Journal of Pharmaceutical Research International

33(61A): 318-326, 2021; Article no.JPRI.77726
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Green Synthesis of Copper Nanoparticles Using Ginger Oleoresin and Evaluation of its Anticancer Activity against Liver Cancer Cell Line

Raja Kumar [a], Anitha Roy [b#*], S. Rajeshkumar [c†], S. Raghunandhakumar [d†] and Lakshmi Thangavelu [c]

[a] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai - 600077, Tamil Nadu, India.
[b] Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai-600077, Tamilnadu, India.
[c] Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
[d] Cancer and Stem Cell Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, 600 077, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i61A35591

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77726

Original Research Article

Received 22 October 2021
Accepted 27 December 2021
Published 28 December 2021

ABSTRACT

Introduction: Nanotechnology is getting used in developing countries to assist, treat disease, and stop health issues. Nanotechnology in addition to nanomedicine is being applied to or developed for application to a spread of commercial and purification processes. Ginger, scientifically known as *Zingiber officinale*, belongs to the family *Zingiberaceae*.
Aim: The aim of the study was to green synthesis copper nanoparticles using ginger oleoresin and to evaluate its cytotoxic activity against liver cancer cells.
Materials and Methods: The Ginger oleoresin was obtained from Synthite Industries Private

[#]Professor,
[†]Associate Professor,
**Corresponding author: E-mail: anitharoy@ saveetha.com;*

Limited, Kerala with a product code: 4010000370 was used for the study. Copper nanoparticles were prepared from oleoresin and confirmed using UV-Visible spectroscopy. The prepared copper nanoparticles were then evaluated for the anticancer effect on liver cancer cells using the method of Mosmann. The cells (1 × 105 cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with replications. Different concentration (control, 10, 20, 30, 40, and 60 µg/ml) of ginger oleoresins mediated copper nanoparticles were tested for the anticancer activity. The percentage of cell viability was calculated.

Results: Dose-dependent anticancer activity was observed with ginger oleoresins mediated copper Nanoparticles. The IC_{50} value was 30 mg / ml

Conclusion: In the present study, ginger oleoresin showed a good activity cytotoxic effect on liver cancer cell lines. However, more research is needed to understand the underlying mechanisms of the cytotoxic property of the ginger plant.

Keywords: Anticancer activity; copper nanoparticles; ginger oleoresin; green synthesis; innovation; liver cancer.

1. INTRODUCTION

Cancer, the most prevalent moderately curable group of diseases involving abnormal growth potential of cells to multiply and spread across the body parts leading to functional impairment of the essential process [1]. It causes significant morbidity and mortality and is a major health problem worldwide. Chemotherapy is widely used for the treatment of cancer but still exhibits low specificity and is restricted by dose-limiting toxicity. It is a challenge to seek out the therapy and medicines for the treatments of varied sorts of cancer. However, conventional methods with a combination of controlled release technology and targeted drug delivery can reduce toxicity. Nanomaterials are expected to revolutionize cancer diagnosis and therapy [2]. Liver cancer cell are employed to understand properties such as regulation of cellular events, uncontrollable cell proliferation, minimal inhibitory concentration, evasion of cell death [3].

Nanoparticles are particles with sizes starting from 1 to 100 nanometers. These have gained popularity in recent years and are shown to possess a good range of technological and biological applications. Among the metallic element nanoparticles, silver nanoparticles have unique properties like chemical stability, anti-inflammatory, antiseptic, antimicrobial, antioxidant, antiviral, and antifungal activity which has made it the particle of interest to the scientific community [4,5]. These particles are usually prepared by physical or chemical methods which are not environment friendly and moreover with various biological risks. Hence, there is a requirement to follow other methods which are non-hazardous to the environment and which follow green synthesis of nanoparticles using varied biological agents like bacteria, fungi, plant extract, or plant biomass [6,7].

Ginger is mainly known as *Zingiber officinale*, it belongs to the family of *Zingiberaceae*. The preparation is done by solvent extraction of dried rhizomes. It contains a volatile oil in a composition of about 30 - 35 ml/100 g. Ginger has been known to have anti-inflammatory effects. Ginger inhibits cyclooxygenase and suppresses prostaglandin secretion, hence bringing about anti-inflammatory effects. Ginger also inhibits 5-lipoxygenase, suppresses leukotriene production, hence inhibits the pro-inflammatory effects of leukotrienes. Ginger extract from the *Zingiberaceae* has been shown to inhibit several genes that are liable for inflammatory responses [8]. 6-gingerol and shogaol are the active constituents of ginger and are responsible, not only for their anti-inflammatory effects but also for the antitumor and antioxidant effects of ginger. The content of the oleoresin may vary with the solvent used for the extraction [9].

Many studies were done using various nanoparticles such as *Symplocos racemosa* bark assisted copper nanoparticles and their antibacterial activity against *Staphylococcus aureus* and *Lactobacilli* species [10], antibacterial activity of nanoparticle prepared from herbs [11] and *In vitro* cytotoxic effects of copper nanoparticles synthesized from avocado seed extract [12], Green synthesis of copper oxide nanoparticles using tamarind extract and its alpha-amylase inhibitory activity [13], cytotoxic activity study of copper oxide nanoparticle mediated through tamarind extract [14]. Our team has extensive knowledge and research experience that has been translated into high

quality publications [15 – 20]. The aim of the study was to prepare copper nanoparticles by green synthesis, using ginger oleoresin and to evaluate its cytotoxic activity against liver cancer cells.

2. MATERIALS AND METHODS

2.1 Study Setting

Cell line Research was conducted in Blue Lab, Saveetha Dental College, Chennai, India.

2.2 Collection and Preparation

The Ginger oleoresin extract is collected from Synthite Industries, with a product code: 4010000370. Ginger, scientifically known as *Zingiber officinale*, belongs to the family *Zingiberaceae*.

2.3 Synthesis of Cu Nanoparticles

20 mM $CuSO_4$ was prepared initially and mixed with ginger oleoresin solution to prepare nanoparticles. The solution was kept in a magnetic stirrer for nanoparticle synthesis. The color change was observed visually and photographed. The solution containing the nanoparticles was centrifuged using Lark refrigerated centrifuge. The Cu nanoparticles solution was centrifuged at 8000 rpm for 10 min and the pellet collected was washed with distilled water twice. The final purified pellet was collected and dried in a hot air oven at 100 – 150°C for 24 hrs, and finally, the nanoparticles powder was collected and stored in an airtight Eppendorf tube.

2.4 Confirmation of Cu Nanoparticles

The synthesized nanoparticles solution is preliminarily characterized using ultraviolet (UV)-visible spectroscopy; 3 ml of the solution is taken in the cuvette and scanned in a double-beam UV-visible spectrophotometer from 300 nm to 700 nm wavelength. UV-Visible spectrometer readings were taken every 2 hours and recorded. The peak in the graph was noticed after 72 hours which coincided with a visible color change from pale pink to brown, indicating the formation of nanoparticles (Fig. 1).The results were recorded for the graphical analysis (Fig. 2).

3. CELL LINE STUDY

3.1 Chemical

DMEM medium,0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO was from Himedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid, and methanol were purchased from Sisco Research Laboratories (SRL) India.

3.2 Cell Structure

The liver cell line was procured from National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T255 culture flasks containing DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using Trypsin-EDTA solution.

4. MTT ASSAY ANALYSIS

In vitro cytotoxic activity assay: The effect of ginger oleoresin on cell viability was measured by MTT assay following the method by Mosmann. Briefly, the cells (1 × 105 cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with replications. Treatment was conducted for 24 hours with different concentrations (control, 10,20,30,40,60,80µg/ml) of ginger oleoresin. After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilized with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance in control cells (0.1 % DMSO) (A570). The IC_{50} was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control

4.1 Cell Viability Estimation

Cell viability is calculated using the formula:

Cell viability (%) = {A570 od of (sample)/A570 od of (control)} x 100

5. MORPHOLOGICAL STUDY

Based on the MTT assay we selected the IC_{50} value of ginger oleoresin for future study. The characterization of morphological changes in liver cancer cell lines before and after treatment with ginger oleoresin was observed under a phase-contrast microscope.

5.1 Statistical Analysis

All data obtained were analyzed and represented as mean ± SE .The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. In all tests, the level of statistical significance was set at $p<0.05$.

6. RESULTS

In the present study, the cytotoxic activity effect of ginger oleoresin against liver cancer cells was evaluated using an MTT assay. MTT assay provided a quick, simple, and cost-effective way for testing the cytotoxic activity of ginger oleoresin. In this study, liver cancer cells were treated with different concentrations of the extract of ginger oleoresin for 24 hours. As the concentration of the extract increased, the percentage of cell viability decreased which depicted significance in the cytotoxic activity of ginger oleoresin against liver cancer cells (Fig. 3). The IC_{50} value was found to be 30 µg/ml. The cell viability was also found to be 10%. Thereby the results show that as the drug concentration increases, the percentage of cell viability decreases proving ginger cytotoxic effect of oleoresins. Ginger oleoresin extract caused a dose-dependent increase in the cytotoxic activity of liver cancer cells. Morphological changes and apoptosis of liver cancer cells were observed using ginger oleoresin under an inverted phase-contrast microscope at 20x magnification (Fig. 4).

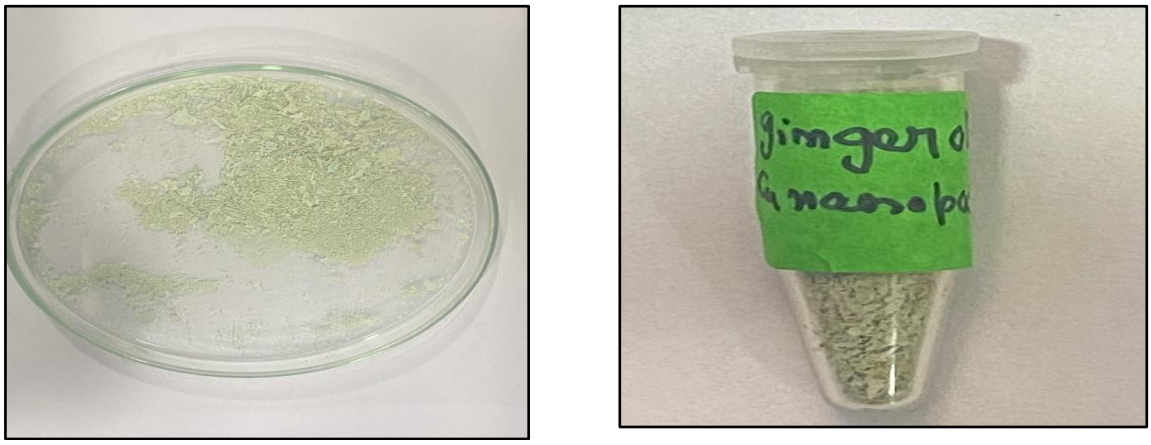

Fig. 1. Ginger oleoresin mediated Copper oxide nanoparticles

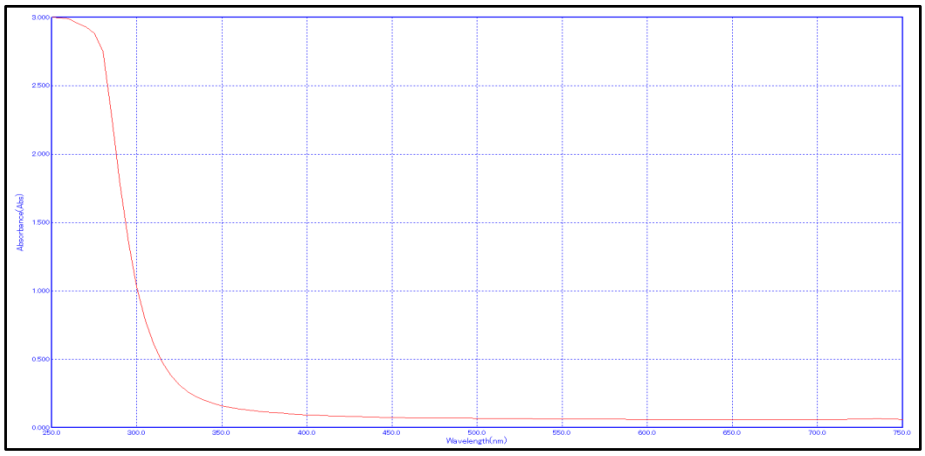

Fig. 2. Graph showing the UV spectroscopy of ginger oleoresin mediated copper nanoparticles

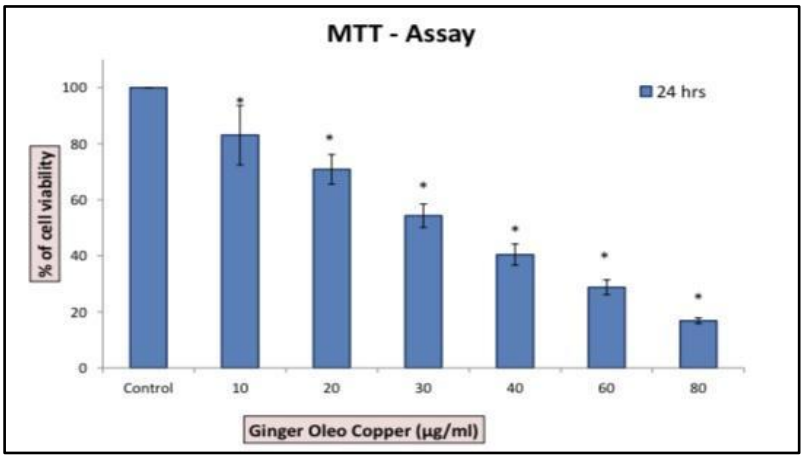

Fig. 3. The graph represents the cytotoxic activity effects of ginger oleoresin mediated copper nanoparticles on HEPG2 cells. Cells were treated with ginger oleoresin (10, 20,30,40,60, and 80 µg/ml) for 24 hours and cell viability was evaluated by MTT assay. Blue denotes the control groups and other concentrations of ginger oleoresin. The X-axis denotes controls of the different concentrations of ginger oleoresin-mediated copper nanoparticles and the Y-axis denotes the percentages of cell viability. Percentage inhibition of cell viability .Data shown as means ± SE (n = 3). * Compared with the control, p < 0.05 .The p value was 0.007 at 40µg/ml and considered as significant as it was < 0.05

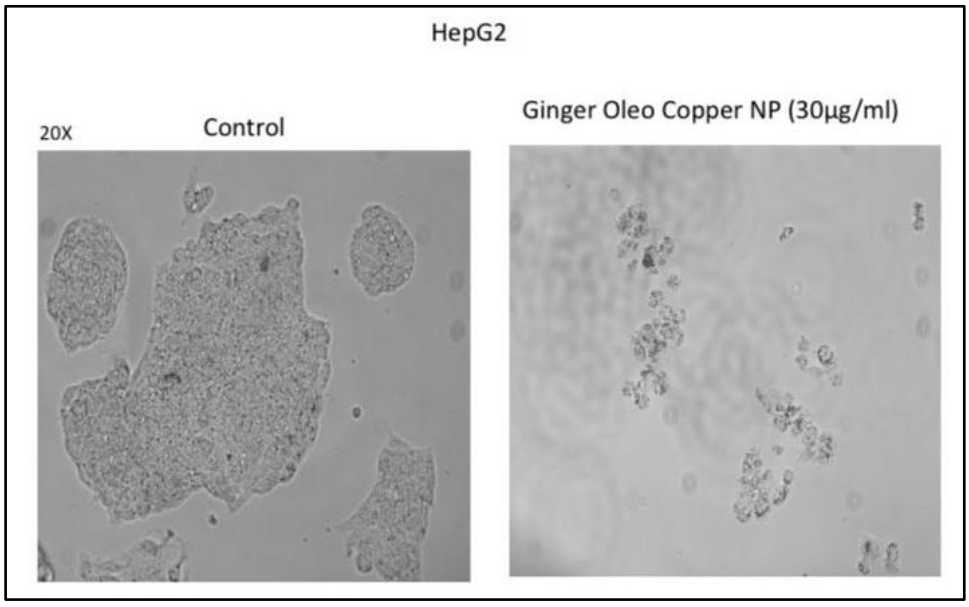

Fig. 4. Assessment of cell morphology of HepG2 treated without or with ginger oleoresin. Cells were treated with ginger oleoresin (30µg/ml) for 24 hours along with the control group. Images were obtained using an inverted Phase contrast microscope at 20x magnification

7. DISCUSSION

In the present study, we evaluated the cytotoxic activity potential of ginger oleoresin in HEPG2 cells by MTT assay. MTT assay was employed to assess the viable cells to measure the growth modulation of cells *in vitro*. The *in vitro* cytotoxic assay is an economical method as well as a reliable method. The HepG2 cells were treated with different concentrations of ginger oleoresin (10µg/ml to 80µg/ml) for 24 hours. The ginger oleoresin caused a dose-dependent increase in concentration.

In the present study, liver cancer cells were treated with ginger oleoresin at different

concentrations and evaluated by MTT assay (Fig. 3, Fig. 4) depict clearly that there was good level of anti-cancer activity exhibited by ginger oleoresin against liver cancer cell line when compared with the previous *in vitro* study performed by [21,22] to analyze the anticancer activity of ginger against cholangiocarcinoma also proved that there is a remarkable anticancer activity for the extract, but both the studies were with different cell line, parameter but both the study results were quite similar.

Shenai et al in the year 2017 stated that the ethanolic leaf extract of *Caralluma fimbriata* is capable of reducing cell proliferation by inducing anticancer activity of COLO 320 cells when we compare with our study we found that 30 µg/ml can lead to apoptosis [23]. The activity of ginger extracts performed by different researchers found that the plant is with good pharmacological activity [24,25]. Jennifer et al have used KB cells to evaluate the anticancer activity with increasing concentrations of the ethanolic extract of *C. fimbriata* (100 – 300µg/m1) and has shown a dose-dependent increase in anticancer activity in KB cell lines. Many studies have shown dose dependent pharmacological activity [26-37].

The Cytotoxic Activity effect of ginger oleoresin on liver cancer cells was proved; when the drug concentration was increased, the percentage of cell viability decreased proving cytotoxic activity effect. The limitation seen in the present study was that the mechanism of cytotoxic activity with ginger oleoresin was not explored and future studies should be done with animal models to take it to the next level of research.

8. CONCLUSION

In the present study, ginger oleoresin showed a good cytotoxic effect on liver cancer cell lines which may be helpful in the treatment of liver cancer. However, more research is needed to understand the underlying mechanisms of the cytotoxic property of the plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by the Scientific Review Board, Saveetha Dental College, Chennai. [Approval number: IHEC/SDC/UG-1926/21/91].

ACKNOWLEDGEMENT

The authors thank Synthite Industries Private Limited, Kerala for providing the extract for this study. The authors extend their gratitude to the management of Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences for all support to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vennila R, Kamalraj S, Muthumary J. In vitro studies on anticancer activity of fungal taxol against human breast cancer cell line MCF-7 cells [Internet]. Biomedicine & Aging Pathology. 2012;2:16–8. Available:http://dx.doi.org/10.1016/j.biomag.2012.01.001
2. Rajeshkumar S. Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells. J Genet Eng Biotechnol. 2016 Jun;14(1):195–202.
3. Youssef AM, Malki A, Badr MH, Elbayaa RY, Sultan AS. Synthesis and anticancer activity of novel benzimidazole and benzothiazole derivatives against hepg2 liver cancer cells [Internet]. Medicinal Chemistry. 2012;8:151–62. DOI:http://dx.doi.org/10.2174/157340612800493719
4. Kanagavalli U, Mohamed Sadiq A, Sathishkumar, Rajeshkumar S. Plant Assisted Synthesis of Silver Nanoparticles Using Boerhaavia diffusa Leaves Extract and Evolution of Antibacterial Activity [Internet]. Research Journal of Pharmacy and Technology. 2016;9:1064. DOI:http://dx.doi.org/10.5958/0974-360x.2016.00201.8
5. 5.Pranati T, Anitha R, Rajeshkumar S, Lakshmi T. Preparation of Silver nanoparticles using Nutmeg oleoresin and its Antimicrobial activity against Oral pathogens [Internet]. Research Journal of Pharmacy and Technology. 2019;12: 2799. DOI:http://dx.doi.org/10.5958/0974-360x.2019.00471.2
6. Kandhan TS, Roy A, Lakshmi T, Rajeshkumar S. Green synthesis of Rosemary oleoresin mediated silver nanoparticles and its effect on Oral

pathogens [Internet]. Research Journal of Pharmacy and Technology. 2019;12:5379. DOI:http://dx.doi.org/10.5958/0974-360x.2019.00933.8

7. M G, Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, et al. Phyllanthus reticulatus mediated synthesis and characterization of silver nanoparticles and its antibacterial activity against gram positive and gram negative pathogens [Internet]. International Journal of Research in Pharmaceutical Sciences. 2019;10:3099–106. DOI:http://dx.doi.org/10.26452/ijrps.v10i4.1603
8. Grzanna R, Lindmark L, Frondoza CG. Ginger—an herbal medicinal product with broad anti-inflammatory actions [Internet]. Journal of Medicinal Food. 2005;8:125–32. DOI:http://dx.doi.org/10.1089/jmf.2005.8.125
9. Bailey-Shaw YA, Williams LAD, Junor G-AO, Green CE, Hibbert SL, Salmon CNA, et al. Changes in the contents of oleoresin and pungent bioactive principles of Jamaican ginger (*Zingiber officinale* Roscoe.) during Maturation [Internet]. Journal of Agricultural and Food Chemistry. 2008;56:5564–71. DOI:http://dx.doi.org/10.1021/jf072782m
10. Das A, Roy A, Rajeshkumar S, Lakshmi T. Anti-inflammatory activity of turmeric oil mediated silver nanoparticles [Internet]. Research Journal of Pharmacy and Technology. 2019;12:3507. DOI:http://dx.doi.org/10.5958/0974-360x.2019.00596.1
11. Sunar S, Rajeshkumar S, Roy A, Lakshmi T. Preparation of herbal formulation and it's application on nanoparticles synthesis and antibacterial activity [Internet]. International Journal of Research in Pharmaceutical Sciences. 2019;10. DOI:http://dx.doi.org/10.26452/ijrps.v10i3.1447
12. Kiran K, Rajeshkumar S, Roy A, Santhoshkumar J, Lakshmi T. In vitro cytotoxic Effects of Copper Nanoparticles Synthesized from Avocado Seed Extract [Internet]. Indian Journal of Public Health Research & Development. 2019;10:3497. DOI:http://dx.doi.org/10.5958/0976-5506.2019.04125.1
13. Rajeshkumar S, Lakshmi T, Tharani M, Sivaperumal P. Green synthesis of gold nanoparticles using pomegranate peel extract and its antioxidant and anticancer activity against liver cancer cell line [Internet]. Alinteri Journal of Agricultural Sciences. 2020;35:164–9. DOI:http://dx.doi.org/10.47059/alinteri/v35i2/ajas20089
14. Rajeshkumar S, Menon S, Venkat Kumar S, Tambuwala MM, Bakshi HA, Mehta M, et al. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract [Internet]. Journal of Photochemistry and Photobiology B: Biology. 2019;197:111531. DOI:http://dx.doi.org/10.1016/j.jphotobiol.2019.111531
15. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 Oct;117:91–5.
16. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. Biocatal Agric Biotechnol. 2019 May 1;19:101138.
17. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res. 2020 Mar 1;27(8):8166–75.
18. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity [Internet]. South African Journal of Chemical Engineering. 2020;32:1–4. DOI:http://dx.doi.org/10.1016/j.sajce.2019.11.005
19. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. Fuel. 2020 Oct 1;277:118166.
20. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021 Mar 18;12(1):192.

21. Sivananda N. Cytotoxic effect of diphtheria toxin in mammalian cell lines [Internet]. Indian Journal of Science and Technology. 2010;3:1177–9. DOI:http://dx.doi.org/10.17485/ijst/2010/v3i12.2
22. Bharath J, Indumathy. R. In vitro Cytotoxic and Apoptotic activities of *Morinda pubescens* leaves on HL-60 Cell Line [Internet]. Research Journal of Pharmacy and Technology. 2019;12:5479. DOI:http://dx.doi.org/10.5958/0974-360x.2019.00951.x
23. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of *Caralluma fimbriata* against human colon cancer cells [Internet]. Pharmacognosy Journal. 2017; 19:204–7. DOI:http://dx.doi.org/10.5530/pj.2017.2.34
24. Begum A, Jeevitha M, Preetha S, Rajeshkumar S. Cytotoxicity of iron nanoparticles synthesized using dried ginger [Internet]. Journal of Pharmaceutical Research International. 2020;112–8. DOI:http://dx.doi.org/10.9734/jpri/2020/v32i2530829
25. Devi RS, Shruthi Devi R, Jeevitha M, Preetha S, Rajeshkumar S. Free radical scavenging activity of copper nanoparticles synthesized from dried ginger [Internet]. Journal of Pharmaceutical Research International. 2020. p. 1–7. DOI:http://dx.doi.org/10.9734/jpri/2020/v32i1930703
26. Suhasini SJ, Jennifer Suhasini S, Roy A, Sosa G, Lakshmi T. The Cytotoxic effect of Caralluma fimbriata on KB cell lines [Internet]. Research Journal of Pharmacy and Technology. 2019;12:4995. DOI:http://dx.doi.org/10.5958/0974-360x.2019.00865.5
27. Dhayanithi J, Rajeshkumar S, Roy A, Lakshmi T. Preparation and evaluation of antifungal activity of arrow root mediated selenium nanoparticles against *Candida albicans* -. Journal of Complementary Medicine Research. 2020;11(5):83–8.
28. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Pharmacognosy Research. 2018;10:343. DOI:http://dx.doi.org/10.4103/pr.pr_46_18
29. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative evaluation of remineralizing potential of a paste containing bioactive glass and a topical cream containing casein phosphopeptide-amorphous calcium phosphate: An in Vitro study. Pesqui Bras Odontopediatria Clin Integr. 2019 Mar 12;19(0):4668.
30. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol [Internet]. 2017 Mar [cited 2021 Sep 15];44(3). DOI:https://pubmed.ncbi.nlm.nih.gov/28004401/
31. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A report of two cases. Case Rep Dent [Internet]. 2019 Jan 22 [cited 2021 Sep 15];2019. DOI:https://pubmed.ncbi.nlm.nih.gov/30805222/
32. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev [Internet]. 2014 Jun 30 [cited 2021 Sep 15];8(1). DOI:https://pubmed.ncbi.nlm.nih.gov/25992232/
33. Menon S, Ks SD, R S, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces [Internet]. 2018 Oct 1 [cited 2021 Sep 15];170. DOI:https://pubmed.ncbi.nlm.nih.gov/29936381/
34. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent [Internet]. 2020 Jan [cited 2021 Sep 15];80(1). DOI:https://pubmed.ncbi.nlm.nih.gov/31710096/
35. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). DOI:https://pubmed.ncbi.nlm.nih.gov/11286449/
36. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability

and push-out bond strength of an epoxy resin root canal sealer. Int Endod J [Internet]. 2011 Jun [cited 2021 Sep 15];44(6). DOI:https://pubmed.ncbi.nlm.nih.gov/21255047/

37. Kumar MS, Vamsi G, Sripriya R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J Periodontol. 2006 Nov;77(11):1803–8.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77726