INTRODUCTION
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1. Introduction:
In recent time use of nanoparticles has gained real pace as they discovered to be useful in a number of applications. Nanoparticles remains novel in properties based on their formed shape, size and morphology by which they can easily interact with plants, animals and microbes for number of instances (Husen and Siddiqi, 2014; Siddiqi, 2016; Siddiqi and Husen, 2017). In today’s research attempts, use of silver nanoparticles finds a special spot since it remains specialized in an antibacterial activity and able to control wide range of microorganism when tested for occasions (Wei et al., 2015; Lara et al., 2011;). AgNPs being an eco-friendly molecule receives its special value in drugs, electronics, catalysis and antimicrobial activity (Lara, 2011; Lokina, 2014). In a general aspect of biology-based AgNPs synthesis use of plant, bacteria, fungi, yeast, actinomycetes is very common (Lokina, 2014; Saifuddin et al., 2009; Shahverdi et al., 2007).

In many of plant research use of flower, leaves and fruits are recommended (Husen and Siddiqi, 2014). In addition to that enzymes are been reported to be useful in the synthesis of silver and gold nanoparticles. Here it is also been noted that features of nanoparticles such as size, morphology and stability remain dependent on nature of the solvent, concentration, temperature and strength of reducing agent (Siddiqi and Husen, 2016).

In today’s scenario use of AgNPs is keeping the special place as they are well characterized for their antimicrobial features. Still number of detailed researches been done on AgNPs but they are yet have remained unexploited (Ahamed et al., 2010; Chen and Schluesener, 2008).

With the earlier discoveries of antibiotics, human for sure was able to control the pathogen growth and reduces the sure mortality with the use of these agents. Now, this controlling mechanism certainly becoming weaker since microbes are developing antibiotic resistance. To tackle this situation, attempts are been made to develop new drugs by using the nanoparticles made up of metals and also the salts of the metals which have been found to inhibit the growth of many bacterial species and other species. In concern now, AgNPs receives special importance as they are inherent in antimicrobial features (Jones et al., 2004; Silver and Phung, 1996). Since silver salts
are already in practice to control bacterial growth which was useful in human treatment especially for catheters, cuts, burns and wounds (Catauro et al., 204; Crabtree et al., 2003).

1.1 Synthesis of silver nanoparticles:
Generally, the nanoparticles of metals are mainly formed by two approaches a) Top down and b) Bottom-up. Here in Bottom-up, the molecule has been built up systematically atom to atom, molecule to molecule and cluster to cluster. In the case of "Top-down" approach, big size molecule will be sliced down to obtain the particle of nano size (Husen and Siddiqi, 2014). In many cases the most preferred approach is “Bottom up” in which the use of catalysts such as reducing agent and enzymes found to be more common. However, the “Top down” approach starting from bulk form, changes by the reduction of size to the nano scale which can be achieved by involving the specialized ablations, cutting, etching and sputtering. Here top-down approach suffers from surface structure defects. These defects affect the properties of the metallic nanoparticles such as the physical properties and surface chemistry. Now in requirement, number of AgNPs are successfully synthesized by involving chemical methods (Zhang et al., 2011; Roldán et al., 2013; Sotiriou and Pratsinis, 2010; Sotiriou et al., 2011); biological methods and physical methods (Abou El-Nour ,2010; Tien,2008; Asanithi ,2012). In the case of chemical-based synthesis of nanoparticles, it involves chemical reduction, irradiation, electrochemical, and pyrolysis methods (Zhang et al., 2007). It is noted that AgNPs synthesis in solution demands metal precursors, stability agent, the reducing agent, or capping agent. In general practices, the use of alcohol, hydrazine compounds, borohydride, ascorbic acid and sodium citrate has been reported. In contrast, the demand of the physical method is highly reactive chemicals that are less lethal and completes reaction in quick time. Here they involve direct current magnetron sputtering (Asanithi et al., 2012), physical vapour condensation, arc-discharge (Tien, 2008). Here physical method puts up another advantage over chemical that physical based AgNPs have narrow size distribution (Asanithi et al., 2012) but it suffers from disadvantage i.e., it requires high consumption of energy (Asanithi et al., 2012).
By all these points, it could be put forward that use of biological synthesis of AgNPs using plant extract and/or involving microbial preparation found to be better alternative approach and also it promotes the number of advantages over chemical and physical preparation. Keeping in mind the potential of biological synthesis of AgNPs such as its simplicity, cost effective features and eco-friendly nature; the AgNPs have been successfully utilised to increase the higher productivity. In many reports, use of Biosynthetic approach in metal and metal oxide nanoparticles using fungi, bacteria, plant and algal extract, yeast promisingly finds application in the field of nanotechnology (Siddiqi and Husen, 2017).

1.1.1 Plants and Silver nanoparticles:
Plant represent its many biomolecules in the form of proteins, fats, carbohydrates, pigments, nucleic acids, and of course the secondary metabolites which play an important role to reduce metal salts in generation of nanoparticles. This process also guarantees less or no toxic compounds formation. Many plants are already been reported to produce silver nanoparticles and/or other nanoparticles as listed below.
Aloe vera plant extract successfully produced AgNPs in the presence of ammonical silver nitrate which was confirmed by involving Fourier transform infrared spectroscopy, UV-Visible spectroscopy, transmission electron microscopy, Scanning electron microscopy and X-ray powder diffraction. They reported the formation of AgNPs with octahedron shape having 5-50nm size. They also found these AgNPs as an antibacterial agent (Logaranjan et al., 2016). Aloe vera once again reported being a reducing agent for better AgNPs formation with the ability to control S. epidermidis and P. aeruginosa (Tippayawat et al., 2016). Mango seed extract has also been used for the synthesis of AgNPs and found to be successfully interacting with bovine serum albumin (Sreekanth et al., 2015). The researcher reported the concept of green synthesis of AgNPs from Erigeron bonariensis aqueous extract and reported them as both stabilising and reducing agent. Formation of spherical AgNPs with the size of 13nm was successfully confirmed via SEM and TEM analysis (Kumar et al., 2015). Use of Nutmeg (Myristica fragrans) has been put forward to produce AgNPs which found applications in drug and pharmaceutical industry (Jelin et al., 2015). Silver nanoparticles of Momordica charantia leaf extractable to showcase surface plasmon resonance peak at 424nm
having spherical nanoparticles formation. For the synthesis of AgNPs the excellent catalytic activity has been confirmed by the reduction of methylene blue (Ajitha et al., 2015). Ability to synthesise AgNPs by using the green approach using the extract of *Carambola* fruit, at room temperature has been reported. It has been confirmed by the high negative zeta potential value summarizes dispersion stability of AgNPs. In a synthesis from fruit extract presence of polyols, aldehydes, amines and organic acid has been confirmed (Chowdhury et al., 2015). *Andean blackberry* fruit extract reported being promising to produce nanoparticles by being the successful reducing and capping agent. The formed AgNPs found to be of spherical shape, crystalline, with 12-50 nm size. These AgNPs found to be a good antioxidant and shares the application in biomedical applications (Kumar et al., 2017). By using the white/visible solar and blue light emitting diode on *Capuli* (*Prunus serotina Ehrh-Var.Capuli*) cherry extract successful AgNPs was synthesized (Kumar et al., 2016). Silver nanoparticles with Green AgNPs synthesis approach formed with *Piper nigrum* when they are readily formed exposing to 3 minutes of sunlight which was confirmed to be stable to form silver nanoparticles and nano-rods (Mohapatra et al., 2015). It has been evident that plant extract used successfully to synthesize AgNPs as well as gold nanoparticles (AuNPs) and they provide features which act as a stabilizing and capping agent. Resultant they produce AgNPs with better antibacterial and anticancer activity (Amooaghaie et al., 2015). Green nanoparticles synthesized from *Acmella oleracea* able to form efficient molecules with its dopamine sensing properties when studied with flower extract based AgNPs (Raj et al., 2016). In a similar manner, AgNPs successfully prepared from the dried *Piper betel* leaves extract. Here plant extract acted successfully as reducing and stabilizing agent having unique spherical configuration and face centred cubic structure of AgNPs (Kamachandran et al., 2015).

Plant *Chelidonium majus* positive for flavonoids and alkaloids in its aerial part able to produce DL S-253.3nm, spherical, quasi-spherical nanoparticles (Barbinta-Patrascu et al., 2016). Flower of *Calotropis procera* rich in triterpenes, tannins, steroids, alkaloids, and cardiac glycosides able to produce nanoparticles of 35nm having a face centred cubic arrangement (Babu and Prabu, 2011). Plant *Sterculia acuminata* having ascorbic acid, gallic acid, phenolic compounds, methyl gallate, pyrogallol and many polyphenolic
compounds in the fruit part able to produce ~10nm spherical shape nanoparticles (Bogireddy et al., 2016). The bark of *Terminalia cuneata* rich in tannins, saponins, triterpenoids, flavonoids, ellagic acid, gallic acid and phytosterols able to produce 25-50nm of spherical shaped nanoparticles (Edison et al., 2016). Plant *Cirsium japonicum* whole part is rich in saponins, proteins and flavonoids with the ability to form nanoparticles of 4-8nm having a special shape (Khan et al., 2016). Plant *Isatis tinctoria* whole plant able to form nanoparticles of 10-15nm having spherical shape is rich in flavonoids and saponins (Ahmad et al., 2016).

Fruit of *Aegle marmelos* is positive with phytosterols, flavonoids, alkaloids, triterpenoids, and amino acids that able to form 22.5nm spherical, hexagonal, roughly circular nanoparticles which are formed by the presence of given reducing agent (Velmurugan et al., 2016).

Plant *Trachyspermum ammi* seed source contains reducing agents such as fatty acids, proteins, flavonoids and alkaloids with 36nm of cubic nanoparticles forming ability (Chouhan and Meena et al., 2015). Plant *Eucalyptus globulus* leaf part is rich in reducing agents such as alkaloids and flavonoids with the ability to form 1.9-4.3 and 5-25nm nanoparticles forming ability (Ali et al., 2015). Plant *Cydonia oblonga* seeds detected for the presence of terpenoids, flavonoids, proteins and amino acids which were found responsible for the formation of 38nm face centred cubic nanoparticles (Zia et al., 2016). Roots of *Chelidonium majus* able to form 15.42nm of spherical shaped nanoparticles with many bioactive compounds detected to be remained present (Alishah et al., 2016). Leaf of *Aristolochia indica* having ability to form spherical shaped 10-50nm of nanoparticles with many reducing compounds present to perform the task (Shanmugam et al., 2016). Leaf of *Cerasus serrulata* rich in alcohol and phenolic compounds and proteins able to form 10-50nm spherical nanoparticles (Karthik et al., 2016).

**1.1.2 Nanoparticles and Plants:**

Nanoparticles are successfully synthesized from the parts of the plants such as roots, stem, barks, leaves, shoots, flowers, seeds and their metabolites (Husen and Siddiqi, 2014; Husen, 2017). By using Spectrophotometer preliminary confirmation of silver nanoparticles reported to be successful. In an example, plant *Pongamia pinnata* able to
given green nanoparticles from its seed extract when absorption maxima recorded at 439nm. Further by well-disseminated nanoparticles with an average size of 16.4nm with Zeta potential of -23.7mV have been recorded. In a recent publication, green synthesis of AgNPs from a plant extract of *Pelargonium endlicherianum* has been recorded. This plant registered the presence of apocynin, gallic acid and quercetin which was reported to reduce silver nanoparticles (Karatoprak et al., 2017). Plant *Sambucus nigra* fruit extractable to synthesise AgNPs using fruit extract which was confirmed by XRD to be crystalline (Moldovan et al., 2016). Plant root of *Thalictrum foliolosum* able to synthesize AgNPs which has been confirmed with the sharp peak at 420nm investigated in UV-Vis spectrophotometer (Hazarika et al., 2016). The ability of Aloe vera to synthesize face centred cubic geometry based AgNPs which was reported to be antimicrobial in nature (Logaranjan et al., 2016). As compared to the common available antibiotic drugs the antibacterial activity of *Piper longum* fruit extracts able to synthesize AgNPs was two to four-fold more. (Reddy et al., 2014). It is put forward that the extract of flower *P. longum* is less effective than their AgNPs to control pathogenic bacteria. Similarly, AgNPs produced by the extract from leaf of *Ceropegia thwaitesii* confirmed when the sharp peak was recorded at 430nm (Muthukrishnan et al., 2015). The number of plants such as *Solanum tricobaactum, Citrus sinensis, Syzygium cumini, Ocimum tenuiflorum and Centella asiatica* able to form AgNPs in colloidal forms with various sizes for effective action (Brayner, 2008). Plant *Artocarpus heterophyllus* found to be effective in the synthesis of AgNPs using seed powder extract which was confirmed by EDAX, SEM, IR spectroscopy, TEM and SEAE. Further, it is confirmed that they showcase irregular shape and size (Jagtap and Bapat, 2013). It is ascertained in UV-spectra of lingonberry with an absorption peak at 486nm and that of Cranberry it was 520nm having AgNPs. As the two absorption peaks are obtained those cannot be assigned to Nano particles only but also partially to a different reducing agent present in the juices. This has indicated that due to this spectral presence of polydispersed silver nanoparticles must be present (Puisoa et al., 2014).
1.1.3 AgNPs and Antibacterial Activity:

It is confirmed that AgNPs found to succeed in controlling many pathogens effectively such as *Vibrio cholera*, *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, *Syphilis typhus*, and other (Rajakumar, 2017). Since these particles are surely been capable of attaching to the bacterial surface or penetrating the cell membrane depending upon the size of particle. Resultant, the particles were found to be more toxic to the strains of bacteria. It is reported that more antibacterial activity attributed to the lowering of particles size (Agnihothri et al., 2014). In many studies, the possibility of prevailing mechanism of action of growth inhibition of microbes has been reported but the most suited remains by the production of free radical has been supported or confirmed with the appearance of a peak at 363.33 in the electron spin resonance (ESR) spectrum of AgNPs (Kim et al., 2007). Since, the formation of free radical is a very common and reported inhibition of growth of microbes is by attacking the membrane lipids which results in its dissociation and damage. (Mendis et al., 2005).

It is also been noted that equal mass of AgNPs and that of Ag ions able to inhibit *S. aureus* and *E. coli*. Further, in the study, it is related to the liberation of silver cation from AgNPs brings about the antibacterial activity (Priyadarshini et al., 2013). It is reported that Ag$^+$ ion is much smaller than neutral AgNPs and by which it is easy to interact with electron-rich biomolecules that are mostly present in bacterial cell wall having P or S and N. It is also been confirmed that Ag$^+$ easily get permeated into bacteria through cell wall (Dibrov et al., 2002; Hamouda et al., 2000), which results in disintegration of cell wall that leads to denaturation of protein and death. Hence it is also been reported that the key factors in inhibiting the growth of microbes are the negative charge on the cell membrane of the microorganisms and present Ag$^+$ ion (Kittler et al., 2010; Hamouda et al., 2000). It is also been put forward that ability of nanoparticles to be inhibitory in its action lies with its concentration (Sondi and Salopek-Sondi, 2004). It is reported that pits are formed by nanoparticles in the cell wall of microbes by which it gets accumulated, and results to permeate into the cell of bacteria which leads to the death of bacteria (Sondi and Salopek-Sondi, 2004). It is also been reported that, besides AgNPs and silver compounds, the presence of other inorganic ions brings about antibacterial properties (Puissoua et al., 2014; Hamouda et al., 2000; Furno et al., 2004). It is
understood that AgNPs get oxidised to Ag\(^+\) but cannot be reduced (Hamouda et al., 2000; Sondi and Salopek-Sondi, 2004). Here the outermost electronic configuration \(4d^{10}, 5s^1\) is showcased by silver and also to become Ag\(^-\) anion it obviates to hold an extra electron. In one success story, in the burn therapy, the silver salt of sulphadiazine used successfully by which skin remain protected from infection caused by \textit{Pseudomonas species}. Here silver gets the slow release from the salt by which it becomes more toxic to microorganisms. It is also been learnt that due to sparingly soluble nature of the salt of silver it acts on the external cell structure. Hence the salts of silver and AgNPs reported to be cytotoxic and find a broad range of antimicrobial feature (Kim et al., 2007). It is put forward that the monodispersed nanoparticles remain uniform in size. Here Graphene oxide able to showcase antibacterial activity against \textit{E. coli} (Akhavan and Ghaderi, 2010; Hu et al., 2010) but AgNPs functionalized graphene-based material able to increase antibacterial activity (Xu et al., 2011; Ma et al., 2011). This ability of Graphene oxide related to its ability to disperse in polar solvents like water and by which easy deposition of nanoparticles certainly remains feasible. As per the study, it is also been put forward that AgNPs and Ag-graphene oxide tested for antibacterial features with concentration such as 6.25 and 1000\(\mu\)g/ml remain more effective to control the Gram negative and Gram positive bacteria (Akhavan and Ghaderi, 2010; Ma et al., 2011).

As per the report, AgNPs release silver ions that penetrate into components of bacterial cells mainly to the DNA, peptidoglycan and protein which prevent the organism from getting replicated further (Chaloupka et al., 2010; Morones et al., 2005). Here by oxidation of elemental silver Ag\(^+\) ions are released in the presence of oxidizing agent occurs like this:

\[
\text{Silver nanoparticles} \rightarrow \text{Ag}^+ + e^-
\]

In a bacterial cell wall presence of organic groups like protein and carbonyl are electron donor rather being electron acceptor due to which they fail in the production of Ag\(^+\) from the Ag atoms nonetheless the Ag\(^+\) ions which are generally been produced confirms the presence of oxidising agent (Ma et al., 2011; Le et al., 2010). Hence Ag\(^+\) ions once formed is able to bind to the protein of bacterial cell and successfully inhibits the vital functions of organisms. It is also been put forward in Green AgNPs prepared from seed
extract of *Nelumbo nucifera* that it remains exceedingly lethal to Gram-negative bacteria when ranged in size as 2.76-16.62nm. The activity is related to disallowing the respiration of the cells due to attachment of AgNPs with the surface of the cell membrane. It has been ascertained that Gram-negative bacteria compose its outer layer with a lipopolysaccharide layer and the inner layer with linear polysaccharide chain able to form a 3D network with peptides. Here AgNPs simply get stored by the charge-based attraction of present weak positive charge on the silver nanoparticles and negative charge available with the polysaccharides. Results to the halt of the cell replication of the microbes. It has also been stated that microbial toxicity via interaction with nanoparticles generally occurs because of the creation of free radicals, like Reactive Oxygen Species (Soenen et al., 2011; Nel et al., 2009). When ROS generated it brings about rupture of membrane which ultimately affect penetrability. During the course of growing the inhibition occurrence of electrostatic interaction, penetration and adsorption of the nanoparticles into the cell wall of bacteria has been reported. Not only is that the toxicity of nanoparticles shows some relationship with surface modification, composition, type of microorganism and intrinsic properties (Lara et al., 2011; Bolla et al., 2011; Thwala et al., 2011; Allahverdiyev et al., 2011; Guzman et al., 2012). It is confirmed that nanoparticles certainly increase the disrupting cell respiration and peroxidation of the lipid layer successfully (Wan et al., 2011).

It is also been confirmed the synergistic activity of biogenetic Silver Nanoparticles with antibiotics such as ampicillin, chloramphenicol, erythromycin and kanamycin by which it enhances its sensitivity against Gram- negative and Gram- positive bacteria (Devi and Joshi, 2012; Juan et al., 2010). Nitrifying bacteria also remain susceptible to the NPs (You et al., 2011).

**1.2 Plant *Syzygium aromaticum*:**

Plant *Syzygium aromaticum* also was known as clove is having the synonyms *Caryophyllus aromaticus* L. and *Eugenia aromatic (L.) Baill.*

The plant has been classified as the cellular organism, *Eukaryota, Viridiplantae, Streptophyta, Streptophytina, Embryophyta, Tracheophyta, Euphyllyphyta, Spermatophyta, Magnoli phyta, Mesangiospermae, Eudicotyledons, Gunneridae, Pentapetalae, rosids, malvids, Myrtales, Myrtaeae, Myroideae, Syzygieae, Syzygium.*
Clove (*S. aromaticum*) categorized into species that finds its tremendous natural power to be useful in food preservatives and for many medicinal applications. Clove originated from Indonesia but now common in all parts of the world. This plant represents itself as a rich source of eugenol, gallic acid and eugenol acetate and represents itself useful in pharmaceutical, food, cosmetic and agricultural applications.

All over the world spices are an integral part of the cooking. Among them, clove in combination with mint, thyme, oregano and cinnamon able to provide best of spices combination altogether. Not only they are important in the kitchen but also, they are responsible for antioxidant and antimicrobial activities. Here clove, in particular, showcases its importance since it possesses potent antioxidant and antimicrobial activities and that makes this plant an important spice to study in detail (Shan et al., 2005).

*S. aromaticum* is a tree from the *Myrtaceae* family which is medium size tree (8-12m) originated from the native place of Maluku Island in eastern Indonesia. For 100's of years, this plant has kept trade special value and remains economic centre point in the Asiatic region.

In many regions cultivation of clove generally been observed at coastal areas with maximum altitudes of 200m above the sea level. Once the plant grows up to 4 years it starts producing flower buds, which is a commercial part of the tree. The collection of the flower buds is generally during the maturation phase before flowering. For the collection of bud generally, manual plucking is adopted or use of chemically mediated protocol by involving phytohormone which majorly releases ethylene in the vegetal tissue (Filho et al., 2013). Many countries like India, Indonesia, Malaysia, Sri-lanka, Madagascar and Tanzania remain the major producer of the clove (Kamatou et al., 2012). In-country Brazil also clove is cultured in the northeast region, with on an average of 8000 hectares of cultivation which is able to produce 2500 tons per year (Oliveira et al., 2007; Oliveira et al., 2009).

As per studies, clove is rich in many bioactive compounds such as hydroxybenzoic acids, hydroxyphenyl propens, hydroxycinnamic acids and flavonoids. Further presence of Eugenol which is one of the bioactive compounds ranges in the concentration as 9381.70 to 14650.00mg per 100g of fresh plant material (Neveu et al., 2010).
In a set of phenolic acid, gallic acid remains in the concentration of 783.50mg/100g fresh weight. Similarly, the presence of hydrolyzable tannins in a concentration of 2375.8mg/100g which is a derivative of gallic acid has been reported (Shan et al., 2005). In addition to the presence of phenolic acids such as feralic, caffeic ellagic and salicylic acid has been reported. Flavonoids such as Kaempferol, quercetin and its derivatives found to be present in clove in lower concentrations. It is confirmed that clove flower buds are rich in 18% of essential oil. Among the essential oil the percentage of eugenol is 89% followed by 5% to 15% is eugenol acetate and β-carifileno (Jirovetz et al., 2006). It is also been confirmed that oil of clove remains positive for α-humulene with concentration up to 2.1% some other volatile moieties recorded in low percentages as part of essential oil are, limonene, farnesol, ethyl hexanoate, benzaldehyde, 2-heptanone and β-pinene.

1.2.1 Features of clove:
Clove is traditionally used in medicinal and spices and now finds a special place in pharmaceuticals. It represents many biological activities as represented below:

1.2.2 Antimicrobial activity:
Better growth controlling the activity of the clove has been demonstrated against a number of fungal and bacterial strains. It is when tested in a comparative model with number of Indian spices such as mint, cinnamon, ginger, mustard and garlic it has been observed that only clove at 3% concentration able to control all the foodborne pathogens such as S. aureus E. coli and B. cereus and it showcase growth inhibitory activity at 1% of clove extract (Sofia et al., 2007). In another study, the antibacterial activity of black pepper, clove, nutmeg, geranium, thyme and oregano tested with 25 strains of Gram- negative and Gram- positive bacteria and it has been deciphered that oils of thyme, clove and oregano able to inhibit bacterial growth strongly (Dorman et al., 2000).

It is put forward that clove, oregano, bay, and thyme based essential oil proving to be inhibitory to the E. coli O157: H7 (Burt et al., 2003). It is also been confirmed that when clove based carvacrol and eugenol encapsulated in a non-ionic surfactant it has been confirmed that this approachable to inhibit the bacterial growth due to eugenol those have remained on the surface in contact with food (Pérez-Conesa et al., 2006).
Ability to inhibit fungal strain also been reported to be positive with sensitive species recorded as *Microsporum gypseum, NCIM/100, Mucor sp., Aspergillus sp., Fusarium oxysporum, Trichophyton rubrum* and *Fusarium monoliforme* (Rana et al., 2011). Based on the chromatographic technique antifungal activity linked with eugenol as it can lyse spore and micelles successfully. It is also been linked with membrane disruption and deformation of macromolecules (Devi et al., 2010). Clove oil found to be effective in controlling dermatophytes such as *Trichophyton rubrum (KCCM 60443), Microsporum gypseum, Trichophyton mentagrophytes (KCTC 6077)*, and *Epidermophyton floccosum* when concentration set at 0.2mg/ml with the inhibition up to 60% (Park et al., 2007). Ability of pure oil or its in mixture with rosemary (*Rosmarinus officinalis spp.*) oil able to control *Staphylococcus aureus, E. coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Bacillus subtilis* and *Proteus vulgaris* with minimum inhibitory concentration between 0.062% and 0.500% (V/V) and recognised as antibacterial agent and a promising candidate for food preservation (Fu et al., 2007).

In the present investigation, the ability of clove (*Syzygium aromaticum*) which is an important medicinal herb as well as it remains the important part in species were tested further to control bacterial pathogens capable of human diseases.

To carry out such a task, clove flower buds were processed for the production of bud extract and it was used further to prepare silver nanoparticles. These nanoparticles were then checked for antibacterial capability by well diffusion assay.

Lastly, structural features of nanoparticles were determined by using scanning electron microscopy and details were reported for the promising AgNPs set at variable physiochemical parameters.

### 1.3 Base literature of the study

Nanotechnology is a science which deals with the synthesis of nanoparticles which remains in control shape size, and dispersity of materials which are measured at nanometre scale length (Kalishwaralal, 2008; Paulkumar, 2014). Since Nanoparticles possess a high volume to area ratio they are recognized as active molecules at low concentration. Nanoparticles produced from silver, gold, cadmium sulphide, zinc oxide, zinc sulphide are the major candidates for the study (Malarkodi, 2014; Vanaja, 2014; Rajeshkumar, 2014; Paulkumar, 2013). In recent time, newly designed silver
nanoparticles from different origin has gain special attention to the fields of biomedicine, anticancer activity (Valodkar, 2011), anti-HIV candidate (Elechiguerra, 2005), bacterial pathogen sensing (Zhao, 2004), and even catalytic activity (Nagy and Mestl, 1999).

In the field of antimicrobial, silver nanoparticles gaining special value since they are found to be controlling the number of human and animal disease-causing bacteria and fungus (Paulkumar, 2013). Number of pathogenic microbes showcased sensitivity to the silver nanoparticles and those are Klebsiella pneumoniae, Bacillus sp., Pseudomonas species, Bacillus subtilis (Vanaja, 2013; Vanaja and Annadurai, 2013), P. aeruginosa, Proteus vulgaris (Prabhu, 2010), Escherichia coli, Fusarium oxysporum, Staphylococcus aureus and Citrobacter species (Musarrat, 2010).

Both the Prokaryote and Eukaryote species are capable of synthesizing silver nanoparticles such as in bacteria both Gram negative and Gram positive like Klebsiella pneumonia, Bacillus subtilis, (Shahverdi, 2007; Paulkumar, 2013), Cladosporium cladosporioides (Balaji, 2009), Marine algae Turbinaria conoides and Padina tetrastrumatica (Rajeshkumar, 2012), the green waste peels of banana fruits (Bankar, 2010), pure carbohydrates like glucose, fructose, maltose and sucrose (Manno, 2008; Filippo, 2010; Panigrahi, 2005).

In a green nanomaterial synthesis number of plants are already been reported to be useful such as Azadirachta indica (Shankar, 2004), Nelumbo nucifera (Karthiga, 2012), Garcinia mangostana (Santhosh Kumar, 2011), Cinnamomum camphora (Huang, 2007), and grapefruit extract (Gnanajobitha, 2013).

Plant possess a number of phytochemicals and that remained varied in parts and hence finds application in various fields. These biochemicals can also play a vital role in nanoparticles synthesis (Santhosh Kumar, 2011). Plants are majorly rich in biochemical like protein/enzymes and secondary metabolites like flavonoids or terpenoids (Shankar, 2003), majority of them are water soluble such as alkaloids, polysaccharose, and flavonoids (Lin, 2010), it is also rich in quinones and organic acid or metabolic fluxes and oxido-reductively labile metabolites such as ascorbates or catechol/photocatachenic acid (Jha, 2009), and remain rich in is over bascoside, luteolin, verbascoside and chrysoeriol-7-o-diglucuronine (Cruz, 2010).
Plant *Syzygium aromaticum* (Taxonomy ID 219868) also known by its synonyms *Eugenia aromatica* (L.) *Bill*, *Caryophyllus aromaticus* L.; *Eugenia caryophyllata* as per NCBI taxonomy database. *S. aromaticum* also was known as clove, is a medium-size tree (8-12m) belonging to *Myrtaceae* family originated from Maluku islands in eastern Indonesia.

Clove is rich in phenolic compounds such as flavonoids, hydroxybenzoic acid, hydroxycinnamic acids and hydroxyphenyl propens and found to be prominent in Eugenol which is in the range of 9381 to 14650 mg per 100g of fresh plant material (Neveu). It is also rich in Gallic acid, tannins, Kaempferol, quercetin and others (Shan, 2005).

Based on these many bioactive compounds clove certainly remains positive for inhibition of bacterial and fungal growth. According to Sofia, clove is rich in antimicrobial activity along with plants like mint, cinnamon, mustard and ginger. It showcases best scored inhibitory activity at 3% concentration to control *E. coli* and *Bacillus cereus* in an aqueous medium (Sofia, 2007).

Worker Dorman and Deans (Dorman et al., 2000) also reported the better antibacterial feature of clove against 25 strains of Gram- negative and Gram- positive bacteria.

*E. coli 0157:H7* strain also found to be sensitive to the clove, oregano, bay and thyme essential oil (Burt et al., 2003). In a similar fashion, purified clove-based eugenol and carvacrol was determined to be effective in controlling foodborne pathogen *E. coli 0157:H7* and *Listeria monocytogenes* (Pérez-Conesa et al., 2006).

Clove oil also found to be effective against dermatophytes as *Microsporum canis*, *Trichophyton rubram*, *Epidermophyton flocossum* and *Microsporum gypseum* at 0.2mg/ml concentration with up to 60% effectiveness (Park et al., 2007).

With this overview in the present examination, an endeavor has been made to synthesize green silver nanoparticles by *S. aromaticum* under the influence of glucose and variable physiochemical parameters to study its effect on the concurrent antimicrobial activity against multiple drug-resistant bacteria isolated from different medical conditions.

According to Poulose P., (2014) Metal nanoparticles represents unique features such as optical, electronic and catalytic properties. Their chemical and physical processes are
very much defined and controlled. The biosynthesis of nanoparticles is often scored better to showcase their application when synthesized from plants, bacteria and fungi. The worker also reported the potent application of metal nanoparticles in antibacterial and antifungal activity.

Basavegowda and Lee, (2014) reported the synthesis of silver and gold nanoparticles by medically supreme leaf extract of Perilla frutescens which has acted as a reducing agent. These nanoparticles are firstly confirmed for their formation by involving techniques like X-ray diffraction, transmission electron microscopy, Fourier transform infrared spectroscopy and UV-Visible spectroscopy. According to surface plasmon resonance spectra, Silver and Gold nanoparticles ranges between 50nm and 40nm, respectively. These nanoparticles are proposed to be favourable in biotechnological and biomedical utilizations.

Dubey et al., (2010) reported the use of Sorbus aucupari aqueous plant extract in the synthesis of silver and gold nanoparticles from their salt solutions. The nanoparticles detailed being triangular, hexagonal and round in shape with an average size of 18 and 16 nm for gold and silver NPs, respectively. A number of techniques are used to define its shape, structure and functional groups and reported their prominent features.

Since the number of bacterial pathogens is transforming themselves into resistance strains the greater challenge has been set in front of us for their future control.

Worker Lateef et al., (2018) reported the use of Petiveria alliacea (PA) in silver nanoparticles (NPs) synthesis and these PA-NPs were recognized effective in controlling Klebsiella pneumonia, E. coli, and Staphylococcus aureus with 100% inhibition. It is also effective as an antifungal agent which can control Aspergillus fumigatus and Aspergillus flavus with 100-150µg/ml concentration.

Worker Amina and Kauskar, (2018) reported the success of silver mesoporous silica nanoparticles having properties to inactivate E. coli, P. aeruginosa, S. aureus upon exposure to red light (620±20nm wavelength) in which instead of plant source light energy was used to increase the action of nanoparticles.

Worker Fernandes RA (2018) reported the use of Pomegranate peel extract to synthesize silver nanoparticles which are able to inhibit the growth of C. albicans and S.
aureus when used in spray formulation system. Further, the stable AgNPs remain low in cytotoxicity, assured its use in the biomedical field.

Mohammed AE, (2018) recorded the synthesis of AgNPs from the concentrate of Ferula asafoetida, Phoenix dactylifera and Acacia nilotica with an average range of NPs size as 67.8 to 155 nm in diameter. These biogenic silver nanoparticles are significant in antibacterial activity with 10 to 32 mm diameter of the inhibition against S. aureus, P. aeruginosa and E. coli.

Synthesis of nanoparticles by involving plant extract certainly has revolutionised the nanotechnology sector. According to Hussain et al., (2018) leaves of Moringa oleifera found to be effective in synthesizing AgNPs having 413-420nm size. These AgNPs is able to control plant pathogens to spread brown spot disease and it also been involved in the production of endogenous enzymes and secondary metabolite antioxidants in plant C. reticulata.

Worker Escarcega-Gonzalez et al., (2018) reported the use of Acacia rigidula in AgNPs synthesis by conforming its synthesis through transmission electron microscopy, electron dispersive spectroscopy, Fourier transform and Ultraviolet-visible spectroscopy infrared spectroscopy. The result of bactericidal activity reported inhibition of clinical of P. Aeruginosa which are multidrug resistant and Bacillus subtilis and hence AgNPs recommended for therapeutic applications in animal models for safety studies.

Worker de Barros, (2018) reported the formation of silver nanoparticles with the use of orange peel (Citrus sinensis) which was recorded to be antibacterial against Xanthomonas axonopodis pv citri (Xac) which is responsible for citric canker in oranges.

Worker Prabukumar et al., (2018) reported that AgNPs synthesis by plant Crescentia cujete L. was greatly affected by the pH, time, temperature and stoichiometric proportion which is in result affects shape, size, dispersity and synthesis rate. The resultant AgNPs profoundly showcased antibacterial activity against human pathogens such as Bacillus subtilis, S. epidermidis, Rhodococcus rhodochrous, Shigella flexneri and Salmonella typhi.

Worker Almertaha et al., (2018) reported AgNPs synthesis by Mentha pulegium leaves to extract at optimum pH 8.6 and confirmed by TEM, UV-Vis spectroscopy, X-ray spectroscopy and other methods.
According to Kumar et al., (2014) synthesis of AgNPs by Alternanthera dentata leaf extract was occurred at room temperature by involving aqueous extract of plant and found to be antibacterial in nature against K. pneumoniae, P. aeruginosa, E. coli and E. faecalis.

Synthesis of Ziziphora tenuior extract based AgNPs was reported at room temperature by the Sadeghi et al., with nanoparticles cubic structure formed having 38nm size (Sadeghi and Gholamoseinpoor, 2015).

According to Mageswari et al., (2015) reported the formation of AgNPs from psychrotolerant bacteria at 12°C without aggregate formation.

Worker Sun et al., (2014) reported the AgNPs formation from the extract of Fagopyri dibotyris rhizoma at 25°C with volume ratio of 0.1g x mL (-1) Fagopyri Dibotysis Rhizoma extract and 1mmol x L (-1) AgNPs was 1 to 10 with reaction time of 3.5 hours and reported to be stable after synthesis.

According to Dhuper et al., (2012) green nanotechnology is featuring a number of advantages such as low cost, eco-friendly, possibility of large scale production, and hence found to be better as compared to chemical and physical methods.

According to Kalishwaralal et al., (2010) large scale nanoparticles synthesis is found to be feasible as compared to microbial nanoparticles which require more stringent conditions and possess some biohazards.

As green synthesis provides natural capping agent for stabilization of nanoparticles; plants are always recommended for the better synthesis of nanoparticles and hence are in great demand for its profound research.

Plants are a miraculous agent as it possesses bio-molecular complex such as enzymes, alkaloids, phenolic, tannins, saponins, vitamins, terpenoids and proteins which are having medicinal values and environmentally benign and complex in nature as suggested by Kulkarni et al., (2014).

Among plants, Acorus calamus found to be effective for the production of silver nanoparticles with better action as antioxidant, anticancer as well as an antibacterial in nature as suggested by Nakkala et al., (2014a).

Nakkala et al., (2014b) reported Boerhavia diffusa plant extractable to form Nanoparticles of 25nm size when confirmed by XRD and TEM analysis and able to
control *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Flavobacterium branchiophilum*.

Suna et al., (2014) related the high level of steroids, carbohydrates, Sapogenins and flavonoids act as reducing agents and as capping agents to synthesize silver nanoparticles.

Nabikhan et al., (2010) synthesized 5 to 20nm size silver nanoparticles as evidenced by TEM with a change in colour of extracts to yellowish brown moderately.

Gopinatha et al., (2012) synthesized silver nanoparticles by using a fruit part in the dried form of the plant, *Tribulus terrestris* with 16-28nm size. They exhibited antibacterial activity by using Kirby Bauer method against multidrug resistant *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*.

Mariselvam R. et al., (2014) synthesized antimicrobial silver nanoparticles from tree *Cocos nucifera* using ethyl acetate and methanol as a solvent in the ratio 40:60. It showcased antibacterial activity against *Salmonella paratyphi; Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Ashok Kumar et al., (2015) studied on an extract of *Abutilon indicum* and synthesized silver nanoparticles and those found to be effective against *E. coli, S. typhi, B. Subtilis* and *S. aureus*.

Sadeghi et al., (2015) reported the success of *Ziziphoratenuior* leaves for silver nanoparticles synthesis with a spherical and uniform distribution of it with 8 to 40 nm size and better characterized with FTIR analysis.

Ulug et al., (2015) able to synthesize silver nanoparticles from an aqueous mixture of *Ficus carica* leaf extract by involving irradiation method.

Geetha et al., (2014) able to synthesize the silver nanoparticles from *Cymbopogon citratus* (DC) *Stapf* (lemongrass) in an aqueous solution of 5mM AgNO₃ and those remain inhibitory to the growth of *P. mirabilis, P. aeruginosa, Shigella flexneri, E. coli, S. sonnei* and *K. pneumonia*.

Krishnaraj et al., (2010) reported the formation of AgNPs from leaf extract of *Acalypha indica* in a rapid synthesis protocol as AgNPs observed within 30 minutes.
Veeraputhiran et al., (2013) involved orange peel (Citrus sinensis) to produce nanoparticles of silver with by varying concentration of AgNO₃ to give nanoparticle ranging from 15 to 50 nm.

Dwivedi et al., (2010) succeeded in reporting rapid biosynthesis of silver nanoparticles by involving obnoxious weed Chenopodium album. The synthesized nanoparticles ranged from 10-30 nanometre. The shape of particle was confirmed by TEM analysis which was found to be spherical.

Ramyal et al., (2012) succeeded in reporting the use of Azadirachta indica leaves aqueous extract in the synthesis of silver nanoparticles with the size of 10-35nm confirmed by TEM analysis. They also reported silver nanoparticles which were spherical in shape with face centred cubic (FCC crystalline structure) synthesized from Hevea brasiliensis with 2nm to 10nm.

Zhang et al., reported a synergistic approach in the formation of silver nanoparticles by Aloe vera and able to control E. coli when studied in standard plate count.

Govindraju et al., (2010) successfully reported the synthesis of silver nanoparticles by Solanus torvum and that were able to control the growth of S. aureus, A. flavus, P. Aeruginosa and A. niger when tested by Disc diffusion method.

Geethalakshmi et al., (2010) synthesized AgNPs by using Trianthema decandra extract which was able to control P. aeruginosa and E. coli by well diffusion method.

Khandelwal et al., (2010) reported the success of AgNPs synthesis by using Argimone mexicana able to control E. coli, P. aeruginosa, A. flavus when tested in Disc diffusion test.

Sadgehi et al., (2015) involved green synthesis approach by using Ziziphora tenuior extract to synthesize silver nanoparticles, at room temperature and those were able to control a number of human bacterial pathogens tested by the disc diffusion method.

Kumarasamyraja et al., (2013) able to synthesize Green silver nanoparticles with the aqueous extract of Acalypha indica and those were found to be controlling, P. mirabilis, P. Aeruginosa, E. coli and others.

According to Klueh et al., (2000) silver nanoparticles antimicrobial activity purely depends upon

a. Capping agent.
b. Size and environmental conditions such as size, pH, ionic strength and

The exact mechanism of antimicrobial or toxicity activities by silver nanoparticles still remained unclear but it has been put forward that Ag\(^+\) charge involved in antimicrobial activities.

According to Cao, (2001); Wright, (1999) and Mathew, (2009) for the antibacterial activity the electrostatic interaction between nanoparticles as positively charged moiety and negatively charged bacterial cells are responsible.

Green Chemistry provides a unique design for the chemical products, which reduces the chances of forming hazardous substances. Since many years natural products mainly the secondary plant metabolites majorly been used to synthesize silver nanoparticles. It is known that plant-based silver nanoparticles provide features which are safer eco-friendly energy-efficient and being less toxic as compared to chemically synthesized counterparts. It is known that secondary metabolites commonly available in plants and mainly plant remain rich in terpenoids which has a significant role in silver nanoparticle synthesis. It has been understood in detail that the involvement of terpenoids certainly making its impact in making silver nanoparticles by giving their capping as well as reducing agents by which stable silver nanoparticles could be formed. In practical form terpenoids are now identified for their functional group as per the FTIR methodology (Mashwani, 2016).

According to Baruah, (2018), plants and their extracts makes an impact on the green synthesis of nanoparticles. The number of metal nanoparticles synthesises successfully. Green technique synthesized gold nanoparticles using aqueous extract of *Alpinia nigra*. This nanoparticle can showcase antibacterial, antifungal and antioxidant activity. As per biochemistry extract found to be rich in flavonoids which has been confirmed by high-performance liquid chromatography. The synthesis of gold nanoparticles by *A. nigra* confirmed by UV-Vis spectrophotometer, FTIR, XRD and TEM.

Further crystalline nature of silver nanoparticles confirmed by powder XRD analysis. The size of the nanoparticles found to be 21-52 nanometres as an average. It has been ascertaining that the presence of polyphenolics acts as a reducing and capping agent.
Research has also been stated that Aloe Vera based nanoparticles confirmed by FTIR, SEM, XRD, and TEM found to be useful in carrying drugs to the target area and reported to be helpful in colon related diseases (Malviya, 2017). It has put forward that metal-based nanoparticles are becoming more popular since it possesses variable biological, optical, physical and magnetic properties. The number of methodologies like physical, chemical and biological is used to synthesize nanoparticles. Among them environmental benign, cost-effective, green technology offered by plants stands vital as plants can act as reducing as well as capping agents for the synthesis of nanoparticles. Plants are rich in alcohols, phenols, terpenes, proteins, saponins and also remain present with microbes those can produce many vital enzymes which can stabilise nanoparticle synthesis (Ovais et al., 2018).

In recent research, nanoparticles also find its application in biomedical studies which has mainly been used in tissue culture and preclinical animal model studies. They are most useful and objects the post-harvest modifications which makes them with the yielding molecules. Those are also useful in cancer cells, and tumour tissue studies once investigated in preclinical mice model (Shukla et al., 2018). Nanoparticles having the dimensions of 1-100 nanometres, showcase number of physicochemical variations once formed by the biogenic reduction which is bottom-up approach and in together plant-based green synthesis of Ag, Au, Pd, Fe, Ru, Cd, CuO, CeO₂, Fe₃O₄, TiO₂ and ZnO NPs has been successfully put forward.

Studies on iron-based nanoparticles which remain affected for their physical, chemical and biological transformation when comes in contact with variable pH, ions, dissolved oxygen and natural organic matter. This nanoparticle finds a special place in environmental studies and found to be less toxic to the biological species. (Lei, 2018). Nanoparticles have also been used to control plant pathogenic fungi when synthesized from oleoyl- chitosan. Particles found to be having size of 2.9 - 6.96 nanometres. These nanoparticles are mainly formed by Nigrospora sphaerica, Nigrospora oryzae, Alternaria tenuissima, and Botryosphaeria dothidea (Xing, 2016). Plants for now providing the number of essential reducing and capping agents which are vital for the synthesis of silver nanoparticles. In a success story leaf extract of Ocimum sanctum and its derivative quercetin which is a flower not present in the tulsi
found to stand vital in the synthesis of silver nanoparticles under variable pH, temperature, reaction concentration and reaction time. Variations in environmental conditions lead to variable size, morphology, shape and stability of resultant silver nanoparticles. The molecules formed could be confirmed by involving methodologies like optical spectroscopy, mainly absorption photoluminescence and Fourier transform infrared. (Jain and Mehta, 2017).

**Mechanism of action of silver nanoparticles**

Use of silver salt is known to human since very long. These are used mainly in the field of agriculture and medicine, especially to control bacterial, fungal growth and to provide its antioxidant activity. It has been reported that silver nanoparticles able to arrest the growth and multiplication of several bacterial species including *Citrobacter koseri, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and fungus such as *Candida albicans*. Innovation has been brought about by binding Ag/Ag⁺ with the cell surface biomolecules result in silver nanoparticles can produce reactive oxygen species and free radicals which ultimately leads to apoptosis and cell death. Silver nanoparticles are remained smaller in size than bacteria which can diffuse through cell wall by rapturing it, which has been confirmed by SEM and TEM images. This study further investigated that smaller nanoparticles are more toxic as compared to bigger ones. This feature can be used selectively for the particular use in nature (Siddiqi, 2018).

In one study, *Pseudomonas aeruginosa* has been treated with silver nanoparticles to understand its exact mechanism of action. As per proteomic response, 59 silver regulated proteins where 27 remains upregulated and 32 downregulated, and 5 silver binding proteins also identified. As per bioinformatics study silver nanoparticle acted on cell membrane interfered with its activity once they form reactive oxygen species (Yan, 2018).

In a related study, silver nanoparticles found to be acting on moulds where silver nanoparticles found to be accumulating in the cell body and remain associated with nucleotide derivative (Coenzyme A), amino acids (phenyl glycine), peptides and lipids. The interaction leads to shortening and condensation of hyphae, cell plasmolysis,
numerous membranous structure, collapsed cytoplasm and other self-degrading features (Pietrzak, 2016).

Antimicrobial resistance increasing worldwide. In recent time field of green synthesis explored the potential of nanoparticles controls the pathogenic microbes. The number of metal-based nanoparticles produced from copper, silver, gold, zinc extract found to be useful to control Gram-positive and Gram-negative bacteria mainly Bacillus subtilis, E. coli, Staphylococcus aureus and others. They are also found to be sufficient to control fungi suggest pathogenic Aspergillus niger, Fusarium oxysporum etc. The possible mode of action for these nanoparticles recorded to be as ability to produce excessive reactive oxygen species inside the microbial cell, disruption of respiratory chain enzymes by damaging the plasma membrane, ability to accumulate metal ions in microbial membranes, presence of electrostatic attraction between metal nanoparticles and microbial cells brings about the destruction of metabolic activity and increase production of hydrogen peroxide leads to innovation of enzymatic activity (Nisar, 2019).

Ipomoea batatas have been used successfully for the synthesis of silver nanoparticles able to control tested pathogenic bacteria with zone of inhibition ranging from 8 to 11 nm; they found to these nanoparticles better in antioxidant activity also (Das, 2019). Synthesis of silver nanoparticles using Senna alata bark extract confirmed by UV-Visible spectroscopy and other methods and found to be producing average diameter of 10 to 30 nanometres these nanoparticles able to control Gram-positive and Gram-negative bacteria as well as fungi with zone of inhibition 11 to 14 nm silver nanoparticle and MIC found to be 31 to 125 microgram per ml. Here nanoparticles found to be involved in potassium leakage through the membrane, which is significant mode of action against microorganisms. The potassium leakage recorded 0.97 and 3.05 ppm which leads to number of morphological changes in the microorganisms and has nominated as antimicrobial agent (Ontong, 2019).

Monodisperse Fe₃O₄ nanoparticles stabilized by citric acid found to be controlling Gram-positive S. aureus and Gram-negative E. coli which is showcasing its involvement as a disinfectant mainly in medical instruments cleaning (Shatan, 2019). In a fungus 5 nm AgNPs able to induce cell cycle arrest as well as reduces the glucose uptake in fungi 30 minutes treatment in a dose-dependent manner has been given this indicates that
nanoparticles also acts on cell cycle proteins to make the innovation of *C. albicans* which remains ROS independent in its action (Lee, 2019). Use of beetroot for the purified betanin used successfully to synthesise silver-betanin core-shell triangular nanodisks. These nanoparticles registered its antibacterial activity of *E. coli* and *S. aureus* probably by inhibiting membrane activity (Kosa, 2019).

Rifampicin conjugated silver nanoparticles registered its success to control methicillin-resistant *Staphylococcus aureus* since it assists in biofilm inhibition and better penetration of Rifampicin (Farooq, 2019). The combined effect of sulphonamides, silver nitrate and nano silvers found to be potential quorum sensing inhibitors when investigated in *Bacillus subtilis*. Action for the inhibition found to be safe to the environment and human health which promotes its utility in the medical field (Yu, 2019).

Many biologically synthesized nanoparticles revealed with ability of downregulation of antioxidant machinery and oxidative enzymes, loss of virulence, disruption of osmotic balance and cellular integrity. This feature increases the chances of using nanoparticles as a better antibacterial and antifungal agent (Kumari, 2019).

Silver nanoparticles able to alter the zeta potential towards bacterial membrane proteins which ultimately leads to change in membrane permeability by which penetration of linezolid into the cell becomes possible. Nanoparticle further binds to the bacterial membrane, thereby reduces the biological activity of the membrane ones remain bound with respiratory chain dehydrogenases. This finding put forward that nanoparticle acts on efflux pump obstruct its operation as well as it alters the membrane integrity at the same time which increases the concentration of linezolid inside the cytoplasm (Anuj, 2019).

Use of nanoparticle also finds this application in purifying drinking water. Here chitosan (CTS)/biochar- nanosilver (C-Ag) antibacterial composite prepared under high-temperature carbonisation process. The composite found to be useful to control *E. coli* when tested on plate count method which proves that use of this composite could be sustainable antibacterial activity and can be reusable for drinking water purposes (Hu, 2019).

Nanoparticles synthesized by *Galega officinalis* extract in presence of silver nitrate as a precursor found to be having variable action as per the reaction parameters set for its
biosynthesis. The best performer nanoparticle recorded to be formed at pH 11, 1.6 mM of AgNO₃ and 15% V/V-1 of G. officinalis. These nanoparticles able to control E. coli, S. aureus and Pseudomonas syringae when the nanoparticles having the 23 nanometres of the size (Manosalva, 2019). Laser-induced graphene doped with silver nanoparticles found to be better antibacterial surface when nanoparticle having the size of 5 to 10 nanometres. The surface used to be toxic to the bacteria censored religious silver ions and hence increases the functionality of laser-induced graphene-coated surface (Gupta, 2019).

Silver nanoparticles find its application antimicrobial and in wound dressing material. They can successfully control pathogens like E. coli, Staphylococcus aureus, Pseudomonas aeruginosa that purely remain dependent on the surface area of silver nanoparticles (Cheon, 2019). Titanium implant-associated infection relieving control by silver nanoparticle loaded TiO₂ nanorods. These nanorods selectively punctured the bacterial membrane by the release of silver ions from the rods and provided antibiofilm activity also. Strategy finds application in reducing clinical infections during implant surgeries (Guan, 2019).

Graphene-based nanomaterial finds special place to tackle antibiotic-resistant pathogens since it remains toxic to the bacteria whose mechanism of action purely remains dependent it on the size and composition of the material (Kumar, 2019). Nanoparticles synthesized by using ultrasound to produce iron oxide nanoparticles in presence of fenugreek seed extract. Ultra-sonication produces smaller size nanoparticles about 20 nm in size as compared to magnetic steering method able to produce 40 nm size nanoparticles (Deshmukh, 2019).

Silver nanoparticles obtained on the variable reducing agents of cellulose found to be having different inhibition potential against associate E. coli and Staphylococcus aureus they are independent on the synthesis conditions set with pH, temperature and reaction (Suwan, 2019). Fruit of Akebia trifoliata var. Australis found to be useful since it contains citric acid extracted pectin, which acts as a reducing agent to produce silver nanoparticles. These silver nanoparticles able to attach to the cell, especially to the sponge thereby act as a surface bacterial disinfectant and finds food value (Yu, 2019).
Silver nanoparticles prepared in the presence of crude ethyl acetate extract of *Ulva lactuca* found to be effective control pathogenic fungi. An extract found to be rich in terpenoids palmitic acid and showcase the minimum inhibitory concentration of silver nanoparticles as 80 micrograms per ml (Sahayaraj, 2019). Medicinal plant-based nanoparticles showcase growth potential in the field of nanomedicine. Since nanoparticles have high surface to volume ratio it remains locally active at low concentration and thereby increases the much required medicinal potential. Silver nanoparticles produced from *Azadirachta indica*, *Syzygium cumini*, able to control prokaryotic as well as eukaryotic cells and able to control *Bacillus subtilis*, *Escherichia coli* along with cancerous (HT1080) and non-cancerous (HEK293) cell lines (Nayak, 2019).

Plant *Ribes Khorassanicum* fruits used in the synthesis of silver nanoparticles which serves as reducing and capping agent also. When silver nitrate used in 1 milli molar concentration the mean diameter of silver nanoparticles. The formation of silver nanoparticle with 1 milli molar silver nitrate in the presence of fruit extract given the nanoparticles of size 20-40 nanometres. These nanoparticles showcased antibacterial effect mainly against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. (Taghavizadeh Yazdi, 2019).

**Silver Nanoparticles and Antibacterial Applications**

Recent advances in the field of nanotechnology and nanoscience made its way in the field of diagnosis treatment and prevention of various diseases occurring in human. Silver nanoparticle stands one of the vital and fascinating nanomaterials which serve in a biomedical application among several metabolic nanoparticles. Silver nanoparticle finds unique space in nanomedicine since it is useful in various purposes; it also showcases define physical-chemical and biological characteristics. Importantly it has now been used in multifunctional bi applications such as antibacterial, antiviral antifungal antiangiogenic, anticancer (Zhang, 2016).

Silver nanoparticles mainly been ranging between 1 to 100 nm. It has been synthesized by chemical and physical methods but having the disadvantage that the process remains expensive and many times toxic in nature. Another alternative biological way has been explored in detail since this method remains environmental friendly less toxic
and those includes plant extracts microorganism and fungi as a reducing agent. Silver nanoparticle finds special place in diagnostic application, antimicrobial activity and therapeutic use also (Mathur, 2018). Silver nanoparticle special place in antimicrobial activity which remains dependent on the release of silver ions and result and cellular damage which is brought about by membrane attachment, DNA damage, free radical generation and others. Till the exact mechanism of action of silver nanoparticle not understood in detail and which remains the area of exploration (Duran, 2016).

Even though nanoparticles mainly silver nanoparticles are majorly studied in our technology industry its safety measure in terms of its toxicology remain a major concern. With the advancement of biotechnological applications of silver nanoparticles, it is essential to study deceptive relationship of the same. Similar to the silver nanoparticles, use of gold silver nanoparticle also finds its application remind coated on cellulose paper and showcase is inhibition towards *Escherichia coli*. The presence of 15 nm silver gold nanoparticle found to be most useful to control is *E. coli* growth and hence find its universal application in food packaging, wound dressing, clothing and other products (Tsai, 2017).

Silver nanoparticles PEGylated when used in hydrogel able to control *S. aureus* and *E. coli* cytotoxic and showcase its application in injectable antibacterial materials (Niu, 2018). Since we are experiencing antibiotic resistance bacteria it is essential to involve alternative medicine which can include in drug and gene delivery, antibacterial activity, tissue engineering and imaging. Nanoparticles are that molecules and among them gold, silver, copper, zinc oxide, and magnesium oxide serve as major molecules (Vimbela, 2017). The silver nanoparticle is now decorated with melamine foams which act as a support for the slow release of silver nanoparticles when used as antibacterial filters able to control *Escherichia coli* and other pathogens present in water (Pinto, 2018).

**Synthesis of Nanoparticles**

Silver nanoparticles synthesised by number of ways such as chemical physical and biological form. In one study silver nanoparticles synthesised in a single step by using a mixture of silver nitrate, starch and distilled water. This is the first method where touch solution found to be useful for the synthesis of silver nanoparticles. This method put
forward that nanoparticles could be form and effortless way with the size of 10 nm which shows good antibacterial activity also (Raghavendra, 2016). Since we know silver nanoparticles gaining worldwide attention especially in medical science and disease treatment use of organic green sources for its synthesis showcase and increase interest. To confirm this synthesis number of methodologies such as UV-Visible spectroscopy, transmission electron microscopy, atomic force microscopy, energy dispersive X-ray spectroscopy and others are readily contributing. Synthesis methodology found to be most preferred since it governs less toxic, eco-friendly, and low-cost methodology (Mousavi, 2018).

Plant extracts are now readily used since it contains proteins, enzymes, amino acids, terpenoids, flavonoids which acts as a reducing as well as a capping agent for the synthesis of silver nanoparticles. Till date, hundreds of plant used to synthesise eco-friendly silver nanoparticles and showcased their potential to be a better antibacterial agent, but very few reported the exact operating conditions involved in the synthesis (Rajesh Kumar and Bharat, 2017).

Silver nanoparticles are not synthesized biological means which finds their typical applications in infertility management, antibacterial effects, burns, cancer treatment, skin damage and others but always remains the concern for its toxicity (Mathur, 2018). Plant endophytes also been involved in the synthesis of silver nanoparticles and recorded for their different mechanism of actions which finds frequent application pharmaceutical activity. However, the exact synthesis procedure still not remain clear (Rahman, 2019).

Now noble metal nanoparticles are being used in electronics, catalysis, optics, environmental and biotechnology applications. In many of these studies, green synthesis showcases its better feasibility and now mainly been used in various fields of industry (Abbasi, 2016). Today’s research focus has been made to synthesise environmental friendly dry table powder silver nanoparticles by involving starch as a reducing and stabilising agent in the vicinity of sodium hydroxide. Sodium hydroxide required as a better reactant along with starch to act as a reducing agent and prepared silver nanoparticles with the methodologies found to form 30 nm sphere-like structure and reported to be highly purified. This methodology can be very easily transformed to
the large scale production and can find easy transportation as per the demand of industries mainly textile and painting industry (Hebeish, 2016).

Synthesis of silver nanoparticles reported in intracellular also and found in *Meyerozyma guilliermondii* KX008616 under aerobic and anaerobic conditions. Particles exhibited the size of 400 metres with spherical shape. They are recorded to form nano aggregates in the cytoplasm or on the cell wall region. This study reported that used yeast biomass could be useful for the industrial fermentation process so that maximum production of silver nanoparticles could be achieved (Alamri, 2018).

The fungus also involved in the synthesis of silver nanoparticles since it remains low cost, simple and energy-efficient process. Fungi have this capacity since they can produce enzymes that acts as both reducing and capping agents to give stable and shape control silver nanoparticles. Fungi origin silver nanoparticles now finding its commonplace as a better anti-HIV, antibacterial, and for catalytic activities but remain suffered from toxicity to be tackled in the next time (Zhao, 2018). It has been said that green synthesis nanoparticle approaches the need for stabilising and capping agent and biological activity remain dependent on shape and size formed during the process. These nanoparticles mainly remain vital for their antibacterial and anticancer activity, and primarily silver nanoparticle and gold nanoparticles are majorly studied (Patil and Kim, 2017).

Different solvent-based plant extracts, for example, methanol and dichloromethane used for the synthesis of nanoparticles by involving plant *Pulicaria gnaphalodes* (Vent.) Boiss aerial parts. This plant developed small and polydisperse nanoparticles with low aggregates and methanol concentrate produced stronger spherical and single nanoparticles when contrasted with dichloromethane methane, capable of producing permeable polyhedral accumulated nanoparticles (Chitsazi et al., 2016).

**Nanoparticles and Plants**

Use of plant and nanoparticles finds variable use right from plant growth and interaction until the use of plants to synthesise silver nanoparticles.

In one study delivery of nanoparticles investigated in vivo to understand the exact interaction of nanoparticles with the plant. Since nanoparticles remain as quantum dots
remains smaller in plant cell wall, with versatile surface chemistry, it is ideal for investigating, its interaction with plant (Wu et al., 2017). As plants based nanoparticles are now by synthesised to use in medical sector mainly for the anticancer and drug carriers but many plant-based Nanoparticles found to be cytotoxic for example gold nanoparticles formed by the Plumbago zeylanica, Cassia auriculata and Commelina nudiflora. In contrast, few metallic nanoparticles derived from plant extract found to be safe with lethal media dose 1-20 μg / ml as prepared from the Abutilon inducum, Butea monosperma, Gossypium hurtum, Melia azedarach and Indonecsiella echioides. These nanoparticles also share their therapeutic index of >2.0 when checked on cancer cells and human cells. In a different report number of metallic nanoparticles found to be cytotoxic and a strict vigilance is required while producing such kind of nanoparticles on a large scale by keeping in mind the environmental impact.

Plants based nanoparticles are finding a new place in the field of medical therapy. Synthesis of nanoparticles purely remained dependent on reaction temperature, iron precursor concentration, concentration of leaf extracts reaction time, and number of statistical factors are understood in detail to stable synthesis nanoparticles so that improved production of it could be achieved (Ebrahiminezhad et al., 2018).

Plant-based nanoparticles mainly investigated in number of literature for their rate of synthesis, their toxicities interactions of NPs and plants, and phytoremediation processes (Arruda et al., 2015).

Gold nanoparticles synthesis from plants remains dependent on metabolites such as flavonoids, alkaloids, terpenoids, Phenols, sugars, alcohol and sugar. It has further mentioned that plant with aroma or colour is their leaves, flowers or roots are the main contributors for the synthesis of nanoparticles since they are rich in chemicals act as a reducing agent to produce metal nanoparticles. Besides, its interaction with metal ion will give variable shape, size and activity based on the incubation time, temperature, concentration and pH of the solution (Siddiqi and Husen, 2017). In today’s era of genetic engineering plant-based nanoparticles used in number of application in addition nanoparticles are also used to transfer the biomolecules delivery directly into the cells since they quickly travel the plant cell walls without external force and hence finding
new possibility of genetic transformation of plant using nanotechnology (Cunningham et al., 2018).

Not only nanoparticles are useful in the number of ways but sometimes proves cytotoxic especially to the plants. Cerium oxide nanoparticles (CeO$_2$ NPs) proves to be contaminant and toxic to the plants. Once in contact with 10, 50, and 100 mg/ kg CeO$_2$ NPs, accelerated biomass recorded in radish plant, root expression recorded at least 2.2 times, further chlorophyll content also increased by 12.2 % as compared to control (Gui et al., 2017).

**Nanoparticles Drug**

Nanoparticles are not only act as a drug itself, but it also been used to carry the drug to the targeted area since they have direct access to the plasma membrane. Nanoparticles encapsulated with drugs found to be increasing the bioavailability of drug which has been used to improve the concentration of drug close to the cancer cells and also been useful in understanding cell differentiation studies (Suk and Gopinath, 2017).

Use of albumin for the synthesis of nanoparticles which can be used for bioimaging and drug delivery has showcased promised. These nanoparticles could be classified as nanocarriers, template, Scaffold, stabilizer and albumin- polymer conjugate. (An and Zhang, 2017).

Since tumour microenvironment plays an important role in tumorigenesis; tumour invasion and metastasis, here it is proposed that nanoparticles can modulate microenvironment for better drug delivery and tumour therapy produced from plant and other sources (Young and Gao, 2017).

Lipid-Polymer-hybrid nanoparticles considered as next-generation molecule for better drug delivery especially in oncology. Its outer surface could be designed in such a way that it can take part in active targeting of anticancer therapy as well as it can be involved in diagnostic imaging (Mukherjee et al., 2019). It is essential to administer the drug through mucosal surface which has been assisted by the presence of nanoparticles for better drug delivery. After mucosal administration component interact with nanoparticles that form the biomolecules corona that modulates their resultant behaviour assist in nanoparticles insertion (Gracia- Diaz et al., 2018).
About Antibiotics
Antibiotics are considered as a wonder drug in the era of 20th century and that finds the way to control deadly bacterial infections. These wonder drugs helped to reduce mortality and morbidity in that period and spread one success series for the infection control (Kapil, 2005; Tiwari et al., 2013). However, as the treatment started continuing for decade, bacteria able to survive antibiotics and that again started to increase morbidity, mortality and economic loss on drugs (Bebell and Muiru, 2014; Chang et al., 2015). As early as in 1910 discovery of Arsenic based drug ‘Salvarsan’ able to treat syphilis then in 1935 Prontosil -a sulpha drug and in 1940 development of penicillin set up the real pace for antibiotics discovery. Thereafter period from 1950 to 1970s reported as golden era of discovery to novel antibiotics (Aminov, 2010). It is also been possible to transmit antibiotic resistant bacteria from one country to another as frequent travelling of patient become feasible (Richet et al., 2001). One of the reasons for the development of the drug resistance and also increased antibiotic resistance is the improper use of the antibiotics by patients. In additions, antibiotics are majorly administered in animal industry and also contribute to increase in antibiotic resistant bacteria (Ahmad et al., 2011; Silbergeld et al., 2012; Wielinga and Schlundt, 2012).
Since the society experiencing an antibiotic resistance in numbers, medical scholars reported the possibility of coming year’s pre-antibiotics era (Davies and Davies, 2010). Majorly few pathogens like, Vancomycin resistance Enterococci (VRE), ESBL (Extended spectrum β-lactamase) Vancomycin intermediate Staphylococcus aureus (VISA) or Methicillin resistant S. aureus are representing the antibiotic resistant features of ever increasing pathogens (Kumar and Singh, 2013). It is highlighted that all pathogens are no longer treatable by the antibiotics. Not only is this as a concern, pathogens like Pseudomonas aeruginosa, S. aureus, Streptococcus pneumonia and Mycobacterium tuberculosis able to showcase multidrug resistance features further increasing the threat of the human (Lowy, 2003; D’Souza et al., 2009; Ballal et al.,
In a hospital environment, prevalence of ESCAPE which is a multiple drug resistant species group formed by *K. pneumoniae*, *Enterobacter sp*, *S. aureus*, *E. faecium*, *P. aeruginosa*, and *Acinetobacter baumannii* also proving to be lethal (Pendleton et al., 2013).

These ESCAPE pathogens represent antimicrobial compound resistance by developing number of mechanisms such as enzymatic inactivation, formation of biofilm, changing cell permeability through porin loss, modification of target sites and increase in efflux pump. The threat of the resistant bacteria is increasing worldwide which is claimed to be at least 50,000 lives annually estimated in Europe and US. It is tough to estimate in other countries or region as data is scare but since the drug resistance going on increasing, it will lead to 10 million deaths annually as per estimation (O'Neill, 2014; Mendelson, 2015). Further, this drug resistance contributes in higher rates of hospitalization, longer hospitalization and higher treatment cost (Kapil, 2005; Grundmann et al., 2006). As per rough estimation by one study, this drug resistant pattern will add additional burden of about 100 trillion USD by the year 2050 (O'Neill, 2014).

**Antibiotic resistance with mechanism**

Since the introduction of new antibiotics in therapy continued to tackle the problem of resistance to the available one, bacteria also becoming capable of developing resistance to new antibiotics (Behera, 2010). In one such example, initially drug penicillin was found to be resistant in *S. aureus* when penicillinase was shown expression. To counteract this resistance, introduction of cloxacillin was done. Later on bacteria also able to develop resistance to cloxacillin by altering the, the penicillin binding proteins which is target site for binding of β-lactam antibiotics and that has started the story of MRSA. This event of resistance now continued to bring resistance to antibiotics chloramphenicol, macrolides, tetracycline, aminoglycosides and lincosamides (Nikaido, 2009).

Generally, origin of the genes that are responsible for the antibiotic resistance occurred naturally. These genes which are encoding antibiotic resistance are actually remain part of own genome in the bacterial chromosome due to the spontaneous mutations. The mutation occurs in an order of $10^{-8}$ to $10^{-9}$ which is responsible for antibiotic resistance,
wherever these mutations are quick to develop resistance bacteria to develop since these bacteria can divide very fast and can achieve absolute population in no time (Tiwari and Tiwari; 2011). Once these resistance strains developed, their mutated genes directly transferred to progeny via replication. When the wild type and mutated strains are allowed to grow in selective pressure, wild type failed to survive where mutated version grows voraciously and able to sustain the pressure of antibiotics (Todar, 2008 http://textbookofbacteriology.net). As a record, first antibiotic resistance especially of penicillin was reported in year 1942 once it was commercialized to use (Davies, 2010). Resistance to antibiotics majorly occurs through genomic alteration such as acquiring resistance plasmids, gene responsible for antibiotic resistance, transposons, or due to activation of efflux pump which withdraw drug from inside of the cell (Nikaido, 2009). Plasmid based multiple drug resistance reported to be the recent phenomenon when the sensitive strain receives it from resistant species as recorded in early 1940s (Behera, 2010). As many as for 400 different categories, 20,000 potential resistance genes have been reported in bacterial genome databases (Liu and Pop, 2009).

Plasmids: As it is known that acquisition of resistance is brought about by genetic transfer and/or chromosomal mutations, here greater threat is of the cases of transferable resistance as it can easily disseminate genes responsible for resistance to other species. In one example, R plasmid is capable of doing this gene transfer for multiple drug resistance. In another example, Horizontal gene transfer able to transmit antibiotic resistance via so called mobile genetic elements (Beceiro et al., 2013).

Antibiotics are inactivated by the expression of enzyme which acts on drug (substrate) to modify or degrade antibiotics which bring about the drugs inactivation (Kumar and Verela, 2013). In one example, S. aureus showcase resistance to penicillin by producing enzyme β-lactamase that is involved in hydrolysing antibiotics β-lactam ring (Kong et al., 2010).

Drug acts on specific molecule in target cell by mutational changes does occur in the binding site that lead to inactivation of the drug target site interaction. In an example, Methicillin resistance Staphylococcus species contains mecA gene which encodes
PBP2A. This protein showcased low affinity for β-lactam antibiotics, together with β-lactamase inhibitor combinations (ampicillin/sulbactam), carbapenems and cephalosporins (Levy, 1998).

Microbes are equipped with five super-families of efflux systems a) QacA major facilitators (MFS), b) QacA major facilitators (MFS) x, ATP binding cassettes (ABC), c) resistance-nodulation cell division (RND) and NorM, multi-antimicrobial extrusion protein family (MATE), d) Mex AB, Qac C small multidrug resistance family (SMR). All these Efflux pumps able to export antibiotics before they can act on target sites (Kourtesi et al., 2013). In one example, Streptococcus pyogenes with active drug Efflux pump represents macrolide resistance. The gene Mec A encodes the pump and remained specific for 14 and 15 membered macrolides (Kaplan, 2003).

Bacteria with this features alters the drug permeability through entry ports (Ang et al., 2004). In one example, P. aeruginosa showcasing mutational loss of porin proteins and able to resist the entry of imipenem by which can alter the outer membrane permeability (Lister et al., 2009).

By microbial aggregation formation of biofilm has been recorded. In this aggregation cells are remaining in together by involving the extracellular polymeric substance. This biofilm assists in early attachment of bacteria to the medical devices and to human tissues which is responsible in increase of the virulence and antibiotic resistance by hampering the entry of drug to target site. It also reduces phagocytosis (Hoiby et al., 2010).

**Action of plant**

Plant based product able to affect the protein-protein interaction and by which defined changes in the mitosis, apoptosis, immune response and signal transduction has been evidenced (Koehn and Carter, 2005).

In an action mechanism plant secondary metabolite works in serially different ways like interruption of RNA/DNA synthesis, function (Anandhi et al., 2014), disturbance in structure and function along with efflux system (Sanchez et al., 2010; Chitemerere and Mukanganyama, 2014), induction of coagulation of cytoplasmic constituents, and inhibition of quorum sensing which is a normal cell-cell communication (Nazzaro et al., 2013).
In a structural comparison to natural products, synthetic drugs remain varied in terms of frequency of different radicals and spatial configuration (Koehn and Carter, 2005). As plants showcase less sulphur, phosphate, nitrogen and represents Stereo-chemical abundance, enhanced scaffold variety, and diversity in ring system and carbohydrate contents makes them effective combination of action (Schmidt et al., 2008). In addition, bacteria remain incapable of developing resistance to plant products as it contains number of complex phytochemicals (Carson and Hammer, 2011). In contrast in recent time attention has been made that microbes are also developing resistance to herbal antimicrobials.

It is in general noted that since plant crude extract remain positive for number of bioactive compounds, its overall activity not only represents its anti-microbial activity but also affects number of vital pathways of the pathogens (Brijesh et al., 2009). In example, leaf extract of guava represents its antibacterial activity and in addition it prevents leaf extract and events of colonization (Birdi et al., 2010). Plant *Alpinia galanga* extract able to control multidrug resistant strains of *M. tuberculosis*. This activity of extracts remained varied under aerobic and anaerobic conditions which decide phytoactive components of plant extract (Cos et al., 2006). In a number of reviews and author reporting list of herbals are involved in antimicrobial activity along with its mechanism of action as botanicals (Nascimento et al., 2000; Verma and Singh, 2008; Almagboul et al., 2011).

In an example, plant *Rhizome copticlis* able to produce alkaloid berberine able to represent its antimicrobial activity. In addition, it showcases anti-herpes effect by inhibiting synthesis of herpes simplex viral DNA (Chin et al., 2010). Berberine at a concentration of 30-45µg/ml does able to showcase antibacterial effect against *Staphylococcus aureus* by inhibiting its biofilm formation (Wang et al., 2009).

In all microbe’s role of Efflux pump has become vital in bringing resistant to antimicrobials, it is becoming increasing important in altering the permeability of the outer membrane. In counteraction use of medicinal plants reported to inhibit Efflux pump along with disturbing the permeability of cytoplasmic membranes. (Sibanda and Okoh, 2007; Stavri et al., 2007; Jin et al., 2010; Li et al., 2015).
In one example, *Nor A* (efflux pump) activity in microbes got inhibited by plant produced flavonolignan 5-methoxyhydnocarpin. It is in combination with berberine able to enhance the activity as antimicrobial of each other when present in same plant. Another compound, farnesol is able to inhibit *Mycobacterium smegmatis* by EtBr as well as alone. This compound successfully possesses and Efflux pump inhibition which actually allow EtBr to retain inside of the cell of *M. smegmatis mc 2155* (Jin et al., 2010).

*Nor A* induced ethidium bromide efflux pump reported to be inhibited by phyto-compounds such as the alkaloid Reserpine (*Rauwolfia Vomitoria*) and terpene carnosic acid (*Rosmarinus Officinalis*), the diterpene tatarol (*Chamaecyparis nootkatensis*) (Holler et al., 2012).

In bringing about drug resistance in *M. tuberculosis* role with putative drug efflux pump belonged to member of the MFS or ABC super families is vital as stated by Kourtesi et al., (2013). Use of piperine in treatment of *M. tuberculosis* makes strains susceptible to rifampicin by allowing modulation of RV1258C, an efflux protein belonging to MFS superfamily (Sharma et al., 2010). Hence by adding piperine along with the rifampicin (Sharma et al., 2010) makes *M. tuberculosis* susceptible by RV1258C modulation. In contrast, when tested with other resistant strains/species, piperine did not showcase any susceptibility probably due to the presence of other efflux pump than RV 1258C (Birdi et al., 2012).
1.4 Need for research

In the present time, the human is surviving against pathogens by involving antibiotics those developed since long. Now it is becoming tough to control these pathogens as they are making a number of genetic changes which makes antibiotics ineffective in its action. In requirement, alternatives like green nanoparticles are put forward as future drugs and which demands its better investigation. A present study attempting the same principle to developed new nanoparticles which could be able to control MDR pathogens which are increasing mortality in human.
1.5 **Scope of the study:**

- The study will put forward a methodology for the synthesis of silver nanoparticles (AgNPs) from *Syzygium aromaticum*
- The study will determine the antibacterial activity of the AgNPs against Multidrug-resistant bacterial pathogens
- The possibility of new drug discovery protocol has been proposed.
1.6 Problem on Hand:

Ever since the human learnt to involve antibiotics in the therapy, life expectancy certainly been increased. But still the pathogen remains survived to the effective action of antibiotics since they started to acquire antibiotic resistance with them by number of means. In today’s scenario we are not sure that given medicine will work to cure patient of infecting pathogens. Instead, now patient reported with increased mortality since we are not able to control pathogen in time in many cases.

In a view, time has arrived where we should look after any alternative for antibiotics. In success stories, researchers are now focusing on the “Smaller miraculous drug-like molecule” called as nanoparticles (NPs). These NPs finds multiple uses and also capable of inhibiting human pathogen by the ability to inhibit pathogen growth.

In today’s scenario, Nanoparticles are formed by top and bottom approaches by involving chemical, physical, and biological features and for the most of the time plant-based nanoparticles find everyday utility in antimicrobial therapy since it remains safe and adopt natural protocol.

Further nanotechnology research gaining pace since all investigational machinery and protocols are readily available by which research could be planned accordingly.
Looking at the present scenarios, it is imperative to research uro-pathogens so that future therapy using Nanoparticles will assure safety, less resistivity and early cure which has not evidenced in immediate antibiotic treatment.

1.7 Objectives:

- Synthesis of silver nanoparticles (AgNPs) by *Syzygium aromaticum* aromatic flower buds.
- Effect of glucose and physiochemical conditions on AgNPs synthesis along with *Syzygium aromaticum* aromatic flower buds.
- Antibacterial activity of AgNPs on multidrug-resistant bacterial species.
- Characterization of AgNPs features by Spectrophotometer and SEM analysis.
- Molecular identification (16S rRNA) of *S. enterica, P. Aeruginosa, S. aureus, K. pneumoniae* and *E. coli*. 
1.8 Research design/Methodology

A. Synthesis of AgNPs by *S. aromaticum* aromatic flower buds.

About 10g of *S. aromaticum* flower buds will be cleaned by washing twice or thrice with distilled water and after that will be disinfected with the assistance of 0.1% Mercury(II) chloride for 1 min so as to limit the microbial defilement. It has been then homogenized into mortar pestle and will be moved into 100ml of twofold distilled water and will be continued bubbling for 15 min at 60°C. The substance will be cooled and transferred through Whatman no. 1 filter paper. The substance will be put away at the temperature of 4°C in a fridge for the time of about fourteen days.

In the subsequent stage, to amalgamate silver nanoparticles, 10ml of bud concentrate will be treated with 90ml of 1mM silver nitrate solution at room temperature. In a test, brown colour obtained will be checked by estimating UV-Vis spectra at 360 to 700nm.

B. Effect of Glucose and physiochemical parameters on AgNPs synthesis.
To contemplate the impact of parameters, for example, reaction pH, time, temperature and silver nitrate concentration alongside glucose on silver nanoparticles synthesis, the accompanying conditions will be set

a) pH- 3.5, 4.5, 5.5, 7.5, and 9.5 with silver nitrate 1mM, temperature 35°C.
b) Temperature- 20°C, 35°C, 45°C, and 70°C with silver nitrate 1mM, pH 5.5.
c) Glucose- 1%, 2%, 3%, 4% with silver nitrate 1mM at pH 5.5.

C. Antibacterial activity of Silver Nanoparticles on multidrug-resistant bacterial species.

To study the antibacterial action of AgNPs of S. aromaticum, medical samples or directly the medical cultures obtained from the testing laboratory will be screened by well diffusion test with lawn culture method and well-having a load of the 100µl concentration of AgNPs.

All isolates will be preliminarily distinguished by culturing, staining on specific media and in few cases by molecular identification (16S rRNA).

Antibiotic sensitivity will be done with all clinical isolates to discover multidrug-resistant pattern on Muller Hinton agar.

D. Characterization of AgNPs.

Better performing AgNPs described before by including the investigation of double beam UV-Vis spectroscopy, at various wavelength 360 to 700 nm will be affirmed for its Crystal structures by analysing them with SEM/TEM to comprehend nanoparticles size and morphology.

1. Statistical Analysis

Whole experimental data set recorded during the study will be compared with standard or control set value by involving ANOVA test with P<0.05. In addition, the decision will be made to apply t-test, Duncan's test, Dunnet test and others test to gain best result analysis possible. In software, Microsoft Excel and Graph Pad Prism will be involved.

2. Alternatives for study
During the course of study proposed methodology may be changed slightly to receive the better result and few techniques may be added or deleted as and when required. For example, EDAX and FTIR spectroscopy may be added in the testing and even concentration of the silver nitrate may be changed from 1mM to 2 mM and other so that better AgNPs could form.

1.9 Limitations

- The study is involving only invitro aspect of investigation and no in vivo testing is involved.
- The study is not checking its cytotoxicity on animal cell line and safety measures.
- The study is not been able to recommend the doses to control bacterial growth in vivo.
- The study will not be able to put forward the exact synthesis pathway/mechanism of the nanoparticles.
- The study is not relating to the effect of every biomolecule on nanoparticles synthesis present in the plant extract.