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Green Synthesis of Metal Nanoparticles using *Ocimum sanctum* (Tulsi)

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Abstract
In recent literature, the area of metal nanoparticles has found considerable attraction due to the versatile role of such nanoparticles in biomedical science, catalysis, antimicrobial study, cosmetics, energy production, pollution control and information storage. The effective use of these metal nanoparticles as material and medicine depend on their shape, size and functional groups surrounding them. The conventional methods used for synthesis of metal nanoparticles are not environment friendly. Green synthesis of metal nanoparticles, being eco-friendly, bio-compatible and less toxic, is an alternative approach in this regard. *Ocimum sanctum* or 'tulsi' is known to be the most sacred aromatic plant found in India. According to Indian traditional medicine such as Ayurveda, Unani and Siddha, almost all the parts of tulsi tree are known to have some therapeutic property. The extracts from different parts of the plant, specifically from the leaf, have been reported for synthesis of different metal nanoparticles including silver, gold, copper, palladium, iron etc. These nanoparticles are potentially applicable in the area of medicine, material science and catalysis. The present review provides a brief account of the synthesis, spectrochemical analysis and potential applications of these nanoparticles.

Keywords
Ocimum Sanctum, green synthesis, metal nanoparticle, silver, antibacterial study

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1. INTRODUCTION:
In recent years, nanotechnology has emerged as one of the most prominent fields of research in material science. As properties of such nanoparticles depend mainly on their size, metal nanoparticles have found miscellaneous potential applications.[1,2] There are various physical and chemical methods for synthesis of metal nanomaterials including hydrothermal, thermal, chemical reduction, photochemical reduction, electrochemical reduction, etc. Although these methods are useful in successful synthesis of nanomaterials, there are some major drawbacks such as requirement of high energy, sophisticated equipment and most importantly use of toxic reagents and solvents. In alternative approach, nanoparticles are synthesized using different plant extracts or microorganisms and also using new energy transfer techniques and equipments like sonochemistry, microwave, etc.[3-16] When plant extracts are used for the synthesis of metal

nanoparticles in place of harmful or toxic chemicals, the process is known as "green nanotechnology".

The efficiency of these metal nanoparticles in various fields of applications depends on their size and the functional groups present on their surface. The concentration and physicochemical nature of the capping agent(s) used during synthesis of the nanoparticles are also very important in this regard. The green reagents used for synthesis of nanoparticles contain numerous biomolecules with diverse chemical structures. The biogenic reagents used so far for synthesis of nanoparticles comprise of extracts from important medicinal plants, bacteria,[17] fungi[18] and enzymes.[17] The use of microorganism and enzyme requires sophisticated instrumentation and complicated cell culture processes. The plant extract-based methods are simple, less costly, and environment-friendly. The green reagents with plant extracts are useful for both micro- and macroscale synthesis of metallic nanoparticles. Most importantly, these plant extracts themselves are inherently useful for pharmacological applications. So, the synthesized metal nanoparticles have additional biomedical functioning. In recent literature, various medicinal plants are mentioned for synthesis of metal nanoparticles. The list of important medicinal plants includes *Azadirachta indica* (Neem),[19] *Boswellia ovalifoliolata*,[20] *Carica papaya* (Papaya),[21] *Catharanthus roseus* (Periwinkle Sadabahar),[22] *Cinnamomum camphora* (camphor tree),[23] *Citrus aurantium* (Sweet Orange, Mosambi),[24] *Datura metel* (Indian Thornapple, Hindu Datura),[25] *Jatropha curcas* (physic nut), *Medicago sativa* (Alfalfa),[26] *Nelumbo nucifera* (lotus),[27] *Pelargonium graveolens* (rose geranium),[28] *Solanum melongena* (eggplant, brinjal), *Tridax procumbens* (tridax daisy), etc.

The holy plant *Ocimum sanctum* (tulsi), an important ingredient in many formulations, is regularly used for the prevention and treatment of different diseases. The extract and essential oil from various parts of the plant including leaves, roots and seeds are recognized for antimicrobial, anti-diabetic, anti-carcinogenic and anti-inflammatory properties. The major phytochemicals in these extracts are of alkaloid, terpenoid, tannin, phenolic, steroid and saponin type. In addition to their antibacterial activity, these classes of molecules have reducing and stabilizing ability for synthesis of metal nanoparticles. Thus, in recent reports, extract from different parts of *Ocimum sanctum* plant have been used for synthesis and stabilization of nanoparticles of different metals including gold, silver, palladium, platinum, nickel, etc. The present review summarizes these green methods for the synthesis of metal nanoparticles with brief account of their properties and potential applications.

2. *Ocimum sanctum*:

Ocimum sanctum or *Ocimum tenuiflorum* is a short-lived perennial shrub with average height of 30–75 cm, hairy stems and sparsely hairy leaves. It belongs to *Lamiaceae* family and it is also known as *Ocimum sanctum* L. or *Ocimum tenuiflorum* L. For *Ocimum* (tulsi), there are approximately 160 different varieties including *Ocimum sanctum*, *Ocimum tenuiflorum* (Krishna tulsi), *Ocimum canum* (Dulal tulsi), *Ocimum gratissimum* (Rama tulsi), *Ocimum americanum*, *Ocimum camphora*, *Ocimum kilimandscharicum* and *Ocimum micranthu*.[29,30] These plants are widely distributed throughout the warm regions of the world and traditionally used for different medicinal purposes. In most parts of India, *ocimum* with green leaves are known as Rama tulsi and with purple leaves as Krishna tulsi. All these plants are aromatic, erect, branched annual or biennial subshrub with simple, opposite, green leaves that are strongly scented. The leaves of the plant are ovate, oblong, obtuse or acute and up to 5 cm in length. The flowers of this holy plant are purple coloured, and the fruits are small reddish–yellow in colour. *Ocimum sanctum* is grown throughout India from the Himalayas to the Andaman and Nicobar Islands. It is also found in several Arab countries, as well as in Malaysia, Australia, and West Africa.

2a. Phytochemistry:

Different parts of *Ocimum sanctum* have very complex chemical composition with a huge number of phytochemicals and their composition may vary in different types of plants. The characteristic aroma of *Ocimum sanctum* is due to volatile essential oil present mainly in the leaves of the plant. The major components of this volatile oil are eugenol (70%) and methyl eugenol (20%). The other minor components of volatile oil are limatrol, linalool, carvacrol, ursolic acid and caryophyllene. The volatile oil extracted from seeds of *Ocimum sanctum* contains sitosterol and fatty acids. In addition to considerable amounts of eugenol, the fresh leaves and stem of the plant contain several other phenolic compounds like cirsilineol, cirsimaritin, isothymusin, apigenin, rosmarinic acid and isothymonin (Figure 1). The leaf extract also contains quite a few flavonoids, including orientin and vicenin, ursolic acid, luteolin and molludistin. Several other compounds like tannins and a number of monoterpenes and sesquiterpenes, including β-elemene, neral, bornyl acetate, β-pinene, stigmasterol, α-pinene and cholesterol are also extracted from *Ocimum sanctum*.[31, 32]

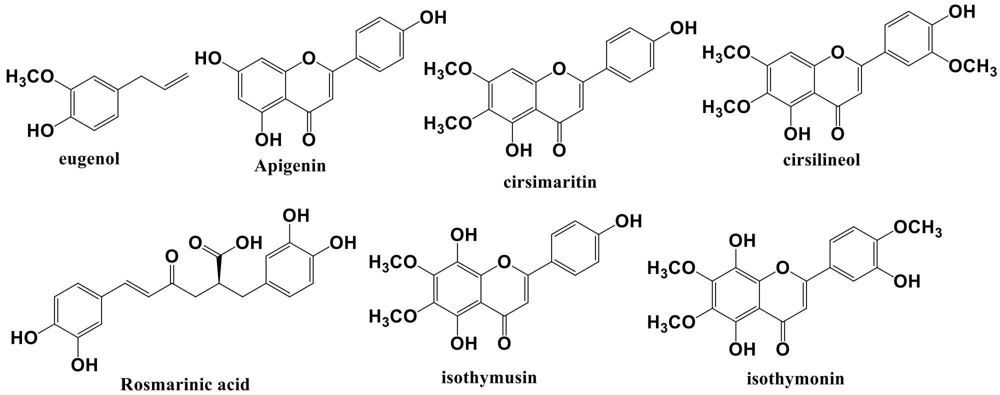


Figure 1. Chemical structures of the major phytoconstituents present in *Ocimum sanctum*

2b. Medicinal Use:

The name of the plant 'tulsi' is derived from the Sanskrit word that means 'matchless one'. Tulsi is also known as 'The Queen of Herbs' as it has been used for treatment of body, mind and spirit in India for more than 5000 years. Different parts of this plant are well known as traditional medicine in Ayurveda, Unani, Siddha, Greek, and Roman. Tulsi is described as 'the elixir of life' in Ayurveda as it is believed to support longevity. In recent years, scientific studies have revealed that the extracts of tulsi and its essential oil have numerous potential medical applications including antimicrobial, anti-inflammatory, analgesic, antiasthmatic, antipyretic etc.[33, 34] Experimental studies have established that the principal phytochemicals of tulsi are responsible for these pharmacological properties.

3. Synthesis of nanoparticles:

Recently, various plant extracts mediated biosynthesis of metal nanoparticles have gained significant attention due to the ease of synthesis and green reaction conditions. A broad variety of phytochemicals extracted from different parts of *Ocimum sanctum* (tulsi) plant are extensively used as safe and nontoxic reagent for synthesis of metal nanoparticles. The extract from different parts of *Ocimum sanctum* act as both reducing and capping agent in synthesis of various metal nanoparticles.

3a. Silver nanoparticle:

Silver is the most diversely used and extensively studied metal in the area of green synthesis of metal nanoparticles. In the initial report, extract from room temperature dried stem and root of *Ocimum sanctum* was used for synthesis of silver nanoparticles (AgNPs).[35] The nanoparticles were studied using transmission electron microscopy (TEM), X-ray diffraction (XRD) and selected area electron diffraction (SAED) spectra. According to TEM study, the silver nanoparticles obtained using root and stem extract were of sizes 10±2 nm and 5±1.5 nm respectively.

In another report, *Ocimum sanctum* leaf extract was used to reduce silver ions into silver nanoparticles with 4–30 nm size and having antimicrobial activity.[36] From DLS study, the Zeta average diameter was found to be 22.38 ± 3.56 nm with polydispersity index (PDI) of 0.310. The size of the nanoparticle measured using DLS is slightly bigger as compared to the particle size measured from TEM micrographs. It is because dynamic light scattering (DLS) method measures the hydrodynamic radius. These nanoparticles have antimicrobial activity against both gram-positive and Gram-negative bacteria.

In another report, AgNPs were prepared with leaf extract of *Ocimum sanctum*, where the scanning electron microscopy (SEM) images showed almost spherical nanoparticle with diameter of 40 to 50 nm.[37] When UV-Visible spectrograph was recorded as a function of time for the colloidal solution of silver nanoparticles, it showed broadening of peak at 430 nm with increase in time. This indicated that the particles are polydispersed in nature. The XRD results suggested crystallization of the bio-organic phase on the surface of the silver nanoparticles.

In a comparative study, silver nanoparticles were synthesized using *Ocimum sanctum* extract and it was compared with silver nanoparticles (AgNPs) that were chemically synthesized and citrate stabilized.[38] The AgNPs synthesized with *Ocimum sanctum* extract have shown better antimicrobial activities against both gram-positive and gram-negative bacteria.

Another report on green synthesis of silver nanoparticles (AgNPs) using *Ocimum sanctum*[39] leaf extract showed that the size of nanoparticles was around 42 nm. AgNPs were rapidly synthesized using aqueous extract of tulsi leaf with $AgNO_3$ solution within 15 minutes. These nanoparticles were compared to conventional formulations as a reducing and stabilizing agent. For these green silver nanoparticles, the zeta potential of AgNPs was found to be -55.0 mV indicating high stability of the nanoparticles.

To understand the impact of other surfactant in green method, silver nanoparticles were synthesized using *Ocimum sanctum* leaf extract as reducing agent in a combination with cetyltrimethylammonium bromide (CTAB).[40] From TEM study, it was found that the average particle size ranged from 18 to 35 nm and most of the particles are sphere-shaped. In UV-Visible spectra, the characteristic peak for AgNPs was observed at 450 nm. The shape and size of the nanoparticle and the position of the absorption maxima was found to be dependent on the concentration of CTAB in solution.

Besides different parts of the plant, the root surface of intact plant *Ocimum sanctum* also have immense reducing strength to reduce silver ion (Ag^+) and produce silver nanoparticle. The roots of the plant can change clear colourless silver nitrate solutions to turbid brown.[41] The formation of silver nanoparticles was indicated by absorption spectra of these turbid brown solutions with specific surface plasmon resonance peak. The X-ray data indicated characteristic of crystalline face-centered cubic structure of Ag^0 and cubic structure of Ag_2O. These results revealed the inbuilt potential of root system to generate Ag^0/Ag_2O-NPs. The roots of intact plants can also reduce triphenyl tetrazolium to triphenyl formazon and root membrane impermeable ferricyanide ion to ferrocyanide This suggested involvement of plasma membrane bound enzyme dehydrogenases in reduction of Ag^+ and formation of Ag^0/Ag_2O-NPs. From UV-Visible study and X-ray data, the size of silver nanoparticle generated by root surface of intact plant *Ocimum sanctum* was calculated to be 20-50 nm.

In another report silver nanoparticles were synthesized using leaf extract of *Ocimum sanctum* extract in presence of direct sunlight.[42] The UV-Visible spectra exhibited the absorption band maxima at 430 nm which is attributed to surface plasmon resonance (SPR) of silver nanoparticles. From TEM study, it was observed that silver nanoparticles were spherical in shape and most of them have particle size of 7-11 nm. These nanoparticles showed prospective antibacterial efficacy against gram-positive and gram-negative bacteria.

The AgNPs synthesized using leaf extract of *Ocimum basilicum* and *Ocimum sanctum* showed excellent enzyme inhibitory activity and antibacterial properties. In addition to this, the phenolic rich plants such as *Ocimum* species have potent antioxidant activity. Thus, green synthesis of AgNp based drugs has huge opportunities for the development of low cost, highly efficient therapy for hyperglycaemia, and prevention of diabetic induced complications such as peripheral neuropathy that predisposes bacterial infections.[43]

Silver nanocarrier mediated targeted delivery and biosensing have a lot of scope in medical research especially in reproductive health care. In an advanced approach, silver nanoparticles were loaded inside the multi-walled Carbon nanotube (MWCNT) which was designed to target the intracellular part of the sperm cell. It was planned to be used for biosensing based infertility diagnosis. AgNPs were photosynthesized using *Ocimum tenuiflorum* or tulsi extract. The AgNPs synthesized exhibited spherical shape with particle size of 5-40 nm and surface plasmon resonance at 430 nm. The loading of freshly prepared AgNPs into emulsified MWCNT, was confirmed with spectroscopic and microscopic methods including FT-IR analysis. Interestingly, heat flow analysis revealed the improved stability of AgNPs after loading on MWCNT and the encapsulated AgNPs has particle size of 10 nm. The homogeneous loading of AgNPs on MWCNT was confirmed by surface enhanced Raman spectroscopy (SERS). Finally, DNA fragmentation and morphological examination confirmed the binding and targetability of AgNPs to the sperm nucleus which is suitable in fertility diagnosis.[44]

Ocimum sanctum leaf extract is also useful for the synthesis of cellulose-silver nanoparticle composite films.[45] Here the plant extract acts as reducing agent in the absence and presence of sunlight and the synthesized nanoparticles were characterized by SEM, FT-IR, XRD and antibacterial tests. Sunlight hastened up the preparation of these composite films. The average size of the *in situ* generated AgNPs was reduced by the sunlight. The antibacterial activity and other properties of the composites were enhanced by the sunlight. The cellulose/AgNP composite films with improved properties by sunlight can be considered for medical purpose as antibacterial dressing materials.

Cobalt ferrite and silver doped cobalt ferrite nanoparticles were synthesized using the seed extract of *Ocimum sanctum* (tulsi) and *Allium sativum* (garlic cloves).[46] Systematic X-ray studies confirmed that the prepared samples are crystalline and have cubic (inverse spinel) structure. Magnetic measurements through Vibrating Sample Magnetometer (VSM) at room temperature showed that the magnetic saturation values for samples obtained using tulsi seed extract are much higher than those obtained using garlic extract. All the prepared silver nanoparticles showed antibacterial activity against gram-positive and gram-negative bacteria.

A further modification around silver nanoparticles was achieved by effective impregnation of silver nanoparticles on the conventional tissue paper for the inhibition of hospital borne pathogenic bacteria growth.[47] The AgNPs were biosynthesized using *Ocimum sanctum* leaf extract. The AgNPs synthesized using the leaf extracts had face centered cubic lattice structure and nano scale dimensions. It gave absorbance maximum at 438 nm in UV-Visible spectrum of the nanoparticles. These nanoparticles were used to engineer tissue paper towels and at the same time they provided biocompatible coating of plant proteins on the tissue paper. The extent of coating by the nanoparticles on the tissue paper was examined by SEM analysis, XRD and FT-IR study. These studies confirmed the presence of AgNPs on the paper fibres and also indicated the appreciable amounts of bio-active functional groups on the modified tissue paper. These nano-engineered tissue paper towels showed antibacterial activity against common gram-positive as well as gram-negative pathogens. From MTT assay, it was revealed that the AgNPs coated paper towels do not pose any unintended cytotoxicity. This result highlighted the prospect of green synthesis of cost-effective sanitizing product for decreasing the risk of health associated infections.

In another approach, silver nanoparticle synthesized with *Ocimum tenuiflorum* leaf extract was impregnated on TiO_2.[48] This nano silver impregnated TiO_2 acted as heterogeneous catalyst for oxidation of thiophene to thiophene sulphone (Figure 2). In the oxidation reaction of thiophene, tertiary butyl hydroperoxide was used as an oxidant. From SEM study, the average particle size of the catalyst nanoparticle was found to be 128.71 nm.

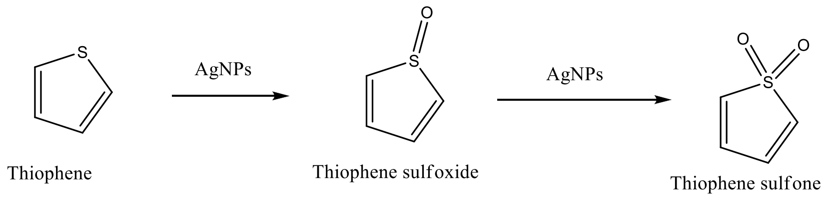


Figure 2. Oxidation of thiophene to corresponding oxide and sulfone

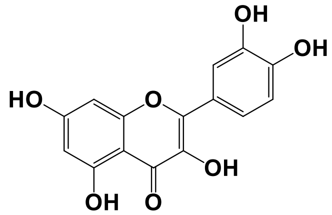
Controlled release of silver nanoparticles and phytochemicals is a useful approach for wound dressing. In a recent report, flexible silver nanoparticles containing nanocomposite hydrogel were prepared using combination of silver modified polyvinyl alcohol (PVA) and chitosan hydrogels with the help of physical cross-linking techniques.[49] It reduced the toxic effects of the ingredients and prevented silver accumulation due to high cross-linking and dense inter-polymeric network. From spectral analysis, morphological studies and particle size analysis the diameter of the silver nanoparticles was found to be 25-30 nm. These studies also indicated the successfully fabricated and homogeneously dispersed silver nanoparticles in the hydrogel network. The highly interconnected porous structure, with uniformly distributed AgNPs, suitable tensile strength and biocompatibility, is responsible for the controlled release of silver nanoparticles and its wound healing property.

The anti-microbial activity of silver nanoparticles can further be improved in a combination with ZnO nanoparticles. Nanocomposite containing silver and ZnO nanoparticles (Ag/ZnO-NCs) was synthesized by one step green reaction using *Ocimum tenuiflorum* plant seed extract.[50] Three different types of Ag/ZnO-NCs were prepared using 0.5%, 1% and 2% of silver nanoparticles. The TEM image of the samples showed the Ag/ZnO-NCs consisting of metallic Ag nanoparticles. Pure ZnO-NPs had flake shape nanoparticles with an average size of 30-40 nm. For 0.5%, 1% and 2% Ag/ZnO-NCs, the size of the nanoparticles was found to be 25-35 nm, 15-22 nm and 50-60 nm, respectively. In all of them, the nanoparticles were having both hexagonal and spherical shapes. These Ag/ZnO-NCs have much better antimicrobial activity as compared to pure ZnO-NPs. The addition of specific amount of silver in ZnO-NPs creates a low energy band gap of 0.1%. The generation of the oxidizing radicals in the nanocomposites was verified by EPR spectroscopy and they are potentially useful for photocatalytic activity.

The green synthesis of silver nanoparticles is not only important in biomedical research but also useful in agricultural field. Silver nanoparticles were synthesized from aqueous solution of silver salts using plant extracts from *Ocimum sanctum* and *Ocimum gratissimum*.[51] The formation of silver nanoparticle was established by the presence of peak between 400-420 nm observed in the UV-Visible spectra. The crystalline nature of the obtained AgNPs was confirmed by X-ray diffraction analysis and the average particle sizes of the AgNPs from *Ocimum* *sanctum* and *Ocimum gratissimum* were 15.31 nm and 17.64 nm, respectively. The XRD and UV-Visible studies indicated that *Ocimum sanctum* has better reducing property. The synthesized silver nanoparticles showed antimicrobial activity when studied on *Vigna radiata* seeds.

For rapid synthesis of silver nano-colloid, silver ions were treated with blend of extracts from two leaves-*Ocimum sanctum* and *Azadirachta indica*.[52] The synthesized colloid was stable with spherical particles of size 10 nm. The blend of leaf extract acted as a better reducing and stabilizing agent. These synthesized nano-colloids exhibited a good colorimetric sensing property towards carcinogenic Cr (VI) in aqueous solution by changing the colour of the solution from ''brown to red''. The change in colour was accompanied with an increase in absorption intensity. The detection limit of the sensor was found to be 10^{-6} M and there was no interference observed for detection of Cr (VI) in the presence of other cations and anions. The proposed sensor is potentially applicable for the detection of Cr (VI) in drinking water samples. Another important feature of these silver nanoparticles was their antidiabetic property as they displayed an enhanced inhibitory effect towards a α-glucosidase and amylase enzyme model. These bioderived AgNPs have the scope for the development of new nano-therapy for the treatment of diabetes.

Quercetin is a flavonoid present in *Ocimum sanctum* (tulsi plant). Silver nanoparticles were synthesized using leaf extracts of *Ocimum sanctum* (tulsi) and its derivative quercetin separately as precursors to investigate the role of biomolecules present in tulsi in the formation of AgNPs.[53] The synthesized AgNPs exhibited strong absorption maximum between 400–450 nm and the size of the AgNPs obtained were between 10–20 nm in both the cases under different physicochemical conditions such as *p*H, temperature, reaction time and reactants concentration. The silver nanoparticles from both the cases have same optical, morphological, and antibacterial characteristics, which indicated that biomolecules like quercetin (Figure 3) present in *Ocimum sanctum* (tulsi) are mainly responsible for the reduction of metal ions to metal nanoparticles.



Quercetin

Figure 3. Structure of quarcetin

Silver nanoparticle synthesized with *Ocimum sanctum* leaf extract can also be used as catalyst at ambient temperature in presence of sunlight. It is useful for the catalytic reduction of 4-nitrophenol to 4-nitrophenol (Figure 4).[54] In this one pot green synthetic process, sunlight was used as the clean source of energy. The average diameter of the silver nanoparticles, as observed from the HR-TEM analysis, was approximately 5–10 nm and with zeta potential value of −11 mV. These AgNPs gave sharp and strong plasmon resonance at 430 nm.

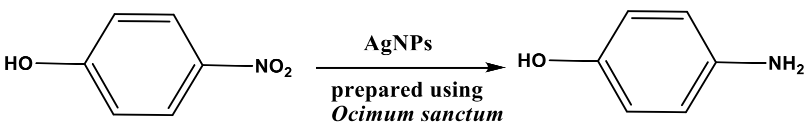


Figure 4. Reduction of 4-nitrophenol in presence of AgNPs

Bio-fabricated silver nanoparticles were synthesized using Carbopol-dependent nanosilver gel.[55] The silver salt was reduced using *Ocimum sanctum* leaf extract. The particle size of the silver nanoparticle was found to be 20 nm. The silver nanoparticles were analyzed with the help of UV-Visible spectra where Plasmon resonance band was observed in the range of 436–446 nm. When the concentration of the plant extract was increased, the absorption spectra changed indicating the change in particle size. From *in vitro* animal study, it was observed that this Carbopol-dependent nanosilver gel has shown superior wound recovering potential compared to conventional formulations as studied.

3b. Gold nanoparticle:

Gold nanoparticles, due to their exceptionally tuneable, unique surface plasmon resonance (SPR), have found potential applications in biomedical science. Gold nanoparticles are useful candidates for application in clinical analysis of pathogens, photothermal therapy and drug delivery. In one of the method reported earlier, rapid single-pot biosynthesis of gold nanoparticles was done using the leaf extract of *Ocimum sanctum*.[56] From TEM study, the morphology and size of the gold nanoparticles were determined where most of the nanoparticles had size of 13 nm with spherical distribution.

Gold nanoparticles can also be synthesized using the root of intact *Ocimum sanctum* plants in aqueous phase under ambient conditions. During this synthesis of gold nanoparticle, the colour of the aqueous solution changed from pale yellow to purple or golden.[57] The gold nanoparticles were actually generated due to reduction of Au(III) to Au(0) by the root surface of the intact plant with strong reducing ability. These roots can even reduce 2,6-dichlorophenol-indophenol (DCPIP) which confirmed their reducing property. Gold nanoparticles with different functional groups and dimension were prepared by *Ocimum sanctum* plant extracts which were prepared by sequential solvent fractionation with different polarity (with hexane, chloroform, n-butanol, and water).[58] For chloroform circular discs with rough edges were obtained. When hexane fraction was used for reduction, spherical nanoparticles smaller than 100 nm were obtained. The aqueous extract gave large, anisotropic platelets with defined edges and n-butanol extract resulted in Au aggregates. These differences are due to presence of different reducing agents in different solvents as analyzed by gas chromatography-mass spectrometry (GC-MS).

3c. Copper Nanoparticle:

The Copper nanoparticles have attracted attention of researchers because copper is less costly, and it has very high thermal and electrical conductivities. Thus, the synthesis of Cu nanoparticles has huge potential in scientific research and industrial application.

Copper nanoparticles were synthesized in poly (vinyl alcohol) (PVA) using *Ocimum sanctum* extract.[59] The average size of the Cu nanoparticles was 13 nm as calculated from UV-Visible spectroscopy and from TEM study, it was found to be 16.01± 4.41 nm. These values were comparable with the particle size calculated from X-ray data i.e. 18.48 nm. In fluorescence spectra, excitation in the range of 220-245 nm wavelengths gave emission peak at 450 nm. The UV-Visible spectra gave surface plasmon band at 591 nm which confirmed the formation of the copper nanoparticles.

In another report, copper nanoparticles were synthesized by bio-reduction of $CuSO_4$ using methanol extract of *Ocimum sanctum.*[60] These nanoparticles were stable even for 24 weeks where copper is present in oxide as well as dioxide form, as confirmed by EDS spectrum. The Z-average diameter of the nanoparticles was 122.7 nm with polydispersity index of 0.116. The antimicrobial study of these nanoparticles showed improved activity against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli.*

In one of the modified methods, CuO nanoparticles (CuONPs) were synthesized by reaction of *Ocimum sanctum* leaf extract at room temperature. The formation of the CuONPs was confirmed by presence of a peak at 586 nm in the UV-Visible spectrum. These CuO nanoparticles were useful catalyst for the *N*-arylation using aryl and styryl halides on nitrogen nucleophiles (Figure 5). These highly active ecofriendly and biogenic CuONPs are useful catalyst for C−N bond formation reaction involving a broad range of *N*-nucleophiles.

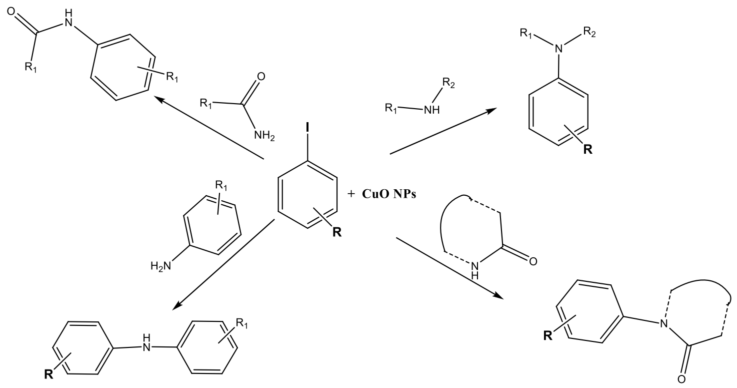


Figure 5. Biogenic CuONPs catalysed C-N cross coupling reaction

In addition to copper metal and copper oxide nanoparticles, copper iodide nanoparticles were also synthesized using *Ocimum sanctum* with copper salt under microwave irradiation in a short period of time (Figure 6).[61] The plant extract acted as reducing agent as well as capping agent. The CuI NPs had a broad absorption band with a peak at 425 nm in the UV-Visible spectra. From X-ray and SEM data the CuI NPs was found to be cubic in packing. The size of nanoparticle was calculated to be 66 nm. These nanoparticles were useful for photocatalytic degradation under visible light irradiation of methylene blue (MB), methyl orange (MO) dyes at a concentration of 20 ppm.

$$2\,Cu(NO_3)_2 \cdot 3H_2O + 2KI \xrightarrow{\text{Plant Extract}} 2\,CuI + 2\,KNO_3 + 6\,H_2O + 2\,NO_2 + 4\,O_2$$

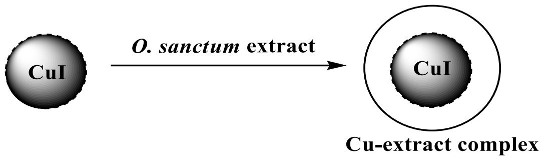


Figure 6. CuI nanoparticle synthesis

3d. Palladium Nanoparticles:

Green synthesis of palladium nanoparticles (PdNP@AT-mont) using aqueous *Ocimum sanctum* leaf extract as a reducing agent was reported on chemically modified montmorillonite solid support.[62] From TEM study, it was observed that most of the palladium nanoparticles are well distributed on the surface of modified montmorillonite and the wide distribution of particle size between 10-80 nm. The modification of montmorillonite was done in order to increase the surface area and also for generation of suitable pores as good support and stabilizer for the synthesized metal nanoparticles. Catalytic hydrodechlorination (HDC) is considered as an appropriate process for the dechlorination of wastewater containing organochlorinated pollutants. The synthesized palladium nanoparticles (PdNPs) on modified montmorillonite were useful in the catalytic HDC of the hazardous pollutant 4-chlorophenol in water under base free mild reaction conditions (Figure 7). This heterogeneous catalyst could easily be recovered by simple filtration and reused several times without any significant loss in its catalytic efficiency.

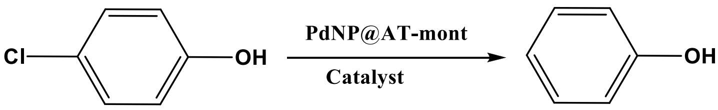
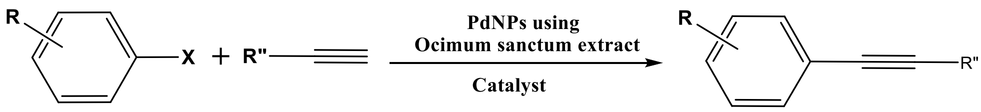


Figure 7. Catalytic hydrodechlorination of 4-chlorophenol

Palladium nanoparticles synthesized with *Ocimum sanctum* leaf extract have huge potential to be used as catalyst in organic synthesis. Palladium nanoparticles synthesized with *Ocimum sanctum* leaf extract (Pd_{OS} NPs) are capable of catalyzing Sonogashira coupling reaction.[63] The tandem synthesis of palladium nanoparticles and C–C cross-coupling reaction was achieved in an one-pot reaction. In the reaction, Pd $(OAc)_2$ was reacted with *Ocimum sanctum* leaf extract to reduce Pd (II) to Pd(0) with a gradual change in colour from light brown to black within one hour. The aryl halide and terminal alkyne were added to the same reaction mixture to produce the coupling product (Figure 8). The UV-Visible absorption spectra of the *in situ*-generated colloidal suspension of palladium nanoparticle initially gave characteristic peak of the Pd(II) ion near 400 nm which disappears on complete reduction of the Pd(II) salts to nanosized Pd(0) after addition of *Ocimum sanctum* leaf extract. From X-ray study the mean diameter of the nanoparticles was calculated to be about 4.41 nm.



R = 4-NO_2, 4-OMe, 4-COMe, 4-OH, 2-Me etc.
R" = 4-Me, 4-OMe, 3-Me, butyl, hexyl, cyclohexyl etc.

Figure 8. Sonogashira coupling reaction using green synthesis of PdNPs

3e. Platinum nanoparticles:

Platinum nanoparticles were synthesized by reduction of aqueous solution of chloroplatinic acid ($H_2PtCl_6, 6H_2O$) with the leaf extract of *Ocimum sanctum*.[64] The colour of the solution initially changed from light yellow to brown due to reduction of platinum (IV) to platinum (II) and then turned black due to formation of platinum (0). A greater extent of conversion of platinum ions to nanoparticles was obtained using *Ocimum sanctum* leaf extract at a reaction temperature of 100 ˚C. From scanning electron microscopy, it was found that the reduced platinum particles aggregate with irregular shape. From X-ray diffraction spectroscopy, the average particle size of platinum nanoparticle was calculated to be 23 nm. The hydrogen evolution potential and catalytic activity of the platinum nanoparticles were studied using linear scan voltammetry. These environmental friendly platinum nanoparticles were potentially useful for electrolysis of water.

3f. Strontium nanoparticle:

Strontium Oxide nanoparticles (SrO NPs) were synthesized by reaction of strontium nitrate with *Ocimum sanctum* extract under microwave condition followed by annealing at 500 °C for 2 hour.[65] For reduction of one mole of strontium metal ion, at least one mole of eugenol is required in the extract. After the complete reduction, strontium metal was oxidized by atmospheric oxygen to form SrO NPs in microwave oven heating. For the SrO NPs synthesized, the UV-Visible absorption peak was observed at 274 nm and in the photoluminescence spectrum, the emission band was observed in the visible region at 455–480 nm. From anti-microbial study, it was found that these SrO NPs are effective against gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus vulgaris*.

3g. Nickel nanoparticles:

Leaf extract of *Ocimum sanctum* was used for reduction of aqueous solution of nickel nitrate to give Nickel nanoparticles.[66] The appearance of absorption peak at 395 nm in the UV–Visible spectrum indicated the formation of nickel nanoparticles. Microscopic studies like SEM and TEM showed that the nickel nanoparticles are almost spherical in shape with particle size ranging between 12 to 36 nm. From adsorption study, it was established that these nickel nanoparticles are capable of removing cationic and anionic azo dyes and some of the anionic pollutants like NO_3^-, SO_4^{2-} etc.

The stability of nickel nanoparticles could be further improved by chemical modification with organic molecules like L-arginine.[67] The L-arginine modified nickel nanoparticles (Arg-Ni NPs) were synthesized by reduction of nickel nitrate with *Ocimum sanctum* extract and stabilized by addition of L-arginine. The synthesized Arg-Ni NPs had higher chemical and thermal stability compared to Ni NPs. Their UV-Visible spectrum showed the corresponding peak at 390 nm and particle size was between 10 to 28 nm. For bacterial transformation, these Arg-Ni NPs were useful as gene transfer vector. Ammonia borane (NH_3BH_3) was used as a safe source of H_2 as it could produce large amount of hydrogen even at room temperature and atmospheric pressure. One mole of ammonia borane could release three moles of hydrogen on hydrolysis. Arg-Ni NPs were used as catalyst for hydrolytic dehydrogenation of ammonia borane for effective hydrogen storage (Figure 9).

$$NH_3BH_3 + 2H_2O \xrightarrow{\text{Arg-NiNPs}} NH_4^+ + BO_2^- + 3H_2$$

Figure 9. Hydrogen production from hydrolysis of ammonia borane in presence of Arg-Ni NPs as catalyst

3h. Iron Nanoparticles:

Hematite (α-Fe_2O_3) is a stable, nontoxic and corrosion-resistant n-type semiconductor where the band gap is 2.1 eV. Economical and ecological synthesis of tulsi treated iron nanoparticles was done in two steps. In the first step, iron (II) chloride was reacted with sodium hydroxide to give hematite nanoparticles. Further modification of the nanoparticles was then done by leaf extract of *Ocimum sanctum*.[68] These nanoparticles were analysed with UV-Visible spectroscopy which gave the characteristic peak at 490 nm. Using TEM study the size of the nanoparticles was determined and was found to be 40 nm. These nanoparticles show better antibacterial property against *Escherichia coli* and *Staphylococcus aureus.* The higher value of antibacterial property of the nanoparticles could be attributed to the use of *Ocimum sanctum* leaf extract for their synthesis.

4. CONCLUSION:

In summary, extracts from different parts of *Ocimum sanctum* plant have found extensive application for the green synthesis of metal nanoparticles. Different phytochemicals that are extracted from various anatomical parts of the plant have different abilities to reduce the metal ion and to stabilize the nanoparticle by capping with organic molecules. Even the root surface of the intact plant can also reduce the metal ions and produce nanoparticle. Extracts from different parts of *Ocimum sanctum* in a combination with several metal ions produced nanoparticles with better morphological features and improved chemical and thermal stability. Further variation in the structure and activity of metal nanoparticle was achieved by addition of diverse molecules like PVA, cellulose, etc. Among the various metal nanoparticles, silver nanoparticles have been studied extensively. There are also sufficient numbers of reports on nanoparticles of other metal ions with distinctive properties. The methods for the synthesis of metal nanoparticles with *Ocimum sanctum* extract use water as solvent, plant extract as reagent, mild reaction condition etc. justifying the green reaction conditions. Analytical techniques such as X-ray analysis, UV-Visible spectroscopy and

TEM study of the nanoparticles have been used to calculate the size of the nanoparticles. These nanoparticles were widely used for antimicrobial study where they show improved results as compared to nanoparticles synthesized with conventional chemical reduction. These nanoparticles have also been reported as catalyst for organic transformations. The application of metal nanoparticles, synthesized with *Ocimum sanctum* extract, have several other aspects which need to be investigated and explored further.

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