Greener synthesis of silver nanoparticles using waste of onion (*Allium cepa*) and quercetin. A comparative study.

Submitted in fulfillment of the requirement for the degree of
Bachelor of Technology in Biotechnology

Submitted by

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CERTIFICATE

This is to certify that Mr Sehaj Singh Gulati has carried out the undergraduate project work on **"Greener synthesis of silver nanoparticles using waste of onion (*Allium cepa*) and quercetin. A comparative study."** under my supervision from July 2018 to May 2019. The work presented in this project report is original and has not been submitted anywhere else for any other degree.

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DECLARATION OF SCHOLAR

I hereby declare that the project titled **"Greener synthesis of silver nanoparticles using waste of onion (*Allium cepa*) and quercetin. A comparative study."** submitted towards fulfilment for the award of degree of Bachelor of Technology in Biotechnology from Jaypee University Of Information Technology is based on the results of studies carried out under the supervision of Dr. Abhishek Chaudhary. This work, in part or in whole, has not been submitted anywhere else for award of any degree or diploma. I am responsible for the contents of this report.

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List of abbreviations

Abbreviation	Full Form
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
SIAA	Society of Industrial-Technology for Antimicrobial Articles
ROS	Reactive oxygen species
XRD	X ray diffraction
JCPDS	Joint Committee on Powder Diffraction Standards
FWHM	Full width at half maximum
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
DLS	Dynamic light scattering
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
FCC	Face centred cubic
OENP	Silver nanoparticles synthesized using onion extract
QTNP	Silver nanoparticles synthesized using quercetin

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Abstract

Silver nanoparticles are one of the most widely used nanoparticles currently, finding applications in fields of nanobiotechnology, diagnostics, bio-sensing, cosmetics and material science. Presently most of the nanoparticles used are synthesized through the chemical route thus creating a threat to the environment. Alternately green synthesis of nanoparticles has become an area of interest for researchers worldwide in the last decade. This study investigates the potential of onion waste extract for green synthesis of silver nanoparticles along with pure biomolecule quercetin, a major component of the onion waste. The present study includes the characterization of synthesized nanoparticles through scanning electron microscopy, X-ray diffraction and UV-VIS spectrophotometry, which revealed that spherical nanoparticles with mean diameter of 10nm were synthesized. As quercetin is a polyphenol molecule, hence change in its antioxidant behaviour in the bound and free form was also determined. Through this study, differences in the properties of nanoparticles synthesized using purified biomolecules and natural extracts was brought into light. The synthesized particles were assessed for their antioxidant and antimicrobial potential. Nanoparticles synthesized using onion scored higher radical scavenging activities in ABTS and DPPH assays. In case of antimicrobial activity, particles synthesized using quercetin were more potent.

Chapter 1:
Introduction

The demand of silver nanoparticles driven services is increasing exponentially in various fields due to their promising results in fields of medicine, biosensors and cosmetics, to name a few [1][2][3]. Although, the most of the demands are full filled by the chemical methods but the operational risks and environmental issues are restricting the use of these kinds of processes. Chemically synthesized nanoparticles are not biocompatible because of the toxic reagents involved in the synthesis [4][5]. Green synthesis of nanoparticles is becoming a widely popular area of research as an alternate to the costly and dangerous chemical processes [6]. Green synthesis of nanoparticles can be performed using fungi, bacteria and plant extracts [7][8]. Out of all these, plant extracts are the most suitable for commercial production due to fast rate of nanoparticle formation, easy extraction and reduced risk of contamination. Green synthesis of silver nanoparticles has already been performed using extracts of various plants like *Camellia sinensis* [9], *Aloe vera* [7], *Allium sativum* [10], *Pistacia atlantica* [11], *Nelumbo nucifera* [12], *Datura metel* [13], *Citrus sinensis* [14] and many other plants. The major problem with using these plants for commercial production of nanoparticles is that availability of these plants in large quantities will lead to change in crop patterns, conflicting interest with food as in the case of using food crops for production of first generation biofuels [15].

Onion is a vegetable used in almost every house daily in India and across the world. India is one of the largest producers of onion in the world. Along with it onion waste, i.e., the crisp outermost skin, is produced in huge quantities. It is not used in cooking and discarded in kitchens. At such a large scale, economic and environment friendly solutions must be looked into for management of this waste. Ironically, it is this waste part of onion bulb that has the highest amount of quercetin, a flavonoid, responsible for most health benefits of onion [16]. The red onion, which is most commonly available and consumed variety in India, has the highest amount of quercetin among the various onion varieties [17]. The outermost scale leaves of red onion can have total quercetin and its glucoside derivatives amounting to 66.33mg/g dry weight of onion, making it one of the richest sources of the flavonoid among the staple food items [17]. In traditional medicine, onion is well known for its antibacterial and anti-inflammatory properties. Compost of onion waste can be used to get rid of white-rot fungi from soil [18].

The availability of biologically active compounds like phenolics has been well documented in onion leaves [19] which can be used as reducing and capping agent for the synthesis of

various metallic nanoparticles. To understand the role of phenolics of onion waste extract in the process of reduction and capping of nanoparticles, pure quercetin was also used along with onion extract to study the dynamics of nanoparticle synthesis. The present study will outline a methodology for the use of polyphenols, particularly flavanoids in the formation of nanoparticles from different metal ions, focusing on silver in this case. On a commercial scale, choice of reducing agent in the form of crude plant extract or purified biomolecule would heavily influence the cost of synthesis. Difference in the amount of flavonoids in the plants varies from season to season and from crop to crop cultivated in different geographical regions can give rise to variations in different batches of synthesized nanoparticles [20].

Among all metallic nanoparticles, silver nanoparticles are one of the most widely used ones due to their antibacterial properties. The silver nanoparticles find commercial applications in textile industries, wound dressings [21], cosmetics[2], toothpastes[22] and water treatment plants[23][24] as anti-microbial agents. The use of silver nanoparticles has been approved by regulatory bodies like the EPA, FDA, Japan's SIAA, FITI Testing and Research Institute and Korea's Testing and Research Institute for Chemical Industry [25]. To meet such a high demand and rate of production, it is imperative and urgent to find sustainable, environment and economically friendly routes of nanoparticles synthesis to reduce the stress on the resources available.

The major attributes of silver nanoparticles that are taken into account in this study are the antibacterial and antioxidant effects. Silver is a universally known anti-bacterial agent, both in ionic form as well as nanoparticle form. [26]. Apart from bacteria, silver can be toxic to humans too. Toxicological studies involving bacteria and human cell lines both indicate that silver nanoparticles are less toxic as compared to silver ions, making them better suited for application in humans [27]. Clear mechanism of this silver toxicity has not yet been elucidated. Among many other reasons, ROS production has been reported to be one of the major causes of cell death [28]. To negate this production of ROS by silver nanoparticles, quercetin has been used as capping agent for the synthesized silver nanoparticles. The flavonoid shows remarkable antioxidant activity in its nascent form. The same activity is measured in this study for quercetin bound to silver nanoparticles as capping agent. Also, it has been previously noticed that nanoparticles can exhibit powerful antioxidant activities [29]. Silver nanoparticles can also play a significant role in combating the rising issue of antibiotic resistance in pathogenic microbes [30].

Chapter 2:
Review of Literature

2.1 History of nanotechnology

Nanotechnology is an old branch of science, tracing its origins in modern history back to 1800s, when Michael Faraday reported the formation of nanoparticles for the first time. He highlighted that a mere decrease in size of gold particles can cause its colour to change from yellow to ruby red. The scientific community worldwide was intrigued and inspired by the idea of manipulating matter at the scale of individual atoms and molecules mentioned in the historic speech "There's plenty of room at the bottom" by the infamous American physicist Richard Feynman at a meeting of the American Physical Society, back in December 29th of the year 1959. The term nanotechnology was officially used for the very first time by Norio Taniguchi in 1974 in a conference at the Tokyo University of Science for describing processes related to semiconductors [31].

2.2 Methods of nanoparticle synthesis

2.2.1 Top down approach

Nanoparticle synthesis strategy can be broadly classified into two categories: top down and bottom up. The top down approach aims at reducing the size of bulk materials to the extent that their dimensions reach the nano-scale. These consist mostly of high energy requiring physical methods which include, but are not limited to sputtering [32], ball milling [33] and laser ablation [34]. These methods require sophisticated machinery and increase the cost of process. Many of the physical methods are based on the principle of evaporation and condensation, which uses a tube furnace. Tube furnace has a lot of disadvantages associated with it, as it occupies a large area and consumes a huge amount of energy during the heating process, heating the surrounding environment as well. Moreover, power in the order of kilowatts is necessary for its functioning along with a pre heating time of few minutes [35].

2.2.2 Bottom up approach

The bottom up approach on the other hand, works by assembling ions of the subject of interest into themselves till they grow to the size of a nanostructure. This is one of the most widely used approaches for metallic nanoparticles in particular. The methods or techniques that fall under this approach can be further mentioned in two separate categories; chemical synthesis and green synthesis or biological synthesis [36]. Both the categories involve the use of three components, namely metal ion source, a reducing agent and a stabilizing or capping agent.

2.2.3 Chemical synthesis

The chemical synthesis usually uses separate substances as reducing and capping agents, whereas in green synthesis, a single substance serves the purpose of both reducing as well as stabilizing agent. Some substances commonly used as reducing and capping agents in chemical synthesis are sodium borohydride, sodium citrate, N,N-dimethyl formamide, and ascorbate [37] [38]. Many chemicals used in chemical synthesis approach are of a toxic and hazardous nature, which limits the use of such synthesized nanoparticles in human applications. They also generate toxic by-products due to which the problem of environmental pollution and disposal of such substances also arises [39]. Due to these disadvantages, scientists started researching about the green route of nanoparticle synthesis.

2.2.4 Green synthesis

Since green synthesis uses bacteria, fungi or plant extracts as reducing and capping agents, the problems of chemical synthesis do not arise in this case. An advantage of using the bottom up approach is that large quantities of nanoparticles can be synthesized in short durations of time. However, among the different biological routes available, use of plant extract has proven to be more advantageous for large scale, industry suited production. This is because use of microbes in an industry would require highly aseptic conditions and the subsequent maintenance. Use of microorganisms brings along with it the tedious task of maintaining cell cultures. Use of plant extracts saves not only from this tedious job, but also reduces costs associated with microbial isolation and culture media, thus giving it an economic advantage over other methods of synthesis. Proteins, polysaccharides, amino acids, alkaloids, polyphenols, saponins, tanins, vitamins and terpinoids are some of the classes of biomolecules involved in reduction and stabilization of nanoparticles. These biomolecules are benign but at the same time biologically complex [39]. Since properties of nanoparticles are largely dependent on their surface composition, these biomolecules have an impact on the biological properties of the nanoparticles while acting as surface stabilizing agents. This depends on the functional groups involved in stabilization of nanoparticles. Rest of the groups interact with the surrounding solvent medium, which confer the biological properties of the nanostructures. Some plants used for synthesis of nanoparticle sare given in the table 1.

Table 1: Silver nanoparticles synthesized through green route and their characteristics

Plant	Plant's part	Size (nm)	Shape	Reference
Aloe vera	Leaves	50–350	Sphere, triangular	[7]
Memecylon edule	Leaves	20–50	Triangle, sphere, hexagon	[40]
Nelumbo nucifera	Leaves	25–80	Sphere, triangle	[12]
Eclipta prostrate	Leaves	35–60	Triangles, hexagons, pentagons	[41]
Ziziphora tenuior	Leaves	8–40	Sphere	[42]
Tea extract	Leaves	5–100	Sphere	[43]
Acorus calamus	Rhizome	31.83	Sphere	[44]
Cocous nucifera	Inflorescence	22	Sphere	[45]
Pistacia atlantica	Seeds	10-50	Sphere	[11]
Vitex negundo	Leaves	5,10-30	Sphere	[46]
Melia dubia	Leaves	35	Sphere	[47]
Boerhaavia diffusa	Whole plant	25	Sphere	[48]
Tribulus terrestris	Fruit	16–28	Sphere	[49]
Datura metel	Leaves	16–40	Quasilinear superstructures	[13]
Allium sativum	Leaves	4–22	Sphere	[10]
Acalypha indica	Leaves	20-30	Sphere	[50]

2.3 Characterization of nanoparticles

Presence of silver nanoparticles in a solution can be detected by the naked eye, as an appearance of yellow to brown colour, depending on the concentration of particles. At higher concentration, the solution turns to a dark brown coloured liquid.

2.3.1 Characterization of crystal lattice and size – X Ray Diffraction

X ray diffraction (XRD) is an important technique associated with the characterization of metallic nanoparticles. This technique yields important information about the 3 dimensional arrangements of atoms within the lattice of the nanoparticles. This technique employs the wave nature of light, which is responsible for the phenomenon of diffraction and interference. In XRD, X-rays of a single wavelength are made to pass through a material. The X-rays interact with the electrons of the atoms of the sample material and undergo diffraction. This results in scattering of the X-rays in multiple directions. Next these scattered X-rays interact with each other. Most of them get cancelled out due to destructive interference, but a few undergo constructive interference, resulting in a higher intensity. The angles at which constructive interference can be observed is given by Bragg's law: $2\,d\sin\theta = n\,\lambda$. Where d = distance between two planes of diffraction, θ = Bragg angle, λ = wavelength of X-ray. The result of an XRD experiment appears in the form of a graph of intensity vs 2θ. The kind of crystal lattice structure of material under consideration can be found out by identifying the Bragg diffraction planes corresponding to the angles (2θ) at which intensity is high due to constructive interference. These findings can be validated by comparing the results to relevant JCPDS files, which are compiled as a database by the International Centre for Diffraction Data. The results of XRD can also be used for calculating the mean diameter of nanoparticles using the Scherrer equation; $t = \frac{K\lambda}{\beta\cos\theta}$ named after the swiss physicist Paul Scherrer. In this equation, t = mean diameter of particles, K = shape factor, having a typical value of 0.9 which may vary with particle shape, λ = wavelength of X-ray source, β = FWHM of peak and θ = Bragg angle.

2.3.2 Size distribution estimation - UV-visible spectrophotometry

Among the analytical techniques, one of the simplest and most widely used techniques for characterizing silver nanoparticles is UV-visible spectrophotometry, which is an instrument

based on the Beer-Lambert's law. For any substance to be detected using this technique, it must absorb light of a particular wavelength. Silver nanoparticles show high absorbance in the region of approximately 400-450nm [51]. This characteristic absorption peak is due to the phenomenon of localized surface plasmon resonance. In case of nanoparticles, due to their small size, incident light of appropriate wavelength is able to cause resonance in the conduction electrons of the molecules, which does not take place in bulk or ionic form of the material. As with all types of resonance, it is a prerequisite that the frequency of excitation source is equal to the natural frequency of the substance in consideration. This resonance does not allow transmittance of light from the solution (i.e. high absorbance) at a wavelength characteristic of the material. In case of silver nanoparticles, the wavelength at which surface plasmon resonance is observed lies in the region of 400-450nm. The limits of this region can vary for different nanoformulations depending on the size of constituting nanoparticles as well as the capping agent used. . The surface plasmon resonance peak shows a red shift with increase in the mean size of nanoparticles in a liquid system, while a blue shift is observed in case of smaller nanoparticles [51][52] . A correlation between nanoparticle size and maximum absorbance wavelength of the absorption spectra of citrate coated silver nanoparticles can be seen in table 2.

The width of the absorption band also yields information regarding the size distribution of nanoparticles in a system. A broad peak indicates that there is a wide distribution of particles in terms of their size. Similarly, when the absorption peak is narrow, it is an indicative of all nanoparticles having their sizes confined in a short range. While using mie theory to calculate the size of nanoparticles using UV-visible absorption spectra, the FWHM (full width at half maximum) of the peak is inversely proportional to the average size of the nanoparticles [53]. Intensity of the absorbance peak is directly proportional to the quantity of nanoparticles resonating at that particular wavelength.

2.3.3 Characterization of size and morphology – SEM, TEM & DLS

As discussed earlier, particles exhibit remarkably different properties at nanoscale, compared to the bulk or ionic forms, is its mere size in the nanoscale. Therefore, size plays a very important role in determining the properties of nanoparticles, both physical and biological. This can be seen clearly in a study carried out by Martínez-Castañón et. al. where silver nanoparticles of different sizes show different levels of toxicity towards *E. coli* and *S. aureus* [51]. It should also be noted that smaller particle size means a larger surface area to volume

Table 2: Size, maximum absorption wavelength and extinction coefficient of citrate coated AgNPs [52]

Size (nm)	λ_{max} (nm)	ε/ M^{-1} cm^{-1} x 10^8
10	392.1	5.56
20	400.8	41.8
30	405.6	145
40	412.3	336
50	420.9	537
60	431.5	739
70	443.8	941
80	458.3	1142
90	474.6	1344
100	492.8	1546

ratio, which again is an important factor influencing the way nanoparticles interact with other substances in their vicinity [54]. Size of nanoparticles in a system can be determined using various techniques. SEM (Scanning electron microscopy) and TEM (transmission electron microscopy) are the gold standard techniques when it comes to determining size of nanoparticles. However, they yield limited information about the size distribution of particles. The small amount of sample captured in an image does not truly represent the entire population of nanoparticles of different sizes. For realizing the entire spectra of size of nanoparticles present in a system, dynamic light scattering is used. While UV-visible spectrophotometry gives an idea of particle size distribution, it does not give quantitative results in terms of particle diameter. SEM also elucidates the surface morphology of nanoparticles under examination. Most of the green synthesis strategies produce spherical nanoparticles. However in some cases, the biological extracts contain certain biomolecules, which do not adhere to the surface of initial cluster of atoms uniformly. Such a case of functionally non uniform surface leads to further nucleation and growth of the nanostructure

along specific directions. This gives rise to triangular, linear, hexagonal and pentagonal nanoparticles [40][41]. Interactions of biologically active moieties among themselves, as well as with the solvent and the nanomaterial of interest play an important role in determining the resulting morphology of nanoparticles. In this study, TEM and DLS were not performed since the necessary information regarding size and size distribution of nanoparticles can be acquired using UV-visible spectrophotometry and SEM.

2.4 Introduction to *Allium cepa*

Onion (*Allium cepa*) is one of the most widely cultivated and consumed crops worldwide. Also known as common onion or bulb onion, it is the most widely cultivated species of its genus. It belongs to the family Amaryllidaceae. Close botanical relatives of this plant include garlic (*Allium sativum*), shallots (*Allium cepa var. aggregatum)* and chives (*Allium schoenoprasum*). In 2016, India was the second largest producer of onion worldwide at 19.6 million tonnes [55].

2.5 Phytochemicals in *Allium cepa* – Rich source of antioxidants

Based on their colour, onion can be classified into yellow, white and red onions [56]. Of these common commercial varieties, red onions contain the highest amount of phytochemicals [17]. In a study carried out by Hertog et al ranked onion as the highest quercetin containing molecule among 9 fruits and 28 vegetables [57]. The bulb of the onion is the part of plant which is used as food, which is the part rich in phytochemicals. However, an interesting pattern can be recognized while studying the distribution of these beneficial molecules in the scales of onion bulb. As we traverse the layers of onion bulb, starting from outside, the content of polyphenols decreases [17] [58]. In other words, the outermost layer of the onion bulb is the richest in phytochemicals. Ironically, this is the part of bulb that is almost never consumed as food. With enough research providing substantial evidence regarding the health benefits of flavonoids, flavanols, and other plant secondary metabolites present in onion skin, it would be an injustice to nature if such valuable materials are simply washed down the drains of our kitchens. Use of polyphenols such as quercetin and gallic acid in synthesis of metallic nanoparticles has been reported earlier [51][59]. A comparative analysis of pure biomolecule and the biomolecule containing plant extract can help

understand the dynamics of synthesis in a better manner, shedding light on the advantages and disadvantages of both strategies. It has already been proposed in case of apple and onion that the numerous phytochemicals present in them act synergistically when consumed in the form of whole food, and not specific isolated biomolecules [60]. Investigation is required to say the same about nascent plant extracts being used for nanoparticle synthesis in contrast with purified biomolecules. List of major phytochemicals found in onions is given in the table 3.

2.6 Silver and its anti-microbial activity

Silver is a popular antimicrobial agent. Before the discovery of antibiotics, silver based ointments and medications were popular means of treating bacterial infection. One of the most well-known examples is the silver sulfadiazine, which was used as a topical medication for treating second and third degree burns. However, it was replaced with better acting antibiotics such as mupicorin. As time has passed, irresponsible and excessive use of antibiotics has led to a global health challenge in the form antibiotic ressistance. All over the world, cases of infections with drug resistant pathogens, showing antigenic shift and/or antigenic drift, are increasing. The real wrath of these nasty pathogens is being faced by the developing countries, where public health infrastructure is not well developed. To combat this threat, research groups all over the world are finding novel antibacterial agents. In such a global scenario, silver nanoparticles have attracted a fair share of attention as promising antimicrobial agents.

The mechanism behind antimicrobial activity of silver nanoparticles has not yet been clearly elucidated. The first barrier of defence for a bacterial cell, i.e. the cell wall is a target for silver ions released by silver nanoparticles, as well as for positively charged silver nanoparticles, due to the presence of negatively charged moieties such as techoic acids on the bacterial cell wall, resulting in electrostatic interactions, leading to lysis of the cell wall [61][62]. At the molecular level, it has been seen that DNA replication does not take place in presence of silver ions [63]. Silver ions also inhibit the expression of ribosomal subunit proteins and other cellular proteins and enzymes essential for ATP production [64]. These biocidal effects of silver ions can also be attributed to silver nanoparticles since significant contribution to their antimicrobial activity is from the slow release of silver ions from their surface. In the complex myriad of functional components in a cell, certain sulphur containing

Table 3: List of major flavonoids found in *Allium cepa*

Sr. No	Flavonoid	Reference
1	Quercetin	[65], [66], [67]
2	Quercetin 4′-glucoside	[68], [69]
3	Quercetin 3,4′-diglucoside	[68], [70]
4	Quercetin 7-glucoside	[71]
5	Quercetin 7,4′-diglucoside	[70], [72]
6	Quercetin 3,7-diglucoside	[72]
7	Quercetin 3-glucoside	[70], [68]
8	Quercetin dimer	[73], [74]
9	Myricetin	[75], [76]
10	Kaempferol	[77]
11	Kaempferol 4′-glucoside	[71]
12	Isorhamnetin	[78]
13	Isorhamnetin 4′-glucoside	[78], [71]
14	Cyanidin 3-glucoside	[79], [80]
16	Cyanidin 3-(6″-malonylglucoside)	[79], [80]
17	Cyanidin 3,5-diglucoside	[81], [79]
18	Delphinidin 3-glucoside	[82]
19	Peonidin 3-glucoside	[83], [80]
20	Taxifolin	[69]
21	Taxifolin 7-glucoside	[69]

proteins, enzymes and phosphorus containing molecules such as DNA seem to be the preferred binding targets for silver nanoparticles [22].

ROS production is one of the causes of cytotoxicity of silver nanoparticles to mammalian cells [84]. Silver nanoparticles can show significant antioxidant activities when stabilized with suitable capping agents [85] [86][87]. Using nanoparticles having high antioxidant potential for their antimicrobial effect might be able to increase their therapeutic window, thus killing microbes and not adversely affecting cells of the host organism at the same time.

2.7 Objectives

- To synthesize silver nanoparticles using quercetin and onion waste extract
- To characterize the synthesized nanoparticles
- To Study the effect of reaction conditions on synthesis of nanoparticles
- To compare the nanoparticles in terms of their biological properties

Chapter 3:
Materials and Methods

3.1 Glassware

Borosillicate glassware was used for all the experiments. Prior to experimentation, all glassware was washed with aqua regia. Aqua regia was prepared by mixing hydrochloric acid and nitric acid in a molar ratio of 3:1. Hydrochloric and nitric acids were obtained from Merck, India. Further washing of glassware was done using labolene, obtained from Fischer Scientific, India.

3.2 Prepration of onion extract

For the preparation of onion extract, outermost scaly leaves of onion waste was collected from food waste of the university mess of Jaypee University of Information Technology in Himachal Pradesh, India. These were washed with distilled water and dried. 10g of leaves were crushed in liquid nitrogen and then boiled in 100ml of distilled water at 100ºC for 30 minutes. The slurry thus formed was filtered through Whatman No. 1 filter paper. The filtrate obtained was the final onion extract used in the experiments. This extract was stored at 4ºC for further use.

3.3 Synthesis of nanoparticles

Analytical reagent grade silver nitrate was obtained from Merck India and used without any further purification. 0.0085g of silver nitrate salt was dissolved in 25ml of distilled water to obtain 2mM stock solution of silver nitrate [13]. Quercetin (99% purity) was obtained from Sigma-Aldrich Chemical Co. and used without any further purification. 0.003g of quercetin was dissolved in 10ml mixture of ethanol and distilled water in 1:1 v/v ratio to obtain 1mM solution of quercetin. This is done because quercetin has very low solubility in pure water. The solution was stored at 4ºC in dark bottle to prevent photo-degradation of quercetin [13]. Synthesis of silver nanoparticles was done by simple mixing of 5ml of 2mM $AgNO_3$ solution with 1ml of 50µM quercetin solution/onion extract [11] and thereafter left to react. The formation of silver nanoparticles can be estimated by the naked eye by checking for the formation of a brown coloured liquid, which is a result of conversion of Ag^+ ions to Ag^0 form.

3.4 X-Ray Diffraction

X-ray diffraction analysis was carried out using Supernova X-ray diffractometer manufactured by Agilent Technologies, USA. The instrument used monochromatic Cu kα radiation of 1.5418 Å wavelength. Scanning was done in the region of 10º to 90º 2θ angle pattern. To prepare the sample for examination, prepared nanoformulation was left over night in oven at 70ºC to evaporate the water. A viscous, dark coloured liquid was obtained which was spread over a glass surface and used for the experiment.

3.5 Scanning Electron Microscopy

SEM image was obtained using Nova NanoSem 450 instrument, manufactured by FEI, USA. The scan was carried out at a pressure of $3.8x10^{-3}$ Pa at an accelerating voltage of 10 kV. Optical density of sample was adjusted to 0.5 prior to the experiment.

3.6 Effect of reaction time on nanoparticle formation

Various synthesis parameters were optimized to achieve better nanoparticles in terms of uniform size distribution, absence of agglomeration and higher amount of nanoparticles synthesized. During the course of reaction, silver ions are converted from Ag^{+} to Ag^{0} form. This removes the interionic repulsion between positively charged ions and a core of silver atoms is formed. This core grows with addition of more silver atoms, a process known as nucleation. As time passes, growth of particles as well as increase in number of particles also takes place. To find out the optimal time for nanoparticles synthesis, samples were taken out of the reaction vessel at regular time intervals and stored at 4ºC, since rate of conversion of silver ions to nanoparticle form is negligible at this temperature. For observing the changes in synthesized nanoparticles under these varying conditions, UV-Vis spectra of the resulting nanoformulations was calculated using Thermo Evolution UV-Vis spectrophotometer.

3.7 Effect of pH on nanoparticle formation

It is a documented fact that pH plays a crucial role in nanoparticles synthesis [88]. The pH of nanoparticles synthesis was optimized by altering the pH of reducing agent, and adding it to

the silver nitrate solution. The pH of silver nitrate solution itself was not altered. pH of the reducing agent was varied by adding to it 0.1M sodium hydroxide solution. Variation in the nanoparticle formation was studied for pH 6, 7, 8, 9 & 10. It is known that the first pKa value for the phenol group of quercetin is 7.17 [89]. Therefore, it is understood that quercetin will act as a better reducing agent in a basic environment, as the polyphenol groups will be in their deprotonated form. For observing the changes in synthesized nanoparticles under these varying conditions, UV-Vis spectra of the resulting nanoformulations was calculated using Thermo Evolution UV-Vis spectrophotometer.

3.8 Effect of reducing agent concentration on nanoparticle formation

During nanoparticle synthesis, concentration of reducing agent in the reaction mixture determines how many molecules or functional moieties are available to act upon the silver ions present in solution. A minimum amount of reducing agent molecules is necessary for efficient stabilization of the silver atoms undergoing nucleation. To study the effect of concentration of reducing on the formation of silver nanoparticles, silver nitrate was added to the reducing agent solution in the varying ratios (v/v) of 5:0.5, 5:1.0, 5:1.5, 5:2.0 and 5:2.5. The amount of reducing agent is kept less than that of silver nitrate owing to the fact that multiple hydroxyl moieties found in individual quercetin and other polyphenol molecules are responsible for reduction of Ag^{+} ions to Ag^{0} form. For observing the changes in synthesized nanoparticles under these varying conditions, UV-Vis spectra of the resulting nanoformulations was calculated using Thermo Evolution UV-Vis spectrophotometer

3.9 Effect of reaction temperature on nanoparticle formation

For studying the effect of temperature on the formation of nanoparticles, the synthesis was carried out in temperatures ranging from 15ºC to 75ºC. As in all chemical reactions, temperature plays an important role in the physical properties of the nanoparticles being synthesized. Temperature dictates the rate of underlying chemical redox reaction that is responsible for reduction and stabilization of silver ions into silver nanoparticles. Thus temperature influences the stabilizing efficiency of the responsible molecules, flavonoids in this case. For observing the changes in synthesized nanoparticles under these varying conditions, UV-Vis spectra of the resulting nanoformulations was calculated using Thermo Evolution UV-Vis spectrophotometer. Properties of the synthesized particles were analysed by studying the characteristic surface plasmon resonance peak at around 400nm-450nm.

3.10 Antimicrobial susceptibility test by well diffusion method

The antimicrobial activity of nanoparticles was determined by well diffusion assay [59] against *E. coli* ATCC 25922 and *E. coli* 2011C- 3573. Luria broth, prepared using dehydrated culture media obtained from HiMedia Laboratories, was inoculated with these bacterial strains for 24 hrs. 100µl of these active cultures were spread onto petri plates of luria agar, prepared from dehydrated culture media obtained from HiMedia Laboratories Pvt. Ltd. Wells were punched in these plates and 20µl of onion nanoformulation, quercetin nanoformulation, onion extract, pure quercetin and silver nitrate solution was transferred into separate wells. After an incubation of 24 hours at 37ºC, the plates were observed for presence of a clear zone, free of bacterial growth surrounding the wells. Aseptic techniques were followed throughout this experiment.

3.11 ABTS radical scavenging assay

Method described by Anna Floegel et. al. was used with some modifications [90]. 7mM solution of ABTS, obtained from Sigma-Aldrich Chemical Co., was mixed with 2.45mM solution of potassium persulfate, obtained from Merck India, in 1:1 v/v ratio. These solutions were prepared in 80% ethanol. Then this mixture was allowed to stand overnight, allowing the formation of ABTS radical cation, which is blue in colour and gives maximum absorbance at 734nm. 50µl of onion nanoformulation, quercetin nanoformulation, onion extract, pure quercetin and silver nitrate solution each was added to 1450µl of the above prepared reagent and left to react in dark for 30 minutes. Scavenging of free radicals by the samples leads to a decrease in absorbance at 734nm, indicating antioxidant potential of the substance. The percentage antioxidant activity can be calculated using the formula

$$Percentage\ antioxidant\ activity = \frac{Absorbance_{blank} - Absorbance_{sample}}{Absorbance_{blank}} * 100$$

Where $Absorbance_{blank}$= absorbance of reagent without sample at time = 0 minutes, $Absorbance_{sample}$ = absorbance of reagent with sample at time = 30 minutes.

3.12 DPPH radical scavenging assay

DPPH is a dark-coloured crystalline powder composed of stable free-radical molecules, that on dissolution gives maximum absorption at 515nm. Method described by Anna Floegel et. al. was used with some modifications [90]. 0.0118g of DPPH, obtained from Sigma-Aldrich Chemical Co. was dissolved in 100 ml of 80% ethanol. This resulted in formation of a deep purple coloured solution. To 1250µl of this solution, 250µl of onion nanoformulation, quercetin nanoformulation, onion extract, pure quercetin and silver nitrate solution each was added and left to react in dark for 30 minutes. Scavenging of free radicals of DPPH resulted in a decrease in absorption at 515nm, which is directly proportional of the antioxidant activity of the sample substance. The percentage antioxidant activity can be calculated using the formula

$$Percentage\ antioxidant\ activity = \frac{Absorbance_{blank} - Absorbance_{sample}}{Absorbance_{blank}} * 100$$

Where $Absorbance_{blank}$= absorbance of reagent without sample at time = 0 minutes, $Absorbance_{sample}$ = absorbance of reagent with sample at time = 30 minutes.

Chapter 4:
Results and Discussions

4.1 Synthesis of nanoparticles

Preliminary synthesis of silver nanoparticles from quercetin and onion extract was assessed based on colour change. Initially colourless aqueous solution of silver nitrate turned slightly brown immediately on addition of onion extract and quercetin. After allowing the mixture to react for three to four hours, the colour of the solution intensified. The colour change in nanoparticles synthesized using onion extract (OENPs) was more intense than that of those synthesized with quercetin (QTNPs). These observations were validated by the UV-vis spectra of OENPs and QTNPs, where intensity of OENP peak is much higher than that of QTNP. Maximum absorption wavelength for QTNPs and OENPs are 421nm and 417nm respectively, indicating synthesis of particles of almost identical size. However the size distribution of particles prepared by these two methods varies significantly. The narrow OENP absorption peak represents nanoparticles in a comparatively smaller size range unlike the broad peak of QTNP which indicates particles belonging to a large range of sizes present in large quantities. With these findings, it can be concluded that onion extract acts as a better reducing agent and stabilizing agent than pure quercetin.

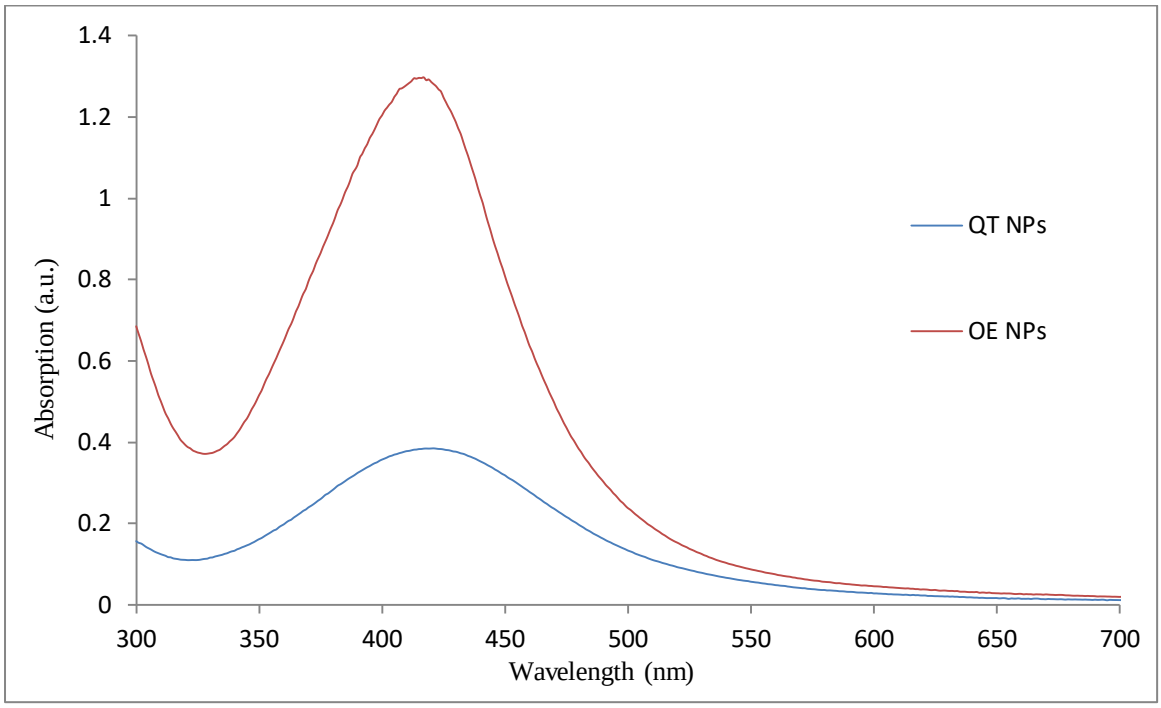

Figure 1: Absorption spectra of QTNPs and OENPs

Table 4: Absorption maximum wavelength of different nanoformulations

Nanoformulation	Absorption maximum wavelength (nm)
QTNP	421
OENP	417

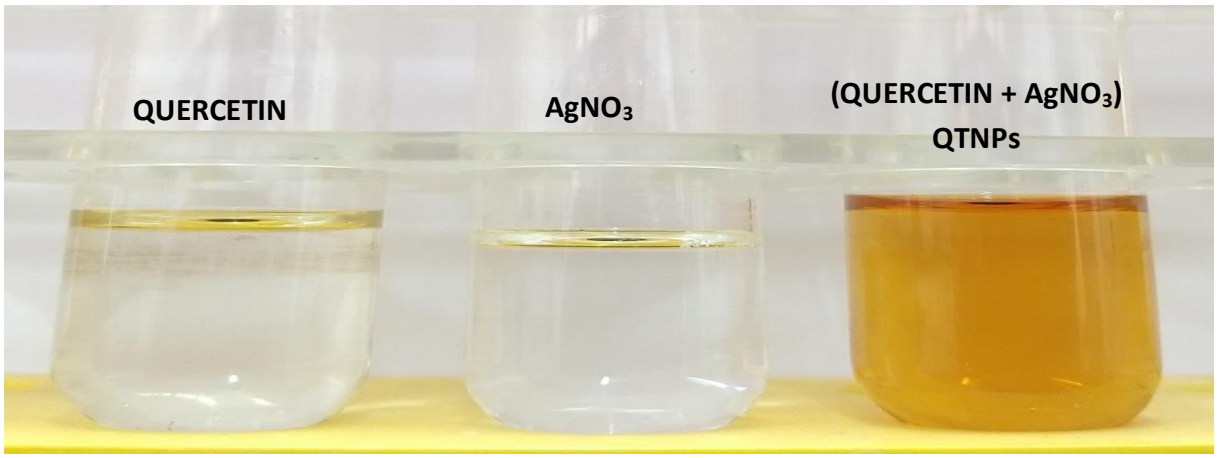

Figure 2: Change in colour of $AgNO_3$ solution after addition of quercetin due to formation of QTNPs

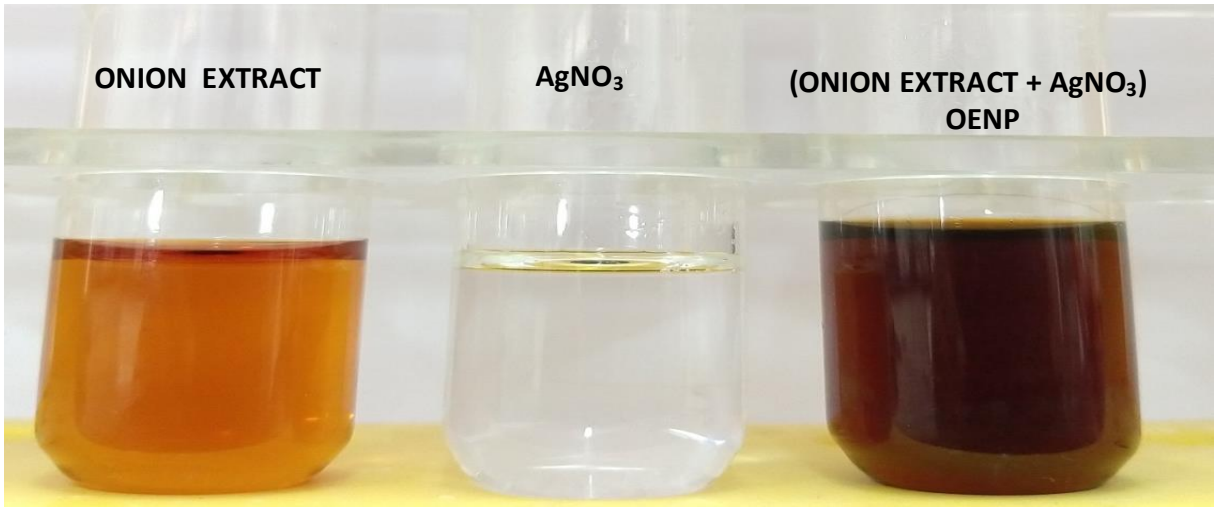

Figure 3: Change in colour of $AgNO_3$ solution after addition of onion extract due to formation of OENPs

4.2 X ray diffraction

Study of XRD pattern is necessary to establish silver as the constituent of nanoparticles as well as its crystal lattice structure. Peaks obtained at 38.06º, 43.94º, 64.46º and 77.38º represented the Bragg reflections (111), (200), (220) and (311) respectively. This pattern closely matched the standard reference values given in JCPDS file 89-3722, implying that the XRD pattern is representative of green synthesized silver nanoparticles having FCC (face centred cubic) crystal lattice structure. Apart from these standard peaks, some other peaks at 29.6º, 32.2º, 27.8º and 46.16º were also recorded. Such peaks have been reported previously in case of XRD analysis of silver nanoparticles synthesized by the green route. Appearance of these peaks could be attributed to the presence of plant phytochemicals in the sample [91][92]. Using the Scherrer equation, mean diameter of nanoparticles in the sample was calculated to be 10.5nm.

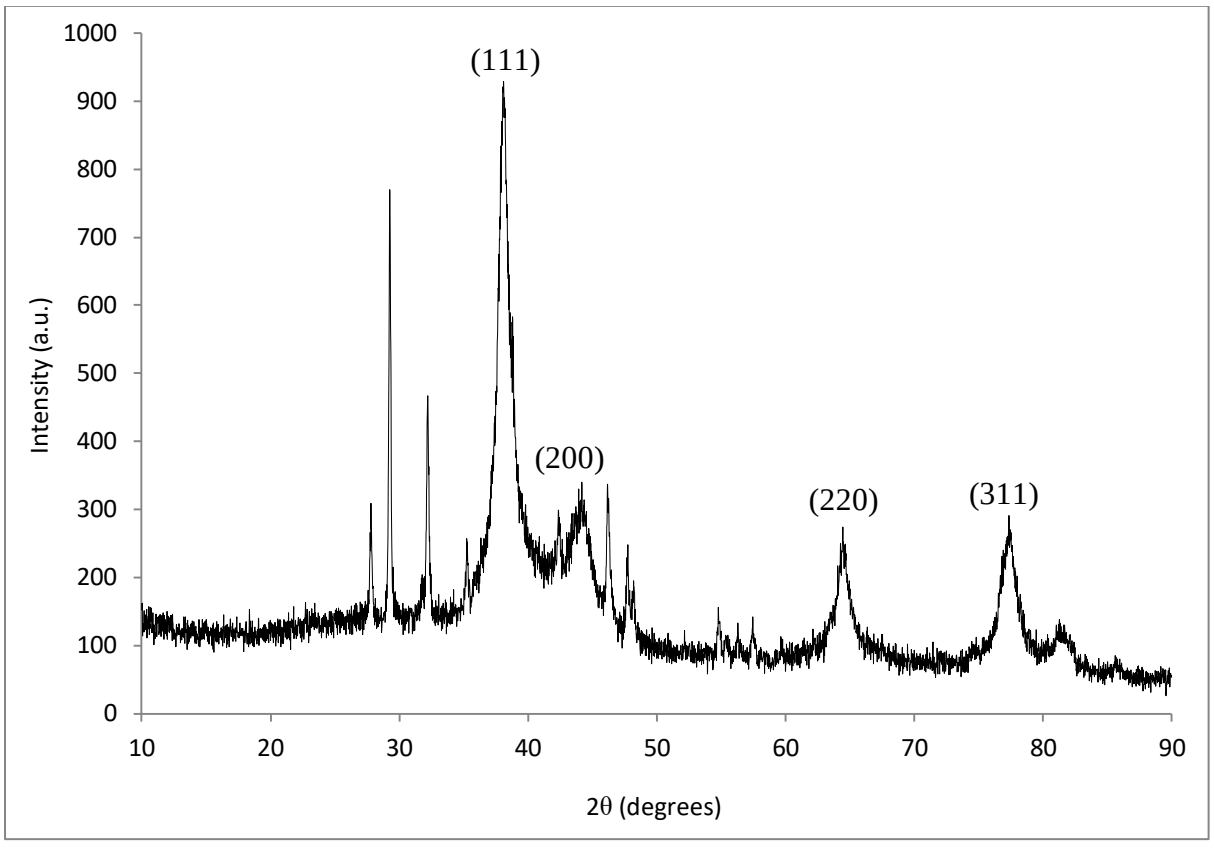

Figure 4: XRD spectra of silver nanoparticles in 10º to 90º range

4.3 Scanning electron microscopy

The SEM image revealed that the synthesized nanoparticles are spherical in shape. Magnification of 70,000X was achieved. The size of these synthesized nanoparticles was measured to be 10nm. This is in good correlation with the particle size calculated by using Scherrer equation.

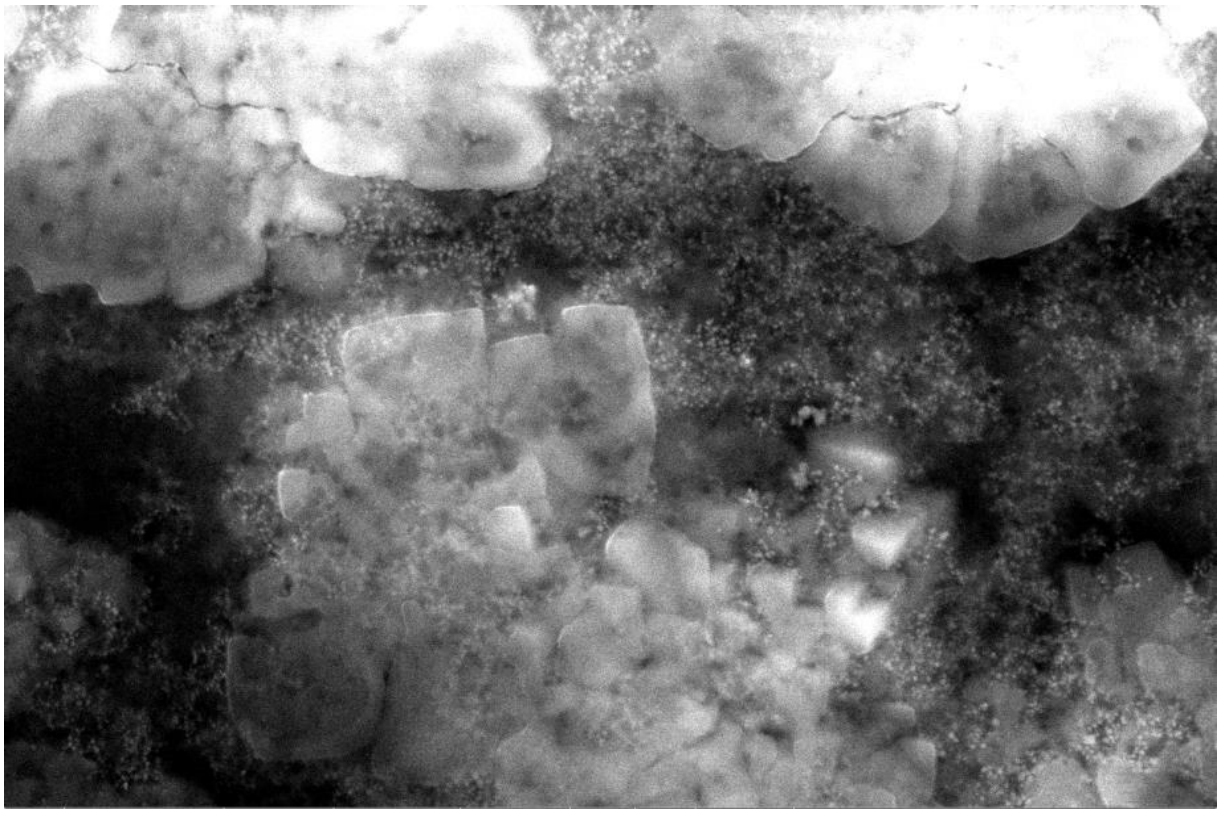

Figure 5: SEM micrograph of silver nanoparticles

4.4 Effect of reaction time on nanoparticles formation

Reaction time is an important parameter in nanoparticle synthesis. Comparative analysis of the UV-vis spectra of nanoformulation collected at different time intervals shows us that there is an increase in the amount of nanoparticles synthesized with increasing time. This can be said on the basis of increasing absorbance of the consecutive spectra. As seen in the graph, a sharp peak is not observed till reaction time of 30 minutes. Moreover, an increase in absorption in the form of a hump can be seen in the region around 550nm wavelength. This is a clear indication of agglomeration of particles. This can be said on the basis of the fact that larger size particles give maximum absorption at higher wavelengths. This also shows that quercetin molecules do not act as effective capping agents till this time period. After the 30 minutes time period there can be seen a noticeable shift in the absorption maxima from 445nm to 432nm, which remains almost constant as time of reaction increases further. After 180 minutes, there was no appreciable increase in the amount of nanoparticles being synthesized, therefore samples were analysed only till the 240 minutes time period.

Onion extract on the other hand exhibited remarkable reducing as well as capping power. A time period of mere one minute was enough to synthesize an appreciable amount of nanoparticles. As we increase the time of reaction, the amount of nanoparticles in the system increases steadily. A look at the absorption maximum wavelengths of the different spectra reveals that nanoparticles formed at different time periods are almost of the same size. This again is an example of the excellent stabilizing property of onion extract. This enhanced stabilizing capacity can be attributed to the large number and types of biomolecules present in the onion extract, majorly consisting of flavonoids. It is evident by the analysis of maximum absorption wavelength that use of onion extract leads to formation of smaller nanoparticles, as compared to pure quercetin. In both the cases, it can be seen that providing more time for synthesis does not mean that the particles will go on increasing in size, but rather the amount of individual particles in the system increases. The intensity of absorption peaks correlate with the intensity of colour of samples obtained for analysis.

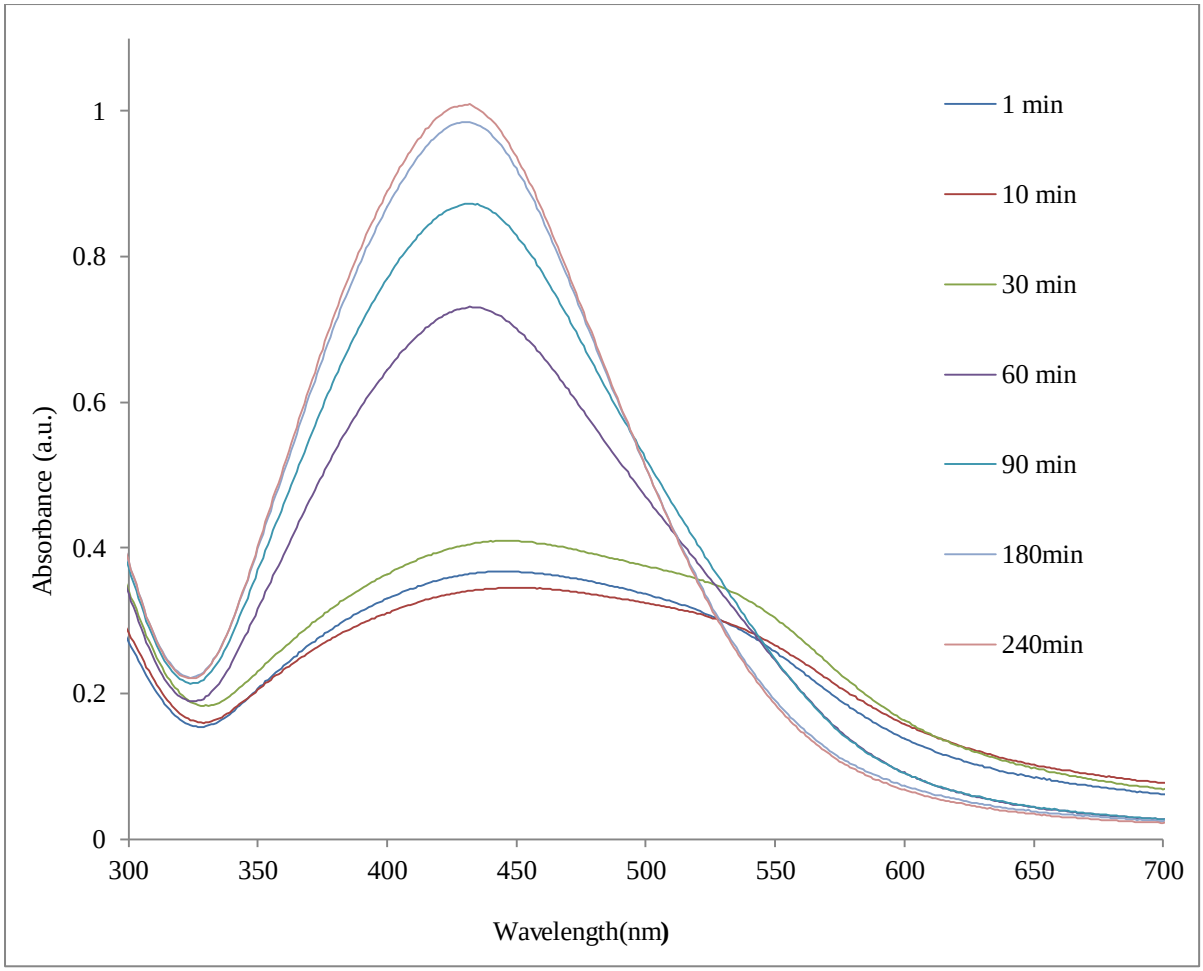

Figure 6: Absorption spectra of QTNPs at varying reaction time intervals

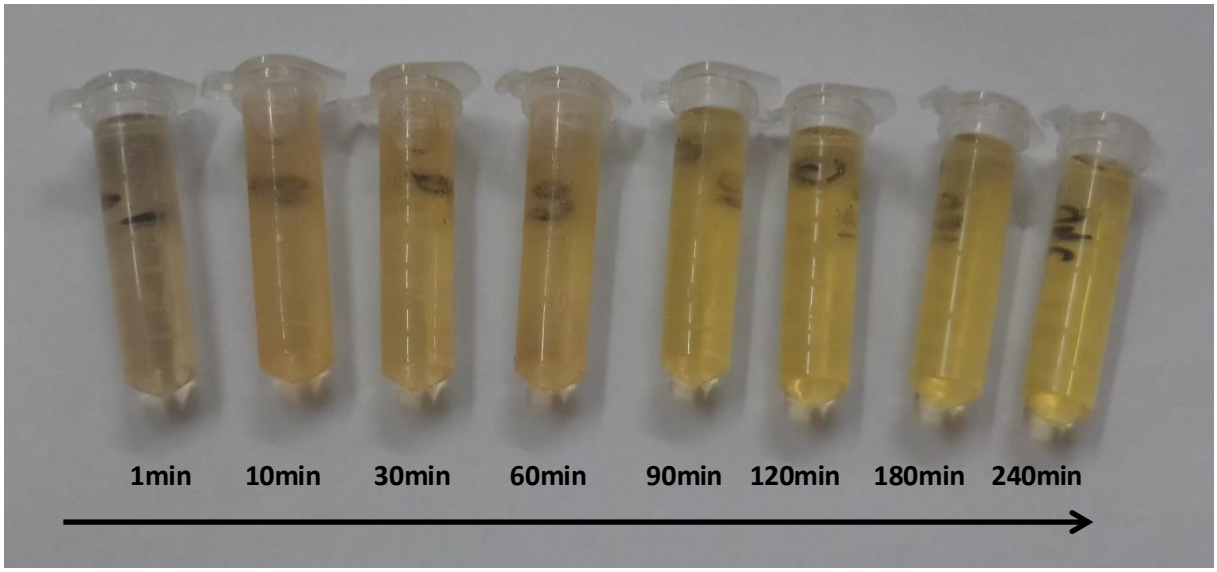

Figure 7: QTNPs collected at varying reaction time intervals

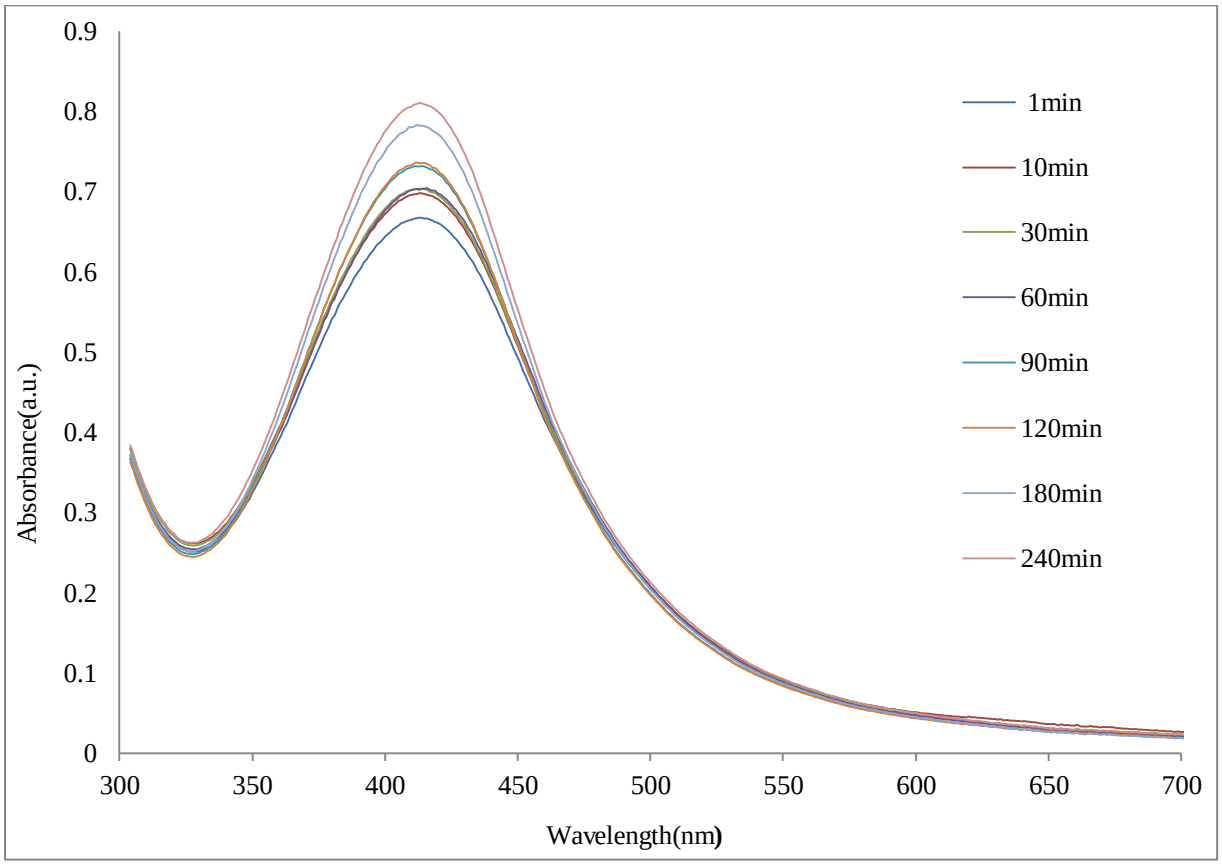

Figure 8: Absorption spectra of OENPs at varying reaction time intervals

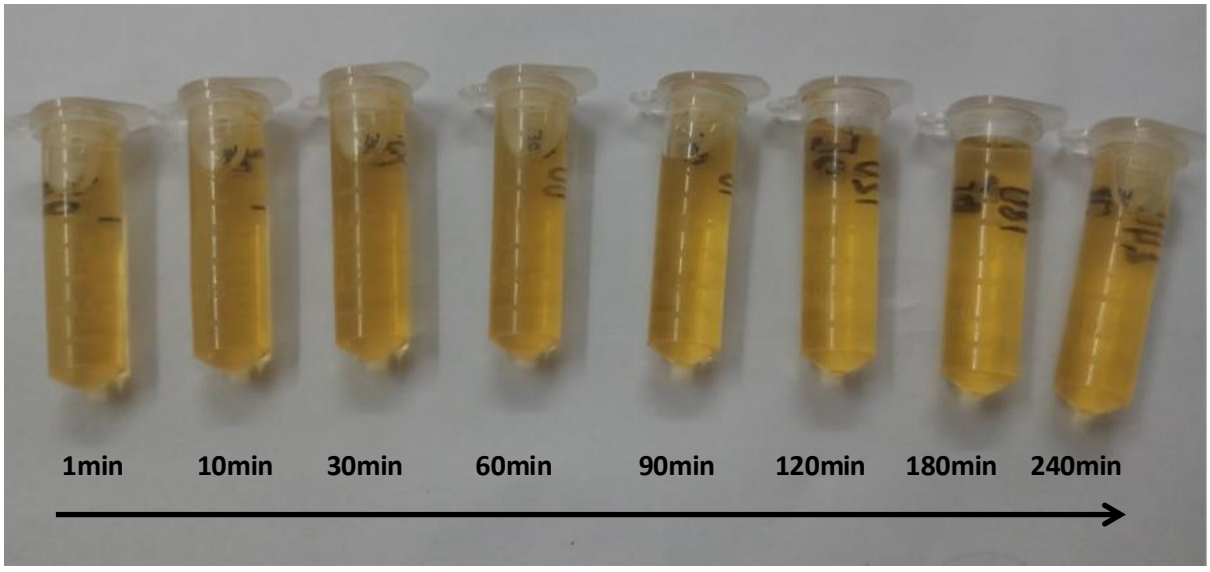

Figure 9: OENPs collected at varying reaction time intervals

Table 5: Absorption maximum wavelength of QTNPs at varying reaction time intervals

Reaction time interval(minutes)	Absorption maximum wavelength (nm)
1	445
10	450
30	445
60	432
90	432
180	431
240	432

Table 6: Absorption maximum wavelength of OENPs at varying reaction time intervals

Reaction time interval (minutes)	Absorption maximum wavelength (nm)
1	413
10	413
30	414
60	416
90	414
120	412
180	412
240	413

4.5 Effect of pH on nanoparticle formation

The underlying mechanism behind the conversion of silver ions to silver nanoparticles is the conversion of Ag^{+} to Ag^{0} form. Therefore this reaction, unlike all redox reactions, depends on the pH of reaction environment. As discussed earlier, the starting point for varying pH was kept at 6. As pH was increased significant trends can be seen in the change in intensity as well as in wavelength of maximum absorbance. In case of quercetin, it can be seen that at pH 6, there is a very broad absorption spectra, which means there is no significant formation of nanoparticles. From pH 7 to 9, the absorption peaks increase in intensity, revealing the increase in number of protonated OH groups of quercetin. However, from pH 9 to 10, a drastic increase in the absorption intensity can be seen. This is because the second pKa of quercetin lies between 9 and 10. Therefore an abnormal increase in its reducing power, resulting in an abnormal increase in amount of nanoparticles formed. An interesting observation here is that at such a high pH, quercetin's stabilizing power cannot remain at par with its reducing power. A hump in the 550nm – 600nm region indicates that agglomeration of particles has taken place. As pH increases, there is a significant decrease in the absorption maximum wavelength of the test samples. This indicates that stabilizing power of the OH groups also increases with rise in pH. It is due to this efficient stabilizing that at higher pH, the nanoparticles do not get enough time to assimilate more Ag^{0} particles to the growing nanostructure, thus limiting their size.

Onion extract in this case outperformed quercetin in terms of both reducing power as well as stabilizing efficiency. At pH 6, the reducing power had to be poor due to reasons discussed earlier, but the stabilizing power was also very less, even less than that of pure quercetin. This can be said on the basis of absorption maximum at 451nm compared to that of quercetin at 430nm. However, things change pH 7 onwards. There is a steady increase in absorption intensity till pH 9. At pH 10, similar to case of quercetin, a sharp rise in absorption intensity is seen due to similar reasons. Even at pH 10, the OENPs showed no sign of agglomeration, which is due to the many polyphenolic compounds involved in the stabilization process. A decreasing trend in the absorption maximum wavelengths with increasing pH can be seen, although the magnitude of change is very small compared to that of quercetin. The intensity of absorption peaks correlate with the intensity of colour of samples obtained for analysis.

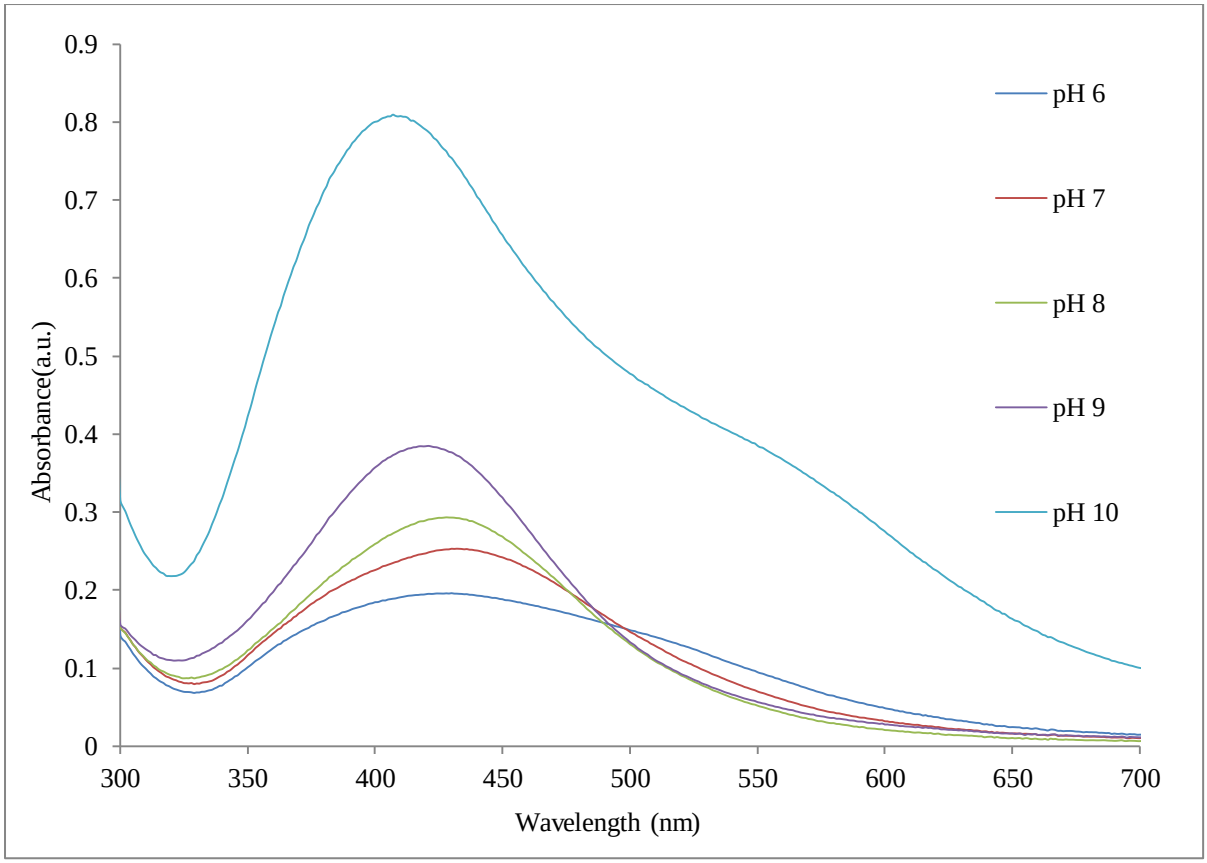

Figure 10: Absorption spectra of QTNPs at varying pH

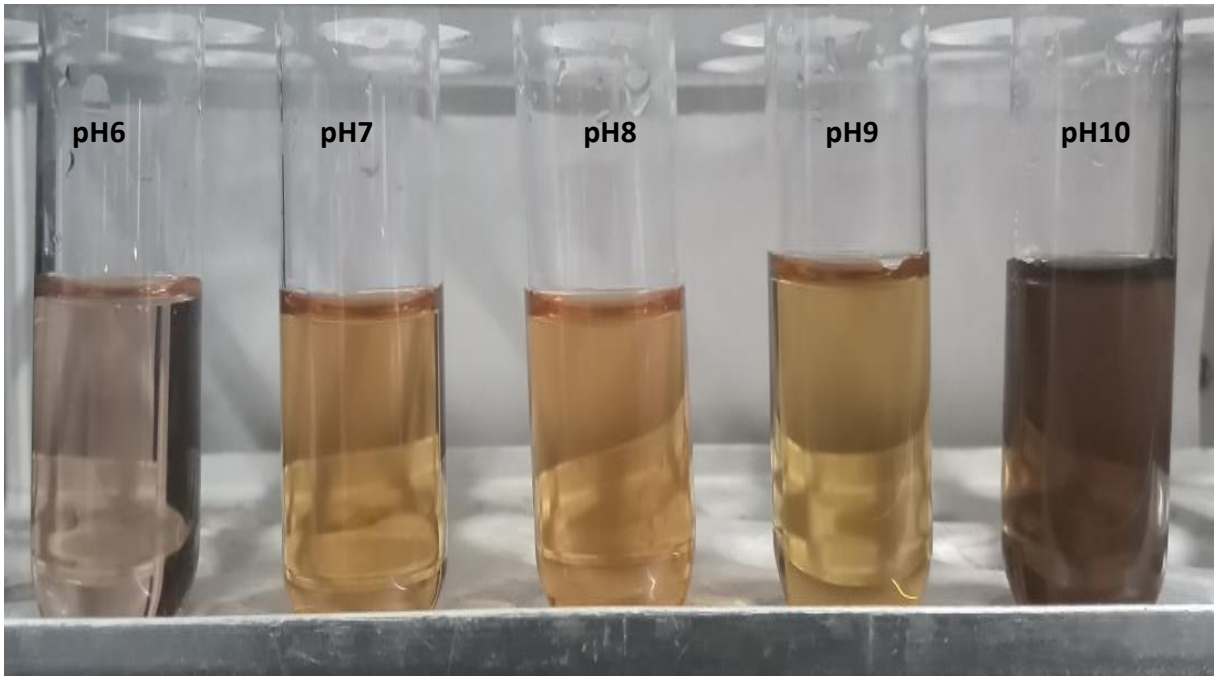

Figure 11: QTNPs formed at varying pH

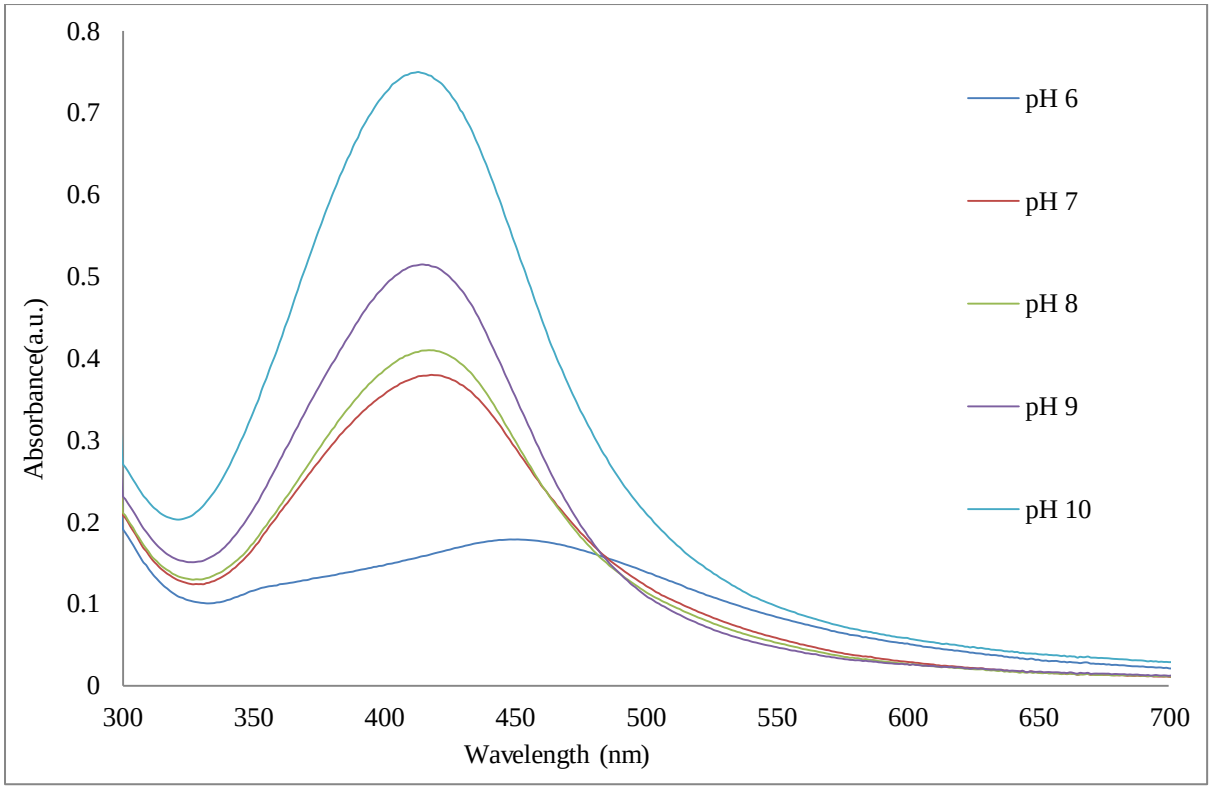

Figure 12: Absorption spectra of OENPs at varying pH

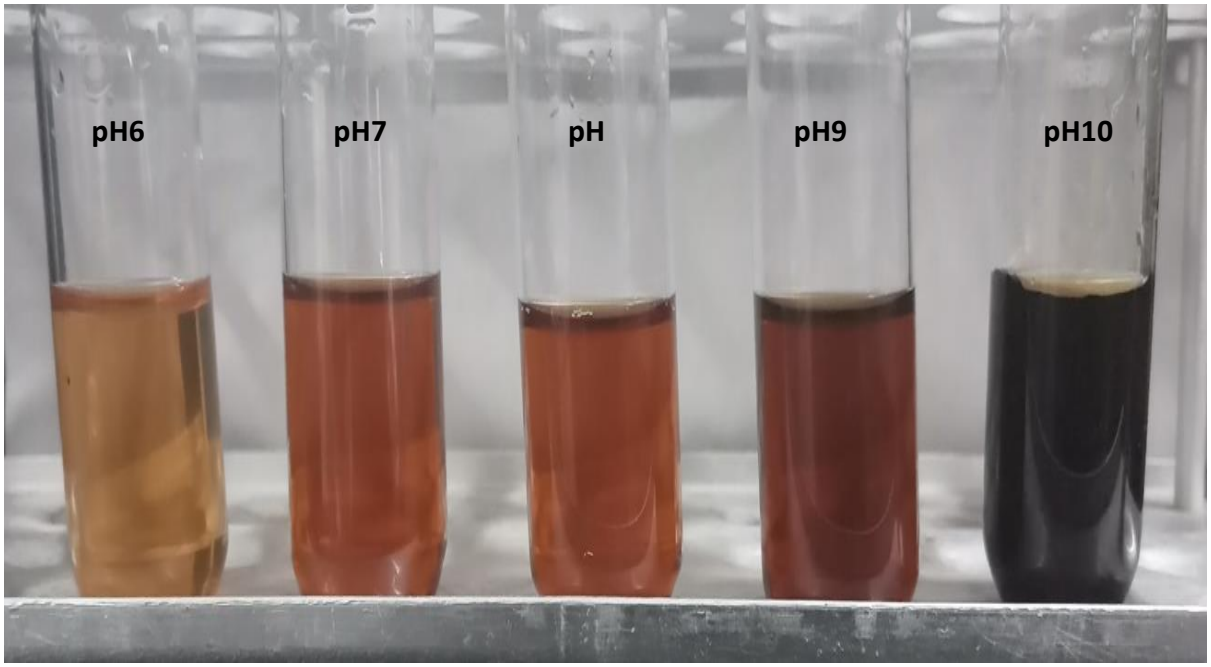

Figure 13: OENPs formed at varying pH

Table 7: Absorption maximum wavelength of QTNPs at varying reaction pH

Reaction pH	Absorption maximum wavelength (nm)
6	430
7	432
8	428
9	421
10	407

Table 8: Absorption maximum wavelength of OENPs at varying reaction pH

Reaction pH	Absorption maximum wavelength (nm)
6	451
7	418
8	417
9	415
10	413

4.6 Effect of concentration of reactants on nanoparticle formation

In case of quercetin, silver nitrate to quercetin ratios of 5:0.5 and 5:0.1 did not give a satisfactory result, as indicated by the UV spectra. The high absorption near 500nm region indicates that a large number of Ag^0 ions could not be assimilated efficiently, as a result they grew to a large size. It can be said that this spectra indicates slight agglomeration. The ratios onwards of 5:1.5 yield good results. As more and more reducing and stabilizing agent molecules are available with increasing concentration, It yields to an increase in the amount of nanoparticles, as indicated by the rise in absorption intensity. From the ratio 5:0.5 to 5:1.5, a significant decrease in the absorption maximum wavelength can be seen. This is because efficient stabilization does not allow nanoparticles to grow in size. However, a slight increase in this wavelength (2nm) can be seen as concentration of reducing agent is further increased. This can be due to the steric hindrances between the stabilizing molecules themselves, providing the nanoparticles to grow in size to a small extent.

In case of onion extract being used as reducing agent, the large amount of polyphenols present in it produced good results even at the low concentration ratio of 5:0.5. As seen in the previous results also, onion extract shows extremely efficient stabilizing capacity for silver nanoparticles. Due to this property of onion extract, the effect of rising concentration of reducing agent on nanoparticle size can also be witnessed clearly. As the ratio of onion extract to silver nitrate is increased, lowering of the absorption maximum wavelength can be clearly seen. The intensity of absorption peaks correlate with the intensity of colour of samples obtained for analysis.

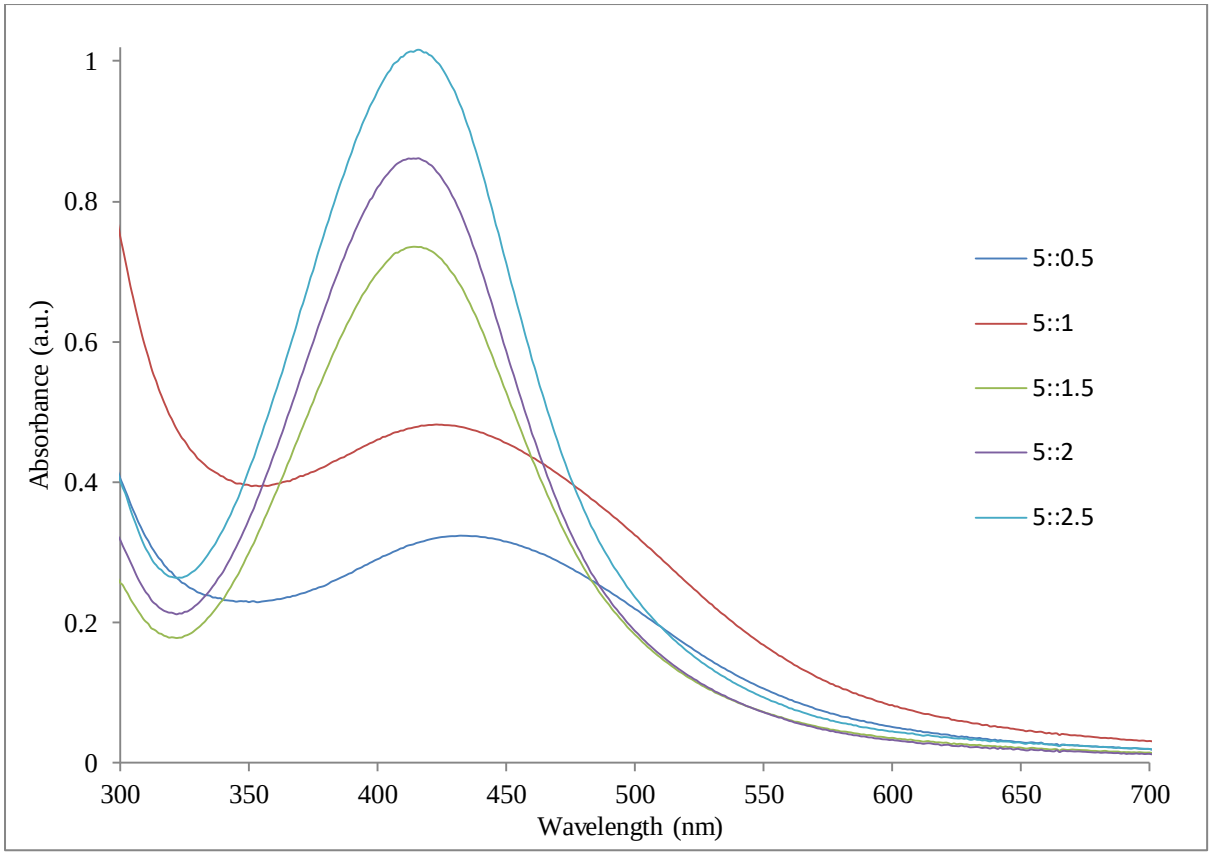

Figure 14: Absorption spectra of QTNPs at varying ratio of silver nitrate to quercetin

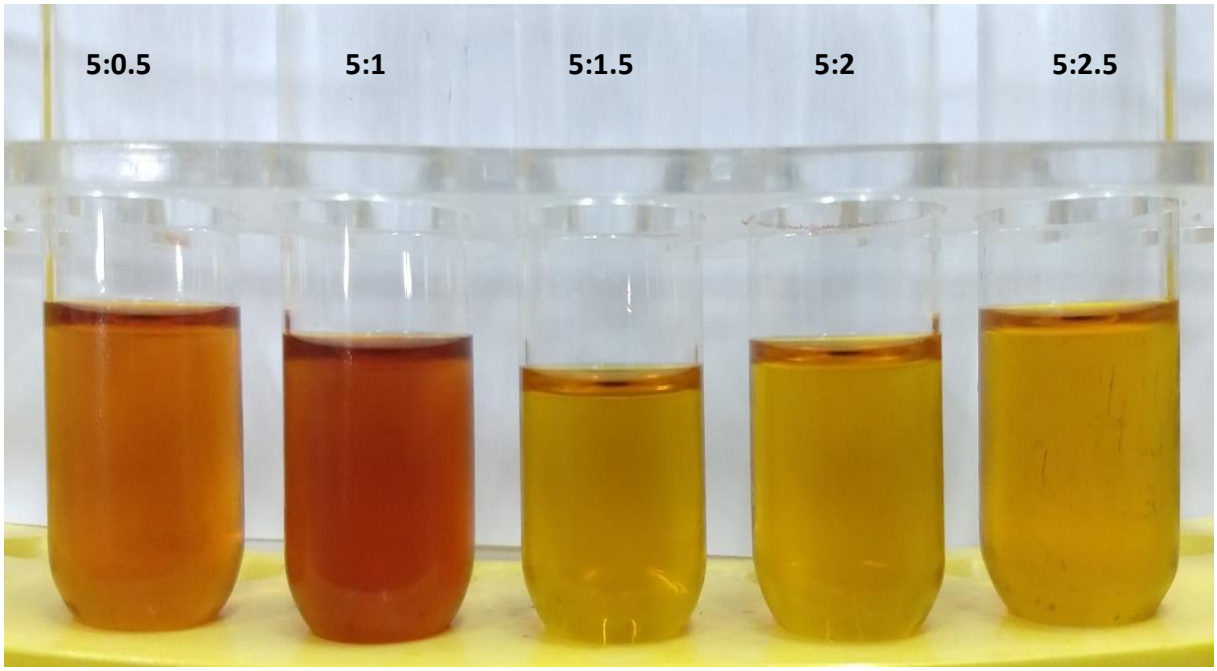

Figure 15: QTNPs formed at varying ratios of silver nitrate and quercetin

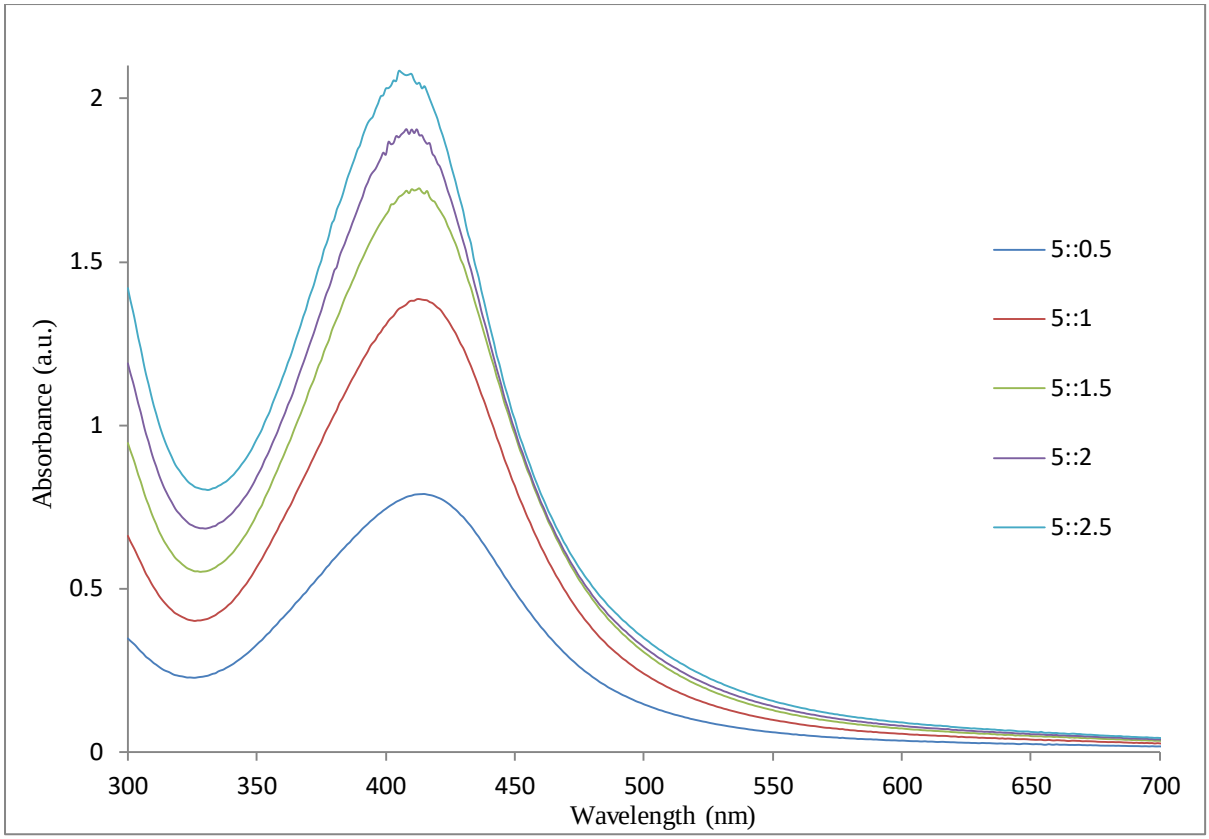

Figure16: Absorption spectra of OENPs at varying ratio of silver nitrate & onion extract

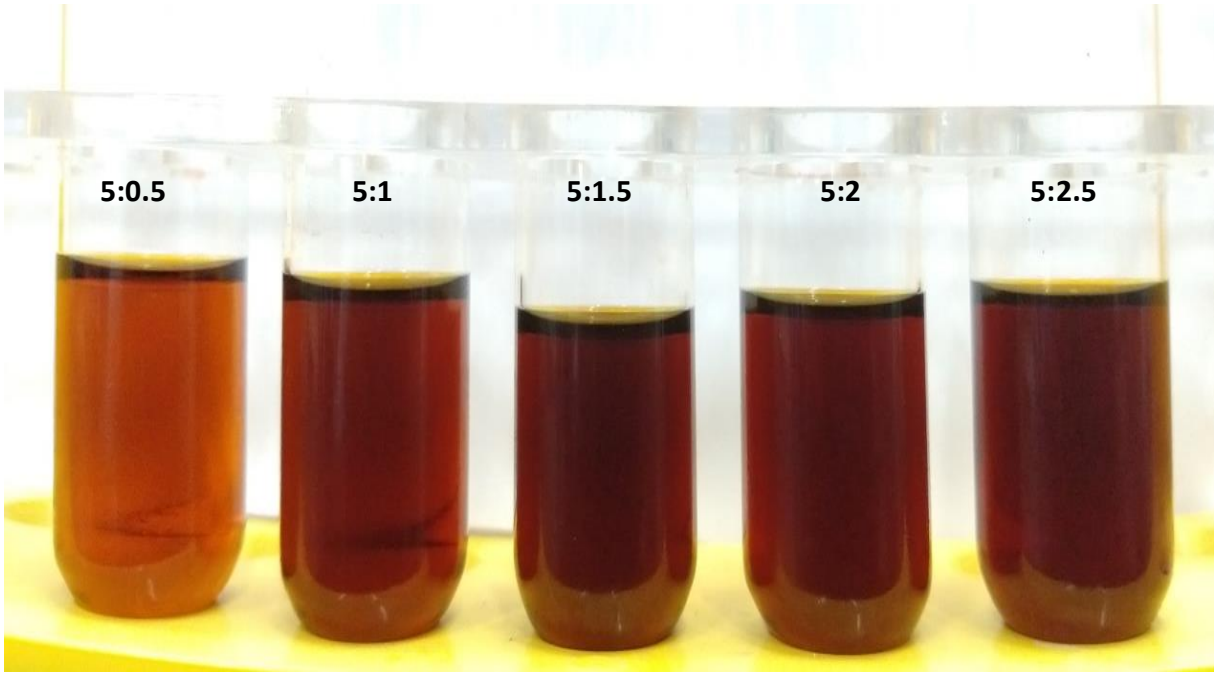

Figure 17: OENPs formed at varying ratios of silver nitrate and onion extract

Table 9: Absorption maximum wavelength of QTNPs at varying ratio of silver nitrate and quercetin

Ratio of silver nitrate & quercetin (v/v)	Absorption maximum wavelength (nm)
5:0.5	432
5:1	423
5:1.5	414
5:2	416
5:2.5	416

Table 10: Absorption maximum wavelength of OENPs at varying ratio of silver nitrate and onion extract

Ratio of silver nitrate & quercetin (v/v)	Absorption maximum wavelength (nm)
5:0.5	415
5:1	413
5:1.5	413
5:2	408
5:2.5	405

4.7 Effect of temperature on nanoparticle formation

At the starting temperature of 15ºC, quercetin shows satisfactory performance in terms of nanoparticle synthesis. But on comparison with spectra for higher temperature of synthesis, it can be seen that width of peak is more than other spectra indicating a wider distribution of nanoparticles of different sizes. Therefore temperature of 15ºC can be called as sub optimal. On increasing the temperature, intensity of spectra increases due to the reactions of reduction and stabilization taking place at a faster rate. This rise in intensity is accompanies by decreasing wavelength of maximum absorption, which is due to increase in efficiency of stabilization. As temperature reaches 65ºC and 75ºC decrease in maximum absorption wavelength still takes place. However, the intensity of spectra starts decreasing, indicating a decrease in the amount of nanoparticles synthesized in the system. This is a result of the high temperature causing dissociation of the stabilizing agent from the silver nanoparticle surface.

A similar trend, although much smaller in magnitude can be seen in case of synthesis employing onion extract. Even at a temperature of just 15 ºC, it has effectively reduced and capped a good amount of silver ions and converted them to nanoparticles. Rise in intensity of the UV-vis spectra can be seen till temperature is raised to 65 ºC. Beyond that, there is a slight reduction in the intensity of spectra, indicating that high temperature inhibited a small amount of Ag^0 particles from being stabilized by the onion flavonoids. The intensity of absorption peaks correlate with the intensity of colour of samples obtained for analysis.

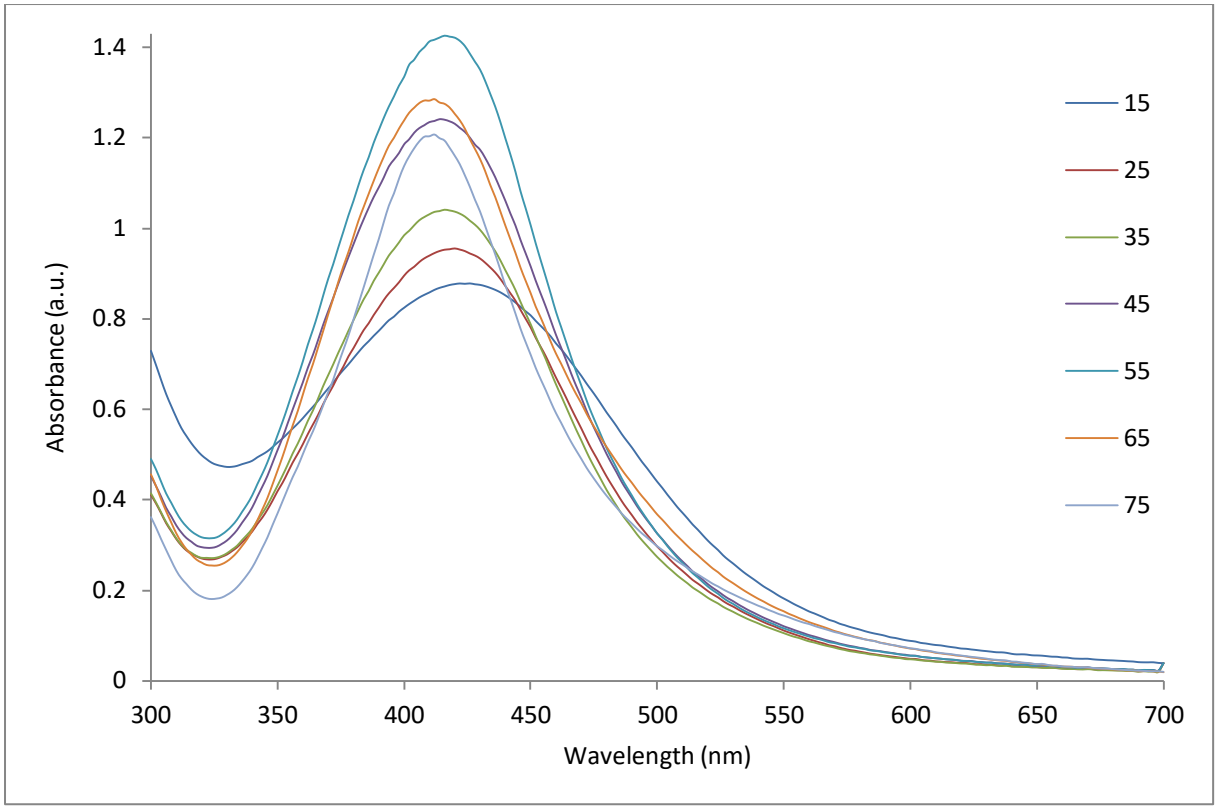

Figure 18: Absorption spectra of QTNPs at varying temperature of reaction

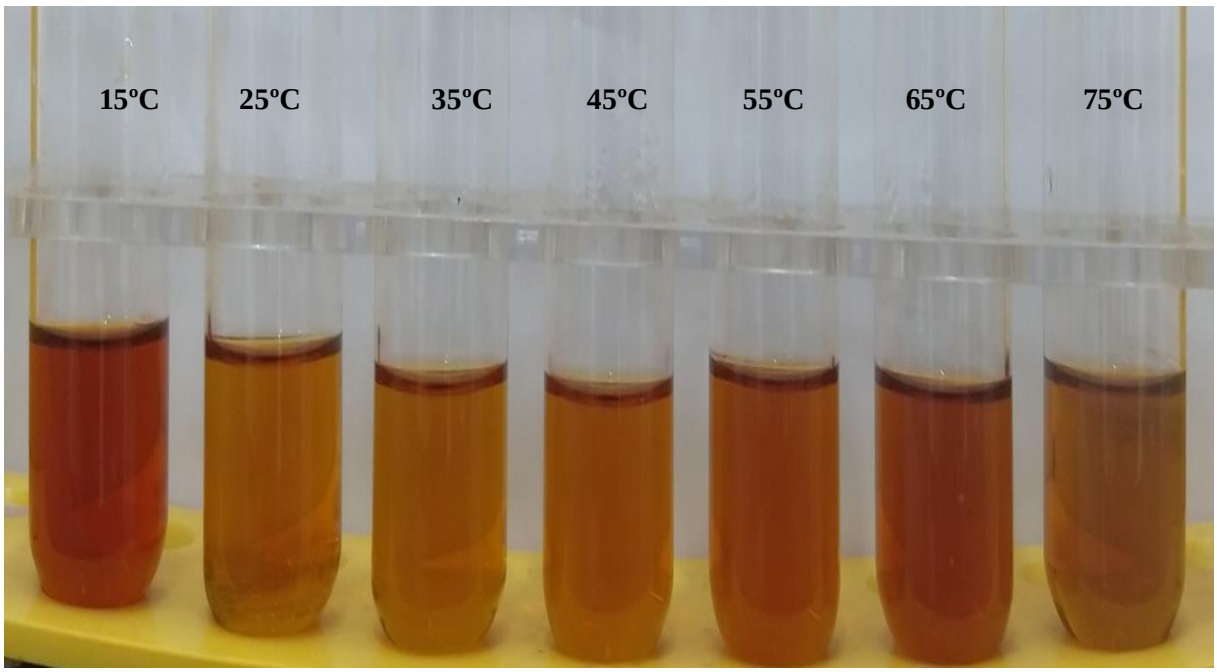

Figure 19: QTNPs formed at varying reaction temperatures

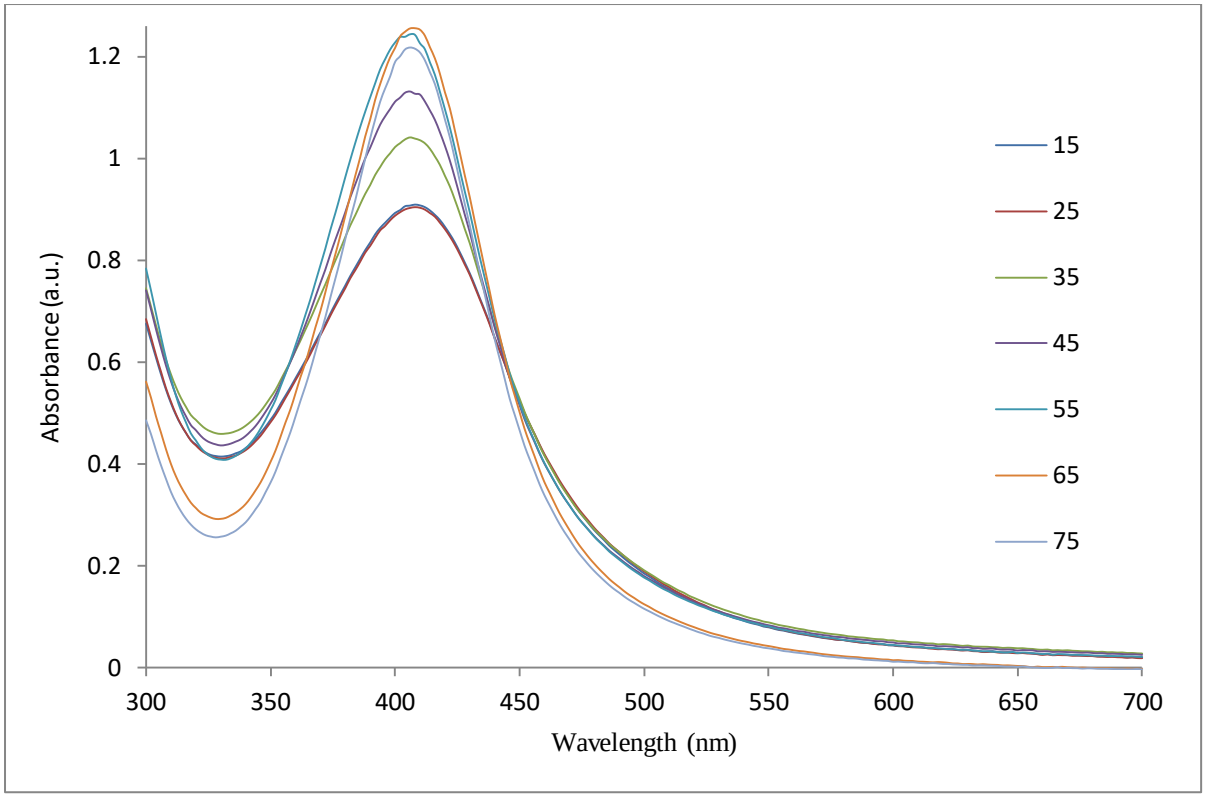

Figure 20: Absorption spectra of OENPs at varying temperature of reaction

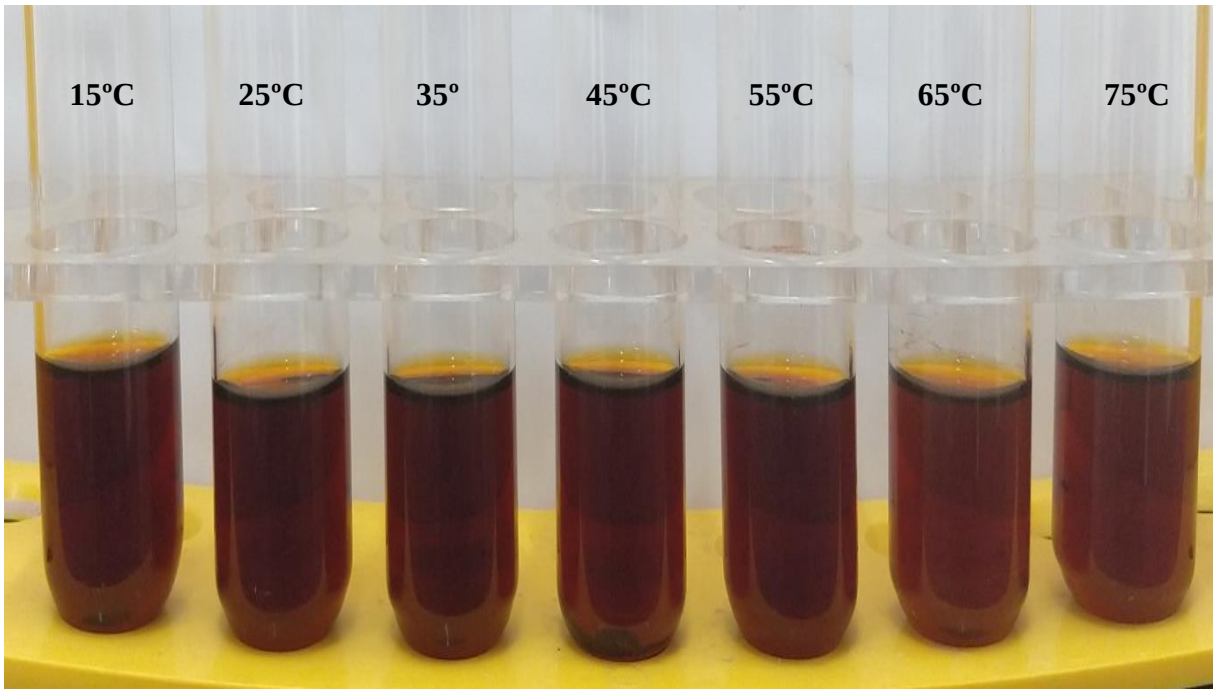

Figure 21: OENPs formed at varying reaction temperatures

Table 11: Absorption maximum wavelength of QTNPs at varying reaction temperature

Reaction temperature (ºC)	Absorption maximum wavelength (nm)
15	426
25	420
35	416
45	414
55	416
65	412
75	412

Table 12: Absorption maximum wavelength of OENPs at varying reaction temperature

Reaction temperature (ºC)	Absorption maximum wavelength (nm)
15	408
25	408
35	406
45	406
55	406
65	408
75	406

4.8 Antibacterial assay using well diffusion method

Antibacterial activity of silver nanoparticles is well known. However, during nanoparticle synthesis, the stabilizing agent used may or may not influence the antibacterial activity of the nanoparticles. When assayed against bacterial strains *E. coli* 25922, and *E. coli* 2011C- 3573, QTNPs had the highest antibacterial activity, followed by aqueous silver nitrate solution and OENPs. These observations can be explained on the basis of silver ion toxicity. One of the major mechanisms of antimicrobial activity of silver nanoparticles is the release of silver ions from their surface. It is also a known fact that silver ions are more toxic to bacteria as compared to silver nanoparticles. Since OENPs have shown very high rate of conversion of silver ions into silver nanoparticles, their antibacterial activity is less than that of aqueous silver nitrate. QTNP formulation on the other hand, has a significant amount of silver ions in free form, along with significant amount of silver nanoparticles. Thus the two agents responsible for antibacterial activity are acting together, forming zones of inhibition greater than those formed by the two components separately.

Table 13: Diameters of zones of inhibition (cm) formed by various components against bacterial cultures *E. coli* ATCC 25922 & *E. coli* 2011C-3573

	Diameter of zone of inhibition formed by samples (cm)				
Bacterial Strain	**Quercetin**	**Onion Extract**	**$AgNO_3$**	**QTNP**	**OENP**
***E. coli* ATCC 25922**	NIL	NIL	0.55	0.7	0.45
***E. coli* 2011C-3573**	NIL	NIL	0.40	0.63	0.35

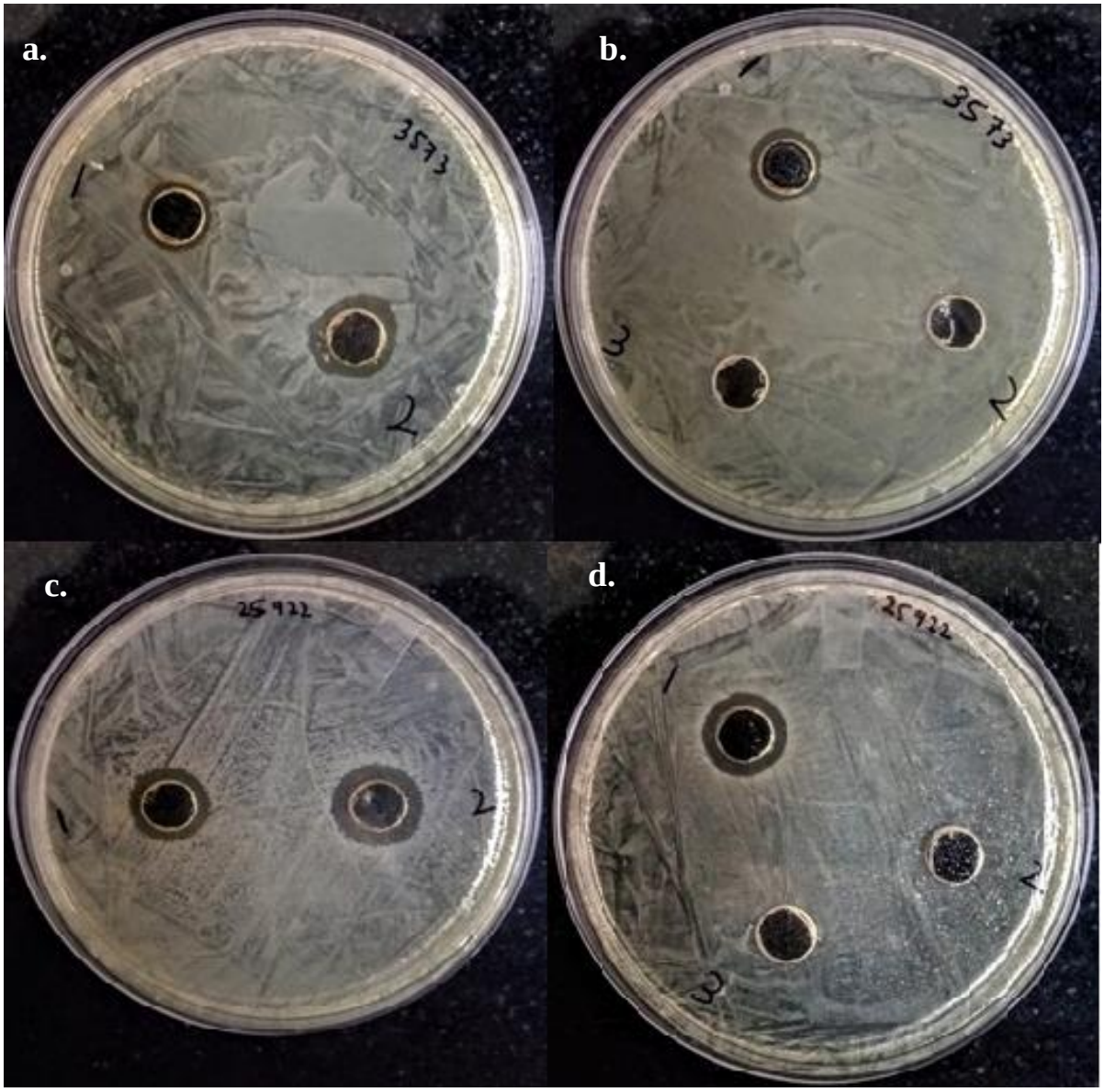

Figure 22: Well diffusion assay performed on luria agar plates to check susceptibility of *E. coli* ATCC 25922 against (a.1) OENPs, (a.2) QENPs, (b.1) $AgNO_3$, (b.2) onion extract, & (b.3) quercetin, and that of *E. coli* 2011C-3573 against (c.1) OENPs, (c.2) QENPs, (d.1) $AgNO_3$, (d.2) onion extract, & (d.3) quercetin.

4.9 ABTS and DPPH radical scavenging assays

Pure quercetin is a standard flavonoid used in antioxidant assays. Onion extract, owing to the large amount of polyphenol compounds, obviously exhibits high in vitro antioxidant activity. However, using the same chemical species to stabilize nanoparticles greatly increases their antioxidant capacity, as measured by the ABTS and DPPH assays. These are based on free radical scavenging mechanisms. On comparing pure quercetin and QTNPs, 8 fold and 11 fold increase in the antioxidant activity was recorded using ABTS and DPPH assays respectively. Similarly in case of onion extract and OENPs, 1.9 fold and 1.8 fold increase in antioxidant activities was observed using ABTS and DPPH assays respectively. While the magnitude of increase in activity is greater in case of pure quercetin and QTNPs, overall greater activity is observed in case of onion extract and OENPs. This increase can be attributed to the functional groups participating in the stabilization of nanoparticles, as well as those which are not. Apparently, the tendency of polyphenol groups to donate electrons increases when they adhere to the surface of silver nanoparticles as compared to their free form. Radical scavenging values obtained from the two tests are in good correlation with each other

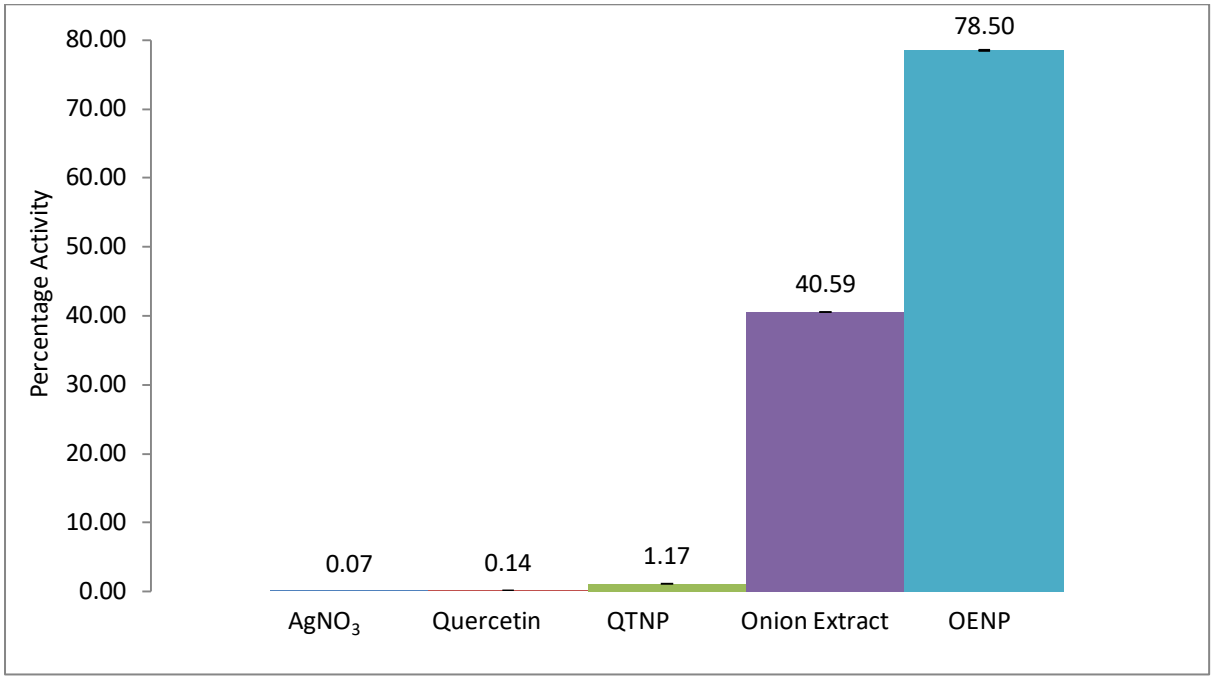

Figure 23: Percentage radical scavenging activity of nanoformulations and other compounds using ABTS assay

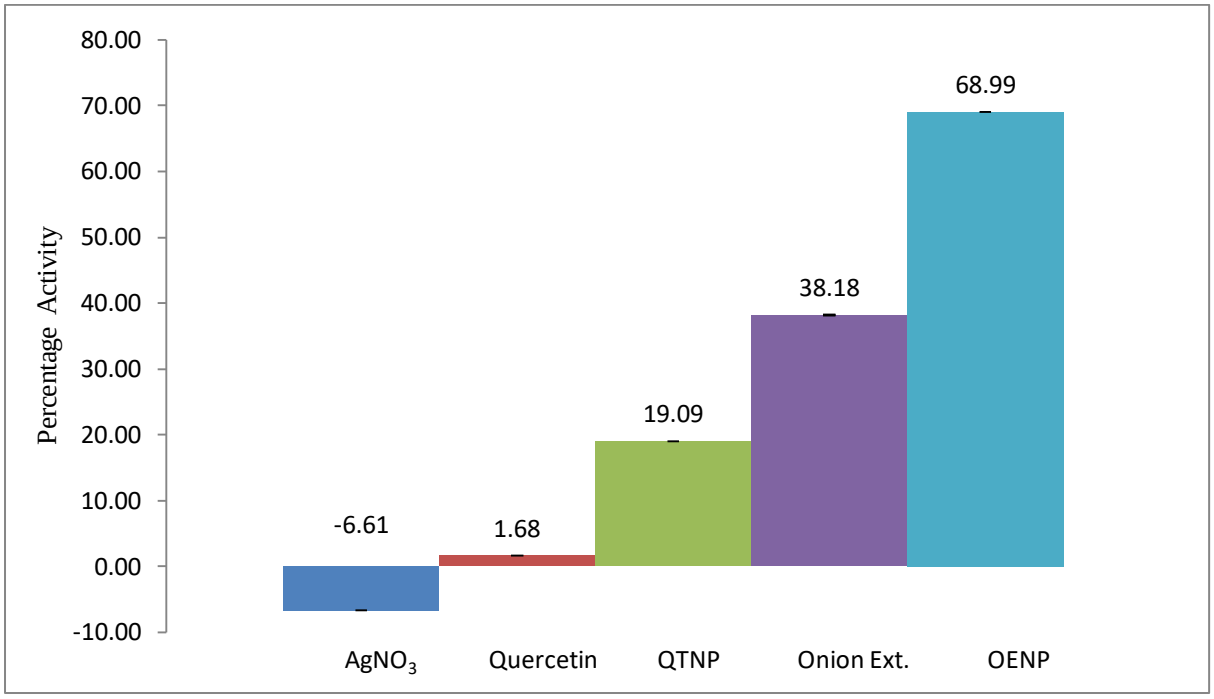

Figure 24: Percentage radical scavenging activity of nanoformulations and other compounds using DPPH assay

Chapter 5:
Conclusion

Through the studies performed, it has been proven that plant extracts can serve as better reducing and stabilizing agents for green synthesis of metallic nanoparticles, in terms of uniform size and total amount of nanoparticles synthesized. By noticing the change in properties of synthesized nanoparticles with changing parameters of time, pH, reactant concentration and temperature, optimized nanoparticle synthesis conditions can be determined for synthesizing different metallic nanoparticles with different reducing agents. Apart from this, use of onion waste extract will also address the problem of agricultural waste management, while not competing with other interests of mankind such as food. Use of plant extract instead of purified biomolecules would lay extra burden on the industry to supply these purified biomolecules, thus taking a toll on the environment. Onion waste extract on the other hand, is easy to prepare and can be prepared quickly without a lot of energy and time being consumed. It would also prove to be advantageous financially. Due to these facts, onion waste extract can be an efficient strategy of producing nanoparticles on an industrial level. The antibacterial activity displayed makes these nanoparticles promising antibacterial agents, showing the synergistic effect of silver nanoparticles along with silver ions. Also, the huge increase in the free radical scavenging capacity of these polyphenol stabilized nanoparticles can find higher chances of use in medicine, since toxicity of silver ions in human cells due to ROS production can be potentially nullified by this enhanced antioxidant potential of theirs.

Chapter 6:
References

1) Q. Suna, X. Cai, J. Li, M. Zheng, Z. Chenb, C.P. Yu. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. Colloid Surf A: Physicochem Eng Aspects, 444 (2014), 226-231
2) Salvioni L., Galbiati E., Collico V., Alessio G., Avvakumova S., Corsi F., Tortora P., Prosperi D., Colombo M. Negatively charged silver nanoparticles with potent antibacterial activity and reduced toxicity for pharmaceutical preparations. International Journal of Nanomedicine, 12 (2017), 2517–2530.
3) Anderson K., Poulter B., Dudgeon J., Li S., Ma X. A Highly Sensitive Nonenzymatic Glucose Biosensor Based on the Regulatory Effect of Glucose on Electrochemical Behaviors of Colloidal Silver Nanoparticles on MoS_2. Sensors 17(2017), 1807.
4) Li X., Xu H., Zhe-Sheng C., Chen G.. Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomaterials, 11 (2011), 1–16.
5) Park Y. New paradigm shift for the green synthesis of antibacterial silver nanoparticles utilizing plant extracts. Toxicol. Res. 30(3) (2014), 169.
6) Kalishwaralal K, Deepak V, Ramkumarpandian S, et al. Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Mater Lett., 62(2008), 4411–4413.
7) S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract Biotechnol Prog, 22 (2006), 577-583.
8) Zhang, Liu Z., Shen W., Gurunathan S.. Silver Nanoparticles: Synthesis, Characterizati-on, Properties, Applications, and Therapeutic Approaches. Int J Mol Sci. 17(2016), 1534 .
9) S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, K. Srinivasan. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochem Acta A Mol Biomol Spectrosc, 79 (2011), 594-598.
10) Ahamed, M., Majeed Khan, M. A., Siddiqui, M. K. J., AlSalhi, M. S., & Alrokayan, S. A.. Green synthesis, characterization and evaluation of biocompatibility of silver nanop-articles. Physica E: Low-Dimensional Systems and Nanostructures, 43(2011), 1266–1271.
11) B. Sadeghi, A. Rostami, S.S. Momeni. Facile green synthesis of silver nanoparticles using seed aqueous extract of *Pistacia atlantica* and its antibacterial activity. Spectrochim Part A: Mol Biomol Spectrosc, 134 (2015), 326-332.

12) T. Santhoshkumar, A.A. Rahuman, G. Rajakumar, S. Marimuthu, A. Bagavan, C. Jayaseelan Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors Parasitol Res, 108 (2011), 693-702.

13) J. Kesharwani, K.Y. Yoon, J. Hwang, M. Rai. Phytofabrication of silver nanoparticles by leaf extract of *Datura metel*: hypothetical mechanism involved in synthesis. J Bionanosci, 3 (2009), 39-44.

14) S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, K. Srinivasan. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochem Acta A Mol Biomol Spectrosc, 79 (2011), 594-598.

15) Rulli M, Bellomi D, Cazzoli A, De Carolis G, D'Odorico. The water-land-food nexus of first-generation biofuels. Sci Rep., 6(2016), 2251

16) K. Downes, G.A. Chope, and L.A. Terry. Economical and environmentally-friendly approaches for usage of onion (*Allium cepa* L.) waste Postharvest Biol. Technol., 54(2016), 80–86.

17) Jung-Ho Kwak, Jeong Min Seo, Na-Hyung Kim, Mariadhas Valan Arasu, Sunggil Kim, Moo Kyoung Yoon, Sun-Ju Kim. Variation of quercetin glycoside derivatives in three onion (*Allium cepa* L.) varieties. Saudi J Biol Sci. 24(2017), 1387–1391.

18) E Coventry, R Noble, A Mead, J.M Whipps. Control of Allium white rot (*Sclerotium cepivorum*) with composted onion waste. Soil Biology and Biochemistry, 34(2002), 1037-1045

19) M. Kim, C.Y. Kim, and I. Park. Prevention of enzymatic browning of pear by onion extract. Food Chem., 89(2005), 181–184.

20) Kaur, C., Joshi, S., & Kapoor, H. C.. Antioxidants in onion (*Allium cepa L*) cultivars grown in india. Journal of Food Biochemistry, 33(2009), 184–200.

21) H.J. Lee, S.Y. Yeo, S.H. Jeong. Antibacterial effect of nanosized silver colloidal solution on textile fabrics, J. Mater. Sci. 38 (2003) 2199–2204.

22) Allaker, R. P. The Use of Nanoparticles to Control Oral Biofilm Formation. Journal of Dental Research, 89(2010), 1175–1186.

23) Theresa A. Dankovich and Derek G. Gray Bactericidal. Paper Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment Environ. Sci. Technol., 45 (2011), 1992–1998.

24) Linhares, A. M. F., Grando, R. L., Borges, C. P., & da Fonseca, F. V. Technological Prospection on Membranes Containing Silver Nanoparticles for Water Disinfection. Recent Patents on Nanotechnology, 12(2018), 3–12.
25) Kholoud M.M., Abou El-Nour Ala'a Eftaiha, Abdulrhman Al-Warthan. Reda A.A., Ammar. Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry, 3(2010), 135-140.
26) Yoojin Choi, Hyun-A Kim, Kyoung-Woong Kim, Byung-Tae Lee. Comparative toxicity of silver nanoparticles and silver ions to Escherichia coli. Journal of Environmental Sciences, 66(2018), 50-60.
27) Foldbjerg, R., Olesen, P., Hougaard, M., Dang, D. A., Hoffmann, H. J., & Autrup, H.. PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicology Letters, 190(2009), 156–162.
28) He W, Liu X, Kienzle A, Müller. In Vitro Uptake of Silver Nanoparticles and Their Toxicity in Human Mesenchymal Stem Cells Derived from Bone Marrow. J Nanosci Nanotechnol. 16(2016), 219-28.
29) Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari M. Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. Int J Nanomedicine. 13(2018), 8013-8024.
30) Diksha Jha, Prasanna Kumar Thiruveedula, Rajiv Pathak, Bipul Kumar, Hemant K. Gautam, Shrish Agnihotri, Ashwani Kumar Sharma, Pradeep Kumar. Multifunctional biosynthesized silver nanoparticles exhibiting excellent antimicrobial potential against multi-drug resistant microbes along with remarkable anticancerous properties. Materials Science and Engineering: C, 80(2017), 659-669.
31) Taniguchi, N. On the Basic Concept of Nanotechnology. Proceedings of the International Conference on Production Engineering, Tokyo, 1974, 18-23.
32) Monu Verma, Vinod Kumar, Akash Katoch. Sputtering based synthesis of CuO nanoparticles and their structural, thermal and optical studies, Materials Science in Semiconductor Processing, 76(2018), 55-60.
33) Santosh Kumar Singh, James W. Lillard, Rajesh Singh. Reversal of drug resistance by planetary ball milled (PBM) nanoparticle loaded with resveratrol and docetaxel in prostate cancer. Cancer Letters, 427(2018), 49-62.

34) Alavi, Atieh, Jafari, Azadeh, Heidary, Sahar, Fayaz, Vahid, Terohid, S. Effect of Laser Pulse Energy on the Structure, Morphology and Optical Properties of Tantalum-Oxide Nanoparticles Generated by Laser Ablation. Journal of Nanoelectronics and Optoelectronics. 13(2018), 1407-1412.
35) M.-C. Daniel, D. Astruc. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev, 104 (2004), 293.
36) Lee, S.H., Jun, B.-H. Silver Nanoparticles: Synthesis and Application for Nanomed-icine. Int. J. Mol. Sci., 20(2019), 865.
37) S. Iravani, H. Korbekandi, S.V. Mirmohammadi, B. Zolfaghari. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci, 9(2014), 385-406
38) Q.H. Tran, V.Q. Nguyen, A.T. Le. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv Nat Sci: Nanosci Nanotechnol, 4 (2013).
39) Shakeel Ahmed, Mudasir Ahmad, Babu Lal Swami, Saiqa Ikram. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, Journal of Advanced Research, Volume 7, Issue 1, 2016, Pages 17-28
40) T. Elavazhagan, K.D. Arunachalam. *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles Int J Nanomed, 6 (2011), 1265-1278
41) G. Rajakumar, A. Abdul Rahuman. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. Acta Trop, 118 (2011), 196-203.
42) B. Ulug, M. HalukTurkdemir, A. Cicek, A. Mete Role of irradiation in the green synthesis of silver nanoparticles mediated by fig (*Ficus carica*) leaf extract Spectrochim Part A: Mol Biomol Spectrosc, 135 (2015), 153-161.
43) Nadagouda, M. N., & Varma, R. S.. Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chemistry, 10(2008), 859.
44) J.R. Nakkala, R. Mata, A. Kumar Gupta, S. Rani Sadras. Biological activities of green silver nanoparticles synthesized with *Acorous calamus* rhizome extract Eur J Med Chem, 85 (2014), 784-794.
45) R. Mariselvam, A.J.A. Ranjitsingh, A. Usha Raja Nanthini, K. Kalirajan, C. Padmalatha, M.P. Selvakumar. Green synthesis of silver nanoparticles from the

extract of the inflorescence of *Cocos nucifera* (Family: Arecaceae) for enhanced antibacterial activity. Spectrochim Part A: Mol Biomol Spectrosc, 129 (2014), 537-541.

46) M. Zargar, A.A. Hamid, F.A. Bakar, M.N. Shamsudin, K. Shameli, F. Jahanshiri. Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L. Molecules, 16 (2011), 6667-6676.

47) V. Kathiravan, S. Ravi, S.A. kumar. Synthesis of silver nanoparticles from *Melia dubia* leaf extract and their in vitro anticancer activity. Spectrochim Acta Part A: Mol Biomol Spectrosc, 130 (2014), 116-121.

48) J.R. Nakkala, R. Mata, A.K. Gupta, S.R. Sadras Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their antibacterial activity. Indus Crop Prod, 52 (2014), pp. 562-566.

49) V. Gopinatha, M.D. Ali, S. Priyadarshini, N. Meera Priyadharsshini, N. Thajuddinb, P. Velusamy. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. Colloid Surf B: Biointerface, 96 (2012), 69-74.

50) C. Krishnaraj, E. Jagan, S. Rajasekar, P. Selvakumar, P. Kalaichelvan, N. Mohan. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces, 76 (2010), pp. 50-56.

51) Martínez-Castañón, G.A., Niño-Martínez, N., Martínez-Gutierrez, F. et al. Synthesis and antibacterial activity of silver nanoparticles with different sizes. J Nanopart Res (2008) 10: 1343.

52) Paramelle, D., Sadovoy, A., Gorelik, S., Free, P., Hobley, J., & Fernig, D. G. (2014). A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra. The Analyst, 139(19), 4855.

53) T.C. Prathna, N. Chandrasekaran, Ashok M. Raichur, Amitava Mukherjee, Biomimetic synthesis of silver nanoparticles by *Citrus limon* (lemon) aqueous extract and theoretical prediction of particle size, Colloids and Surfaces B: Biointerfaces, 82(2011), 152-159.

54) Anu Mary Ealias and M P Saravanakumar. A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf. Ser.: Mater. Sci. Eng. 263(2017).

55) Onion (dry) production in 2016: Crops/World Regions/Production Quantity from pick lists". Food and Agriculture Organization, Statistics Division (FAOSTAT) (2017).
56) Miean K.H., Mohamed S.. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. J. Agric. Food Chem., 49(2001), 3106–3112.
57) Hertog M.G.L., Hollman P.C.H., Venema D.P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem., 40(1992), 1591–1598.
58) Sidhu, J. S., Ali, M., Al-Rashdan, A., & Ahmed, N.. Onion (Allium cepa L.) is potentially a good source of important antioxidants. Journal of Food Science and Technology (2019).
59) Siddhant Jain & Mohan Singh Mehata. Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. Scientific Reports, 7(2017) 15867.
60) Rui Hai Liu. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American Journal of Clinical Nutrition, 78(2003), 517S–520S
61) Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Cho, M.-H.. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine, 3(2007), 95–101.
62) Sondi I, Salopek-Sondi B. Silver nanoparticles as an antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275(2004), 177-182.
63) Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res, 52(2000), 662-668.
64) Yamanaka M, Hara K, Kudo J. Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl Environ Microbiol 71(2005), 7589-7593.
65) Perkin, A. G., Hummel, J. J. Occurrence of quercetin in the outer skins of the bulb of the onion. J. Chem. Soc. 69 (1896), 1295– 1298.
66) Herrmann, K. Flavonols and phenols in onions (Allium cepa L.). Arch. Pharm. Ber. Dtsch. Pharm. Ges. , 291(1958), 238–247.

67) Herrmann, K. Quercetin glycosides of the onion (Allium cepa). Naturwissenschaften, 43(1956), 158.

68) Ferreres, F., Gil, M. I., Tomás-Barberán, F. A. Anthocyanins and flavonoids from shredded red onion and changes during storage in perforated films. Food Res. Int., 29(1996), 389–395.

69) Corea, G., Fatturusso, E., Lanzotti, V., Capasso, R., Izzo, A. A. Antispasmodic Saponins from Bulbs of Red Onion, Allium cepa L. Var. Tropea. J. Agric. Food Chem., 53(2005), 935–940.

70) Kiviranta, J., Huovinen, K., Hiltunen, R. Variation of phenolic substances in onion. Acta Pharm. Fennica, 97(1988), 67–72.

71) Tsushida, T., Suzuki, M. Isolation of flavonoid-glycosides in onion and identification by chemical synthesis of the glycosides. Nippon Shokuhin Kagaku Kaishi 1995, 42, 100–108.

72) Tsushida, T., Suzuki, M. Content of flavonol glucosides and some properties of enzymes metabolizing the glucosides in onion. J. Jpn. Soc. Food Sci. Technol., 43(1996), 642–649.

73) Furusawa, M., Tanaka, T., Nakaya, K. I., Iinuma, M., Tuchiya, H. A new flavonol glucoside from onion. Heterocycles, 57(2002), 2175–2177.

74) Ly, T. N., Hazama, C., Shimoyamada, M., Ando, H., Kato, K., Yamauchi, R. Antioxidative compounds from the outer scales of onion. J. Agric. Food Chem., 53(2005), 8183–8189.

75) Franke, A. A., Custer, L. J., Arakaki, C., Murphy, S. P. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. J. Food Comp. Anal., 17(2004), 1–35.

76) Sellappan, S., Akoh, C. Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. J. Agric. Food Chem., 50(2002), 5338–5342.

77) Sood, A. R., Joshi, K. Flavonoid components of Allium cepa. J. Indian Chem. Soc., 51(1974), 975.

78) Park, Y.-K., Lee, C. Y. Identification of isorhamnetin 4′-glucoside in onions. J. Agric. Food Chem., 44(1996), 34–36.

79) Fossen, T., Slimestad, R., Andersen, Ø. M. Anthocyanins with 4′-glucosidation from red onion Allium cepa. Phytochemistry, 64(2003), 1367–1374.

80) Donner, H., Gao, L., Mazza, G. Separation and characterization of simple and malonylated anthocyanins in red onions, Allium cepa L. Food Res. Int., 30(1997), 637–643.

81) Fossen, T., Andersen, Ø. M., Øvstedal, D. O., Pedersen, A. T., Raknes, Å. Characteristic anthocyanin pattern from onions and other Allium spp. J. Food Sci., 61(1996), 703–706.

82) Gennaro, L., Leonardi, C., Esposito, F., Salucci, M., Maiani, G., Quaglia, G., Fogliano, V. Flavonoid and carbohydrate content in Tropea red onions: effect of homelike peeling and storage. J. Agric. Food Chem., 50(2002), 1904–1910.

83) Fuleki, T. Anthocyanins in red onion Allium cepa. J. Food Sci., 36(1971), 101–104.

84) Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, et al.. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 8(2009), 543-557.

85) K.L. Niraimathi, V. Sudha, R. Lavanya, P. Brindha. Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities. Colloids and Surfaces B: Biointerfaces, 102(2013), Pages 288-291.

86) Mohamed S. Abdel-Aziz, Mohamed S. Shaheen, Aziza A. El-Nekeety, Mosaad A. Abdel-Wahhab. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. Journal of Saudi Chemical Society, 18(2014), 356-363.

87) N. Jayachandra Reddy, D. Nagoor Vali, M. Rani, S. Sudha Rani. Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by *Piper longum* fruit, Materials Science and Engineering: C. 34(2014), 115-122.

88) Shashi Prabha Dubey, Manu Lahtinen, Mika Sillanpää, Tansy fruit mediated greener synthesis of silver and gold nanoparticles, Process Biochemistry, 45(2010), 1065-1071.

89) National Center for Biotechnology Information. PubChem Database. Quercetin, CID=5280343, https://pubchem.ncbi.nlm.nih.gov/compound/5280343 (accessed on Apr. 19, 2019)

90) Anna Floegel, Dae-Ok Kim, Sang-Jin Chung, Sung I. Koo, Ock K. Chun.Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of Food Composition and Analysis. 24(2011), 1043-1048.

91) Suvith VS, Philip D. Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc, 118(2014), 526–532.
92) Kumar V, Yadav SK. Plant mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol 84(2009), 151–157.