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Formation of Simple to Highly Complex Molecular Structures by Combining with Biosynthesized Silver Nanoparticles from Parts of *Grewia tiliaefolia* Vahl

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Abstract: At present interdisciplinary sciences are playing a crucial role in research in which “Nanotechnology” is one which is now in the most advanced phase; especially in the field of biological sciences, it has been doing a good deal of wonders. As of now, Nanoparticles showing extraordinary results in all fields of science. The plant-mediated silver nanoparticles were endowed with quality and have different shapes and sizes; they were proved to be the best in their way of curing the diseases. But certain unwanted aspects were (identified) happening during the synthesis process of SNPs and also after synthesis of SNPs. All these might be because of the presence of certain unwanted impure metal ions (toxic) in the extracts and their bonding with the 'Ag' ions at the same time among themselves. The present study was mainly focused on the different types of Simple to Highly Complex Molecular Structures (S–HCMS) formed along with synthesized Silver Nanoparticles (SNPs) from leaf, bark and fruits of *Grewia tiliaefolia* a Tiliaceae species. The study was done by using standard methods by combining XRD peaks of unwanted impurities present along with ‘Ag’ peaks with ICP-OES method and analyzed by using different standard procedure. This study gives a disquisition on the presence of nine S–HCMS and with schematic illustrations.

Keywords: *Grewia tiliaefolia,* ICP-OES, Silver Nanoparticles (SNPs), UV-VIS, XRD, Simple to Highly Complex Molecular Structures (S–HCMS)

1. Introduction

The statement about Nanotechnology "The Principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It was not an attempt to violate any laws; it was something, in principle, that can be done; but in practice, it has not been done because we were too big_ *Richard Feynman* 1959. This principle was axiomatic. Nano [1], Nanotechnology [2, 3], and Nanoparticle [4], with large surface area to volume ratio, came into existence by the thoughts of scientists a few decades ago and that was improved a lot in the course of time. Now it was proved to be the best in the field of science and to give the best results in solving human and environmental problems and they filled the gap from large to atomic level [2], it absorbed all the pure sciences and changed them into interdisciplinary [5, 6]. At present all the industries were joined with Nanotechnology [7, 8], from that a new field Nanobiotechnology was emerged and developed by covering all the fields of science like electronics, pesticides, medicine, parasitology, biotechnology, material science, physics, and chemistry [1]. This opened new areas in research such as bionanotechnology, quantum dots, surface-enhanced Raman scattering (SERS) [7], biomedical nanotechnology, nanomedicine (hybrid fields) [9]. A string of Chemical and physical methods have been used for a long time in the production of NPs, nowadays all types of biological systems were used in the production of metal nanoparticles [7]. Physical methods are including plasma arcing, ball milling, thermal evaporate, spray pyrolysis, ultrathin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of nanoparticles. The chemical methods are used to synthesize NPs by electro-deposition, sol-gel process, chemical solution deposition, chemical vapour deposition, soft chemical method, Langmuir Blodgett method, catalytic route, hydrolysis, co-precipitation method and wet chemical method [10]. As a part of biosynthesis, plant-mediated nanoparticles synthesis was identified as bedrock; it was cost-effective, environmentally friendly and safe for human therapeutic use [11]. The activation phase, the growth phases (Ostwald ripening) & the termination phase were three phages identified in bio-reduction of metal nanoparticles [12]. Scholars were reported different metals for the biogenic synthesis of nanoparticles [13]. By using this interdisciplinary version of science it was easy to get the required size, morphology along with required properties of Nanomaterials (NMs) and they can also be used as multidisciplinary [6]. Evaluation of characteristics of NMs such as shape, size distribution, surface area, solubility, and aggregation was a must before assessing toxicity or biocompatibility [14]. The verisimilitude was metal particles at nano-level were showing extraordinary characteristics in curing and controlling a number of diseases, hence used in a myriad of applications in different fields such as biomedical, sensors, antimicrobial, catalysts, electronics, optical fibres, agricultural, bio-labelling, in other important areas of science [15], clinical diagnostic and therapy [13], mechanics, optics, chemical industry, space industries, drug-gene delivery, energy science, optoelectronic devices, photoelectrochemical applications and nonlinear optical devices. Used in single-electron transistors (SETs) and light emitters, small particles such as iron oxide, fullerenes encapsulating Gd^{+} ions (gadofullerenes) and single-walled carbon nanotube nanocapsules encapsulating Gd^{3+} ion clusters are nothing but advanced materials synthesized in nanotechnology. Other applications ranging from bio-sensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, a chemical sensor, and wireless electronic logic and memory schemes all these are again applicable to different fields such as medical imaging, nanocomposites, filters, drug delivery and hyperthermia of tumours [7]. Targeted delivery of anticancer drugs by using fluorescent and magnetic nanocrystals detection and monitoring of tumour biomarkers can be possible more easily [16]. In biomedical research and applications, NMs were also used *in vitro* and *in vivo* conditions. The nanomaterial along with biological sciences has opened new diagnostic devices, contrast agents, analytical tools, physical therapy applications and drug delivery vehicles [9]. Nanocubes, nanoplates, nanorods, spherical nanoparticles, flower, etc. nanostructures developed and have been used in the biomedical field [14]. Nanotechnology has also improved our food quality, healthfulness, and self-life also food products monitoring by online, now a day's nanotechnology techniques were helping us to know the changes taking place during food processing method [17]. Other important NMs were used in the diagnostic as well as in treatment they were metallic nanoparticle, quantum dots, carbon nanotubes, magnetic nanoparticles, fullerenes, liposomes, dendrimers and engineered hybrid nanoparticles [9]. Speedy spreading of the pathogen can be detected by the potential to raise sensitivity, specificity with the help of biosensors [18]. Quantum dots, nanostructured platforms, nano-imaging and nanopore DNA sequencing tools, these can be given high-quality analysis and monitoring and helps in crop protection [19]. In a score of biological applications of nanoparticles include target drug discharge, gene therapy, identify effective tissues, biological molecules and cells separation and purification, bio-labelling, repair of damaged tissues by tissue engineering, DNA probing and microsurgical techniques [20]. The steep increase of Nanomaterial application in biology and medicine, fluorescent biological labels, bio-detection of pathogens, detection of proteins, probing of DNA structure, separation and purification of biological molecules and cells, MRI contrast enhancement, phagokinetic studies [21]. The most effective plant protection methods such as nanoemulsions, nano encapsulates, nanocontainers and nanocages have some of the nano pesticides delivery techniques were also developed. The chitosan nanoparticles were used as both degradable antibacterial material and also for the slow release of NPK fertilizers, for cementing and as a coating material for control release of fertilizers nanolayers were developed with Kaolin clay [19] and Nanosuspensions [22]. The selected plant parts are showing various medicinal properties in their raw form, but SNPs synthesized from the extracts of plant parts show more accurate medicinal properties against various harmful microbes and showing more positive results in other applications, while synthesis of SNPs there are certain metal particles or impurities also identified along with the SNPs forming various combination bonds and finally Simple to Highly Complex Molecular Structures (S-HCMS). It is necessary to study the structures formed by these impurities or unwanted metal particles and their effects on SNPs, etc. help to improve the quality of SNPs by that possible to the production of more accurate medicines for controlling various harmful microbes and highly advanced medicines for human welfare, etc. in this context, there is a scope to do further research on this work in the future.

2. Review of Literature

'Lepis infermale' is the name to the silver in Latin, *'Lunar caustic'* in English and *Pierre infermate* in Fench [23]; Transitional metal and rare, present in earth's crust [24]. Historically metallic silver was known to be third metal after gold and copper by Caldeans about 4,000 B. C. E. Infected corneal ulcers, interstitial keratitis, blepharitis, and dacrocystitis were can be successfully cleared by the

colloidal form of silver not only that colloidal silver was also effective against puerperal sepsis, staphylococcal sepsis, tonsillitis, acute epididymitis, and other infectious diseases [25]. In ionized form silver not only react with tissues proteins to bring structural changes cell walls of bacteria but also binds with DNA and RNA and stops bacterial replication by denaturing them [26]. The silver compound is used in photographic plates, in artificial rain falling, as an antimicrobial agent, as Ag^+ easily penetrates living organisms [24]. From the earlier periods as an anti-infectious agent, silver nanoparticles have proved that it was invigorating metal in the field of science, and it was also used to progress in a natural way. Silver nanoparticles were proved to be a safe bet in biological as well as in other material fields [27]. Production of SNPs can be done by physio-chemical techniques such as chemical reduction, gamma-ray radiation, microemulsion, an electrochemical method, laser ablation, autoclave, microwave, photochemical reduction and green synthesis of SNPs reported [28]; UV- radiation therapy & Ultrasonic assisted synthesis [29]; high energy radiation, microwave irradiation and inert gas condensation [30] and Plasma catalysis [23]. Sonication, ball milling, flame pyrolysis, radiation, electric arc discharge, etc. were physical methods for synthesizing SNPs, Physical methods need a very high quality of instruments, they also need a high temperature, pressure, and they need high energy for synthesis process and also Wizardry persons [31]. Other important physical and chemical methods of SNPs synthesis includes surface deposition, arc discharge, plasma polymerization, laser Chemical Vapour Deposition (CVD), emulsion polymerization and chemical reduction, thermal decomposition in organic solvents, photoreduction in reverse micelles [32] and sequential injection [27]. The synthesis of SNPs by green chemistry [33] or green synthesis methods differentiated into polyoxometallates, polysaccharide, Tollen's method, irradiation and biological methods [32]. The green synthesis of SNPs depends on a few major physical and chemical parameters includes reaction temperatures, metal ion concentration, extracts contents, pH of the reaction mixture, time taking while reaction has taken place and agitation. The size, shape and morphology of the SNPs can be change by concentrations of metal ion and extract concentrations used. This was reported that better stability of SNPs occurs in basic medium and Sparking pace growth rate shown in basic pH. At this pH, excellent yield and monodispersity can be observed and increasing the pH of a reaction mixture gives small and uniform-sized nanoparticles. By changing pH, nearly spherical SNPs were transformed to complete spherical SNPs. It was hard evidence that unstable SNPs with agglomeration were formed at high pH i.e., pH>11 [28]. All the phytochemicals like Polyphenols, flavonoids, reducing sugars, sterols, essential oils, starch, cellulose, pectin’s, gums, resins, lactins, etc., here and now playing both the reducing agents and capping agent’s role in the SNPs synthesis process [34]. SNPs synthesized by biological methods show high yield, solubility and high stability [14]. SNPs were used against a wide range of microorganisms in an appreciable number of fields like biomedical, drug delivery, water treatment and agricultural allied sectors. SNPs were applied in inks, adhesives, electronic devices, pastes, etc. due to high conductivity, therapeutic agents, as glyconano sensors for disease diagnosis, as nanocarriers for drugs delivery, in radiation therapy, in H_2O_2 sensor, in ESR-Dosimetry, as heavy metal ion sensors, as a catalyst for reduction of dyes such as methylene blue [28], gene delivery, artificial implants [35], cosmetics, food storage, textile coatings, in some environmental applications, as effective anti-tumour, in drug-delivery systems, acting either as passive or active, nanocarriers for anticancer drugs, great optical properties, in cotton fabrics, natural and artificial fibers, thin polymer films and wound pads [36], air sanitizer sprays, socks, pillows, slippers, respirators, wet wipes, detergents, soaps, shampoos, kinds of toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones, etc. [37]. Dye removal and anti-corrosion agent [38], antimicrobial deodorant fibres and cell electrodes [7]. In water and air treatment, mirrors, medical, dentistry, and food packaging [32]. Nanocrystalline silver particles were proved to be sterling, hence applicable to fields like high sensitivity biomolecular detection and diagnostics, anti-inflammatory, anti-angiogenesis, antiplatelet activity, molecular imaging, biomedicine, photonics and microelectronics [39, 40]. It was flabbergasted to know that, 320 tons or more of SNPs was producing per year these were used in mainly nanomedical imaging, biosensing and food products [41, 37]. As a part of the antimicrobial activity of SNPs, they were showing potential activity against Gram –ve and +ve bacterial strains such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans* and *Staphylococcus*. These SNPs were also showing strong antifungal activity against *Candida albicans, C. glabrata, C. parapsilosis, C. krusei,* and *Trichophyton mentagrophytes*. SNPs were also showing antiviral activity against HIV-1, Hepatitis-B virus, Herpes simplex, monkeypox and respiratory syncytial virus [42].

The SNPs were shows a score of advantages as well as uses but some adverse effects were also noticed regarding the SNPs which were previously discussed by a number of researchers. The aim of this work was trying to find out certain ignoramus reasons with a candid approach. SNPs were showing parlous effects when released into nature, changes fate, and toting up toxicity to the natural aquatic environment was noticed; this toxicity comes by façade paints; this toxicity stands next to physicochemical parameters like particle number concentration and particle size distribution (PSD) and in the environment. Presence of dissolved ‘Ag’ might be from the oxidation of the SNPs [43], was the reason and showing the undesirable influence on the environment. Especially showing downbeat effects on aquatic organisms such that fishes, invertebrates and including algae [44].

Work on the toxicity of silver ions and nanosilver at cellular and protein level was carried out by many researchers at present, but precious little work has been done regarding effects of silver and nanosilver on fluorescence inner filter effect in fluorescence experiments. This may be shown the effect on the accuracy and meticulousness of fluorescence assays. Little work has done on the study the interaction of Ag^+ and protein by Isothermal Titration Calorimetry (ITC) and mechanism of toxic interaction between lysozyme and Ag^+ by spectroscopic and ITC method, in the meanwhile by using "Lakowicz" method the identified fluorescence inner filter effect was corrected. By understanding the toxic effects of silver ions at the molecular level it is possible to improve the medical diagnosis and targeted therapy [24]. SNPs were used variously in our daily life due these humans were exposing to them hence there was a toxicological menace in the coming future or already facing the danger. These nanomaterials were entering into the cells of our body through ingestion, dermal contact, inhalation and penetration. Researchers were now interested in noxious studies caused by all nanoparticles including SNPs. In recent in vitro studies proved the toxic effects of SNPs in cells including NIH 3T3 fibroblast cells, Hela cells and human glioblastoma cells. The system of the reaction of SNPs with mitochondrial damage, oxidative stress, DNA damage and induction of apoptosis [45]. Toxicity of NMs to organisms including airborne (inhalation) routes evaluation has become essential. This was a serious part started by the Nanomaterials Toxicity Screening Working Group (NTSWG -Project). Health and Environmental problems were arising unknowingly by nanoparticles, when consumer exposing themselves by handling consumer products on the application, during testing and in the disposal. By utilizing consumer products consumers were exposing severely to nanoparticles because of this, undesirable health effects were coming. These adverse effects were due to the consumer products having nanoparticles with various size, surface area and chemistry, solubility, possibility shape, at the same time location and different concentrations. In the meanwhile, certain nanoparticles were having the property such as partially combing nature or do not combine with the main consumer product and they would like to exist independently these shows special potential adverse effects when exposed to them by inhalation and dermal route. This provides valuable information and makes a foray of research regarding the adverse effects of nanomaterials [46].

From the ancient time's plants and their products has been used in curing different diseases. A number of herbalists were mentioned about different plants and their usage in their prestigious works and made it into valuable literature. The genus was named by "Carolus Linnaeus" in honour of "Nehemiah Grew", known as the *"Father of Plant Physiology"*(1641-1712 AD.) from England [47]. *Grewia tiliaefolia* was a Tiliaceae member found in different areas of tropical and subtropical climatic conditions of the world with 150 species. The researchers found that species contains shrubs and trees were also growing in warmer regions and around 40 species were distributed in sub-continent they include G. *tenax, G. hirsuta, G. damine, G. Lasiodwascus, G. optiva, G. biloba, G. bicolor, G. tiliaefolia, G. flavescens* [48, 49]. In the subcontinent, these species were commonly known by the names of "Dhamini and Dhaman". In Ayurvedic system, it was used in vitiated conditions of pitta and Kapha, burning sensation, hyperdipsia, rhinopathy, pharyngopathy, cough, skin diseases, pruritus, wounds, ulcers, diarrhoea, haematemesis, and general debility [50]. Members of this genus were being used as a traditional medicine in treating a large number of diseases [48]. Parts of these trees have been used as astringent, expectorant, antipruritic and aphrodisiac in India by well known traditional medicinal practitioners and at the same time by local tribal people [49]. The VC. Prof. Jehandar Shah first Identified a chemical "friedelin" from the *Grewia tiliaefolia* in the year 1965 [51]. More work has been done and identified more chemicals like D-erythro-2-hexenoic acid γ-lactone, Gulonic acid γ- lactone, betulin, friedelin, lupeol, tannins, flavonoids, hemicelluloses, phenolics, lupenol and lignin [52]. The leaves of certain species in *Grewia* were consumed as vegetables [53]. The species *G. tiliaefolia* leaves were used as antibiotic in skin eruptions, the fresh leaves were mostly used as fodder. It was found that the ether extract of *G. tiliaefolia* leaves was having the antibiotic nature. The studies of physicochemical and phytochemicals have carried out on this species. The leaves were having the adsorptive nature due to this the constituents in the leaves were getting more significance [52]. It was detected that the leaves of *G. tiliaefolia* Vahl in aqueous extract condition showing an extraordinary analgesic and antipyretic properties in combination with morphine and paracetamol at the doses of 10 and 150 mg / Kg IP as standard drugs and it was noted that the effect was comparable to that of paracetamol. As the part of Ayurvedic medicine, the bark was used to control the conditions like Pitta and Kapha, burning sensation, cough, skin diseases, wounds, ulcers, diarrhoea, seminal weakness, general debility, and hypertension [51]. The bark was having specialized uniqueness like sweetness, acrid, refrigerant, oleaginous, vulnerary, constipating, emetic, styptic, tonic, haematemesis [54]. The mucilage formed of bark mixed along with hot water was an antidote for opium poison in human adults. The bark was also used to treat spermatorrhoea, in Bahraich, U. P., India, in human adults. A number of biological properties were studied regarding the bark by several researchers. Betulin, friedelin and lupeol belong to triterpenoid metabolite were isolated from the bark of *G. tiliaefolia* [55]. The bark was medicinally used to heal the infected wounds, to cure a cough, throat complaints, dysentery and other infectious diseases [51, 56]. The bark extract and pastes were used for curing pneumonia, bronchial infections, urinary infections and to heal old wounds. lupeol was isolated from the methanol extract of the stem bark of *Grewia tiliaefolia* and evaluated the cytotoxic properties on in-vitro cell lines [57]. Overall study about the plant, SNPs and S-HCMS provides a new path of study in synthesis of nanoparticles.

3. Material and Methods

3.1. Collection of Plant Material

The leaf, bark and fruits of this species were collected from the University of Hyderabad of Telangana State. Only the fresh leaves and fruits were washed twice with tap water (but not the bark) and with distilled water to remove foreign particles. These materials placed on normal filter papers to absorb the water drops and leaves and bark were cut into small pieces, but the fruits were dried as it was in the shade on filter papers under careful observation for 20 days and confirmed that all the three materials were completely dried then they were separately powdered and stored in an airtight jar.

3.2. Mineral Analysis by ICP-OES

The leaf, bark and fruit of all three powders were submitted to the ICP-OES for the mineral analysis. It was a very important test to know the minerals present in the parts of the plant or tree this helps us to know the presence chemical in the highest and the lowest percentage. Every plant, as well as trees, as well as trees, were having certain chemicals, we call them phytochemicals or phytomedicines in which few were having the toxic nature used by the plant in protecting themselves and others having nutritional values which were taken by the humans and animals. Scientists have investigated the reasons for the development of these phytochemicals and their toxic and dietary formulations. It was found that the phytochemicals need a superior controlling capacity. By knowing potentiality of the toxic and essential elements and their harmful effects used in excess it was possible to develop the doses of intake. To meet the requirements it was necessary to select the finest method for sample preparation and testing on specific parameters like concentration of the analyzed metals, nature of the sample in solid, liquid, etc., and type of material matrix. In performing these tests certain spectrometry techniques such as FAAS. FAAS was used in large extent. ICP-OES, ICP-MS was used for the detection of elements present in samples. Certain elements were detected in percentages and others in PPM, due to their biological nature and their high sensitivity, versatility, multi-element analysis, ruggedness and speed in the analysis [58]. The aim of the study about the mineral content present in the plant was done in three ways, such as Dry ashing (DA), two wet digestion procedures, conventional (CD) and assisted by microwave radiation (MW) under ICP-OES. The elements evaluated for macro (Ca and Mg) and microelements (Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, V and Zn).

3.3. Synthesis of Silver Nanoparticles from Leaf, Bark and Fruit Extracts of G. tiliaefolia

5ml of aqueous leaf extract was taken into the 250 ml conical flask and added 1mM $AgNO_3$ solution under controlled conditions such as temperature and pH.

3.4. Characterization of SNPs

The synthesized silver nanoparticles were subjected and characterized by UV- VIS (UV-VIS -NIR spectrophotometer, Jasco Make, Japan Model: (V-670), XRD (Bruker D8 advance, Germany).

4. Results

4.1. ICP-OES Analysis of Minerals Present in Leaf, Bark and Fruit Powders of G. tiliaefolia

This ICP-OES [59] method was one of the basic parameter used to know about the minerals present in the parts of the tree and this information helps a lot in knowing the characteristic features including the toxicity of phytochemicals in crude form, the formation of phytochemicals were profoundly affected by the minerals. The health condition of a plant and plants showing resistance to the pests, insects other microorganisms responsible for different diseases somewhat depends on mineral content. The mineral ions were showing a great effect on the physiological aspect of every plant life. In this ICP-OES analysis, it was noticed that the leaf, bark and fruit powders have the macro and micro mineral elements N, P, K, Ca, Mg, Zn, Fe, Mn, B and Mb (Table 1).

Table 1. *Shows the results of ICP-OES mineral analysis (dry powders) from leaf, bark, and fruits of G. tiliaefolia.*

Parameter	Units	Readings		
Plant Reference	***G tilifolia***	***G. tiliaefolia leaf***	***G. tiliaefolia bark***	***G. tiliaefolia fruit***
Nitrogen	%	1.72	1.68	1.66
Phosphorus	%	0.35	0.11	0.31
Potassium	%	1.51	0.89	1.26
Calcium	%	2.36	3.73	1.13
Magnesium	%	0.38	0.22	0.24
Zinc	ppm	37.16	25.56	49.48
Iron	ppm	326.5	263.5	280.3
Copper	ppm	--	429.5	142.3
Manganese	ppm	33.65	15.11	44.50
Boron	ppm	41.67	--	--
Molybdenum	ppm	1.235	1.911	3.201

4.2. UV -VIS Graph Analysis of Peaks Formed from Synthesized (SNPs) Extracts of Leaf, Bark and Fruits from Grewia tiliaefolia

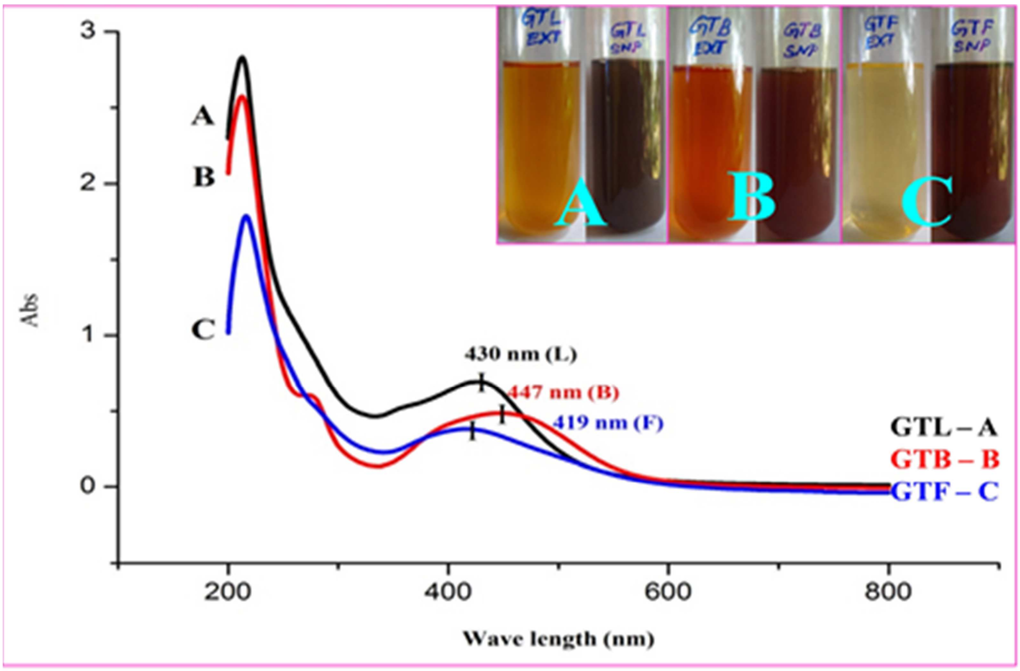

Figure 1. *Shows the results of UV-VIS graphs of synthesized SNPs from leaf (A), bark (B) & fruits (C) of G. tiliaefolia. Inset images show the difference in leaf (A), bark (B), and fruit (C) extracts in test tubes before synthesis (controls - pale in color), and color change in extracts after synthesis of SNPs (A, B & C - dark brown in color).*

This is also a preliminary parameter it helpful to know the synthesis SNPs. It gives the peak between 390 – 450 nm for SNPs if the synthesis has taken place and confirms the formation SNPs. The three parts of synthesized extracts of the leaf (Figure 1A), bark (Figure 1B) & and fruit (Figure 1C) were submitted to the basic parameter i.e., UV-VIS. The synthesized extracts were showing peaks leaf (a) at 430 nm, bark (b) at 447 nm and fruit (c) at 419 nm showing the formation of silver nanoparticles (SNPs) by reduction from $Ag^{+} \rightarrow Ag^{0}$ (Figure 1).

4.3. XRD Graph Analysis of Synthesized SNPs and Other Unwanted Peaks from Leaf, Bark and Fruits of Grewia tiliaefolia

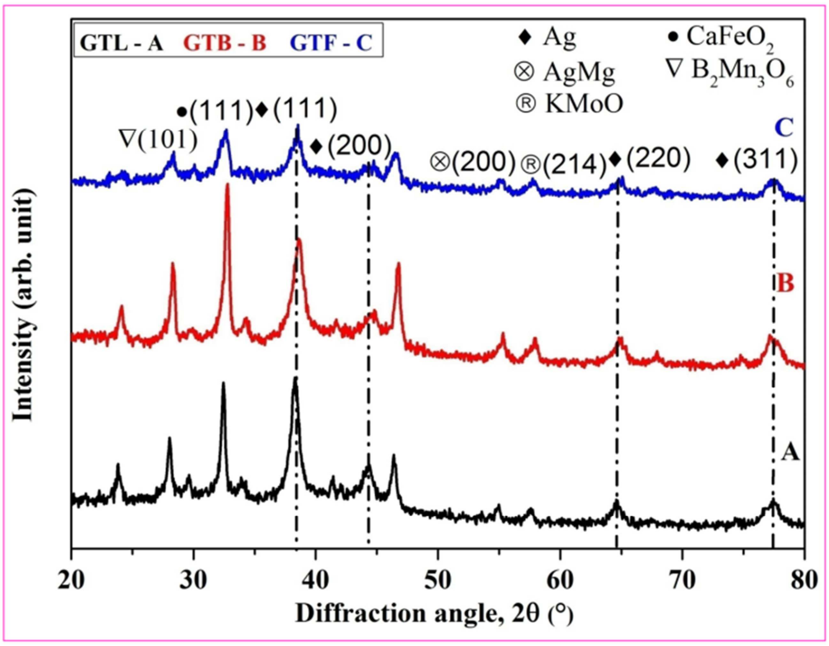

Figure 2. *Shows the results of the analysis of SNPs and other unwanted elemental peaks (impurities) with diffraction angles at 2θ (°) (JCPDS file no.) in the XRD graph after the synthesis process from extracts of the leaf (A), bark (B), and fruits (C) of G. tiliaefolia.*

***Table 2.** Show results of XRD peaks with diffraction angles at 2θ (*) JCPDS file No. and FWHM of SNPs synthesized from leaf, bark, and fruits of G. tiliaefolia.*

Plant parts of	**Diffraction angle position at 2θ(°) Peaks formed a lattice planes**				**JCPDS File No.**	**D=Kλ/β Cosθ**
G. tiliaefolia	**38 (111)**	**44 (200)**	**64 (220)**	**77 (311)**		**FWHM**
Leaf	38.29	44.26	64.49	77.56	04-0783	12.77 nm
Bark	38.45	44.51	64.81	77.33	87-0720	10.14 nm
Fruit	38.44	44.51	64.80	77.43	87-0719	43.37 nm

In the analysis of the SNPs graph of leaf showed the peak positions at 2θ (°) with lattice planes were at 38.19 (111), 44.26 (200), 64.45 (220) and 77.51 (311). In the bark, the peaks were 38.45 (111), 44.51 (200), 64.78 (220) and 77.33 (311) and in the fruit, the graph shows the peaks at 38.35 (111), 44.51 (200), 64.80 (220) and 77.51 (311). These were main peaks for the identification of the formation of the SNPs. The FWHM value was calculated with the help of the standard equation Debye–Scherrer equation $D=K\lambda/\beta \cos\theta$ (Table 2). The impurities were identified as sub-peaks and the peaks were calculated and structures were drawn by using standard methods.

5. Discussion

5.1. ICP-OES Analysis

This method was designed to know the minerals present in the given sample in dry powdered form. The system gives the total information about the mineral elements and their total quantity at the same time they may be in minute quantity. These elements might show negative effects on SNPs from the beginning of the synthesis process to after the formation of SNPs. Due to this SNPs efficiency may affect in a broad range. In this ICP-OES analysis (Table 1), it was noticed that the leaf powder has the macro and micro mineral elements to lead its daily life N (1.72%), P (0.35%), K (1.51%), Ca (2.36%) and Mg (0. 38%) were mentioned in percentages due to their high usage in their daily physiological life and Zn (37.16 ppm), Fe (326.5 ppm), Mn (33.65 ppm), B (41.67 ppm) and Mb (1.235 ppm) in ppm due to their usage was limited. In bark N (1.68%), P (0.11%), K (0.89%), Ca (3.73%) and Mg (0.22%) was mentioned in the percentages and Zn (25.56 ppm), Fe (263.5 p.m.), Cu (429.5 ppm), Mn (15.11 ppm), and Mb (1.911 ppm) were mentioned in the ppm-level. The fruit powder results showed that the N (1.66%), P (0.31%), K (1.26%), Ca (1.13%) and Mg (0.24%) was mentioned in the percentages and Zn (49.48 ppm), Fe (280.3 ppm), Cu (142.3 ppm), Mn (44.50 ppm), and Mb (3.201 ppm) were mentioned in the ppm. The same types of tests of results were found in *Maytenus ilicifolia* Mart.

5.2. UV – VIS Analysis

The extracts change their colour from pale yellow or white colour or colourless to brown and reddish-brown indicates the formation of SNPs by excitation with SPR and UV-VIS provides the information about the formation of SNPs along with size, shape in a controlled condition in aqueous condition [39]. The formation of the perfect peak was Tangible proof for SNPs, it was between 390nm of 450 nm. The peak formation also depends on certain important factors like pH and temperature. The UV results give peaks at different nanometers for the different material of the same plant shows the reduction of silver depends upon the various organic materials present in it; even after the pH and temperature maintained, the formation of the peak occurred at different nanometers [60] because surface Plasmon Resonance (SPR) – Strong absorption in the visible region observed [61]. SNPs particle size, shape, and the dielectric constant of the solvents and adsorption of particles on SNPs surfaces are the reasons for the position and shape of Plasmon absorption of SNPs [3]. The parts contain a number of bio compounds it was said that tannins might involve in the reduction of $AgNO_3$ to SNPs [45].

5.3. XRD Analysis of SNPs

XRD graph (Figure 2) was used to determine the phase distribution, crystallinity, and purity of the synthesized nanoparticles. It was concluded that the nanoparticles were crystalline, having a cubic shape. The average particle size of SNPs synthesized by the present green method can be calculated using the Debye–Scherrer equation $D=K\lambda/\beta\cos\theta$ and found the average size (FWHM) values of SNPs, similar results were reported by other authors [62].

5.4. Novel Approach in Identification and Analysis of Adverse Effects of Impurities Present Along with SNPs Peaks in XRD Graph

Recent Hypothesis told that plants were having a number of elements when plant extracts used for SNPs synthesis these elemental ions becomes impurities on the surface of the SNPs [63]. An Infinitesimal amount of impurities may ready to mar the accuracy of results. XRD graph was giving the SNPs data in the form of peaks; this data was exactly matched to standard reference files of JCPDS file numbers. All peak positions for silver nanoparticles probably forms the diffraction angles at the 2θ value at 38°, 44°, 64° and 77°. The XRD analysis was also used to determine the phase distribution, crystalline nature (FWHM), the purity of the synthesized silver nanoparticles. The crystal structures for SNPs were FCC most probable [64]. While analyzing the XRD graph, generally the peaks other than SNPs were fall into disfavour and treated as Fiddling about sub-peaks made of other elements and called them as impurities. But these peaks formed impurities might play diametrically opposite results as well as shows telling effect against SNPs at different stages of synthesis in a chain process and also after the formation of SNPs. There is a disarmingly frank study need here to solve the problem of impurities and all these were a clear case of different work.

These telling effects include such as impurities might be bottleneck of synthesis; breakneck the synthesis of SNPs; different pH adjustment conditions for different extracts synthesis; the SNPs synthesized were having very crummy nature; these impurities might undermine the SNPs; formation of SNPs in different temperatures; difference in reaction time and delay in colour change sometimes showing different colour formation were observed in inter and intra-species and among species or different parts of the same tree or plant [29]; these may de escalate the colour even after SNPs were synthesized; certain plants are not able to produce SNPs; production of unwanted shapes and sizes; agglomeration of particles [65]; viscous nature of particles; immediate reaction with air even after made into dry powder; poor solubility of SNPs in water once made into dry powder; in long standing SNPs in dried powdered form (in refrigeration) shows decreased in antimicrobial efficacy; change in surface area of absorption on nanoparticles; taking very long time in synthesizing process; reducing the capacity of long standing storage; conversion of SNPs to harmful or poisonous at present or at long standing due to the reactions taking place and formation of unwanted bonds of impurities with SNPs (by S–HCMS); these may change or show a negative effect on the structures of SNPs by that, the effect of binding nature might be decreased; these might be one of the reasons for not getting enough negative charge (in graph) in Zeta potentials by increasing particle sizes [36]; these might slow down the direct drug delivery process present in the SNPs by decreasing SNPs speed and might alter the movement by attracting other unknown molecules. It was noted that these impurities have become Inalienable part of the SNPs. To stop Incalculable damage caused by these impurities and to produce ebullient SNPs to solve the problems caused by the micro and other organisms it needs a lot of research work.

The above mentioned adverse conditions were showing by impurities before and after combining with SNPs, up to now, researchers did work to produce SNPs by the concentration of adding $AgNO_3$ solution as well as a plant extract and maintaining the reaction time and temperature, finally, the results in getting the SNPs and these also have certain drawbacks those were founded by the researchers [66]. Silver ions and SNPs were showing adverse effects against microbes such as bacteria (*Escherichia coli, Staphylococcus aureus*) and yeast. But it was noticed that silver ions were forming complex bonds in a limited way by forming their complexes with these microbes and also found that silver ions effect was remaining for short period, other researcher found that this drawback can be resolved by applying the undivided SNPs which were having highest antimicrobial activity. These can be produced by reactive oxygen species include H_2O_2.

In the recent past in toxicology research about SNPs has shown utterly different adverse effects of SNPs caused oxidative stress and cease of mitochondrial function by SNPs in minute levels (10– 50 μg ml^{-1}). But significant cytotoxicity and caused abnormal cellular morphology, cellular shrinkage, and acquisition of irregular shape was observed at higher doses (>1.0 mg L^{-1}). Principally cytotoxic mechanism of SNPs is based on the initiation of reactive oxygen species (ROS). Hence exposure to silver nanoparticles generates weakening in glutathione level, rising of ROS levels, lipid peroxidation, and increased expression of ROS responsive genes, leading to DNA damage, apoptosis, and necrosis. As a result of large surface area to volume ratio of SNPs, these penetrate all parts through ingestion, inhalation or skin as well as all systems of the human body such as circulatory system, nervous system, etc. Silver nanoparticles can induce adversely toxic effect to the male reproductive system, on the proliferation and cytokine expression by peripheral blood mononuclear cells. In case of male and female Sprague–Dawley rat bone marrow in vivo in his gastrointestinal toxicology studies, it was noticed that SNPs at the size of 60 nm for 28 days have shown no such genetic toxicity and no significant changes were observed regarding body weight of both the rats. But exposing to over more than 300 mg of SNPs, alkaline phosphatase and cholesterol values were altered resulting in little damage to the liver was observed [12]. In another study 0.4-30 μg amount of silver was daily accumulated in humans it comes from natural food and water. The recent studies revealed different types of results regarding the toxic effects of SNPs within biological systems such as bacteria, viruses, human cells as well. Generally, SNPs were known as effective antimicrobial agents, with non-toxic effects to healthy mammalian cells. But different in vitro studies have shown the nano-silver-related toxic effects in rat hepatocytes and neuronal cells, murine stem cells, and human lung epithelial cells. Alveolar regions leading to lung injuries and can also cause significant changes within the nervous system, also in liver and kidney tissues when SNPs breathed in. SNPs when deposited in intratracheal region start affecting functions of vascular system additionally exacerbate cardiac reperfusion or in chemia injury. Changes in permeability in the membrane of mitochondria and damage to DNA were caused by nanosilver intracellular accumulation of ROS. Exposing in-vitro conditions to SNPs shows increases in oxidative stress, genotoxicity and apoptosis levels were reported. The activation of innate immunity and increasing the permeability of endothelial cells were due to the inflammatory responses of oxidative stress caused by SNPs. Chromosomal abnormality, DNA damage and required mutagenicity were reported even SNPs vaccinated at minute non-cytotoxic doses. Dispersion rate, concentration, surface charge, size, morphology and surface fictionalization were the physicochemical features of SNPs which influence genotoxicity and cytotoxicity. Till now the accurate toxic effects of SNPs and their related toxicity mechanisms reports were inadequate [36].

The other things have to note that the directly consumed plant parts were also showing negative effects. All herbs, shrubs as well as trees were made of organic and mineral contents, these minerals were present in different concentrations depending upon the different environmental or ecological conditions like soil texture types, pH of the soil, availability of water, mineral contents present in that area [67].

These conditions were used by the trees and they grow either against or by adapting the conditions there. Most of the trees were accumulated certain unimportant, sometimes heavy elements, reports were shown that a few were having detoxification capacity but few are having the hyperaccumulation of toxic metals examples are *Arabidopsis halleri* and *Thlaspi caerulescens*. Various plants such as Acanthopanax sciadophylloides, Maytenus founieri, Brassica juncea, Ilex crenata, Sesbania drummondii, and Clethra barbinervis have enormous potential for phytoremediation of heavy metals [7]. These unwanted minerals may be harmful to all living beings, those consuming them, this may be most directly sometimes the harness is adumbrating. The elements present in the plant material were tested with standard method ICP-OES. The list of elements, mostly present in the plant parts was appeared on the XRD graph as sub-peaks along with the SNPs. These elements were not in the single-molecule form but forming complexes with other elements molecules.

The above mentioned were of immense statements about the toxicity of the SNPs in different ways and the reasons. This was related to study about the mineral or elements in all plant parts and their characteristics were mentioned in previous works. But these elemental ions were showing very interesting characteristics either individually or among interlinking themselves or by combing with SNPs and become nub of the toxicity characteristics of SNPs synthesized from plants. Even though elements were present in different concentrations i.e. in a minute or maximum concentrations can deviate the main source i.e., SNPs and their characteristics by attracting and forming bonds with them because these mineral or elementals were having magnetic properties. Recently, researchers have been working on the impurities appeared as low-intensity peaks in the XRD graph [ICDD - International Centre for Diffraction Data] creating starting revolution along with starting results [68]. These impurities might have magnetic properties, and these can be measured. This statement was supporting the above-mentioned work. The study was completed by examining and with the comparison of leaf, bark and fruit synthesized extracts. Generally speaking, a tree was having the same type of minerals in their parts but it depending upon the utilization a few elements were confined to certain parts of that tree and absent in other parts.

In the present analysis of XRD graph, it was noticed that the leaf, bark and fruits of this plant or tree were having the nearly same type of mineral or elemental ions and rarely a few mineral ions were confined to only certain parts. Any tree species parts were mostly having the same type of minerals, but sometimes concentration was increased depending upon the utilization in the physiological activities due to this they may become most important for that part only. Certain unknown, unwanted or unnecessary minerals were also accumulating into certain parts especially it depends on the growing areas of those plants or trees (Table 1) in either smaller or larger quantity may influence the total SNPs during formation and also after synthesis has taken place. These mineral ions in XRD were reported as impurities, these were noticed in the same conditions in three parts.

Table 3. *Show results of Simple to Highly Complex Molecular Structures (S-HCMS) formed by mineral elements mentioned in ICP-OES system and peaks at different levels with standard files matched with PCPDWIN peaks.*

S.No.	Elements shows in ICP-OES	Standard Peaks shown by elements in XRD	Matched Peak in PCPDWIN	File No.	Complex formed
1	Ca	*32.51*	32.51	21-0917	$CaFeO_2$
2.	Mg/Mn	*55.25/55.25*	55.41/55.27	29-0871/76-0718	$AgMg/B_2Mn_3O_6$
3.	K	*28.43*	28.29	50-0149	$KMgPO_4$
4.	K	*24.02*	24.77	50-0149	$KMgPO_4$
5.	K	*57.87*	57.89	50-0149	$KMgPO_4$
6.	K	*67.62*	67.46	50-0149	$KMgPO_4$
7.	Zn	*46.76*	46.75	37-1486	ZnB_2O_6
8.	Ca	*74.93*	74.91	47-1148	Ca_2Zn
*		*57.87*	57.87	24-0879	KMo

Table 4. *Shows the results of COD matching files and Simple to Highly Complex Molecules (S-HCM Structures) references.*

S.No.	COD CIF - File No.	Complex molecule formed	Reap	GOD	References from Crystallographic data
1.	1509596	AgCaMg	6.30	9.44	Nowotny, 37, 31–34, (1946).
2.	4119331***	AgBMn	6.30	11.50	Holger Braunschweig *et al.* 135, 2313 – 2320, (2013).
3.	8103396	AgPK	6.30	11.46	Somer M,; Asbrand, M.; Eisen-mann, B., 212, 78–78, (1997).
4.	2105354	AgZnMo	6.30	9.35	Komura, Y.; Tokunaga, K., 36, 1548-1554, (1980).
5.	2105820 **	AgNFe	6.30	11.75	Rodriguez-Velamazan *et al.* 70, 436-443, (2014).
6.	1531911	AgNB	6.30	11.18	Malinina *et al.* 47, 1275-1284, (2002).
7.	1509463	AgNMn	6.31	6.86	Fruchart *et al.* 259, 392-393, (1964).
8.	4061733***	AgFeB	6.30	4.53	Franken *et al.* (2005).
9.	1509802	AgFeMn	6.30	6.73	Chouaibi *et al.,* 159, 46-50, (2001).

G. tiliaefolia. Generally, these mineral ions were not considered along with main peaks of Ag and these peaks were come by unknown elements at the same time their functions were not known. But by keen observation of the peaks, it was noticed that the elements appeared in ICP-OES was related to XRD unwanted or impure peaks. These ICP-OES elements were tested in crystallographic data and found certain cards with file number having a possible group forming elements and 'Ag' was taken as the core element in all the categories (Table 3) and correlated with previous literature such as combination. The unknown peaks or impurities peaks in XRD were tested with PDPCWIN files, certain values are nearly and few values are matched exactly with crystallographic data card no. But they combined with other elements also it shows multi-elemental bondings [69-77] (Table 4). Nine S–HCMS (Figure 3) with schematic illustrations (3D view) were drawn by the advanced software's and in this, it was represents the number of atoms present in the compound and its Bond length also. A few elements like Potassium have polycrystalline nature. The molecular structures are the element Boron with Fe (Ag Basic), Boron with Mn are showing highly complex structures with different shapes. The complex formed by the N with Fe is also forming complex structures. Zn with Mo is also forming a slight complex structure. But Fe with Mn is forming a simple structure. Fe with N and Fe with B are forming more complex structures.

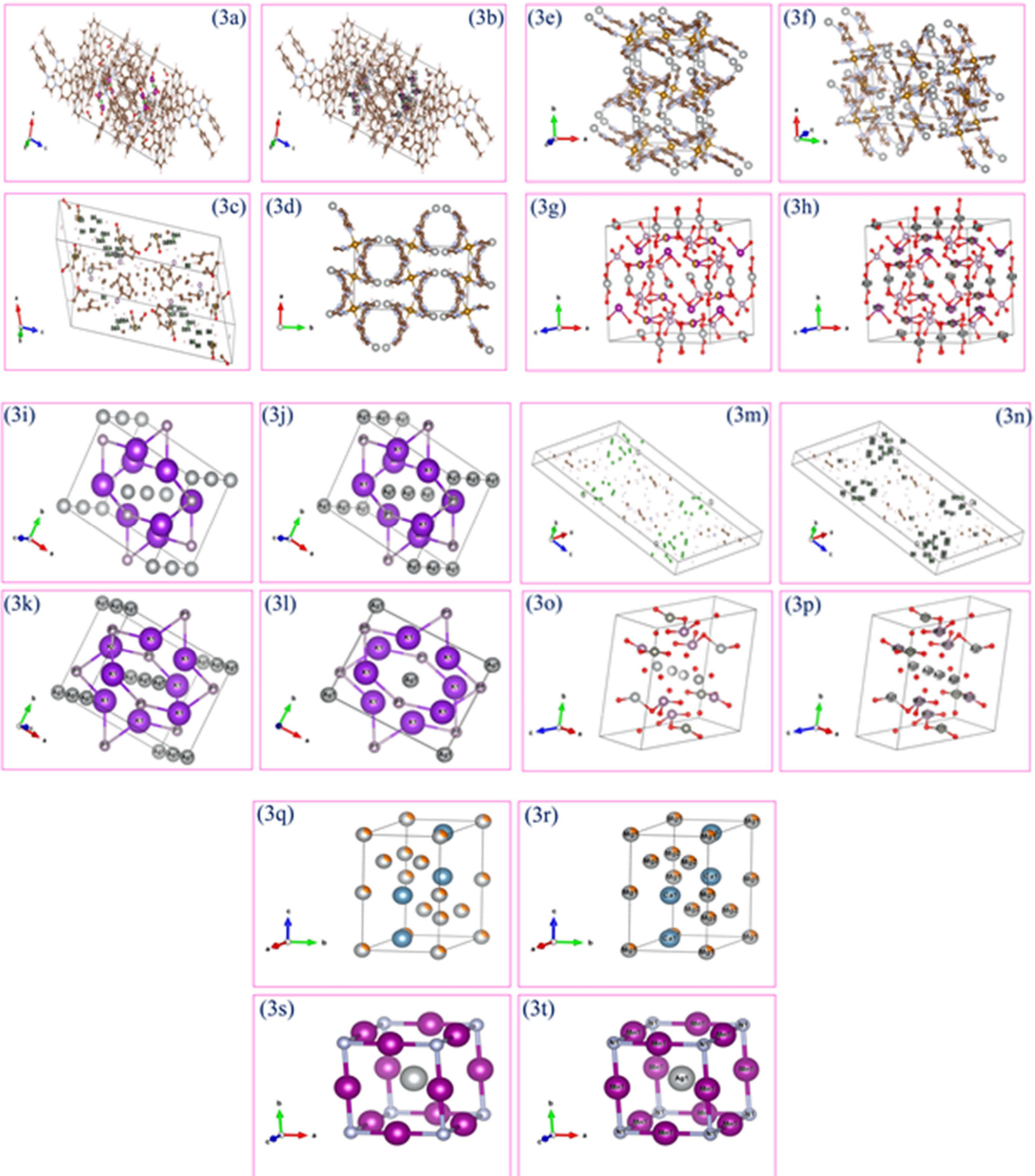

Figure 3. *Show results of Simple to Highly complex Molecular Structures (S-HCMS in 3D view) formed after synthesis of SNPs along with silver (Ag) with strong bonding includes 3a&b – AgBMn; 3c – AgFeB; 3 d,e&f – AgNFe; 3g&h – AgFeMn; 3i,jk&l – AgPK; 3m&n – AgNB; 3o&p –AgZnMo; 3q&r – AgCaMg; 3s&t - AgNMn.*

6. Conclusion

The present drugs are not causing enough damage against most of the dangerous microbes causing diseases. Microorganisms becoming more resistance to present drugs, not only that these impurities might helps in acquiring resistance against the drugs and these impurities might help in the increasing the strength of enzymes of microbes due to this they are causing more damage even after using novel drugs. In this paper, work about impurities and a number of negative impacts were discussed. Several research workers have been working on some of these problems and have shown certain important solutions to prevent agglomeration of nanoparticles [32]. A number of researchers have discussed the adverse effects of SNPs. Synthesis of nanostructures with fewer defects, the more homogenous composition of different chemicals (organic and inorganic as well) and better short and long-range ordering [32]. Improving the health of humans, animals and useful plant sources can be done by providing improved novel drugs which should be eco-friendly. The production of improved and novel drugs can be possible by removing these impurities otherwise these show repercussion and try to revamp the novelty of drugs. The work going on impurities might be a nugget. At present there is a need to produce costly drugs at the same time there is a possibility in improving the present drugs in lieu of production of costly drugs hence there is need to do more research.

Abbreviations

ICP-OES: Inductively Coupled Plasma Optical Emission spectroscopy
SNPs: Silver Nanoparticles
GT/ *G. tiliaefolia*: *Grewia tiliaefolia* Vahl
FWHM: Full Width at Half Maximum
FCC: Face Centered Cubic structure
COD: Crystallography Open Database
PCPDWIN: XRD pattern analysis software
JCPDS: Joint Committee on Powder Diffraction Standards
ICDD: International Centre for Diffraction Data

Declaration

Availability of Data and Material

Not applicable. As a corresponding author I read and approved the final manuscript.

Competing Interests

The author declares that there are no competing interests.

Authors' Contributions

PRM collected plant material, and performed experiment, data analysis and wrote the manuscript; NS supervised this work and given necessary suggestions.

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