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GREEN SYNTHESIS OF COPPER NANOPARTICLES USING POMEGRANATE JUICE AND ASSESSMENT OF THEIR ANTIMICROBIAL EFFICACY

S. E. Naina Vinodini, Suvarna Ravi Bathe
Department of Physics, Govt. City College(A), Nayapul, Hyderabad

ABSTRACT

Study of structural and antimicrobial properties of copper nanoparticles synthesized by green synthesis process with pomegranate juice as stabilising and capping agent. This process is the promising approach in the field of nanotechnology with their effective antimicrobial activity and also it is a cost-effective and environment friendly method, as it eliminates the need for hazardous chemicals and reduces waste generation. The biomolecules present in plant extracts, such as flavonoids, proteins, tannins, phenols and terpenoids act as both reducing and stabilizing agents during synthesis process. Pomegranate juice contains phytochemical components that can efficiently convert metal ions into copper nanoparticles quickly. The green synthesized copper nanoparticles were characterized by UV-Visible, XRD, SEM, EDX and effective antimicrobial activity. The characteristic UV absorption peaks were observed at 220 nm and 232.5 nm. From the XRD it was found that the average particle size were found to be 43,9 nm. The CuNps have demonstrated the antimicrobial activity against a range of bacteria (Pseudomonas, proteus, staphylococcus and basillus)

Keywords: *CuNPS; green synthesis; antimicrobial activity*

INTRODUCTION

Nanoparticles are building blocks of nanotechnology. Nanotechnology reveals the atomic and molecular analysis of materials. Synthesis part of Nanoparticles is the most active area of research in modern material science [2] ,because of their unique properties such as optical, electromagnetic, electrical, catalytic, and mechanical, which enable them to be used in various sectors such as medical diagnostics, therapy, electronics, clothing, agriculture, solar energy conversion and even in cosmetics[1,2]. The present period has been nanotechnology exploring, in which researchers are focused efforts on discovery of new properties, transformation of matter, devices and applications [12].

Copper has been the most widely used metal since ancient times for its unique properties. Copper nanoparticles have a great significance in as they often display distinctive and considerable modified physical, chemical and biological properties as compared to their counterparts at macro

level.[2]. Stability and reactivity are two important factors that impede the use and development of metal cluster in new generation materials [8]. Metal compounds generally reduce into their respective nanoparticles because of microbial enzymes or the plant phytochemicals with antioxidant or reducing properties [9].

Green synthesis is the most sought method for present researchers because of its simple methodology in preparation, cost effective, non-toxic, ease of availability, reduces time consumption and ecofriendly nature. The extracts from plants can behave as capping and reducing agents for the synthesis of nanoparticles[7]. The green synthesis can be conducted using extracellular and intra cellular approaches. The extracellular method involves synthesis using plant extracts by different methods and intra cellular involves making development of plants metal-rich natural media [1].

Punica granatum L (pomegranate) fruit is an ancient unique fruit which is used in several systems of medicine for a variety of ailments [14]. Many researchers have presented their studies on antioxidant, anticarcinogenic and anti-inflammatory properties of pomegranate fruit extracts, focusing on treatment and prevention of different illnesses of human race [14]. In Ayurvedic medicine the pomegranate is considered " a pharmacy unto itself " [15]. The edible part of fruit contains acids, sugars, vitamins, polysaccharides, polyphenols and minerals [17]. The chemical composition of a ripened pomegranate fruit has moisture – 72.6 % - 86.4 %, protein 0.05 % - 1.6 %, fat 0.01 % - 0.9 %, mineral elements 0.36 % - 0.73 %, fibre 3.4 % - 5 %, carbohydrates 15.4 %- 19.6 % , calcium 3.0 – 12 mg, phosphorous 8- 39 mg, Iron 0.3- 1,2 mg, sodium 3 mg, Magnesium- 9 mg, ascorbic acid (Vitamin C) – 4-14 mg, Thiamine (vitamin B1) – 0.01 mg, Riboflavine (Vitamin B2)- 0.012- 0.03 mg and Niacine – 0.18- 0.3 mg [16]. The high mineral content in pomegranate fruit juice can contribute to the daily intake of these constituents in diet [16]. The sun dried rind of immature fruit of pomegranate for the therapy and prophylaxis of malaria [17].

The study of antimicrobial effects of CuNPs using E.Coli and Bacillus subtilis revealed the fact that CuNPs are exhibited superior activity over silver nanoparticles specially in antibacterial activity [4]. The CuNPs obtained from green synthesis using z-spina Christi fruits as green reducing agent shown to be antibacterial against pathogenic bacteria was assessed [7].CuNPs have exhibited potential antimicrobial activity over E.coli, Bacillus subtilis, salmonella Typhi, Klebsiella pneumonia and Staphylococcus aureus [10].

In the present study efforts were made to prepare the Copper Nanoparticles (Cu NPs) are synthesized using green synthesis process in one step and cost efficient method. For the synthesis of CuNPs using pomegranate juice as stabilizing and capping agent. The amount of pomegranate juice was changed to form different morphologies of copper nanostructures. The green synthesized

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CuNPs are tested for antimicrobial activity for a range of microbes and were also characterized with the aid of SEM,XRD, UV-vis and EDS.

MATERIALS AND METHODS

The chemical cuprous oxide was purchased from Sigma Company and deionized water was used for preparation of solutions. Punicagranatum (Pomegranate fruit) was collected from local market cleaned thoroughly with tap water and then with distilled water, dried and the arils (seeds) were separated carefully and kept in a ziplock cover. The juice was collected by pressing arils gently and the juice was filtered using 20-25 µm filter paper. The juice was collected in a beaker kept at 3^0 C for further use. The process was shown in Fig.1.

Fig.1 Green Synthesis Process of CuNPs.

In another beaker 200 ml distilled water was taken and kept on stirrer and heated upto 80^0 C. Then 1.59 gms (0.02 mole) copper oxide was added and continued stirring at 500 rpm. The copper oxide solution was prepared. Then 3 ml of freshly prepared pomegranate juice was diluted with 30 ml of distilled water, stirred well and taken in thoroughly cleaned burette. Then the diluted pomegranate juice was added drop wise to copper oxide solution. Slowly the colour of copper oxide solution was changed indicating the formation of CuNPs. The solution was collected in vials for checking the formation of CuNPs through UV- Vis technique. The same procedure was repeated for 6 ml pomegranate juice also. The solution was taken to rest of the solution was centrifused and dried for some time and collected the powder was collected in vials as shown in fig.2.and sealed with paraffin paper to avoid the oxidation of CuNps. The CuNps were characterized for XRD, SEM and EDX and tested antimicrobial activity through well diffusion.

RESULTS AND DISCUSSION

Characterisation

The formation of CuNPs nanoparticles was confirmed using UV-Visible spectroscopy which allowed for the Surface Plasmon Resonance (SPR).U.V visible spectra were obtained using a UVJACO V-750 spectrometer, scanning the nanoparticle solution over a 200-800 nm wavelength range. The analysis identified synthesized CuNPs based on their characteristic absorption peaks. The surface morphology and size of the synthesized CuNps were investigated using scanning electron microscope (SEM). Thin films of the nanoparticles were prepared on carbon –coated copper grids and examined using a ZEISS Merlin Compact instrument operating at an accelerated voltage of 20kV. The SEM analysis allowed for the visualization of the nanoparticles and the determination of their size distribution and morphology.

The crystalline structure of the CuNPs was determined by X – ray diffraction (XRD) with a Bruker D8 Advance instrument. This technique provided information about the synthesized nanoparticle's crystalline planes and crystallographic structure. The XRD patterns were obtained using Cu-Kα radiation.

Overall, the combination of UV- visible spectroscopy , SEM and XRD measurements facilitated a comprehensive characterization of the synthesized CuNPs which included the optical, morphology and crystal structure. The elemental composition in the reaction mixture was determined by EDX analysis. For antimicrobial activity well diffusion method was used.

UV – Visible Spectroscopy Analysis

The formation of CuNps, was confirmed using UV-Vis spectroscopy. The location of the absorption band peak of CuNps is characterized by factors such as size, reaction time, morphology, temperature, pre-cursor salt concentration and aqueous pomegranate juice extract. Bio-phytochemicals found in pomegranate juice extract play a vital role in stabilizing and forming CuNPs. Figure 2 shows the UV-Vis absorption spectrum of CuNPs from a wavelength range of 200-800 nm. The absorption peak was observed as characteristic peak of CuNPs wavelengths 220 and 232.5 nm, which is possibly due to the electron transition from the valence band to the conduction band (inter- band transition). The energy gap is around 2.19eV [4]. The result obtained from UV-Visible spectroscopy analysis of the samples are presented in the figure. It is the important technique to detect the Surface Plasmon Resonance property of nanoparticles. The peaks reveal that the CuNPs are formed within the range of 200 nm to 800 nm. Both 3 ml and 6 ml concentrations of pomegranate juice were observed and confirmed at the peaks at 220 nm and 232.5 nm respectively. The results are in agreement with [1,4, 5,9,10].

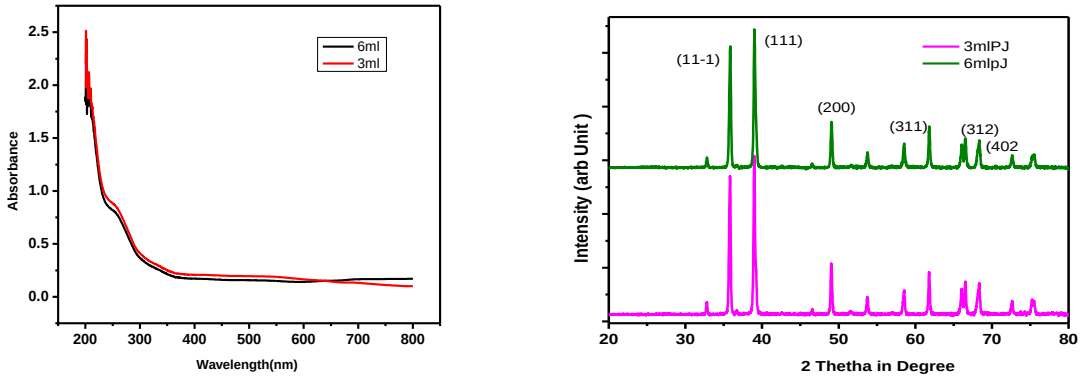


Fig. 2 UV-Vis Absorption peaks and XRD pattern for 3 ml and 6 ml Compositions of pomegranate Juice.

XRD Analysis

The XRD spectrum analysis was used to study and identify the crystalline structure of CuNP materials. The XRd patterns reveal that the synthesized CuNps were identified on three high-intensity peaks at 2θ = 32.88,36,39,50,54,60,62.5,66.5,68.5,68,73,76 corresponding with their crystal planes [11-1],[111], [200],[311] [312][420] of CuNps with the monoclinc structure of CuNps (Fig.). The most intense peak is observed at (111) and the lattice constant was calculated using the following equation. $a = \lambda \left(\frac{\sqrt{h^2+k^2+l^2}}{2\,sin\theta}\right)$ Å

The results are in agreement with [8, 3,4,18, 7, 9]. To determine the average particle size of the CuNps, the Debye- Scherrer equation $D = \frac{K\lambda}{\beta cos\theta}$ is used. Where D- crystalline size of CuNPs λ – wavelength of X Ray radiation used in XRD,K – Scherrer Constant β – full width at half maxima of the diffraction peak. θ – Bragg's angle

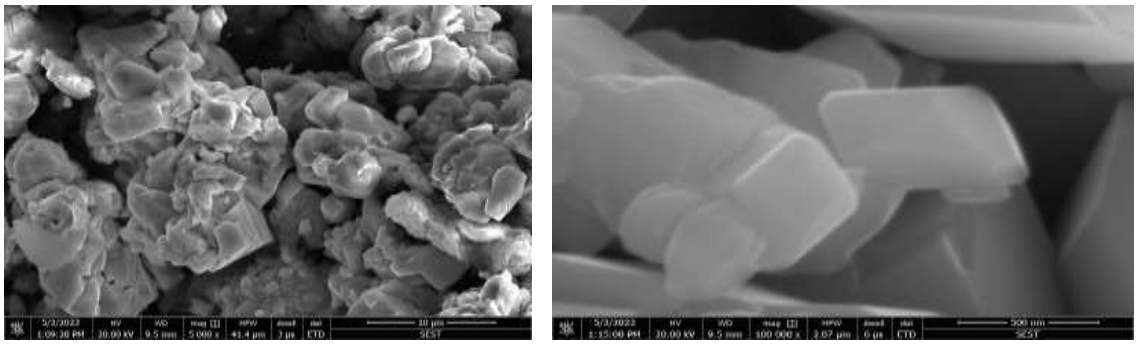
According to Debye-Scherrer equation the average particle size of CuNPs was found to be 46.30 nm and the size is in agreement with [4,1,8,3]. The XRd indicates several characteristic peaks of cubic crystalline structure for CuNps as per standard JCPDS data card 00-001-1117

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to examine the surface morphology and structural features of the synthesized CuNps. the observed images at different magnifications are depicted in Fig ……. . The micrograph images of the as-synthesized CuNPS reveal an accumulation of CuNPs in the form of fluffy agglomerates and flaky cubes are formed reasonably distributed. The CuNPs tend to form aggregates to minimize their surface energy, possibly due to vander- waal forces acting between the CuNpPs . the initial mechanism of Cu atom favours isotropic growth in

the early stages might be due to primarily atoms favouring relatively poor thermal energy. the lower thermal energy favours relatively large numbers of CuNPs which might be due to having more active sites for deposited Cu-nanoparticles. When reaction time increases , Cu atoms acquire high thermal energy and deposit the CUNPs cluster to minimize surface energy, The shape of CuNps was observed as irregular –shaped structures with sizes ranging from 50 nm- 100 nm. The agglomeration clusters were formed, which might be due to the varying crystallization growth of CuNPs. The particle sizes are in agreement with XRD[8,7,4,19,9].

3 ml



6 ml

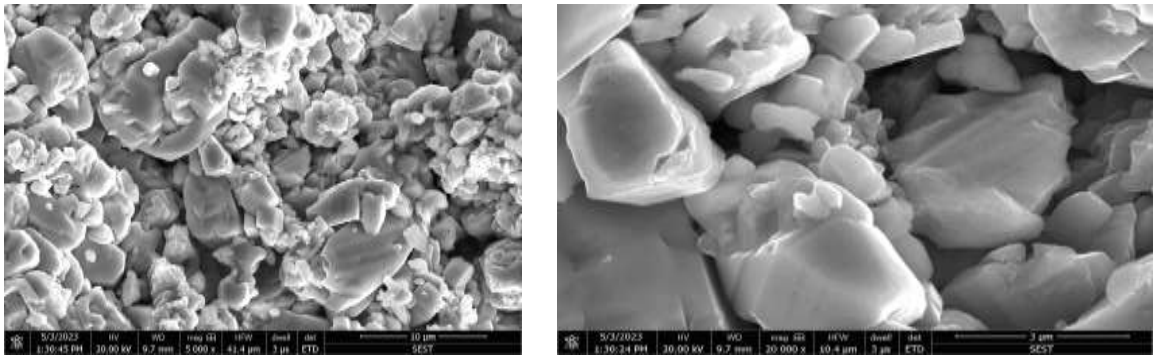
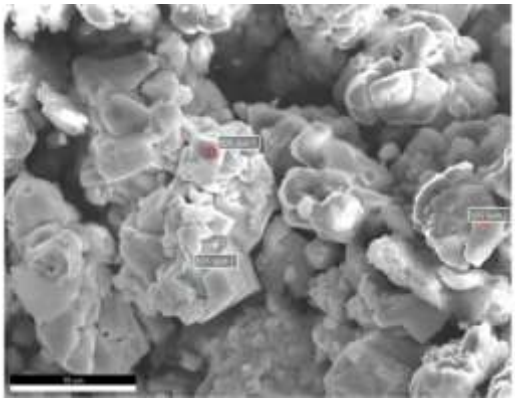
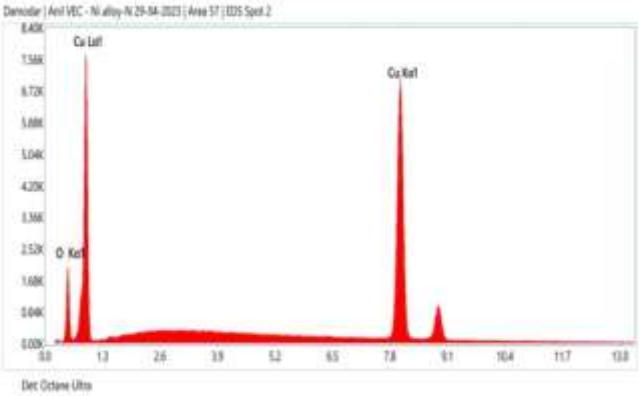


Fig. 3. SEM analysis of CuNPs.

Energy Dispersive X-ray (EDX) Analysis: EDX when combined with electron microscopes can provide elemental analysis on areas as small as several nanometers. The effect of the electron beam on the surface and penetrating into the depth of the particle produces X- rays that are characteristic of the element found on or near the surface of the sample. X- rays are generated in approximately 2μm depth of the sample. In the present sample EDX analysis data confirms the existence of CuNPS to an extent of CuLα. The weight percentage of CuNPS are shown in the graphs.

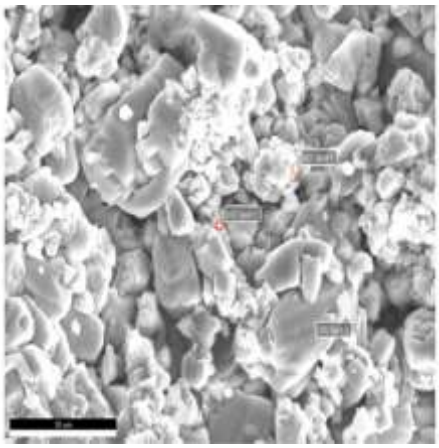
3ml

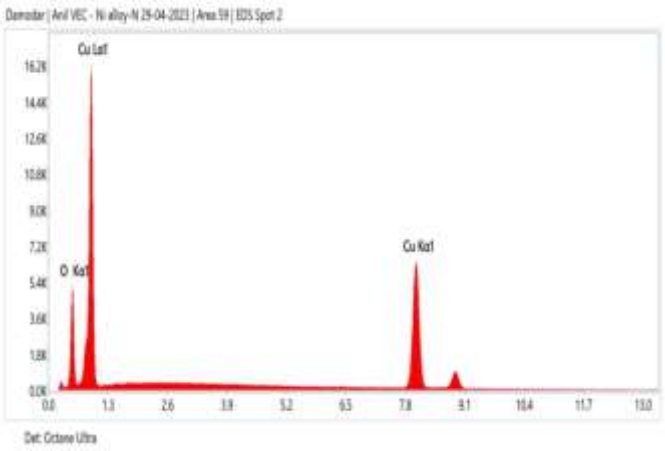




Element	Weight %	MDL	Atomic %	Error %	R	A	F
O K	11.2	0.28	33.3	10.3	0.8039	0.1922	1.0000
Cu K	88.8	0.39	66.7	2.3	0.9171	0.9807	1.0397

6 ml





Element	Weight %	MDL	Atomic %	Error %	R	A
O K	26.2	0.22	58.5	9.4	0.8278	0.2177
Cu K	73.8	0.33	41.5	2.3	0.9319	0.9834

Fig. 4 EDX analysis of CuNPs.

The peaks corresponding to elemental Cu, C,O were clearly identified and no additional peaks were present which demonstrates the purity of the synthesized CuNPs and this was consistent with XRD studies. Both the concentrations 3ml and 6 ml pomegranate juice in cuprous oxide solution reduced copper particles in to nanoparticle size with good percentage of copper components in EDX studies. The results are in good agreement with [5,4,9].

Anti-Microbial Activity:

Antimicrobial activities were determined by well diffusion method. The antimicrobial was carried out using Agarwell diffusion method. For this preseeded disc diffusion plates were by adding 5% incoculum of the test organism *Pseudomonaus, proteu, staphylococcus and Basillus*, in broth (having an O.D of 0.5 and containing 10^5 cfu/mL) to nutrient agar and then pouring the plates (pour plate method). Concentrated, wet and different dilutions of pomegranate juice, seeds powder and peel powder) extracts of various samples (50µL) were introduced into the wells using micropipettes and allowed to diffuse at room temperature. The plates were incubated at room temperature . After the incubation period, the mean diameters of the zones of inhibition around the wells were recorded and the zone inhibition for *Pseudomonaus, Proteus, staphylococcus and Bacillus* are found to be 23 mm, 17mm,19mm and 22mm respectively. The experiments were conducted in triplicates along with control and the average values were recorded for antimicrobial activity. Results are in agreement with [7,4,9,10,18,20].

Fig. 5 Antimicrobial activity of CuNPs.

CONCLUSION

In this study, we successfully synthesized copper nanoparticles(CuNPs) using one-step ,eco-friendly and cost effective green synthesis method. The bio-components present in punica granatum (pomegranate) juice served as excellent reducing and stabilizing agent during the synthesis process. The UV-Vis and XRD analysis confirmed the high purity and cubic crystal structure of the CUNPs phase, with a crystalline size of 46.39 nm and it is in agreement with SEM morphology. The green-synthesised CuNps reveal cubic crystal clusters with different lattice constants. The biosynthesized route is more important than the chemically because it offers a new

means for researchers to produce cost-effective ,highly active sites with high purity, non-toxic, eco-friendly and have low energy consumption. These efforts will definitely facilitate the translation of synthesis of CuNPs from the laboratory scale to large –scale industrial production with the goal of benefitting human health and for sustain the protection of environment with chemicals. The absorption phenomenon in copper nanoparticles, due to surface plasmon resonance is found to be, prominent at about 220 and 232.5 nm .The XRD indicated the presence of CuNPs with significant peaks at [111]. EDX shows the elemental composition is above 80% in the sample. Through antimicrobial activity CuNPs have explored the long term mechanism in antimicrobial efficacy which would open up new avenues in the field of nanomedicine.

REFERENCES

1. J. Santhosh Kumar, Happy Agarwal, Soumya Menon, S.Rajesh Kumar and Venkat Kumar "A biological synthesis of Copper nanoparticles and its potential applications Green Synthesis, Characterization and Applications of Nanoparticles" . https://doi.org/10.1016/B978-0-08-102579-6.00009-5© 2019Elsevier Inc
2. P. NagaPadma, Syed Thanveer Banu and S.Chaitanya Kumari "Studies on Green Synthesis of Copper Nanoparticles Using *Punicagranatum"ARRB,23(1): 1-10,2018; Article no. ARRB. 38894*
3. Farnosh Tavakoli, Masoud Salavati Niasari and Fatemeh Mohandes "Green synthesis of flower like CuI microstructures composed of trigonal nano structures using pomegranate juice " Materials letter 100 (2013) 133-136. https://doi.org/10.1016/j.matlet.2013.02.114
4. Anand Murthy, Tegane Deaslegn, Mebrattu kassa, Buzuayehu Abebe and Temesgen Assefa "Synthesis of Green Copper nanoparticles using medicinal plant Hagenia abyssinica (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties" published in Hindavi Journal of nanomaterial Volume 2020, Article ID 3924081, 12 pages https://doi.org/10.1155/2020/3924081.
5. M. T. Delma and M. Jaya Rajan, "Green synthesis of copper and lead nanoparticles using Zingiber Officinale stem extract," *International Journal of Scientific and Research Publications*, vol. 6, no. 11, pp. 134–137, 2016.
6. S. Shende, A. P. Ingle, A. Gade, and M. Rai, "Green synthesis of copper nanoparticles by *Citrus medica* Linn. (Idilimbu) juice and its antimicrobial activity," *World Journal of Microbiology and Biotechnology*, vol. 31, no. 6, pp. 865–873, 2015.
7. R. Khani, B. Roostaei, G. Bagherzade, and M. Moudi, "Green synthesis of copper nanoparticles by fruit extract of *Ziziphusspina-christi* (L.) Willd.: application for adsorption of triphenylmethane dye and antibacterial assay," *Journal of Molecular Liquids*, vol. 255, pp. 541–549, 2018.
8. P.K.Khanna, S.Gaikwad, P.V.Adhyapak, N.Singh and R.Marimuthu "Synthesis and characterization of Copper Nanoparticles published in Material letters 61(2007 4711-4714.

9. G. Caroling, M. Nithya Priyadarshini, E. Vinodhini, A. Mercy Ranjitham, P. Shanthi "Biosynthesis of Copper nanoparticles using aqueous Guava extract – Characterisation of antibacterial effects" International Journal of Pharmacy and Biological Sciences (e-ISSN 2230-7605).
10. S. Thakur, S. Sharma, S. Thakur, and R. Rai, "Green synthesis of copper nano-particles using Asparagus adscendensRoxb. root and leaf extract and their antimicrobial activities," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 4, pp. 683–694, 2018.
11. P.NagaPadma[1*],SyedThanveerBanu[1]and S. Chaitanya Kumari "Studies on Green Synthesis of Copper Nanoparticles Using *Punicagranatum" Annual Research & Review in Biology* 23(1):1-10,2018; Article no. ARRB.38894 ISSN:2347-565X,NLMID:101632869
12. Hutchison JE. Greener nanoscience :A proactive approach to advancing applications and reducing implication soft nanotechnology.ACSNano.2008;2(3):395-402.
13. Sangiliy and iG, etal. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichiacoli*. Colloids and Surfaces B:Biointerfaces. 2009;74:328-335.
14. Jurenka, Julie "Therapeutic Applications of Pomegranate (Punicagranatum L.): A Review. Published in Alternative Medicine Review . Jun2008, Vol. 13 Issue 2, p128-144. 17p.
15. Naqvi, SA, Khan M S Vohora SB 1991" Antibacterial , Antifungal and Antihelmenthic investigations on Indian medicinal Plants " Published in Fitoterapia 62 : 221-228.
16. A Allwyn Sundar Raj, Swathi Rekha, suvedha and Renupriya " Pomegranate: Constituents biological properties, therapeutic applications and its safety – A review" published in International Journal of Advanced Life Sciences ; ISSN 2277-758X.Vol 7 issue (4) 2014.
17. Singh and Singh " An ethnobotanical study of medicinal plants in Chandauli district of Uttar Pradesh" published in Journal of Ethnopharmacology vol 121 issue 2, 2009 324-329.
18. Wisam M Mohammed, Tahseen H Mabark and Raad, M,S Al-Haddad "Effect of Copper nanoparticles on Antimicrobial activity prepared by Sol-gem method " published in International Journal of Applied Engineering research ISSN 0973-4572 Volume 13 Number 12 (2018) pp 10559- 10562.
19. Maria G Miguel, Maria A Neves and Maria D Antues "Pomegranate (Punica granatum L) : A medicinal Plant with myriad biological properties – A short Review"
20. Shivsharan Utkarsha, Ravva Sony " Antimicrobial activity of Pomegranate Juice " published in Indian Journals .com Volume 11 issue 10 page nos 4329-4331.