DOI: https://doi.org/10.53350/pjmhs2023171641

ORIGINAL ARTICLE

Green Synthesis of Silver Nanoparticle from Brassica Oleracea for Determination of Paracetamol in the Presence of Tyrosine, Leucine and Alanine by Derivative Method

MUHAMMAD ISMAIL[1], WANG XIANGKE[1], SAIMA[2]
[1]*College of Energy Dynamics and Mechanical Engineering, North China Electric Power University, Beijing, China.*
[2]*Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan.*
Correspondence to: Saima, Email: Saimaup204@gmail.com, Cell: 03419880744

ABSTARCT

In this work, biogenic silver nanoparticles (Ag NPs) from Brassica oleracea (Bo) leaf extract (AgNP-Bo) were prepared and utilized in synthetic mixtures for the accurate, effective, and sensitive spectrophotometric detection of Paracetamol. Measurements in the UV-Visible, X-ray diffraction (XRD), and scanning electron microscopy (SEM) have been used to confirm the synthesis of AgNP-Bo. These statistics indicate that the average particle size is 26.39 nm, the crystallinity has an aggregated structure, and the typical absorption wavelength is 445 nm. To decrease the possible influence of the listed water-soluble interference analytes, namely Tyrosine, Alanine, and Leucine, the 2nd order derivative method was used for our investigation. Using complexation with AgNP-Bo, the linear range, limit of detection (LOD), and relative standard deviation (RSD) for Paracetamol were calculated. The obtained values were 0.1-2, 0.09 µM, and 1.06%, respectively. With average percent recovery values of 93.33, 96.67, and 90.00%, respectively, AgNP-Bo also demonstrated excellent performance for Paracetamol detection in synthetic combinations including Tyrosine, Alanine, and Leucine. The suggested technique applied effectively for the quantitative analysis of synthetic mixtures, which makes it possible to employ Ag NPs going forward to determine biological molecules when interfering analytes from actual samples are present.

Keywords: Silver nanoparticles; Brassica oleracea; Paracetamol; 2nd order derivative spectrophotometry; Interferences.

INTRODUCTION

As an analgesic and antipyretic, paracetamol (N-acetyl-p-aminophenol) is a safe alternative to aspirin when used at therapeutic levels [1]. It lowers fever by preventing the central nervous system's generation of prostaglandins, and it relieves pain by preventing the hypothalamus's ability to regulate body temperature [2, 3]. Being a weak acid with a pKa value of 9.5, paracetamol is easily absorbed after ingestion and broadly dispersed across most bodily fluids. It is then swiftly eliminated by urination [4]. The majority of the time, paracetamol has no negative side effects, but in rare cases, hypersensitivity or overdoses may cause the production of several liver and nephrotoxic metabolites [5]. For the relief of minor pain, fever, lumber pain, backache, migraine, and other nonspecific symptoms, paracetamol is often self-prescribed without any medical supervision and taken orally or as a rectal suppository. It has been discovered to be a potent treatment for osteoarthritis and, more recently, postoperative pain [6 , 7]. The accumulation of dangerous metabolites after an overdose of paracetamol may cause significant, even fatal, liver and kidney damage. Nevertheless, frequent paracetamol use in late pregnancy may increase the likelihood that the unborn child would suffer persistent wheezing [8 , 9 , 10]. Children typically get 120 to 250 mg for ages one to five, and 250 to 500 mg for ages six to twelve. With a daily maximum of 4 g, the normal adult dose by 30 days is 0.5-1 g every four to six hours. These quantities may be administered as needed every four to six hours, with a daily maximum of four doses [11].

In the early years of this decade, interest in the study of nanoparticles increased due to the efficient use of nanoparticles in optoelectronic devices, clinical applications, and the medical field in general—particularly in the areas of diagnosis, optical switching, drug delivery, and the treatment of cancer [12, 13]. In precisely, silver-based nanomaterials made of noble metals have drawn an excessive amount of attention [14]. This is due to the fact that Ag NPs have pronounced surface plasmon resonance (SPR) [15]. The integration of synthetic advancements with SPR permits controlled and methodical alterations in the geometry of nanomaterials as a sensitive function of the nanocomposite structure [16]. These qualities enable the use of Ag NPs in the development of novel, enzyme-free procedures for the detection of paracetamol [17]. Moreover, it is essential to know how silver nanoparticles are produced [18]. Since several physical and chemical preparation techniques that are often unaffordable and harmful to the environment have been documented [19]. For this aim, scientists have developed an alternative way to make silver nanoparticles, namely from plant extracts, which has been demonstrated to be more affordable, efficient, and secure [20].

Brassica oleracea, a plant used for food and medicine, was chosen in this instance for the synthesis of the Ag NPs on the grounds that its polyphenolic components would act as a reducing and stabilizing mediator and would also be able to interact with the paracetamol molecules on the surface of the AgNP-Bo [21].

In this study, the biosynthesis of Ag NPs was carried out by Brassica oleracea leaf extract and used in the quicker, easier, and more convenient 2nd order derivative method for the spectrophotometric determination of paracetamol in synthetic samples containing water-soluble interference molecules, such as Tyrosine, Leucine, and Alanine.

Our study's objective was to develop a more reliable, efficient, and accurate method for detecting paracetamol using Ag NPs in both laboratory and clinical settings, as well as to open up new avenues for future research on methods to monitor the blood paracetamol levels of human beings.

Experimental: Chemicals and reagents: The chemicals used in our study were of a very high purity and didn't need to be further purified. Tyrosine and Leucine were purchased from Sigma-Aldrich, whereas silver nitrate ($AgNO_3$), Paracetamol, and Alanine were purchased from MERCK. Brassica oleracea plant leaves were purchased from a plant nursery in Mardan (KPK), Pakistan. Standard solutions of 1 M silver nitrate, Paracetamol, Tyrosine, Leucine, and Alanine were made in deionized water and kept as stock samples.

Instrumentation: With a double beam UV-1602 spectrophotometer, all absorbance measurements were made. JEOL JSM-7800F performed the SEM examination and measured the particle sizes for AgNP-Bo morphology. The AgNP-Bo crystallinity XRD study was performed using the Bruker D8 Advance Cu Kα (λ = 1.5418 Å).

Preparation of AgNP-Bo: The 40 g of Brassica oleracea leaves were washed, dried, powdered, boiled in 500 mL of deionized water for 30 minutes, cooled in a water bath, and then processed by filtering. The filtrate was stored of further processing, along with measuring absorbance in the 300-800 nm range.

A 300 mL solution of silver nitrate (1 µM) was stirred at 50 °C for 30 minutes and 50 mL of Brassica oleracea leaf extract was added dropwise while being stirred until a yellow color solution was developed. The solution was heated for approximately two hours,

giving it a brownish orange color, a UV-visible spectrophotometer scanning at 300-800 nm wavelength range was performed. The AgNP-Bo was produced, centrifuged for 20 minutes at 5000 rpm, washed with deionized water and distilled ethanol, and dried at 25 ^{o}C.

Determination of Paracetamol by AgNP-Bo: 1 µM of AgNP-Bo were stirred for 30 min with 1 µM each of Paracetamol, Tyrosine, Leucine, and Alanine solutions in a 50:50 ratio, absorbance spectra in the 300–800 nm range were taken, using the Ag NPs as a blank. The following stage was computing the zero-crossing point (ZCP) and obtaining the 2nd order derivative spectra of AgNP-Bo with Tyrosine, Leucine, and Alanine (interferences-AgNP-Bo). Next, 2nd order derivative spectra of each solution were computed using the absorbance spectra of the various solutions containing 0.1-2 M Paracetamol combined with AgNP-Bo in a 50:50 ratio (Paracetamol-AgNP-Bo). In the last step, a calibration curve of interferences-AgNP-Bo at ZCP wavelength (nm) was constructed, and then correlation coefficients and straight-line equations were calculated [22 , 23]. The calibration curve was employed to determine the unknown Paracetamol quantities using a regression equation.

Recovery experiments were carried out by testing different known quantities of Paracetamol in triplicate of each formulation in order to accurately and precisely assess the suggested approach. Then the limits of detection (LOD), the limits of quantification (LOQ), the percentage of recovery, the standard deviation (SD), the relative standard deviation (RSD), and the confidence interval/Limit are calculated.

Paracetamol was also measured using AgNP-Bo in manufactured synthetic mixtures. To accomplish this, five mixes were made by mixing 1 µM of tyrosine, leucine, and alanine with paracetamol at concentrations ranging from 0.2-1.7 µM.

RESULTS AND DISCUSSION

Confirmation of AgNP-Bo preparation: When leaf extract was added to $AgNO_3$ solution, the solution's color changed from translucent to brownish orange, confirming that AgNP-Bo had been successfully synthesized. UV-Visible spectra were used for further validation. The UV-Visible absorbance spectra of Brassica oleracea leaf are shown in Figure 1 together with AgNP-Bo, which displays a distinctive peak of Ag NP at 445. The fact that this peak is the distinctive SPR of metallic Ag NP indicates that AgNP-Bo has probably been produced. Also, owing to a plant's lack of metallic SPR, no absorption bands of pure Brassica oleracea leaf extract was seen at a certain wavelength range [24].

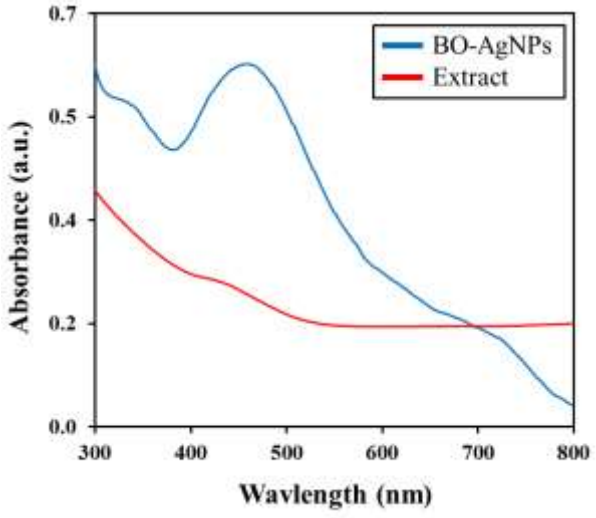

Figure 1: UV-Visible absorbance spectra of Brassica oleracea extract and prepared AgNP-Bo

XRD analysis: As shown in Figure 2, the XRD examination supported the purity and crystallinity of the AgNP-Bo phase. The JCPDS standard powder diffraction card was used to analyze the observed XRD diffractogram, and it revealed a connection to the silver 04-0783 [25]. It may be explained by the 2 values of AgNP-Bo matching the lattice planes at 38, 44, 64, and 77 degrees, which have been determined to be caused by the face-centered cubic crystal structure of silver metal giving the nanocrystalline size was found as 2.12 nm using the Debye-Scherrer equation,.

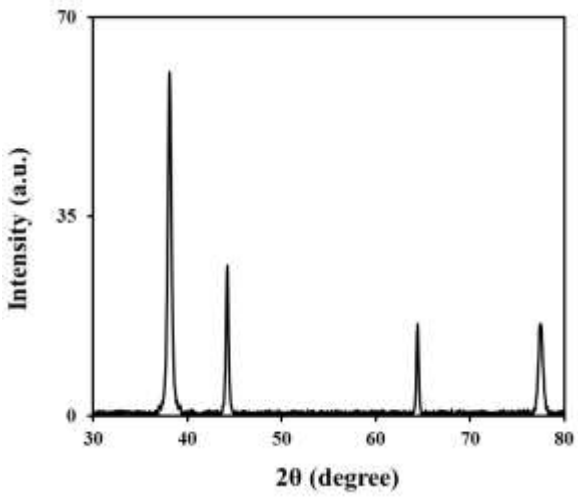

Figure 2: XRD pattern of AgNP-Bo.

Surface morphology analysis: As-prepared AgNP-Bo underwent SEM investigation, which revealed the presence of extremely tiny nanoparticles with granules dispersed randomly over rough surfaces, as seen in Figure 3. Moreover, ImageJ software was used to calculate the size of the Ag NPs [26]. The aspect ratio was computed due to an unequal distribution of particles, and the resultant mean particle size was found as 26.39 nm [27].

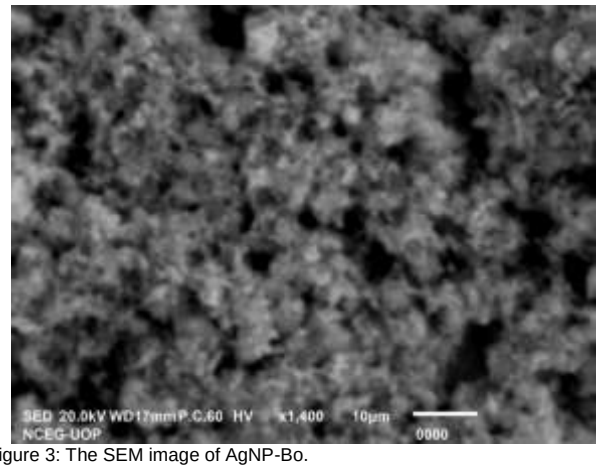

Figure 3: The SEM image of AgNP-Bo.

Determination of Paracetamol by prepared silver nanoparticle: Complexation of AgNP-Bo with Paracetamol: AgNP-Bo was combined with Tyrosine, Leucine, Alanine, and Paracetamol. The complexation has been confirmed by comparing the analyte spectra in Figure 4 to those of pure AgNP-Bo. The Figure demonstrates the changes in absorbance maxima on wavelength that influenced the analytes' absorbance bands. Leucine, Alanine, and Paracetamol each showed a blue shift in contrast to Tyrosine's red shift. These peak movements might be explained by analytes

interacting with nanoparticles changing the Ag NPs dielectric constant [28].

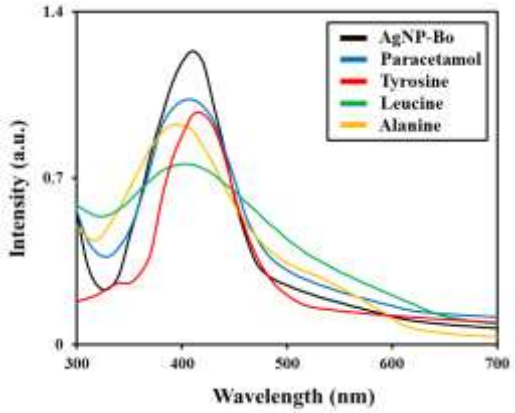

Figure 4: UV-Vis. spectra of pure AgNP-Bo and complexed with analytes.

Analysis of Paracetamol by 2nd order derivative method: It is particularly challenging to identify a single analyte at a given wavelength in a mixed environment that contains interferences since these analytes invariably provide insensitive absorption measurements. Derivative spectroscopies are one of the techniques that may be used in these situations to reduce interference effects and aid in the precise identification of an analyte. Two distinct solutions are required for the measurement of paracetamol using the second order derivative spectrophotometry technique in mixed samples. A solution comprising interference analytes (Tyrosine, Leucine and Alanine) and a pure analyte (Paracetamol) that has to be determined. As shown in Figure 5, the first computation of the 2nd order derivative spectra of AgNP-Bo with Tyrosine, Leucine, and Alanine (interferences-AgNP-Bo) from their respective spectra shows that all three derivative spectra intersect at zero absorbance at a wavelength of 370 nm. Given that there is currently no concentration of these three interference analytes, this wavelength was chosen as the ZCP.

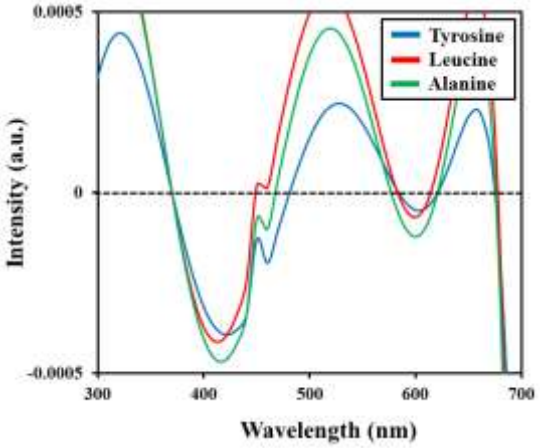

Figure 5: 2nd order derivative UV-Vis. spectra of AgNP-Bo complexed with Tyrosine, Leucine and Alanine.

Thereafter, 2nd order derivative spectra of each of the absorbance spectra of 0.1-2 µM Paracetamol with AgNP-Bo (Paracetamol-AgNP-Bo) were computed. These measurements were used to create the calibration curve at 370 nm, which showed the slope, intercept, and correlation coefficient (R^2) as 1.15, -0.85 and 0.98, respectively (Figure 6). This calibration curve was used to estimate the paracetamol concentrations in synthetic combinations that were unknown. The linear range, LOD, LOQ, and SD are also shown in Table 1, and they were discovered to be 0.1-2, 0.09, 0.29, and 0.06 µM, respectively. In contrast, 1.06% of RSD was attained. In conclusion, the LOD, LOQ, and SD values are all under 1%, while the RSD value is within 10%, demonstrating the method's efficacy and sensitivity.

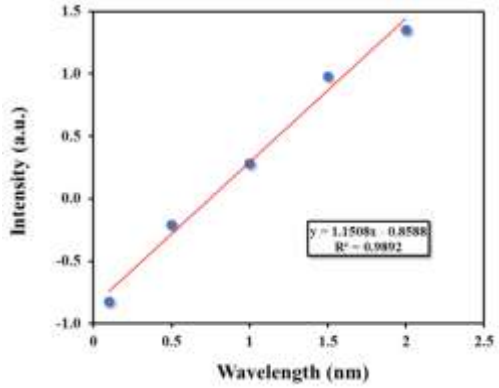

Figure 6: Standard calibration curve for Paracetamol determination.

Table 1: Analytical parameters for the determination of Paracetamol

Parameter	Values
ZCP (nm)	370
Linear range (µM)	0.1-2
LOD (µM)	0.09
LOQ (µM)	0.29
Regression equation (y)	y = 1.1508x - 0.8588
Slope (b)	1.15
Intercept (a)	-0.85
Correlation coefficient (R^2)	0.98
SD (µM)	0.06
RSD (%)	1.06

Precision and accuracy determination: Recovery study processes were carried out to ensure that the suggested method was carefully assessed. After the evaluation of known doses of pure paracetamol three times, recovery examinations were carried out. The absorbance spectra of each solution were recorded. The regression equation predicts the paracetamol concentration. According to Table 2, the accuracy and precision for the triplicate measurement of paracetamol yielded average recovery percentages of 93.33, 96.67, and 90.00%, respectively. RSD for triplicates was found to be, respectively, 5.6, 3.9, and 5.3%. The percent recoveries and RSD values are close to 100 and 10%, respectively, according to the data, showing results of proximal precision. Also, the three samples' confidence intervals are 10.33, 14.67, and 19.00, respectively, whereas the samples' confidence limits were determined to be 0.12, 0.12, and 0.20, respectively, which is close to 1. These results demonstrate the sensitivity and selectivity of the suggested technique.

Table 2: Accuracy and precision for Paracetamol determination

S.No	Taken (µM)	Found (µM)	Recovery (%)	Av. Recovery (%) ± RSD	Confidence interval/Limit
1	1.1	1	90.00	93.33 ± 5.6	10.33 ± 0.12
	1.1	1.1	100.00		
	1.1	1	90.00		
2	1.5	1.5	100.00	96.67 ± 3.9	14.67 ± 0.12
	1.5	1.4	90.00		
	1.5	1.5	100.00		
3	2	1.9	90.00	90.00 ± 5.3	19.00 ± 0.20
	2	1.8	80.00		
	2	2	100.00		

Determination of Paracetamol in synthetic samples: The suggested approach has also been used to assess the usefulness of AgNP-Bo for detecting paracetamol in synthetic mixes. Five

concentrations of paracetamol, 0.2, 0.3, 0.7, 1.3, and 1.7 M, were measured by AgNP-Bo utilizing a calibration curve in solutions comprising interference analytes. Table 3 displays the data, with the average percent recovery being 100.15 and the RSD being 7.80%, with a confidence interval of 8.42 and a confidence limit of 1.31.

Table 3: Analysis of Paracetamol in synthetic mixtures

Amount taken (µM)	Amount found (µM)	% Recovery	Av.% Recovery ± RSD	Confidence interval/Limit
0.20	0.21	105.00	100.15 ± 7.80	8.42 ± 1.31
0.30	0.29	96.67		
0.70	0.68	97.14		
1.30	1.31	100.77		
1.70	1.72	101.18		

CONCLUSION

The function of silver nanoparticles in the latest research on their synthesis in determination of Paracetamol is highlighted by a simple, sensitive, low-cost technique. The prepared AgNP-Bo was successfully employed to detect Paracetamol in synthetic mixtures using the spectrophotometric 2nd order derivative method, with good results. The sensitivity of utilizing Ag NPs in the detection of Paracetamol in synthetic mixtures, especially in the presence of interfering molecules, has enhanced due to the successful use of the recommended technique with the help of spectrophotometry. Because to its high percentage of recovery and low RSD, the suggested approach for assessing paracetamol is very accurate. The recommended method's standard deviations were around 10, and its % recoveries were very near to 100, demonstrating superior reproducibility. For the examination of real serums, such as blood or other live and clinical formulations, this technique is more suitable and sensitive. A biomarker for diagnosing and identifying specific illnesses linked to variations in the amount of Paracetamol in living things may also be created using the suggested method. This approach might be enhanced and applied in upcoming studies for general and industrial analyses.

Declaration of Interests: The author declares no conflict of interests.

Funding: This research work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement: Muhammad Ismail: Conceptualization, Formal analysis & Roles/Writing - original draft; Wang Xiangke: Methodology, Project administration, Supervision; Aneeba Amjad: Data curation; Qaisar Khan: Validation; Abdul Ahad Khan: Investigation & Software.

Acknowledgment: The authors appreciate the College of Energy Dynamics and Mechanical Engineering, North China Electric Power University, Beijing, China for the support and facilitation during this Research work.

REFERENCES

1. *Freo U, Ruocco C, Valerio A, Scagnol I, Nisoli E. Paracetamol: a review of guideline recommendations. J. Clin. Med. 2021 Jul 31;10(15):3420.*
2. *Przybyła GW, Szychowski KA, Gmiński J. Paracetamol–An old drug with new mechanisms of action. Clin. Exp. Pharmacol. Physio. 2021 Jan;48(1):3-19.*
3. *Chiew AL, Reith D, Pomerleau A, Wong A, Isoardi KZ, Soderstrom J, Buckley NA. Updated guidelines for the management of paracetamol poisoning in Australia and New Zealand. Med. J. Aus. 2020 Mar;212(4):175-83.*
4. *Offor SJ, Amadi CN, Chijioke-Nwauche I, Manautou JE, Orisakwe OE. Potential deleterious effects of paracetamol dose regime used in Nigeria versus that of the United States of America. Tox. Rep. 2022 Apr 27.*
5. *Pacheco-Álvarez M, Benítez RP, Rodríguez-Narváez OM, Brillas E, Peralta-Hernández JM. A critical review on paracetamol removal from different aqueous matrices by Fenton and Fenton-based processes, and their combined methods. Chemosphere. 2022 May 13:134883.*
6. *Alwan S, Conover EA, Harris-Sagaribay L, Lamm SH, Lavigne SV, Lusskin SI, Obican SG, Romeo AN, Scialli AR, Wisner KL. Paracetamol use in pregnancy—caution over causal inference from available data. Nature Rev. Endoc. 2022 Mar;18(3):190.*
7. *Juujärvi S, Saarela T, Hallman M, Aikio O. Trial of paracetamol for premature newborns: five-year follow-up. J. Mat. Fet. Neonatal Med. 2022 Dec 12;35(25):5210-2.*
8. *Gautam S, Singhal J, Lee HK, Chae KH. Drug delivery of paracetamol by metal-organic frameworks (HKUST-1): Improvised synthesis and investigations. Mat. T. Chem. 2022 Mar 1;23: 100647.*
9. *Rajamehala M, Pandian AM, Rajasimman M, Gopalakrishnan B. Synthesis of metal-based functional nanocomposite material and its application for the elimination of paracetamol from synthetic wastewater. Chemosphere. 2022 Dec 1;308: 136530.*
10. *Coelho AM, Queiroz IF, Lima WG, Talvani A, Perucci LO, Oliveira de Souza M, Costa DC. Temporal analysis of paracetamol-induced hepatotoxicity. Drug Chem. Toxi. 2022 Mar 250:50-0.*
11. *Gao YQ, Zhou JQ, Rao YY, Ning H, Zhang J, Shi J, Gao NY. Comparative study of degradation of ketoprofen and paracetamol by ultrasonic irradiation: Mechanism, toxicity and DBP formation. Ultrason. Sonochem. 2022 Jan 1;82: 105906.*
12. *Samuel MS, Ravikumar M, John J A, Selvarajan E, Patel H, Chander PS, Soundarya J, Vuppala S, Balaji R, Chandrasekar N. A review on green synthesis of nanoparticles and their diverse biomedical and environmental applications. Catalysts. 2022 Apr 20;12(5):459.*
13. *Kaur M, Gautam A, Guleria P, Singh K, Kumar V. Green synthesis of metal nanoparticles and their environmental applications. Curr. Opin. Environ. Sci. Health. 2022 Aug 28:100390.*
14. *Taha RH. Green synthesis of silver and gold nanoparticles and their potential applications as therapeutics in cancer therapy; a review. Inorg. Chem. Comm. 2022 Jul 9:109610.*
15. *Rakib-Uz-Zaman SM, Hoque Apu E, Muntasir MN, Mowna SA, Khanom MG, Jahan SS, Akter N, R. Khan MA, Shuborna NS, Shams SM, Khan K. Biosynthesis of silver nanoparticles from Cymbopogon citratus leaf extract and evaluation of their antimicrobial properties. Challenges. 2022 May 5;13(1):18.*
16. *Chatterjee S, Lou XY, Liang F, Yang YW. Surface-functionalized gold and silver nanoparticles for colorimetric and fluorescent sensing of metal ions and biomolecules. Coord. Chem. Rev. 2022 May 15;459: 214461.*
17. *Kumar V, Minocha N, Garg V, Dureja H. Nanostructured materials used in drug delivery. Mater. Today: Proc. 2022 Jan 1;69: 174-80.*
18. *Nathanael K, Pico P, Kovalchuk NM, Lavino AD, Simmons MJ, Matar OK. Computational modelling and microfluidics as emerging approaches to synthesis of silver nanoparticles–a review. Chem. Eng. J. 2022 Feb 9:135178.*
19. *Lomelí-Rosales DA, Zamudio-Ojeda A, Reyes-Maldonado OK, López-Reyes ME, Basulto-Padilla GC, Lopez-Naranjo EJ, Zuñiga-Mayo VM, Velázquez-Juárez G. Green synthesis of gold and silver nanoparticles using leaf extract of Capsicum chinense plant. Molecules. 2022 Mar 4;27(5):1692.*
20. *Abasi F, Raja NI, Mashwani ZU, Amjad MS, Ehsan M, Mustafa N, Haroon M, Proćków J. Biogenic Silver Nanoparticles as a Stress Alleviator in Plants: A Mechanistic Overview. Molecules. 2022 May 24;27(11):3378.*
21. *Bilal A, Kasi JK, Kasi AK, Bokhari M, Ahmed S, Ali SW. Environment friendly synthesis of nickel ferrite nanoparticles using Brassica oleracea var. capitate (green cabbage) as a fuel and their structural and magnetic characterizations. Materials Chemistry and Physics. 2022 Oct 15;290: 126483.*
22. *Eissa AS, Attia K, Abdel-Monem A, Abdel-Raoof A. Three different chemometric approaches coupled with uv spectroscopy for analysis of amlodipine and olmesartan in presence of their acidic degradation products. L-Azhar J. Pharm. Sci. 2022 Oct 13;66(2):148-62.*
23. *Gupta D, Bhardwaj S, Sethi S, Pramanik S, Das DK, Kumar R, Singh PP, Vashistha VK. Simultaneous spectrophotometric determination of drug components from their dosage formulations. Spectrochim. Acta A Mol. Biomol. 2022 Jan 6:120819.*
24. *Lubis FA, Malek NA, Sani NS, Jemon K. Biogenic synthesis of silver nanoparticles using Persicaria odorata leaf extract: Antibacterial, cytocompatibility, and in vitro wound healing evaluation. Particuology. 2022 Nov 1;70: 10-9.*
25. *Crisan CM, Mocan T, Manolea M, Lasca LI, Tăbăran FA, Mocan L. Review on silver nanoparticles as a novel class of antibacterial solutions. App. Sci. 2021 Jan 26;11(3):1120.*
26. *Ural N. The significance of scanning electron microscopy (SEM) analysis on the microstructure of improved clay: An overview. Open Geosci. 2021 Jan 1;13(1):197-218.*
27. *Heath GR, Kots E, Robertson JL, Lansky S, Khelashvili G, Weinstein H, Scheuring S. Localization atomic force microscopy. Nature. 2021 Jun 17;594(7863): 385-90.*
28. *Nguyen VP, Qian W, Li Y, Liu B, Aaberg M, Henry J, Zhang W, Wang X, Paulus YM. Chain-like gold nanoparticle clusters for multimodal photoacoustic microscopy and optical coherence tomography enhanced molecular imaging. Nature Comm. 2021 Jan 4;12(1): 34.*