Synthesis, Characterization and Antimicrobial activity of Silver Nanoparticles by Green synthesis

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Biomedical Engineering and Sciences

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Declaration

I certify that this research work titled "*Synthesis, Characterization and Antimicrobial activity of Silver Nanoparticles by Green synthesis"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.

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Dr. Adeeb Shehzad

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Abstract

Green synthesis of metallic nanoparticles has been developed as environment friendly and cost effective alternative over other methods such as physical and chemical, thus gaining significant attraction in nanomedicine. In present study silver nanoparticles have been synthesized using silver nitrate and various concentrations of methanolic root extract of *Rhazya stricta.* The color change (yellowish to orange brown) in reaction mixture was observed during synthesis process. Stability and dispersion of nanoparticles was improved by adding xylitol. Synthesized nanoparticles were confirmed using UV–vis spectroscopy, scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), X-ray diffractometer (XRD), and Fourier transforms infrared spectroscopy (FTIR). Furthermore, the antibacterial activity was determined against strains of gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria for crude plant extract and synthesized nanoparticles.

Key Words: *Silver nanoparticles, Rhazya stricta, Antibacterial activity, Xylitol, Dispersion of nanoparticle*

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Abbreviations

NPs	Nanoparticles
AgNPs	Silver nanoparticles
R. stricta	*Rhazya Stricta*

CHAPTER 1: INTRODUCTION

Nanobiotechnology is new, fascinating and fast growing field for research in science and engineering. Nanotechnology involves creation and manipulation of materials at the nano scale. It is a relatively new area for researchers, with commercial applications. Nanotechnology involves the synthesis of different metal nanoparticles (NPs) like silver, gold, iron, titanium, zinc, magnesium, alginate, copper etc. with various physical, chemical and biological methods**.** Synthesis of NPs through green processes is a good substitute over the physical and chemical methods because of the synthesis of highly stable NPs that are environment friendly, biocompatible, cost effective, less toxic, one step method, ease of scale up and safe for human therapeutic use (Rajasekharreddy and Rani, 2014, Ponarulselvam et al., 2012, Verma and Mehata, 2016).

Silver nanoparticles (AgNPs) playing a major role in the field of nanomedicine and nanotechnology (Logeswari et al., 2015). They are proved to be most effective in therapeutic and pharmaceutical industries because of good antifungal and antimicrobial activity against bacteria, viruses and other microorganisms. AgNPs have a wide range of applications in various fields like in diagnostic biomedical optical imaging (Vo-Dinh et al., 2005) molecular labeling, cancer therapy (Wu et al., 2008), wound dressings, coating of catheters, dental works, scaffold, medical devices and sensors for refractive index (Saha et al., 2012, Guo and Tao, 2007).

Rhazya stricta (*R. stricta*) is one of the important medicinal plant. It is abundantly present in different hilly areas of Pakistan. It is a small, glabrous, erect shrub, that contains some biomolecules such as alkaloids, flavonoids and phenolic compounds (Bukhari et al., 2015, Iqbal et al., 2006). It is used for the treatment of numerous diseases like obesity, diabetes mellitus, sore throat, cardiovascular, metabolic, neurodegenerative diseases as well as cancers (Gilani et al., 2007, Ali et al., 2000). It possess anti-oxidant, anti-carcinogenic, antimicrobial, anti-dramatist, antihypertensive (Verpoorte, 1998), antidepressant, anti-inflammatory (Baeshen et al., 2010, Lantero et al., 2014), antipyretic (Marwat et al., 2012), antifungal (Reddy et al., 2016) and herbicidal activities (Gilani et al., 2007, Marwat et al., 2012).

Several studies exhibit the antimicrobial activity of AgNps against several pathogenic and multidrug-resistant microorganisms (Singh et al., 2016).

In this study, we have synthesized the AgNPs by using root extract of *R. stricta* plant along with xylitol in which plant act as a reducing agent. Sugars are used to increase the dispersion of NPs in various experiments. We used the xylitol as a capping agent and to increase the dispersion of.NPs NPs synthesized with *R. stricta* plant were characterized with techniques including UV-Vis spectroscopy, X-Ray Diffraction (XRD), Scanning electron microscopy(SEM), Fourier Transform Infra-Red Spectroscopy (FTIR) and Energy Dispersive X- Ray Spectroscopy (EDX). We test the antibacterial activity of synthesized AgNPs against gram-positive (*Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*).

Research objectives:

- AgNPs synthesis with *R. stricta* plant root extract
- Characterization of synthesized NPs
- Evaluation of antimicrobial activity of AgNPs
- Improvement of NPs dispersion

CHAPTER 2: LITRETURE REVIEW

The concept of nanotechnology was first given by Richard Feynman in 1959. Nanotechnology is a new emerging field of science that involves the production, design, manipulation and use of materials in nanometer range. It is a multidisciplinary approach that includes different disciplines such as biology, physics, chemistry, biochemistry engineering and medicine (Iravani et al., 2014). NPs have significant advantages with wide range of applications in the field of bio-medical, antimicrobials, catalysts, sensors, chemical industries, optical fibers, agricultural, electronics, bio-labeling and in other areas (Iravani et al., 2014).

Synthesis of NPs is classified into two processes that are “Top-down” process and “Bottom-up” process. In Top-down approach small particles of nanoscale are formed by the breakdown of bulky materials with different techniques such as milling, grinding etc. In Bottom-up approach atoms combined together by self-assembly and form new nuclei that grow into a particle of nanoscale. With the advancement in scientific knowledge and technologies in the field of medicinal plant biology, now plants are employing in synthesis of NPs (Kavitha et al., 2013).

Synthesis of NPs can be done with different methods that includes chemical (Sun et al., 2002), radiation (Dimitrijevic et al., 2001), electrochemical, phytochemical (Callegari et al., 2003) and biological techniques (Roy and Das, 2015).

Chemical and physical methods involve in the synthesis of AgNPs are mostly very expensive and cause some toxic chemical agents to absorb on NPs surface in biomedical applications. Laser ablation and evaporation-condensation are the most commonly used physical methods to synthesize NPs. Most chemical methods are not environment friendly that involve the use of chemical reagents like sodium citrate, Tollens reagent, ascorbate, elemental hydrogen, sodium borohydride ($NaBH_4$) and N, N-dimethylformamide (DMF) are used for silver ions (Ag^+) reduction in both aqueous and non-aqueous solutions (Jawaad et al., 2014, Iravani et al., 2014).

This enhances the need to use biological approaches for synthesis of NPs. Biological method includes the use of plants and microorganisms (bacteria, fungi, algae). Synthesis of NPs by using plants is getting benefit over other biological processes because microorganism mediated synthesis is not favorable for industrial needs and require complex procedure for maintenance of microbial culture (Iravani, 2011).

Plant mediated synthesis is best platform for NPs synthesis because of its simplicity, free from toxic chemicals, speedy process and act as both capping and stabilizing agents itself. Plants contain various reducing agents like ascorbic acids, citric acid, flavonoids, dehydrogenases, alkaloids and reductases that is the reason for choosing plants for NPs synthesis (Iravani, 2011, Iravani et al., 2014).

Metallic NPs have distinctive properties like excellent conductivity, catalytic activity, chemical stability etc. that are dependent on the particle shape, size and its distribution. The core properties of metal NPs are determined by their shape, size, composition, structure and crystallinity. Among all metals, silver has the highest thermal and electrical conductivity (Jawaad et al., 2014).

Plants have been used for cure of various diseases from ancient times. 3000 plants are officially documented as medicinal plant but more than 6000 plants use by traditional practitioners (Ahmed and Ikram, 2015). Plants are used as medicines by 60% of the world's growth for treatment of various human diseases because 70-80% of the global population cannot afford modern medication (Kumar et al., 2013). Plant extracts efficiently cure group of diseases with a minute toxicity further they are also effective cost, readily available and have better therapeutic effects (Semwal et al., 2014). Pakistan has a significant heritage of herbal remedies but like most developing countries, its rural population still depends on the traditional natural system of medicine to a large extent (Begum et al., 2012).

Several experiments have been done for the synthesis of silver NPs through using medicinal plants such as *Capsicum annuum, Ananas comosus* (Ahmad and Sharma, 2012), *Azadirachta indica*, *Oryza sativa, Camellia sinensis, Saccharum officinarum, Sesbania drummondii*, *Olea europaea* (Nasir et al., 2016) *Sorghum bicolour, Erythrina indica* (Kalainila et al., 2014) *Zea mays, Helianthus annus, Terminalia arjuna,* (Ahmed and Ikram, 2015) *Basella alba*, *Rhododendron dauricum* (Mittal et al., 2012) *Aloe vera*, *Lysinibacillus sphaericus (Gou et al., 2015)* Magnolia kobus, Adhathoda vasica (Nazeruddin et al., 2014) *Medicago sativa*, *Cinamomum camphora* and *Polyalthia longifolia* (Kaviya et al., 2011) in the domain of nanopharmaceutical (Geoprincy et al., 2013).

2.1 *Rhazya stricta (R. stricta)*

R. stricta is a medicinal plant that used in traditional eastern medicine and possesses important pharmacological activities. The genus Rhazya contains two species that are *Rhazya stricta* and *Rhazya orientalis.* Rhazya species were called so because of the name of a Muslim scientist Abu Bakr Mohammed bin Zakariya Ar-Razi who was known in Europe commonly under the Latinized name of Rhazes (Marwat et al., 2012, Baeshen et al., 2015). *R. stricta* belongs to Apocynaceae family and is widely distributed in Pakistan, India, Afghanistan, Saudi Arabia, United Arab Emirates, Iraq and Iran (Marwat et al., 2012). It is a small, glabrous, erect shrub (Iqbal et al., 2006) that contains milky sap with toxic compounds and many other materials that is used to treat various diseases with low toxicity (Verpoorte, 1998).

R. stricta is used for the cure of various inflammatory diseases such as cardiovascular, metabolic, neurologic disorders as well as cancers (Gilani et al., 2007). It has anti-oxidant, anti-carcinogenic, antimicrobial, anti-dramatist, antihypertensive (Verpoorte, 1998) antidepressant, anti-inflammatory (Baeshen et al., 2010, Lantero et al., 2014), antiacetylcholinestrase (Shahat et al., 2015), antineoplastic, Antipyretic (Marwat et al., 2012), antifungal (Reddy et al., 2016), antispasmodic (Gilani et al., 2007) and herbicidal activities. (Marwat et al., 2012).

2.2 Photochemistry and Therapeutic Properties of *R. stricta*

Phytochemical analysis of *R. stricta* shows Alkaloids, coumarins, flavonoids, phenols, resins, saponins, steroids, glycosides, tannins, diterpenes and triterpenes present in R. stricta plant that exhibits several pharmacological proerties (Mohamed et al., 2014, Khan et al., 2012, Al-Homidan et al., 2002).

The antimicrobial property of glycosides ensures its use as an anti-infective agent. Coumarins which was reported in *R.stricta* extracts, are well known for its antitumor, antibacterial and anthelmintic properties (Reddy et al., 2016). Phytochemical assay of the *R. stricta* shows that it has high concentrations of phenols which are known to be potent antioxidant (Khan et al., 2012). Flavonoids contain antiviral, antimicrobial, anti-inflammatory, anti-allergic, antioxidant, antiproliferative and anticancer activities (Elkady et al., 2012).

R. stricta plant has more than 100 alkaloids that (El Gendy et al., 2012) have several pharmacological properties (Mohamed et al., 2014). Its effective use is because of the indole alkaloids in the plant that make its contribution to treat inflammatory diseases like diabetes mellitus, different types of cancer, neurodegenerative, metabolic problems (Baeshen et al., 2010), hypertension, chronic rheumatism, sore throat, and fever (Iqbal et al., 2006). Further two indole alkaloid 16-epi-Z-isositsirikine and didemethoxycarbonyl-tetrahydrosecamine that are obtained from *R. stricta* shows antineoplastic activity that make it effective for the treatment of neurodegenerative diseases (Elkady, 2013). Many monomericand dimeric terpenoid indole alkaloids (TIAs) and few non-alkaloid component are also present in R.stricta and is worth noting that some TIAs, e.g. vallesiachotamine have anti-cancer properties (Akhgari et al., 2015).

A phenolic indole alkaloid sewarine has recently identified from *R. stricta*, that is the first naturally derived κ opioid peptide (KOP) antagonist. It has strong apoptotic effects because of activation of caspase pathways, modulation of anti-apoptotic and pro-apoptotic proteins and inhibitory effect of NF-κB activation (Lantero et al., 2014).

2. 3 Antimicrobial Activity

Currently, bacterial infections particularly caused by multi-drug resistant (MDR) bacteria have become a great challenge for modern healthcare. Antimicrobials used in various areas that includes medicine, crop production, food animals and as disinfectants in hospitals, farms and households (Darabpour et al., 2011).

Human are always prone to infectious diseases throughout the world. Different antibiotics are although being used for bacterial infections but resistance to antibiotics is a major problem in treatment of bacterial infections. Therefore, we need to find novel antibacterial agents with improved activity (Monte et al., 2014).

AgNPs as antibacterial agent has gained significant application in biomedical sciences. The AgNPs of smaller size show improved properties when compare with the bulk material. AgNPs use as antiseptic agent for few years. Silver nitrate solution is used as an ointment for the treatment of burn and infections since 19th century. Literature shows that antimicrobial activity is consequence of +ve charge on silver ion. The antibacterial activity is because of the electrostatic attraction between +ve charged NPs and -ve charged cell membrane of bacteria (Nazeruddin et al., 2014).

NPs become attached to the bacterial cell wall and disrupt its permeability. It also effects bacterial cellular respiration. The NPs invade in the cell wall that cause cellular damage while interacting with sulfur and phosphorus containing compounds, like DNA and protein that are present in the cell. AgNPs possess bactericidal properties that are because of the discharge of silver ions from NPs (Dwivedi and Gopal, 2010). Efficacy of silver nanoparticles significantly depends on size of the synthesized nanoparticles. Smaller NPs will display strong antibacterial activities because of equivalent silver mass content (Geoprincy et al., 2013).

To verify the formation of AgNPs UV-Vis spectrophotometer monitored in the range of 300-800nm. Then various characterization techniques are used to completely understand the properties of synthesized nanoparticles. These techniques include the X-Ray Diffraction (XRD), Scanning electron microscopy(SEM), Energy Dispersive X- Ray Spectroscopy (EDX) and Fourier Transform Infra-Red Spectroscopy (FTIR) (Nazeruddin et al., 2014, Forough and FAHADI, 2011)

CHAPTER 3: METHODOLOGY

3.1 Selection and collection of plant material

R. stricta natural plants was selected for the AgNPs synthesis. It was collected from khushab district of Punjab, Pakistan. The roots from *R. stricta* were washed 2–3 times with de-ionized water.

3.2 Extraction

The shade-dried roots of *R. stricta* plant 147 g was extracted thrice with methanol at room temperature. Then rotary evaporator is used to evaporate the solvent. The resulted extract is 13gram after drying.

3.3 Biosynthesis of silver nanoparticles

For synthesis of AgNPs 10 gram of the dried extract is dissolved in 18ml methanol and place at 4°C. 1mM solution of silver nitrate ($AgNO_3$) was prepared in deionized water. 6ml methanolic plant extract is added drop by drop in 100ml $AgNO_3$ (1mM) with continuous shaking. Another reaction is carried out with same amount of extract as 6ml is added in 100ml $AgNO_3$ along with 6ml xylitol (10-2 M). Then both the reactions heated separately using magnetic stirrer at 60 °C for two hours**.** The change in colored is the indication for synthesis of NPs**.**

For characterization of AgNPs, centrifuge the sample at 12000rpm for 20 minutes. The supernatant was discorded to get rid of any uncoordinated materials. Then washed the sample 3 times with distilled water to remove extra proteins or enzymes that are not capping the AgNPs. Then after sonication, the sample dried with vacuum drier over the night at 50°C. The schematic presentation of the methodology is shown in Figure 1.

Antimicrobial activity measures against gram negative and gram positive bacteria. *Escherichia Coli* and *Bacillus subtilis* bacterium are used for study.

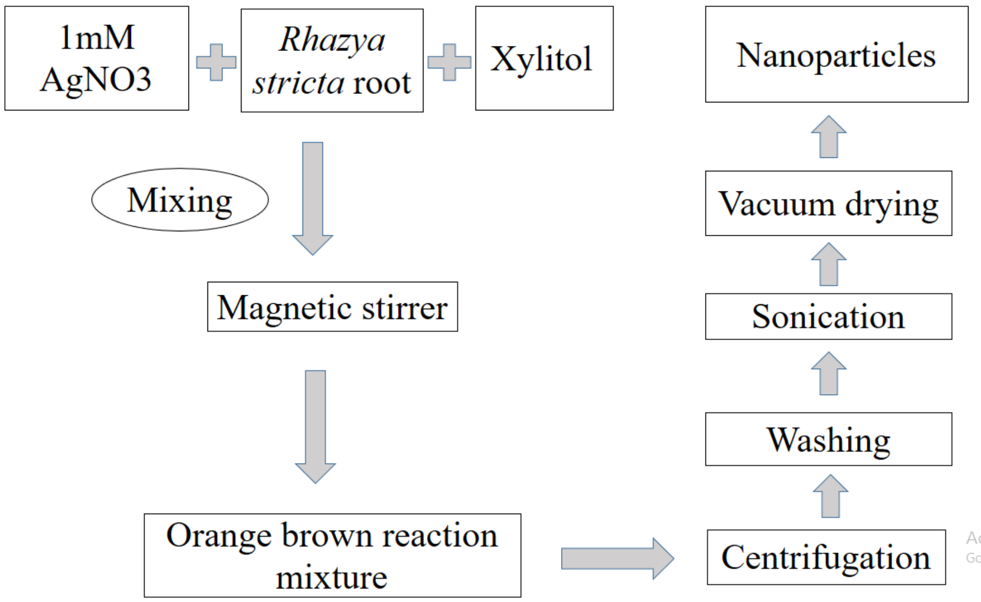

Figure 1: schematic presentation of methodology for silver nanoparticle synthesis

3.4 UV-Visible Absorbance spectroscopy

Synthesis of AgNPs solution with root extract of the plant *R. stricta* could easily observed by ultraviolet-visible (UV-Vis) spectroscopy. UV spectrum for 250µl, 350µl, 450µl,550µl, 650µl concentrations of plant extract in 10ml of AgNO3 solution are observed. The UV-VIS absorption spectra for AgNPs were observed in a range of 300-800nm with uv-2800 spectrophotometer.

3.5 Fourier Transforms Infrared Spectroscopy (FTIR)

Biomolecules that are responsible for reduction of silver salt were studied by using Fourier transform infrared (FTIR) spectrometer (Verma and Mehata, 2016).

3.6 X-Ray Diffraction

The structural characterization and the crystalline nature of AgNPs were determined by X-ray diffractometer for the vacuumed dried AgNPs.

3.7 Scanning Electron Microscopy and Energy Dispersive X-Ray (EDX)

Scanning Electron Microscopy conforms the presence of synthesized AgNPs. The surface morphology of AgNPs was investigated using JEOL-6490A-JSM for Scanning Electron Microscopy (SEM). Two or three drops of the sample placed on the grid and drying it under a lamp. Energy dispersive spectroscopy (EDS) is used to identify the elemental composition of AgNPs.

3.8 Antibacterial Activity

The antibacterial activity of the extract and Ag NPs was measured for *Escherichia coli*, and *Bacillus subtilis* by the disk diffusion method. The disks were soaked with distilled water, standard antibiotic Cefixime, *R. stricta* extract and the AgNPs solution with and without xylitol addition separately. 50µl concentrations of the AgNPs were used to ensure identification of the antibacterial activity. The disks were autoclaved before being placed in agar media containing the microbial cultures. Culture media plates were divided into equal parts and already prepared disks were placed on every part of plate. The disk soaked in distilled water was utilized as the negative control, and disk soaked with cefipime was used as the positive control. Then plates were incubatedf or 24–48 hours at 37°C. The zone of inhibition was measured for both bacterial strains.

CHAPTER4: RESULTS

4.1 Visual Observations

The colour of solution changes from pale yellow to orange brown after 2 hours heating on magnetic stirrer that is shown in Figure 2. It is the first indication for NPs synthesis.

Figure 2: Change in colour after synthesis of AgNPs

4.2 UV-VIS Spectroscopy of synthesized nanoparticles using *R. stricta* root extract.

Figure 3. shows the absorption peaks of AgNPs synthesized by *R. stricta* root extract at 380, 392, 402, 414 and 427nm wavelength. As the concentration of plant extract increases the peak also increased but after a specific concentration further increase in concentration cause the broadening of the peak indicating the increased size of NPs. Previous studies indicated that the spherical AgNPs have absorption bands at around 400 nm in the UV visible spectra (Nasir et al., 2016).

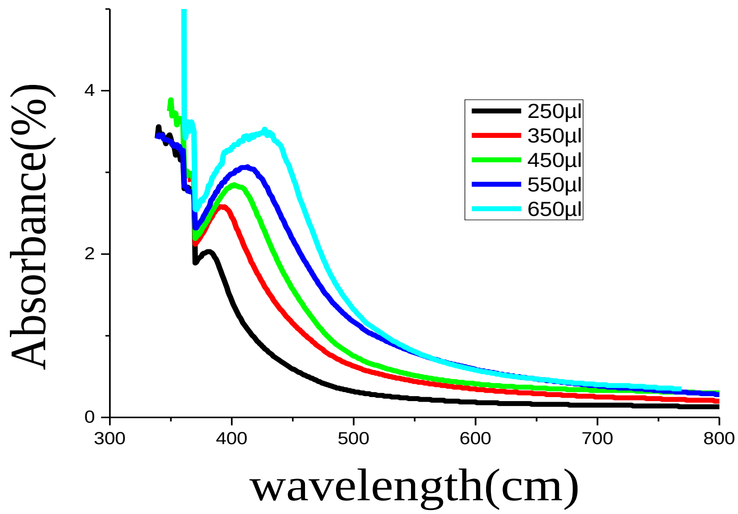

Figure 3: Concentration dependent UV-Vis Spectra of AgNPs of *R. stricta*

4.3 Scanning electron microscopy of synthesized nanoparticles using *R. stricta* root extract

The Scanning Electron Microscopy (SEM) images as shown in figure 4 and figure 5 depicts the formation of spherical shape NPs. Xylitol act as a capping agent and prevents the NPs from aggregation and results in uniform dispersion (Figure 4). Whereas the image of synthesized AgNPs only with plant extract shows more agglomeration (Figure 5).

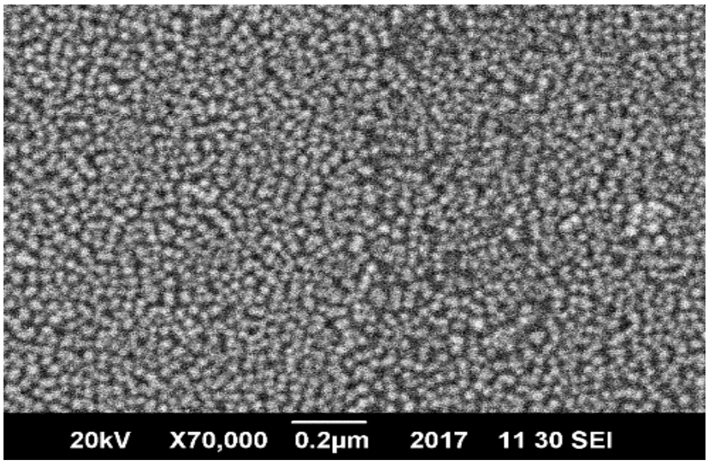

Figure 4: Image of Scanning Electron Microscopy (SEM)of AgNPs synthesized by using *R. stricta* with xylitol

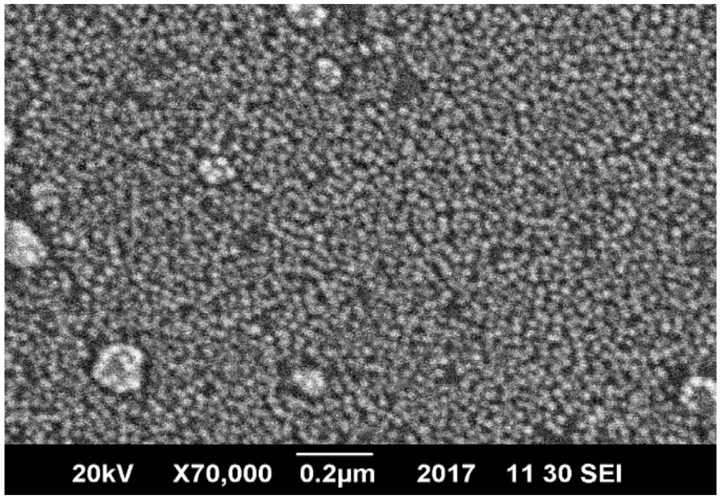

Figure 5: Image of Scanning Electron Microscopy (SEM) of AgNPs by using *R. stricta*

The size distribution of the AgNPs synthesized using *R. stricta* plant root extract along with xylitol is shown in figure 6 and with only *R. stricta* extract is shown in Figure 7.

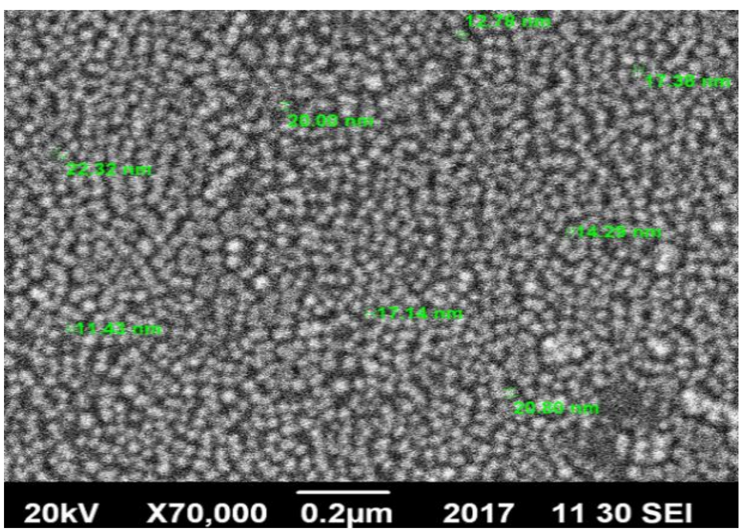

Figure 6: Image of Scanning Electron Microscopy (SEM) of AgNPs synthesized using *R. stricta* with xylitol

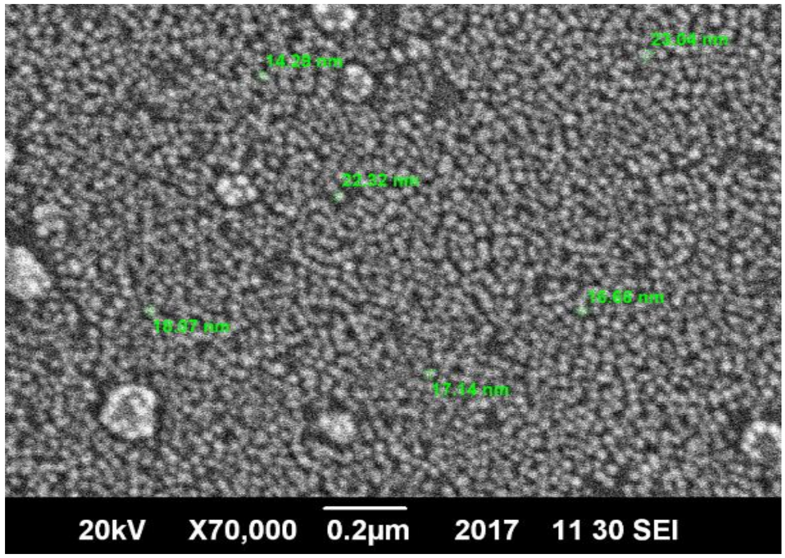

Figure 7: Image of Scanning Electron Microscopy (SEM) of AgNPs synthesized with *R. stricta*

4.5 Elemental analysis of of synthesized nanoparticles using *R. stricta* root extract with energy dispersive X-ray (EDX)

The AgNPs synthesized using *R. stricta* are also characterized by EDX analysis. The optical absorption peak is recorded almost at 3 keV, that is obvious for silver nanocrystals absorption because of the surface Plasmon resonance.

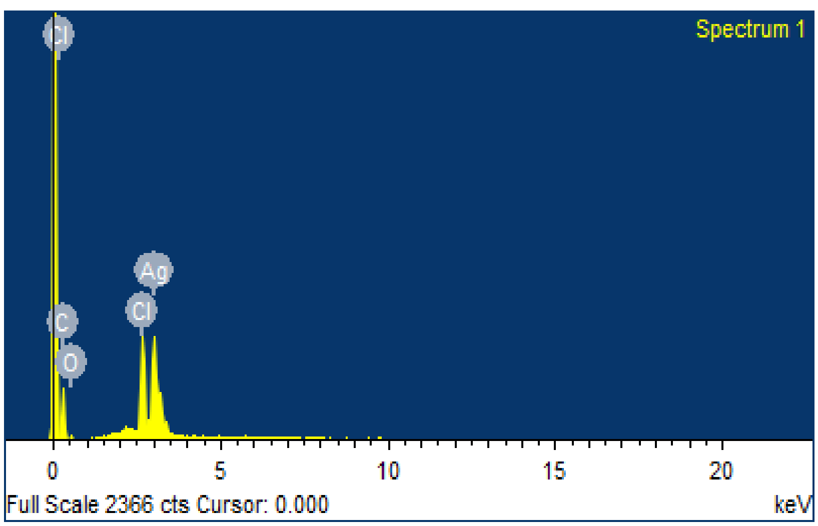

Figure 8: Energy Dispersive X-Ray image of AgNPs synthesized using *R. stricta*

The EDX spectrum shows silver peak along with oxygen and chlorine peaks. The chlorine and oxygen peaks are may be due to glass biomolecules that are present on the surface of silver nanoparticles or may be chlorine come from the glass slides that used for sample preparation (Kalainila et al., 2014).

4.5 Structural characterization of AgNPs synthesized using *R. stricta* root extract with X-ray diffraction (XRD)

The dry powders of the AgNPs were used for XRD analysis. XRD patterns of synthesized AgNPs are shown in Figure 9. The synthesized NPs are of crystalline nature. The XRD pattern is showing the intense five peaks of NPs at 31.99, 45.5, 54.85, 57.52 and 67.24 are indexed to the face centered cubic structures of AgNPs. The sharpening of the peaks reveals the spherical shape of NPs.

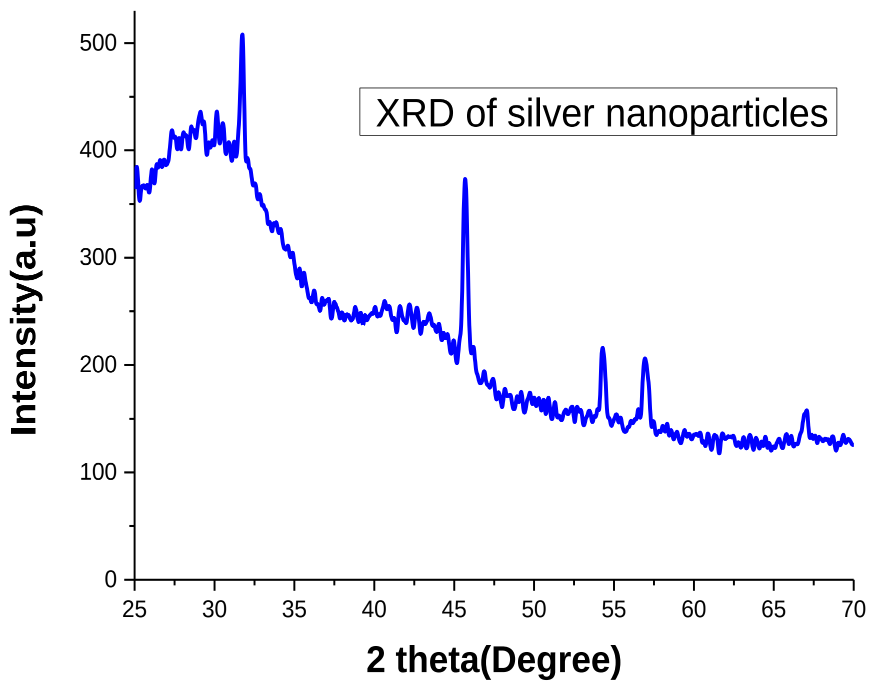

Figure 9: X-Ray Diffraction (XRD) image of the AgNPs synthesized using *R. stricta*

4.5 Fourier transform infra-red spectroscopy (FTIR) analysis of synthesized nanoparticles using *R. stricta* root extract.

FTIR spectroscopy is used to investigated the surface chemistry of the biosynthesized AgNPs. FTIR measurements were done to identify the biomolecules that are responsible for Ag+ ions reduction and capping of biologically reduced AgNPs synthesized by *R. stricta* root extract along with xylitol. Figure 10 shows the FTIR spectrum of xylitol and gives bands at 3362.59 (OH of alcohol) and 1417.88 (C-H bending of alkane).

Figure 11 shows the FTIR spectrum of root extract of *R. stricta* plant and gives peaks at 724,1024, 1455, 1687, 2123, 2921 and 3374cm−1. A peak at 3374cm−1 indicates the stretching of the N–H bond of amino groups and show presence of bonded hydroxyl (-OH) group. The absorption peak at 2921 cm−1 could be due to –CH stretching vibrations of –CH3 and –CH2 functional groups. The absorption peaks at 1687 cm−1 is due to the presence of C–O stretching in carboxyl coupled to the amide linkage in amide I (Kalainila et al., 2014).

The absorption peaks at 1455 indicated the presence of C-N stretching in amide. The two bands present at 1024 and 742cm−1 can be indicative of the –O– stretching vibrations of aliphatic and aromatic amines (Gou et al., 2015).

FTIR spectrum of the *R. stricta* roots extract reveal that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of *R. stricta* roots extract were responsible for reduction of silver ion to metallic silver nanoparticles (Nasir et al., 2016). Plant extract contain proteins that binds with the silver nanoparticles through carboxyl or amino group present in protein (Kaviya et al., 2011). Figure 12 and Figure 13 shows the FTIR spectra for silver nanoparticles synthesized using only *Rhazya stricta* plant and *Rhazya stricta* plant along with xylitol.

The presence of the residual plant extract in the sample is due to the similarity of the spectras with little marginal shifts in peak position (Ahmed and Ikram, 2015). Experimentally, Xylitol does not contain the ability to reduce the silver ions, but it possibly involves in the capping of the synthesized AgNPs by electrostatic attraction or bind with the protein groups present in the extract through hydrogen bonding and enhance the silver nanoparticle's stability. It also protected the NPs from agglomeration (Kaviya et al., 2011).

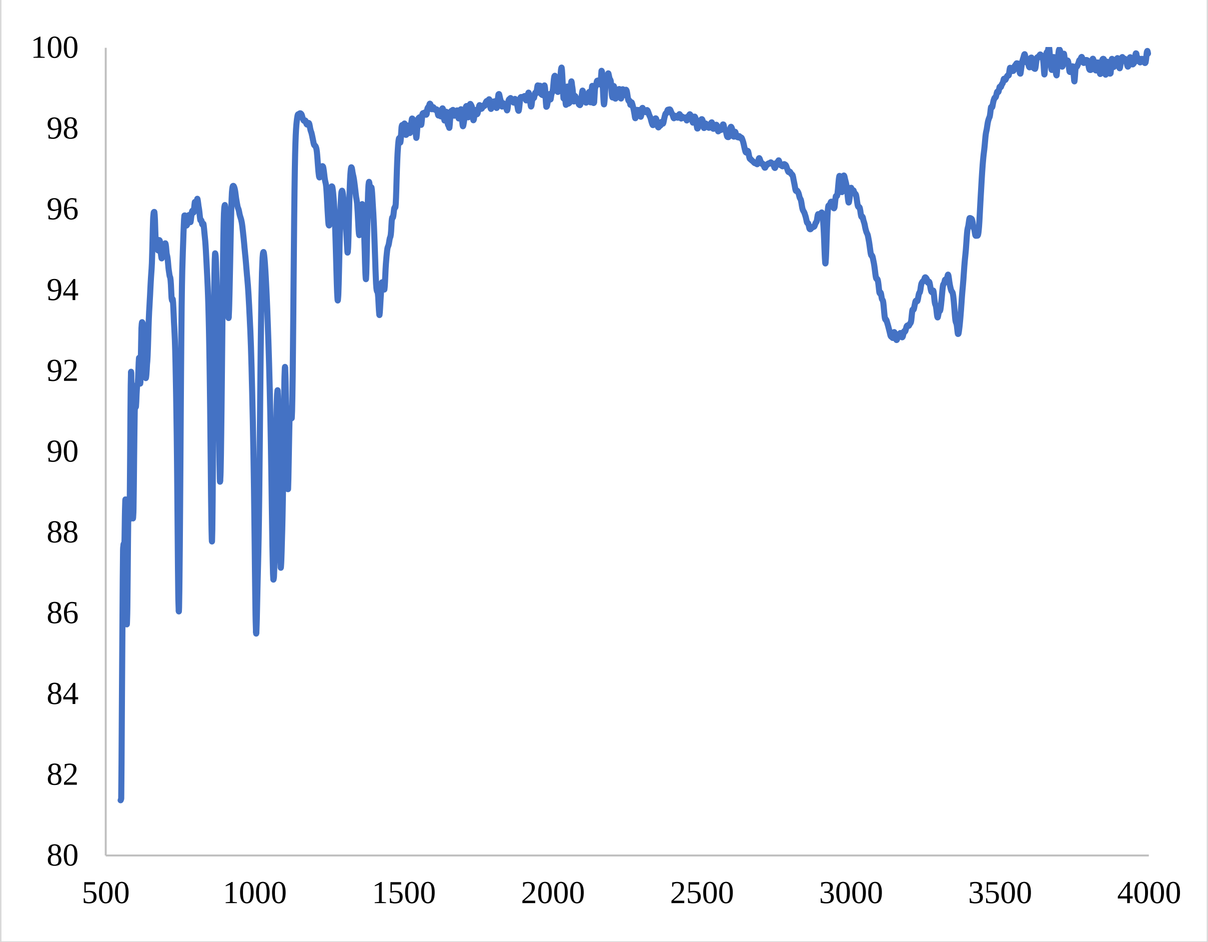

Figure 10: FTIR of Xylitol

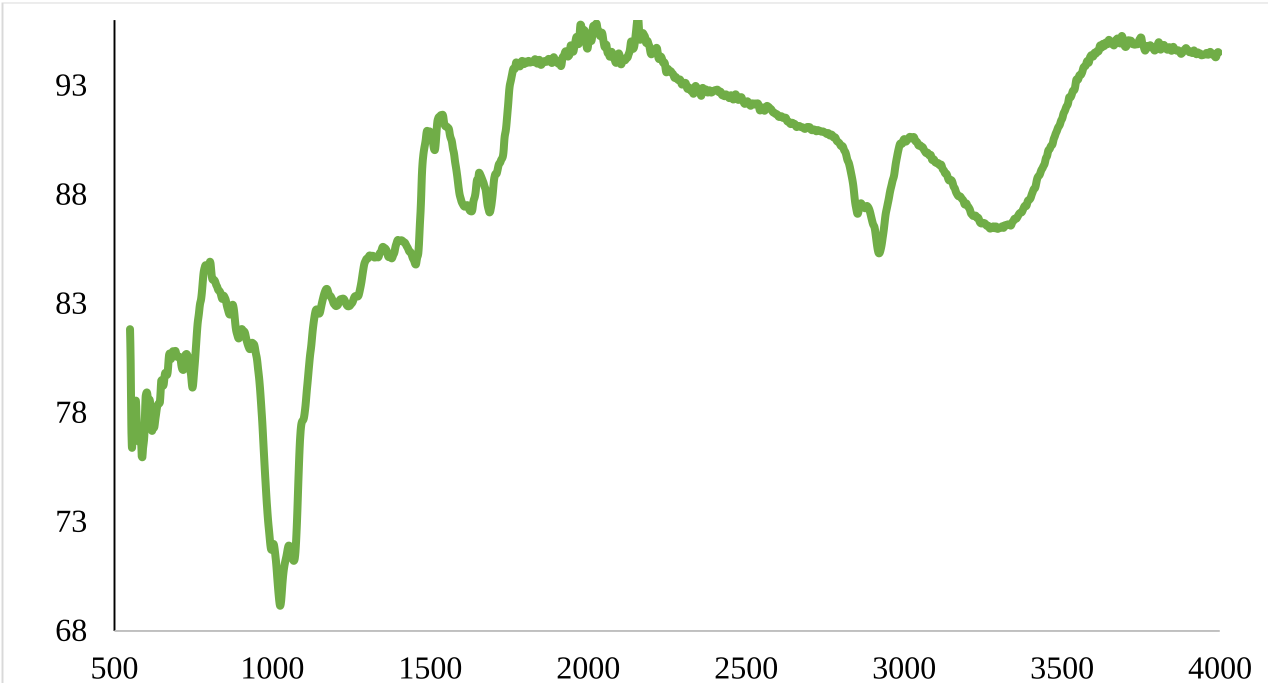

Figure 11: FTIR of medicinal plant *R. stricta*

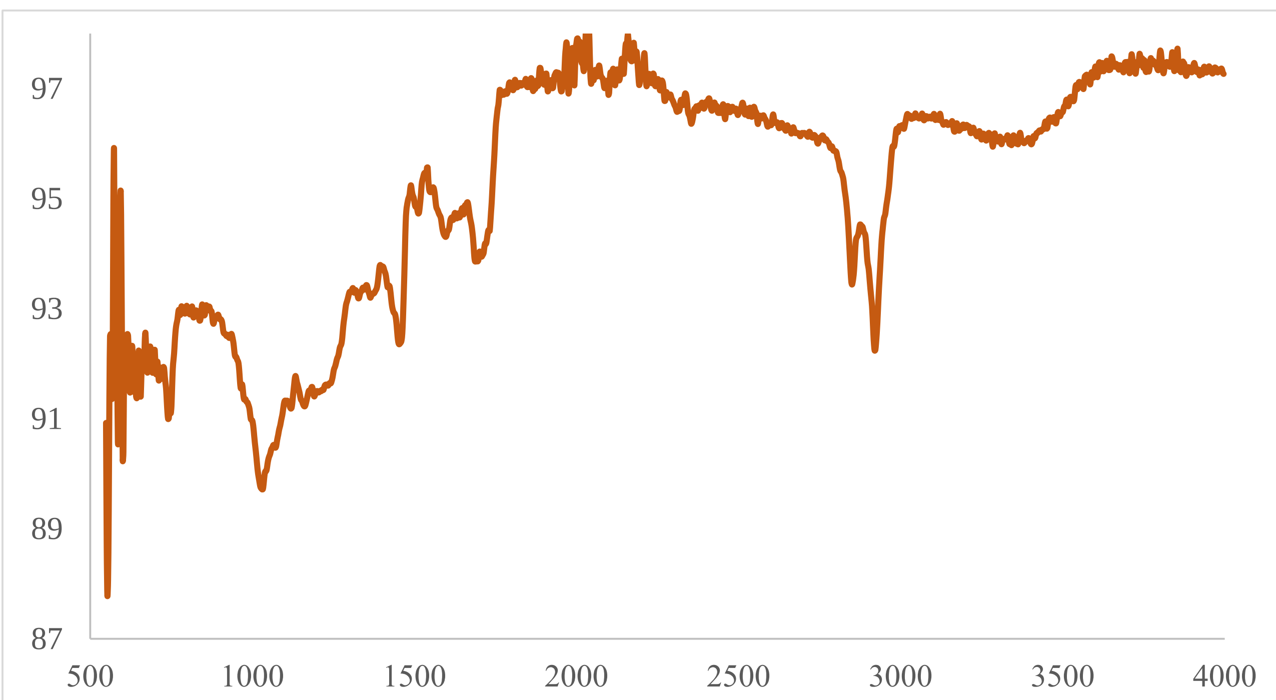

Figure 12: FTIR of AgNPs using *R. stricta*

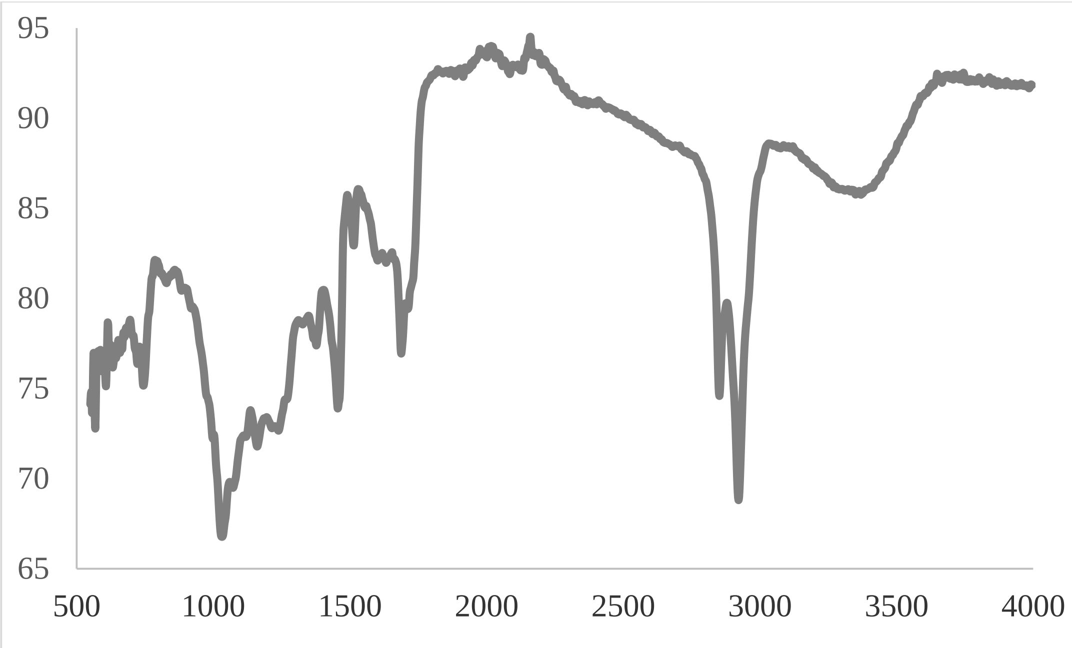

Figure 13: FTIR of AgNPs using *R. stricta* along with xyliyol

4.6 Antimicrobial activity of AgNPs synthesized using *R. stricta* root extract

Plant mediated synthesis of AgNPs exhibited tremendous antibacterial activity against bacterial strain *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). The concentration dependent effect of *Bacillus subtilis* and *Escherichia coli* in dramatic and graphical representation is shown in Figure 14, 15, 16 and 17.

4.6.1 Concentration dependent effect of AgNPs synthesized using *R. stricta* root extract

Three different concentration that are 450µl,550µl and 650µl of plant extract in 10ml of AgNO3 are used to check the antibacterial activity. When concentration is increased from 450µl to 550µl zone of inhibition is increased, but at 650µl zone of inhibition is smaller than 550µl because of the increase in the size of the synthesized NPs that is also indicated by the broader peak of UV spectroscopy at 650µl concentration. Smaller sized AgNPs give better antibacterial activity.

Cefipime is an antibiotic that is used as a positive control in the experiment and distilled water is used as a negative control. Cefipime gives maximum zone of inhibition and distilled water shows no inhibition of bacteria.

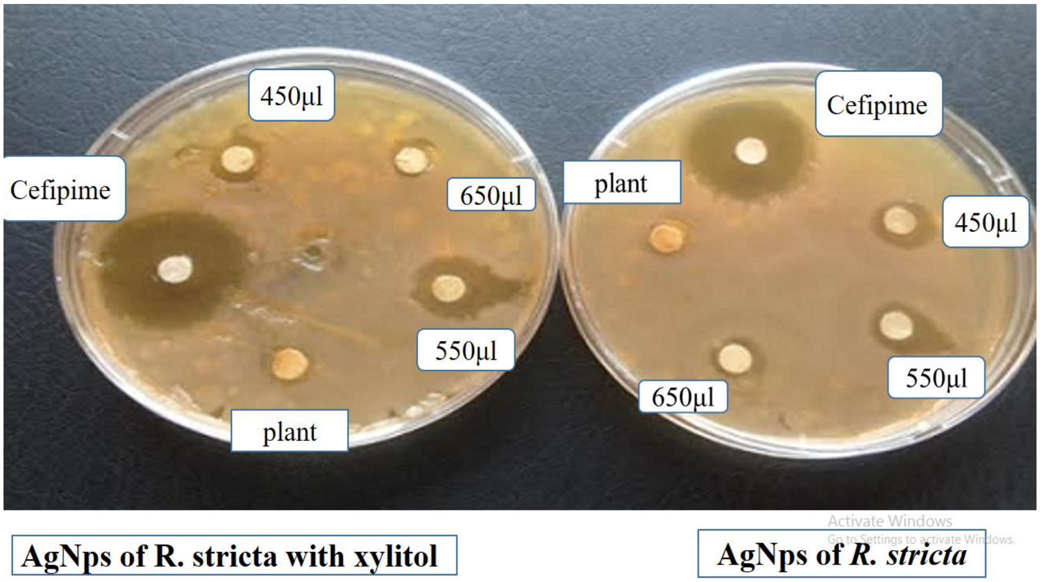

Figure 14: Concentration dependent effect *Bacillus subtilis*

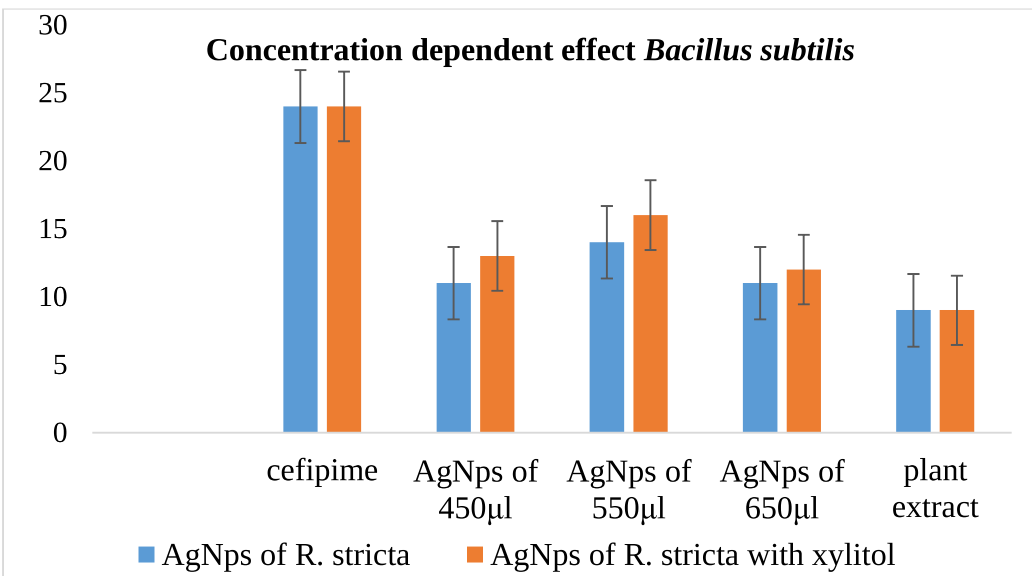

Figure 15: Graphical representation of Concentration dependent effect *Bacillus subtilis*

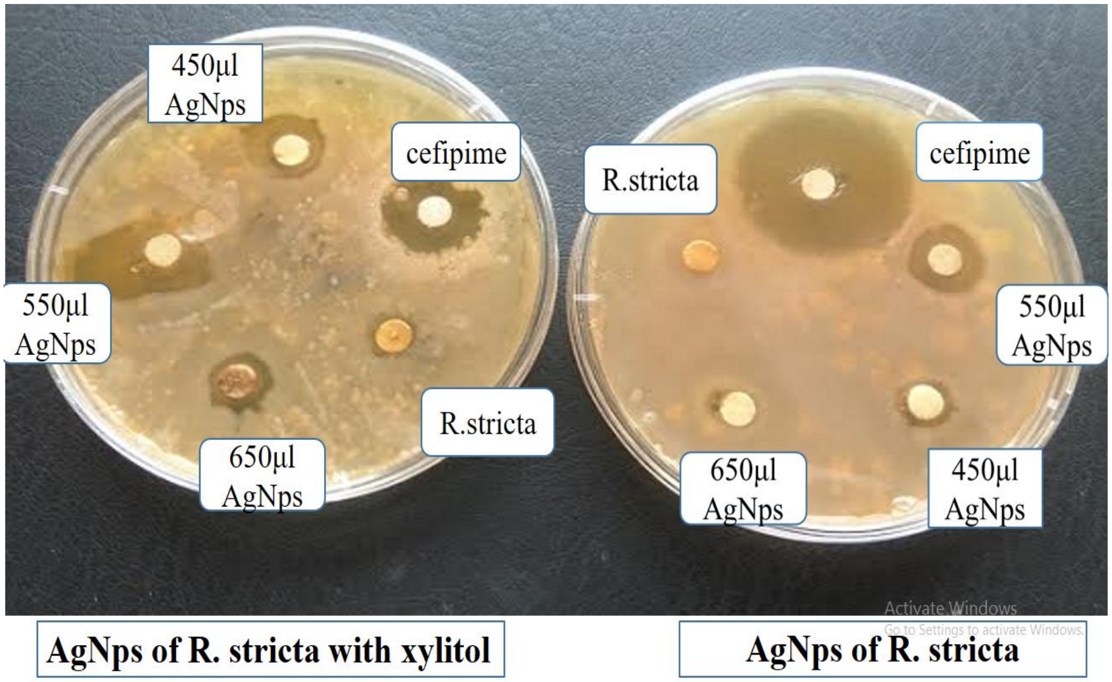

Figure 16: Concentration dependent effect *Escherichia Coli*

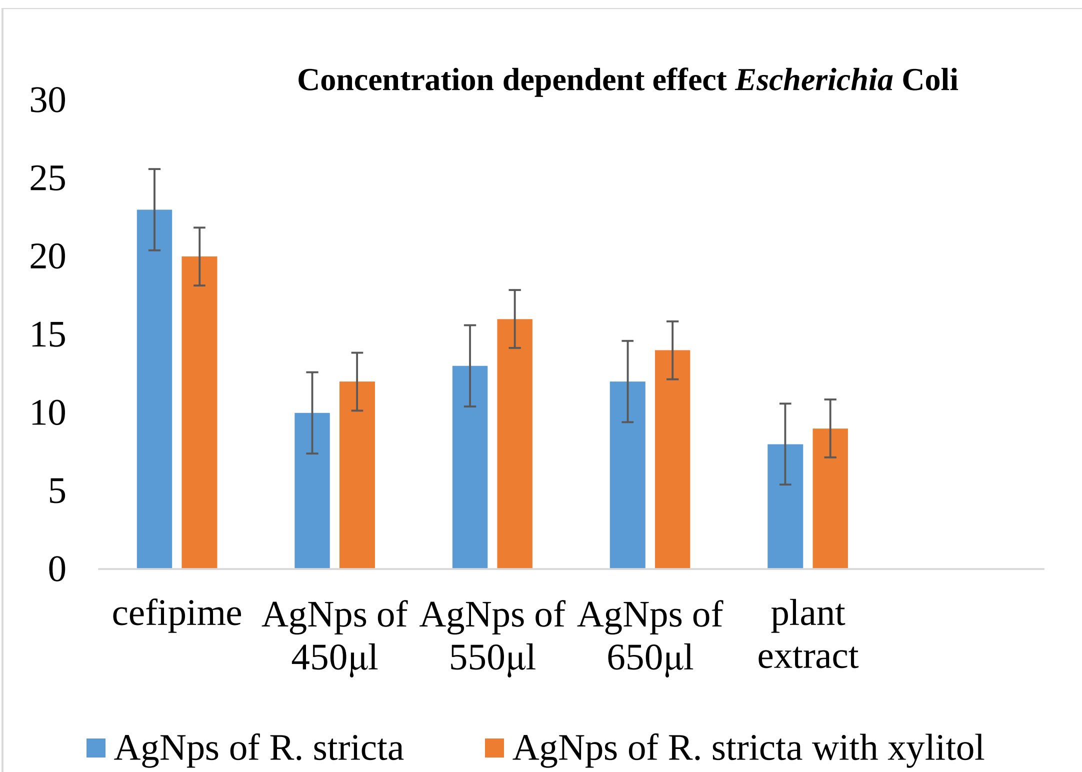

Figure 17: Graphical representation of Concentration dependent effect *Escherichia Coli*

The AgNPs synthesized by *R. stricta* root plant extract along with xylitol better antibacterial activity than with only plant extract because xylitol increases stability and decreases the agglomeration of synthesized AgNPs. As a results uniformly distributed NPs are formed that enhance the antibacterial activity of AgNps of plant root extract of *R. stricta*

4.6.2 Time dependent effect of AgNPs synthesized using *R. stricta* root extract

Time dependent activity of AgNps synthesized with *R. stricta* extract and extract along with xylitol for *bacillus subtilis* is shown in figure 18 and of *Escherichia coli* in figure 19. Only 550µl concentration of plant extract in 10ml silver nitrate solution is used to check time dependent activity of synthesized AgNps because this concentration gives best result in concentration dependent effect study. After 48 hours' zone of inhibition increases by 1mm than 24 hours' zone. Figure 18, 19, 20 and 21 shows that plant also has antibacterial activity but its activity increase after synthesizing NPs.

Figure 18: Time dependent effect of *Bacillus subtilis*

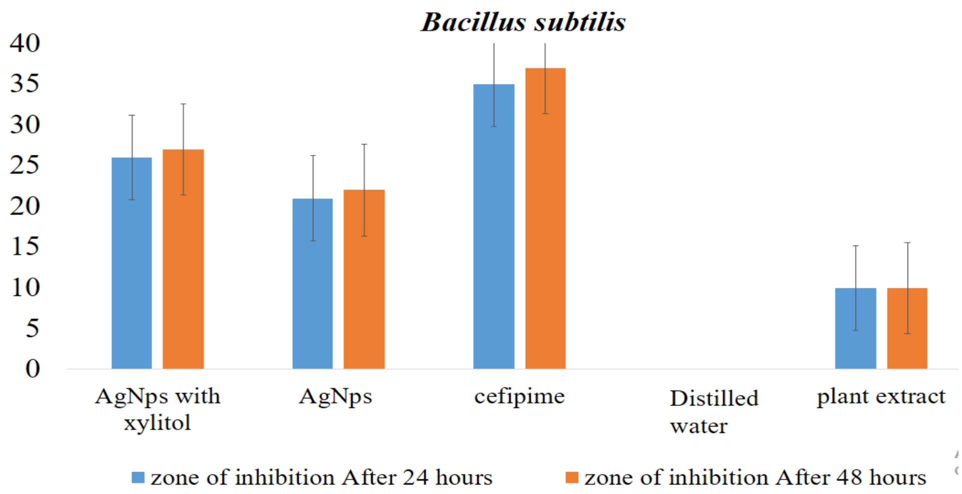

Figure 19: Graphical presentation for time dependent effect of *Bacillus subtilis*

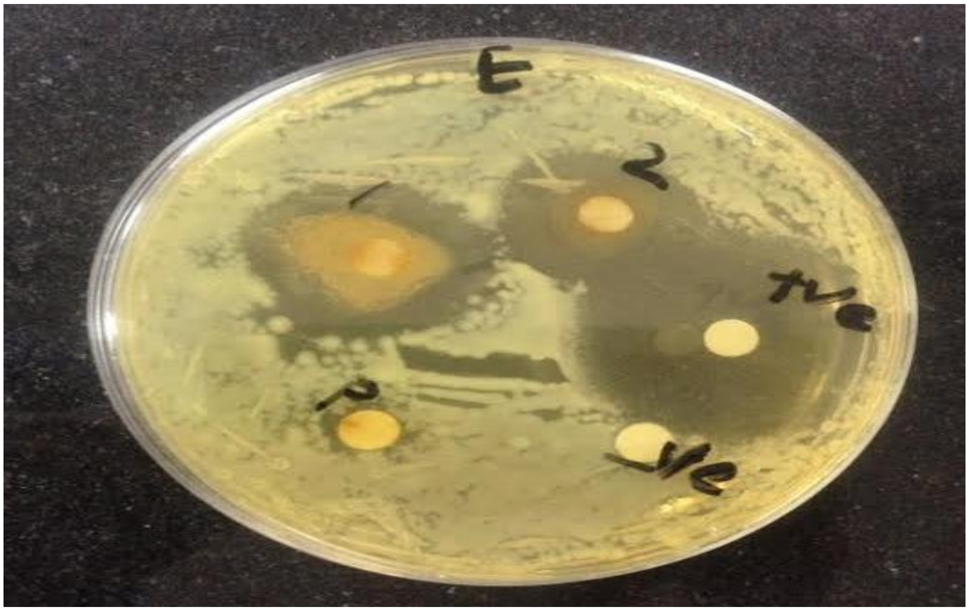

Figure 20: Time dependent effect of *Escherichia Coli*

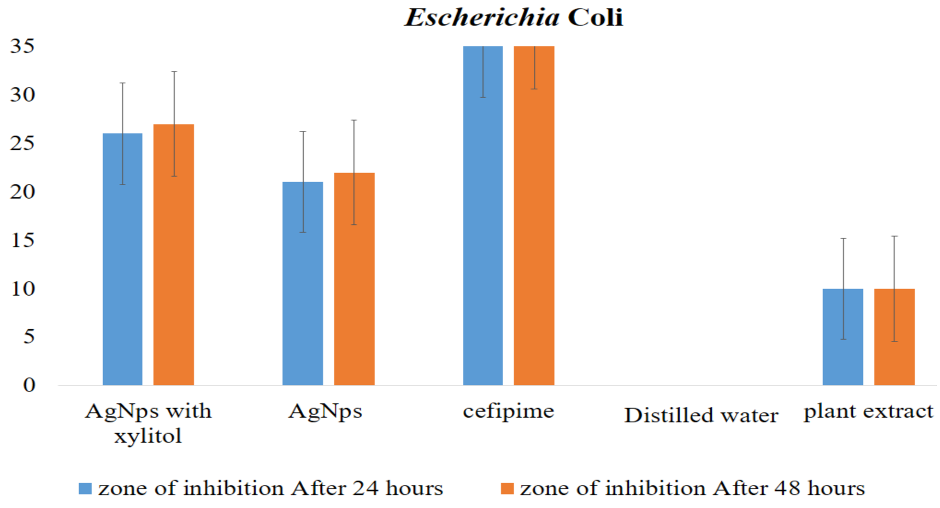

Figure 21: Graphical presentation for time dependent effect of *Escherichia Col*

4.6.3 Difference between effect of AgNPs of *R. stricta* along with xylitol on *Escherichia* Coli and *Bacillus subtilis*

The synthesized silver nanoparticles gives better results for *Escherichia* Coli than *Bacillus subtilis* that is shown in figure 22 and figure 23.

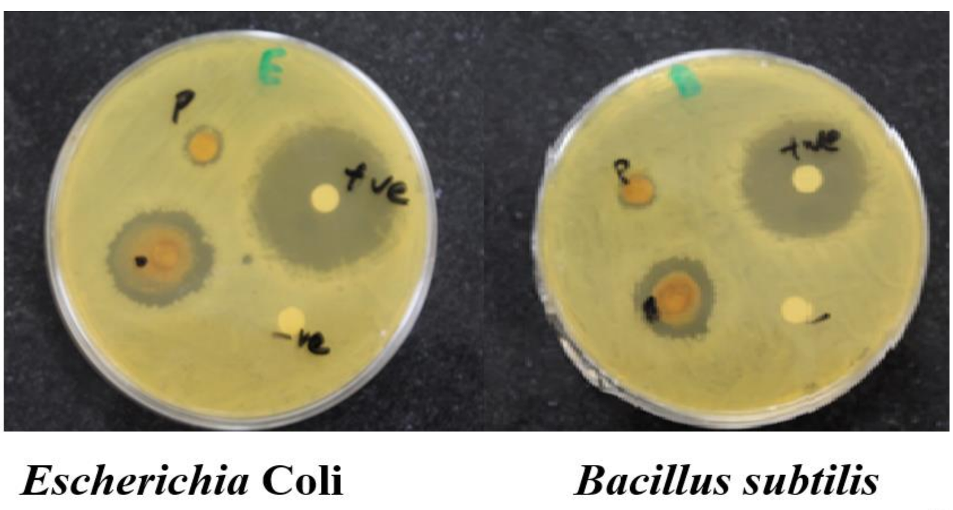

Figure 22: Difference between effect of silver nanoparticles of R. stricta along with xylitol on *Escherichia Coli* and *Bacillus subtilis*

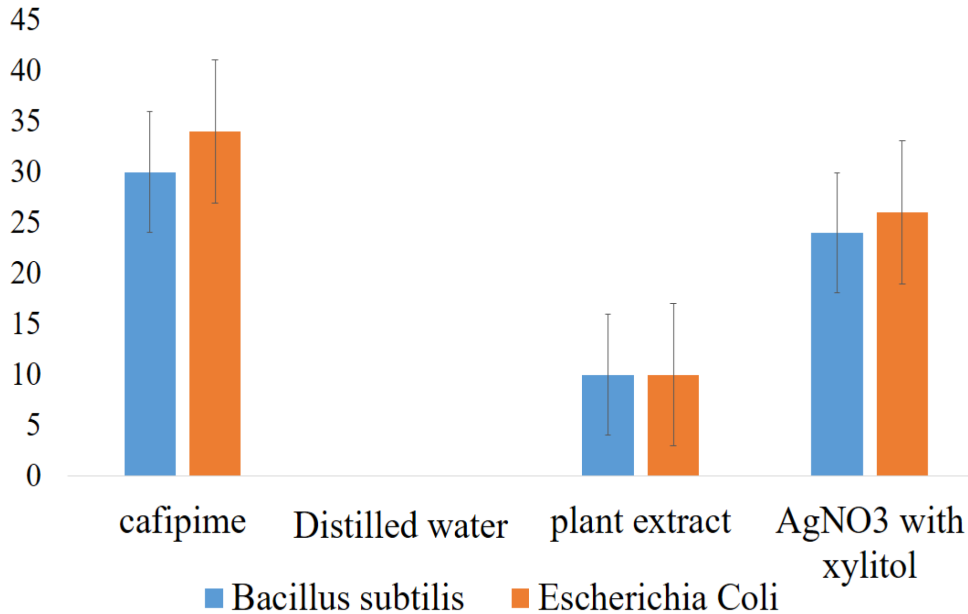

Figure 23: Graphical representation of difference between effect of silver nanoparticles of *R. stricta* along with xylitol on *Escherichia Coli* and *Bacillus subtilis*

CHAPTER 5: DISCUSSION

Green synthesis of AgNPs decreases the problem of chemical agents that causes various adverse effects, therefore making NPs more compatible with the environment friendly approach. *R. stricta* plant roots possess antibacterial activity that is considerably increased after synthesis of NPs with it. The synthesized NPs are characterized with techniques including UV-Vis spectroscopy, X-Ray Diffraction (XRD), Scanning electron microscopy (SEM), Fourier Transform Infra-Red Spectroscopy (FTIR) and Energy Dispersive X- Ray Spectroscopy (EDX).

AgNPs using *R. stricta* plant root extract gives better result at 550µl concentration of plant extract in 10ml silver nitrate solution. When concentration is further increases, larger nanoparticles are synthesized. Broader peak of UV-Vis spectroscopy at 650 µl concentration of plant indicated the large size of NPs. Further increase in plant extract concentration gives noisy peak along with broadening of peak at UV-Vis spectrum.

There is comparable difference of nanoparticle dispersion between the nanoparticle synthesized with and without xylitol addition. Xylitol prevent the agglomeration of nanoparticles and result in enhanced uniform distribution and small size of particles, that increases the antibacterial efficacy of silver nanoparticles.

NPs synthesis with 550µl concentration of plant extract gives largest zone of inhibition because when further concentration is increased (650µl concentration of plant), size of the synthesized NPs also increased that result in declines of the antibacterial activity of NPs. It indicated that smaller size nanoparticles gives better antibacterial activity against reference bacterial strains that are *Escherichia Coli* and *Bacillus subtilis.*

Time dependent study of antibacterial activity for the synthesized AgNPs measured after 24 and 48 hours. The zone of inhibition measured after 24 hours increases about 1mm after 48 hours.

The synthesized silver nanoparticles give better results for gram negative bacteria (*Escherichia Coli)* than gram positive bacteria (*Bacillus subtilis)*.

CHAPTER 6: CONCLUSION

AgNPs synthesised using *R. stricta* root extract along with xylitol possess good antimicrobial activity because they are monodispersed, stable and have less aggregation. Plant act as a reducing agent to synthesized the silver nanoparticles and xylitol enhanced the dispersion of nanoparticles. Smaller size NPs gives better antibacterial activity. Antibacterial activity of *R. stricta* plant root increases with AgNPs synthesis. The synthesized AgNPs increases the therapeutic efficacy and strengthen the medicinal values of plant, *R. stricta* and provide a potent source of antimicrobial agent against *Escherichia Coli* and *Bacillus subtilis*.

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