

## ANTI-PROLIFERATIVE EFFECT OF CISSUS QUADRANGULARIS STEM EXTRACT ON HUMAN BREAST CANCER CELLS

- **KAUSHIK VISHNUR**

Graduate, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences Chennai, Tamilnadu, India

- **Dr. S.Raghunandhakumar\***

Associate Professor, Cancer and stem cell research laboratory Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, No.162, PH Road, Saveetha University, Chennai, Tamil Nadu, India-600 077. Email Id: raghunandhakumars.sdc@saveetha.com

- **Dr.D.Ezhilarasan**

Associate Professor, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences Saveetha University, Chennai-600077, Tamilnadu, India. Email Id:ezhilarasand.sdc@saveetha.com

- **Dr. T. Lakshmi**

Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India.

**Corresponding Author**

- **Dr. S.Raghunandhakumar\***

Associate Professor, Cancer and stem cell research laboratory Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, No.162, PH Road, Saveetha University, Chennai, Tamil Nadu, India-600 077.  
Email Id: raghunandhakumars.sdc@saveetha.com

### ABSTRACT:

**AIM:**The purpose of this research was to investigate whether an aqueous extract of *CissusQuadrangularis* might inhibit the cell proliferation on MCF-7 human breast cancer cells.

**METHODS:**The anti-proliferative properties of *CissusQuadrangularis* were evaluated using the MTT assay on a human breast cancer cell line. In addition, phase contrast microscopy was used to study the herbal extract-induced morphological changes. All collected data were statistically examined and calculated (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA.

**RESULTS:**The cytotoxic potential of the aqueous extract of *CissusQuadrangularis* against the MCF-7 cell line was substantiated by a higher level of anti-proliferative activity after 24 hours of treatment. The MTT test clearly demonstrated that *C.quadrangularis* treatment drastically decreased cell viability in different dose and time for 24 hours respectively. The P value is <0.001

**CONCLUSION:**The current study demonstrates that an aqueous extract of *C. quadrangularis* has a cytotoxic impact on the MCF-7 human breast cancer cell line at a concentration of 18 g/mL, which represents the IC50 value.

**Key words:** *C.quadrangularis*, Anti-Proliferation, Breast Cancer, Apoptosis

### INTRODUCTION:

Cancer is the uncontrolled growth of ailments associated with abnormal cell development that has the ability to spread to other parts of the body. These distinctions with benign tumours, that do not really unfold. Possible signs and symptoms include the presence of a lump, atypical injury, protracted cough, unexplained weight loss, and changes in the regularity of bowel motions. Although these symptoms could point to cancer, there are other possible explanations for why they are occurring. There are more than a hundred different types of cancer that may affect people. (1)Breast cancer is the second most common malignancy among women in the United States. There are many subtypes of carcinoma that may manifest as breast cancer (2).

Breast cancer may arise in a variety of breast tissues. The breast consists of three major components: lobules, ducts, and animal tissue. The lobules are the milk-producing glands. The ducts consist of tubes that transport milk to the teat. (3) The animal tissue (comprised of fibrous and fatty tissue) encompasses and supports everything. The majority of breast cancers start in the ducts or lobules. (3) Breast cancer may spread beyond the breast through blood arteries and fluid-carrying vessels. Once carcinoma has spread to other parts of the body, it is said to have metastasized. Invasive ductal malignant neoplastic disease and Invasive lobe malignant neoplastic disease are the most prevalent kinds of cancer.

*Cissusquadrangularis*, also known as grassland grape, adamant creeper, and devil's backbone, is a member of the family of grapes. *Cissusquadrangularis*, which is indigenous to certain regions of Asia, Africa, and Arabia, has been used as a natural medicine for a multitude of diseases for a very long time. It is one of the most often used medicinal herbs in Asian countries, as well as in ancient African and Ayurvedic medicine. All parts of the plant are utilised for medicinal purposes. Common uses for *Cissusquadrangularis* include bone health and weight reduction. It is also used for polygenic illness, excessive cholesterol, haemorrhoids, and several other ailments, however there is no scientific evidence to support these applications. There's not enough data to understand however *Cissusquadrangularis* may work for medicative functions in folks. *In vivo* and *in vitro* studies demonstrate its inhibitory, analgesic, and anti-inflammatory effects. It would be active against the organism responsible for protozoal illness. (5)

*C. quadrangularis* may grow to a height of 1.5 metres (4.9 ft) and has branches that are sectioned in a quadrangular pattern. The internodes on these branches are between 8 and 10 centimetres (three to four inches) long and 1.2 and 1.5 centimetres (0.5 and 0.6 inches) broad. There may be leathery edges on all sides. The nodes of toothed trilobe leaves are 2–5 cm (0.8–2.0 in) broad. Every node has a plant structure that grows from the other side. Racemes of yellowish or multicoloured cabbage butterfly blooms; spherical berries become red when mature. *Cissusquadrangularis* is an evergreen climber that grows quickly to five metres (16 feet) by 5 metres (1.6 feet). It is hardy to zone ten (UK). Suitable for light (sandy), medium (loamy), and heavy (clay) soils. It likes soil that drains well and can grow in soil that doesn't have much nutrition. Acid, neutral, and basic (alkaline) soils are all suitable, but it may even thrive in very acidic and alkaline soils. It is unable to thrive in the shade. It enjoys moist or dry soil and can withstand drought. (6) The goal of this research is to see whether *C. quadrangularis* leaf extract has antiproliferative properties in human breast cancer cell lines.

## **MATERIALS AND METHODS:**

### **CELL VIABILITY ASSAY**

The cytotoxic activity of *C. quadrangularis* stem extract was evaluated using the MTT test, which was performed in accordance with Mosmann's methodology (7). In a brief, the cells were planted in a 96 well microtiter plate with replications at a concentration of  $1 \times 10^5$  cells per millilitre. Each well contained 100 microliters of medium. Different concentrations of *C. quadrangularis* (10, 20, 30, 50, 100, 200 M) were used for a 24-hour treatment. After incubation, each well was replaced with 20  $\mu$ l of 5 mg/ml MTT stock solution and incubated for 4 hours at 37 °C. In order to quantify the absorbance at 570 nm using a microplate reader, the produced formazan crystals were first dissolved in DMSO (SpectraMax M5, Molecular Devices, USA). Cell viability (%) was calculated as a ratio of absorbance ( $A_{570}$ ) in treated cells to absorbance (0.1% DMSO) in control cells ( $A_{570}$ ). Based on comparisons between the treated control (DMSO) and the sample concentration required to lower absorbance by 50%, the IC<sub>50</sub> value was determined. The following equation was used to determine the percentage of cells that were viable:

$$\text{Cell viability (\%)} = \left\{ \frac{A_{570} \text{ of (sample)}}{A_{570} \text{ of (control)}} \right\} \times 100.$$

## **RESULTS:**

The MTT assay was used to determine the vitality of the cells. In addition, the measurement of IC<sub>50</sub> value indicated that the *C. quadrangularis* stem extract has anticancer activity. This indicates that the active component concentration should lower cell viability to 50%. At 30 g/ml, however, the stem extract inhibits 50% cell growth (IC<sub>50</sub> value). Based on the findings, it seems that herbal extracts may possess a cell cytotoxic action that is effective against breast cancer cell lines.

Similarly, morphological alterations with specific apoptotic hallmarks such as membrane blebbing, nuclear condensation, fragmented nuclei, and cell wall breakdown were identified under phase contrast microscopy. Which clearly demonstrates the anticancer potential of *C. quadrangularis* stem extract treatment for 24 hours on MCF-7 cell line.

Control

*C. quadrangularis* stem extract

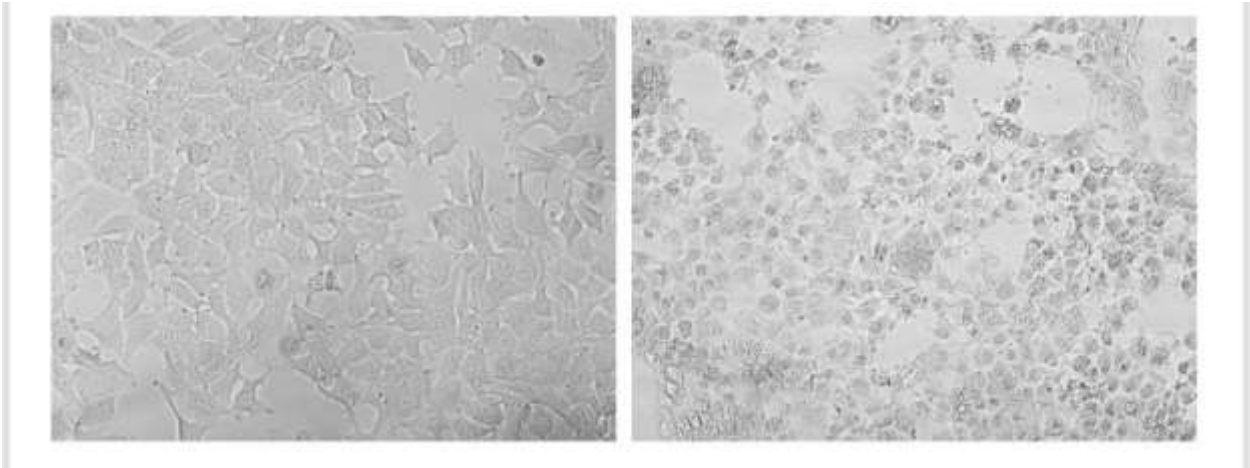


Figure 1: Represents morphological changes that have taken place in a breast cancer cell line before and after treatment with *Cissus quadrangularis* at a concentration of 18 g/mL for 24 hours by Phase contrast microscopy at 20x magnification

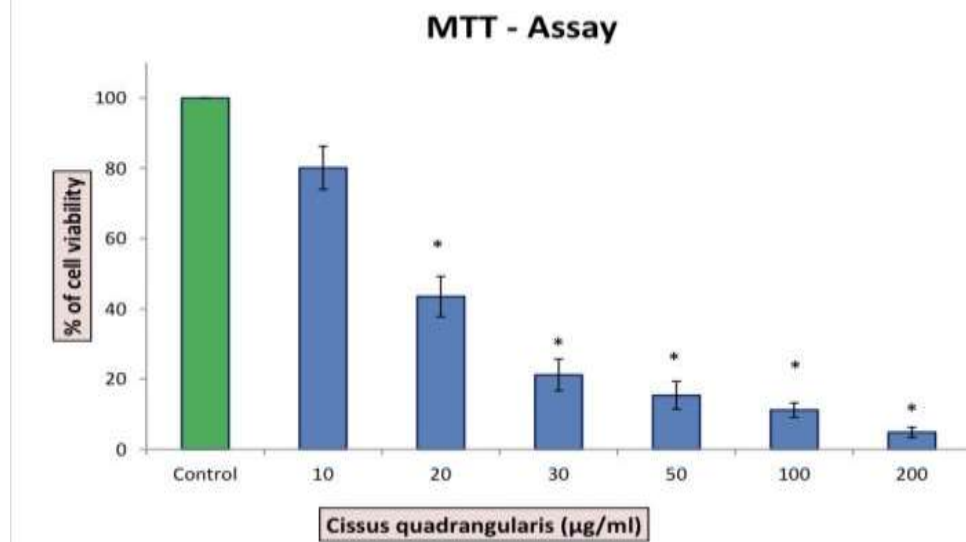


Figure 2: The MTT assay was used to evaluate the cytotoxic effects of *C. quadrangularis* stem extract on MCF-7 cells. The cells were treated with different concentrations (10, 20, 30, 50, 100, and 200 g) for 24 hours. The IC<sub>50</sub> value of 18 g/ml, which was shown to have 50% inhibition, has been established and used in further research. The data are presented as means standard deviations (n = 3), and a value of p < 0.001 indicates statistical significance when compared to the control-blank group.

## DISCUSSION:

The cytotoxic activity of CQ extract against carcinomatous cells suggests that it might be used to treat skin cancer. Several studies demonstrated that the cytotoxic activity of the herb *Cissus quadrangularis* in multiple cell lines such as Narse deity, Vero, MCF7, computer memory unit oral epidermoid malignant neoplastic disease cells, and EAC (Ehrlich Pathological Carcinoma) cell line clearly suggests that it will be useful for the treatment of cancer such as hepato cellular carcinoma and cervical cancer.(8)This bioactive component found in plants will prevent carcinogenesis by inhibiting metabolic activation, enhancing detoxification, or providing alternative targets to electrophonic metabolites.(9,10). They will act by preventing the cooperation of substance cancer-causing agents or endogenous free revolutionaries with desoxyribonucleic acid, thereby reducing the degree of harm and materialising changes that contribute not only to tumor progression but also to reformist genomic shakiness and overall growth change. (9). This antiproliferative activity might be related to the nature of the chemicals contained in each crude extract and how they interact with the metabolic characteristics of each kind of cancer cell, or it could be due to the efficacy of specific enzymes that serve as antioxidants, particularly in cancer cells (3). Due to the absence of an *in vivo* investigation, the study's effectiveness cannot be determined. This opens the door for a variety of future investigations, such as evaluating

the activity of the medicine in in vivo tests and determining the potential adverse effects of the extract, among other things. Our team has extensive research experience as well as a depth of knowledge, both of which have culminated in the production of high-quality papers.(11–15),(16),(17),(18),(19),(20),(21),(22,23),(24–28),(29),(30).(31)(32)(33)(34)(34,35)(36)(37)(38)(39)(40)(41)(42)(43)(44)(45)(46)(47)

#### CONCLUSION:

The anticancer activity of *Cissusquadranqularis* (L) stem extracts was assessed in vitro using MTT assays in the current study. The investigation revealed that the MCF-7 breast cancer cells exhibited significant antiproliferative activity. Furthermore, future research should focus on elucidating the molecular mechanism behind these herbal extracts' cytotoxic and antiproliferative actions on cancer cells.

#### Reference:

1. Murthy KNC, Chidambara Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishankar GA. Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L [Internet]. Vol. 6, Journal of Medicinal Food. 2003. p. 99–105. Available from: <http://dx.doi.org/10.1089/109662003322233495>
2. Balasubramanian G, Sarathi M, Rajesh Kumar S, Sahul Hameed AS. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp [Internet]. Vol. 263, Aquaculture. 2007. p. 15–9. Available from: <http://dx.doi.org/10.1016/j.aquaculture.2006.09.037>
3. Lopez-Nava G, Asokkumar R. Reply to Katakwar et al [Internet]. Vol. 53, Endoscopy. 2021. p. 340–340. Available from: <http://dx.doi.org/10.1055/a-1337-2500>
4. Lewandowska U, Szewczyk K, Owczarek K, Hrabec Z, Podsędek A, Koziółkiewicz M, et al. Flavanols from evening primrose (*Oenothera paradoxa*) defatted seeds inhibit prostate cells invasiveness and cause changes in Bcl-2/Bax mRNA ratio. J Agric Food Chem. 2013 Mar 27;61(12):2987–98.
5. Sotgia F, Martinez-Outschoorn UE, Lisanti MP. Mitochondrial oxidative stress drives tumor progression and metastasis: should we use antioxidants as a key component of cancer treatment and prevention? [Internet]. Vol. 9, BMC Medicine. 2011. Available from: <http://dx.doi.org/10.1186/1741-7015-9-62>
6. Srisook K, Palachot M, Mongkol N, Srisook E, Saraputit S. Anti-inflammatory effect of ethyl acetate extract from *Cissus quadrangularis* Linn may be involved with induction of heme oxygenase-1 and suppression of NF- $\kappa$ B activation [Internet]. Vol. 133, Journal of Ethnopharmacology. 2011. p. 1008–14. Available from: <http://dx.doi.org/10.1016/j.jep.2010.11.029>
7. Suresh P, Xavier AS, Karthik VP, Punnagai K. Anticancer Activity of *Cissus Quadrangularis* L. Methanolic Extract Against MG63 Human Osteosarcoma Cells – An In-Vitro Evaluation using Cytotoxicity Assay. Biomedical and Pharmacology Journal. 2019 Jun 25;12(2):975–80.
8. Rana SVS. Metals and apoptosis: Recent developments [Internet]. Vol. 22, Journal of Trace Elements in Medicine and Biology. 2008. p. 262–84. Available from: <http://dx.doi.org/10.1016/j.jtemb.2008.08.002>
9. Raghunandhakumar S, Paramasivam A, Senthilraja S, Naveenkumar C, Asokkumar S, Binuclara J, et al. Thymoquinone inhibits cell proliferation through regulation of G1/S phase cell cycle transition in N-nitrosodiethylamine-induced experimental rat hepatocellular carcinoma. Toxicol Lett. 2013 Oct 23;223(1):60–72.
10. McArthur G, Eve PM, Jones K, Banales E, Kohonen S, Anandakumar T, et al. Phonics training for English-speaking poor readers. Cochrane Database Syst Rev. 2012 Dec 12;12:CD009115.
11. 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.
12. 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.
13. 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.
14. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using *Gymnema sylvestris* leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, South African Journal of Chemical Engineering. 2020. p. 1–4. Available from: <http://dx.doi.org/10.1016/j.sajce.2019.11.005>
15. 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.
16. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*. S Afr J Chem Eng. 2019 Jul;29:17–23.
17. Raj R K, D E, S R.  $\beta$ -Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A. 2020 Sep;108(9):1899–908.
18. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from

- Phenerochaetechryso sporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. *MicrobPathog*. 2018 Apr;117:68–72.
19. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. *Hum Exp Toxicol*. 2019 Jun 1;38(6):694–702.
  20. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. *Hepatobiliary Pancreat Dis Int*. 2018 Jun;17(3):192–7.
  21. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. *Arab J Gastroenterol*. 2018 Jun;19(2):56–64.
  22. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of *Tamarindus indica* on MCF-7 human breast cancer cell line. *J Drug Deliv Sci Technol*. 2020 Feb 1;55:101376.
  23. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. *Drug Dev Res*. 2019 Sep;80(6):714–30.
  24. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol*. 2018 Oct;89(10):1241–8.
  25. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol*. 2021 Feb;122:105030.
  26. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2021 Jul;132(1):118–9.
  27. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygiumcumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med*. 2019 Feb;48(2):115–21.
  28. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprakasam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments. *Implant Dent*. 2019 Jun;28(3):289–95.
  29. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. *Aquaculture*. 2019 May 30;507:1–6.
  30. Markov A, Thangavelu L, Aravindhana 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.
  31. Rajeshkumar S, Ezhilarasan D, Puyathron N, Lakshmi T. Role of supermagnetic nanoparticles in Alzheimer disease. In: *Nanobiotechnology in Neurodegenerative Diseases*. Cham: Springer International Publishing; 2019. p. 225–40.
  32. 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. *Alinteriziraibilimderg*. 2020 Nov 27;35(2):164–9.
  33. Rajeshkumar S, Tharani M, Sivaperumal P, Lakshmi T. Synthesis of Antimicrobial Silver Nanoparticles by Using Flower of *Calotropis Gigantea*. *Journal of Complementary Medicine Research*. 2020;11(5):8–16.
  34. Lakshmi T, Ezhilarasan D, Nagaich U, Vijayaragavan R. *Acacia catechu* Ethanolic Seed Extract Triggers Apoptosis of SCC-25 Cells. *Pharmacogn Mag [Internet]*. 2017 Oct [cited 2021 Aug 31];13(Suppl 3). Available from: <https://pubmed.ncbi.nlm.nih.gov/29142391/>
  35. Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*. *Biochemistry and Biophysics Reports*. 2019 Mar 1;17:208–11.
  36. Rajeshkumar S, Sivaperumal P, Tharani M, Lakshmi T. Green Synthesis of Zinc Oxide Nanoparticles by *Cardiospermum* -. *Journal of Complementary Medicine Research*. 2020;11(5):128–36.
  37. Rajeshkumar S, Tharani M, Sivaperumal P, Lakshmi T. Green Synthesis of Selenium Nanoparticles Using Black Tea (*Camellia Sinensis*) And Its Antioxidant and Antimicrobial Activity. *Journal of Complementary Medicine Research*. 2020;11(5):75–82.
  38. R. Jagadheeswari RJ, T. Lakshmi TL, Balusamy SR, David S, Kumar SR. Biosynthesis of silver nanoparticles using *Withania somnifera* (L.) Dunal extract and its antibacterial activity against food pathogens. *Ann Phytomed [Internet]*. 2020 Jun;9(1). Available from: [http://www.ukaazpublications.com/publications/?smd\\_process\\_download=1&download\\_id=9526](http://www.ukaazpublications.com/publications/?smd_process_download=1&download_id=9526)
  39. Molecular docking analysis of compounds from *Lycopersicon esculentum* with the insulin receptor to combat type 2 diabetes [Internet]. [cited 2021 Aug 31]. Available from: <http://www.bioinformation.net/016/97320630016748.htm>
  40. Anticancer effects and lysosomal acidification in A549 cells by Astaxanthin from *Haematococcus lacustris* [Internet]. [cited 2021 Aug 31]. Available from: <http://www.bioinformation.net/016/97320630016965.htm>
  41. Akshayaa L, Lakshmi, Thangavelu, Devaraj, Ezhilarasan, Roy, Anitha, Raghunandhakumar, S, Sivaperumal P, David, Sheba, Dua, Kamal, Chellappan, Dinesh Kumar. Data on known anti-virals in combating CoVid-19. *Bioinformation*. 2020;878–878.
  42. Rajeshkumar S, Agarwal H, Sivaperumal P, Shanmugam VK, Lakshmi T. Antimicrobial, anti-inflammatory and

- anticancer potential of Microbes mediated zinc oxide nanoparticles. *Journal of Complementary Medicine Research*. 2020;11(5):41–8.
43. Thangavelu L, Balusamy SR, Shanmugam R, Sivanesan S, Devaraj E, Rajagopalan V, et al. Evaluation of the sub-acute toxicity of *Acacia catechu* Willd seed extract in a Wistar albino rat model. *Regul Toxicol Pharmacol* [Internet]. 2020 Jun [cited 2021 Aug 31];113. Available from: <https://pubmed.ncbi.nlm.nih.gov/32169672/>
  44. Cytotoxic potentials of silibinin assisted silver nanoparticles on human colorectal HT-29 cancer cells [Internet]. [cited 2021 Aug 31]. Available from: <http://www.bioinformation.net/016/97320630016817.htm>
  45. Shaker Ardakani L, Surendar A, Thangavelu L, Mandal T. Silver nanoparticles (Ag NPs) as catalyst in chemical reactions. *Synth Commun*. 2021 Mar 8;1–21.
  46. Hashim IM, Ghazi IF, Kuzichkin OR, Shakirova IA, Surendar A, Thangavelu L, et al. Effects of Primary Stored Energy on Relaxation Behavior of High Entropy Bulk Metallic Glasses Under Compressive Elastostatic Loading. *Trans Indian Inst Met*. 2021 Mar 14;74(6):1295–301.
  47. Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. *J Adv Pharm Technol Res* [Internet]. 2013 Apr [cited 2021 Aug 31];4(2). Available from: <https://pubmed.ncbi.nlm.nih.gov/23833747/>