RAPID SONOCHEMICALLY-ASSISTED GREEN SYNTHESIS OF HIGHLY STABLE AND BIOCOMPATIBLE PLATINUM NANOPARTICLES AS NOVEL CONTRAST AGENT FOR COMPUTED TOMOGRAPHY

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by

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Mahmood Subhi Jameel

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LIST OF SYMBOLS

^{o}C	Degree Celsius
µL	Microliter
θ	Angle
λ	Wavelength
ωo	Larmor Frequency
ζ	Zeta Potential
Bo	Magnetic Field
d_{hkl}	Interplanar Spacing
D_p	Crystal Size
ε	The Strain
a	Lattice Constant
h,k,l	Miller Indices
γ	Gyromagnetic Ratio
Hz	Hertz
rpm	Revolution Per Minutes
a.u	Arbitrary Unit
FOV	Field Of View

LIST OF ABBREVIATIONS

P. farcta	*Prosopis Farcta* Fruits Extract
V	Volts
L	Liter
mg	Milligram
µg	Microgram
nm	Nanometer
DLS	Dynamic Light Scattering
Au	Gold
Ag	Silver
WCR	Wet Chemical Reduction
CVD	Chemical Vapour Deposition
K	Kelvin
atm	Atmosphere
FTIR	Fourier-Transform Infrared Spectroscopy
s	Second
$PtCl_6^{2-}$	Platinum Hexachloride
W	Watt
cm	Centimeter
min	Minute
$H^{\bullet}$	Hydrogen Free Radical
$OH^{\bullet}$	Hydroxyl Free Radical
SDS	Sodium Dodecylsulphate
µM	Micromolar
Ar	Argon
CO	Carbon Monoxide

MRI	Magnetic Resonance Imaging
CT	Computed Tomography
HU	Hounsfield Unit
NPs	Nanoparticles
PSB	Polybutylene Succinate
Pt	Platinum
Å	Angstrom
Pt-I NPs	Preconcentrate Cetylpyridinium Complexed Hexaiodoplatinum Nanoparticles
PDl	Polydispersity Index Values
HT-29	A Human Colon Cancer Cell Line
MTT	dye compound 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
pH	Potential of Hydrogen
IC50	Half-maximal inhibitory concentration
$AgNO_3$	silver nitrate
rpm	Revolutions per minute
DPPH	1,1-diphenyl-2-picryl-hydrazyl
TEM	Transmission Electron Microscopy
FRAP	ferric reducing antioxidant power
CAs	contrast agents
Z	atomic mass number
MSNs	mesoporous silica nanoparticles
XRD	X-ray Diffraction
NIR	near-infrared
OI	optical imaging
ICPMS	Inductively coupled plasma mass spectrometry
PEG	Thiol-terminated polyethylene glycol

EDX	Energy dispersive X-ray spectroscopy
FESEM	Field emission scanning electron microscopy
ROI	regions of interest
HEK	Human embryonic kidney
NIR	Near-infra red
Fe	iron
$H_2PtCl_6.6H_2O$	Hexachloroplatinic acid hexahydrate
BSA	bovine serum albumin
SPR	Surface plasma resonance
UV-vis	Ultraviolet – visible spectroscopy
WST – 1	Water soluble tetrazolium - 1
MPa	Pascal
MHz	Mega hertz
ART	algebraic reconstruction technique
HRTEM	high-resolution transmission electron microscopy
GC-MC	Gas chromatography-mass spectroscopy
K_2PtCl_4	potassium tetrachloroplatinate (II)
PDI	Polydispersity index
ζ	zeta potential
λ,	wavelength
d	lattice spacing
θ	diffraction angle
FWHM	full width at half maximum
SAED	selected area diffraction
DMEM	Dulbecco's Modified Eagle Medium

LIST OF APPENDICES

SINTESIS HIJAU BANTUAN SONOKIMIA PANTAS NANOPARTIKEL PLATINUM YANG AMAT STABIL DAN BIOSERASI SEBAGAI AGEN KONTRAS BARU UNTUK TOMOGRAFI BERKOMPUTER

ABSTRAK

Nanopartikel Platinum (Pt NPs) mempunyai beberapa sifat intrinsik, termasuk rintangan yang baik terhadap kakisan, serangan kimia, kawasan permukaan yang tinggi untuk jumlah nisbah dan mempunyai potensi besar untuk menjadi pesaing yang kuat untuk ejen kontras pengimejan CT kerana kelebihannya dalam penyerapan X-ray yang tinggi. Walau bagaimanapun, kaedah sintesis Pt NPs sebagai ejen kontras untuk pengimejan CT memerlukan penyediaan yang rumit, penggunaan bahan kimia berbahaya dan mahal dan serta perlu diubahsuai dengan menggunakan bahan kimia yang pelbagai (inorganik dan organik) untuk meningkatkan kestabilan dan kebioserasiannya. Di sini, penyediaan nanopartikel platinum (Pt NPs) yang ringkas dan biasa telah dicapai untuk meningkatkan nilai Hounsfield (HU) untuk pengimejan CT. Pt NPs yang sangat stabil, bioserasi dan teramat kecil dengan saiz teras min 5 ±1.5 nm diperolehi melalui kaedah satu-langkah, satu-periuk, mesra-pengguna dan kaedah terus menggunakan ekstrak buah *farcta Prosopis* (*P. farcta*) sebagai ejen penurunan dan penstabil melalui sintesis sonokimia. Tanduk padat ultrasonik Vibra-Cell dengan ukuran hujung ½ inci, frekuensi 20 kHz, output kuasa 750 watt dan amplitud 40% digunakan dalam sintesis sonochemical Pt NPs. Ketulenan amorfus atau habluran Pt NPs adalah disebabkan oleh kitaran pemanasan / penyejukan pesat mikrobuih peronggaan sonikasi. Kesucian, keseragaman dan penghabluran Pt NPs yang disintesis melalui kaedah sonokimia telah menunjukkan keputusan yang lebih baik daripada kaedah konvensional (kaedah penurunan). Pt NPs juga mempamerkan saiz zarah yang

lebih kecil dengan kestabilan koloidal yang lebih tinggi dan saiz hidrodinamik yang sekata. Keputusan mengesahkan bahawa kuasa iradiasi kaedah sonokimia meningkatkan sifat-sifat hablur nanopartikel dan menghalang penggumpalan, terbukti dengan nilai potensi zeta sebanyak −38.9 mV. Kestabilan yang tinggi dan bioserasi Pt NPs boleh dikaitkan dengan sebatian organik yang terkandung dalam ekstrak *P. farcta*. Pt NPs konjugasi sebatian organik juga dikenal (Oxirane, 2,3-dimethyl-, trans-, Oxirane, 2.3-dimethyl-, cis-, asid asetik, diisooctyl phthalate, phthalic acid, dan di[2-propylpentyI]ester). Tenaga tinggi yang dihasilkan oleh penyinaran ultrasound dipercayai dapat memudahkan interaksi ligan antara ekstrak *P. farcta* dan Pt NP walaupun jangka masa pendek (30 minit) sintesis. Keputusan menunjukkan bahawa kebolehidupan sel dalam Pt NPs yang disintesis tidak terjejas pada kepekatan yang agak tinggi (150 μg mL^{-1}). Attenuasi sinar-X untuk NPs yang disintesis adalah 70% lebih tinggi daripada Omnipaque (agen kontras CT berasaskan iodin yang popular yang kini digunakan di klinik), nilai HU NP Pt yang disintesis adalah 246 pada 7.2 mg mL^{-1}, sementara nilai HU Omnipaque ialah 178 pada 7.2 mg mL^{-1}. Kajian ini memberi gambaran baru mengenai penggunaan tumbuh-tumbuhan untuk menyediakan agen kontras baru dalam pengimejan molekul pada masa depan dan kaedah yang berpotensi untuk sintesis Pt NPs yang berkaitan untuk aplikasi perubatan.

RAPID SONOCHEMICALLY-ASSISTED GREEN SYNTHESIS OF HIGHLY STABLE AND BIOCOMPATIBLE PLATINUM NANOPARTICLES AS NOVEL CONTRAST AGENT FOR COMPUTED TOMOGRAPHY

ABSTRACT

Platinum nanoparticles (Pt NPs) have some intrinsic properties, including good resistance to corrosion, chemical attacks, high surface area to volume ratio and hold massive potential to be strong contenders for CT imaging contrast agent due to their advantages of high X-ray absorption. However, the synthesis methods of Pt NPs as a contrast agent for CT imaging require elaborate preparation, use of hazardous and expensive chemicals and modified Pt NPs using different chemical materials (inorganic and organic) to improve their stability and biocompatibility. Herein, a simple and facile plant-directed preparation of Pt NPs was achieved to improve the Hounsfield (HU) values for CT imaging. A highly-stable, biocompatible and ultrasmall Pt NPs with a mean core size of 5 ±1.5 nm was obtained through one step, one-pot, user-friendly, and straightforward method using *Prosopis farcta* fruits extract (*P. farcta*) as reducing agent and stabilizer using a sonochemical synthesis. A Vibra-Cell ultrasonic solid horn with a ½ inch tip size, 20 kHz frequency, 750 watts power output and 40% amplitude was used in the sonochemical synthesis of Pt NPs. The formation of amorphous or crystalline Pt NPs is attributed to the sonication's cavitation microbubbles' rapid heating/cooling cycles. The purity, uniformity and crystallinity of Pt NPs synthesized by the sonochemical method were better than those obtained from the conventional (reduction) method. The Pt NPs also exhibited smaller particle size with higher colloidal stability and uniform hydrodynamic size. The results confirmed that the sonochemical method's irradiation power enhances the crystalline properties

of nanoparticles and hinders their agglomeration, evident by a zeta potential value of −38.9 mV. The high stability and biocompatibility of the Pt NPs can be attributed to the organic compounds contained in the *P. farcta* extract. The organic compound-conjugated Pt NPs are also identified (Oxirane, 2,3-dimethyl-, trans-, Oxirane, 2.3-dimethyl-, cis-, acetic acid, diisooctyl phthalate, phthalic acid, and di[2-propylpentyI]ester). The high energy produced by ultrasound irradiation is believed to have facilitated a ligand interaction between the *P. farcta* extract and the Pt NPs despite the short duration (30 minutes) of synthesis. Results showed that the cell viability of the synthesized Pt NPs is unaffected (nontoxic) at relatively high concentrations (150 μg mL^{-1}). The X-ray attenuation of the as-synthesised NPs was 70% higher than Omnipaque (a popular iodine-based CT contrast agent currently used in the clinic), HU value of as-synthesized Pt NPs was 246 at 7.2 mg mL^{-1}, while HU value of Omnipaque was 178 at 7.2 mg mL^{-1}. This work gives new insights into plants' use to prepare novel contrast agents in future molecular imaging, thereby making it a promising method for synthesizing Pt NPs relevant for medical applications.

CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is a multidisciplinary field that includes the design and engineering of functional systems on a molecular scale. It is an applied science field focusing on the synthesis, characterization and application of materials and devices at the nanoscale. In general, nanotechnology can be defined as the art and science of manipulating matter on a nanoscale to create new and unique materials [1]. The prefix "nano" comes from the Greek word "nanos", meaning "dwarf" (one billionth of 10^{-9} meters). Today, "Nano" is a popular term widely used in modern science, but also included in dictionaries: for example, nanoscience, nanowire, nanotube, nanotechnology, nanostructure, nanoscale, nanometer, nanorobot, nanomaterial, among others. Richard Feynman first introduced the idea of nanotechnology with his famous speech titled “There is plenty of rooms at the bottom” during the Annual Meeting of the American Physical Society at Caltech, USA [2]. The term "nanoparticle" is used to describe a particle having a size in the range of 1-100 nm, having one of at least four possible sizes (zero, one, two and three-dimensional) (Figure 1.1).

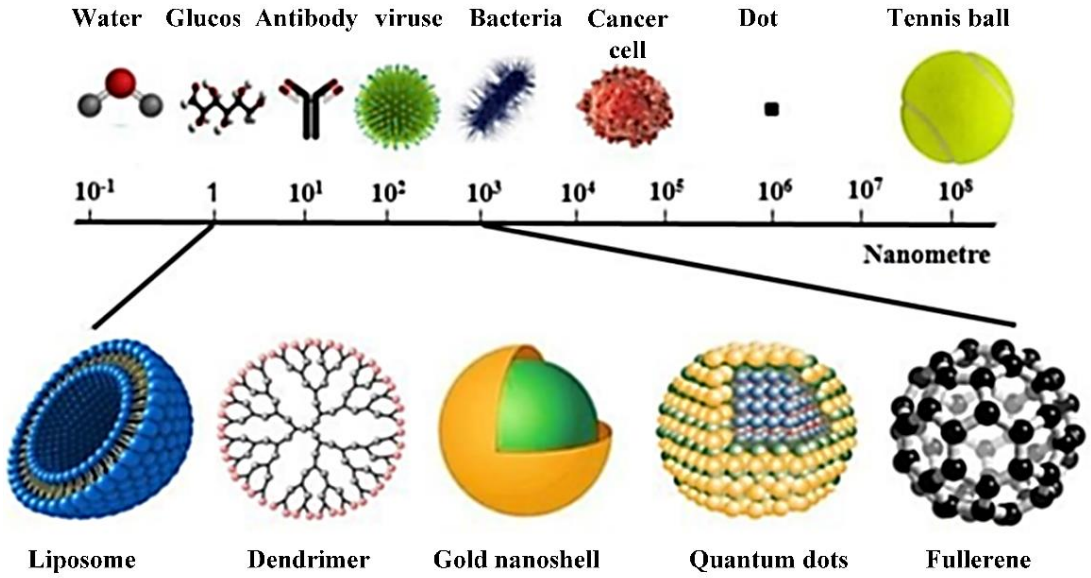

Figure 1.1 Nanoparticle sizes and comparison with other biological molecules [3].

In this size range, nanoparticles' physical, chemical and biological properties vary due to the properties of each atom/molecule and related materials [4, 5]. Numerous technologies are used in the production of nanomaterials from various sources, such as physical, chemical, and biological methods, and different techniques are used to maximise the production of nanomaterials, such as the use of different raw materials, temperature, and pH [6]. Nanoparticles are commonly classified based on their morphology, dimensions, shape, composition, uniformity, and agglomeration. Three morphologies of NPs were recently identified: spherical, crystalline, and flat (Figure 1.2). Nanoparticles are also classified into four types based on electron movement of dimension: zero dimension (0D); 1D, which includes thin films that are mainly used in electronic devices sensor mechanisms; 2D, which includes second-generation NPs such as carbon nanotubes, which provide high absorption and stability; and 3D, which includes dendrimers and quantum dots [7, 8]. Thanks to their attractive

properties, nanoparticles have a wide range of uses such as energy, physics, chemistry, biology, biotechnology, medicine, industry, technology and industry [9, 10].

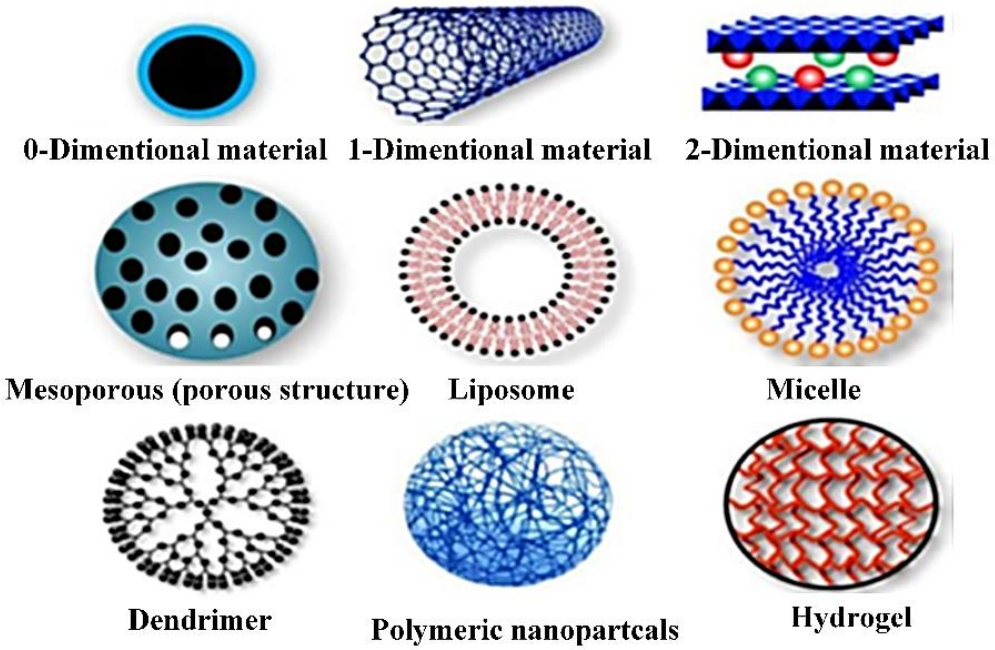

Figure 1.2 Different types of nanoparticles commonly used for various applications [11].

Nanoparticles are generally grouped as organic nanoparticles and inorganic nanoparticles containing carbon nanoparticles. Inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (such as gold, platinum, palladium, silver, among others) and semiconductor nanoparticles (such as titanium oxide and zinc oxide) [12, 13]. The interest in inorganic nanoparticles, noble metal nanoparticles is growing, as they provide superior material properties with functional versatility. Due to its size and magnificent properties, nanoparticles are potentially used in nano type fields such as in-body drug delivery, treatment and diagnosis of diseases, biosensors, medical imaging, tissue engineering, bioelectronics, and bone regeneration. Because

of their leading biology applications, these nanoparticles are called nano biomaterials [14-18].

1.2 Platinum Nanoparticles (Pt NPs)

Platinum NPs (Pt NPs) have led to a new revolution in nanotechnology, including the chemical industry, automotive sector, biomedical applications, and therapeutic span. They are also used in numerous biomedical fields, including diagnostics with different agents for imaging, medical implants, drug delivery, and photothermal therapy [19-21]. Recent years have seen significant developments in molecular imaging as an effective disease prevention and diagnostic inspection method that could provide detailed reports on both anatomical and functional imaging with remarkably improved signal sensitivity and specificity [22, 23]. It is worth noting that CT, one of the diagnostic methods most commonly used in clinical applications, has gained significant interest because of its relatively low cost and high resolution. [24-28]. Contrast agents are of high importance for enhanced CT imaging analysis. Until now, almost all contrast agents that have been approved for clinical use is based on the use of iodine small molecule agents which face several challenges, such as low circulation time, difficult modifications and possible toxic and side effects in some cases [29, 30]. Inspiringly, nanomaterials have now emerged as ideal imaging agents for in vivo biomedical diagnosis and imaging applications, exhibiting superior properties and in many ways outperforming conventional small-molecule agents. Among the versatile inorganic contrast agents studied in this area, platinum-based nanoparticles hold intense potential to be strong contenders for CT imaging due to their advantages of high X-ray absorption, simple synthesis control and feasible thiol-metal bond surface functionalization. Pt NPs and their alloys exhibit excellent catalytic

properties because of their large surface areas. They are mainly used to reduce pollutants and play a major role in the chemical reactions for the synthesis of various chemicals in the chemical industry such as oxidation in the process of organic acid production [31] dehydrogenation [32, 33] and hydrogenation reactions for the synthesis of biofuel [34], vitamins and fats [35]. They are also used in green technologies, such as highly polluting aromatic compound [36], solar energy harvesting [37] and water treatment [38], and to maintain a clean environment. Currently, both the research and industrial fields have shown considerable interest in the synthesis of catalytic Pt NPs for therapeutic applications because of their low toxicity, high stability, and fewer side effects.

1.3 Research Gap

Various studies on synthesis Pt NPs by employing conventional methods with physical, chemical and biological approaches to investigate the preparation of Pt NPs for medical application, the chemical approaches being the most popular. However, those methods are subject to a few critical limitations, including the use of hazardous and expensive chemicals, complex and laborious preparation requirements [20, 39]. To overcome these constraints, the plant-assisted synthesis of noble metal NPs, especially Pt NPs, has recently garnered interest because of its nontoxicity, eco-friendliness, and simplicity of usage [40-44]. Based on the literature, the conventional methods exhibit some disadvantages that include lengthy synthesis duration (5 – 48 hours) [40, 45, 46], high temperature (high temperature will lead to too high reaction kinetics. It is impossible to control the growth step of the crystallization process in reactions with fast kinetics; on the other hand, an explanation for this is that cherry extract is a reducing agent which is rich in ascorbic acid [47] and this acid becomes

slightly unstable at higher temperatures [48] and leads to a poor reduction process, uncontrolled and fast aggregation) [49, 50], and poor stability [51, 52]. The nanoparticles' efficacy for a biomedical application depends on their stability and biocompatibility [53]. In addition, the toxicity of NPs is dependent on their stability [54], which has also led to a need to understand the behavior of NPs suspensions to be settled. Importantly, the nanoparticles, which change particle stability over time, could potentially cause toxic effects [55].

Among the plants, *Prosopis farcta* fruits (*P. farcta*) has a widespread distribution and displays antibacterial [56, 57], anticancer [58, 59], antidiabetic [60-62], antihyperlipidemic [63, 64], and antioxidant capabilities [58, 62, 65]. In addition, a number of studies utilized *P. farcta* extract as a reducing agent in the synthesis of metallic NPs, such as Ag [66] and Au [67]. However, no studies have synthesis Pt NPs using *P. farcta* fruit as a reducing and stabilizer agent by sonochemical method. Furthermore, to date, the synthesis methods of Pt NPs as a contrast agent for CT imaging are complicated, requires more than one step or the need to modify Pt NPs using different chemical materials (inorganic and organic) to improve their stability and biocompatibility [39, 68, 69]. (Table 1.1).

Table 1.1 Previous studies of the influence of conventional method for green synthesis Pt NPs.

No.	Green Method	Plant	Reaction time	Reaction temperature (°C)	Size (nm)	Zeta potential (mV)	Ref.
1	Conventional	*Garcinia mangostana*	10 min	100	20 -25	-13	[49]
2	Conventional	*Black Cumin Seed*	48 h	75	3.47	-	[40]
3	Conventional	*Sechium edule*	24 h	Room temp.	28.56	-21	[45]
4	Conventional	*Ononidis radix*	5 h	80	20	-	[46]
5	Autoclaving	*Gum olibanum*	30 min	121	3.7-5	-12.7	[50]
6	Conventional	*Water hyacinth*	1 h	90	3.74	-0.0536	[52]

1.4 Problem Statement

The synthesis methods strongly influences the physiochemical properties of Pt NPs, for medical application. However, no study is available that comparatively analyses Pt NPs synthesized sonochemically and conventionally, using *P. farcta* fruits extract as a reducing agent and stabilizer for CT-scan imaging application.

1.5 Objectives of Work

The main objectives of this thesis are summarized in the points below:

1. To synthesise high stabile Pt NPs using *P. farcta* fruit as reducing and stabilizer agent by conventional (reduction) and sonochemical method.

2. To compare the green synthesizing methods of Pt NPs and to select the optimum sample with better physicochemical properties.

3. To identify the organic compounds, exist in *P. farcta* responsible for reducing and stabilizing the optimum sample.

4. To examine Pt NPs toxicity on human embryonic kidney (HEK293) and evaluate the optimum sample's sensitivity as a contrast agent for CT-scan.

1.6 Scope of Work

This study's main scope is to synthesize highly stable and biocompatible Pt NPs promising for medical application (CT-scan imaging) using a rapid, simple, environmentally friendly, and economical approach method. The *P. fracta* used will

be limited to the supplied material from one source in Iraq, while the variations of power used to generate the ultrasonication process are limited to a maximum of 700W and 40kHz (equipment limitation), respectively. This study also attempts to find out the organic compounds in *P. farcta* fruit extract responsible for reducing and stabilizing Pt NPs. In addition, the toxicity of Pt NPs will be only tested against normal cell (HEK293) and the sensitivity of the Pt NPs for CT-scan as contrast agent are evaluated using Agar media as the container for nanoparticles.

1.7 Contribution of the Thesis

This thesis contributes to the synthesis of a highly stable and biocompatible Pt NPs by rapid, simple, environmentally friendly, and economical approach method used *P. farcta* fruit extract as a reducing and stabilizer agent for CT-scan imaging propose.

1.8 Organization of the Dissertation

Chapter 1 describes a brief background of nanotechnology and Pt NPs, research problem, aim and objective, significance and implication of the research. **Chapter 2** discusses the literature review of some properties of Pt NPs, various methods of Pt NPs synthesis, as well as a background of *P. farcta* as reducing agent and Pt NPs as contrast agent for ST-scan. Also this chapter presents the various theories used to support this research. **Chapter 3** describe the whole experimental procedures, characterization techniques and application of the as-synthesized Pt NPs for application as a contrast agent for CT imaging. **Chapter 4** depicts details of the various results observed in this work are highlighted and discussed. Finally, the conclusion and possible future research were presented in **Chapter 5**.

CHAPTER 2

LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1 Introduction

Pt NPs are distinguished by certain inherent properties, including strong resistance to corrosion and chemical attacks, and a high surface-to-volume ratio [70]. To date, various studies have used physical, chemical and biological techniques to synthesis Pt NPs. It can be made with sizes between 2 and 100 nm, depending on the process and the reaction conditions [71, 72]. Pt NPs come in a wide range of shapes, including spheres, rods, cubes and tetrahedral [73, 74]. This chapter also covers the fundamental theories that govern the experimental setup and related diverse mechanisms and conditions such as cavitation, acoustic cavitation, shockwaves, high temperature and pressure generated by ultrasonic irradiation. The theories that underpin the application of CT-scan as a contrast agent were also covered.

2.2 Pt NPs and Their Properties

Due to its unique properties (optical, electrical and magnetic properties) [75-77], Pt NPs can be used in catalyst applications, energy, biotechnology, anticancer/antioxidant/antimicrobial applications, as well as in the fabrication of nano and biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products and electronics. Different physical and chemical techniques are utilized for the synthesis and stabilization of Pt NPs. Ionic or molecular forms of platinum are used as metal precursors in the synthesis of Pt NPs. Various chemical methods that include chemical and physicochemical reduction approaches,

electrochemical techniques, and radiolysis are extensively utilized to synthesize Pt NPs using diverse organic and inorganic reducing agents[78, 79]. However, these methods are constrained by several factors such as high cost, and the use of toxic chemicals that are deleterious to humans and the environment [80]. Hence, it is imperative to synthesize nanoparticles by means of eco-friendly techniques. Green syntheses via biological systems are typified by high yields of spherical, small-sized and highly stable nanoparticles.

The use of plant/plant extracts is the most preferred green synthesis approach because of the non-toxic nature of plant extract, low-cost and easy availability of herbs. Phytochemicals derived from plants are effective in the synthesis of nanoparticles. For instance, the water-soluble organic component of medicinal plants is utilized to reduce nanoparticles and stabilize the synthesized nanoparticles. The plant extracts are more efficacious in the synthesis of metal nanoparticles compared to other conventional methods. Therefore, this chapter reviews the use of bacteria, fungi, algae, plants and other biologically developed products in the biogenic synthesis and biomedical applications of Pt NPs [79, 81-83].

2.3 Synthesis of Pt NPs

The size, morphology, composition and structure of Pt NPs, as well as the presence of a capping agent, control their application for industrial and biomedical purposes [75, 84-88]. These physicochemical properties demand continuous development of novel synthesis methods for the optimization of these intrinsic properties. The modification and functionalization of Pt NPs for biomedical uses are directed by recently obtained data, which revealed that the physicochemical properties, dispersivity and stability of the NPs in a biological environment play key roles in

determining their safety or toxic levels. The critical challenges encumbering the potential use of Pt NPs as drug carriers and antioxidant materials include biocompatibility, specifically defined properties and contamination-free production (e.g., endotoxin, Pt precursors, toxic unreacted reagents, organic solvents.) [89]. The several synthesis techniques are discussed in the underlying subsections.

2.3.1 Chemical Method

The chemical synthesis methods, including wet chemical reduction (WCR) [90], electrochemical reduction [91, 92]**,** galvanic displacement [93, 94], and chemical vapour deposition (CVD) [95, 96], which are all employed to precisely define the physicochemical properties of NPs. Mainly, WCR is frequently utilized because of its ability to control the features of NPs successfully. WCR synthesizes Pt NPs from Pt precursor solutions with the aid of a reducing agent [90], which enables the stringent control of features of NPs such as size and morphology by altering the precursor concentration [19], reaction temperature [75], and incorporation of organic or inorganic ligands [97-100]. The use of WCR methods to synthesize Pt NPs with enhanced catalytic performances has been extensively explored. Many shapes modifying agents (polymers, surfactants and capping agents) have also been used to facilitate the asymmetric synthesis of NPs. Multiphase synthesis set-ups have also been developed [101-103], which include utilizing gaseous reducing agents to attain enhanced control of reaction parameters [104]. However, this approach is constrained by the need for vast quantities of organic solvents, surfactants and capping agents that could increase the toxicity levels of NPs. Besides, a huge volume of production (large-scale) can create an environmental threat. Hence, the use of microwave heating and glycerol, as a reducing agent and solvent, have been developed to allow for industrial

up-scaling and reduced environmental impact, [105, 106]. Thiol-chemistry is also frequently used to synthesize NPs that are stable in an aqueous environment or inorganic media. Thiol ligands, such as alkane thiols [107-109] or thiols bearing polar groups [100, 110, 111] have been used to synthesize stable Pt clusters with controlled size and precise morphology. These techniques enable a further decrease in the size of NPs, thereby elevating the surface/volume ratio. Despite the issue of decreased catalytic performance with the addition of thiols, their presence on the surface can transform the properties of the NPs such as the combination of hydrophobic and hydrophilic ligands on the same NP to support selective contact with substrates and the environment [112].

Conversely, these chemicals (aromatic, aliphatic and amino-terminated thiols) exhibit potential hazardous effects and can be lethal both in vitro and in vivo [113]. To synthesize Pt NPs that are biocompatible, the approach of using ‘‘green reagents’’ like sodium citrate and ascorbic acid is most promising, due to the fact they allow robust control on solvent and guarantee the purity of reagents [113-116]. This synthesis approach can also assure precise control on size, morphology, and catalytic properties, in addition to reasonably high yield [113]. For example, citrate capped Pt NPs are reported to display excellent cytocompatibility and high antioxidant capability [114]. Furthermore, the use of green reagents enables the simple surface functionalization of NPs, which is essential to the synthesis of Pt NPs for biomedical purposes, since nanomaterials' biological characteristics are highly dependent on their bare surface area. Therefore, since the surface coating of NPs affects their toxic levels and specific targeting, biogenic synthesis techniques using biomolecules as templates have been initiated [117]. For instance, apoferritin protein encapsulated Pt NPs was proven to enhance the cellular uptake and considerably decrease membrane damage [118-120].

Similarly, Wang et al. [121] developed dendrimers encapsulated Pt NPs to replicate the catalytic centres within natural enzymes. Yamamoto et al. [122] posited that Pt clusters' catalytic activity was significantly improved when encapsulated with dendrimers, enabling the controlled growth of the nanomaterial. The utilization of dendrimers as encapsulating agents for the synthesis of Pt NPs and clusters has also been explored in other studies [122, 123].

2.3.2 Physical Method

Other synthesis methods that have recently gained attention include laser ablation [124, 125], aerosol-assisted deposition [126], electron-beam-induced reduction [127], and flame synthesis[128, 129]. These physical methods were developed to deal with certain constraints of the chemical methods (such as toxic reagents, organic solvents). The laser ablation technique utilizes the continuous or pulsed application of a high-power laser beam for the evaporation of NPs from solid source. This versatile technique is anchored in the control of pulses, reaction temperature, and atmospheric gas pressure to attain a particular set of NP properties. The key benefit of using this approach is the absence of redundant coatings, solvent contaminations and stabilizers which are problematics usually associated with synthesis in nanomedicine [130]. However, the mechanism of NP production has not been entirely elucidated. Moreover, the high dilution required in addition to the complexities of modifying the size, morphology, and production yield of NPs has constrained their use [113, 131, 132]. Furthermore, the stability of these NPs in aqueous solutions is a complex procedure. However, in the absence of stabilisers, laser ablation is capable of synthesising stable NPs due to intrinsic electrical repulsion effects arising from the presence of surface charges on NPs [133, 134]. Even so, this

could pose a problem in biological systems, because clustering/agglomeration and precipitation might simultaneously arise during incubation of NPs under intricate conditions, such as cell culture media and high ionic strength solutions. Cathodic corrosion is one more basic physical technique of synthesizing Pt NPs, which entails the conversion of a bulk alloy electrode in a suspension of NPs with similar constituents [135]. However, this approach is similarly constrained by relatively low production yield and size modification.

2.3.3 Biological Method

Bio-assisted synthesis techniques were developed as viable substitutes of chemical and physical techniques. Their advantages include the absence of unwanted reaction solvents. The application of biological syntheses has focused on the production of noble metal nanomaterials, because of their ease of reduction using weak reducing agents [136-144]. Few studies have explained the production of Pt NPs by means of the green synthesis approach [145-147]. Nonetheless, procedures have been successfully developed to synthesize monodisperse and stable Pt NPs via the biosynthesis approach, using bacteria [148-151], cyanobacteria [152, 153], seaweeds [154, 155], fungi [156-158], plants [159-161], in addition to bio-derived products which include honey [52, 162]. Some studies exploited the activity of specific hydrogenase enzymes in sulphate-reducing bacteria to reduce Pt (IV) into Pt NPs [163, 164]. Like the WCR, the concentrations of Pt salt and protein serve vital roles in tuning the morphology and dimension of the NPs during biogenic synthesis [165]. The synthesis of Pt NPs using fungi, such as *Neurospora crassa* [158] and *Fusarium oxysporum* [156, 157, 166], is a valuable ‘‘scale-up’’ approach. The phytochemical constituents of plant extracts [167] and wood [168, 169] have also been exploited as

capping agents in the biogenic synthesis of metal NPs. The biosynthesis of Pt NPs via phytoreduction was first reported in 2009, where 2–12 nm-sized Pt NPs were synthesized using leaf extracts with 90% yield and extremely low leaf biomass [170]. Presently, studies have reported the use of a wide and diverse range of vegetable-derived products such as *Diospyros kaki* [171], *Ocimum sanctum* [172], *Medicago sativa* and *Brassica juncea* [173] to synthesize Pt NPs. Using the root extract of *Asparagus racemosus Linn*, Raut et al. [174] developed a quick procedure of synthesizing monodisperse, spherical Pt NPs with a size range of 1–6 nm in an aqueous solution under ambient conditions.

The key benefits of synthesizing Pt NPs from plant extracts are simplicity, convenience, inexpensiveness, easy scalability, low energy requirement, environmental friendliness, and minimum use of hazardous substances and maximized the synthesis process's effectiveness. It is particularly pertinent that the synthesis process of NPs is devoid of toxic materials. The plant extract-based synthesis increases the stability of NPs in terms of size and shape, and produces a higher yield compared to other physical and chemical methods.

2.3.4 Sonochemical Method

Wood and Loomis (1927) were the first to elucidate the phenomenon of sonic wave propagation in liquids media. They reported that the sound travels faster in water than in air. However, due to the higher density of water than the earth’s atmosphere, it is difficult to have sonic waves in the water. Nonetheless, the sonic waves were dispersed in water by creating bubbles and loud noises in the water. However, this process was complicated by the length ratio of the lower frequency waves to spread through the walls of the bubbles and to make contact with the water [175].

In the 1980s, the field of sonochemistry was regenerated by introducing steady generators capable of generating high-intensity ultrasounds to overcome the constraints as mentioned above [176]. Sonochemistry entails the process of synthesizing nanoparticles using ultrasound radiation through the impact of acoustic cavitation (formation, expansion and implosive collapse of the bubbles) in liquid media (Figure 2.1). The ultrasound radiation generated by the sonication amplitude (%) is related to the power (dBm) produced by the sonicator, as expressed in Eqn. 2.1. Following the bubbles' implosive collapse, shockwaves, microstreamers, and microjects are produced in the area, which increases turbulence and shear forces that can enable mass transport [177].

$$power\ (dbm) = 10\ log_{10} A\ \ and\ 1dBm = 0.0013\ W \qquad (2.1)$$

Where A denotes the sonication amplitude and 1 watt = 30 dBm

The bubble collapse results in massive production of energy with very high temperature and pressure of approximately 5000K and 500 atm, respectively, at a hot-spot point, and heating and cooling rate of over 10^{11} K/s concurrently within the sonicated liquid [178]. As the bubbles rapidly collapse inward, chemical excitation of substances within or adjacent to the bubbles results in high temperatures and pressures. Acoustic cavitation can lead to an array of results that include sonoluminescence, increase in chemical activity in the solution via the formation of novel, relatively stable chemical species or secondary and primary radical reactions.

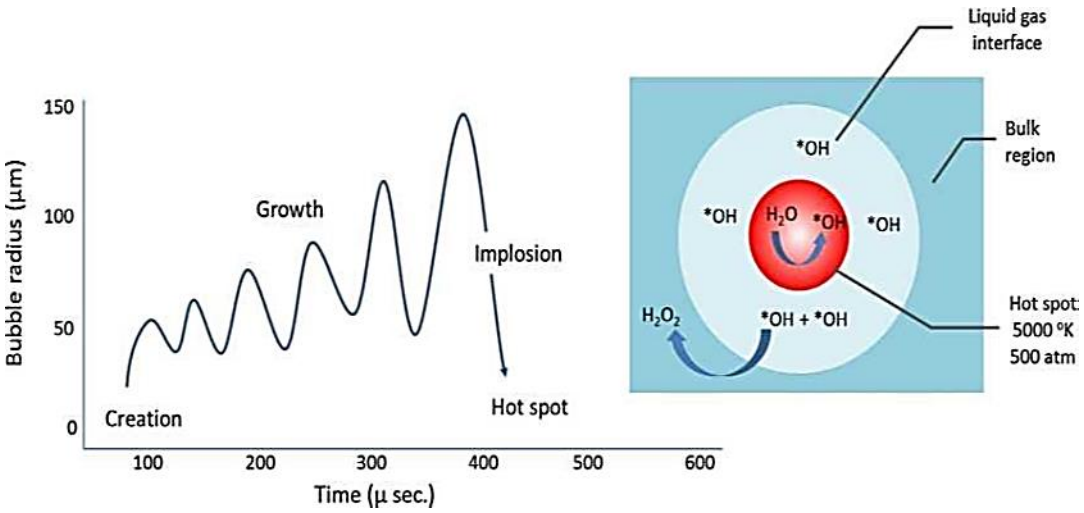

Figure 2.1 Schematic representation of cavitation bubbles displaying stable and transient cavitation and reaction zones in the cavitation process [179].

The sonochemical approach has been established as a valuable technique that requires less quantity of chemicals, non-toxic to the environment, and capable of producing novel materials with remarkable properties [180]. Moreover, it generates no wastes and eliminates the high expenses of recycling chemical waste [181].

There are a number of fabrication techniques for synthesizing nanostructured materials. Narrowly sized nanomaterials with high surface areas have been produced using the sonochemical method [179, 181]. In contrast to conventional techniques, ultrasound can easily control the particle size by tuning the critical factors such as irradiation time, reaction temperature, sonication power and concentration of the precursor [180, 181]. The fundamental theory that governs the sonchemical approach is the acoustic cavitation phenomenon, which is referred to as hot spot theory [182].

In recent times, it has been demonstrated that ultrasound irradiation of aqueous solutions that contain noble metal salts can produce the equivalent colloidal noble metals including gold [183], palladium [184], silver [185] and platinum [186]. Caruso

et al. [187] investigated the relationship between the ultrasound-induced cavitation and the eventual synthesis of colloidal Pt via sonochemical reduction of $PtCl_6^{2-}$. The study discovered a direct relationship between the degree of reduction of $PtCl_6^{2-}$ in the presence of alcohol and Gibbs surface energy of the alcohol at the air-water interface. To synthesize Pt colloids, a 20 kHz ultrasound Sonifier (standard 450 Branson Probe) with a high gain stepped horn (19 mm diameter tip) was used to transmit ultrasound waves to the 40 mL solution of $PtCl_6^{2-}$ for 5 min. Using calorimetry, the power absorbed by the solution was determined to be 10 W cm^{-2}. The diameter of the synthesized colloidal Pt is approximately 3 nm. The reduction of Pt (IV) in the presence of alcohol is explained as follows: the primary $H^{\bullet}$ and $OH^{\bullet}$ radicals are consumed by the alcohol that is adsorbed at the interface, which then diffuse away from the bubble to react with Pt (IV) in bulk solution. The follow-up reactions after this initial reduction step as well as the factors that determine the final size of the colloid Pt are unknown. Undoubtedly, the adsorption of Pt (IV) and onto alcohol Pt (0) clusters plays a key role in determining the final size of the colloid Pt.

Radziuk et al. [188] reported the effect of ultrasonic treatment on the crystal properties and catalytic activity of Pt NPs. The simultaneous heating/cooling cycles of the cavitation microbubbles explains the melting and precipitation/crystallization of amorphous or crystalline Pt NPs. Mizukoshi et al. [189] employed high-intensity ultrasound (200 kHz, 6Wcm^{-2}) for the preparation of Pt NPs in an Pt (II)-SDS aqueous system at the rate of 26.7 μM min^{-1}. The particles formed in the presence of a surfactant (sodium dodecyl sulfate) were stable, evenly spherical, and relatively monodispersed with an average diameter of 2.6 nm. The reducing species that were sonochemically produced in the hot bubbles' proximity would react with the $PtCl_4^{2-}$ complexes to form the Pt NPs. Chave et al., [190] sonochemically deposited Pt on the surfaces of

polystyrene beads. The synthesized Pt NPs were then delivered into a porous silica matrix using the polystyrene beads as a disposable template. The deposition of Pt NPs was facilitated by the sonochemical reduction of Pt (IV) under ambient conditions in latex solutions containing polystyrene beads in the presence of formic acid under Ar or Ar/CO atmosphere without any additives. Following ultrasonic irradiation for a few hours, the Pt NPs were well-dispersed on the PSB polystyrene beads within the range of 3–5 nm.

Zin et al. [191] reported the synthesis of Pt NPs from aqueous chloroplatinic solutions in the presence of low-frequency high-power ultrasound (20 kHz) irradiation. The morphology and structure of the synthesized Pt NPs confirm the successful application of the sonoelectrochemical approach. The smallest particle has a mean diameter of approximately 13 nm and exhibits face-centred cubic structure with a lattice parameter of 3.91Å. In general, it can be inferred that sonication via ultrasound irradiation enables the synthesis of highly pure Pt NPs with controlled structure and uniform nanometric crystalline size.

Kora et al. [192] created a facile and novel cloud point process (single pot method) to simultaneously synthesize and preconcentrate cetylpyridinium complexed hexaiodoplatinum nanoparticles (Pt-I NPs) through the catalytic conversion of the leachate using sonication. The synthesis and preconcentration of Pt-I NPs was achieved at a diluted metal precursor concentration. Hence, the study develops an approach of simplifying the synthesis of Pt-I NP with an average size of 5.6 nm, which is hitherto an inaccessible and intricate procedure using conventional methods [193]. Remarkably, the demonstrated dual solubility of the preconcentrated Pt-I NP supports their use in both chemical and biological applications.

Recently, several studies have sonochemically synthesized NPs using plant extract as a reducing agent and stabilizer. For instance, Fatih et al. [70] employed the sonochemical method to synthesize monodisperse Pt NPs using *Punica granatum* extract as a reducing agent. The solution containing $PtCl_4$ and pomegranate extract was placed on a magnetic stirrer under ambient conditions for 24 hours. The color of the solution transformed from yellow to brown, indicating the formation of Pt NPs. The acquired Pt NPs were then centrifuged at 4 °C and 15,000 rpm for 30 minutes to purify the sample. This procedure was repeated twice. Afterwards, the final pellet was washed thrice with ethanol and aerated in a vacuum oven. The Pt NPs have an average particle size of 20.12 nm with high dispersivity in water. This study substantiated the effectiveness of biologically synthesized Pt NPs for the treatment of cancer cells, thus further research was conducted to extend their application to drug administration via conjugation with metabolites.

Nonetheless, the traditional techniques (using plant extract without sonication) are constrained by several limitations such as long synthesis duration [40, 45, 46], high temperature [49, 50], and poor stability [51, 52]. Anyik and Oluwafemi utilized water *hyacinth* leaves extract as a reducing and capping agent in the synthesis of Pt NPs with a low value of zeta potential which accounted for their aggregation [194]. Likewise, Chelli and Golder conducted a plant-mediated synthesis of Pt NPs, using *Sechium edule* fruit extract as a reducing agent. The Pt NPs exhibited low zeta potential value, indicating the agglomeration of the nanoparticles [45]. Thus, the sonochemical method is an interesting alternative approach for the synthesis of NPs, since it can reduce the time of preparation and produce monodisperse NPs with pure crystallinity and high stability [195-197]. For this study, Pt NPs were synthesized both sonochemically and conventionally, with *P.farcta* fruits extract utilized as a reducing agent and stabilizer.

The structural and morphological properties as well as colloidal stability of Pt NPs synthesized by both methods were determined and compared.

2.4 Prosopis Farcta Fruits

Prosopis farcta is prevalent in the southwestern parts of Asia, and is an invasive plant in the eastern region of USA (Figure 2.2)[198]. It is a member of the Fabaceae family and the genus of *Prosopis* that comprises 44 species[199]. Archeological discoveries infer that ancient populace of the eastern Mediterranean basin utilized this plant, essentially for food [198, 200]. Prosopis farcta has been harnessed in the Middle East to make traditional medicines, notably for the treatment of diabetes[201]. In Pakistan, the plant is customarily used for several purposes that include medicinal (humans and animals), animal food, and fencing [202]. In herbal medicine of Jordan, *P. farcta* is utilized as an antispasmodic and analgesic agent [203]. It is well known that Iran is at the forefront of using this plant as traditional medicine. The uses include blood thinner, antidiabetic treatment, sterilizer (hands), acne treatment, anti-atherosclerosis and menstruation pain [198, 204-208].

Figure 2.2 *Prosopis farcta* plant (leaves and fruits).

2.5 *Prosopis farcta* Fruits as Reducing and Stabilizer Agent

Majority of the therapeutic activities of *P. farcta* have been studied with several publications available on the subject. In addition to conventional activities such as antioxidant, antibacterial and antidiabetic, many other aspects have been explored, such as conjugation with nanoparticles. *P. farcta* contains different compounds that include apigenin 5-hydroxytryptamine (alkaloids), l-arabinose, quercetin (flavonoids) tryptamine, lectin, and phenolic compounds, such as caffeic acid derivative, vicenin-2, apigenin c-glycoside, and tannins [67]. These compounds can reduce metal ions and stabilize the NPs. *P. farcta* exhibits antibacterial [56, 57], anticancer [58, 59], antidiabetic [60-62], antihyperlipidemic [63, 64], and antioxidant [58, 62, 65] competencies. Moreover, the root extract of *P. farcta* has been reported to have the capability to lower blood pressure [209].

Numerous studies used *P. farcta* extract as a reducing agent in the synthesis of metallic NPs, e.g., Ag [66] and Au [67]. Prior research has explored the reducing effect of *P. farcta* extract on the synthesis of metal NPs. Sarani et al. [67] utilized the extract from the leaves of *P. farcta* to biosynthesize Au NPs. The extract was obtained through the addition of 50 mL of water to 5 mg of leaf powder. The resulting mixture was stirred at 150 rpm for 4 h, and afterwards filtered using Whatman paper (No. 1). 5 mL of the aqueous leaf extract was then added to 50 mL of aqueous gold chloride solution (1 mM). The final mixture was stirred at 150 rpm for 30 min under ambient conditions. The resulting biosynthesized Au NPs were homogeneously and spherically shaped with an average particle size of 25 nm. The cytotoxic effect of AuNPs was explored on a cancerous colon (HT-29) cell line via the MTT assay, and the IC_{50} of Au-NPs was calculated to be 419.7 μg/mL. The results inferred that the apoptotic effects on cells increased when the concentration of AuNPs attained 200 μg/mL. Using *P. farcta* extract, the synthesis of AuNPs becomes facile, cost-effective and fast.

Salaria et al. [210] utilized the extract from the fruit of *P. farcta* to synthesize Ag NPs. The fresh fruits were meticulously rinsed several times with deionized water and then dried. 2.5 g of fruit was then added to 100 mL of distilled water boiled for 30 min. The resulting suspension was filtered with Whatman filter paper (No. 40). Afterwards, the solution was centrifuged at 4000 rpm for 30 min to remove impurities and residues. 9 mL of 0.001 M aqueous solution of silver nitrate ($AgNO_3$) was prepared in a Stoppard Erlenmeyer flask followed by different volumes (100, 110, 120, 130, or 140 μL) of fruit extract (2.5 g/100 mL). The flask was stored at room temperature under dark conditions. To determine the effect of temperature on synthesis of the nanoparticles, the reaction mixtures were stirred in a rotary shaker at 150 rpm at 50 °C, 60 °C or 70 °C for a period. After completion of the reaction, the mixture

was centrifuged for 20 min at 15000 rpm. The precipitates were washed with distilled water several times to remove impurities like precursor materials or natural products from the extract. The reaction time effect was assessed by incubating the optimized content of the reaction mixtures for 25 to 45 min. The particle size of the synthesized AgNPs varied from 10.26 nm to 14.65 nm. In addition, the total phenolic and flavonoid contents of fruit extract and AgNPs were evaluated. The antiradical scavenging activity of AgNPs-conjugated with plant extract was determined using 1,1-diphenyl-2-picryl-hydroxyl (DPPH) and ferric reducing antioxidant power (FRAP) assay while their antimicrobial activity was evaluated against *Staphylococcus aureus, Streptococcus pneumonia, Escherichia, Salmonella typhi* using the disk diffusion method. The total phenols and flavonoids in AgNPs-conjugated with plant extract were 462.69 (mgGAE/g extract) and 386.94 (mgQE/g extract), respectively, which were considerably higher than fruit extract. Biosynthesized AgNPs showed a comparably higher antioxidant and antibacterial activity than *P. farcta* fruit extract alone. It could be deduced that *P. farcta* fruit extract can be widely utilized in the making of potential antioxidant and antibacterial AgNPs for biomedical applications.

Furthermore, Majid et al. [66] synthesized Ag NPs at room temperature using leaf extract of *P. farcta*. The leaf extract was obtained by initially stirring a mixture of 5.0 g of dry leaf powder and 50 mL of distilled water in an orbital shaker for 4 h and then filtering the solution through Whatman filter paper No. 1. To synthesize AgNPs, 5 mL of the leaf extract was added dropwise to 95 mL of silver nitrate (1 mM) solution. The solution was subsequently stirred in an orbital shaker for 1 h under ambient conditions. The solution's color transformed from light brown to dark brown, indicating the formation of Ag NPs. The final solution was centrifuged at about 10,000 rpm for 15 min. XRD spectra and TEM analyses revealed the Ag NPs were spherically