"BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING FUNGAL EXTRACT *ASPERGILLUS NIGER* ISOLATED FROM *AGLAIA ELAEAGNOIDEA* AND ITS CHARACTERIZATION".

Dr Pooja R*

Abstract

Biogenic synthesis of silver nanoparticles (AgNPs) using plants has become a promising substitute to the conventional chemical synthesis method. However, the desired size and form of NPs can be produced by optimising the microbe-mediated synthesis by changing their reaction conditions. AgNPs are manufactured by a variety of chemical and physical processes. But so far, these techniques are costly and harmful to the environment. The study here elaborates on the potential of biologically synthesized silver nanoparticles of fungal extract samples from Western Ghats of Agumbe region, Karnataka. In this study, we report the low-cost microorganisms based "eco-friendly" efficient synthesis of silver nanoparticles by using the metal-tolerant fungi *Aspergillus niger*. On the other hand, the appearance of white precipitate at the end of incubation time confirms the production of AgNPs. The physicochemical characterization of silver nanoparticles was performed by employing, SEM, XRD, and FTIR to investigate the stability and structure of AgNPs. SEM result revealed that the particles were irregular spherical, granules. In addition, FTIR confirms the functional group of the silver oxide nanoparticle. The XRD pattern of synthesized nanoparticles confirms the diffraction peaks of AgNPs at 2θ = 31.82°, 34.51°, 36.27°, 47.71°, 56.76°, 62.95°,66.53°, 68.12°, 69.13°, 72.45°, and 77.09°can be assigned to (100), (002), (101), (102), (110), (103), (200), (201), (004) and (202). Indeed, nanotechnology based microbiological active molecules develops new opportunities for us to explore novel applications in terms of green technology.

Keywords: *Aglaia elaeagnoidea,* silver nanoparticles, SEM, XRD, FTIR, Green Technology.

*Department of Biotechnology, Surana College Autonomous, South end Road, Bangalore-560004.
Email: pooja.bio@suranacollege.edu.in ; poojaravi2015@gmail.com.

***Corresponding author**: Dr Pooja R
*Department of Biotechnology, Surana College Autonomous, South end Road, Bangalore-560004.
Email: pooja.bio@suranacollege.edu.in ; poojaravi2015@gmail.com.

DOI: 10.48047/ecb/2023.12.si10.0029

Introduction

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date, these medicinal plants have been extensively utilized in Ayurveda and also in latest technology. Recently, many such plants are gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Plants and various plant products are now being used crucially in the synthesis of nanoparticles (NPs), making nanobiotechnology one of the most active study fields in modern material science. Several methods for creating silver nanoparticles (AgNPs) have been developed because of the scientific revolution in nanotechnology. In this case, bio reducing agents and biological stabilisers of silver ions, such as whole organisms or plant extracts, algae, mushrooms, yeasts, bacteria, fungus, and viruses, are becoming more and more ingrained in green chemistry approaches. In fact, this is because these substances contain a variety of secondary metabolites, including proteins, enzymes, amino acids, vitamins, lipids, and nucleic acids, all of which can participate in the manufacture of AgNPs (1,2,3,4).

This environmentally friendly nanotechnological technique offers benefits including speed, low cost, and ease of use that make it practical and efficient for many procedures. Furthermore, it frequently incorporates a one-step procedure with biocompatible and non-toxic materials, little silver ion concentration, low levels of reactivity, and the usage of water-soluble compounds. As a result, all of these variables lower the danger of toxicity to various living species and the environment (5,6). The creation of nanoparticles (NPs) uses plants and various plant products, making nanobiotechnology one of the most active fields of research in modern materials science. NPs are generally understood to be particles having a size of under 100 nm. These particles, in compared to the bigger particles of the mass material from which they were formed, have exhibited entirely novel and increased features in terms of size, distribution, and shape (7). NPs of noble metals like gold, silver and platinum are well recognized to have significant applications in electronics, magnetic, optoelectronics and information storage (8-11).

Nanobiotechnology and their derived products are unique not only in their treatment methodology but also due to their uniqueness in particle size, physical, chemical, biochemical properties and broad range of application as well. This current emerging field of nanobiotechnology is at the primary stage of development due to lack of implementation of innovative techniques in large industrial scale and yet has to be improved with the modern technologies. Hence, there is a need to design an economic, commercially feasible as well environmentally sustainable route of synthesis of AgNPs in order to meet its growing demand in diverse sectors.

Various approaches available for the synthesis of silver NPs include chemical (12), electrochemical (13), radiation, photochemical methods (14) and Langmuir-Blodgett (15-16) and biological techniques (17,18). In this race of Ag NP preparation, plant-mediated green biomimetic synthesis of silver nanoparticle is considered a widely acceptable technology for rapid production of silver nanoparticles for successfully meeting the excessive need and current market demand and resulting in a reduction in the employment or generation of hazardous substances to human health and the environment.

In the current study, we aimed to isolate and screen the fungi using plant extract. Further, the leaf samples were isolated and screened for identification of fungi using standard manuals. In which *Aspergillus niger* were identified and used for biosynthesis of AgNPs for a broad range for application process. The NPs were well characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) analysis. Despite this, only a few microorganisms are capable of producing AgNPs. So, other microorganisms with the capacity to synthesize AgNPs must be investigated. Therefore, this research was performed to find a new fungal strain that synthesis AgNPs and also to study its properties.

Materials and Methods

Collection of plant material

The plant material *Aglaia elaeagnoidea* was collected from Western Ghats of Agumbe region, Karnataka.

Isolation of fungi

Leaves were washed under running tap water and then with teepol solution to ensure for dust free and clean. Then, leaves were washed thoroughly with sterile double distilled water (SDDW). Under aseptic conditions, leaves were surface sterilized with 10 % H_2O_2 and then with 80 % alcohol followed by thorough rinsing with SDDW for three

to four times. Leaves were dried on sterile blotting paper and then cut into small segments. Leaf segments were placed on solidified potato dextrose agar (PDA) media plates. PDA plates were incubated at 24 ± 2 °C for 10 days. After 10 days, fungal mycelia were harvested and transferred onto fresh PDA plates.

Preliminary phytochemical screening
Various chemical tests were carried out on the Methanolic extract using standard procedures to identify the preliminary screening (19,20).

Alkaloids (Mayer's test)
To 2ml of leaf fungal extract, 1ml of dilute HCl is added and a few drops of Mayer's reagent were added along the slide of the test tube. The formation of yellow colour indicates the presence of alkaloids.

Flavonoids (NaOH test)
The leaf fungal extract was dissolved in 2ml of 2% NaOH and further observed for the formation of intense yellow colour which becomes colourless on addition of few drops of dil. HCl indicates the presence of Flavonoids (21).

Phenol (Lead acetate test)
The leaf fungal extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate solution. The formation of white precipitate indicates the presence of Phenolic compounds (22).

Lignin (Labat test)
To the leaf fungal extract gallic acid is added. The appearance of olive-green colour indicates the presence of lignin (23,24).

Sterols (Salkowski's test)
Few drops of chloroform are added to the methanolic fungal extract along with few drops of sulfuric acid. The appearance of greenish yellow fluorescence indicates the presence of Sterols.

Saponin (Foam test)
1ml of methanolic fungal extract was added to distilled water and shaken vigorously until the formation of honeycomb like foam for 10 to 15minutes indicates the presence of Saponin.

Terpenoids (Salkowski's test)
Few drops of chloroform are added to the methanolic fungal extract along with few drops of sulfuric acid. The appearance of radish brown colour indicates the presence of Terpenoids (25).

Tannins (Ferric chloride test)
To 2ml of leaf fungal extract, the ferric chloride solution was added. The formation of black precipitate indicates the presences of tannins.

Glycosides (Keller Killani test)
To 1ml of fungal extract, 1.5ml of glacial acetic acid was added to this 1 drop of 5% Ferric Chloride and concentrated H_2SO_4 along the sides of test tube. Appearance of reddish brown indicates the presence of Glycosides.

Reducing Sugar (Benedict's test)
1ml of methanolic fungal extract and 2ml benedict's reagent solution was heated in boiling water bath 3 to 5 minutes. The presence of reducing sugar is indicated by the formation of green colour (26).

Carbohydrate (Molisch's test)
To 2ml of methanolic fungal extract α-napthol is added. Further concentrated H_2SO_4 along the sides of the test tube. The violet ring formation at the junction indicates the presence of Carbohydrates (26,27).

Proteins (Ninhydrin test)
To 2ml of leaf fungal extract, few drops of 2% ninhydrin solution is added. The violet colour formation indicates the presence of Proteins (28).

Biosynthesis of AgNPs
The synthesis of silver nanoparticles was carried out according to the method (29). The fungal isolate *Aspergillus niger* was cultured in 100 ml of potato dextrose broth and was incubated at 26 ± 2 °C in shaker incubator (LABLINE) at a speed of 100 rpm. After 21 days of growth, the fungal biomass was harvested and washed thoroughly with SDDW to prevent the contamination of medium components. 10 g of fungal biomass was taken in 250 ml Erlenmeyer flask containing 100 ml SDDW and incubated at 28 ± 4 °C for 72 h in shaker incubator at the speed of 100 rpm. After incubation, the aqueous solution was separated by filtration through Whatmann No.1 filter paper. This solution, namely, fungal filtrate, used for the synthesis of AgNPs. 100 ml of 1 mM of silver nitrate was then added to 100 ml of fungal filtrate and incubated at 28 ± 4 °C for 24 h in the dark condition.

Characterization of Biosynthesized AgNPs

Surface morphology and elemental detection of synthesized AgNPs (30,31).

The surface morphology and elemental detection of synthesized AgNPs were studied by using a scanning electron microscope (SEM).

Phase identification of AgNPs using X-Ray Diffractometer (32).

Phase identification of synthesized and standard AgNPs was measured using by X-Ray Diffractometer (XRD) (M/s. Rigaku, Ultima 4, Tokyo, Japan).

The interaction of the incident rays with the sample produces constructive interference when conditions satisfy Bragg's law. Inter-planar spacing between atoms (d-spacing) was calculated by using Bragg's Law (1912) as given below

Where,

λ = Wave length of X-Ray (0.1540 nm)

θ = Diffraction angle (deg)

n= Integer called order of diffraction

Functional group analysis (33)

The functional group in the synthesized AgNPs was analyzed by using a Fourier Transform Infrared spectroscope (FT-IR) (IRTracer 100 AH, Shimadzu, Japan). The instrument was initialized with Lab Solution software (Version, 100).

Results

Collection of plant material and Isolation of fungi

The plant material *A. elaeagnoidea* was collected from Western Ghats of Agumbe region, Karnataka (Fig 1). The area being at a latitude and longitude of 13.5087°N 75.0959°E. After collection, leaves were separated and used for inoculation. *Aspergillus niger* were identified and used for characterization of AgNPs (Fig 2).

Fig 1: ***Aglaia elaeagnoidea***

Fig 2: ***Aspergillus niger***

Qualitative detection of secondary metabolites

Leaf fungal methanolic extract of *A. elaeagnoidea* plant, were found to be able to produce all the functional metabolites (table 1). All the isolates showed more or less efficient (as observed from the intensity of colour) for the production of alkaloids, flavonoids, saponins, phenol, lignin, sterols, carbohydrates, terpenoids, glycosides and reducing sugars (Fig 3).

Table 1: Qualitative detection of secondary metabolites from *A. elaeagnoidea* (leaf fungal extract)

Phytochemical Test	Methanolic leaf extract
Alkaloids	+
Flavonoids	+
Phenol	+
Lignin	+
Sterols	+
Saponins	+
Terpenoids	+
Tannin	-
Glycosides	+
Reducing Sugar	+
Carbohydrates	+
Protein	-

Present **(+);** Absent **(-)**

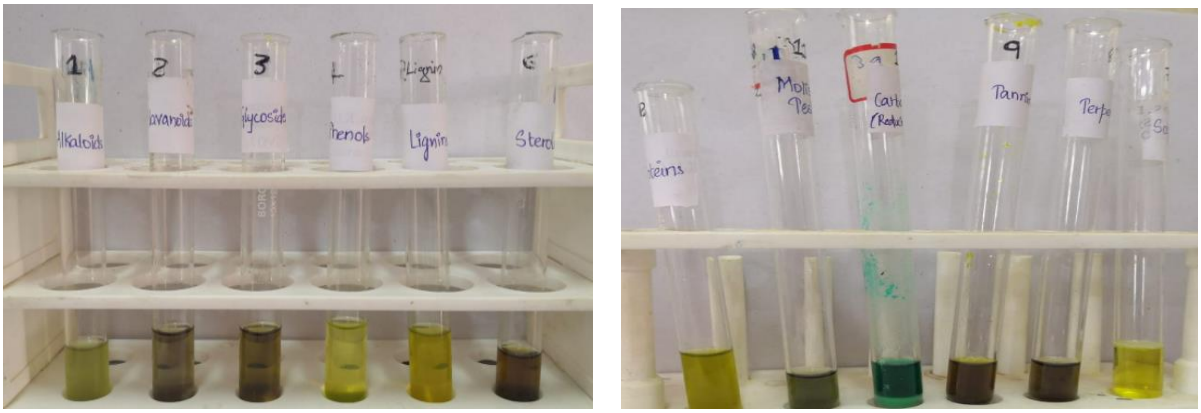

Fig 3: Isolation and identification of secondary metabolites from *A. elaeagnoidea* (leaf fungal extract)

Biosynthesis of Silver Nanoparticles

After incubation of cultured flasks at room temperature on a rotatory shaker *Aspergillus niger* was grown successfully in liquid broth and sufficient biomass was obtained (Fig 4). After 48 h of incubation of the experimental flasks with 1 mM of $AgNO_3$, color change was seen from yellow to brownish which confirms the synthesis of silver nanoparticles.

Fig 4: *Aspergillus niger* biomass during incubation period.

Characterization of silver nanoparticles

XRD Studies

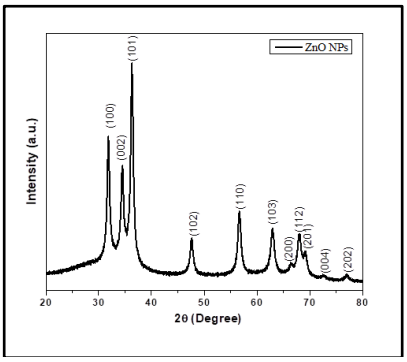

Fig 5: XRD pattern of Ag nanoparticles.

The XRD pattern of *Aspergillus niger Ag*NPs is shown in Fig.5 .The diffraction peaks of Ag NPs at 2θ = 31.82°, 34.51°, 36.27°, 47.71°, 56.76°, 62.95°,66.53°, 68.12°, 69.13°, 72.45°, and 77.09°can be assigned to (100), (002), (101), (102), (110), (103), (200), (201), (004) and (202) was confirmed.

FT-IR Spectrum

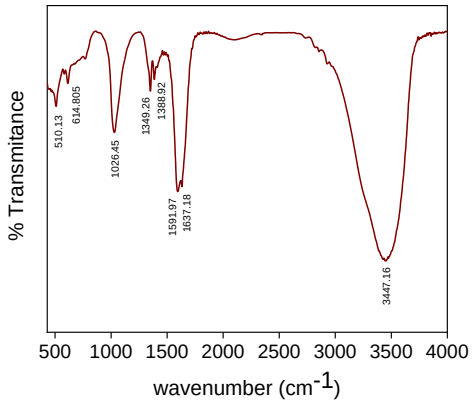

Fig 6: FTIR Spectrum

Fig 6 shows the FTIR spectrum analysis. The hydroxyl (O-H) group of water stretching and banding modes frequently results in large bands of around 3447.16 and 1637.18 cm-1.

SEM analysis

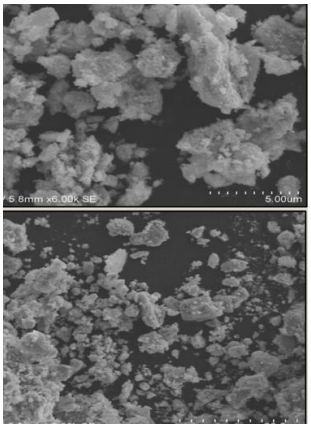

Fig 7: SEM of AgNps

The SEM images exhibited irregular aggravated morphology with the different-sized particles due to agglomeration. It shows a large surface area (Fig 7). It confirms the purity of AgNPs and no other impurity was detected in the sample spectrum.

Conclusion

Medicinal plants have medicinally important compounds in their different parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phytochemical content, special adaptation, and medicinal importance. In this study, we investigated eco-friendly and cost-effective green synthesis of silver nanoparticles using leaf and its fungal extract of medicinal plant *A. elaeagnoidea.* XRD, SEM and FTIR studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape and stable. This green synthesis is inexpensive and simple method can be used as alternative to chemical, physical, and microbial mediated methods used for production of silver nanoparticles.

References

1. Singh, P., Kim, Y.J., Zhang, D., Yang, D.C.: Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. (2016). https://doi.org/10.1016/j.tibtech.2016.02.006
2. Al-Bahrani, R., Raman, J., Lakshmanan, H., Hassan, A.A., Sabaratnam, V.: Green synthesis of silver nanoparticles using tree oyster mushroom *Pleurotus ostreatus* and its inhibitory activity against pathogenic bacteria. Mater. Lett. (2017). https://doi.org/10.1016/j.matlet.2016.09.069
3. Ovais, M., Khalil, A.T., Raza, A., Khan, M.A., Ahmad, I., Islam, N.U., Saravanan, M., Ubaid, M.F., Ali, M., Shinwari, Z.K.: Green synthesis of silver nanoparticles via plant extracts: beginning a new era in cancer theranostics. Nanomedicine 15, 85 (2016). https://doi.org/10.1016/j.matlet.2016.09.069
4. Chokshi, K., Pancha, I., Ghosh, T., Paliwal, C., Maurya, R., Ghosh, A., Mishra, S.: Green synthesis, characterization and antioxidant potential of silver nanoparticles biosynthesized from de-oiled biomass of thermotolerant oleaginous microalgae *Acuto desmus dimorphus*. RSC Adv. (2016). https://doi.org/10.1039/c6ra15322d
5. Teimouri, M., Nejad, F.K., Attar, F., Saboury, A.A., Kostova, I., Benelli, G., Falahati, M.: Gold nanoparticles fabrication by plant extracts: synthesis, characterization, degradation of 4-nitrophenol from industrial wastewater, and insecticidal activity—a review. J. Clean Prod. (2018). https://doi.org/10.1016/j.jclepro.2018.02.268
6. Siddiqi, K.S., Husen, A., Rao, R.A.: A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnol. (2018). https://doi.org/10.1186/s12951-018-0334-5.
7. van den Wildenberg W: Roadmap report on nanoparticles. W&W Espana sl, Barcelona, Spain; 2005.
8. Gratzel M: Photoelectrochemical cells. Nature 2001, 414: 338–344. 10.1038/35104607.
9. Okuda M, Kobayashi Y, Suzuki K, Sonoda K, Kondoh T, Wagawa A, Kondo A, Yoshimura H: Self-organized inorganic nanoparticle arrays on protein lattices. Nano Lett 2005, 5: 991–993. 10.1021/nl050556q
10. Dai J, Bruening ML: Catalytic nanoparticles formed by reduction of metal ions in multilayered polyelectrolyte films. Nano Lett 2002, 2: 497–501. 10.1021/nl025547l
11. Murray CB, Sun S, Doyle H, Betley T: Monodisperse 3d transition-metal (Co, Ni, Fe) nanoparticles. MRS Bull 2001, 26: 985–991. 10.1557/mrs2001.254.
12. Sun Y, Yin Y, Mayers BT, Herricks T, Xia Y (2002) Uniform form silver nanowires synthesis by reducing $AgNO_3$ with ethylene glycol in presence of seeds and poly(vinyl pyrrolidone). Chem Mater 14:4736–4745
13. Yin B, Ma H, Wang S, Chen S: Electrochemical synthesis of silver nanoparticles under protection of poly (N-vinylpyrrolidone). J Phys Chem B 2003, 107: 8898–8904. 10.1021/jp0349031
14. Dimitrijevic NM, Bartels DM, Jonah CD, Takahashi K, Rajh T: Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane. J Phys Chem B 2001, 105: 954–959. 10.1021/jp0028296
15. Callegari A, Tonti D, Chergui M: Photo-chemically grown silver nanoparticles with wavelength-controlled size and shape. Nano Lett 2003, 3: 1565–1568. 10.1021/nl034757a
16. Zhang L, Shen YH, Xie AJ, Li SK, Jin BK, Zhang QF: One-step synthesis of monodisperse silver nanoparticles beneath vitamin E Langmuir monolayers. J Phys Chem B 2006, 110: 6615–6620. 10.1021/jp0570216
17. Swami A, Selvakannan PR, Pasricha R, Sastry M: One-step synthesis of ordered two

dimensional assemblies of silver nanoparticles by the spontaneous reduction of silver ions by pentadecylphenol Langmuir monolayers. J Phys Chem B 2004, 108: 192 69. 10.1021/jp0465581

18. Naik RR, Stringer SJ, Agarwal G, Jones S, Stone MO: Biomimetic synthesis and patterning of silver nanoparticles. Nat Mater 2002, 1: 169–172. 10.1038/nmat758
19. Harborne J. Phytochemical methods London. Chapman and Hall, Ltd. Iinuma M, H Tsuchiya , M Sato ,J Yokoyama, M Ohyama and Y Ohkawa , 1973.
20. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Journal Ethnopharmacology, 1993; 90(2-3):P.249-252.
21. Arusle C S., and K.V Sable. 2017. Preliminary Phytochemicals analysis of Parthenium Hysterophorus leaves. International Journal of recent scientific research .Vol.8(8).19353-19355.
22. Raaman N. Phytochemical techniques. New India Publishing agency, New Delhi,2006,19-24.
23. Bhatt S and S. Dhyani. Preliminary Phytochemical screening of Ailanthus excels Roxb. International journal of current Pharmaceutical research. 2012;4(1):87-89
24. Nanna RS, M Banala, A Pamulaparthi , A Kurra and S kajithoju. Evaluation of phytochemicals and Fluorescent analysis of seed and leaf extracts of Cajanus cajan L. International Journal of Pharmaceutical sciences review and Research. 2013;22 (1):11-18.
25. Das Kunatal and Tribedi sourav.2015 Effect of Zn, Fe and Cu content on Phytochemical investigations and antimicrobial potential of alternanthera brasiliana(L.) O. Kuntze leaf Extracts procured from two different states of India. Terk Journal of pharmaceutical 12(3),345-356.
26. Raaman N. Phytochemical techniques. New India Publishing agency, New Delhi,2006,19-24.
27. Singh V and R Kumar. Study of Phytochemical analysis and antioxidant activity of allium sativum Of Bundelkhand region. International journal of life sciences scientific research .2017;3(6):1451-1458.
28. Silva GO, AT Abeysundara and M M Aponso. Extraction methods Qualitative and Quantitative techniques for screening of Phytochemicals from Plants. American Journal Of Essential Oils and Natural Products . 2017;5(2):29-32.
29. Jaidev LR, Narasimha G. Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. Colloids Surf B. 2010;81:430–433. doi: 10.1016/j.colsurfb.2010.07.033.
30. Guo, Z., Li, Z., Farquhar, J., Kaufman, A. J., Wu, N., Li, C., ... & Wang, P. (2010). Identification of sources and formation processes of atmospheric sulfate by sulfur isotope and scanning electron microscope measurements. Journal of Geophysical Research: Atmospheres, 115(D7).
31. Dhillon, P., Govila, V., & Verma, S. (2014). Evaluation Of Various Desensitizing Agents In Reducing Dentin Hypersensitivity Using Scanning Electron Microscope: A Comparative In Vitro Study. Indian Journal of Dental Sciences, 6(5).
32. Djangang, C. N., Mlowe, S., Njopwouo, D., & Neerish, R. (2015). One-step synthesis of silica nanoparticles by thermolysis of rice husk ash using nontoxic chemicals ethanol and polyethylene glycol. Journal of Applicable Chemistry, 4(4), 1218-1226.
33. Faizan, M., Bhat, S. A., Alam, M. J., Afroz, Z., & Ahmad, S. (2017). Anharmonic vibrational and electronic spectral study of 2-amino-4-hydroxy-6–methylpyrimidine: A combined experimental (FTIR, FT-Raman, UV–Vis) and theoretical (DFT, MP2) approach. Journal of Molecular Structure, 1148, 89-100.