EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 10
123-128
Year: 2020

PHYTOASSISTED SYNTHESIZED COPPER NANOPARTICLES FROM *FICUS KRISHNAE* FOR IT'S ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

Amarvani P. Kanjikar[1] and Ramesh L. Londonkar[2]*

[1]Department of PG Studies and Research in Biotechnology, Vijayanagara Sri Krishnadevaraya University Ballari, Karnataka, India.
[2]Department of Biotechnology, Biopharmaceutical and Nanobiotechnology Gulbarga University, Kalaburagi, Karnataka, India.

***Corresponding Author: Ramesh L. Londonkar**
Department of Biotechnology, Biopharmaceutical and Nanobiotechnology Gulbarga University, Kalaburagi, Karnataka, India.

Article Received on 03/08/2020 **Article Revised on 23/08/2020** **Article Accepted on 13/09/2020**

ABSTRACT
Today the use of copper in different fields of science is due to its properties such as high conductive, good potential reduction and more economic compared to silver and good. Based upon recent survey of research articles on cupper nanoparticle shows potential prevention of infection and antimicrobial agent. In this paper, the copper nanoparticle has been synthesized based on green synthesis method using *Ficus krishane* stem bark aqueous extract without external and capping energy. It was characterized by UV-Visible spectrophotometry, X-ray diffraction and transmission electron microscopy analysis. Copper nanopartcles formation was recognized by colour changes from dark brown and was further proven by UV-visible spectrophotometer analysis based upon the absorption of band at 350-370 nm. The XRD photograph indicated that the face-centered cubic structure of the synthesized CuNPs. SEM study analyzed that the size from 160-228 nm with interparticle distance, whereas shape is spherical. X-ray Diffraction pattern performed three sharp peaks specifically referred to face-centered cubic structured of metallic copper. The antibacterial activity of copper nanoparticles was studied against disease causing pathogens like *Staphylococcus aureus* and *Enterococcus fecalis*. Antioxidant activity of copper nanoparticle by DPPH method was carried out. The present study confirms that the biosynthesized CuNPs from *Ficus krishnae* stem bark extract have a great affiance as antibacterial and antioxidant agent.

KEYWORDS: *Ficus krishnae*, Copper nanoparticles, XRD, SEM, UV-visible spectroscopy and antibacterial activity.

INTRODUCTION
Nanotechnology is the revolutionary area of research in the field of material science, life science, and medical science, physical and chemical science. Today the metal nanoparticles have drawn the attention of scientists due to their extensive application in the development of new technologies in different areas. Synthesis of drugs for diagnosis, monitoring, control, prevention and treatment of various diseases without any side effect by using nanomaterials, in medical it can be defined as nanomedicine.[1] The physicochemical properties and application of nanomaterial formulation leads to the alteration of pharmacokinetic, namely absorption, elimination, distribution and metabolism, has the potent to cross the biological barriers, toxicity and persistence in human body and environment.[2] NPs can be categorized broadly as inorganic and organic NPs. Inorganic NPs incorporate metallic NPs (like Au, Ag, Cu, Al), and magnetic NPs (like Co, Fe, Ni), semi conductor NPs (ZnO, ZnS, CdS).[3]

Chemical, physical methods of synthesis of nanopartical have the health hazardous, difficult in persistence from environment and side effect. Green production of production of NPs in vivo reported using plants and plant parts.[4] Mostly biological extracts are used for in vitro synthesis, which involves the purification of bio-reducing agent mixing it into an aqueous solution of therelevant metal precursor in controlled manner.[5] Among all the biological entities plants and plant extracts seem to be the best agents because they are easily available, suitable for mass production of NPs and their waste products are eco-friendly.[6]

Ficus krishnae also known as Krishna fir of krishna's butter cup in English and makkhann katori in hindi. It is fast growing plant has 10 m in height with spreeding branches and aerial roots. The leaves are simple dorsoventral with acute, apex reticulate venation and unique feature of the plant is that the pocket like folding at the base of leaf.[7] Different parts of the plant used in treatment of various diseases like ulcers, vomiting, fever, inflammation and laprosy [amar diabetic]. The aerial roots are styptic; useful in syphilis, biliousness, dysentery and inflammation of liver, these properties indicates the medicinal importance of this species. The plant having rich secondary metabolites, promising antimicrobial, antioxidant, anti-inflammatory and anti-diabetic activities of *Ficus krishnae* stem bark extract in both invitro and invivo studies.[8,9,10] Based upon previous evidence further research has been under taken to synthesis of CuNPs from *Ficus krishnae* stem bark for the enhancement of infection and antioxidant potential. The present manuscript describes the synthesis of CuNPs from aqueous extract *Ficus krishnae* and characterized by UV-Vis spectroscopy, SEM, XRD analysis. Synthesized CuNPs were evaluation of antibacterial and antioxidant activity.

MATERIAL AND METHODS

Collection and Preparation of Plant extracts

The *Ficus krishnae* stem bark was collected from Dev Dev Vana, Botanical garden Bidar, Karnataka, India. The collected stem bark was shade dried in lab, fine powdered and stored for future use. For preparation of aqueous extract *Ficus krishnae*, stem bark powder was weighed about 8-10g in 250 mL Erlenmeyer flask containing 200 mL of sterile deionised water. The sample was heated at 80˚C for 40-50 mins, then flask were cooled and extract were filtered with Whatman No. 1 filter paper twice to get clear solutions of aqueous extract, then which is stored in refrigerated (4 °C) for further use. In this whole extract preparation the aseptic conditions were maintained for the effective and accurate in results without contamination.

Synthesis of CuNPs from stem bark of *Ficus krishnae*

For the Cu nanoparticle synthesis, 1ml of *Ficus krishnae* stem bark extract was added 100ml of 1mM aqueous $CuSO_4$. $5H_2O_2$ solution in Erlenmeyer flask. Then the solution mixture is incubated overnight at room temperature. After incubation Cu nanoparticles solution were purified by repeated centrifugation at 12000 RPM for 15 Mins and pallet was washed repeatedly with deionized water to remove impurity. Then the Cu nanoparticles pallet was dried in oven at 80˚C.[11]

Characterization of synthesised Copper nanoparticles of *Ficus krishnae*

The color changes have observed after incubation mainly due to surface plasma resonance (SPR) that indicates the formation of nanoparticles.[12] as seen in Figure-1.

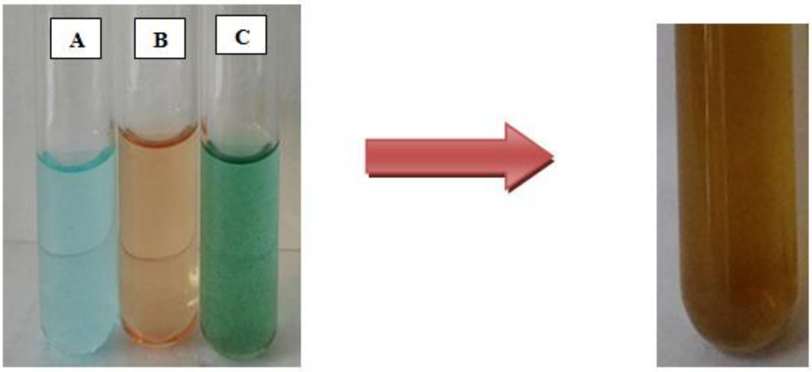

Figure.1: Synthesis of copper Nanoparticles exhibits light colour to dark colour (A-$CuSO_4$, B-Aueous extract and C-CuNPs light colour).

UV-Spectrophotometer analysis

The synthesized copper nanoparticles of *Ficus krishnae* were characterized by UV-Visible spectrophotometer. The reduction of copper nanoparticles was monitored at 200-750 nm absorbances in UV- spectrophotometer.

XRD and SEM analysis

Morphology of the nanoparticles was studied by using SEM analysis, from XRD studies of Cu nanoparticles reported the formation of face centered cubic (FCC) crystalline structured CuNPs of *F krishnae* stem bark.

Antibacterial activity by Agar well diffusion method

The well diffusion method of antimicrobial activity was slightly modified with Ahmed et al.[13] from synthesized Ag nanoparticles. All the glassware, media, and reagents used were sterilized in an autoclave at 121°C for 20 minutes. The *Enterococcus faecali* (ATCC 29212) and *S. aureus* (ATCC 29122) was taken in 25 ml of nutrient broth and incubated over night. Nutrient agar plates were plated and 6 mm wells are made with gel puncture and 10 µL of culture was spreaded on plate, 25 µg and 50 µg (100 µL) of Cu nanoparticles were added to the well, deionized water was used as control. Antibiotic such as Nalixic acid and Ambikacin was used as a positive control. These plates were incubated at 37°C for 24 hours in a bacteriological incubator, and the zone of inhibition (ZOI) was measured by subtracting the well diameter from the total inhibition zone diameter. All tests were performed for three times.

DPPH Radical-Scavenging Activity

The free radical-scavenging activity of the synthesized copper nanoparticles from *Fucus krishnae* was measured using the DPPH method. Take different concentrations 10, 20, 40, 60, 80, 100µg/mL of CuNPs and add 3 mL of 0.1mM DPPH in each test tube. The resultant mixture was incubated in the dark for 10 min at room temperature. The absorbance was measured at 517 nm in UV-visible spectrophotometer. Water, DPPH solution and ascorbic acid (AA) were used as blank, control and reference standard respectively.[14] Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and it is calculated using the following formula.

%Inhibition = [(Ac − At)/Ac] ×100

Where Ac is the absorbance of the control (blank, without sample) and it is the absorbance of the test sample. All tests were performed in triplicate and the graph was plotted with the mean value.

RESULT AND DISCUSSION

The stability of copper nanoparticles was analyzed by UV-Vis spectrophotometer based on time function (Figure-2). The peak green colored shows aqueous extract of *Ficus krishnae* (Sample-3) and $CuSO_4$ (sample-1) in wavelength of 270 nm and 250 nm respectively. After the incubation of copper sulfate and extract, the peaks were shifted and a new peak (sample-2) was formed at wavelength of 350 to 360 nm, this describes the formation of CuNPs.[15] synthesized from *Ficus skrisnae*. The presence of plasmon ribbons on the colloidal surface of Cu helps in the formation of CuNPs absorption peaks, which clearly suggested that reduction process of Cu^{2+} to Cu^0 was successfully occurred by the contain of stem bark extract.[16] A similar study was reported by M Gopinath.[17] about CuNPs synthesized from *Nerium oleander* Leaf aqueous extract by green methods which showed an absorption peak at 350 nm.

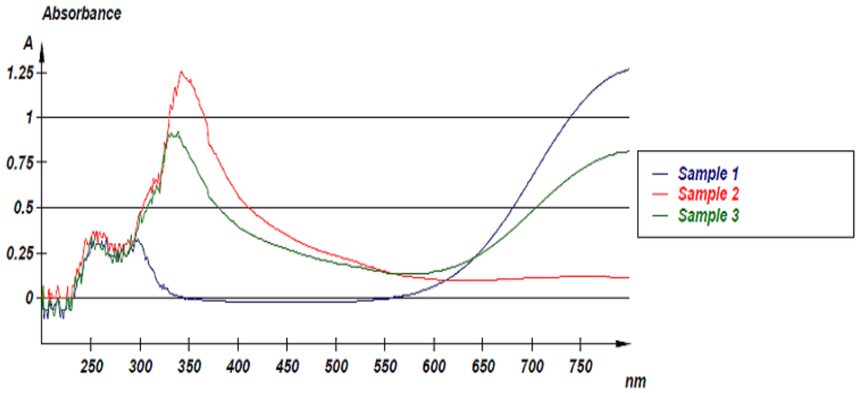

Figure 2: UV-Vis Spectrophotometer Analysis of (sample-3) *Ficus krishnae*. Stem bark Extract, (sample-1) CuSO4, (sample-2) CuNps.

The morphology dimensions of synthesized Cu nanoparticle of *Ficus karishnae* stem bark extract study demonstrated that the average size was ranging from 160-228 nm with interparticle distance, whereas shapes are irregular and spherical as presented in Fig.3.

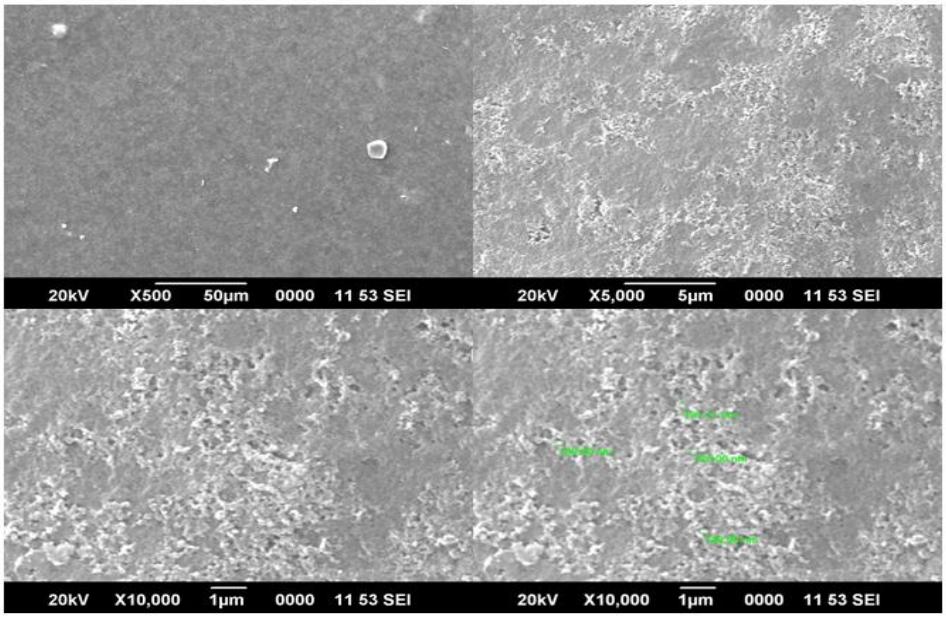

Figure-3: SEM analysis of synthesized nanoparticle.

X-Ray diffraction (XRD)

The XRD pattern of synthesized copper nanoparticle from *Ficus krishnae* stem bark is shown in Figure-4. It is observed the there are broader and less intense peaks in the spectrum. Nano-particles sample is made up of the very small crystallite. The broadness of the peak can be used to calculate the average crystalline size of the Cu NPs using (D=0.90 λ/βcosθ) 0.90 is shape factor, λ is the wavelength of the X-rays and taken as 0.15 Å **(Table 1)**. The absorption increases as the nanoparticle concentration increases Figure-4 shows relationship between Cu NPs and absorption.[18]

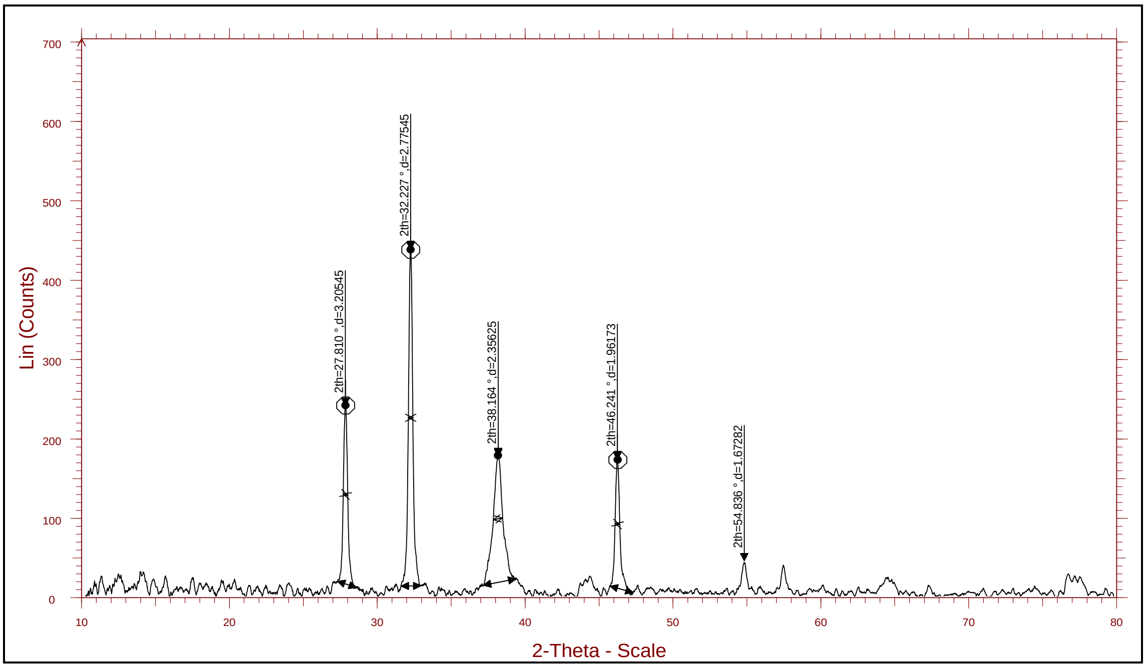

Figure-4: XRD of copper nanopartcles synthesized from stem bark of *Ficus krishnae*.

Table 1: Solvent deasphalting (SDA) of copper nanoparticles.

Experimental diff.angle (20 degrees)	Standard diff. in angle (20 in degree)	HCl (Planes)
28.1	30.19	-110
32.5	35.06	-111
46.5	50.11	-200
55.0	60.15	-220

Antibacterial activity by Agar well diffusion method

The copper nanoparticles pretence to have a good bactericidal activity so in these work the two different organism like *Staphylococcus aureus* and *Enterococcus fecalis,* in these Ambikacin and Nalixic acid used as positive control respectively. The maximum zone of inhibition was 1.8 mm seen in *Enterococcus fecalis* at 50 µg and minimum zone of inhibition was 1.5 mm seen at 50 µg in *Staphylococcus aureus.* Even at 25 µg of concentration the zone of inhibition were 1.5 mm and 1.0 mm in *Enterococcus fecalis* and *Staphylococcus aureus* respectively **(Figure-5).** Whereas positive control Ambikacin and Nalixic acid shows 2.2 mm and 1.6 mm for *Enterococcus fecalis* and *Staphylococcus aureus* respectively as shown in **Table-2.** Previously our work was published that synthesized Ficus krishnae silver nanoparticle shows the good antimicrobial and antioxidant activity.[19]

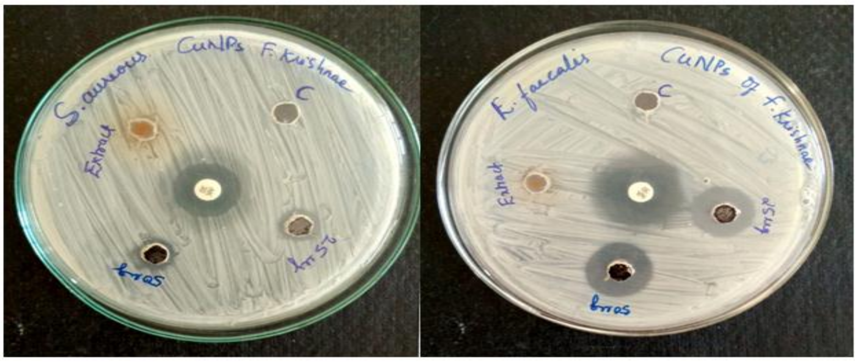

Figure-5: Showing antibacterial activity of copper nanoparticles of *Ficus krishnae*.

Table 2: Zone of inhibition (mm) of micro organisms by well diffusion method of *F.krishnae.*

Test Organism	Strains	C	Standr	CuNPs(25µg)	CuNPs(50µg)
Enterococcus faecalis	ATCC 29212	00	2.2 mm	1.5 mm	1.8 mm
Staphylococcus aureus	ATCC 29122	00	1.6 mm	1.0 mm	1.5 mm

DPPH Radical-Scavenging Activity

In the DPPH assay, DPPH is stable free radical color it has the capacity to reduce completely when react with reducing agents and electron become paired off and solution loses the colour from purple to colorless [Murthy *et al*., 2003 18]. Radical scavenging activity of CuNPs of Ficus krishnae and aqueous extract ranging from 43.5% to 76.65% and 39.09% to 72.01% and respectively as shown in Figure-6. Whereas standard ascorbic acid ranging from 70.4% to 98.59%. The IC_{50} values are 0.7µg, 20µg and 0.3 µg of CuNPs, aqueous extract and standard ascorbic acid respectively. The scavenging activity of synthesized nanoparticle is more potential compared to aqueous extract; study reveals that CuNPs had comparatively significant antioxidant activity as that of the standards.

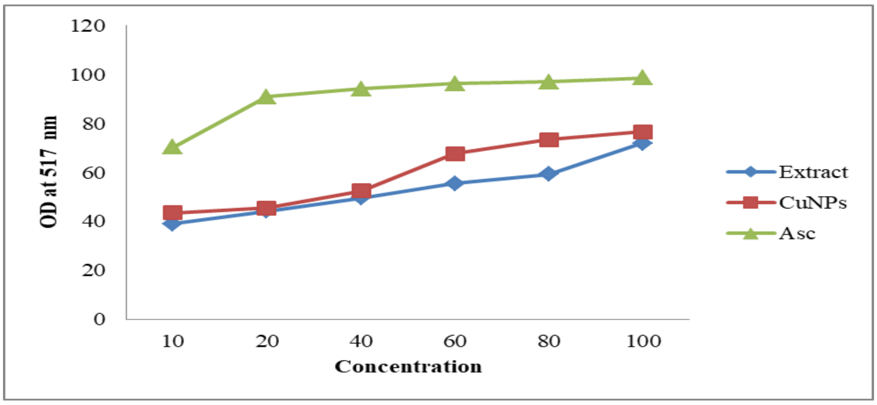

Figure 6: DPPH Radical-Scavenging Activity of Aqueous extract, CuNPs and ascorbic acid.

CONCLUSION

Green route of synthesis of nanoparticle is very easy and simple without any pollution conducted successfully. The characterization was done by UV-Vis analysis confirmed the formation of copper nanoparticles with an absorbing peak at 350 to 360 nm. The XRD analysis showed the formation of well crystallite face centered cubic structyure of copper nanoparticles. This reveals that *Ficus krishnae* stem bark can be used to synthesize stable small sized copper nanoparticles with good antibacterial and antioxidant agent. This method is very significant due to its eco-friendly, low cost, short implementation time and use of unharmful chemical material.

Disclosure statement

No potential conflict of interest was reported by the authors.

REFERENCES

1. Tinkle S, McNeil, S. E., Mühlebach, S., Bawa, R., Borchard, G., Barenholz, Y. C. Nanomedicines: addressing the scientific and regulatory gap. Ann. N. Y. Acad. Sci, 2014; 1313: 35–56. doi: 10.1111/nyas. 12403
2. Bleeker, E. A., de Jong, W. H., Geertsma, R. E., Groenewold, M., Heugens, E. H., Koers-Jacquemijns, M.,. Considerations on the EU definition of nanomaterial: science to support policy making. Regul. Toxicol. Pharmacol, 2013; 65: 119–125. doi: 10.1016/j.yrtph.2012.11.007
3. Vadlapudi V, Kaladhar D.S Review: green synthesis of silver and gold nanoparticles. Middle East J Sci Res, 2014; 19: 834–842.
4. Kharissova OV, Dias HVR, Kharisov BI, Perez BO, Victor M, Perez J. The greener synthesis of nanoparticles. Trends Biotechnol, 2013; 31: 240–248.
5. Rajakumara G, Rahumana AA, Roopan SM, Khannac VG, Elangoa G, Kamaraja C. Fungus-mediated biosynthesis and characterization of TiO2 nanoparticles and their activity against pathogenic bacteria. Spectrochimica Acta Part A, 2012; 9: 123–129.
6. Lee HJ, Lee G, Jang NR, Yun JM, Song JY, Kim BS. Biological synthesis of copper nanoparticles using plant extract. Nanotechnology, 2011; 1: 371–374.
7. Kanjikar Amarvani P, Londonkar Ramesh L. Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Stem Bark of Ficus Krishnae International Journal of Pharmacognosy and Phytochemical Research, 2017; 9(5): 733-738.
8. Kanjikar Amarvani P, L H Aruna, Ramesh L. A novel investigation of in-vitro anti-inflammatory and antioxidant activity of Ficus krishnae. European journal of biomedical and pharmaceutical sciences, 2017; 4(10): 313-317.
9. Kanjikar Amarvani P and Londonkar Ramesh L. Hypoglycemic and tissue-protective evaluation of *Ficus krishnae* in alloxan-induced diabetic rat.

European Journal of Biomedical and Pharmaceutical sciences, ejbps, 2020; 7(4): 312-317.
10. Kanjikar Amarvani P, L H Aruna, Londonkar Ramesh L. Novel efficacy of invitro anti-haemolytic and anti-cancer activities of Ficus krishnae. Der Pharmacia Littre, 2017; 9(12): 16-22.
11. Vasudev D. Kulkarni, Pramod S. Kulkarni. Green Synthesis of Copper Nanoparticles Using Ocimum Sanctum Leaf Extract. International Journal of Chemical Studies, 2014; 1(3): 1-4.
12. Antariksh Saxena , R.M. Tripathi , Fahmina Zafar , Priti Singh. Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity. Materials Letters, 2012; 67: 91–94.
13. Ahmed S, SIkram S. Silver Nanoparticles: One Pot Green Synthesis Using *Terminalia arjuna* Extract for Biological Application. J Nanomed Nanotechnol, 2015; 6: 309. doi:10.4172/2157-7439.1000309.
14. Chidambara **Murthy**, K.N., Vanitha, A., Mahadeva Swamy, M. and Ravishankar, G.A. Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L. J Med Food, 2003; 6(2): 99-105.
15. A. N. Pestryakov, V. P. Petranovskii, A. Kryazhov, O. Ozhereliev, N. Pfänder and A. Knop-Gericke, *Chem. Phys. Lett*, 2004; **385(3-4)**: 173. **DOI:**10.1016/j.cplett, 2003.12.077
16. T. M. D. Dang, T. T. T. Le, E. Fribourg-Blanc and M. C. Dang, *Adv. Nat. Sci. Nanosci.Nanotechnol*, 2011; **2(1)**: 1. **DOI:** 10.1088/2043-6262/2/1/015009
17. M.Gopinath and R.Subbaiya, M.Masilamani Selvam and D.Suresh. Synthesis of Copper Nanoparticles from *Nerium oleander* Leaf aqueous extract and its Antibacterial Activity. *Int.J.Curr.Microbiol.App.Sci*, 2014; 3(9): 814-818.
18. Batoool M, Masood B. Green Synthesis of Copper Nanoparticles Using *Solanum Lycopersicum* (Tomato Aqueous Extract) and Study Characterization. J Nanosci Nanotechnol Res, 2017; 1(1): 5.
19. Amarvani P. Kanjikar, Aruna L. Hugar & Ramesh L. Londonkar Characterization of phyto-nanoparticles from *Ficus krishnae* for their antibacterial and anticancer activities, Drug Development and Industrial Pharmacy, 2017. DOI: 10.1080/03639045.2017.1386205.