoncides are volatile compounds released by plants to protect themselves against some insects and animals. The major constituents of phytoncides are volatile terpenoids; for example, α-pinene, carene and myrcene. Another example of phytoncide is 1,8-cineole (also known as eucalyptol), a major compound of many plants' essential oils, mainly the Eucalyptus globulus oil. In a double-blind, placebo-controlled trial, the therapeutic use of 1,8-cineol was studied in patients with steroid-dependent bronchial asthma [18]. Patients receiving 1,8-cineol, 200 mg thrice daily, have remained clinically stable despite a reduction of their oral steroid administration. Meanwhile, most patients in the placebo group could not tolerate the decrease in their oral steroids. Thus, this was the first study to suggest the clinical relevance of the compound in bronchial asthma with a significant steroid-saving effect in patients. One of its possible mechanisms of action has been associated with the inhibition of cytokines production in Der p-stimulated bronchial epithelial cells (BEAS-2B) by suppressing the activation of p38 mitogen-activated protein kinase (MAPK), protein kinase B signalling [19] and the expression of toll-like receptor 4 (TRL4) [20]. The demonstrated anti-inflammatory effect could be an alternative to steroids in the management of airway inflammation in asthma patients.

b) Phytochemicals used in lung cancer

Lung cancer is the most common source of cancer death worldwide, contributing to 1.8 million deaths in 2020 [21]. Lung cancer can be divided into two groups based on its major pathological distinctions, i.e., small cell lung carcinoma (SCLC) and non-small cell lung

carcinoma (NSCLC). The latter accounts for 80-90% of lung cancer cases [22]. Apart from the standard pharmacological treatment, phytochemicals have been widely studied for their roles in the prevention and supporting treatment of lung cancer. The compounds that have shown remarkable activity include green tea polyphenols, genistein, curcumin, fisetin, quercetin, resveratrol and capsaicin [23, 24].

Tea is a common refreshment enjoyed worldwide, which is extracted from the dried leaves of Camellia sinensis. (-)-epigallocatechin-3- gallate (EGCG), a major compound in green tea, has shown anticancer activity in the mouse lung tumour model [25]. ECGC was shown to induce the microRNA (miRNA) profile changes which further targets genes that are responsible for the anticancer activity of ECGC. Another study reported that one of the miRNAs involved was miR-210, a major miRNA regulated by hypoxia-induced factor 1α (HIF-1α) [26]. Over expression of miR-210 could cause the inhibition of proliferation and anchorage-independent growth in human (i.e., H1299, H460, A549) and mouse (i.e., CL13) lung cancer cells.Despite the anticancer potential, EGCG was found to be a subject of extensive methylation in cells which may limit its activity. However, in a study by Wang and co-workers, a combination of EGCG with quercetin was shown to reduce the methylation of EGCG in lung cancer cell lines (i.e. A549) through the inhibition of catechol-O-methyltransferase (COMT) and multidrug resistance proteins (MRPs) by quercetin [27].

Quercetin is a flavonoid which is abundantly found in fruits, such as berries and citrus fruits, and vegetables, including broccoli and onions. This compound was shown to inhibit the proliferation of HCC827, an NSCLC cell line, via the inhibition of the Src-mediated Fn14/NF-κβ pathway [28]. Src is a protein kinase identified as a pro-tumour factor for various cancers, including NSCLC. The finding supported the ability of quercetin to suppress the proliferation and metastasis of the NSCLC cells. Further, quercetin has also shown its potential in the treatment of tyrosine kinase inhibitor (TKI)-resistant NSCLC. In a recent study by Huang and co-workers, quercetin demonstrated a potent cytotoxic activity on NSCLC cell line with EGFR C797S mutation via inhibition of tyrosine kinase receptor, AXL, and apoptosis induction [29]. Quercetin also showed synergistic activity with brigatinib in the inhibition of tumour growth in a tumour-mice model, which was likely attributed to each molecule's inhibitory activities in different pathways.

c) Phytochemicals used in lung injury

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is a common life- threatening lung disease with high morbidity and mortality [30]. It involves rapid inflammation of the lung that happens in less than seven days, disrupting the lung endothelial and epithelial barriers. The risk factors include genetics, age, smoking history and chronic alcohol abuse, which could induce inflammation and excessive accumulation of macrophages, neutrophils, leukocytes and platelets [31, 32]. The presence of excessive white blood cells will further induce the production of pro-inflammatory factors such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-9 (IL-9) and interleukin-8 (IL-8) as well as inflammatory mediators, which can damage the vascular and alveolar endothelium, causing pulmonary edema and difficulty in air exchange. In addition, oxidative stress could also exacerbate the ALI/ARDS condition [32].

Until now, there has been little progress in the development of therapies for ALI/ARDS. A number of corticosteroids, such as dexamethasone and prednisolone, are largely used for anti-ALI/ARDS activity. However, they are associated with undesirable side effects, including peptic ulcer, osteoporosis and coagulation dysfunction [33], making the treatment for ALI/ARDS an unmet medical need. Therefore, the alternative supporting treatment of the disease using phytochemicals, including flavonoids, alkaloids and terpenoids, has been explored, with plenty of the phytochemicals showing potential activity in ALI/ARDS therapy [31].

It has been widely reported that flavonoids have anti-inflammatory activity via the downregulation of several signalling pathways and the prevention of oxidative stress [31]. This is important in the treatment of ALI/ARDS. In one study, jaceosidin, a flavonoid isolated from *Eupatorium lindleyanum* DC, attenuated the inflammatory responses in a lipopolysaccharide (LPS)-induced ALI mouse model [34]. Its anti-inflammatory activity was attributed to the downregulation of pro-inflammatory mediators (i.e., TNF-α, IL-6 and IL-1β), together with upregulation of anti-inflammatory factors (i.e., IL-4 and IL-10) in bronchoalveolar lavage fluid (BALF). Treatment with jaceosidin also inhibited the activity of myeloperoxidase (MPO) and enhanced the activity of catalase (CAT), which suggested oxyradical scavenging during the process. The same finding was also reported for eriodictyol, a flavonoid isolated from *Dracocephalum rupestre* [35]. Apart from its ability to downregulate the proinflammatory factor, its protective effect in ALI was believed to be related to the suppression of NF-κB signalling and activation of the Nrf2 pathway. This function could further cause a reduction in inflammatory responses and oxidative injury. Another study demonstrated the anti-ALI activity of a third flavonoid, trilobatin, via the activation of AMPK/GSK3β-Nrf2 and the inhibition of the NF-κB pathway [36].

Aside from flavonoids, alkaloids also possess anti-inflammatory and antioxidant properties that could be favourable against ALI. Capsaicin, an alkaloid isolated from chilli peppers, has been shown to protect the lung against LPS-induced ALI in animal models [37]. The protection was achieved via downregulation of the high-mobility group protein B1 (HMGB1)/NF-κβ and PI3K/AKT/mTOR pathways which led to the inhibition of oxidative stress, inflammatory responses, and apoptosis. In another in vivo study done by Lu and co-workers, treatment with sophocarpine resulted in an anti-inflammatory effect against LPS-induced ALI [38]. The effect was achieved by the inhibition of TLR4 expression that could lead to inflammation, inhibition of NF-κB and activation of mitogen-activated protein kinases (MAPKs).

d) Phytochemicals used in pneumonia

Pneumonia is an infection that affects the lower part of the respiratory tract, specifically the alveoli of the lungs. It is commonly due to infection by several bacteria species. Among these, *Staphylococcus aureus* and *Streptococcus pneumoniae* are the most common causing agents for community-acquired pneumonia. Each of these bacteria affects the lung through different mechanisms, leading the lung to be filled with pus and fluid. This eventually leads to painful breathing and limited oxygen intake [39, 40].

Eradicating the causative microorganisms has been the mainstream treatment approach. The use of antibiotics such as penicillin faces challenges, especially with the development of resistant strains that hinder effective control of the disease. Hence, the potential of phytochemicals in the treatment of pneumonia, especially as a complementary medicine, is actively explored as an additional measure to conventional drug treatment. Among the many phytochemicals reported in literature, curcumin and its derivatives have been shown to have activity against all three strains of *Streptococcus pneumoniae* (i.e. penicillin-susceptible, -intermediate and -resistant) [41]. The lowest MIC was recorded for curcumin monoglucoside and curcumin diglucoside at 0.005 $mg.ml^{-1}$ and 0.007 $mg.ml^{-1}$, respectively, approximately three times lower than penicillin G (control). This finding was further validated using molecular simulation works, where the interaction between the derivatives and Penicillin Binding Protein of *S. pneumoniae* showed conformational stability in the dynamic situation. Similar findings were also reported in other studies. Curcumin and its derivatives have been shown to exhibit anti-bacterial activity against *P. aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumonia* and *S. aureus* [42, 43].

Piper nigrum L. is another plant that has been used traditionally in managing pneumonia. A number of studies have pointed out the activity of piperine, a phytochemical from this plant, against *S. aureus*. In one study, Khan et al. described the enhanced antibacterial activity of piperine in combination with ciprofloxacin against *S. aureus* and methicillin-resistant *S. aureus* (MRSA). It was suggested that piperine was involved in the inhibition of ciprofloxacin efflux pumps shown by the accumulation of ethidium bromide (i.e. a substrate of the efflux pump) in the bacteria [44].

e) Phytochemicals used in pulmonary fibrosis

Fibrosis is a condition manifested by an over-production of fibrous connective tissue in the lung that interferes with its normal function. It may be caused by an unsatisfactory repair process of the lung tissue following injury due to internal or external factors. The internal or host factors include the genetic and epigenetic issues that lead to the formation of susceptible and dysfunctional epithelium, whilst the external factors could be the micro-injuries that happen in the lung due to cigarette smoke, wood and metal dust, gastro-oesophageal reflux, and also viral infection, such as COVID-19. Exposure to these factors may lead to the production of reactive oxygen species and subsequent inflammatory reaction in the lung, which causes lung injury. The treatment options available are not satisfactory, with lung transplantation as the only cure in critical conditions, and the available drugs could only reduce the symptoms experienced by patients [45].

Tahir and co-workers (2016) evaluated the activity of the methanolic extract of *Phyllanthus emblica* leaves (PELE) against pulmonary fibrosis in vitro and in vivo [46]. They found that the polyphenols and flavonoids such as gallic acid, rutin, kaempferol and caffeic acid in the extract of PELE may play important roles in the recovery against pulmonary damages in fibrosis through elevation in the activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione (GSH), whilst reducing the level of hydrogen peroxide and nitric oxide. Their finding reiterated that PELE has the ability to help in the recovery from pulmonary damage and, at the same time, maintain the functional integrity of the tissues. This finding was similar to the work done by Özyurt et al., who have also reported the inhibition of pulmonary fibrosis by caffeic acid phenethyl ester (CAPE). CAPE is structurally similar to naturally occurring flavonoids with free radical scavenging and antioxidant characteristics [47].

Flavonoids are a group of phytochemicals that can play an important role in lung fibrosis. In one study, luteolin has been shown to prevent lung fibrosis and airway mucus overproduction, with evidence that it was generally more effective than prednisolone at 10 $mg.kg^{-1}$ in mice [48]. It downregulated the expression of pro-inflammatory cytokines and promoted the production of anti-oxidases and GSH, besides reducing

the expression of microRNA MiR-132 that inhibited the activation of pro-inflammation NF-κB [31]. Other phytochemicals have also been reported to have good activity against pulmonary fibrosis, including cryptotanshinone from *Salvia miltiorrhiza* Bunge [49], naringin from grapefruit and citrus fruits [50], corilagin from *Terminalia chebula* Retz [51] and juglanin from *Juglans mandshurica* [52].

In general, it can be said that the phytochemicals that showed activity in pulmonary fibrosis mainly have anti-inflammatory and antioxidant activities that contribute to their protective effect. As fibrosis is one of the after-effects of COVID-19 infection, these phytochemicals may also have high potential to be developed as single-use or combined therapy in the prevention and/or treatment support of COVID-19 induced pulmonary fibrosis.

NANOFORMULATIONS OF PHYTOCHEMICALS TO TREAT LUNG DISEASES

Phytochemicals have undeniably proven their potential benefits in the prevention and supporting the treatment of many diseases. However, their usage has been restricted by their low solubility, which further affects the formulation stability and bioavailability. In this regard, nanotechnology can be one of the solutions

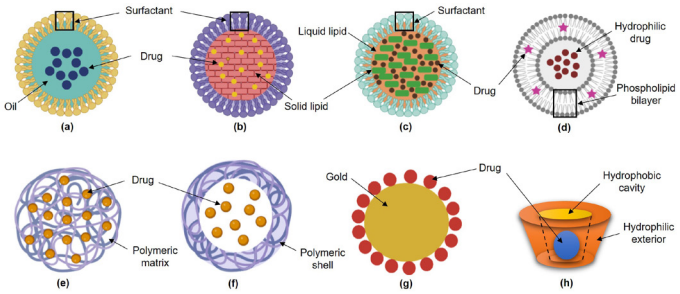

Figure 1 : Schematic representation of various nanocarriers for the delivery of phytochemicals against lung diseases; (a) nanoemulsion, (b) solid lipid nanoparticles, (c) nanostructured lipid carrier, (d) liposomes, (e) nanospheres, (f) nanocapsules, (g) gold nanoparticles and (h) cyclodextrin complex. Created with BioRender.com

to increase their solubility and further enhance their absorption and bioavailability. Encapsulation of the phytochemicals inside the carriers could also provide protection from premature degradation and further extend their circulation in the body [9].

Nanocarriers can be of organic sources, which include nanoemulsion, liposomes and polymeric nanoparticles, as well as inorganic sources such as gold/silver nanoparticles (Fig. 1). In this section, different nanoformulations used in the formulation

Table I : Summary of selected nanoformulations of phytochemicals used for the treatment of lung diseases with their key findings

Nanoformulations	Phytochemicals	Lung diseases	Key Findings	References
Nanoemulsion	Jojoba oil	Acute lung injury	The nanoemulsion showed suitable characteristics for deposition in the deep lung region and effectively reduced the total protein concentration and inflammation markers when inhaled in LPS-induced ALI rat models.	[56]
Nanoemulsion	Curcumin	Lung cancer	Treatments using curcuminoid nanoemulsion caused apoptosis on lung cancer cells (i.e. A549 and H640) via dose-dependent increment of caspase-3, -8, and -9 enzymes, increment in cytochrome C expression, and reduction of cyclin-dependent kinase 1 (CDK1) expression.	[57]
Nanoemulsion	Curcumin	Lung cancer	Oral administration of curcumin-loaded nanoemulsion-based lipid nanosystems resulted in enhanced cytotoxic activity against the A549 cells, enhanced gastrointestinal absorption, and an increase in the relative bioavailability when compared to non-formulated curcumin.	[58]
Solid lipid nanoparticles (SLN)	Grape seed extract (GSE)	Chronic respiratory diseases	The encapsulation of GSE containing proanthocyanidins in SLN showed sufficient stability in the simulated lung fluid by maintaining the particle size and providing a controlled release of the GSE.	[60]
Nanostructured lipid carrier (NLC)	Curcumin	Lung cancer	The curcumin-loaded NLC formulation improved the pharmacokinetics and tissue distribution of curcumin and also improved the *in vitro* anticancer activity against the A549 cells.	[63]
Nanostructured lipid carrier	Oleuropein	Lung cancer	The NLC formulation containing oleuropein showed a loading of up to 50 %w/w with a sustained release profile. The NLC improved the antioxidant activity of oleuropein on the A549, NuLi-1, and CuFi-1 cell lines with the absence of toxicity.	[64]

Liposomes	Genistein	Lung cancer	The nebulised liposomes co-loaded with genistein and erlotinib showed a high fine particle fraction when nebulised using a jet nebuliser.	[71]
Liposomes	Curcumin	Lung cancer	A dry powder of curcumin-loaded liposome showed favourable characteristics for inhalation and a higher uptake into the A549 cells.	[72]
Polymeric nanoparticles	Andrographolide	Asthma	Pulmonary delivery of PLGA nanoparticles loaded with andrographolide showed an improved bioavailability compared to the free drug with better anti-asthmatic activity.	[75]
Polymeric nanoparticles	Ferulic acid	Asthma	Aerosolisation of ferulic acid-loaded chitosan nanoparticles with hyaluronic acid surface functionalisation demonstrated high deposition in the lower respiratory tract. This formulation enhanced the anti-asthmatic effects and has been proven safe and well-tolerated in vivo.	[76]
Metallic nanoparticles	Syringic acid	Lung cancer	Zinc oxide nanoparticles loaded with syringic acid showed cytotoxicity against the A549 cell line and were effective in treating lung cancer in a mouse model.	[80]
Metallic nanoparticles	*Artemisia oliveriana* extract	Lung cancer	Silver nanoparticles synthesised using *Artemisia oliveriana* extract exhibited antibacterial activity on gram-positive bacteria and demonstrated cytotoxicity on the A549 cell line.	[82]
Metallic nanoparticles	*Moringa oleifera* leaf extract	Lung cancer	*Moringa oleifera* leaf extract was used to produce gold nanoparticles which exhibited cytotoxicity against the A549 cell lines but were not cytotoxic to normal peripheral blood mononuclear cells (PBMC).	[83]
Cyclodextrin complexes	Tetrandrine	Pulmonary fibrosis	Lung delivery of tetrandrine-hydroxypropyl-β-cyclodextrin complex was found to alleviate inflammation and fibrosis in bleomycin-induced pulmonary fibrosis rat model.	[87]
Cyclodextrin complexes	Paeonol	Acute lung injury	Spray-dried γ-cyclodextrin metal organic frameworks (CD-MOFs) containing paeonol showed a good fine particle fraction and exhibited rapid absorption, high absolute bioavailability, and reduced lung inflammation in LPS-induced ALI-bearing rats.	[88]
Cyclodextrin complexes	Andrographolide	Pneumonia	Intratracheal administration of andrographolide-β-cyclodextrin complex resulted in a significant anti-pneumonic effect and reduced inflammation in *Staphylococcus aureus*-pneumonic rat models.	[89]

of phytochemicals (Table I) will be discussed for their application in lung diseases via different administration routes; oral and pulmonary.

a) Lipid-based nanoformulations

i) Nanoemulsions

Nanoemulsions were first introduced in the 1950s for the purpose of parenteral nutrition. The formulation uses vegetable oils (e.g. soy oil) or medium-chain triglycerides for the lipid phase, which accounts for 10 – 20 percent of the emulsion [53]. Nanoemulsions are usually in droplets of water-in-oil or oil-in-water, with a mean diameter ranging from 20 to 1000 nm, with a translucent or transparent appearance (Fig. 1(a)). The application of nanoemulsions as a delivery vehicle may avoid concerns associated with traditional dosage forms of anticancer drugs, including non-targeted distribution, systemic toxicity, low bioavailability, and instability [54]. Nanoemulsions have also been shown to protect a phytochemical from degradation, offering long-term stability during storage and site-specific drug delivery [55]. This may allow administration via various routes, including intravenous, topical, and oral.

In a recent study, jojoba oil nanoemulsion powder was found to be a potential natural oil-based inhalable medicine for the treatment of acute lung injury caused by LPS or hydrogen peroxide (H_2O_2) [56]. The dried powder showed a mass median aerodynamic diameter (MMAD) of 4.17 μm and fine particle fraction (FPF) of 39 %, which would be suitable for deposition in the deep lung region. The effectiveness was proven when given by inhalation to the LPS-induced ALI rat models whereby the formulation reduced total protein concentration and down-regulated TNF-α, IL-1, IL-6 and NF-κB p65.

Curcumin is another interesting phytochemical used in lung delivery, with few studies exploring nanoemulsion as a carrier for the compound. A study by Chang and Chen showed that treatments using curcuminoid nanoemulsion on lung cancer cells (i.e., A549 and H640) led to cell apoptosis in both cells [57]. The treated cells showed a dose-dependent increase in caspase-3, caspase-8, and caspase-9 activities, as well as an increment in cytochrome C expression. Besides, the cyclin-dependent kinase 1 (CDK1) expression was reduced in a dose-dependent manner, which

suggested that the apoptosis might be caused by both mitochondria and death receptor pathways. Another group of researchers explored the possibility of administering curcumin using nanoemulsion-based lipid nanosystems via the oral route for the treatment of lung cancer [58]. In the study, curcumin was first encapsulated in water-in-oil nanoemulsion prior to loading into a lipid matrix. Apart from the enhanced cytotoxic activity against the A549 cells by the nanosystems, it also showed improvement in the absorption constant and effective permeability in the gastrointestinal tract when compared to non- formulated curcumin. This led to an increase in the relative bioavailability of the formulated curcumin to non-formulated curcumin by 734%.

ii) Solid lipid nanoparticles (SLN)

Besides nanoemulsion and other traditional colloidal carriers, an alternative carrier was introduced in 1991 with the first development of solid lipid nanoparticles (SLN) by Müller [59]. SLN is essentially similar to the nanoemulsions system, except that it uses solid lipids instead of liquid lipids and is stabilised by emulsifiers (Fig. 1(b)). Since its introduction, SLN has been widely studied in various applications such as pharmaceutical, cosmetic and agriculture. Castellani and co-workers reported the encapsulation of grape seed extract (GSE) containing proanthocyanidins in SLN for the treatment of chronic respiratory diseases [60]. In the study, the GSE-loaded SLN showed sufficient stability by maintaining the particle size, 243 nm, in the simulated lung fluid for up to 48 hours. The in vitro results in airway epithelial cells, H441, also suggested a controlled release of GSE from the SLN, which was exhibited by a longer duration of antioxidant activity of the formulation compared to the free GSE. However, SLN has several limitations, including low loading capacity and potential expulsion of the encapsulated active compound during storage. This has made SLN a less popular choice compared to the other delivery systems.

iii) Nanostructured lipid carrier (NLC)

Nanostructured lipid carrier (NLC) was introduced as a second-generation lipid nanocarrier [61]. NLC consists of both solid and liquid lipids and is stabilised by emulsifiers (Fig. 1(c)). In contrast to the SLN, the carrier can provide a higher active loading and firmer incorporation of the active inside the particle matrix during the shelf life [62].

Curcumin has been fabricated into the NLC system by Wang et al. for the treatment of lung adenocarcinoma [63]. The study found that NLC formulation improved the pharmacokinetics and tissue distribution of curcumin, as well as outperforming free curcumin in the in vitro anticancer activity against A549 cells. The latter activity was caused by a greater proliferation inhibition and apoptosis induction by the NLC system compared to the free curcumin. Oleuropein, a polyphenol from olive leaves (*Olea europaea L.*), is another phytochemical that has been incorporated into NLC formulation for its antioxidant activity in the lung [64]. The formulation showed effective loading of up to 50 %w/w and a sustained release characteristic of the compound from the lipid core. The NLC was shown to improve and maintain the antioxidant activity of oleuropein on A549, normal bronchial epithelial (NuLi-1) and cystic fibrosis (CuFi-1) cell lines. The carrier also demonstrated the absence of toxicity in the tested cells. The effectiveness of the carrier and its biocompatibility with the lung cells suggest that it has good potential to be developed for pulmonary administration.

iv) Liposomes

Liposomes were first introduced by Bangham et al., which can be described as tiny spherical- shaped vesicles made of phospholipids, cholesterols and non-toxic surfactants [65] (Fig. 1(d)). The unique characteristics of the carrier that could mimic cell membranes have enabled them to overcome biological barriers, resulting in improved pharmacodynamics [66].

Liposomal drug delivery system has widely been studied as a vehicle for targeted delivery to reduce the cytotoxic effect of therapeutic molecules on normal cells [67]. Genistein-loaded liposomes have been shown to possess chemopreventive activity in vitro and in vivo [68]. In another study, genistein was encapsulated in simple and stealth liposomes. The formulations were able to preserve the antioxidant activity of the compound, as well as significantly improve the anticancer activity in murine and human cancer cell lines when compared to free molecules [69]. Further characterisation revealed that genistein-loaded liposomes triggered apoptosis in the cancer cells via strong depolarisation of mitochondria. Incorporation of genistein with another anticancer agent was also possible using nanoliposomes, as reported by Song and co- workers [70]. The combined liposomal formulation of genistein and plumbagin demonstrated similar release kinetics patterns for both agents, which could explain their synergistic activity on the targeted cancer cells. The liposomal formulation was also shown to inhibit the growth of xenografted prostate tumour by inhibiting PI3K/AKT3 signalling pathway and reducing the glucose transporter 1 (Glut-1).

In terms of feasibility for lung targeting, a study conducted by Nimmano and colleagues has explored the production of nebulised liposomes co-loaded with genistein and another anticancer agent, erlotinib [71]. The liposomes were prepared by thin-film hydration method and probe sonication. In this study, a jet nebuliser has been shown to be more suitable for the

formulation as it showed a higher fine particle fraction in the in vitro aerosol characterisation, as compared to a mesh nebuliser. Apart from pulmonary administration in solution form, liposomal dry powder inhalation is also possible. In one study, curcumin-loaded liposome dry powder formulation was prepared with favourable characteristics for inhalation; mean aerodynamic diameter (MMAD) of 5.81 μm and fine particle fraction of 46.71% [72]. The curcumin liposomes showed a higher and faster uptake into A549 cells, as well as low cytotoxicity on normal human bronchial epithelial cells (i.e., BEAS-2B), as compared to free curcumin. Thus, localised delivery of the formulation to the lung, combined with high therapeutic efficiency, may be promising as a future therapy for lung cancer.

b) Polymeric nanoparticles

Polymeric nanoparticles are particles of the size between 1 to 1000 nm, packed with active ingredients either assembled on the interior or exterior part of a polymeric core. Nanocapsules and nanospheres are two types of polymeric nanoparticles with different structures that can be produced by different methods, including solvent evaporation and nanoprecipitation [73]. Nanocapsules use the principle of a reservoir system (Fig. 1(f)). These particles are made up of the aqueous or oil-based inner core and a polymeric outer shell, with the active ingredients loaded in the inner core. Nanospheres, on the other hand, function on the concept of matrix systems (Fig. 1(e)), in which the active substances are incorporated within or on the surface of the polymeric chains [73, 74].

Chakraborty and colleagues have fabricated PLGA nanoparticles, encapsulating andrographolide, to compare its bioavailability and anti-asthmatic efficacy via oral and pulmonary routes [75]. When compared to free andrographolide, the nanoparticles showed greater anti-asthmatic impact by lowering histamine levels, eosinophil counts, and interleukins IL-4, IL-5, and IL-13 levels in the broncho-alveolar lavage fluid of ovalbumin-induced mouse asthma model. Although both administration routes demonstrated improved bioavailability compared to free andrographolide, pulmonary administration showed a higher percentage of andrographolide-loaded nanoparticles in the lung compared to oral administration. Thus, pulmonary delivery was suggested as the optimum route of administration for the nanoparticles in the treatment of asthma due to its superior therapeutic results.

In another investigation of anti-asthmatic properties of a phytochemical formulation, ferulic acid was encapsulated in chitosan nanoparticles with hyaluronic acid surface functionalisation [76]. Aerosolisation of the nanoparticles using a mesh nebuliser has produced droplets with MMAD of 1.81 μm, which allow high deposition of the formulation to the lower respiratory tract. The presence of hyaluronic acid, a second-generation mucoadhesive agent, has increased the therapeutic efficacy of ferulic acid nanoparticles. Bronchoconstriction, hypersensitivity, and the number of cells responsible for the inflammation in asthma have been reduced. Further, the formulation has been proven safe and well-tolerated in vivo.

c) Metallic nanoparticles

Metallic nanoparticles are submicron (10 – 1000 nm) colloidal particles made of pure metals (e.g., gold, silver) or metal oxides (e.g., silver oxide, zinc oxide) (Fig. 1(g)). These nanoparticles have been used in various fields, including bioimaging [77], photothermal therapy [78] and drug delivery [79]. In terms of drug delivery, a therapeutic molecule can be either dispersed or covalently attached to the surface or encapsulated within the structure.

As an example, zinc oxide nanoparticles loaded with syringic acid, a natural polyphenolic compound, were synthesised and tested for their anticancer activity in vitro and in vivo [80]. The nanoparticles were found to be cytotoxic to the A549 cell line via induction of ROS, disruption of mitochondrial membrane potential and morphological alteration. The syringic acid-loaded nanoparticles were also shown to be effective in treating lung cancer in a mouse model and have also been shown to reduce cancer marker enzymes such as aryl hydrocarbon hydroxylase (AHH) and lactate dehydrogenase (LDH).

Apart from that, various plant extracts were also used in the synthesis of metal nanoparticles using the biosynthesis method. Biosynthesis is a green technology that uses biological precursors such as fungi, bacteria, algae and plant extract that offers a more energy-efficient process and avoidance of hazardous chemicals. The abundance of secondary metabolites in plant extracts allows them to act as reducing and stabilising agents during the synthesis of metallic nanoparticles [81]. In one study, *Artemisia oliveriana* extract was used in the synthesis of silver nanoparticles [82]. Besides showing antibacterial activity on gram positive bacteria, the produced nanoparticles also showed significant cytotoxicity on the A549 cell line via the expression of apoptosis-related genes, including Bax, caspase-3 and caspase-9. Similar findings were also reported by Tiloke et al., who reported on the application of *Moringa oleifera* leaf extract to produce gold nanoparticles [83]. The nanoparticles were found to be cytotoxic and have the ability to induce apoptosis in A549 cell lines but were not cytotoxic to normal peripheral blood mononuclear cells (PBMC).

d) Cyclodextrin complexes

Cyclodextrins are cyclic oligosaccharides that are produced through the activity of cyclodextrin

glucanotransferase enzyme on starch. The natural cyclodextrins contain six (α-), seven (β-) and eight (γ-) (α-1,4)-linked α-D-glucopyranose units [84]. Random substitution of multiple hydroxyl groups on the β- and γ-cyclodextrins with several functional groups, such as hydroxypropyl and sulfobutylether, improves the aqueous solubility and toxicological profile of the molecules [85].

Cyclodextrin has truncated cone structure with a hydrophilic exterior and a hydrophobic cavity [86]. This is an interesting design in drug delivery as it allows the formation of a reversible inclusion complex or host-guest complex between cyclodextrin and a lipophilic drug/molecule (Fig. 1(h)). Various phytochemicals have been explored and incorporated into the structure. In one recent study, an inclusion complex of tetrandrine with hydroxypropyl-β-cyclodextrin was prepared using the freeze-drying method [87]. Intratracheal instillation of the complex in a bleomycin-induced pulmonary fibrosis rat model showed alleviation of inflammation and fibrosis, besides enabling the regulation of protein expression that occurs during disease development. Additionally, localised delivery to the lung has also limited the systemic distribution of tetrandrine as compared to intravenous administration.

In another study, paeonol, a natural phenolic compound, was complexed with γ-cyclodextrin to form γ-cyclodextrin metal organic frameworks (CD-MOFs) [88]. The complex was spray dried to form dry powder with good fine particle fraction (~28%). In vivo inhalation of the dried complex in rats showed rapid absorption and high absolute bioavailability compared to oral administration due to an increase in the permeability of the complex in bronchial and alveolar epithelial cell monolayers. A reduction in lung inflammation in LPS-induced ALI-bearing rats was also observed.

Andrographolide, a natural diterpenoid, was complexed with β-cyclodextrin and administered to *Staphylococcus aureus*-pneumonic rat models via intratracheal instillation [89]. The treatment showed a significant anti pneumonic effect and alleviation of inflammation through the regulation of immune responses. The improvement in the therapeutic activities may be attributed to the solubility improvement of andrographolide, which further accelerated its dissolution rate.

CONCLUSION AND FUTURE PERSPECTIVE

Herbal medicine, either as a single compound, extract, or whole part, has been studied and suggested to be beneficial in supporting the treatment of many diseases. Occasionally, the selection of the plants is based on ethnobotany, which observes the use of indigenous plants by people from certain cultures or religions as remedies for their injuries and diseases [90]. While the activity of certain plants has been proven in that community, pharmacological and pre-clinical studies are important to understand the efficacy, as well as establishing the toxicities related to the extracts or compounds. In modern medicine, phytochemicals have not only shown their potential as a single compound but also when combined with existing pharmacotherapy.

They can offer synergistic activities by targeting different pathways of disease, especially in lung cancer therapy, in which the phytochemical could potentiate the activity of available anticancer agents and prevent or reverse resistance towards the therapy.

Although pre-clinical activities of these phytochemicals have been proven, many of them are hydrophobic in nature which has proven to be a challenge in formulation, besides having low bioavailability when given orally. Thus, formulating the compounds into nanoformulations is an attractive approach to improve bioavailability as nanoformulations usually have a higher area-to-volume ratio and are able to encapsulate hydrophobic molecules. They can also provide sustained and controlled release of the entrapped molecule. Furthermore, the decoration of the nanoparticle surfaces with targeting moieties will allow the application of active drug targeting, which could potentially increase the therapeutic activity at the target cells. When treating diseases involving the lung, inhalable formulation would be an attractive option, together with the advantages of nanoformulations. The pulmonary route will further ensure higher concentration at the diseased site and reduce the systemic side effect.

The studies presented in this review would surely contribute to the advancement of the treatment of lung disorders, especially in lethal diseases such as lung cancer. Despite the promising benefits, the upscaling of these formulations is another challenge that needs to be addressed. One example is the scaling up of nanoliposomes formulation. Although in the laboratory, the thin film rehydration method is a popular choice, it is difficult to be scaled up. The scaling-up method might not be able to produce homogenous and appropriate particle size with the lipid film [91]. This would be one of the issues that need to be taken into consideration as unnecessarily sophisticated formulation processes would become a hurdle at a later stage of development. This could also hinder the advancement of this drug delivery system into clinical trials, which need a large batch size of quality-controlled raw materials and formulation process.

Currently, clinical trials involving phytochemicals for treating lung diseases are still limited and are using simple formulations such as tablets (searched on

clinicaltrials.gov on 10 February 2023). Further studies are needed to evaluate the cost-effectiveness and long-term safety of nanoformulations for phytochemicals. Since an abundance of pre-clinical studies has shown the potential of these phytochemicals, the authors are optimistic that these phytochemical-based formulations will surely find their place alongside conventional therapy in the future.

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