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Synthesis of PdNPs Biochemically using Moringa Olifera Leaf Extract and its Applications

A Project Report Submitted to
Deenbandhu Chhotu Ram University, Murthal
In partial fulfillment of the Degree of Master of Technology in Biotechnology
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It was a really good experience with them. Last but not least, endless gratitude to the Divine GOD who does the impossible every day.

Abstract

In recent years green chemistry is playing important role for synthesizing organic compounds. Because of its eco-friendly nature, and low-cost technique. Metal nanoparticles exhibited some physical properties (such as particle aggregation, photo emission, electrical and heat conductivity) and chemical properties (catalytic activity) and some biological applications. Here we have done the green synthesis of palladium nanoparticles successfully by using Moringa olifera leaf extract as biomaterial. The synthesized nanoparticles were characterized by UVvisible spectroscopy, X-ray diffraction, and TEM analysis. In this paper we have employed the photo catalytic applications by using green approach of synthesized palladium nanoparticles.

***Keywords*: Moringa olifera leaf, Palladium acetate, UV analysis, TEM and Direct brown dye**

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INTRODUCTION

Eco-Chemistry

As we all know that the concept of "green chemistry" was coined in 1990's by mass-media in terms of latest theories of biochemistry to overcome the pollution causing factors and enhancements of nature quality indexes with respects to industries waste management. And soon these conceptual theories were extremely famous in the different research organization. So that nowadays the working area in green chemistry in vast and growing on large scale.

Green chemistry has been defined as "the use of chemistry techniques and methodologies that reduce and eliminate the use or generation of feedstock, products and by-products that are hazardous to human health and the environment". In a short definition, "Green chemistry is using chemistry for pollution prevention".

Some important areas of green chemistry are as follows

- Use of alternative synthetic pathways
- Alternative reaction conditions, or raised specificity & decrease wastage ratio.
- Design of eco-compatible chemicals

Eco-chemistry describes the complete life span of chemical concept just like occasion in order to creation of new concepts and theories. Eco-Chemistry argues with new inventors for creating new concept & employ substance for in terms of enhancement of path to raise the efficiency particles.

Nanoparticles

The nano particle is defined as the smallest entity that can act as a whole with respect to properties and transport nanoparticles are particles with dimension of 100 nanometer or less. The properties of many conventional materials change when formed as nanoparticles. This is because nanoparticle typically have more surface area per weight than larger particles. This makes them more reactive towards certain other molecules.

Metallic and semiconducting nanoparticle are of great importance due to their unusual size and shape dependent properties for biological and chemical reorganization of single molecule, control release of biological relevant molecules catalysis and immunoassays.

Palladium

Palladium is a metal with excellent hydrogen and oxygen adoption capacity and is widely used in the catalyst industries.

Various types of Pd catalysts mainly used in

- petrochemical industry
- catalytic hydrogenation
- oxidation reactions

Such as the preparation of acetaldehyde, pyridine derivatives, vinyl acetate and a variety of chemical reaction products.

International Journal of Engineering Research & Technology (IJERT)
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Vol. 14 Issue 02, February-2025

Morniga olifera

Drumstick tree and its variant redirect here this name is also used for cassia fistula the golden rain tree the poured of M.olifera are found in Nepal.

Moringa oleifera is a fast-growing drought tolerant tree belonging to the morning family, native to the Indian subcontinent. Common name includes morning or drumstick tree. It is wildly cultivated for its young seed pod and leaves used as a vegetable end traditional herbal medicine. It is also used for water purification although listed as an invasive species in several countries M.olifera has not been observed to invade intact habitat or displace native plants and therefore should contribute currently be considered a widely used for medicines.

M.olifera is a fast-growing deciduous tree reaching 10 to 12 meter in height with a trunk diameter of 45 centimeters. The bark is whitish Gray surrounded by the cork. Young shoots have purple or greenish white hairy bark. The tree has an open crown of drooping fragile branches and the leaves from pinnate lobes and Tripinnate lobes.

The flowers are fragrant and hermaphroditic surrounded by 5 regulars fine leave yellow white petals. The flower is about 1 to 1.5 centimeter long and two centimeter wide. They grow on slender, hairy stems in spreading or drooping inflorescence 10 to 25 centimeter long.

In this plant flowering starts from 1st 6 months after planting the M.olifera. The flowering is only occurring once as annually in end of the springs, initially in summer & during specific season in cold areas. The flowering periods is twice or even in whole year where the temperature range is constant.

In this plant fruits are hanging downwards direction i.e., 3-sided brown in colour and capsule structure upto 20-45 cm long in length which having dark brown globular seed with a dia of 1 cm. Also, these seed having 3 whitish paper like wing. These seeds are dispersed in distinct areas with the help of air & water.

The cultivation of these plant is 3 to 6 feet cut per & permitted to grow again that's why the pods and leaves always remain within the arms reach.

Acc to quantitative data analysis the average yields are 6 ton/ha/year in fresh quality. The difference of harvest output is much higher in monsoon & drought seasons i.e. 1120 KG/ha to 690 KG/ha at per harvest outcomes. In this plant different segment of plants are harvested in different time periods like, leaf & stems are harvested from the young plant after the 60 days seedling intervals & respectively follow this harvested logic in next 7 years. During per harvest the 60 cm length of the plants are cut back of the ground. Meanwhile during the few productions system, the leaf is harvested weekly.

The cultivation of M.olifera can also be done intensively with proper irrigation and fertilization. Trials in one million plants per ha & 9 cuttings per year for 4 years resulted in average fresh materials yield of 580 tons/ha/year, or about 174 tons of fresh leafs.

Under on growing nationalities the M.orniga tree has ability to enhancement of nutrition, enhance food privacies, promote villages infrastructure, & support sustainable soil conservation. It can be used as animal feed, liq. Micronutrients, natural dewormers & adjuvants.

M.orniga has been used to combat malnutrition, specially into infants & nursing mothers. Due to M.olifera plant grows in arid & semi-arid environment, it can provide a versatile & nutritious food resource year-round in many different geographical regions. Around 140 unions around the international have inaugurated programs to grow M.olifera to reduce malnutrition, purify water or production of cooking oil.

M.olifera powder of leaf is used for washing hand i.e., its leaf powder is used for soap making. In advanced it has anti-septic detergent properties in the form of phytochemical present leaves. Therefor seeds of M.olifera & press cake has been implemented as waste water conditioners to dewatering & drying fecal sludge.

The seed cake of M.olifera was obtained as a side product of pressing seeds to carry oil, this obtaining oil is also used for the filtration of H_2O with the help of flocculation to producing of pure water for utilizing by animal & human for drinking purpose. The seeds of M.olifera is having dimeric cationic proteins that absorb & neutralize colloidal particle charges in disturbed water, thus the colloidal particles are clump together, similarly the suspended particles are easily removed as sludge by settle down and filtration. Most of the impurities from water are removed by Morniga seed cake. Hence, we can also say that the Morniga olifera is effectively nontoxic and sustainable alternate to purify the drinking water which is affected by several pollutants.

Biosynthesis of PdNPs

While we are talking about the synthesis of PdNPs by chemical procedure like Chemical co-precipitation, Hydrolysis, thermal dissociation & sol-gel technology. But these techniques are hazardous to environment because these methods employed by using chemical solvents was used for synthesize of

International Journal of Engineering Research & Technology (IJERT)
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PdNPs.

Biosynthesis is best method to reduce the toxicity from chemicals.
Palladium nanoparticles were synthesized using Moringa olifera leaf extract on room temp. without the use of any surfactant, capping agent or template. PdNPs have numerous of uses in various issues i.e., used in device sensors, used as catalyst or in making active membrane. So, they are synthesized by Chemical, Electrochemical, and Sono-chemical methods.

The PdNPs broadly uses like catalyst within several reaction to enhancement of rate of that reactions. It is also used in chemiresistor type as a sensing device. Numerous of Pd are released along with waste water i.e., needs to be minimize to reduce the complete operational costs of industries. An intermetallic compound is also present in Pd and PdNPs which possessed unique application i.e., Catalytic active membrane and sensors.

Catalytic activity of PdNPs
The Pd is must important to formation of C-C bond in the Suzuki cross-coupling reaction b/w the organoboronic acid & halides due to versatility & ability of the formation of product.
Methodology for the generation of C-C bond formation is extremely versatile in terms of Suzuki cross-coupling reaction is employed most effective in formation of bi-aryls, the reaction is very important because the stability of precursors, boronic acid, & facility of work up. These NPs catalyst can help to address for the separation of & recycling of issues countered with in numerous of homo & heterogeneous catalyzed reaction. The much more important thing is MNPs supported for catalyst show not just only high catalytic reactivities. Also it shows the high degree of chemical balancing & don't swell in organic solvents.

Applications of nanoparticles
Numerous of applications are shows by NPs. Here are the few of application of nanomaterials into biology & medicine mentioned as:

A labeled Biological Fluorescent
This meant that tracking way and detection of biological molecules by fluorescent used in life science generally in terms of non-destructive. Natural fluorescent are tiny molecules and few proteins with in the cells i.e., termed as intrinsic fluorescence and auto fluorescence.

Genes and drug delivery
The aim of our investigation is to develop a generic platform for super paramagnetic iron oxide nanoparticles drug delivery systems (SPION-DDS) in which transport, imaging, transfection, and heating are all possible.

Pathogens Bio detections
All around the international to complete sequence microbial genomes fast and effectively with in advance DNA sequencing techniques has been make it legal and possible for the scientists. The complete genomes offering new chances for analyzing & understand access to the DNA sequence for m/o at molecular stage.

Detection of proteins
To detecting of protein analysis an ultrasensitive technique has been developed. Magnetic micro particles are feeling the system with respect to antibodies which is specifically attached to a target of interest or NPs probes which is encoding together with DNA. Also, it is unique to target protein interest or antibodies which can target sandwich captured by micro probes particles.

DNA structure Probing
A DNA probe is a kind of scientific process of extracting genetic information from a DNA sample. This is to identify the connections and similarities between two DNA samples. A DNA probe is used to find a sequence of nucleotides in a segment of DNA.

Engineering of Tissue
It is the application of cells combination, materials or methods also an engineering, that is also appropriate biochemical or physiochemical factor for enhancement and replace biological activities. Meanwhile once it was categorized in terms of sub-field of living matter, that have grown in scope with importance's are considering as field in its own authority.

International Journal of Engineering Research & Technology (IJERT)
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Vol. 14 Issue 02, February-2025

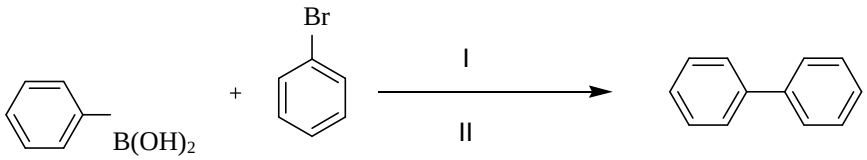
The reliable technique is approach to addressing lacking of organ present for transplantation with help of tissue engineering. The tissue engineering is most exciting area in biotech, due to its involvement of state-of-the-art technique from engineering section and biology.

OBJECTIVES

- Biosynthesis of Palladium nanoparticles by using agricultural waste M. olifera leaf extract.
- To characterize the NPs by various characterization methods like TEM, X-ray diffraction and UV-vis spectra.
- Photocatalytic activity of synthesized PdNPs with the different dyes.

REVIEW OF LITERATURE

M. Mora et al., 2006, Acc. to this the Pd catalysts is a heterogeneous Suzuki cross-coupling reaction as shown in fig. A great outcome was given by a catalyst that containing acetate-pyridine complex Pd which support the hydrotalcite that give conversion values. The situation of reaction is extremely mild. Selectivity and conversion is also given by catalyst.



Reagents: I. Pd- hydrotalcite, II. K_2CO_3 reflux at 80 ^{0}C, 12 hr

Fig. 2.1 Heterogeneous Suzuki cross-coupling reactions over palladium catalysts

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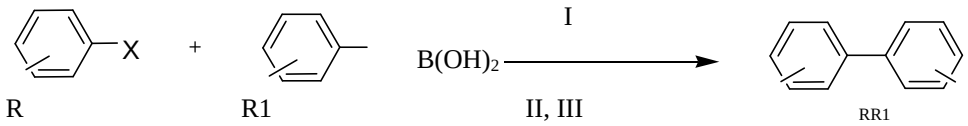
J. H. Li et al., 2006, reported the PEG-400 promoted Pd $(OAc)_2$/DABCOcatalyzed cross-coupling reactions in aqueous media **Fig.2.2**. Accordance with the effect of PEG on Pd catalyzed reaction is aqueous i.e., Suzuki-Miyura reaction of p-bromoanisole acid. And that find $Pd(OAc)_2$ or DABCO. The reaction that efficiently work in aqueous medium is Stille & Heck when PEG-400 was used as phase-transfer catalyst.

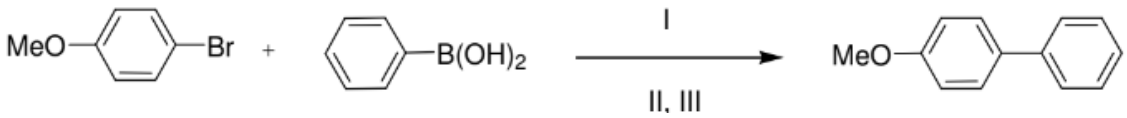


R= H, CH_3, NO_2, Ph R1 = H, NO2 X= I, Br

Reagents: (I) Pd $(OAC)_2$, (II) Na_2CO_3 and (III) 80 ^{0}C, 4hrs

Fig. 2.2 Pd $(OAC)_2$-catalyzed cross-coupling reactions in aqueous media

K. K. Senapathi et al., 2010, Stated that the PdNPs supported on Co ferrite, which is an efficiently magnetic separated catalyst for the ligand free Suzuki reaction shown in fig. reported the PdNPs supported on cobalt ferrite, an efficient magnetically separable catalyst for ligand free Suzuki reaction Fig.2.3. Acc. to their conclusion the magnetic catalyst of PdNPs was designed and developed for the reaction i.e., Suzuki cross-coupling. This reaction can be carried out through the uses of ethanolic dispersion of catalyst with the ligand free situation with loading catalyst. And hence, such MNPs catalyst are updated and new development in the use of heterogenous catalyst for the coupling reaction.

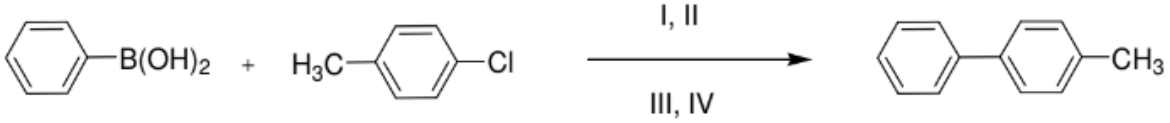


Reagents and condition: (I) Pd (OAC)2, (II) Na2CO3 and (III) 80 0C, 4h
Fig .2.3 A sufficient magnetic separator catalyst for the ligand free reaction.

M. Bagherzadeh et al., 2012, reported the Pd & Cu complex substance together with O_2-N_2 mixed as a donor sufficient catalyst to heck coupling reaction as shown in fig. They are resulted the C-C bond formation in heck reaction. In this reaction they are followed by the reaction was carried out.

Reagents and condition: (I) Pd (OAC)2, (II) Na2CO3 (III) EtOH reflux at 80 0C, 10h.
Fig.2.4 Complex of Pd & Cu with O_2-N_2 with donor like sufficient catalyst for heck coupling reaction

Y. Seat et al., 2008, reported the both reaction of Ar-X catalyzed by 1, 3- dialkylimidazolium or palladium Fig.2.5. They synthesized the C-C coupling reaction with methyl substitution; this is carried by using phenyl boronic acid with the aryl chloride when the $Pd(OAc)_2$ is present as a catalyst.

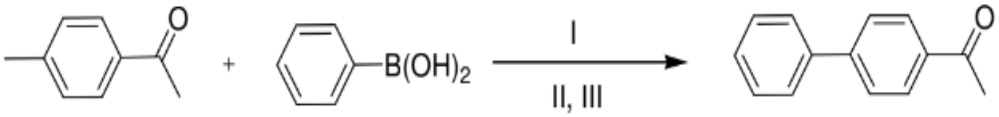


International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

Reagents and condition: (I) Pd (OAC)2, (II) K2CO3 (III) H2O in DMSO reflux at 80 0C, 5 h.

Fig. 2.5 Ar-X of Heck and Suzuki reactivity was catalyzed using $Pd(OAc)_2$.

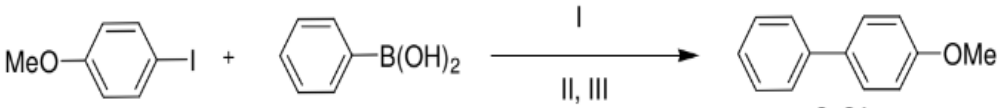
J. Qiu et al., suggested a sufficient easy guideline for a $PdCl_2$ ligand free & additive substance of coupling reaction of Ar-Br mentioned in fig. In this protocol they are done superficial sufficient, ecofriendly & easy developed to catalyzed additive & ligand free reaction of Ar-Br with aryl boronic acid in presence of H_2O & C_2H_5OH.



Reagents and condition: (I) Pd catalyst, (II) K2CO3 (III) H2O in DMSO reflux at 80 0C, 5 h.

Fig. 2.6 Additive & ligand free Suzuki reaction of Ar-Br

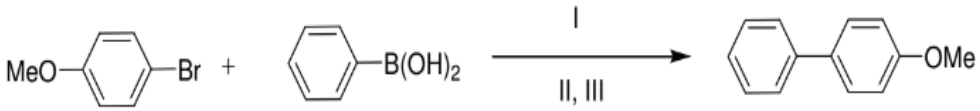
R. Cano et al., 2011, reported incorporated Pd with magnetite, cross coupling reaction of ligand free for catalyst mentioned in fig. These were demonstrated with good catalyst for the reaction incorporated Pd on magnetite when the lack of extra additives & ligand with catalyst elimination at broad range of iodinated substrate i.e., aromatic.



Reagents and condition: (I) Pd (0) catalyst, (II) Na2CO3 (III) EtOH reflux at 130 0C, 5 h.

Fig.2.7 Ligand free cross-coupling reaction of Pd catalyst

D. Zhengyin et al., 2011, reported that the PdNPs during of in-situ generation, PdNPs that are ligand free catalyzed by ultra speed cross coupling in aqueous phase upto 25℃ as in fig. These are applied the chemical reaction using PdNPs with Ar-Br & phenyl boronic acid.



Reagents and condition: (I) PdNPs, (II) Na2CO3 (III) EtOH, tolune and H2O reflux at 130 0C for 5 h.

Fig.2.8 PdNPs at 25℃ catalyzed the extremely fast reaction in aqueous.

Roopan et. al., reported the acaricidal, insecticidal & larvicidal effect of aqueous remove Annona squamosa L peel like biomaterial to reduce of Pd salts with into NPs. They are synthesized the Pd nanoparticles in green synthesis way, & the synthesized NPs was characterized by UV & XRD or TEM.

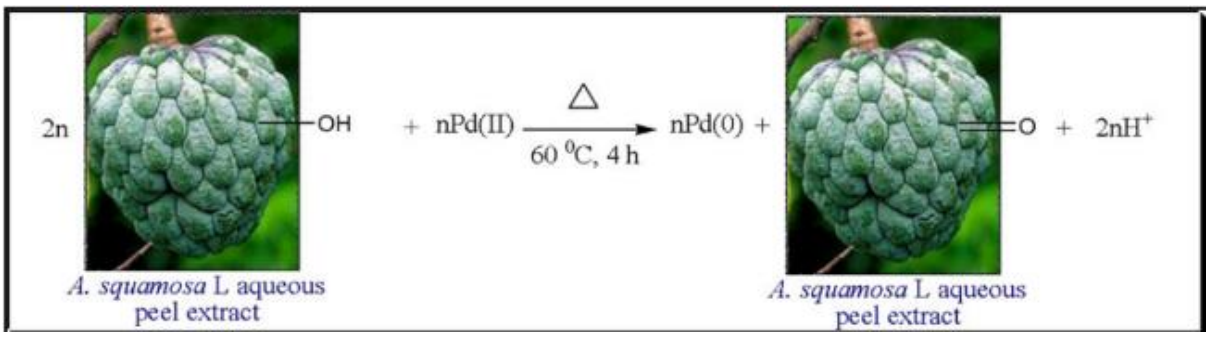


Fig.2.9 Formation of PdNPs by A. squamosa. A squamosa experience of peel extract.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

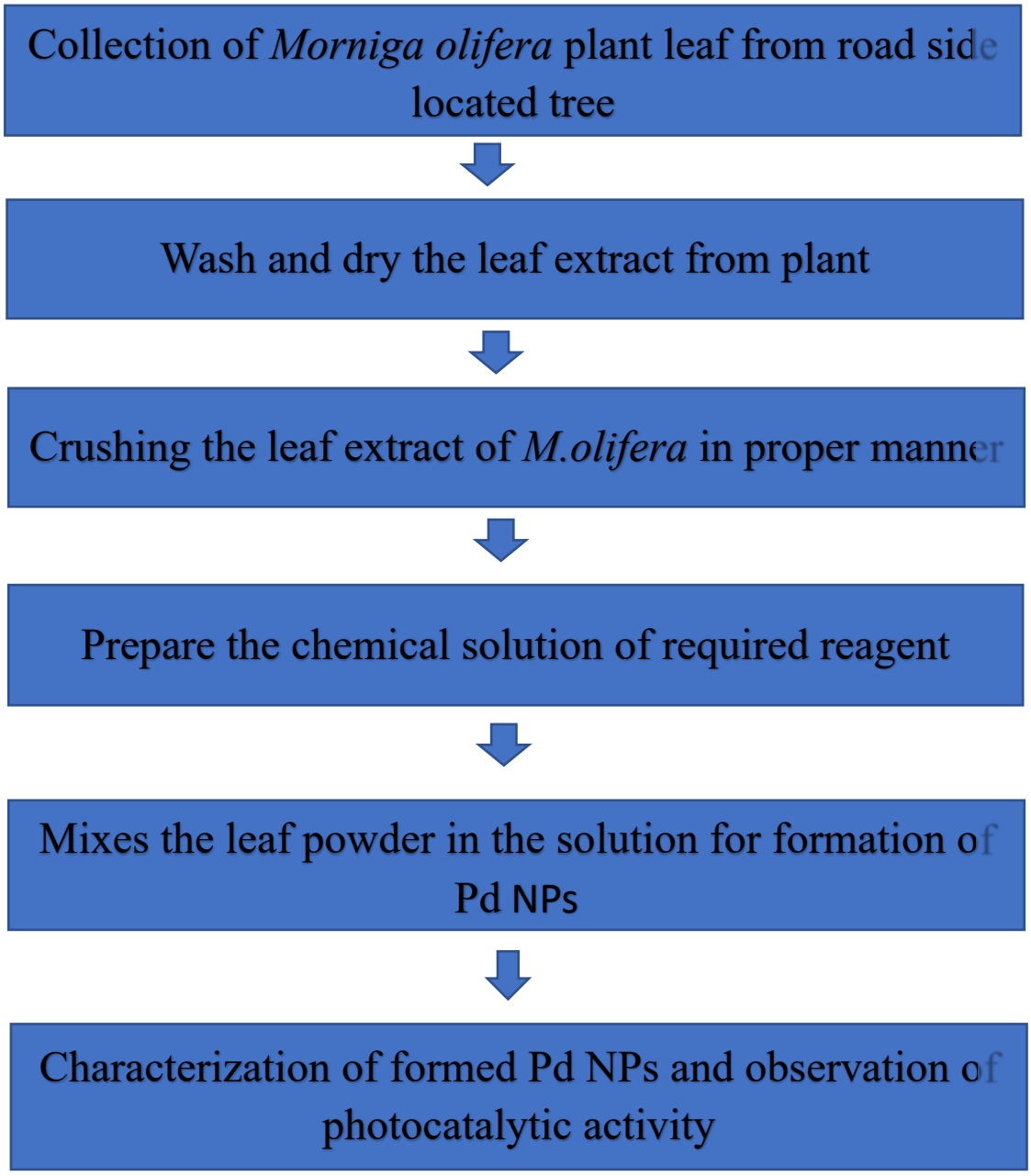
Bankar et. al., 2010 that is able to study leaf with extracted mediated novel note for the synthesis of PdNPs was characterized using UV, TEM & XRD.

Wei Chen et al., 2001, suggested the sonochemical approach to the confined synthesis of palladium nanoparticles in mesoporous silica. They are synthesized the PdNPs by sonication for the purification of the extract and synthesized nanoparticles was characterized by UV, XRD and TEM.

According this literature survey we were going to synthesis the Palladium nanoparticles by using M.olifera leaf extract in the pattern of green chemistry and catalytic applications of the synthesized PdNPs.

PLAN OF WORK

Following chemicals and reagents will be use for employing my project work



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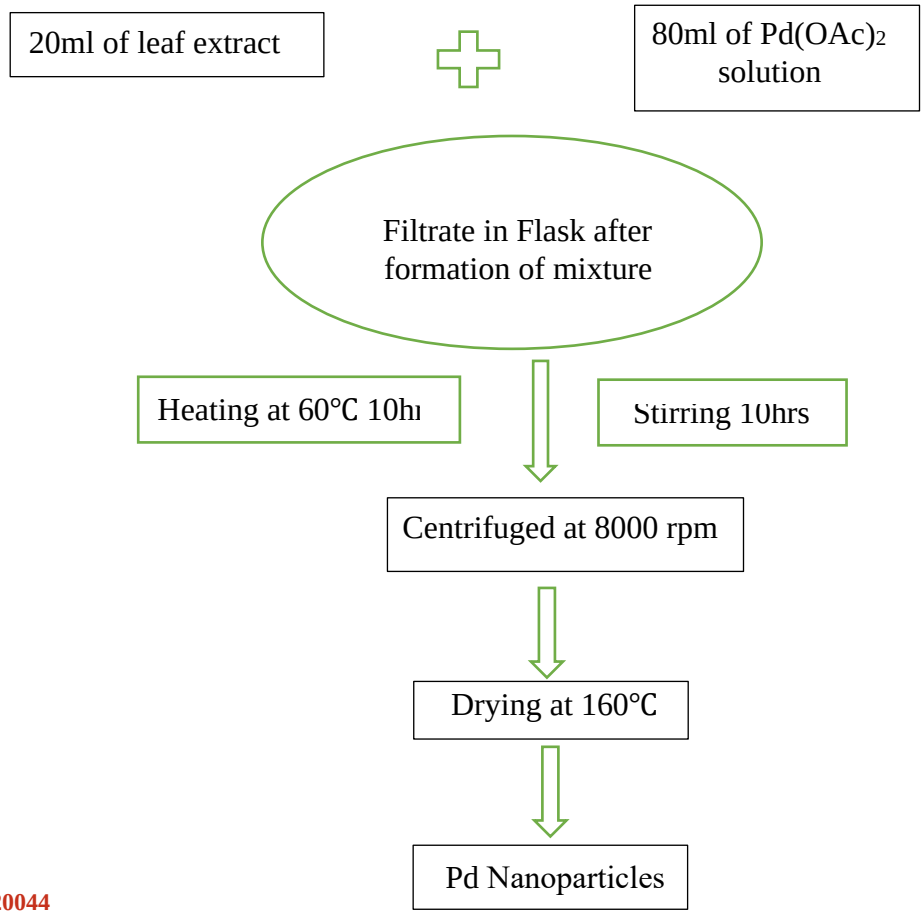
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

MATERIALS AND METHOD

- ✓ Ethanol, $Pd(OAc)_2$, Tolune, Ethyl acetate, Petroleum ether, Dichloro methane, DMSO, Chloroform, CaCl2, Na2CO3, K2CO3 Distilled H2O, Direct brown dye Alizarin red Rhodamine B. All these chemical are procured by Belco Pharma From their dealing partner
- ➢ Firstly we were collected of *M.olifera* from road side tree located on N.N. road Bahadurgarh near outer area of village Bhamnoli.
- ➢ Then we washed the *M.olifera* with distilled water and kept for air drying for a week properly in Quality lab of Belco Pharma
- ➢ After that we crushed properly collected *M.olifera* with the help of mortar and pestle and prepared a leaf powder in lab.
- ➢ And take up about 10g of *M.olifera* leaf powder that was transferred into a 250ml conical flask which having 90ml of distilled water.
- ➢ Mixed the leaf powder in water properly upon rotary shaker for 1hr extract was filter with filter paper & filtrates was collected in different flsk.
- ➢ On other we have prepared a metal solution of $Pd(OAc)_2$ by adding 0.1mM of $Pd(OAc)_2$ into 100ml of pure H_2O. Also mixed good of distilled water. And mixed properly such solution also on a rotary shaker for 1hr.

Synthesis of PdNPs:
Both the solution i.e. aqueous extract of leaf powder and metal solution of $Pd(OAc)_2$ were mixed together to making of PdNPs. Solution is aqueous of 1m molarity $Pd(OAc)_2$ was newly and fresh prepared with the water up to Q milli or used for the formation of PdNPs. The 10ml solution from aqueous leaf extract was mixed into 80ml of 1m molarity of palladium acetate solution and making PdNPs at the 60℃ temp. and stirring up to 10 hours.



International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

Following instruments were used for synthesis and Characterization of PdNPs and its compounds

- UV-spectroscopy (Schimadzu UV spectrophotometer, model UV-1800)
- X-ray diffraction device
- TEM

Characterization:
Here the UV-visible spectrometer is used for monitoring the bio reduction of palladium acetate in the solution. Moreover, this characterization was also done by using X-ray diffraction with different model like diffractometer and bruker etc. & TEM analytical study using 120kV accelerating potential with methanol solvent. These final results observed from microbiology lab of Belco Pharma located in MIE Part- A, Bahadurgarh.

RESULTS AND DISCUSSION

Aqueous extract of M.Olifera was prepared by using M.Olifera leaf powder and distilled water. We have taken GCMS report for secondary metabolites of M.Olifera extract and identified the hydroxy group containing secondary metabolites in M.Olifera aqueous extract.

Morniga Olifera Leaf

Wash and dried

leaf Powder of Leaf

100ml Water

Stirring 1hr and filter

250ml flask

Filterate of leaf extract

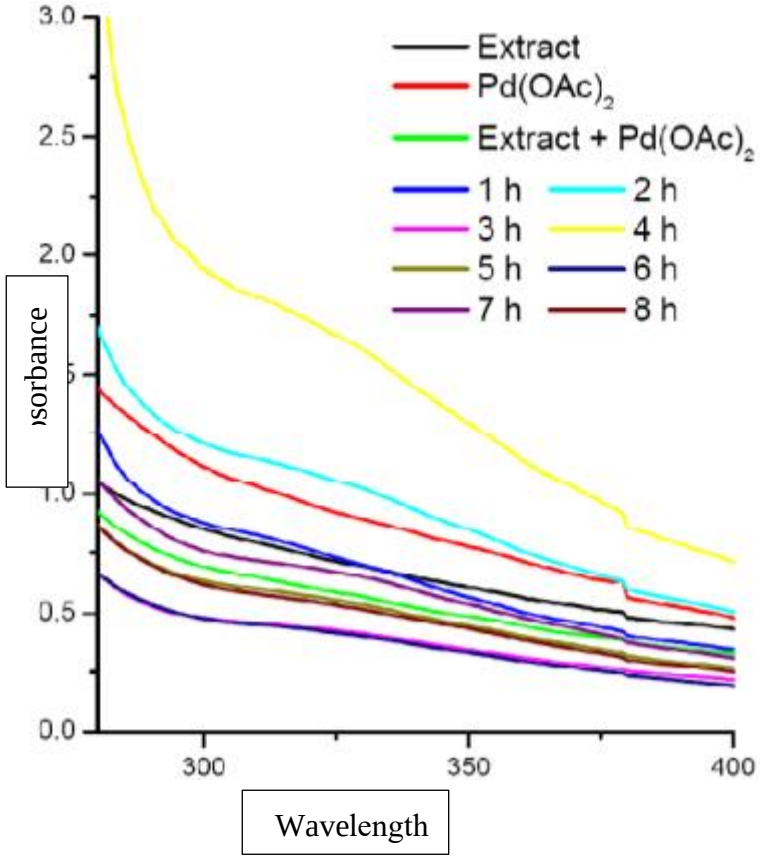
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

GCMS report for secondary metabolites of *M.Olifera* extract

Sr. No.	R. T	COMPOUND NAME	M. F	M. W	%peak
1	23.9	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430	30.45
2	23.8	α-Tocopherol-β-D-mannoside	$C_{35}H_{60}O_7$	594	22.02
3	22.1	Tetradecahydro-3,11-dihydro-10,13-dimethyl-2Hcyclopenta[a]phenanthrene-17(14H)-one	$C_{19}H_{30}O_3$	306	12.74
4	18.9	Trimesitylbroane	$C_{27}H_{33}B$	368	10.58
5	`19.2	2,2':4',2"-Teriophene	$C_{12}H_8S_3$	248	5.48

Every 1h M.Olifera aq leaf $Pd(OAc)_2$ solution was taken for the UV analysis. Also we have taken the UV analysis for extract, $Pd(OAc)_2$ solution separately. As we mentioned in my literature survey PdNPs will show the absorbance around 300-350nm. Here got the absorbance near about peak at 300nm. So that I concluded the PdNPs by pre-UV analysis. The overall formation is monitored by UV spectroscopy in 250-800nm range.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025



The presence and appearance of crystalline Pd was confirmed by X-ray diffraction that was comparing with the previous results of reports. And compared our X-ray diffraction spectrum with existing report of previous about nanoparticles or crystals. So, as per the graphical representation the synthesized PdNPs was shows a little hike due to the biomaterial at 56.8 was noticed with impurities.

Then the estimation of particle size as done with the formula called Scherrer's i.e., d= $0.94\lambda/\beta\ cos\theta$ here d termed as mean diameter for the nanoparticles, wavelength of XRD source of radiation is λ , angular FWHM for XRD hike at diffractive angle is β. Then the mean diameter was determined to be 80$\pm$5nm.

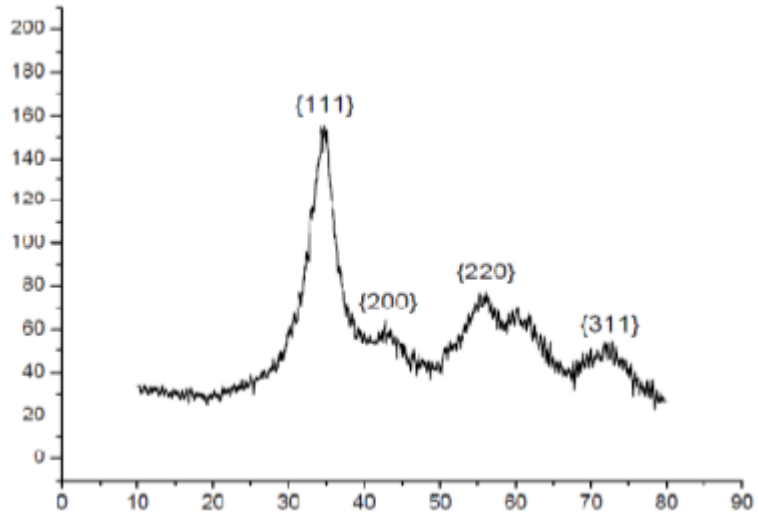


Photo catalytic activity:

Decolourization and growth assay

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 14 Issue 02, February-2025

To measure decolourization, sampling was done after 24 hrs from the inoculated decolorizing media. For the analysis of degradation dye was an solution of aqueous medium of dye and palladium nanoparticles was mixed together and the solution was kept in mechanical shaker for 24 h. And UV-visible spectra were used to monitoring for the successive decrease the absorbance of reaction mixture. Direct brown, alizarin red and rhodamine b dyes solution are used as controls.
The decolonization efficiency of different nanoparticles was expressed as

$$\text{Decolorization (\%)} = \frac{A_0 - A}{A_0} \times 100$$

Where A_0 = initial absorbance; A = absorbance of medium

We have done direct brown for dye degradation with PdNPs, different concentration (50, 100, 150, 200 and 250 mg/ml) of direct brown standard was prepared to that 0.1 Mol% of PdNPs solution was added and kept mixing on mechanical shaker for 3 hrs. Every 15 min solution was collected for UV analysis. Decolourization was observed and percentage of decolourization was decreased.

Sr. No.	Dye Conc mg/ml	Decolourization %
1	50	35.1
2	100	31.7
3	150	26.8
4	200	23.9
5	250	23.5

- The PdNPs were used a photo catalyst in dye degradation.

1. Direct brown,
2. Alizarin red,
3. Rhodamine B dyes are used.

- PdNPs shows good result in dye degradation and these PdNPs were given successful result as a photo catalyst.
- We have done direct brown for dye degradation with PdNPs, at different concentration (i.e. 50, 100, 150, 200 and 250 mg/ml) of direct brown standard was prepared to that 0.1 Mol % of PdNPs solution was added and kept mixing on mechanical shaker for 3 hrs. Every 15 min solution was collected for UV analysis.
- Decolourization was observed and percentage of decolourization was decreased.

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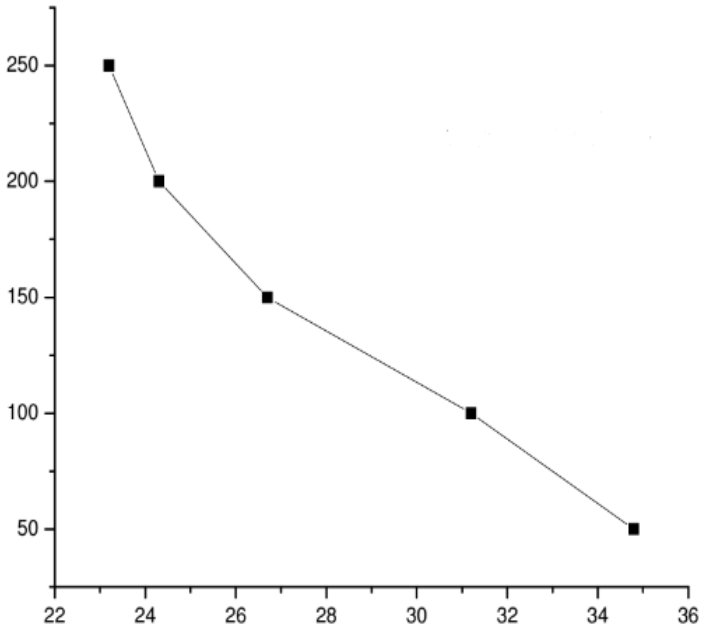
Vol. 14 Issue 02, February-2025



SUMMARY AND CONCLUSIONS

A clean & non-toxic ecofriendly procedure for the formation of PdNPs in the present study. Wrapping around each and every seed provides a contant chemical environment formed by the bio-organic compound present in the broth of M.olifera leaves, which may be primarily responsible for stabilizing the seeds. This technique provide us with a simple and efficient means of synthesizing nanoparticles with catalytic properties regulated by the size of the particles. From the point of view of nanoparticles, it is a development to synthesize economically. In this we concluded green chemistry approach towards PdNP synthesis has several advantages, namely, an easily scalable process, economic viability, etc. The application of these environmentally friendly nanoparticle in biochemistry and other medical and chemical application makes it possible for these methods to stimulate on large scale synthesis of organic materials. On the other hand such as nanomaterials results concluded that M.olifera leaves could be used for PdNP synthesis and showed moderate dye-degrading bleaching activity with photocatalytic activity.. The result concludes that M. olifera leaves can be utilized for synthesis of PdNPs and it shows the moderate activity in decolourization through dye degradation with photocatalytic activity.

SIGNIFICANCE

- Agricultural waste M.Olifera leaf was utilized in a proper manner.
- Green synthesis was adopted for the preparation of palladium nanoparticles.
- Spherical Palladium nanoparticles ranging from 80 ± 5 nm are obtained using M.olifera leaf.
- Ultra low cost technique for the production of palladium nanoparticles, as it does not involve any extreme operation conditions such as high pressure or temperature.
- The photo catalytic activity was evaluated using the photo degradation of aqueous direct brown solutions in the presence of UV irradiation.

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International Journal of Engineering Research & Technology (IJERT)
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