A SYSTEMATIC REVIEW ON CYTOTOXICITY OF PLANT-MEDIATED METALLIC NANOPARTICLES

By

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DEDICATION PAGE

Special praise be to Allah,
The Sustainer of the creation,
The Compassionate,
The Merciful.

Highest and profound gratitude to my husband,
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whom always be my spirit burner and driving force,
in striving me for excellence.
I cannot thank you enough for all the support,
tolerance, and love that you have given me.
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Seek knowledge from the cradle to the grave.

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and the difference you each made in my life.
Go forward in our lives and make the most for every moment.

This quest for knowledge
has been challenging but incredible from its beginning.
May this study and knowledge from it propagate us towards excellence!

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293	Embryonic human kidney (293) cells
3T3-L1	Murine adipocyte cells
5-FU	5-fluorouracil
A375	Human melanoma cells
A549	Human epithelial lung carcinoma cells
Ag	Silver
AgNp	Silver nanoparticles
AGS	Gastric adenocarcinoma cells
Au	Gold
AuNP	Gold nanoparticles
C26 (murine)	Mouse colon carcinoma cells
Caco-2	Human epithelial colorectal adenocarcinoma cells
Colo 205	Human colorectal adenocarcinoma cells
CV-1	Normal African Green monkey kidney fibroblast cells
DAL	Dalton's ascites lymphoma
ED_{50}	Median effective dose
GBD	Global Burden of Diseases
GI_{50}	Median growth inhibition concentration
HaCaT	Human keratinocytes
HBL100	Human breast epithelial
HCT15	Human colon adenocarcinoma
HCT-116	Human colon carcinoma
HeLa	Human cervical cancer cells

Hep-2	Human laryngeal carcinoma cells
Hep3B	Human hepatocellular carcinoma cells
Hep-G2	Human hepatoma cells
HL-60	Human promyelocytic leukemia cells
HSFs	Human skin fibroblast cells
HT1080	Human fibrosarcoma cells
HT29	Human colorectal adenocarcinoma cells
IC_{50}	Median inhibitory concentration
Jurkat	T-lymphocyte cells
KG-1A	Acute myeloid leukemia cells
L929	Mouse fibroblast cells
LD_{20}	Lethal dose 20 percent
LD_{50}	Median lethal dose
LNCap-FGC	Human prostate carcinoma cells
MaAgNP	*Melia azedarach* silver nanoparticles
MCF7	Human breast cancer cells
MDA-MB-231	Human adenocarcinoma mammary gland
MDCK	Madin Darby canine kidney cells
MG63	Human osteoblast-like cells
MKN28	Gastric cancer cells
NA	Not applicable / Not available
NCDs	Non-Communicable Diseases
NO	Nitrogen oxide
PBMC	Peripheral blood mononuclear cells
PEG	Poly ethylene glycol
PRISMA-P	Preferred Reporting Items for Systematic review and Meta-Analysis Protocols
RAW 254.7	Murine macrophage cells
RoBANS	Risk of Bias Assessment tool for Non-randomized Studies
ROS	Reactive oxygen species
TI	Therapeutic index
TNF-α	Tumor Necrosis Factor alpha
U87	Human glioblastoma

Vero	African green monkey kidney cells
WHO	World Health Organization
WI-38	Human fetal lung

ABSTRAK

Kajian sistematik terhadap kesitotoksikan nanopartikel logam berperantara tumbuhan

Nanoteknologi menawarkan kegunaan yang meluas dalam pelbagai bidang seperti kejuruteraan, telekomunikasi, pengiklanan, tekstil, kosmetik, dan juga perubatan. Keunikan nano-bahan membolehkannya digunakan sebagai agen anti-kanser mahupun penghantar ubat dalam bidang perubatan, terutamanya nano-bahan yang diperoleh daripada tumbuhan. Walau bagaimanapun, isu bio-keselamatan nanopartikel logam yang digunakan sebagai agen anti-kanser harus dititikberatkan. Oleh itu, kajian ini dijalankan bagi membandingkan kesitotoksikan nanopartikel logam berperantara tumbuhan, berdasarkan potensi, kecenderungan jenis sel kanser, serta indeks terapeutik agar calon agen anti-kanser yang terbaik dan selamat dapat di temui. Kajian ini juga cuba mengaitkan faktor saiz dan rupa bentuk dengan potensi kesitotoksikan suatu nanopartikel. Pencarian artikel kajian dijalankan menggunakan pangkalan data elektronik seperti Science Direct, Elsevier, PubMed, Springer Link dan Google Scholar. Selain itu, laman rangkaian sosial akademik, ResearchGate turut digunakan sebagai sumber artikel kajian rujukan. Kata kunci seperti biosintesis, sintesis tumbuhan, berperantara tumbuhan, nanopartikel logam, kesitotoksikan dan anti-kanser digunakan semasa proses pencarian dijalankan. Semua jenis kajian yang memenuhi kriteria rangkuman dan pengecualian telah dipertimbangkan tidak kira apa jua keputusannya. Maklumat dan data daripada 76 artikel kajian yang terpilih telah diambil kira dan dikaji. Indeks terapeutik digunakan dalam kajian ini sebagai panduan keselamatan bagi sebatian yang

dikaji. Kebanyakan kajian yang terpilih menunjukkan bagaimana kesitotoksikan nanopartikel logam berperantara tumbuhan bergantung pada masa dan/atau dos. Nanopartikel perak didapati lebih sitotoksik berbanding nanopartikel emas daripada tumbuhan yang sama; *Cassia auriculata, Commelina nudiflora,* dan *Plumbago zeylanica* digunakan bagi sintesis tersebut tanpa mengira jenis sel kanser yang terlibat. Kesitotoksikan didapati berkadar songsang berbanding saiz nanopartikel. Sebahagian besar nanopartikel logam berperantara tumbuhan didapati berbentuk sfera, dengan bentuk sfera dan hampir-sfera yang paling kerap di temui dengan mod populasi LD_{50} 1–20 µg/mL, diikuti bentuk lain; segitiga, heksagon dan rod yang menunjukkan potensi sitotoksik yang lebih rendah. Nanopartikel logam daripada *Abutilon inducum*, *Annona squamosa, Butea monosperma, Couroupita guainensis, Gossypium hirsutum, Indoneesiella echioides,* dan *Melia azedarach* didapati selamat digunakan sebagai agen anti-kanser kerana mempunyai indeks terapeutik bernilai 2.0 dan ke atas apabila diuji terhadap sel kanser mahupun sel normal manusia. Hasil keputusan kajian ini mencadangkan agar kajian lebih mendalam dijalankan terhadap agen antikanser berpotensi yang disenaraikan untuk mengetahui lebih lanjut tentang fungsi farmakodinamik/toksikodinamik dan farmakokinetik/toksikokinetik tumbuhan tersebut dalam usaha untuk mengurangkan penyakit kanser, iaitu Beban Penyakit Global (Global Burden of Diseases/GBD) dan juga penyebab kematian kedua tertinggi di dunia.

ABSTRACT

A systematic review on cytotoxicity of plant-mediated metallic nanoparticles

Nanotechnology has undeniably offered a variety of applications, ranging from engineering, telecommunication, advertising, textile, cosmetics and even medicine. Due to its unique properties, nanomaterials suggest a promising use in medicine such as being anti-cancer agents along with drug-carriers, especially those which are derived using the green method. However, concern arises on the bio-safety of metallic nanoparticles used as anti-cancer agents. Therefore, this systematic review aims to compare the cytotoxicity of plant-mediated metallic nanoparticles based on its potency, cancer cell type susceptibility, and therapeutic index in the hopes of finding the most promising anti-cancer agents. This study also correlates nanoparticle size and morphology with the potency of cytotoxicity. A literature search was conducted on electronic databases including Science Direct, Elsevier, PubMed, Springer Link and Google Scholar. In addition, an academic social networking site, ResearchGate was also used to obtain research articles. Keywords such as biosynthesis, plant synthesis, plant-mediated, metallic nanoparticle, cytotoxicity and anticancer were used in the literature search. All types of research which met the inclusion and exclusion criteria were considered regardless of the results being positive, negative or null. Data and information from the 76 selected articles were extracted and synthesised. Therapeutic index was used as a safety measure for the studied compound of interest. Most research showed the cytotoxicity property of plant-mediated metallic nanoparticles and cytotoxicity being time

and/or dose-dependent. Silver nanoparticles demonstrated a higher cytotoxicity potency as compared to gold nanoparticles of the same plants; *Cassia auriculata, Commelina nudiflora* and *Plumbago zeylanica* were used for the synthesis irrespective of the cell types used. Cytotoxicity is inversely proportional to size. Plant-mediated metallic nanoparticles were predominantly spherical in shape where spherical and quasi-spherical appeared most often with LD_{50} of 1–20 µg/mL being the mode of populations, followed by other shapes; triangular, hexagonal and rods showing less potency. *Abutilon inducum*, *Annona squamosa, Butea monosperma, Couroupita guainensis, Gossypium hirsutum, Indoneesiella echioides,* and *Melia azedarach* metallic nanoparticles were acceptably safe as anti-cancer agents, having a therapeutic index of 2.0 and above when tested on both cancer cells and normal human cells. Results from this study suggest a focus on the listed potential anti-cancer agents for further investigations on their pharmacodynamic/toxicodynamic and pharmacokinetic/toxicokinetic actions in hopes of reducing cancer, the Global Burden of Diseases (GBD) and the second leading cause of mortality.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Nanoparticles are the fundamental component in nanotechnology. It is intentionally designed and engineered with desired physical, chemical and biological properties moulded to meet the needs of specific applications. To date, nanotechnology has been embraced by industrial sectors due to its tremendous applications of nanoparticles and nanomaterials in diverse fields from engineering, telecommunication, advertising, electronics, textile, sports and toys, space and defense, cosmetics, domestics and even in medical.

The use of nanoparticles and nanotechnology, additionally offer great potential in medicine ranging from diagnostic imaging, cancer therapy, gene therapy as well as drug delivery system. In diagnostic imaging, nanoshell-enhanced optical coherence tomography has improved detection of disease (Low et al., 2006). As drug delivery system, gold nanoparticles coated with polyethylene glycol (PEG) were loaded with cytokine, Tumour Necrosis Factor alpha (TNF-α) to maximise tumour killing (Visaria et al., 2006). Additionally, gold nanoparticles were used with paclitaxel, a chemotherapy drug in cancer treatment to promote anti-proliferative effect and apoptosis in human hepatoma (Hep-G2) cells (Wei et al., 2007).

1.2 Problem statement and rationale of the study

Nanotechnology suggests promising applications in wide areas of medicine such as new anti-bacterial agents in finding better treatment and strategy in combating cancers. Numerous studies have been conducted to develop anti-cancer agent using metallic nanoparticles via various methods; mechanical attrition, laser ablation, photo reduction, chemical electrolysis and also with the use of organisms like bacteria and plants. Plant-mediated metallic nanoparticles have become increasingly famous and researched as cytotoxic agents for the past 10 years as a better substitute for metallic nanoparticles which are chemically or physically derived.

Multiple studies have shown on the potential of plant-mediated metallic nanoparticles as effective cytotoxic agents against various cancers such as lung cancer (A549) cells (Kuppurangan et al., 2016), liver cancer (Hep-G2) cells (Selvarani, 2015), colorectal cancer (HT29) cells (Bupesh et al., 2016; Mata et al., 2016), breast cancer (MCF7) cells (Rashidipour et al., 2014), and brain cancer (U87) cells (Mishra et al., 2016). Despite all these thrilling potentials, concerns arise on the safety issue of developed anti-cancer agents since a potent anti-cancer agent may not only kill cancer cells but also other normal healthy cells when used as a treatment.

An ideal drug or treatment should have properties which include selective, specific to target site, potent (<100mg/day), safe and effective other than minimal food/drug interaction, with convenient dosing frequency and no requirement for blood level monitoring (Spector, 2002). It is desirable to develop

an anti-cancer agent which is stable, specific towards the target site, has a wide therapeutic index, bio-compatible, biodegradable with minimal side effects, reasonably simple reproductive and cost effective. Based on previous studies, these plant-mediated metallic nanoparticles are effective as cytotoxic agents and have reasonably simple production method at low cost but its safety as cytotoxic agents has not been extensively addressed. Additionally, which novel plant-mediated metallic nanoparticles to be investigated further as anti-cancer agents also becomes a question. Thus, this project systematically reviews and compares the efficacy and safety of plant-mediated metallic nanoparticles as cytotoxic agents in aiding the associated decision making plus hoping to reduce the numbers of medicine development failures.

1.3 Study objectives

This systematic review aims to:

- **i.** Compare the cytotoxicity potency of plant-mediated metallic nanoparticles based on recent studies; 2006 to 2017.
- **ii.** Determine the most promising plant-mediated metallic nanoparticles as anti-cancer agents based on its potency, cancer cell type susceptibility, and therapeutic index.
- **iii.** Correlates nanoparticles size and morphology with the potency of cytotoxicity.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanoparticles

A particle is defined as a small object which behaves as a whole unit while nanoparticle refers to organic or inorganic particle having nanoscale size ranging from 1 to 100 nanometers (nm) with one or more dimensions (Williams, 2008). Nanoparticles exhibit significant differences in physical as well as chemical properties based on its size, distribution, and morphology in comparison with bulk material or fine particles in which the nanoparticles are made of (Cao, 2004). Gold per se is yellow-orange in colour, while gold nanoparticles in liquid solution with the size of less than 100 nm have an intense red colour. The melting point differs, 1064 °C and 300 °C for gold slabs and gold nanoparticles respectively. The synthesised nanoparticles display properties which are new or greatly improved and are unique due to their large surface area in respect to their microscopic size. Moreover, nanoparticles also have exclusive optical and electrical properties besides the previously mentioned thermal properties.

2.2 Nanoparticles synthesis

The production of nanoparticles is achieved by several means. Nanoparticles synthesis methods are classified into three main categories namely physical, chemical, and biological (Iravani et al., 2014). Physical methods include mechanical attrition, thermal decomposition, and laser ablation. In 2006, a study conducted by Kim et al. has successfully synthesised copper nanoparticles

using thermal decomposition. Sylvestre et al. (2004) produced gold nanoparticles using laser ablation yielding extremely small, stable and homogenised colloids with a size range of 2.0 to 2.4 nm and dispersion size of 1.0 to 1.5 nm. This technique was also used in an attempt to generate silver nanoparticles which result in highly pure nanoparticles being produced, thereby eliminating solvent contamination (Tsuji et al., 2002). The nanoparticles are also found to be uniformly distributed. However, physical methods require the use of large production space and a huge amount of energy to ensure that the desired temperature at which reduction occurs can be reached on top of additional time required to maintain thermal stability in ensuring homogeneous nanoparticles being produced (Iravani et al., 2014).

Chemical method, on the other hand involves metal salts reduction, microemulsion, radiation, photo-reduction, chemical electrolysis, microwave assisted synthesis and/or sonochemical reduction. Metal salt reduction is the most common chemical method used. Various reducing agents are used such as ascorbate, elemental hydrogen, sodium citrate, sodium borohydride, Tollens reagent, and polyethylene glycol (PEG) copolymers to perform reduction reactions. Chemical methods allow nanoparticles synthesis to occur at room temperature in contrast to thermal decomposition method (Troupis et al., 2002). Furthermore, a short time is required for metallic nanoparticles to be produced. Nonetheless, use of surfactant or surface passivator reagent or stabiliser is necessary to prevent nanoparticles from aggregation as reported by Nayak et al. (2011) and Crooks et al. (2001). Surface passivator thiourea was used in producing agglomerate-free cadmium nanoparticles (Pattabi et al., 2000) while

stabiliser mercaptoacetic acid was used in producing agglomerate-free zinc oxide nanorods (Song et al., 2008). Disappointingly, these chemical methods use chemical substances which are toxic to human health and the environment especially when used in large quantities.

Biological method uses organisms ranging from simple prokaryotic bacterial cells to eukaryotic fungi and plants to synthesise nanoparticles (Mohanpuria et al., 2008). Exploitations on microorganisms and plants as nanofactories has been recognised as green, efficient and convenient (Singh et al., 2016). In contrast to physical and chemical methods, synthesis of nanoparticles using biological methods is environmentally friendly, inexpensive, simple and cost-effective. Biologically synthesised metallic nanoparticles are favoured as chemical and physical methods are fraught with many drawbacks including the use of toxic solvents, generation of hazardous by-products and high energy consumption (Thakkar et al., 2010). Stable silver nanoparticles were successfully developed in a rapid, inexpensive and energy-efficient method using tannic acid from plant extracts at room temperature and this method is free from the use of toxic solvents which are hazardous to the environment (Sivaraman et al., 2009). Furthermore, narrow distribution size and reduction of polydispersity can be manipulated with the use of microorganisms in generating metallic nanoparticles (Singh et al., 2016).

Although stable nanoparticles are produced using various known biosynthesis methods, novel plant-mediated nanoparticle synthesis is preferred over others in addition to biologically active plant compounds could be exploited as key

resources of the green synthesis (Naheed Ahmad et al., 2010). Consistent with the previous statement, metallic nanoparticles produced by medicinal plants are found to be pharmacologically active possibly due to the pharmacologically active compound incorporated into it (Singh et al., 2016). Various plant parts such as leaves, stems, roots, fruits (Singh et al., 2016), latex (Valodkar et al., 2011; Rajkuberan et al., 2016), flowers (Remya et al., 2015; Mata et al., 2015b) even fruit peel (Zhou et al., 2014; Yang et al., 2014) and its extracts could be used. Apart from that, plant-mediated nanosynthesis does not involve complicated process of intracellular synthesis, multiple purifications, and maintenance of microbial cells (Mohanpuria et al., 2008). Several mechanisms were suggested on plant-mediated nanosynthesis but up to this date, the exact mechanism is yet to be completely elucidated (Baker et al., 2013; Singh et al., 2016).

Combination methods involving both biological and chemical have also been used in synthesising nanoparticles (Saifuddin et al., 2009). In recent time, monodispersed biogenic gold nanoparticles were derived using *Areca catechu* nut under microwave irradiation which showed anti-cancer effect on HeLa cells and also anti-bacterial activity against a broad spectrum of pathogens (Rajan et al., 2015). Although various methods are used to synthesise nanoparticles, new and improved methods are still being investigated and developed to create a better device or ideal process in obtaining highly purified stable nanoparticles and higher mass production with lower cost involved. In addition to that, a method which has enhanced control over the produced nanoparticle size, size distribution, shape, aggregation, and stability are also desired for higher

reproducibility purpose in developing metallic nanoparticles for various uses and applications such as biocompatible new anti-cancer agents and nanosize drug delivery systems.

2.3 Cancer and its current treatment

Cancer is a major health problem worldwide. According to the National Cancer Institute of United States, cancer is defined as a collection of diseases involving abnormal cell growth beyond control which has the potential of invading other parts of the body. It is listed as one of the Non-Communicable Diseases (NCDs) in Global Burden of Diseases (GBD) with the second leading cause of mortality after cardiovascular disease, accounting deaths of 8.8 million people worldwide in 2015, according to the GBD 2015 Study and World Health Organisation (WHO). From the same sources, common cancer-related mortality comprises of lung cancer as the main lead with approximately 1.69 million deaths, followed by liver cancer with 788 000 deaths, colorectal cancer with 774 000 deaths, stomach cancer 754 000 deaths and breast cancer with 571 000 deaths.

Currently, there are several strategies used to combat cancer. Choices of treatment include surgery, radiation therapy, chemotherapy, hormonal therapy, targeted therapy, angiogenesis inhibition and also immunotherapy such as monoclonal antibody therapy, depending on the type/location of the cancer, its stage as well as patient's general health status. The main goal of cancer treatment is to eradicate cancer cells and prolong life. Even though multiple treatment options are present, the success rate of these strategies varies between each type/location of the cancer and its stage. In addition, currently

available treatments may or may not be curative without the exception of numerous challenges arises from the selected treatment courses ranging from complications and adverse reactions. Patients who survived cancer surgery faces risk of complications such as sepsis, cognitive impairment as well as chronic pain. Radiation therapy comes with the risk of cataract, infertility and secondary cancer such as skin cancer. Chemotherapy, on the other hand, comes with multiple adverse reactions including bone marrow toxicity, irreversible nerve damages, and even secondary cancer formation. Furthermore, multidrug resistance has become a major reason why chemotherapy fails to eradicate cancer (Donnenberg et al., 2005; Ozben, 2006; Housman et al., 2014). Targeted therapy, angiogenesis inhibition, and immunotherapy are still under research phases and yet to be available in healthcare facilities.

Since nanotechnology's arising, the use of nanoparticles as potential strategies in combating cancer have been extensively studied for the past decade. Nanoparticles are used as drug delivery system for chemotherapy drugs to overcome the issue of multidrug resistance due to its ability to permeate and accumulate in cells without being detected by the multidrug resistance mediator, P-glycoprotein (Cho et al., 2008). Multiple studies have shown the potential of metallic nanoparticles as effective cytotoxic agents against various cancers such as human epithelial lung carcinoma (A549) cells (Mukundan et al., 2015; Kuppurangan et al., 2016), human hepatocellular carcinoma (Hep-G2) cells (Selvarani, 2015), human colorectal cancer (HT29) cells (Bupesh et al., 2016; Sengani et al., 2016), human breast cancer (MCF7) cells (Jeyaraj et al., 2013;

Mittal et al., 2015), human cervical cancer (HeLa) cells (Dipankar et al., 2012; Chanthini et al., 2015) and also human glioblastoma (U87) cells (Mittal et al., 2015; Mishra et al., 2016). Yet, safety issue of these synthesised cytotoxic nanomaterials remains huge concerns before research in the further stage is carried out in an attempt to reduce the numbers of medicine development failures.

2.4 Research Gap

Drugs are not always effective and completely safe. The properties of an ideal drug includes selective, specific, localised effect, potent, and consistent efficacy with minimal adverse reactions (Spector, 2002). Therapeutic index is one of the measures of toxicity. It is often used to measure the safety of drug for a particular treatment. Therapeutic index shows a quantitative relationship of a drug efficacy (pharmacology) and safety (toxicology) using a range of endpoints (Muller et al., 2012). It is defined as the ratio of the median lethal dose (LD_{50}) to the median effective dose (ED_{50}). Median lethal dose (LD_{50}) is the dose required to kill half (50%) of the tested population after a specified test duration whereas median effective dose (ED_{50}) is the dose required to achieve the desired outcome or response in half (50%) of the tested population.

In clinical practice, therapeutic index is regarded as the range of doses in which a medication appeared to be effective in clinical trials for a median of participants without causing unacceptable adverse effects (Tamargo et al., 2015). Drugs with narrow therapeutic index have small ratio or differences in dose which may lead to dose-dependent, serious therapeutic failures and/or

adverse drug reactions such as persistent, irreversible, slowly reversible, disability or even fatality (Yu et al., 2015). Cytotoxic agents are among the examples of drugs with narrow therapeutic index other than aminoglycosides, digoxin, levothyroxine, warfarin, phenytoin, theophylline, and lithium carbonate.

A potent cytotoxic agent may not only kill the rapidly growing cancer cells effectively but may also kill other normal healthy cells efficiently during the treatment course. Thus it is highly desirable to develop cytotoxic agents which have high selectivity in eliminating cancer cells yet safe enough not to eradicate rapidly growing normal cells when administered. Therefore, estimation of therapeutic index for newly engineered plant-mediated metallic nanoparticles as cytotoxic agents could help in determining its safety profile. Improvement in identification and assessment of drug safety is important during drug development and pharmacovigilance (Ray, 2009). Therapeutic index determination is one of the improved strategies aiding to identify both suitable and unsuitable drug candidates in the early research phase for associated decision making so that it may reduce the number of expensive research failures in the late stage of clinical trials (Muller et al., 2012).

A drug having therapeutic index of less than one (< 1) is regarded as acceptable for oncology indications. However, a drug which is initially developed for oncology indications having a reasonable therapeutic index of more than one (> 1) holds potential expansions to be studied and used for other indications due to its sensible safety profile (Muller et al., 2012). One example is Imatinib, an antineoplastic drug for leukaemia and gastrointestinal stromal

tumour which has been explored for use in treating pulmonary arterial hypertension. Thus, identification of therapeutic index for plant-mediated metallic nanoparticle may also estimate whether these compound of interests may be use safely for indications other than cancer treatment.

CHAPTER 3

METHODOLOGY

3.1 Search process

Search strategy was planned and constructed. The literature search was executed on full-text electronic databases such as Science Direct, Elsevier, PubMed, and Springer Link. In addition to the stated databases, a general search engine such as Google Scholar was also used. An academic social networking site, ResearchGate, was in addition used to obtain the research articles. Keywords such as biosynthesis, plant synthesis, plant-mediated, metallic nanoparticle, cytotoxicity and anticancer were used during the literature search. A reviewer (author) independently screened the titles and abstracts of the citations and retrieved relevant articles from the mentioned full-text electronic journal databases.

With the objective of complementing the database searches, non-automated manual searches were conducted on the references within the selected articles. After the application of the search strategy, two examiners (supervisor and co-supervisor) reviewed on the screened research titles and abstracts. Documentation on the whole search process was carried out to ensure transparency, replicability and feasibility to reanalyse. Endnote software Version X7 was used as a reference manager. Full text of each identified study was retrieved by author.

3.2 Study selection

All types of research specifically *in vitro*, *in vivo* and *ex vivo* studies and reviews which met the inclusion and exclusion criteria were considered regardless the results are positive, negative or null to diminish selection bias. Pre-clinical and clinical trials were not included as there were no such studies conducted to this date.

3.2.1 Inclusion criteria

For inclusion in this review, literatures fulfilling below-mentioned criteria were collected:

i. Metallic nanoparticles; gold (Au), silver (Ag)
ii. Biologically synthesised using plant
iii. Recent published studies conducted between 2006 to 2017 (including in-press articles)
iv. All types of studies; *in vitro*, *in vivo*, *ex vivo*, review

3.2.2 Exclusion criteria

All searched literatures collected having any of these criteria were excluded from this study:

i. Metallic nanoparticles derived from non-vascular plant (fungus, seaweeds, mushrooms)
ii. Metallic nanoparticles derived from undefined plant (specific pure compound from unknown plant used in the biosynthesis)
iii. Combined synthesis of metallic nanoparticle with chemical or physical method

iv. Combined metallic nanoparticles

v. Studies without available data

vi. Articles which were written in language other than English

The flow chart demonstrates the results from the literature search obtained from all databases mentioned on the association between plant-mediated metallic nanoparticles and cytotoxic activity.

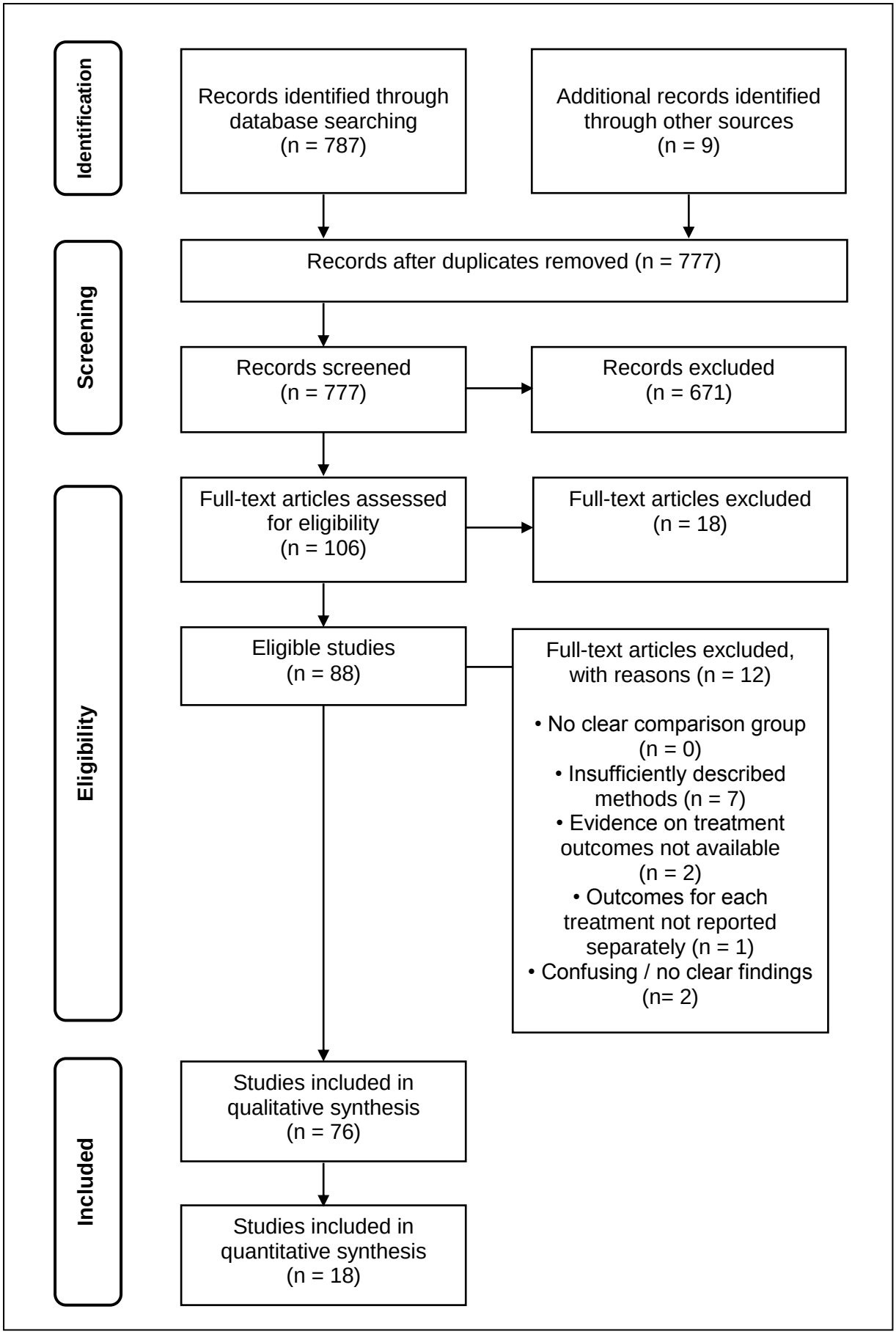

Figure 3.1: Methodology Process

3.2.3 Study quality assessment

Titles and abstracts for all 796 articles were screened with only 88 articles related to the research question. For all the 88 potentially eligible articles, the following criteria were used to assess the quality of the study:

i. Clearly defined control/comparison group
ii. Duration of exposure clearly stated
iii. Method conducted at 37°C to mimics human body temperature
iv. Clearly defined measured outcomes (LD_{50}, IC_{50}, GI_{50}, cell viability, cell death)
v. Ethical standards carried out and maintained, if relevant
vi. Reliable method used to measure outcomes
vii. Multiple measurements of outcome conducted (at least triplicates)
viii. Actual data or evidence available on treatment outcomes
ix. Outcomes reported separately for each treatment
x. Clear statement of findings
xi. Appropriate statistical analysis used

The above study criteria were used aiming at eliminating attrition biases, raised from incomplete reporting of data for each outcome, in addition to reporting biases from selective reporting of positive outcomes. Several appraisal guidelines and checklists were used in developing the study criteria including:

i. Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist
ii. Risk of Bias Assessment tool for Non-randomized Studies (RoBANS)

3.3 Data extraction

Data and information extraction from the selected studies was carried out by author. The following items were gathered for the cytotoxicity activity of plant-mediated metallic nanoparticles:

i. Plant used
ii. Plant part used
iii. Type of normal cells used
iv. Type of cancer cells used
v. Duration of cells exposed to synthesised nanoparticle
vi. Median lethal dose (LD_{50}) or median inhibition concentration (IC_{50}) for each cell type tested
vii. Response relationship (Dose / Time-dependent)
viii. Mechanism of cytotoxicity
ix. Synthesised nanoparticle size
x. Synthesised nanoparticle morphology

3.4 Data synthesis

The descriptions of evidence were presented in tabular format. In addition, therapeutic index for the synthesised plant-mediated metallic nanoparticles was calculated by author for studies which conduct cytotoxicity testing on both cancer and normal cell types. The therapeutic index is often used to measure the safety of a drug for a particular treatment. It is defined as the ratio of the median lethal dose (LD_{50}) to the median effective dose (ED_{50}) (Clark et al., 2012).

$$\textbf{Therapeutic Index (TI)} = \frac{\textbf{Median Lethal Dose } (LD_{50})}{\textbf{Median Effective Dose } (EC_{50})}$$

3.5 Data analysis

Correlation between plants synthesised metallic nanoparticles size and its morphology to cytotoxicity potency were analysed in the current study. Results were presented in graphical format. Based on the availability of reasonable therapeutic index, potential plant-mediated metallic nanoparticles with most promising as anti-cancer agents were selected.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Search results

This systematic review preliminary search results using Science Direct database started on November 11, 2016 yields 319 articles. Subsequently, search results from PubMed database on November 30, 2016 yielded 17 articles. On December 8, 2017 another search using Springer Link database was conducted producing 291 articles. A general search engine, Google Scholar was also used to search for articles related to keywords on December 16, 2017 and 160 articles were retrieved. Lastly, an academic social networking site, ResearchGate was used enabling 9 articles retrieved. Afterward, all articles were imported to Endnote software Version X7, which was used as the reference manager. After the importation, results from databases were merged with a total of 796 articles retrieved and 19 duplicates were removed. Abstract screening of articles yielded 671 articles which were unrelated to the research question. A remaining of 106 articles was potentially eligible.

A total of 18 studies were later excluded due to the pre-set exclusion criteria:

i. Non-vascular plants (n = 10)
ii. Combined synthesis (n = 1)
iii. Combined metallic nanoparticles (n = 0)
iv. Undefined plant used (n = 7)
v. Language other than English (n = 0)