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Green Synthesis of Silver Nanoparticle: It's Characterization and Role Of Drug Delivery in Diabeties

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ABSTRACT: In nanotechnology the synthesis of nano particles from plant source is becoming favourite. Researchers are taking more interest in green nano biotechnology. Different properties of nanoparticles like biological and physiochemical have many applications like in drug delivery, sensor, optoelectronics and magnetic devices. In this article the plant based microwave synthesis of silver nano particles (AgNPs) is discussed. Green nanotechnology for the synthesis of nano particles is eco-friendly. Plant based nano technology should be further studied for the synthesis of nanoparticles from different plant and different parts of plants. The size range of nanoparticles varies from 1 to 100nm. Different techniques done for the depiction of synthesized nanoparticles were UV-spectroscopy, X-ray diffraction, Fourier Transform Infrared spectroscopy (FT-IR), transmission electron microscopy and scanning electron microscopy. The synthesized nanoparticles also pocesses the ability of killing microbs. The plant based production of nanoparticles can be facilely excersized in future for different purposes in engineering and medical fields. Photonanotechnology can deal with various forms of cancer. This article proffer an amprehensive analysis of plant based synthesis of silver nanoparticles with specific focus in their application of drug delivery in diabeties treatment.

Key words: Green synthesis, silver nanoparticles, Microwave heating, characterization, biomedical application, Fraxinus excelsior.

Introduction:

The utilization of nanotechnology for the production of nanoparticles is of great Importance in research site (Albrecth et al.2006). Many different type of nanotechnology and they can be used in different fields of life. Size of nanoparticles are less than the 1mm. The word Nano is infer from Greek term "nanos", which means "very small, tiny or dwarf "(Rai et al.2008). Nanotechnologies is of three different types i.e dry, wet and computational. In wet nanotechnology material like enzymes, tissues , membranes and other cellular components are subjected. Dry nanotechnology subjected to physical chemistry and production of inorganic molecules like silicon and carbon. Computational nanotechnology refers to stimulation of nanometer structure (Sinha et al.2009). For the optimal working in the respective field these three types of nanotechnology work together as shown in Fig.1 Different fields of electronic industry, pesticides, medicine are supported by nanobiotechnology (Bhattacharyya et al.2010). Nanobiotechnology gives an example where the study and development combine various scientific sectors including nanotechnology, biotechnology, material science, physics and chemistry(Rai et al.2008; Huang et al.2007).(Fig.1)

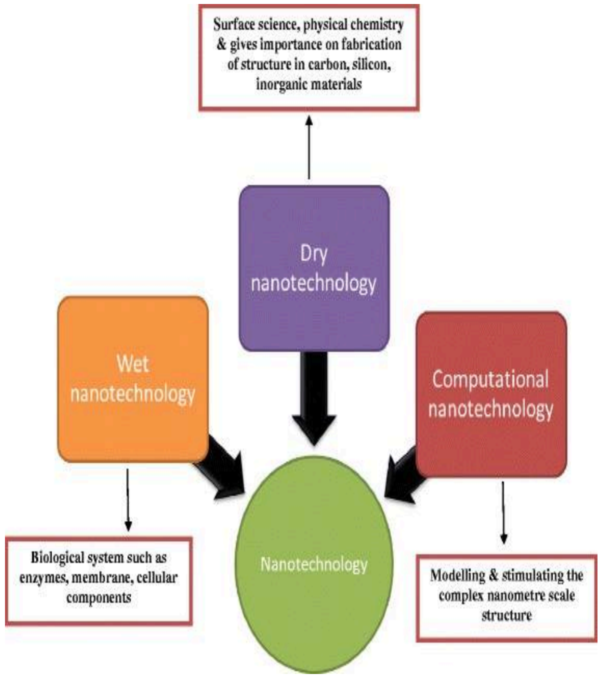

Fig.1 Types of Nanotechnology.

With the collaboration of different natural sciences to nanotechnology produce nanoparticles of various properties like antimicrobial, antioxidant and anticancer. Such nanotechnologies are provoking a new way for the development of effective, easy, and safe drug manufacturation (Dipankar et al.2102). The heating process in microwave shows many advantage over traditional methods. The different advantage of microwave processing includes heightened reaction of kinetics, fast initial heating which gives higher yield. Nucleation processes are well controlled by microwave heating as it can manage the high temperature. Nanaoparticles produced through microwave heating process are of high degree of crystallinity and gives dominion at the of nanostructure (Tsuji et al.2005). Many review shows microwave heating formation of nanoparticles is facile. It has become most effective and attractive process of nanoparticle synthesis (Nadagouda et al.2011 ; Tsuji et al.2005)

Fraxinus excelsior belong to Family Oleaceae and is ordinarily called as “ash” or “Eurpeon ash”. It’s a native plant of temperate Asia and Europe (Hemmer et al.2000 ;Eddooks et al.2002). Many compounds like frouxin, fraxetin, scopoletin, esculin and excelsiosida a secoiridoidglucosides are reported extracts from Fraxinusexlcelsior. Different parts of plant Fraxinus excelsior are very effective in the treatment of various diseases.for the long time , the bark of plant have been used as antibacterial . the seed shows the activity of hypoglycemia . the alcoholic extract of bark processes anti-inflamatory property. hypertensive and obese disorder can also be controlled by the use of extracts of Fraxinus excelsior (Monto et al.2014)

Different modes of nanoparticle synthesis:

Nanoparticle poceeses a particular position because of size, distribution and structure. For the faster bone recovery in 1970s, silver particles were used by R.O.Becker in infection caused by microorganism during orthopedic treatment (Becker et al.1978). Now different physical, chemical, biological and hybrid methods used to synthesize nanoparticles (Liu et al.2011; Mohanpuria et al.2008). Traditionally the synthesis of AgNPs is done by two methods i.e physical and chemical. Both of these methods are based on different techniques like ion sputtering, solvothermal synthesis, reduction and sol-gel. Reduction of chemicals is done in chemical, electrochemical and photochemical. Plant based nanoparticles have gain more attention as they are produced by eco-friendly method (Goodsell.DS.2004). Other advantages of plant based method are pursuing of safe solvents, decrease use of dangerous reagents, tepid responses condition, expeidency and other effective use in different fields like medicinal, surgical and pharmaceutical. Physical condition pressure, energy, temperature and constituent material are trivial (Abdel-Halim et al.2011) as shown in Fig.2.

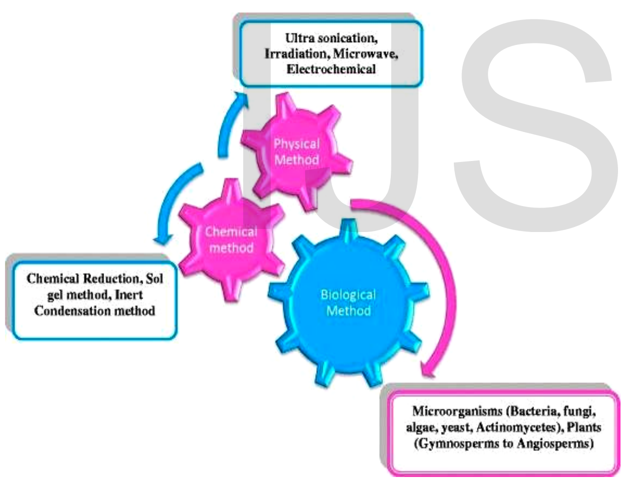

Fig.2. Methods involved in nanoparticles synthesis.

Nanoparticles of nobel metals have gain much importance as they can be used in medicine , biology, natural science, physics and chemistry (Yokohama et al.2007). In many nobel metal nanoparticles, silver nanoparticles is of great importance due to their unique properties like electrical conductivity, chemical stability, catalytic and antimicrobial activity (Sharma et al.2009). Silver as silver nanoparticles processes peculiar properties from bulk silver. The green synthesis of AgNPs is done through plants, microorganism and other biopolymers (Sharma et al.2009). Wet chemical synthesis is use for large scale synthesis of AgNPs but is not safe for biomedical application as toxic chemical are used in this method.the large scale production of silver nanoparticles through physical method is costly and inconvenient. So the green synthesis method is environment friendly, energy-efficient, facile and avoid use of toxic chemicals has gain much importance. As AgNPs are antimicrobial, they are also used in clothing (Vigneshwarna et al.2007), sun-screen(Martinez-gutierrez 2010), cosmetic and food (chaudhary 2011).

Synthesis of silver nanoparticles:

Silver nanoparticles from plant source is of great benefit as no maintenance of cell culture is required and support for large scale synthesis. Extracellular synthesis of nanoparticle are inexpensive due to easier down stream processing. The extract from leaf are utilize rather than whole plant. For the synthesis of silver nanoparticles plants were utilized. Different plant parts are utilize to make plant extract for synthesis of nanoparticles (Sana et al.2015: Bannala et al.2015: kumar et al.2014) are shown in table 1. Hence the Microwave heating of silver nanoparticles is discussed. Microwave heating is of great significance as compared to other heating methods . It gives rapid and controlled heating.

Table 1. Green synthesis of silver nanoparticles using different plant extract.

Plants	Plant parts	Size (nm)	Shape
Prunus yedoensis	Leaf	20–70	Circular, smooth edges
Tephrosia tinctoria	Stem	73	Spherical
Grewia flaviscences	Leaf	50–70	Spherical
Skimmia laureola	Leaf	46	Hexagonal
Clerodendrum serratum	Leaf	5–30	Spherical
Averrhoa carambola	Leaf	14	Spherical
Rosmarinus officinalis	Leaf	10–33	Spherical
Carica papaya	Leaf	50–250	Spherical
Plukenetia volubilis	Leaf	4–25	Optical
Cucurbita maxima	Petals	19	Crystalline
Moringa oleifera	Leaf	11	Rectangle
Acorus calamus	Rhizome	19	Spherical
Aristolochia indica	Leaf	30–55	Spherical or cubical
Euphorbia helioscopia	Leaf	2–14	Spherical
Datura metel	Leaf	40–60	Spherical
Momordica cymbalaria	Fruit	15.5	Spherical
Prosopis farcta	Leaf	10.8	Spherical
Mukia maderaspatana	Leaf	13–34	Spherical
Ficus carica	Leaf	21	Crystalline
Sinapis arvensis	Seed	14	Spherical
Ziziphus Jujuba	Leaf	20–30	Crystalline
Calotropis gigantea	Latex	5–30	Spherical
Nelumbo nucifera	Root	16.7	Polydispersed
Aerva lanata	Leaf	18.62	Spherical
Myrmecodia pendan	Whole plant	10–20	Spherical
Piper longum	Fruit	46	Spherical
Enteromorpha flexuosa	Seaweed	2–32	Circular
Lansium domesticum	Fruit	10–30	Spherical
Onosma dichroantha	Root	5–65	Spherical
Crataegus douglasii	Fruit	29.28	Spherical
Vitex negundo	Leaf	≥20	Cubic
Alstonia scholaris	Bark	50	Spherical
Lycopersicon esculentum	Fruit	10–40	Spherical
Musa balbisiana	Leaf	50	Spherical
Azadirachta indica	Leaf	20	Triangular
Ocimum tenuiflorum	Leaf	50	Cuboidal
Artocarpus heterophyllus	Seed	10.78	Spherical and irregular
Cocos nucifera	Coir	22	Spherical

Fresh Leaves of *Fraxinusexcelsior* were collected and rinsed with Distilled water to remove any dust .The extract of leaf was used with in fourteen days of forming . Leaves were chopped in 25g and were transferred with 100ml of distilled water into Erlenmeyer flask of 250-ml, the solution was boiled for 30 min to remove impurities. Using the Whatman NO.1 filter paper. The filtrates was refrigerated

SYNTHESIS OF SILVER NANO-PARTICLES:

Microwave synthesis reactor was used to synthesized silver nanoparticles . For the synthesis of silver nano-particles 20 ml of 1×10^{3}M aqueous silver nitrate was used . The 1ml of filtrate and 20ml of 1×10^{-3} of aqeous silver nitrate is thoroughly mix with constant stirring . The solution mixture was irradiated at temperature of 90^{0} celsius in a microwave of frequency of 2.45ghz . The solution is cooled at room temperature . The synthesis of silver nanoparticles appears when mixture solution changes into colour from yellow to brown. To remove the non required material silver nanoparticle were centrifugedat 500rpm for 5 min .

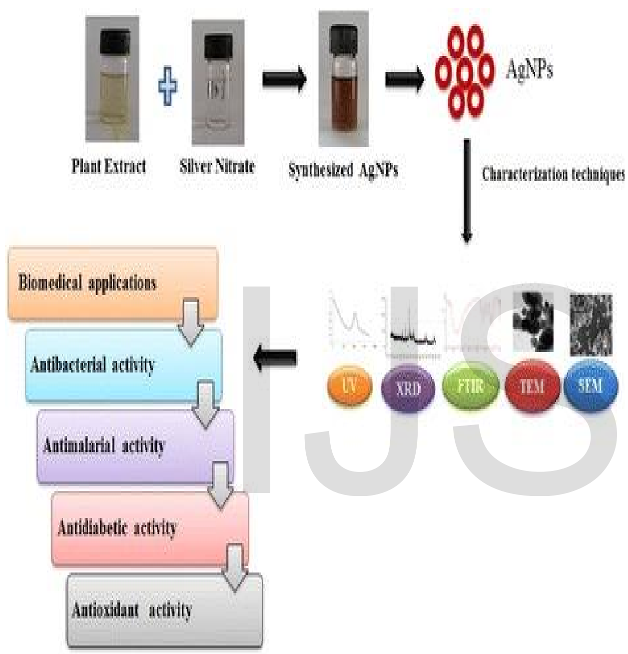

Fig.3. production of silver nanoparticles: their characteristic, properties and applications.

CHARACTERIZATION TECHNIQUES:

For characterization of AgNPs dried powder was used. For the characterization of silver nano particles different techniques were applied i.e..

1. Uv-vis spectral analysis,
2. FT-IR analysis
3. TEM
4. SEM and EDX spectroscopic.

Uv-Vis Spectral Analysis:

For the confirmation of silver nanoparticles by UV-vis spectroscopy is subjected to different aliquotsof reaction solution. Because of excitation in surface plamon vibration of AgNPs pocesses brownish color in aqueous solution. (Kresh-Naranget al.2010). The duration effect of microwave irradiation on uv-vis spectral analysis shows increasing absorbance Fig.4 inferred and that rapidly silver nano particles get reduced it can be seen as 94% of silve rnano particles in experiment get reduced in 90 seconds . As rapid reduction of nanoparticles occur it also reduces the kInectics of reaction . The irradiation time of 90 to 150 seconds shows maximum reduction . But above than 150s irradiation does not show any further reduction. The behavior of medium show variation with the time kineatics . By the exposure of reaction medium to microwave shows increase in color intensity. Concentration variation extract show is examined to get the concentration. For the concentration of silver nanoparticles. To get the optimum concentration different aliquates of leaf extract and silver nitrate solution were made by using 10m $AgNo_3$ different fraction of extract were used . In Fig.5 graph shows at 425nm yellowish brown silver nanoparticles give maximum absorbance at 425nm the band is clear fraction of 2ml extract salute was used .

Above and Low concerntration of extract solution from 2ml show irregular band fraction . Below the 2ml extract solution band was less apparent and and above the 2ml extract the solution become hazy . In second step different concentration of $AgNo_3$ against 2,l of extract solution was observed (Parveen et al 2015). The increase concerntration of $AgNo_3$ from $1\times10^{-3}$to 5×10^{-3} was examined the result in Fig5.shows increase in intensity of band fraction 425nm.

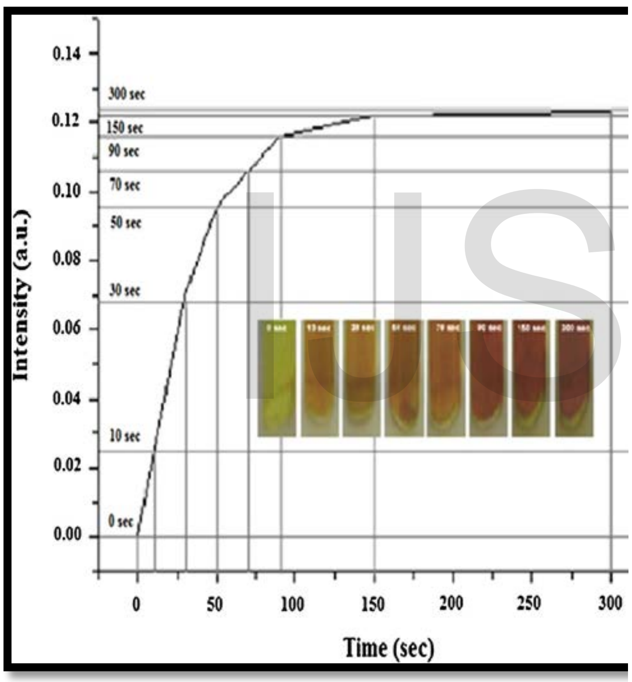

Fig.4. Intensity graph of silver nitrate solution and leaf extract versus time indicating that the 90% of AgNP biosynthesis completed in 90 s. Inset increase in the color intensity of the reaction mixture withtime.

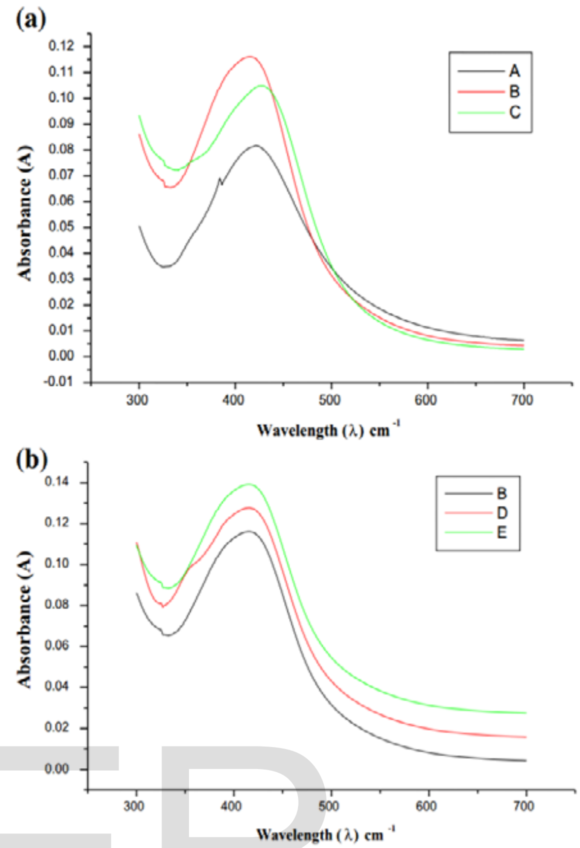

Fig5 (a) UV-Vis absorption spectra of AgNPs synthesized by treating 10^{-3} M aqueous $AgNO_3$ solution with different leaf extract concerntrations(A 1Ml, B2 ml and C3 ml), (b) UV-Vis absorption spectra of AgNPs synthesized by treating 2ml leaf extract solution with different concerntration of $AgNO_3$ solutions B(10^{-3} M), D(3×10^{-3} M) and E (5×10^{-3} M).

FT- TR – ANALYSIS

Fourier transform- infrared analysis was derived to check the molecule for the reduction and capping of AgNP's.FT-IT spectra shows that there is no observable change in absorption bands of Fraxinus excelsior leaf extract and synthesized AgNPs(Fig.5).The FT-IR spectrum of leaf

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extract Fig.6.(a) showed strong absorption peaks at 3438,2923,1637,1376,and 1078cm^{-1}. The FT-IR spectrum of synthesaized silver nanoparticles in Fig.6.(b) show peaks at wavelength og3412, 2926, 1627,1384 and 1075cm^{-1}(Parveen et al.2015).The FT-TR spectra shows the following results

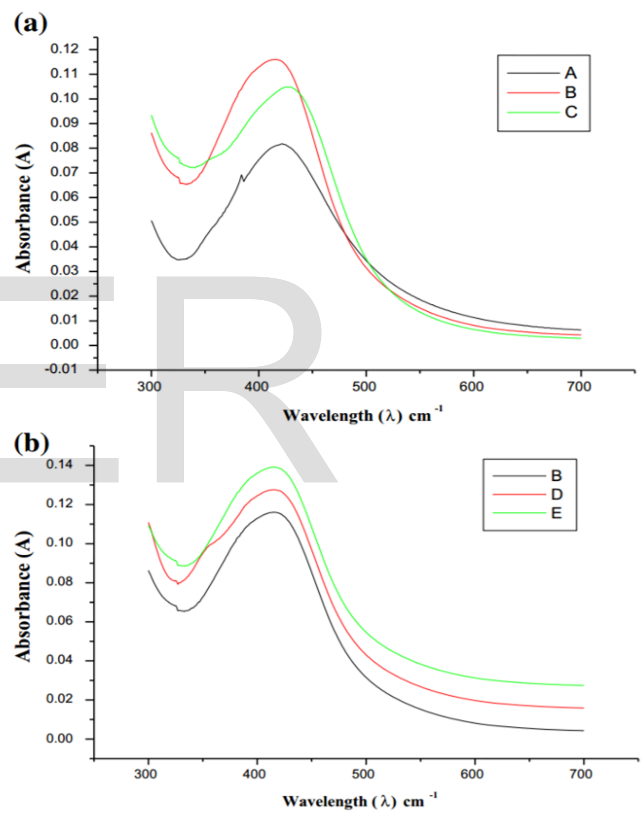
Table.2. The FT-TR spectrum of aqueous leaf extract

Wave Length (cm^{-1})	**group**	**Band**
3438	oH	Broad
2923	CH	Broad
1637	amide	Broad
1376	Methyl	Weak
1078	efliver	Weak

Table.3. The FT-IR spectrum Of Synthesized silver nanoparticles

Wave length (cm^{-1})	**Group**	**Band**
3412	OH	Broad
2926	Aliphatic-CH	Weak
1627	CO(NH)	Broad
1384	Methyl	Narrow
1075	Ether	Weak

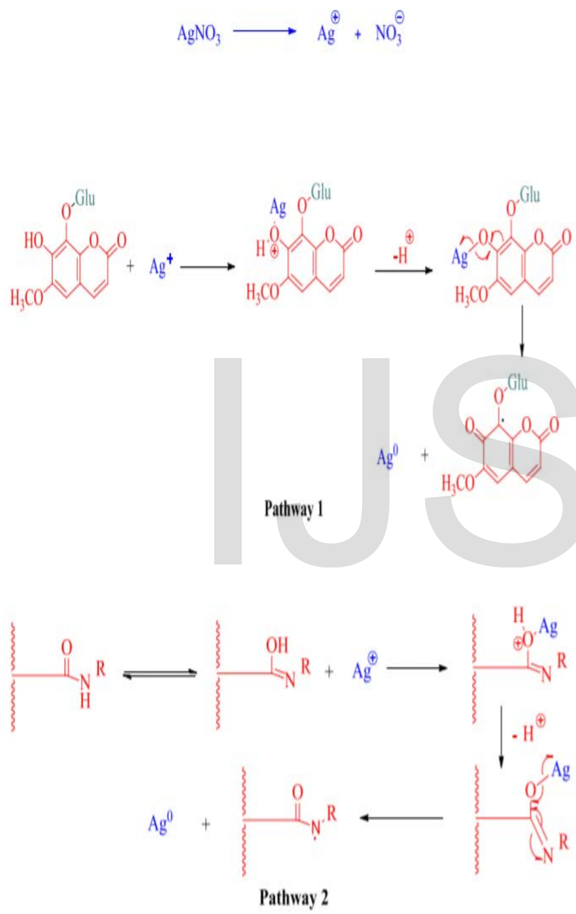
Between 3438 to 3412 cm^{-1} there is a decrease in band intensity of 26 cm^{-1}. It is because of reduction of silver nanoparticles.The change between 1637 to 1627cm^{-1} is due to CO(NH) group and reduction of silver nanopartiocles.The FT-IR analysis shows that organic molecules such as proteins and metabolites like terpenoids with functional group of amines, alcohol, keto aldehyde. And carboxylic acid encapsulate the synthesized AgNP's extracted from *Fraxinusexcelsior*(Parveen et al.2015)



<u>Fig.6.FT-IR spectrum of (a) aqueous leaf extract (b)syenthesizedAgNPs</u>

<u>Mechanism for the reduction of Ag ions</u>

Reduction of silver nanoparticles is because of natural biomolecules present in extract. Mostly involve bio-molecules are amino acids residues, Phenolic compound, terpenoids, alkaloids, flavonoids and glycosides. It is thought that majorly phenolic compounds reduces the silver ions(Arunachalam et al.2013). In scheme 1 proposed reduction process is shown. The phytochemical analysis indicates the presence of phenolic coumarinfraxin in *Fraxinusexcelsior*(Renwart et al 1992).



heme 1 Possible mechanistic pathway for the reduction of Ag^{+} ion by the plant extract

The phenolic hydroxyl group form an intermediate complex with Ag^{+}.after complex formation phenol get oxidized and reduces the Ag. They bythem selves act as the oxidizing agent in the pathway 1. It is seen that the phenolic group stabilize the AgNPs through carbonyl group.The change in IR frequencies to amide functional group suggest In fig.8 its side role in reduction process of Ag^{+} ion as shown in pathway 2.(Parveen et al.2015)

SEM AND EDX ANALYSIS:

SEM technique was used to characterized the morphology and size of silver nanopareicles. The analysis showed that silver nanoparticles are almost all spherical in shape, as uniformly distributed in Fig.7. The size ranges from 25 to 40 nm. The morphology was smooth. At some places the accumulation of silver nanoparticles forms a large sized particle.

Energy Dispersive-X-Ray spectroscopy shows the chemical nature of of synthesized silver nanoparticles(Fig.7). The EDX profile suggests a high peak of 3KeV at silver and small peaks at C, O , Cl. These molecules are supposed to be arrived from surface of AgNPs. The emission of energy at 3KeV indicates the reduction of elemental silver (Varma et al.2003).

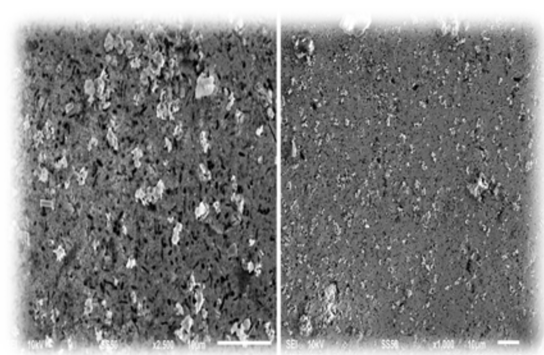


Fig.**7**:SEM micrograph of AgNPs synthesized from 2ml leaf extract treated with 5×10^{-3} M $AgNO_3$ solution.

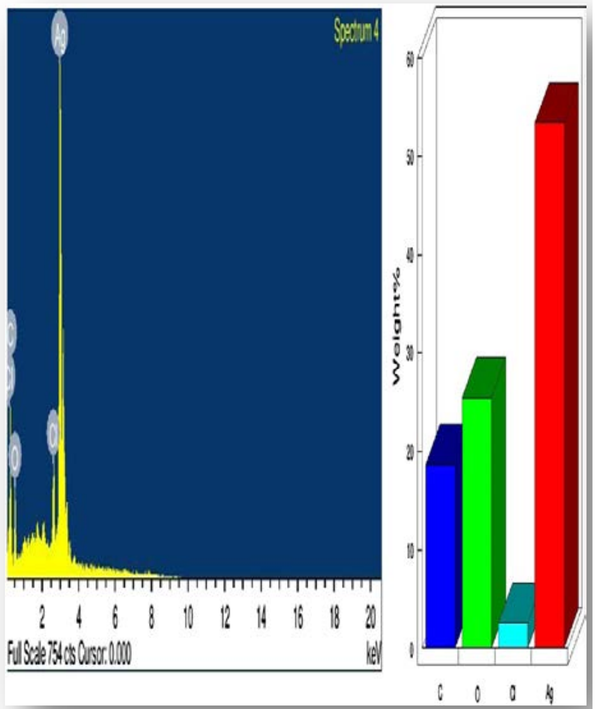


Fig.8.EDX spectrum of synthesized AgNPs with 2ml leaf extracts solution and 5×10^{-3}M $AgNO_3$ solution.

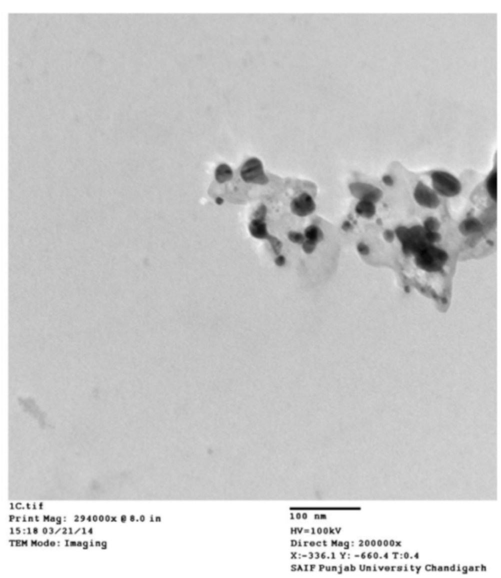


Fig.9: TEM image of AgNPs synthesized from 2ml of leaf extract treated with 5×10^{-3} M $AgNO_3$ solution.

TEM ANALYSIS:

Transmission electron microscopy further characterized the size shape and morphology of ion. The TEM images shows that the shape of silver nanoparticles were spherical. The spherical nanopartocles were of 25 to 40nm in diameter. It also shows that nanoparticles were coated by a faint thin layer of other molecule, present in solution of silver nitrate along the leaf extract of Fraxinus excelsior.(Fig.9).the image by TEM shows that synthesized molecule of AgNPs were spherical and polydispersal. The capping material was organic. It enhances the life time in solution upto four weeks after synthesis. (Shanker et al.2004 ; Song and Kim 2009). The coating molecule must be in the extract solution. The coating stops the silver nanoparticles to accumulate at one place , it also increases their life span by stabilizing them(Prveen et al.2015).

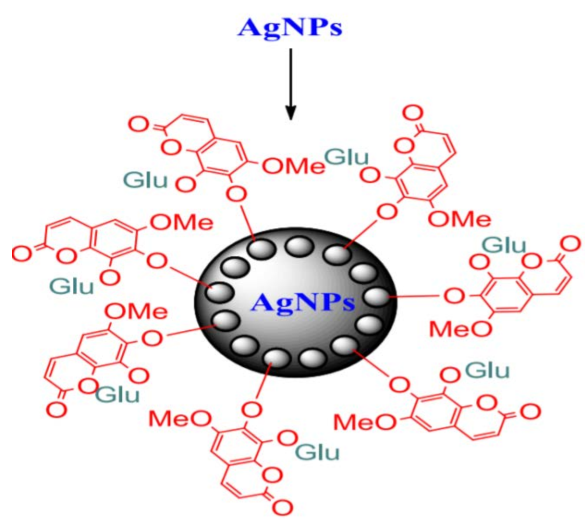


Fig.10. Stabilized structure of synthesized AgNPs

DPPH Scavenging Assay:

By using the 1,1diphenylpicrylhydrazyl (DPPH) method the antioxidant activity was examined. For the dilution of 2,4,6,8,10,12 ug/ml from prepared drug solution of stock (1mg/ml). It was was prepared in methanol. In different concerntrations of drug solution the 3.0ml of the methanolic DPPH was added. The reaction solution was placed for 30 min at room temperature. The OD was taken at wavelength of 517nm. The percentage of SA was calculated as.

%Scavenging activity(SA) =

$\{(A_{control}-A_{Sample})/A_{control}\}\times100$

$A_{Control}$is the OD of L-ascorbic acid (standard).$A_{Sample}$is the OD of various samples. Control was methanolic DPPH. The lowest inhibitory value of extract was 9.83. AgNPs inhibitory value was 18.32for 2ug/ml. For the 10ug/ml AgNP inhibitory value was 86.18 (Parveen al.2015).

Application of silver nanoparticles in Diabetes:

The nanoparticles are used to deliver drug in diabetic patient. Following different forms of nanoparticles are in use.

1. Polymeric biodegradeable nanoparticles i.enano-sphere and nano-capsule.
2. Ceramic nanoparticles
3. Polymeric micelles
4. Dendrimer
5. Liposomes

Insulin delivery routes of nanocarrier

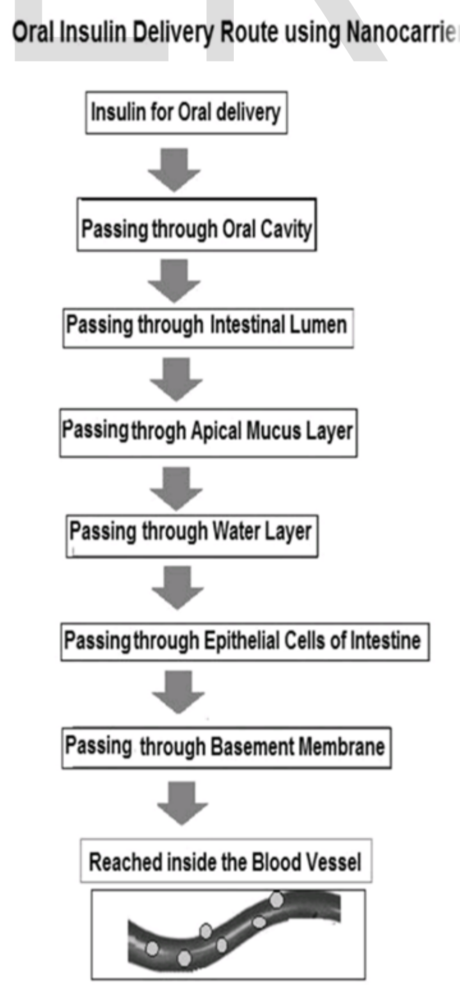
Silver nano-carrier could take the insulin by two different routes.

1. ORAL insulin delivery route using nano-carrier.
2. PULMONARY insulin delivery using nano-carrier

Mechanism of nanoparticles in drug delivery system

Drugs physio-chemical properties like solubility, pKa, size etc effect bioavailability of orally administered drugs. Depending on size particle absorption sites are differently located in the gastrointestinal tract (GIT). Particles of 1µm in diameter are absorbed by the intestinal macrophages via phagocytosis, where as particle of ≤10 µm in diameter are absorbed via peyer's patches. And the nanoparticles of size≤200 nm are absorbed through enterocytes by endocytosis.(O' Hagan DT. 1996). The drug absorption and excretion is effected by the transporter efflux like P-glycoprotien (Pgp) and enzymes present of exterior of enterocytes. Nanotechnology has created complex of size scale in different fields. Nanotechnology is extensively studied in the medicine field for drug delivery system(Varma et al.2003).Nanomedicine must processes the properties like effectiveness, non-toxic, biodegradable, non-inflamatory, non-thrombogenic, non-immunogenic and must release from reticulo-endothelial system (singhal et al.2011; Singhal et al.2012) . Advantages of nanotechnology in drug delivery system includes the tolerability, specificity increase efficiency and therapeutic index of the drugs. Nanomedicine can be applied on different substance like small drugs, proteins, vaccine or nucleic acid. The range of nanoparticles for the therapeutic and imaging usage must be 2 to 1000 nm (Singh and Lillard 2009). Nanotechnology helps in the drug delivery of difficult mechanisms like less water soluble drugs, transcytosis of drugs across the intestinal barrier, targeting of drugs to the specific part of the gastrointestinal tract and in the intracellular and transcellular delivery of bulky macromolecules (Farokhzad and Langer 2009). The limitations of oral administration of drug could be taken by using bio-adhesive polymers. Such polymers assist nanocarrier in adhesion to mucosal epithelial membrane. Effective alternative to oral route is the pulmonary route for drug administration .Fig 11, 12.

The biodegradable polymeric nanoparticles shows better alternate to the oral/pulmonary administrated proteins and peptide drugs (Florence 1997). The strength of nanoparticle related to stability and functionally activeness can be regulated. Pharmaceutically accepted excipients like cyclodextrin, chitosan, PLGA, TPGS/Vitamin E TPGS etc can regulate the pH responsitivity and Pgp effect of nanoparticle (Guo et al.2013). Lowman et al.(1999) formulated pH sensitive nanoparticles to blows-away limitation of oral administration of insulin delivery. He notices the observable decline of blood glucose in diabetic rats for more than eight hours at dose of 25 IU/Kg of loaded insulin(Lowman et al.1999). The release of encapsulated insulinin an organism can be controlled by the various combination of polymers and targeted molecule, as it can increase the uptake and bioavailability of drug (Singh and Lillard 2009). Some of thr pH sensitive biodegradable polymer in usage are PMAA (Sajeesh and Sharma 2006), HPMCP(HP55) (Cui et al.2007), dextran sulphate (Reis et al.2007), alginate(Reis et al.2007), PGA(Sonaje et al.2009) etc.



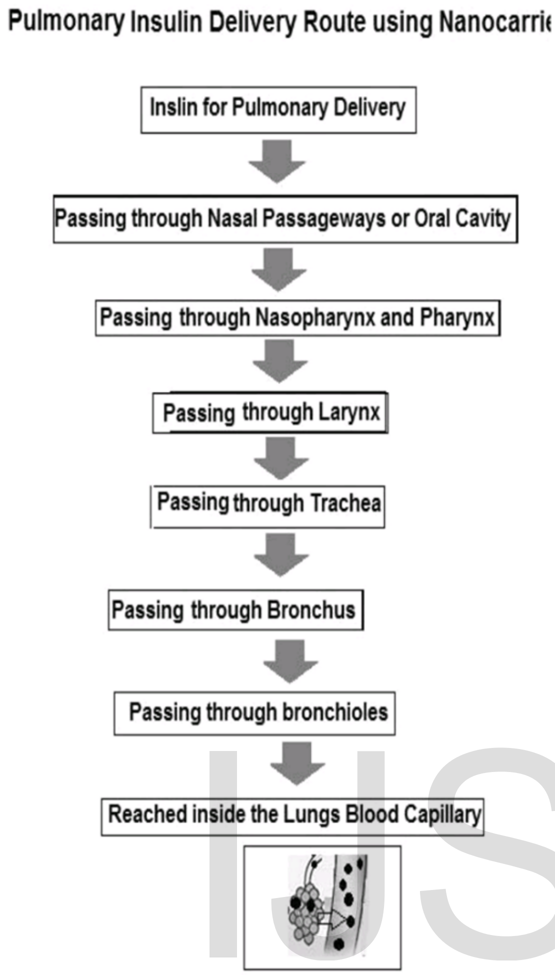


Fig.**11.**Nanocarrier based oral route insulin delivery. **Fig**.**12**. Nanocarrier based pulmonary route insulin delivery.

Conclusion:

The above mentioned method is eco-friendly for the extraction of silver nanoparticle . The different characterization techniques cofirms the presence and formation of silver nanoparticles. The synthesized silver nanoparticles processes the property of diffusing free radicals, can be used as food additives and of biopharmaceutical industry usage. Presently nanoparticle based drug delivery method is in practice. It is providing better results as compared to conventional ways. This nanoparticle system of medication is providing effective treatment. In the coming era it gona be the most efficacy method and control of disease like diabities, TIDM in a better way.

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