



## NANO PHYLOGENETIC CONCEPT: NANOPARTICLE SYNTHESIS FROM PLANT EXTRACT AS TOOL FOR DEDUCING PHYLOGENETIC RELATIONSHIP

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### ABSTRACT:

Phylogenetic studies based diversification of plants species has been carried out using morphological, phytochemicals, secondary metabolites and molecular characterization that are exploited substantially for interpreting the morphological differences, genetic similarities and diversity. Now scientist extensively research for designing, characterisation, fabrication and controlling shape and size at nanometer for claiming application of nano-materials, in cross disciplinary fields. Thus, in light of above scenario, we would like to propose the use different size and shape of nanoparticles fabricated by particular plant extract for deducing inter-intra classification amongst plants. In taxonomic view, the variable size, shape, numbers and concentration of nanoparticles that are synthesized using plants ability to reduce metallic salt to nanoparticles would be used for definite classification among various families, different genus and at species level. The plant material contents such as phytochemicals example flavonoid, which are present in various concentrations in plants (plant specific), and the oxidation-reduction potentials of metals and flavonoids could play major role. Thus in that case is it possible to use plant (flavonoids) based nanoparticles for phylogenetic analysis. Beside, the type of metallic salt used for synthesis would produce particular types size, and shape of NP. The various range of extract could be utilized by keeping the other parameters such as temperature, light, experimentation set up constant. The algorithm for external classification, similarly the algorithm for biochemical compound, molecular data, phyto based nanoparticles could be combine into single algorithm to classify the plants into different important categories. In short, the nanoparticles formed using various plant extract are assessed for their capability to classify plants and nano based classification will support current biochemical, molecular aspect and improved our understanding in plant taxonomy.

**Keywords:** nano scale, silver salts, data science, algorithm, nano based phylogenetic studies.

### INTRODUCTION:

#### Nano

In the previous few decades, human have applied biotechnology in every scientific field for getting a possible solution of every question that arises before us. The Feynman (1960) stated pre nano science concept "[There's Plenty of Room at the Bottom](#)" which was before the introduction of term nanoscience. From there the scientist have identified, improvised and characterized the invention of new nanotechnology (Taniguchi 1974). The nano means very minute thing that comes in nano scale of 100nm or less dimension, in specific nano particles nano are 100-1nm, nano particles fine are 2,500-100nm and nano particles coarse are 10,000-2,500nm (Gopinath et al., 2013; Dimetry and Hussein. 2016).

#### Phylogenetic

The phylogenetic is the study that includes the important elements of plants to conclude the similarity and differences in plants in order to

differentiate among valuable and unimportant plants. To conquer this aim the various parameters of plants haven been considered such as There are many plant species, which have characteristic features related to specific traits. These include food, fruit quality, life shell, sweetness of fruit, color of leaves, color of flowers, growing habitat etc (Agrawal et al., 2008). Further, the phytochemical has been extracted and the isolated biochemical compounds (allelic variants of enzyme isozyme) become the main component for describing classification in plants (Vanderplank. 1991). The taxonomists found the phenotypic data confusing and mostly reliable on phyto compounds aspect to identify plants (Fajardo et al., 1998). However, the discovery of DNA have drastically changed the scenario by usage of initially, non specific markers such as RAPD, ISSR, RFLP, AFLP and later on gene specific markers for examples ITS, rbcL, mat K genes (Hollingsworth et al., 2009) and SSR markers (Lade and Lade. 2018) are applied to confirm similarity and difference in plants. The breeding

program to develop hybrids of spectacular potential as well as natural hybridization led's to progression of plants with different morphology but have similar genetic makeup.

Thus the combining effect of morphological, biochemical and molecular data is eventually utilized for defining plant position in phylogenetic of evolutionary tree. Moreover, the extensive use of plants compounds or flavonoids for nanoparticles have elevated in decoding the potential of phyto nanoparticles for various applications.

#### **MATERIALS & METHOD:**

##### **Nanoparticle Synthesis**

Recently, several high impact factor journal publication shows that medicinal plants have been constantly use to drive full use for synthesis of nanoparticles suggesting possible ecofriendly alternatives to chemical and physical methods (Lade et al., 2017). Equally, It has advantage of stable structure formation, capping support, slow kinetics, easy scale up, and generation of eco environmental byproducts.

##### **Metals for Nano particles Synthesis**

There are several sources used for synthesis of nano particles such as, noble metals i.e. Silver, Platinum, Gold and Paledium. However, the noble silver (Ag) could be best of other in the field of biological system and living organisms (Lade and Patil. 2017). Thus the idea of using Ag salt that could be capped by plant extract consisting of chemicals, compound, and secondary such as flavonoids as reducing agent to form Ag nanoparticles.

##### **Nano Based Phylogenetic**

To overcome the deficiency of scientific reason or supportive data to clarify the diversity and similarity among plant species the comprehensive integration of plant science, green chemistry, taxonomy and nanotechnology could give proper direction. Thus we would like to put forward our concept of classifying the plants on the basis of the nanoparticles that are fabricated using the different plants extract.

##### **Discussion**

The Nanoparticles fabricated in experimentation are used for characterization in order to know about their structure, form, size and shape. The large numbers of instrumentents are available to characterise nanoparticles.

##### **Nanoparticle Characterization Techniques**

The elucidation of exact synthesis, mechanism of nanopaticles by plant extract and characterization could be done viz, UV Spectroscopic analysis for confirmation of Nanoparticles synthesis, Atomic Force Microscopy (AFM) used for 3D visualization of Nanoparticles. Similarly, Dynamic Light Scattering (DLS), Scanning Tunneling Microscope (SEM) and HR-TEM Transmission are used to get average size, particle distribution, size range in nm and shape of nanoparticles. Moreover, X-ray diffraction (XRD) will determine the crystallographic structure and crystallite size (grain size) based on the angle of diffraction of the X-ray beam by the atoms in the crystalline planes. The FTIR will indicate functional group that is identified by standard IR absorption frequencies for nanoparticles synthesis and Zeta Potential analysis will assist for determining the surface charge of Nanoparticles.

##### **Critical Points to Consider**

The plants extract contents specific phytochemicals, secondary metabolites inherent to particular plants, thus it is vital to consider

- a. The phytochemicals profiling
- b. Secondary metabolites fingerprinting
- c. The flavonoids are plant specific
- d. Flavonoid profiling

The plant source from its natural environment or the grown in green house may have different results. Thus the location of plant will affect the phylogenetic analysis.

- a. The plants in natural condition in specific geographical area have unique secondary metabolites.
- b. The green house or lab controlled condition grown plants have different secondary metabolites.

The silver nanoparticles synthesis must be performed in controlled conditions, because the various parameters could have different effect on nano particles production.

- a. The synthesis may be affected by the heat.
- b. The synthesis could be effect by temperature.
- c. The light could have different effect.
- d. The pressure, microwave, sun light etc,

The quantity of the leaf extract will have certain effect on nanoparticles synthesis.

- a. The various range of concentration needs to be study and the selected concentration need to finalized for nanoparticles synthesis.

- b. The range could be optimized as 1mL, 2mL, 3mL, 4mL, 5mL or 10mL.

The silver salt AgNO<sub>3</sub> in millimolar will produce some effect on nanoparticles synthesis.

- a. The AgNO<sub>3</sub> of different millimolar such as 1mM, 2mM, 3mM, 4mM, 5mM or 100mM need to standardized and set for nanoparticles synthesis.
- b. The constant specific mM concentration will produce particular nanoparticles.

Similarly, pH and methodology could be crucial for development of protocol. The variety of metallic salts can be used for synthesis of nanoparticles and their comparative data could provide essential conclusion.

#### **Databank Creation**

The data base of morphological, biochemical, molecular and phyto based nanoparticles formation will enable to deposit the extracted information in authenticated form. The nanoparticles formed using plant extract information such as what plant part used for example leaf, stem, flower, root, seed and tendril. Also the fabricated nanoparticles size, shape, charge, average distribution etc are more factors to be considered and safely deposited in data bank. The various factors discussed above must be standardized and the same protocol could be applied to different plant material extract and a definite size and shape of nanoparticles could be obtained. The computer programmer or data scientist could develop program to count every possible elements about plant and assemble them in form of information in online database. This programme will process, algorithms and systems to utilize every piece of information at macro, micro and nano level and gives the knowledge and insights from data in various forms, both structured and unstructured. The data analyst can be applied to come up with best results in form of graphs. The programme could actually detect and correct the errors beside removing duplicate information from data from database (Mallah et al., 2013). The data exploration method will also help us to examine the data and classify the plants into different categories depending on the internal, external structure, morphology, chemical compounds, secondary metabolites, molecular markers, DNA sequences and plant mediated synthesized nanoparticles. Based on these explorations, we could apply different algorithms which could be used to differentiate important characteristics of distinctive plants.

#### **Tree building method**

The created database for morphological, phytochemicals, molecular as well as phyto based nanoparticles will have all the information and a computer programming could be created by a data scientist that will fetch or consider all stages level characters to compare with the related plant species to make definite classification. The characters that will be considered in programming could be phenotypic, biochemical compounds (allelic variants of enzyme isozyme), genetic and nanoparticles that synthesized, the size, shape, numbers, dimension, its form etc properties will help to generate phylogenetic tree. The statistical method that considers all stages data specific to plants could be developed that have 90% bootstrap values to evaluate reliability of a tree. If the branch point score is around 90%, then the predicted tree is considered accurate (Patwardhan et al., 2014). The algorithm such as K-Nearest Neighbour (K-NN) algorithm is proved to be the algorithm for the external classification of the leaf from different species of plant. The 'k-Nearest Neighbour' (k-NN) classifier is a fundamental tool in pattern analysis, providing a straightforward means of determining class membership for an unseen sample vector. The k-NN classifier is reached out to yield data for all classes in the database collection; thusly this could utilize to evaluate the posterior likelihood of the leaf species; or, can be joined with the posterior likelihood figured utilizing an alternate element or an arrangement of numerous traits or characters. Various different shape extraction techniques have been applied to leaves. Du et al (2007) explore leaf classification with a data set of 20 species of leaf and 20 samples of each. Fifteen conventional digital morphological shape features were calculated per sample and used to classify the plant species automatically. Contrary to this approach, Wang et al (2000) explores the classification of leaf species using a single relatively complex feature: The Centroid Contour Distance Curve (CCDC). After a long duration of research, we found that the more we consolidate these elements, the more exact the distinction between tests is and that is the thing that gives better outcomes in the analysis. Such algorithm, methods, statically approach needs to develop that can use more scientific data to deliver final output.

The identification, compound profiling, molecular characterization and phyto nanoparticles uploaded

in online database and application of novel program, algorithm, statical methods will really render the base for plant classification.

#### CONCLUSION :

The plants based nano particles (100-1nm) dimension are may be effective tools for considering classification in plants. The specific biochemical peculiar to plant species could be profiled and identified, and use for capping and stabilizing the metal salts to obtained particular size, shape, numbers, dimension of nanoparticles. The variety of parameters such as pH, temperature, light, heat, metallic salts, need to optimized and keep constant for experimentation which can be use for synthesis of nanoparticles. The profiled biochemical, its sub type or sub class, morphology of host plant, genetic data together with phyto nanoparticles data deposited in online database will make availability to scientific community. The computer programming to compare all the defined aspect at nano to morpho level shall predict definite classification. However, the experimentation, sampling working, programming will need a lot of time and extensive study to conclude perfect positioning in plant tree. The K-Nearest Neighbour (K-NN) algorithm can be use to best describe, the external classification, similarly the algorithm for biochemical compound and molecular data along with the algorithm for phyto based nanoparticles could be integrated to develop parent algorithm possessing multi algorithm. In Future, particle swarm optimization (PSO) can be used to clean dataset and keep the imperative data with a specific end goal to improve the acquired outcomes and abstain from over fitting issue postured by decision tree algorithm. Based on these decisions we could further classify the plants into different important categories.

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#### REFERANCE :

Agarwal M, Shrivastava N, Padh H (2008). Advances in molecular marker techniques and their

applications in plant sciences. *Plant Cell Reporter*. 27: 617-631.

Dimetry NZ and Hussein HM (2016). Role of Nanotechnology in Agriculture with Special Reference to Pest Control. *International Journal of PharmTech Research*. 9 (10): 121-144.

Du JX, Wang FX and Zhang GJ (2007). Leaf shape based plant species recognition. *Applied mathematics and computation*, 185 (2):883-893.

Fajardo D, Angel F, Grum M, Tohme J, Lobo M, Roca WM, Sanchez I (1998). Genetic variation analysis of the genus *Passiflora* L. using RAPD markers. *Euphytica*. 101: 341-347.

Feynman R (1960). There is plenty of room at the bottom. An invitation to enter new field of Physics. Annual meeting of American Physical Society at Caltech. *Engineering and Science*. 22-36.

Gopinath K, Gowri S and Arumugam A (2013). Phytosynthesis of silver nanoparticles using *Pterocarpus santalinus* leaf extract and their antibacterial properties. *Journal of Nanostructure in Chemistry* 2013, 3: 68.

Hollingsworth ML, Clark AA, Forrest LL, Richardson J, Pennington RT, Long DG, Cowan R, Chase MW, Gaudeul M, Hollingsworth, PM (2009). Selecting barcoding loci for plants: evaluation of seven candidate loci with species level sampling in three divergent groups of land plants. *Molecular Ecology Resources*. 9: 439-457.

Karlstad University (2018). Nanoscience lecture. Lecture1. <http://www3.kau.se/kurstorg/files/n/C10B9C4709ddc16A23JOTTJF6D8D/NanoI-Lecture1%202012.pdf>.

Lade BD, Gogle DP, Nandeshwar SB (2017). Nano Bio Pesticide to Constraint Plant Destructive Pests, *Journal of Nanomedicine Research*

- Lade BD, Patil AS (2017). Silver nano fabrication using leaf disc of *Passiflora foetida* Linn. [Applied Nanoscience](#) Springer. 7(5): 181-19.
- Lade DB, Lade BD (2018). Hybrid Testing in Pigeonpea Using DNA Fingerprinting by SSR-Markers, *Haya: The Saudi Journal of Life Sciences*. 3 (7): 535-540.
- Mallah C, Cope J and Orwell J (2013). Plant Leaf Classification Using Probabilistic Integration of Shape, Texture And Margin Features. *Proceedings of the IASTED International Conference Signal Processing, Pattern Recognition and Applications*. February 12 - 14, 798-098. Innsbruck, Austria, DOI: 10.2316/P.2013.
- Patwardhan A, Ray S, Roy A (2014). Molecular Markers in Phylogenetic studies- A review. *Phylogenetics & Evolutionary Biology*. 2 (2): 1-9.
- Taniguchi N (1974). On the Basic Concept of Nanotechnology. *Proceedings of the International Conference on Production Engineering*, Tokyo, 18-23.
- Vanderplank J (1991). Passion flowers. Cambridge, Massachusetts: The MIT Press, *Massachusetts Institute of Technology*.
- Wang Z, Chi Z, Feng D, and Wang Q (2000). Leaf image retrieval with shape features. *Advances in Visual Information Systems*. 41-52.