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GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *ACER PENTAPOMICUM* LEAVES EXTRACT ITS CHARACTERIZATION, ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT BIOASSAY

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Over recent years biogenic synthesis of gold nanoparticles from plant extracts has gained much interest from the researchers world widely. Therefore, there is need to divulge the cost effective, nonhazardous and ecofriendly procedures and sources for the synthesis of nanoparticles utilizing the plants. *Acer* family is well adorned for its medicinal potency. The aim of the study was to investigate an efficient and stable procedure for the synthesis of gold nanoparticles(AuNps) from aqueous leaves extract of *Acer pentapomicum* which acts as reducing as well as stabilizing agent. AuNps were synthesized by reacting 1mM gold chloride solution with aqueous leaves extract. The synthesized nanoparticles were confirmed by observing a visible change in color of the solution mixture and characterized by UV-visible spectrophotometer, SEM, EDX, XRD and FTIR. The synthesized nanoparticles were also investigated for their antibacterial, antifungal and antioxidant activity. AuNps were successfully synthesized and characterized. The morphology, size, and structural properties of obtained nanoparticles were determined by SEM, EDX and X-ray diffraction (XRD) techniques. Spherical AuNps, as depicted by SEM, were found to have a size range of 19- 24 nm. FTIR analysis confirmed the coating of phenolic and alcoholic compounds on AuNPs, indicating their possible role in the capping and stabilization of AuNPs. X-ray powder diffraction highlighted the crystalline nature of the AuNps. The synthesized AuNps were found highly toxic against *K. pneumonia* followed by *P. aeruginosa B. subtilus, E. coli, S. aureus and X. compestris*. These were also found to possess potent fungicidal and candidacidal activities against various tested species. Additionally, the antioxidant potential against DPPH revealed that green synthesized AuNPs exhibited good antioxidant activity.

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Keywords: Gold nanoparticles, Green synthesis, SEM, Antimicrobial, Antioxidant

1. Introduction

Nanotechnology is the most progressive area of research in the present era. Green nano-technology, green chemistry and biogenic synthesis of different medicinal products are capturing more deliberation instead of typical chemical procedures [1]. Green Nano-technology and green chemistry, the two different practices of science are used in combination to generate biogenically synthesized metal nanoparticles [2,3,4].

Nano-particles are synthesized by both chemical and physical methods, which are quite hazardous. The development of an eco-friendly, procedure for the synthesis of highly stable metal nanoparticles of numerous size, shapes and chemical composition is the most challenging hindrance in Nanotechnology field [5]. Initially metal Nano particles were synthesized by chemical means using chemicals as reducing agents. But because of its toxicity and potential risk on human health and environment, the chemical methods were replaced by biological methods using biological systems (plants, fungi etc.). The plant bioactive compounds act as best reducing agent for the reduction of metal ions to metal nanoparticles with distinct size, shapes and significant anti-microbial efficacy [6]. Metallic Nano-particles are extensively used in almost

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every field of science. Because of its small size and anti-microbial significance scientist are more interested in evaluating different protocols for the synthesis of highly mono-dispersed Nano-particles with proven antimicrobial activities. [6,7].

Acer pentapomicum, a deciduous small tree or shrub belongs to family *Aceraceae*. The genus *Acer (Aceraceae)*, commonly known as maple family, contains about 200 species mainly distributed in Asia, Europe and North America [8]. *A. pentapomicum* is locally known as Tarkana and is native to northern areas of Pakistan [9]. Maple syrup is the largest commercially available and consumed natural product that is obtained entirely from the sap of maple trees. Several *Acer* species are known for their biological activities [10]. Due to the medicinal importance of *Acer* family plants, and the use of these plants commercially and also in folk medicine make us to evaluate the *Acer pentapomicum* specie of this family for its different important biological activities.

In this research, we investigated an efficient and stable procedure for the synthesis of AuNps using *Acer pentapomicum*. Furthermore, their characterization and pharmacological activities were evaluated.

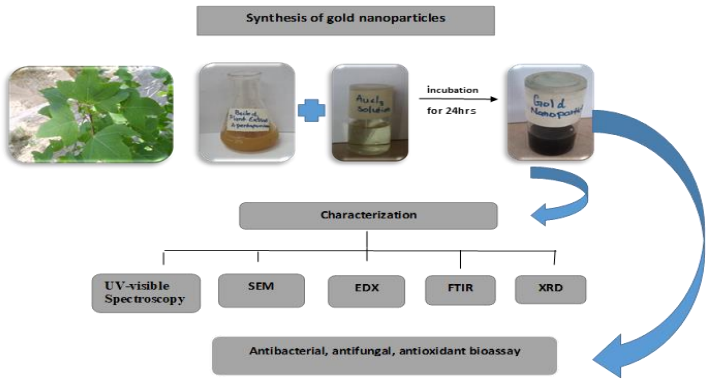
2. Materials and methods

2.1 Preparation of leaves extract

Acer pentapomicum plant was collected from Swat valley, Northern area of Pakistan and were shaded dried. The shade dried leaves were powdered using Anex blender grinder (AG-6040). About 15-20 g of dried leaves powder of *A. pentapomicum* was added to 150 ml of de-ionized water and boiled for 20 mins. The boiled plant extract was filtered (Whatman filter paper No.1) and cooled at room temperature. This extract was further used for the synthesis of gold nanoparticles.

2.2 Biosynthesis of gold nanoparticles

Gold nanoparticles were synthesized following the method of [11]. In a typical procedure, 1 ml of boiled plant extract was added with different ratios of 1 mM solution of gold chloride with constant stirring at room temperature. The yellowish color of the extract solution was immediately changed to purplish color which confirmed the synthesis of gold nanoparticles. The reaction mixture was incubated for 24 hours for complete bio reduction of gold chloride to gold nanoparticles from plant extract.



Scheme 1. Schematic representation of green synthesis of AuNps from A.pentapomicum leaves extract, its characterization by different techniques and its antimicrobial antioxidant activity.

2.3 Stability analysis of biosynthesized AuNPs

The stability test of synthesized nanoparticles was carried out against temperature and pH

2.3.1 Effect of Temperature

The bio-synthesized AuNPs from aqueous plant extract were subjected to heat stress by placing the flask on hot plate. Thermometer was dipped in the nanoparticles solution to observe rise in temperature. The sample was isolated at different temperatures intervals ranging from 25-100°C and checked by UV-visible spectrophotometry. The data was noted in the form scanning graphs.

2.3.2 Effect of pH

The biosynthesized AuNPs were also subjected against pH stress (acid and basic pH). One molar HCl solution and 1M NaOH solutions were prepared in de-ionized water. Few drops of these solutions were added for adjusting different pH range. The pH was adjusted from acidic to basic (3-8). The solutions were kept overnight and the results in the form of scanning graph were obtained by running the samples through UV-vis spectrophotometer.

2.4 Characterization of green synthesized gold nanoparticles

The stabilized biosynthesized nanoparticles were characterized by different techniques described as follows.

2.4.1 UV-visible spectrophotometry

Biosynthesized AuNPs were characterized by UV-Visible spectrophotometer for the confirmation of complete bio reduction of Gold ions to Gold nanoparticles in aqueous plant extract. The UV-visible spectra were recorded from 300 -800 nm wavelengths at 2 nm interval.

2.4.2 Scanning electron microscope

The morphology and average size of AuNPs was determined by SEM (JEOL Japan, JSM 5910) [11]. The resulting images were analyzed by Image J software.

2.4.3 Energy dispersive X-ray

The presence of elemental gold in synthesized AuNPs was confirmed by Energy Dispersive X-ray analysis [11]. Double sided carbon coated glass cover slip was used, lower side of which was used to fixed to the stab, while on upper side sample was loaded and was examined by EDX (OXFORD, UK, Model No. INCA 200).

2.4.4 Fourier Transform Infrared spectroscopy

Fourier transform infrared spectroscopic (FTIR) analysis was carried out for the identification of functional groups involved in the synthesis. The freeze-dried samples of plant extract and AuNPs were grounded separately with KBR (FTIR grade) and analyzed by FTIR (IR-Prestige-21, SHIMADZU), using transmittance mode at $4cm^{1}$ resolution. The resulting spectrum was recorded from 400-4000 cm1 [11].

2.4.5 X-ray diffraction

The crystalline nature, average crystalline size and quality of compounds was checked by X-ray diffraction pattern (JEOL X-Ray diffraction system JDX-3532). The AuNPs were freeze dried (Snidjer Sci., LYSME, Holland). The dried AuNPs were then analyzed by XRD using copper Kα radiation (1.5404 A°) and operated at 40 kv voltage. The XRD pattern was recorded at 2ϑ Bragg's angle ranging from 10 theta to 70 theta and the crystalline size was calculated using Debye- Scherer equation.

$$D = 0.94\ \beta \cos \theta$$

2.5 Antibacterial bioassay of gold nanoparticles

The antibacterial bioassays of AuNPs were investigated against different pathogenic bacteria and using standard well diffusion method [12]. Fresh overnight cultures were taken and spreaded on sterilized nutrient agar plates sterile swabs. Wells of 6mm diameter were punctured in agar plates using sterile cork borer and each well was loaded with different concentration of AuNPs solution (1mg and 2mg per 6 and 12 ul. and an antibiotic (as positive control). The plates were then incubated at 37 ºC for 24 hrs. and the antibacterial activity was calculated by measuring the zone of inhibition around the well impregnated with AuNPs and the percent zone of inhibition was calculated.

2.5.1 Antifungal bioassay

The antifungal efficacy of green synthesized AuNPs from *A. pentapomicum* was determined by fungal growth inhibition assay, the method described by [13]. Sterilized SDA was poured in sterile petri dish. After media setting, 2 wells (6mm in diameter) were punctured in agar plates using a sterile gel puncture. Each well was then introduced with 6, 12µl/well concentrations of the AuNPs. Fungal discs (6mm in diameter) were then inoculated in inverted position on each well. The plates were then incubated at 28 ºC for 72-96hrs. SDA-media plates with a fungal disc but without plant extracts served as a negative control. The fungal colony diameter was measured after 72-96 hrs. and percent growth inhibition was determined in relation to fungal diameter in control [14, 15]. % Growth inhibition was determined by the following formula.

$$GI = (DC-DT)/DC \times 100$$

GI = Percent growth inhibition, DC = fungal colony diameter in control,
DT = fungal colony diameter in treatment

2.5.2 Antioxidant bioassay

The antioxidant bioassay of AuNps was determined by following the methods of [16] by determining its DPPH radical scavenging activity. Different concentrations of 5, 10, 25, 100, 125 and 250 µg/ml AuNPs were prepared in analytical grade methanol. Two and half ml solution of the diluted plant extracts and 1 ml of a 0.3 mM DPPH solution was mixed and kept for 30 min under dark at room temperature. The absorbance of the solution after the reaction was taken at 518 nm using UV visible spectrophotometer. The readings were converted to percent antioxidant.

$$AA = 100(Ao-As)/Ao$$

where; AA = % antioxidant activity
Ao =Absorbance of pure DPPH and
As = Absorbance of the DPPH + extract

3. Results and discussion

3.1 Visual confirmation

The synthesis of AuNPs was first monitored and confirmed visually. Upon adding the different volumes of 1 mM gold chloride (AuCl3) solution with the yellowish plant extract, the color of the solution immediately changes to purplish color, which is the indication of reduction and synthesis process. [17,18,19]. The color of the reaction mixture gets darker after 24 hrs. suggesting the complete bio reduction and formation of larger amount of AuNPs.

3.2 UV-Vis spectrophotometry

Different combination of leaves extract solution and 1mM gold chloride solution were analyzed by UV- VIS spectrophotometry. The absorption spectrum of synthesized AuNPs (Fig 1) was found to have maximum absorption band in the range of 564-570 nm which is due to the surface plasmon resonance of AuNPs [20,21,22,23. Moreover, it was found that 1:12 combination

of aqueous plant extract and 1 mM Gold chloride solution showed maximum absorption band at 568 nm.

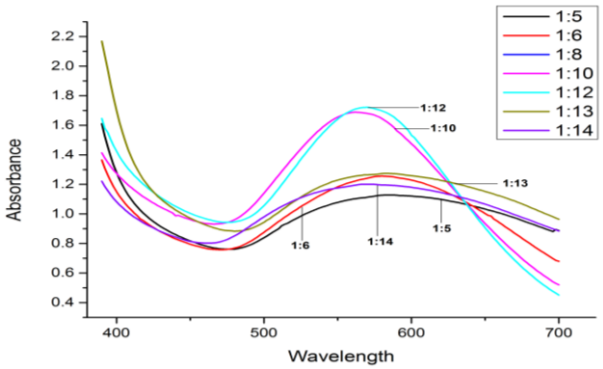


Fig. 1. UV-VIS spectrum of AuNps produced by reacting A. pentapomicum leaves extract (1ml) with different ratios of 1mM (AuCl3) solution, depicting highest peak at 1:12.

3.3 Stability studies

The stability of synthesized gold nanoparticles was tested by studying the effect of different parameters such as temperature andpH.

3.3.1 Effect of temperature

The UV- vis spectrum reveals that the AuNPs were more stable at lower temperature of 25-35 ºC (Fig 2). These results are in harmony with the research conducted by [24]. Our findings also concluded that by increasing the temperature, there is a gradual decrease in the stability of AuNPs. This may be due the loss of bioactive components presents in synthesized AuNPs[25]. At temperature from 70 to 100 °C, almost all the synthesized nanoparticles were degraded, thus showing no detectable absorbance in the specific region.

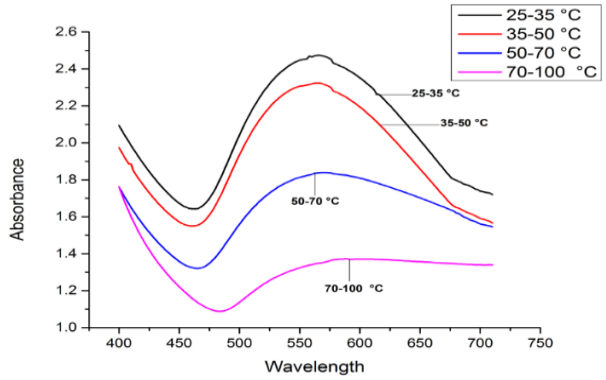


Fig. 2. UV-VIS Spectrum of AuNPs isolated at different temperature ranges.

3.3.2 Effect of pH

pH is another important parameter that affects the nanoparticle synthesis because of its effect on the size and shape of AuNPs. The absorption spectra of AuNPs at different pH of (3-4, 4-5, 5-6, 6-7, 7-8) were observed under UV-VIS spectrophotometer (Fig 3). Enhanced AuNPs synthesis was observed at alkaline pH of 7-8. By increasing the pH from acidic 3-4 to basic 7-8, the absorption intensity also increases, suggesting that the bio-reduction process for the synthesis of AuNPs was completed at alkaline pH. Thus, neutral to basic pH of the reaction mixture was

found to be the most stable and optimal for AuNPs synthesis. [26,27] also reported enhanced AuNPs synthesis at higher pH of 6-8.

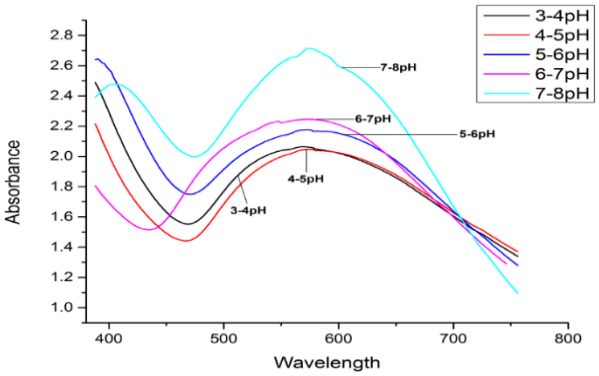


Fig. 3. UV-VIS Spectrum of AuNPs at different pH levels ranging from 3-8.

3.4 Characterization studies

3.4.1 Scanning electron microscopy

SEM micrographs revealed the spherical morphology of our AuNPs (Fig 4). Furthermore, the size of these AuNPs was calculated and found to be 18 -25 nm in range. Similar SEM results for AuNPs from different studied plants were also reported by [18, 28-30].

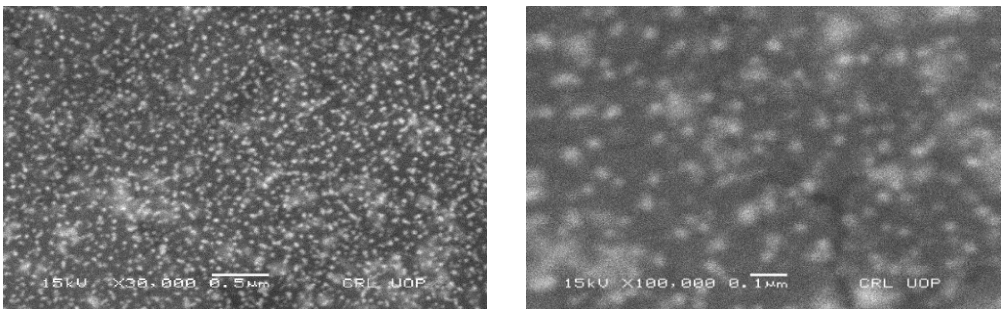


Fig. 4. Scanning electron micrographs of synthesized AuNPs at different magnification revealing spherical morphology and size of 18-24 nm.

3.4.2 Energy dispersive X-ray

EDX spectrum of the synthesized AuNPs reveals strong signals in the gold region and affirms, the formation of AuNPs. A clear, strong peak was observed around 2.40 keV approximately which is the characteristic of gold nanoparticles (Fig. 5). There were also some weak signals for carbon and oxygen atoms which may be due to the X-ray emittance from the enzymes/protiens of the bio-molecules involved in the formation and capping of gold nano-particles. These results are in complete conformity with the results concluded by [18,28].

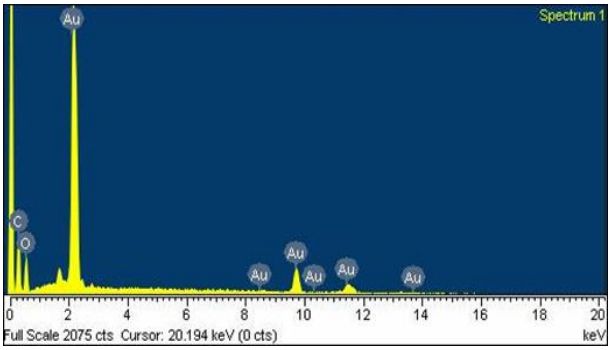


Fig. 5. EDX spectrum of green synthesized AuNPs.

3.4.3 X-Ray diffraction

The synthesized AuNPs were also characterized by X-ray diffraction. Fig 6 represents the XRD pattern. XRD analysis of green synthesized AuNPs reveals the crystalline size and nature of the nanoparticles [23]. The diffracted intensities for biosynthesized AuNPs were recorded at 2º angle from 10°-70°, which confirmed the presence of three major peaks at 38.4°, 44.55°and 64.85°. These major peaks, known as Bragg's reflection affirms the typical diffraction pattern for gold. These peaks correspond to (111), (200), and (220) planes of brag's reflection of gold with FCC (Face centered cubic) structure of gold, which is in accordance with ICCD No. 040784 [27,31]. The recorded XRD pattern indicated and confirmed that the biosynthesized gold nanoparticles using aqueous plant extract were highly crystalline in nature [27].

The crystalline size of AuNPs was calculated by Debye-Scherer equation on the basis of Full width half Maximum (FWHM) of the intense peaks [32], which came out to be 10.35 nm for the most intense peak of 38.4°. The crystalline size for other peaks was 6.4 and 7.56 nm, respectively. The absence of other peaks for crystallographic impurities clearly declared that the synthesized nanoparticles were highly pure and in Nano region [33].

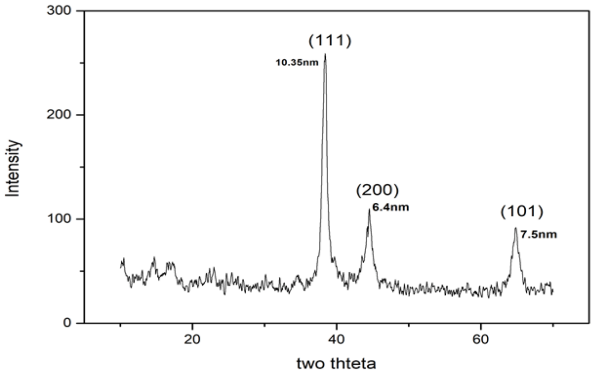


Fig. 6. XRD pattern of biosynthesized AuNPs.

3.4.4 Fourier Transform Infrared spectroscopy

The FTIR spectra in Fig. 7-8 reveals that absorption band of 2677.19 cm-1 originally present in plant extract completely disappeared in synthesized AuNPs sample. The disappearance of this peak which represent the carboxylic group reveals that the carboxylate content present in the plant extract might be responsible for reduction of gold ions to AuNPs. A major shift of 3525.87 cm-1 from 3448.72 cm-1 was also observed indicating the phenolic and alcoholic compounds of plant extract to be involved in the bio-reduction process [17]. Further closer examination and comparison of the FTIR spectra of gold nanoparticles and plant extracts revealed small shifts in the wavenumbers of different absorption bands affirmed the biosynthesis of AuNPs mightinvolve these functional groups [11].

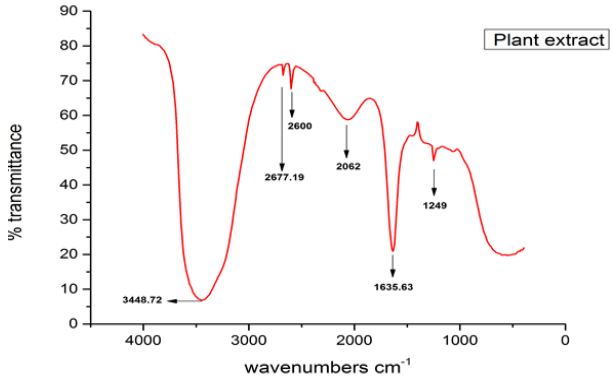


Fig. 7. FTIR spectrum of Aqueous plant extract.

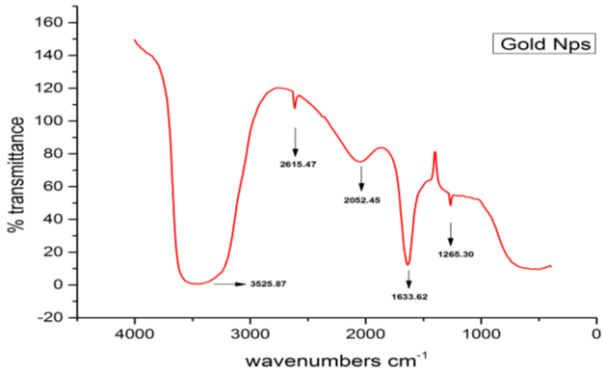


Fig. 8. FTIR spectrum of AuNPs.

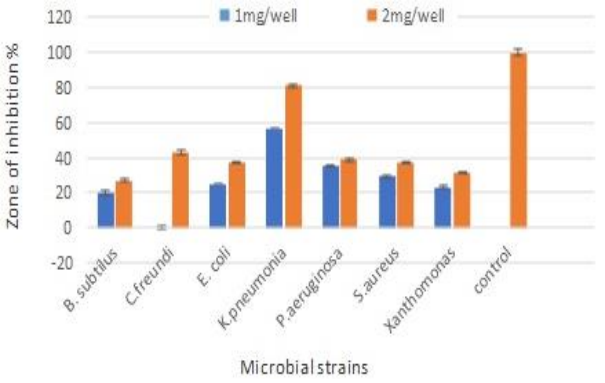


Fig. 9. Antibacterial activity ±SD of green synthesized AuNPs against different microbes.

3.5 Activity studies

3.5.1 Antibacterial bioassay

Plant mediated metal nanoparticles play a vital role in drug delivery and can be used against many microbes due to their efficient antimicrobial properties reported by previous studies [34]. In the present study, we conducted the antibacterial activities of bio-synthesized AuNPs from *A. pentapomicum* plant extracts against various microbes (Figure 9). It is evident from the data that AuNps were toxic against all microbes at different concentrations. *K. pneumonia* was found to be the most susceptible microbe to AuNps at both concentrations of 1 and 2 mg/well. *K. pneumonia* exhibited maximum of 81%, 56.8% at different treated concentrations, respectively. *P. aeruginosa*

on the other hand was found to be the second most susceptible microbe to AuNps. *B. subtilus*, *E. coli*, *S. aureus* and *Xanthomonascompestris* also showed moderate susceptibility. *C. freundi* was found to be resistant at lower concentration, however, showed moderate sensitivity when treated with higher concentration of green synthesized AuNps. AuNPs with efficient antibacterial activity against these different microbes were also reported by [29,35-37].

3.5.2 Antifungal bioassay

The biosynthesized AuNps were also evaluated for their antifungal potency against different fungal species such as *Aspergillus niger*, *Fusarium oxysporium*, *Rizopusoryzae*, *Penicillium chrysogenum*. AuNps were found effective against the tested fungal species (Fig 10) The antifungal evaluation results of AuNPs from different studied plants showed notable activity against different pathogenic human fungi [38]. According to our results, highest growth reduction (43%) was recorded against fusarium followed by *A. niger* with 41.6% of growth inhibition, respectively when tested with different concentrations of AuNPs. *Penicillium chrysogenum* and *R. oryzae*, however, were found to be moderately sensitive to gold nano-particles [26,38-39].

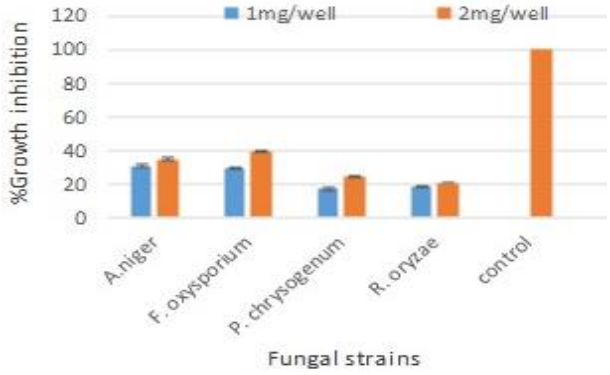


Fig. 10. Antifungal activity ±SD of green synthesized AuNPs against different fungal strains

3.5.3 Antioxidant bioassay

The reactive oxygen species and other free radicals released during various biological processes are the main factors involved in causing pathogenicity. Antioxidant compounds can be defined as any substance that inhibits or obstruct the oxidative damage to the cells [40]. The antioxidant activity of AuNPs was carried out against DPPH. AuNps were found to possess good DPPH radical scavenging activity at all tested concentrations as shown in Fig. 11. AuNps showed a dose dependent activity. Maximum 96% of antioxidant potential was exhibited by gold nanoparticles at 250 µg/ml followed by 93%, 92% and 90% at 125, 100 and 50 µg/ml, respectively. [41,42] also reported the antioxidant activity of green synthesized gold nanoparticles.

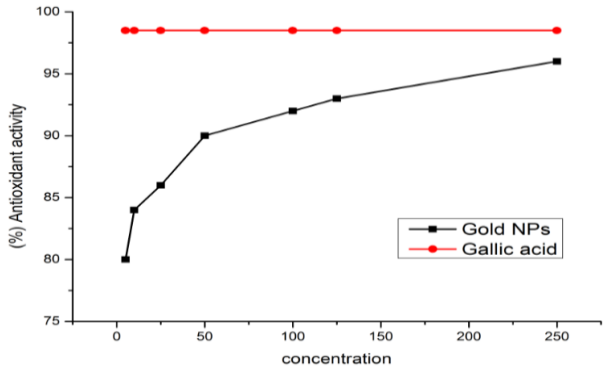


Fig. 11. Antioxidant activity of green synthesized AuNPs.

4. Conclusions

To the best of our knowledge we are the first one to evaluate *Acer pentapomicum* (Maple) plant for bio-synthesis and characterization of AuNPs, its anti-bacterial, anti-fungal, anti-oxidant potential.

In the current investigation, *A. pentapomicum* plant extract presents its potency to produce stable gold nanoparticles at optimum temperature of 25-35°C and with a size range of 18-25 nm which is in typical Nano region, confirmed and identified by different characterization techniques. The synthesized nanoparticles also showed potent antimicrobial activity against different pathogenic bacterial and fungal species and good antioxidant activity against DPPH.

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References

[1] M. Zhang, K. Zhang, B. D. Gusseme, W. Verstraete, The Journal of Bio adhesion and Biofilm Research **30**, 347 (2014).
[2] X. Zhang, Cell Biochemistry and Biophysics **72**(3), 771 (2015).
[3] J. Weingart, P. Vabbilisetty, X. L. Sun, Advance Colloidal Interface Science **10**, 68 (2014).
[4] K. M. Amit, Y. Chisti, U. C. Banerjee, Biotechnology Advances **31**, 346 (2013).
[5] J.Singh, G. Kaur, P. Kaur, R. Bajaj, M. Rawat, World Journal of Pharmacy and Pharmaceutical Sciences **5**(7), 730 (2016).
[6] S. Ahmed, M. Ahmad, B. L. Swami, S. Ikram, Journal of Advanced Research **7**(1), 17(2016).
[7] P. M.Mishra, L. Sundaray, G. K. Naik, K. M. Parida, Optimization of Process Variables Nanoscience and Nanotechnology Letters **6**(5), 409 (2014).
[8] D. M.Van-Gelderen, P. C. De-Jong, H. J. Oterdoom, Maples of the World. Timber press, Portland **1**: 15 (1994).
[9] Mehboob-u-Rahman, Government Jehanzeb college Swat. **1**, 52 (2012).
[10] C. Jiang, M. Chang, C. Wen, Y. Lin, F. Hsu, M. Lee, Journal of Food and Drug Analysis **14**(4), 346 (2006).
[11] P. Banerjee, M. Satapathy, A. Mukhopahayay, P. Das, Bioresources and Bioprocessing **1**, 3 (2014).
[12] D. Mubarakali, N. Thajuddina, K. Jeganathanb, M. Gunasekaranc, Colloids and Surfaces B: Biointerfaces **85**, 360 (2011).
[13] K. Ramdas, G. Suresh, N. Janardhana, S. Masilamani, Journal of Pest Science **52**, 145 (1998).
[14] R. K Grover, D. J. Moore, Phytopathology **52**, 876 (1962).
[15] Y. Rusman, Isolation of new secondary metabolites from sponges associated and plant derived endophytic fungi, PhD thesis, Heinrich Heine University Dusseldorf (2006).
[16] L. I. Mensor, F. S. Menezes, G. G Leitao, A. S. Reis, T. dos Santos, C. S. Coube, S. G. Leitao, Phytotherapy Research **15**, 127 (2001).
[17] A. Dzimitrowicz, G. C. Dicenzo, I. Sergiel, T. Kozleck, P. Pohl, Arabian Journal of Chemistry, in press (2016).
[18] J. Anuradha, T. Abbasi, S. A. Abbasi, Journal of Advanced Research **6**, 711 (2015).
[19] C. Krishnaraj, P. Muthukumaran, R. Ramachandran, M. D. Balakumaran, P. T. Kalaichelvan, Biotechnology Reports **4**, 42 (2014).
[20] A. Sett, M. Gadewar, P. Sharma, M. Deka, U. Bora, Nanoscience and Nanotechnology **7**, 25005 (2016).

[21] P. Kumar, S. Dhiman, R. P.Bhatt, L. Singh, Journal of Applied Pharmaceutical Science **1** (9), 108 (2011).
[22] A. Ravindran, A. Singh, A. M. Raichur, N. Chandrasekaran, A. Mukherjee, Colloids Surface **76**, 32 (2010).
[23] D. Jain, H. K. Daima, S. Kachhwaha, S. L. Kothari, Digest Journal of Nanomaterials and Biostructures **4**(3), 557 (2009).
[24] V. E.Golmoraj, M. R. Khoshayand, M. Amini, K. M. Moghadamd, G. Amin, A. R. Shahverd, Journal of Experimental Nanoscience **6**(2), 200 (2011).
[25] K. Paulkumar, S. Rajeshkumar, G. Gnanajobitha, Vanaja, C. Malarkodi, G. Annadurai, International Journal of Green Chemistry and Bioprocesses **3**, 12 (2013).
[26] R. Sarwar, U. Farooq, M. R. Shah, S. Khan, N. Riaz, S. Naz, A. Ibrar, A. Khan, Rapid Applied science **7**, 29 (2017).
[27] A. K. Singh, O. N. Srivastava, Nanoscale Research Letters **10**,353 (2015).
[28] S. N.Correa, A. M. Naranjo, A. P Herrera, Journal of Physics: Conference Series **687**: 012082 (2016).
[29] B. S. Bhau, S. Ghosh, S. Puri, B. Borah, D. K. Sarmah, R. Khan, Advanced Materials Letters **6**(1), 55 (2015)
[30] M. H. MKhalil, E. H. Ismail, F. E. Magdou, Arabian Journal of Chemistry **5**(4), 431 (2012).
[31] S. P.Dubeya, M. Lahtinenb, M. Sillanpää, Colloids and Surfaces A: Physicochemical and Engineering Aspects **364**, 34 (2010).
[32] S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, S. Thangamani, Asian Pacific Journal of Tropical Biomedicine **2**(7), 574 (2012).
[33] U. R.Pathipati, P. Rajasekharreddy, Colloids and Surfaces A: Physicochemical and Engineering Aspects **389**, 188 (2011).
[34] J. Mittal, A. Singh, A. Batra, M. M. Sharma, An International Journal **1**, 8 (2016).
[35] M. Hosseini, M. Mashreghi, H. Eshghi, Biosynthesis and antibacterial activity of gold nanoparticles coated with reductase enzymes **11**(9),484 (2016).
[36] F. Benakashani, A.R. Allafchian, S. A. H. Jalali, Karbala International Journal of Modern Science **2**(4), 251 (2016).
[37] S. Tvm, P. C. Nagajyothi, K. D. Lee, Journal of Computational and Theoretical Nanoscience **6**(1), 63 (2012).
[38] R. R.Thanighaiarassu, P, Sivamai, R. Devika, B. Nambikkairaj, Journal of Nanomedicine and Nanotechnology **5**, 229 (2014).
[39] C. Jayaseelana, R. Ramkumar, A. A. Rahumana, P. Perumal, Industrial Crops and Products **45**, 423 (2013).
[40] S. Yamagishi, T. Matsui, Nitric oxide, Pharmacological Research **64**, 187 (2011).
[31] H. Shabestariana, M. H. Tabrizib, M. Soltanic, F. Namvard, S. Azizif, R. Mohamad, Material research **20**(1), doi.10.1590/1980-5373 (2017).
[42] K. Saritha, U. Saraswathi, World Journal of Pharmaceutical Sciences **2**(11), 1545 (2014).