RASĀYAN *J. Chem.*
Vol. 19 | No. 3 | 1615-1622 | July-September | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ECO-FRIENDLY SYNTHESIS OF GOLD NANOPARTICLES AND THEIR MULTIFACETED THERAPEUTIC APPLICATIONS

H.P. Spoorthy[1], N. Vijendra Kumar[2], D. Geetha[3], N.D. Rekha[4], G. Karthik[2], B.K.Kendagannaswamy[5], and L. Mallesha[2]✉

[1]Department of Microbiology, JSS College of Arts, Commerce and Science, Ooty Road, Mysuru-25, Karnataka, India

[2]PG Department of Chemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysuru-25, Karnataka, India

[3]Department of Chemistry, Maharani's Science College for Women (Autonomous), Mysuru-05, Karnataka, India

[4]PG Department of Biotechnology, JSS College of Arts, Commerce and Science, Ooty Road, Mysuru-25, Karnataka, India

[5]Department of Chemistry, JSS College of Arts, Commerce and Science, Nanjangud-01, Karnataka, India

✉Corresponding author: mallesha83@gmail.com

ABSTRACT

The biosynthesis of AuNPs was performed efficiently using the bacteria *Azospirillum brasilense*, making it a green method for producing gold nanomaterials. The characterisation of AuNPs was performed through UV-visible spectroscopy, FT-IR, SEM, and XRD analysis, which showed that AuNPs are stable spherical particles with a particle size of about 39 nm. Biological studies found that AuNPs displayed notable antibacterial, anti-inflammatory, anti-proliferation, and anti-angiogenesis properties. Remarkably, the AuNPs exhibited a moderate phospholipase A2 (PLA2) inhibitory capacity, when compared to traditional drug diclofenac. In addition, the nanoparticles also showed strong anti-angiogenesis ability. Overall, these results indicate the excellent bioactivity of biosynthesised AuNPs. This study establishes a foundation for the development of eco-friendly, nanotechnology-based therapeutics with broad-spectrum efficacy.

Keywords: *A. Brasilense,* Gold Nanoparticles, Antimicrobial, Anti-Inflammatory, Antiproliferative, Anti-Angiogenic.

RASĀYAN *J. Chem.*, Vol. 19, No.3, 2026

INTRODUCTION

Nanoparticles (NPs) exhibit a wide spectrum of applications in various fields of physical and biological sciences. This is because of its unique properties, such as magnetic, electric, biological and catalytic functions due to its distinctive size and shape.[1,2] An arsenal of NPs is synthesized such as nano-pores[3], nano-clusters[4], green-NPs[5], nano-tubes[6], etc., for numerous applications. In the nanotechnology era, gold NPs are gaining attention towards combating solutions for the problem associated with multidrug-resistant (MDR) bacterial pathogens.[7] It is established that the NPs kill the bacterial pathogens through multiple ways, such as generating free radicals, DNA damage, potassium efflux, membrane damage, and gene regulations, etc., which is a suitable mode of action for biomedical applications.[8,9] There are several reports available for the synthesis of NPs by chemical methods using chemicals as capping agents, which may raise several environmental concerns and cause dramatically effect on both humans and microbes to get resist in future treatments.[10-13] Much research has presented an effect of inorganic, polymer, carbon, chemically synthesised NPs and pure natural compounds such as thymol, cinnamaldehyde, curcumin, etc. against dangerous pathogens such as *Staphylococcus aureus*, *Bacillus ceruse*, *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri,* etc. to kill and solve the problem of infectious diseases.[14]

Anti-inflammatory involves the regulated production of signaling molecules such as interleukins and cytokines, with keratinocytes actively contributing to the modulation and coordination of these responses.[15] Numerous anti-inflammatory mediators, along with enzymes and antibodies, are produced and released through coordinated interactions between the immune and endocrine systems. Biosynthesised AuNPs perform wound repair and tissue regeneration and treat inflammation very well.[16] Cancer is a disease wherein a cell displays uncontrolled division, which results in a tumour. These cells sometimes invade the surrounding tissues, resulting in metastasis. The nitrogen mustard can be considered the initial step of antineoplastic chemotherapy focused on all tumour cells.[17] Chronic granulocytic leukaemia is a disorder of hematopoietic stem cells characterized by the way of hyper proliferation of frequently immature cells of the myeloid lineage. Certain naturally occurring biomolecules can function as anti-angiogenic agents.[18] Various inorganic nanoparticles have also been shown to inhibit angiogenesis in ocular diseases.[19] Among these, gold nanoparticles have demonstrated particularly promising outcomes.[20] Organic nanoparticles are prepared from the plant source, bacteria, fungi and other methods.[21-23] Guided by this understanding, the present study aims to synthesize gold nanoparticles using *Azospirillum brasilense* and evaluate their multifunctional biological properties, such as antimicrobial, anti-inflammatory, antiproliferative, and anti-angiogenic properties.

EXPERIMENTAL

Chemicals and Bacterial Strains

All chemicals used were of analytical grade. Tetrachloroauric acid ($HAuCl_4$) and dimethyl sulfoxide (DMSO) were used as received. The bacterial strain *Azospirillum brasilense* was procured from NCL, Pune.

Synthesis of Gold NPs

The bacteria were grown on nutrient broth media and shaken incubated for 48 h. The cells were then centrifuged to obtain cell-free supernatants. The supernatants were incubated with $HAuCl_4$ solution at 25 ^{0} C. Formation of the nanoparticles was monitored by the visual detection of a colour change from yellow to dark brown.

Optimisation of Nanoparticles

Media/pH/Temperature

The synthesis process was optimized by varying culture media, pH (3, 7, 11), and temperature (30–50 °C). The yield of nanoparticles was estimated under each condition.

Characterisation of Synthesised Nanoparticles

UV–Vis Spectroscopy: AuNPs formed was monitored using a UV–visible spectrophotometer (Agilent Cary 60).

FT-IR Analysis: Functional groups associated with the synthesised AuNPs were characterised using an FT-IR spectrophotometer (Agilent Cary 630 ATR) over the range of 4000–400 cm^{-1}. [24]

SEM Analysis: AuNP was analysed using Hitachi S-3400N, Japan, operated at 10 kV. [25]

XRD Analysis: A Rigaku MiniFlex II diffractometer was used to determine the XRD of AuNP.[26]

Biological Activity

Antibacterial Assay

Antibacterial activity of the AuNP was determined using the disc diffusion method against selected bacteria.[27]

Antifungal Activity

Antifungal efficacy was evaluated using the poisoned food technique, measuring inhibition of fungal growth.[28]

Anti-inflammatory Assay
Anti-inflammatory activity was assessed using an indirect hemolytic assay, measuring inhibition of PLA2 activity.[29]

Antiproliferative Activity
The cytotoxic potential of AuNPs was analysed employing the MTT assay on human cancer cell lines.[30]

Anti-angiogenic Activity
The CAM assay was employed to evaluate the inhibition of blood vessel formation in developing embryos.[31]

RESULTS AND DISCUSSION

Biosynthesis of AuNPs
The culture supernatant of *A. brasilense* was employed as a bioreductant for the conversion of $HAuCl_4$ to gold nanoparticles (AuNPs). Reduction was completed within 24 h at room temperature. A visible colour change was observed. Brown colour was observed from the initial pale yellow under the light exposure, indicating the formation of colloidal AuNPs, attributed to surface Plasmon resonance.

Characterization
Optimisation of Biosynthesis of AuNPs
Media
The yield of **AuNPs** was observed to be more in Luria-Bertani (LB) media, and least in the case of Elliker Broth (EB) (Table-1).

Table-1: Yield of AuNP (µg/ml) in Various Media

Nanoparticle	LB	NB	EB
	Yield (µg/ml)		
Gold	87.4	85.1	82.0

pH
At pH 3, 7 and 11, the yield of NP was determined. At pH 7 in LB media, the highest yield was observed (Table 2).

Table-2: The Effect of pH

Nanoparticle	pH- 3	pH- 7	pH- 11
	Yield (µg/ml)		
Gold	85.2	87.0	82.3

Effect of Temperature
The influence of temperature on nanoparticle yield was evaluated at 30, 40, and 50 °C. Maximum production of gold nanoparticles was observed at 40 °C in Luria–Bertani medium (Table-3), indicating that temperature significantly influences nanoparticle synthesis.

Table-3: Variation of Temperature

Nanoparticle	30 ºC	40 ºC	50 ºC
	Yield (µg/ml)		
Gold	82.2	87.4	85.3

UV–Visible Spectral Analysis
UV–visible spectroscopy was employed as a preliminary tool to confirm nanoparticle formation through colour change. At 543 nm, an absorption peak was observed (Fig.-1), characteristic of AuNPs. This colour development is attributed to surface Plasmon resonance (SPR), a property absent in bulk metals.[32]

FT-IR Analysis
Functional groups associated with biomolecules involved in AuNP formation were determined by FT-IR. Characteristic absorption bands were observed at 527, 1053, 1399, 1522, 1619, 2113, 2920, and 3267cm^{-1} (Fig.-2). Peak at 1399 cm^{-1} was attributed to C–N stretching vibrations. The band at 1619 cm^{-1}

corresponded to amide carbonyl stretching. Broad peak at 3276 cm^{-1} was due to O–H stretching, indicating the presence of alcohol groups.

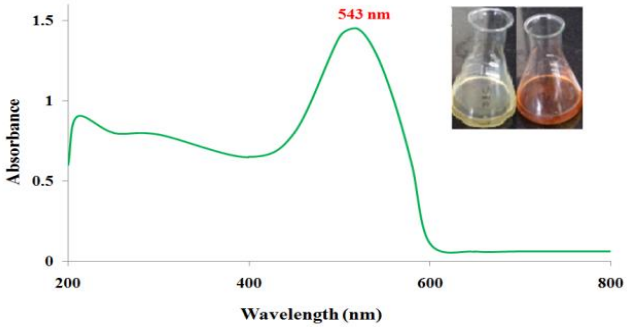

Fig.-1: UV-Visible Spectrum of AuNPs and Colour Change was observed in the Subfigure to Confirm the Nanoparticle Synthesis

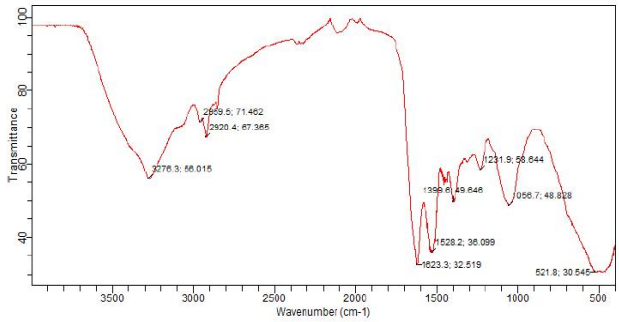

Fig.-2: FT-IR Analysis of Gold Nanoparticles.

Scanning Electron Microscopy

Surface morphology of the AuNPs was examined by SEM. The micrographs (Fig.-3) revealed predominantly spherical nanoparticles with a non-agglomerated distribution and powder-like appearance. The SEM micrograph confirms the formation of gold NPs and was comparable to the other previous reports.[33-34]

Fig.-3: SEM Analysis of AuNPs

X-ray Diffraction

X-ray diffraction analysis confirmed the presence of a face-centred cubic crystal structure characteristic of AuNPs.[35]

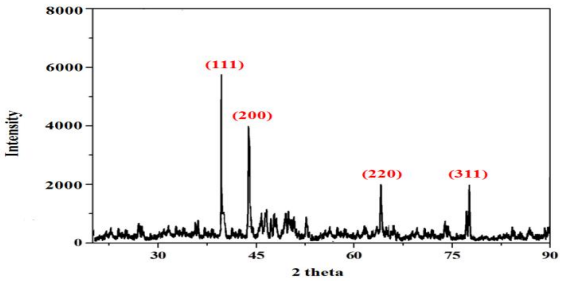

Fig.-4: X-ray Diffraction Patterns of AuNPs

Table-4: Antibacterial Activity of AuNPs

Test sample	Conc. µg/disc	Zone of Inhibition in mm										
		B. cereus	*Staph. aureus*	*Strep. mitis*	*Strep. oralis*	*E. coli*	*Kl. pneumonia*	*Pr. mirabilis*	*Ps. aerogenosa*	*Salm. paratyphi*	*Salm. typhimurium*	*Sh. flexneri*
Gold	Control	7.3 ± 0.58 ^{i}	8.7 ± 0.58 ^{hi}	8.0 ± 0.00 ^{ij}	9.3 ± 0.58 ^{hi}	7.0 ± 0.00 ^{k}	7.7 ± 0.58 ^{j}	8.7 ± 0.58 ^{hijk}	9.3 ± 0.58 ^{i}	7.7 ± 0.58 ^{jk}	7.0 ± 0.00 ^{j}	8.3 ± 0.58 ^{hi}
	50	11.3 ± 0.58 ^{gh}	15.0 ± 0.00 ^{de}	16.3 ± 0.58 ^{c}	15.0 ± 1.00 ^{cde}	13.7 ± 0.58 ^{fgh}	12.3 ± 0.58 ^{hi}	11.0 ± 1.00 ^{gh}	22.0 ± 1.00 ^{c}	18.3 ± 0.58 ^{cde}	16.7 ± 1.15 ^{c}	14.3 ± 0.58 ^{de}
	100	15.7 ± 1.15 ^{e}	18.0 ± 1.00 ^{c}	19.7 ± 0.58 ^{b}	15.3 ± 0.58 ^{cd}	15.0 ± 1.00 ^{fg}	15.3 ± 0.58 ^{ef}	17.3 ± 0.58 ^{c}	28.7 ± 0.58 ^{ab}	20.7 ± 0.58 ^{ab}	19.3 ± 0.58 ^{b}	17.0 ± 1.00 ^{c}
Gentamicin	10	21.0 ± 1.00 ^{cd}	23.0 ± 1.00 ^{a}	19.3 ± 0.58 ^{b}	21.3 ± 0.58 ^{a}	22.7 ± 0.58 ^{b}	18.3 ± 0.58 ^{cd}	21.3 ± 1.53 ^{b}	22.3 ± 1.53 ^{c}	19.3 ± 0.58 ^{bc}	20.3 ± 1.53 ^{b}	20.0 ±1.00 ^{a}
Culture filtrate[36]	25 µL	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -	- NIL -

Results are expressed as means of three independent experiments ± standard deviation (SD). The significance of differences was assessed using one-way analysis of variance (ANOVA) ($p < 0.001$), and further post hoc testing was performed using Tukey's test. Data within a column without common superscripts show no significant difference, whereas data with different superscripts show significant differences ($p < 0.05$).

Biological Activity

Antibacterial Assay

AuNPs prepared were found to possess significant antibacterial and antifungal properties. This may be because AuNPs can cause disruption of the membrane structure in addition to inhibiting metabolic functions. Enhanced efficacy is seen at higher concentrations.

Antifungal Activity

Table-5: Antifungal Activity of AuNP

Test Sample	Conc. (µg/ml)	% Inhibition		
		Aspergillus niger	*Fusarium oxysporum*	*Helminthosporium solani*
Gold	50	69.79 ± 0.26 ^{g}	43.83 ± 0.30 ^{i}	71.75 ± 0.40 ^{g}
	100	72.73 ± 0.26 ^{f}	61.76 ± 0.38 ^{fg}	74.62 ± 0.51 ^{f}
	200	75.84 ± 0.23 ^{d}	73.95 ± 0.32 ^{b}	76.67 ± 0.17 ^{e}
Nystatin[36]	50	81.76 ± 0.36 ^{c}	68.32 ± 0.41 ^{c}	83.17 ± 0.63 ^{c}
	100	84.89 ± 0.49 ^{b}	73.97 ± 0.38 ^{b}	86.79 ± 0.46 ^{b}
	200	88.86 ± 0.69 ^{a}	80.07 ± 0.33 ^{a}	88.90 ± 0.31 ^{a}

Results were reported as means ± standard deviations from triplicate analyses (n = 3). ANOVA was applied for statistical analyses, and Tukey's test was conducted as a post hoc test. Significant differences were established at the $p < 0.05$ level. In the same column, data labelled with different letters indicate significant differences.

Anti-inflammatory Activity

AuNP has shown effective inhibitory action on PLA2 activity, suggesting good anti-inflammatory properties. Such an effect is likely due to the inhibition of inflammation mediators and enzymes working in the arachidonic acid pathway. Higher PLA2 activity plays a role in inflammation activity. As reported by Yamashita *et al.,* the concentrations of PLA2 were significantly higher in patients suffering from different forms of malignancies, especially in breast cancer.[37] The IC_{50} of AuNPs was found to be significantly higher than that of the reference drug diclofenac. The IC_{50} of AuNPs was found to be 18.65 ± 1.21 µg/mL compared to that of diclofenac sodium (IC_{50} = 12.80 ± 1.38 µg/mL). This shows that AuNPs moderately inhibit the PLA2 and prevent the COX and LOX pathway in the arachidonic acid cascade when compared to diclofenac.

Table-6: The IC_{50} values for phospholipase A_2(PLA_2) inhibition

Test sample	IC_{50} µg/ml
Gold	18.65±1.21
Diclofenac sodium[36]	12.80±1.38

Antiproliferative Activity

AuNPs showed cytotoxic properties on cancer cell lines by decreasing cell viability. This can be attributed to oxidative stress and apoptosis induced.[38,39]

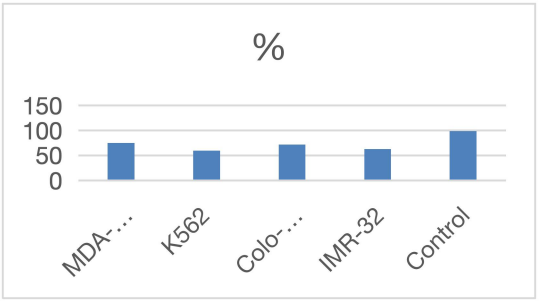

Fig.-5: Antiproliferative Activity (in %) of AuNPs

Anti-angiogenic Activity

The inhibitory effect of gold nanoparticles on the process of angiogenesis was tested through the chorioallantoic membrane (CAM) assay.[40] An important reduction in the number of capillaries was noted in areas exposed to nanoparticles in comparison to the vehicle control (0.1% polyethylene glycol). The nanometallic particles were found to be highly angio-inhibitory when using this test. The chorioallantoic membrane assay is an accepted in vivo model used to assess the role of angiogenesis.

CONCLUSION

Nanoparticle-based technologies have gained increasing attention due to their diverse applications. The current study demonstrates that gold nanoparticles (AuNPs) can be easily prepared through a bio-reducing agent, *A. brasilense* extract, which is both economical and eco-friendly. Characterisation results confirmed the use of biomolecules in capping and stabilisation of nanoparticles. Gold nanoparticles (AuNPs) possessed good antimicrobial, anti-inflammatory, and antiproliferative properties against various cancer cells. Additionally, AuNPs demonstrated promising anti-angiogenic potential in the CAM model. These outcomes reveal the potential of microbially synthesised AuNPs as a multifunctional therapeutic agents. Future efforts should focus on large-scale production and optimization for biomedical applications, particularly in addressing multidrug-resistant infections. Continued advancements are expected to facilitate the development of simplified, multifunctional nanoparticle systems with improved translational potential for clinical applications.

ACKNOWLEDGMENTS

The authors are thankful to the University of Mysore and JSS Research Centre, JSS College of Arts, Commerce and Science, Ooty Road, Mysuru-25, India, for providing infrastructure facilities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

N. Vijendra Kumar https://orcid.org/0000-0002-7791-1545
H.P. Spoorthy https://orcid.org/0000-0002-6952-6524
D. Geetha https://orcid.org/0009-0000-6818-8706
N.D. Rekha https://orcid.org/0000-0001-8259-8568
G. Karthik https://orcid.org/0009-0003-9114-0374
B.K. Kendagannaswamy https://orcid.org/0009-0001-0309-8213
L. Mallesha https://orcid.org/0000-0002-7609-9532

Cite this article as: N. V. Kumar *et al.*, *Rasayan J. Chem.*, 19(3), 1615-1622(2026), https://doi.org/10.31788/RJC.2026.1939803

REFERENCES

1. S. R. Vijayan, P. Santhiyagu, R. Ramasamy, P. Arivalagan, G. Kumar, K. Ethiraj, B. R. Ramaswamy, *Enzyme Microbiology Technology*, **95**, 45(2016), https://doi.org/10.1016/j.enzmictec.2016.06.009
2. V. Harish, M. M. Ansari, D. Tewari, A. B. Yadav, N. Sharma, S. Bawarig, A. Barhoum, *Journal of the Taiwan Institute of Chemical Engineers*, **149**, 105010(2023).
3. M. G. Günay, U. Kemerli, C. Karaman, O. Karaman, A. Gungor, Karimi-Maleh, *Environmental Research*, **217**, 114785(2023), https://doi.org/10.1016/j.envres.2022.114785
4. L. Sun, H. Liu, Y. Ye, Y. Lei, R. Islam, S. Tan, L. Cai, *Signal Transduction and Targeted Therapy*, **8(1),** 418(2023), https://doi.org/10.1038/s41392-023-01642-x
5. S. Vijayaram, H. Razafindralambo, Y. Z. Sun, S. Vasantharaj, H. Ghafarifarsani, S. H. Hoseinifar, M. Raeeszadeh, *Biological Trace Element Research*, **202(1),** 360(2024), https://doi.org/10.1007/s12011-023-03645-9
6. P. Yadav, S. Tyagi, S. Negi, *Arabian Journal for Science and Engineering*, 1-12(2024).
7. X. Li, S. M. Robinson, A. Gupta, K. Saha, Z. Jiang, D. F. Moyano, *ACS Nano*, **8,** 10682(2014), https://doi.org/10.1021/nn5042625
8. H. M. Manukumar, B. Chandrasekhar, K. P. Rakesh, A. P. Ananda, M. Nandhini, P. Lalitha, *Medicinal Chemistry Communications*, **8**, 2181(2017).
9. C. S. Karthik, H. M. Manukumar, A. P. Ananda, S. Nagashree, K. P. Rakesh, L. Mallesha, *International Journal of Biological Macromolecules*, **108**, 489(2018), https://doi.org/10.1016/j.ijbiomac.2017.12.045
10. N. I. Hulkoti, T. C. Taranath, *Colloids and Surfaces B: Biointerfaces*, **121**, 474(2014), https://doi.org/10.1016/j.colsurfb.2014.05.027
11. R. Javed, M. Zia, S. Naz, S. O. Aisida, N. U. Ain, Q. Ao, *Journal of Nanobiotechnology*, **18,** 1(2020), https://doi.org/10.1186/s12951-020-00704-4
12. J. Y. Song, B. S. Kim, *Bioprocess and Biosystems Engineering*, **32**, 79(2009), https://doi.org/10.1007/s00449-008-0224-6
13. G. Wang, H. Mitomo, Y. Matsuo, K. Niikura, M. Maeda, K. Ijiro, *Journal of Colloid and Interface Science*, **452**, 224(2015), https://doi.org/10.1016/j.jcis.2015.04.033
14. H. M. Manukumar, S. Umesha, *Food Research International*, **102**, 144(2017), https://doi.org/10.1016/j.foodres.2017.09.095
15. S. J. P. Jacob, J. S. Finub, A. Narayanan, *Colloids and Surfaces B: Biointerfaces*, **91**, 212(2012), https://doi.org/10.1016/j.colsurfb.2011.11.001
16. S. Gurunathan, L. Kyung-Jin, K. Kalishwaralal, S. Sheikpranbabu, R. Vaidyanathan, S. H. Eom, *Biomaterials*, **30**, 6341(2009), https://doi.org/10.1016/j.biomaterials.2009.08.008
17. R. J. Papac, *Journal of Biology and Medicine*, **74**, 391(2001).
18. K. Y. Han, B. S. Hong, Y. J. Yoon, *Journal of Biological Chemistry*, **406**, 49(2007), https://doi.org/10.1042/BJ20061542
19. J. Chen, S. Patil, S. Seal, J. F. McGinnis, *Nature Nanotechnology*, **1**, 142(2006), https://doi.org/10.1038/nnano.2006.91
20. M. M. Kemp, N*anotechnology*, **20**, 455104(2009), https://doi.org/10.1088/0957-4484/20/45/455104
21. S. R. Vijayan, P. Santhiyagu, R. Ramasamy, P. Arivalagan, G. Kumar, K. Ethiraj, B. R. Ramaswamy, *Enzyme and Microbial Technology*, **95**, 45(2016), https://doi.org/10.1016/j.enzmictec.2016.06.009
22. H. M. Manukumar, B. Chandrasekhar, J. Shivakumar, Sri Raghava, S. Umesha, *Critical Reviews in Food Science and Nutrition*, **57**, 2712(2016), https://doi.org/10.1080/10408398.2016.1143446
23. P. D. Shankar, S. Shobana, I. Karuppusamy, A. Pugazhendhi, V. S. Ramkumar, S. K. Arvindnarayan, G. Kumar, *Enzyme and Microbial Technology,* **95**, 28(2016), https://doi.org/10.1016/j.enzmictec.2016.10.015
24. S. Sandeep, A. S. Santhosh, N. K. Swamy, G. S. Suresh, J. S. Melo, P. Mallu, *Advanced Materials Letters,* **7**, 383(2016), https://doi.org/10.5185/amlett.2016.6067
25. C. S. Karthik, H. M. Manukumar, S. Sandeep, B. L. Sudarshan, S. Nagashree, L. Mallesha, K. P. Rakesh, K. R. Sanjay, P. Mallu, H. L. Qin, *Medicinal Chemistry Communications*, **9**, 713(2018).

26. S. Sandeep, A. S. Santhosh, N. K. Swamy, G. S. Suresh, J. S. Melo, P. Mallu, *Advanced Materials Letters,* **7**, 383(2016), https://doi.org/10.5185/amlett.2016.6067
27. D. Pascal, K. Stanich, B. Girard, G. Mazza, *International Journal of Food Microbiology*, **74(1)**, 101(2002), https://doi.org/10.1016/S0168-1605(01)00734-6
28. N. Khandelwal, A. Singh, D. Jain, M. K. Upadhyay, H. N. Verma, *Digest Journal of Nanomaterials and Biostructures*, **5(2)**, 483(2010).
29. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *Journal of Biological Chemistry*, **193(1)**, 265(1951).
30. T. Mosmann, *Journal of Immunological Methods*, **65**, 55(1983), https://doi.org/10.1016/0022-1759(83)90303-4
31. P. Nowak-Sliwinska, T. M. L. Segura, *Angiogenesis,* **17(4)**, 779(2014), https://doi.org/10.1007/s10456-014-9440-7
32. C. S. Karthik, L. Mallesha, S. Nagashree, P. Mallu, V. Patil, S. Kumar, *Current Chemistry Letters*, **5**, 71(2016).
33. H. M. Manukumar, S. Umesha, H. N. Kumar, *International Journal of Biological Macromolecules*, **102**, 1257(2017), https://doi.org/10.1016/j.ijbiomac.2017.05.030
34. N. Suganthy, V. S. Ramkumar, A. Pugazhendhi, G. Benelli, G. Archunan, *Environmental Science and Pollution Research,* **1**, 12(2017), https://doi.org/10.1007/s11356-017-9789-4
35. R. P. Singh, P. Ramarao, *Toxicology Letters*, **213**, 249(2012), https://doi.org/10.1016/j.toxlet.2012.07.009
36. H. P. Spoorthy, N. C. Mohana, B. R. Nuthan and S. Satish, *International Journal of Pharmacy and Biological Sciences*, **9**, 551(2019)
37. C. T. Pham, D. G. Thomas, J. Beiser, L. M. Mitchell, J. L. Huang, A. Senpan, G. Hu, M. Gordon, N. A. Baker, D. Pan, G. M. Lanza, D. E. Hourcade, *Nanomedicine,* **10**, 651(2014), https://doi.org/10.1016/j.nano.2013.10.012
38. S. Yamashita, J. Yamashita, M. Ogawa, *British Journal of Cancer,* **69**, 1166(1994), https://doi.org/10.1038/bjc.1994.229
39. E. Paszek, J. Czyz, O. Woznicka, D. Jakubiak, J. Wojnarowicz, W. Lojkowski, E. Stępien, *Journal of Biomedical Nanotechnology*, **8,** 957(2012), https://doi.org/10.1166/jbn.2012.1463
40. T. Patil, R. Gambhir, A. Vibhute, A. P. Tiwari, *Journal of Cluster Science*, **34(2),** 705(2023), https://doi.org/10.1007/s10876-022-02287-6

[RJC-9803/2026]