ANTIPROLIFERATIVE POTENTIAL OF ERYTHRINA INDICA LEAF AQUEOUS EXTRACT AGAINST HUMAN BREAST CANCER CELLS

Running title: Effect of Erythrina Indica leaf extract on cancer cells.

ABSTRACT

Introduction :Breast cancer is cancer that develops in breast cells. The cancer forms in either the lobules or the ducts of the breast.Cancer can also occur in the fatty tissue or the fibrous connective tissue within your breast.

MATERIALS AND METHODS:

In vitro cytotoxicity assay The effect of Erythrina indica 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 with different concentrations (0, 5, 7.5, 15, 30, 45, 60, 75 and 100 µg) of Erythrina indica. After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and

Results: The crude extract obtained from *Erythrina indica* greatly inhibits the cancer cell proliferation in different time and dose manner, respectively. At 100 µg/ml of *E.indica* for 24 hrs incubation disrupting the cancer cells undergoing apoptosis mediated cell death. Which was confirmed by morphology evaluation using phase contrast microscope. .

Conclusion: The results suggest that the *E.indica* extract shows the potent antiproliferative activity against breast cancer cells, and it might be a novel new anticancer drug for cancer therapy.

Key words: Anticancer, Sea grass, Breast cancer cell line, Erythrina indica, innovative technique, eco friendly.

INTRODUCTION:

Erythrina indica is a medium-sized, spiny, deciduous tree usually developing to 6-nine m (once in a while 28 m) tall and 60 cm dbh.(1,2) Young stems and branches are thickly armed with stout conical spines up to eight mm lengthy, which fall off after 2-four years; rarely, some spines persist and are retained with the corky bark. Bark easy and inexperienced while young, exfoliating in papery flakes, turning into thick, corky and deeply fissured with age(3,4). Leaves trifoliate, alternate, shiny emerald -inexperienced, on lengthy petioles 6-15 cm, rachis 5-30 cm lengthy, prickly; leaflets easy, shiny, broader than lengthy, eight-20 with the aid of using 5-15 cm, ovate to acuminate with an obtusely pointed end(5)(6). Leaf petiole and rachis are spiny.Flowers in shiny red to scarlet erect terminal racemes 15-20 cm lengthy; stamens barely sticking out from the flower.(7,8) Fruit a cylindrical torulose pod, inexperienced, turning black and wrinkly as they ripen, thin-walled and constricted across the seeds.There are 1-eight easy, oblong, darkish pink to nearly black seeds consistent with pod.

Breast cancer is one of the most common styles of cancers internationally and yet, its pathophysiology is poorly understood. Single-mobileular electrophysiological research has furnished proof that membrane depolarization is implicated withinside the proliferation and metastasis of breast most cancers(9). However, metastatic breast most cancers cells are exceedingly dynamic microscopic structures with complexities past a single-molecular level. There is a pressing need for electrophysiological research and technology able to decipher the intercellular signaling pathways and networks that manage proliferation and metastasis, especially at a populace level. Hence, we gift for the primary time non-invasive in vitro electric recordings of strongly metastatic MDA-MB-231 and weakly/non-metastatic MCF-7 breast most cancers lines.(10)Erythrina indica incorporates glycosides and phenol compounds which can be capable of behaving as antifungal and anticancer, and even incorporates steroid compounds which act as antibacterial and anticancer(11). It has been said that crude extract from Erythrina

indica had excessive phenolic content material. Moreover, suggested the cytotoxicity of crude extract from *E.indica*. The maximum phenolic content material is at the leaves part. One that may be located in tidal coastal regions in Indonesia is *Erythrina indica*. Since different sorts had been suggested to include anticancer bioactive compounds, any other studies to decide the capability of *Erythrina indica* as a supply of anticancer bioactive compounds ought to additionally be conducted.(1) The purpose of these studies was to decide the capability of *E.indica* leaves extract as an anticancer agent.

MATERIALS AND METHODS

CHEMICALS:

SDMEM 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 were 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.

PREPARATION OF EXTRACT:

Erythrina indica herbal powder commercially purchased IMPCOPS - Chennai (Indian Medical Practitioners Co-operative Pharmacy and Stores Limited). 200g of sample was soaked in double distilled water and kept for 3 days at 37°C temperature in continuous intervals of shaking the flask. Further, the solution was filtered and placed in a rotary vacuum evaporator to concentrate fine filtered samples and leftover solvent was evaporated to dryness in a hot air oven. 2 grammes of material was obtained and immediately sorted at 4°C, for further experiments.

The required quantity of the herbal extract was weighed and dissolved in DMSO with concentration of 1mg/ml as a stock solution. This solution was subsequently diluted to a series of concentrations ranging from 50 to 300 µg/ ml for cell viability assay.

CYTOTOXIC ASSAY:

The cytotoxic effect of *Erythrina indica* on MCF-7, were measured with MTT (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) assay by Alam (12) Cells were seeded in 96-well plates at the density of $5x10^3$/100µl and treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) of S.virginianum for 24hrs. After 24hrs 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 (A570) in control cells (0.1 % DMSO). The IC_{50} was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. Percent cell viability was calculated following the equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

inhibition (%)= 100- cell viability (%)

STATISTICAL ANALYSIS:

All data obtained were analyzed and computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. In all tests, the level of statistical significance was set at $p<0.05$

RESULT AND DISCUSSION :

During the recent decades, a number of anticancer compounds derived from natural sources, such as vincristine, vinblastine, taxol, and bleomycin, have been identified and are now extensively utilised to treat various kinds of cancer. Many researchers reports, phenolic

compounds have anti-carcinogenic action and alter the bioenergetic processes of MCF-7 breast cancer cells. Edible plant material includes a large number of micro-constituents, all of which are active in biological systems (13). The present study aims to identify the antiproliferative effect of *Erythrina indica* for breast cancer therapy. The results showed potential cytotoxic effects by MTT assay and morphometric analysis using phase contrast microscopy in Breast cancer cell lines are presented in figure 1 & 2, demonstrating the bioactivity of *E.indica* extract in MCF-7 cells. *E.indica* at a concentration of 250 µg ml−1 hindered the growth of MCF-7 cells. (14–18),(19),(20),(21),(22),(23),(24),((16,25,26),(27–31) .,(32),(33)

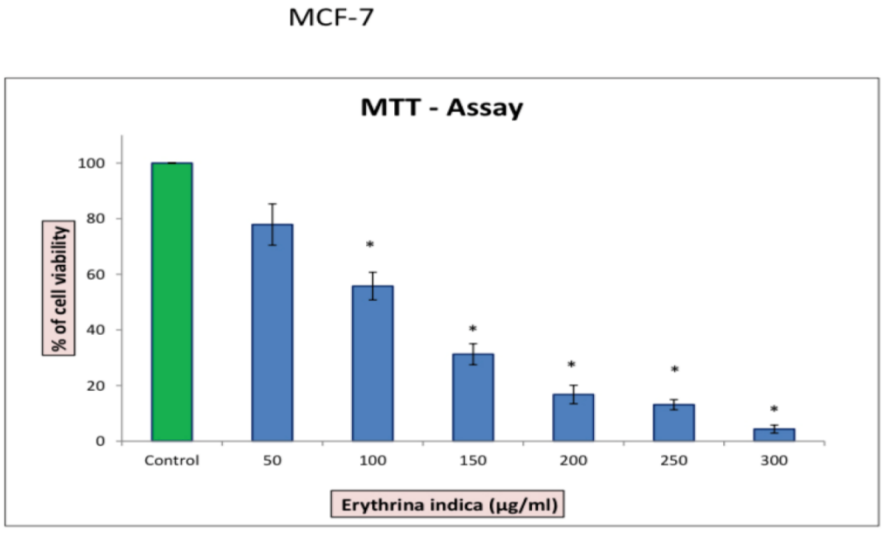

Fig.1 Represent the cytotoxic effect of *Erythrina indica* against breast cancer cells for 24hrs. The X axis represents different concentrations of *E.indica* and Y- axis represents the percentage of cell viability. Green colour denotes control and blue colour represents the different levels of *E.indica* in µg/ml. Data are shown as means ± SD (n = 3) compared with the control-blank group, $p < 0.001$. At 100 µg/ml of *E.indica* only 50 % of the cells were viable, which shows the good cytotoxic activity of the herb.

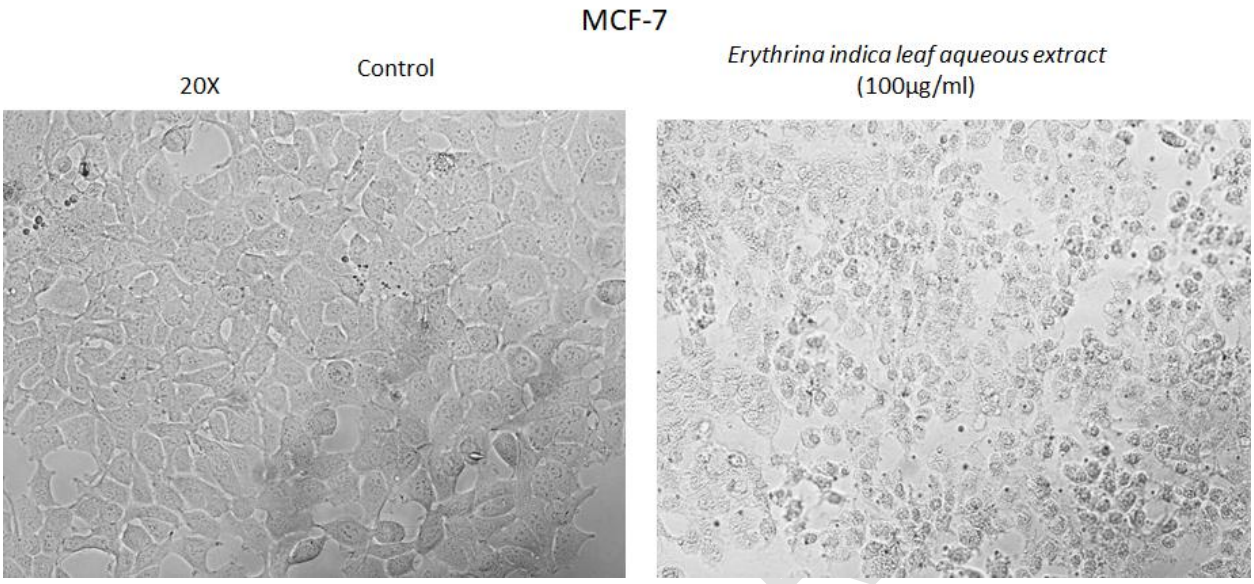

Fig.2. *E.indica's* antiproliferative activity was evaluated by morphological changes with control and treated (100µg/ml) breast cancer cells. Cellular characteristics were disrupted upon herbal treated cells with membrane blebbing, nuclear condensation, fragmentation were observed under phase contrast microscopy 20x magnification,

Breast cell lethality level by semi polar extract was higher than polar extract, but not significantly different with cancer medicine doxorubicin. The extract of C. serrulata reveals the presence of phytocompounds that are biologically active. According to the chromatogram obtained by GCMS ethanol extract of erythrina indica consists of palmitic acid, myristic acid, and pentadecanoic acid as a major component. They may be produced by the plant defense itself from stress as secondary metabolites(34). These cytoprotectants proved to possess pharmacological activity in a similar way as synthetic drugs. The palmitic acid reported possessing anticancer activity, antimicrobial, and nematicide activity(35). The palmitic acid increases the number of probiotic bacteria in the gut; thus, they are involved in the development of the intestine. It is required in the biosynthesis of lung lecithin, which is related to fetal maturation as well as it has been reported that presence of palmitic acid in the Nigerian meal can

partly be related to the low incidence of respiratory disease. Palmitic acid reported inhibiting human hepatoma cell growth in a dose dependent and time-dependent manner. Thus, they possess anticancer. Since other types were reported to contain anticancer bioactive compounds, another research to determine the potential of Erythrina indica as a source of anticancer bioactive compounds should also be conducted (36).(37) (38) (39) (40) (40,41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53)

CONCLUSION:

This study aimed to reveal the antiproliferative effect of *Erythrina indica* aqueous extract against breast cancer cells. The results show that the herbal extract has greatly inhibited cell proliferation at 100 µg/ml (IC_{50} Value) concentration for 24hrs. Further, morphological changes like membrane blebbing, nuclear condensation and fragmentation have been observed upon *E. indica* treatment showing antitumor activity against cancer cells(30) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67). These promising results suggest that *Erythrina indica* as a promising source of natural ingredients, and pave the way to develop novel anticancer drugs for treating cancer, including breast cancer.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES:

1. Mujahid M, Hussain T, Siddiqui HH, Hussain A. Evaluation of hepatoprotective potential

of Erythrina indica leaves against antitubercular drugs induced hepatotoxicity in experimental rats [Internet]. Vol. 8, Journal of Ayurveda and Integrative Medicine. 2017. p. 7–12. Available from: http://dx.doi.org/10.1016/j.jaim.2016.10.005

2. Erythrina indica Lam [Internet]. SpringerReference. Available from: http://dx.doi.org/10.1007/springerreference_68517

3. Sre PRR, Sheila T, Murugesan K. Phytochemical screening and "in–vitro" anti–oxidant activity of methanolic root extract of Erythrina indica [Internet]. Vol. 2, Asian Pacific Journal of Tropical Biomedicine. 2012. p. S1696–700. Available from: http://dx.doi.org/10.1016/s2221-1691(12)60480-8

4. Kalva S, Professor A, Sri Venkateshwara College of Pharmacy, Hyderabad-81. Preparation and Evaluation of Mangifera Indica Loaded Ethosomal Gel for Anti-Inflammatory Activity in Animal Model [Internet]. International Journal of Ayurvedic and Herbal Medicine. 2018. Available from: http://dx.doi.org/10.18535/ijahm/v8i1.06

5. Vadivel V, Biesalski HK. Phenolic Content in Traditionally Processed Erythrina indica L. Seeds: Antioxidant Potential and Type II Diabetes Related Functionality [Internet]. Vol. 7, Current Nutrition & Food Science. 2011. p. 200–8. Available from: http://dx.doi.org/10.2174/157340111797264831

6. Khare CP. Erythrina indica Lam [Internet]. Indian Medicinal Plants. 2007. p. 1–1. Available from: http://dx.doi.org/10.1007/978-0-387-70638-2_580

7. Wankhede S, Juvekar M, Juvekar A, Sakat S, Gambhire M. Study of in vitro and in vivo anti-inflammatory activity of aqueous extract of leaves Erythrina indica [Internet]. Vol. 75, Planta Medica. 2009. Available from: http://dx.doi.org/10.1055/s-0029-1234879

8. Ratnasooriya WD, Dharmasiri MG. Aqueous extract of Sri Lankan Erythrina indica leaves has sedative but not analgesic activity [Internet]. Vol. 70, Fitoterapia. 1999. p. 311–3. Available from: http://dx.doi.org/10.1016/s0367-326x(99)00027-1

9. Jabbar DK. Antidiabetic Activity of Erythrina indica [Internet]. Vol. 14, Research Journal of Applied Sciences. 2019. p. 91–6. Available from: http://dx.doi.org/10.36478/rjasci.2019.91.96

10. Sreelekha TT, Vijayakumar T, Ankanthil R, Vijayan KK, Krishnan Nair M. Immunomodulatory effects of a polysaccharide from Tamarindus indica [Internet]. Vol. 4, Anti-Cancer Drugs. 1993. p. 209–12. Available from: http://dx.doi.org/10.1097/00001813-199304000-00013

11. Reddy TP, Reddy Prasad Reddy T. Exploring the Anti-inflammatory and Anti-cancer compounds from the leaves of Acalypha indica [Internet]. Vol. 4, IOSR Journal of Pharmacy and Biological Sciences. 2012. p. 1–7. Available from: http://dx.doi.org/10.9790/3008-0420107

12. Alam MS, Poonam NS, Koka K, Vijay V, Ganesh S. Intracanalicular antibiotic ointment

loading as a management option for canaliculitis. Orbit. 2021 Aug;40(4):295–300.

13. Zingue S, Gbaweng Yaya AJ, Cisilotto J, Kenmogne LV, Talla E, Bishayee A, et al. Abyssinone V-4' Methyl Ether, a Flavanone Isolated from , Exhibits Cytotoxic Effects on Human Breast Cancer Cells by Induction of Apoptosis and Suppression of Invasion. Evid Based Complement Alternat Med. 2020 Jul 22;2020:6454853.

14. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 Oct;117:91–5.

15. 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.

16. 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.

17. 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]. 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

18. 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.

19. 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.

20. Raj R K, D E, S R. β-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.

21. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog. 2018 Apr;117:68–72.

22. 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.

23. 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.

24. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental

perspective. Arab J Gastroenterol. 2018 Jun;19(2):56–64.

25. 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.

26. 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.

27. 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.

28. 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.

29. 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.

30. Ezhilarasan D, Apoorva VS, Ashok VN. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med [Internet]. 2019 Feb [cited 2021 Sep 15];48(2). Available from: https://pubmed.ncbi.nlm.nih.gov/30451321/

31. 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.

32. 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.

33. 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.

34. Cao Z-W, Zeng Q, Pei H-J, Ren L-D, Bai H-Z, Na R-N. HSP90 expression and its association with wighteone metabolite response in HER2-positive breast cancer cells. Oncol Lett. 2016 Jun;11(6):3719–22.

35. Rathi Sre PR, Reka M, Poovazhagi R, Arul Kumar M, Murugesan K. Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam. Spectrochim Acta A Mol Biomol Spectrosc. 2015 Jan 25;135:1137–44.

36. Grumezescu A. Nanobiomaterials in Cancer Therapy: Applications of Nanobiomaterials. William Andrew; 2016. 588 p.

37. 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.

38. 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. Alınteri zirai bilim derg. 2020 Nov 27;35(2):164–9.

39. 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.

40. 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/

41. 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.

42. 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.

43. 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.

44. 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

45. 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

46. 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

47. 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.

48. 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.

49. 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/

50. 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

51. 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.

52. 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.

53. 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/

54. Danda AK, Krishna TM, Narayanan V, Siddareddi A. Influence of primary and secondary closure of surgical wound after impacted mandibular third molar removal on postoperative pain and swelling--a comparative and split mouth study. J Oral Maxillofac Surg [Internet]. 2010 Feb [cited 2021 Sep 15];68(2). Available from: https://pubmed.ncbi.nlm.nih.gov/20116700/

55. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig [Internet]. 2019 Sep [cited 2021 Sep 15];23(9). Available from: https://pubmed.ncbi.nlm.nih.gov/30552590/

56. Sathivel A, Raghavendran HR, Srinivasan P, Devaki T. Anti-peroxidative and anti-hyperlipidemic nature of Ulva lactuca crude polysaccharide on D-galactosamine induced hepatitis in rats. Food Chem Toxicol [Internet]. 2008 Oct [cited 2021 Sep 15];46(10). Available from: https://pubmed.ncbi.nlm.nih.gov/18706469/

57. Panda S, Doraiswamy J, Malaiappan S, Varghese SS, Del Fabbro M. Additive effect of autologous platelet concentrates in treatment of intrabony defects: a systematic review and meta-analysis. J Investig Clin Dent [Internet]. 2016 Feb [cited 2021 Sep 15];7(1). Available from: https://pubmed.ncbi.nlm.nih.gov/25048153/

58. Neelakantan P, Varughese AA, Sharma S, Subbarao CV, Zehnder M, De-Deus G. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal

sealer to root dentine. Int Endod J [Internet]. 2012 Dec [cited 2021 Sep 15];45(12). Available from: https://pubmed.ncbi.nlm.nih.gov/22612994/

59. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. Clin Oral Investig [Internet]. 2017 Mar [cited 2021 Sep 15];21(2). Available from: https://pubmed.ncbi.nlm.nih.gov/27469101/

60. Sekhar CH, Narayanan V, Baig MF. Role of antimicrobials in third molar surgery: prospective, double blind,randomized, placebo-controlled clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available from: https://pubmed.ncbi.nlm.nih.gov/11286448/

61. DeSouza SI, Rashmi MR, Vasanthi AP, Joseph SM, Rodrigues R. Mobile phones: the next step towards healthcare delivery in rural India? PLoS One [Internet]. 2014 Aug 18 [cited 2021 Sep 15];9(8). Available from: https://pubmed.ncbi.nlm.nih.gov/25133610/

62. Nasim I, Neelakantan P, Sujeer R, Subbarao CV. Color stability of microfilled, microhybrid and nanocomposite resins--an in vitro study. J Dent [Internet]. 2010 [cited 2021 Sep 15];38 Suppl 2. Available from: https://pubmed.ncbi.nlm.nih.gov/20553993/

63. Danda AK, Muthusekhar MR, Narayanan V, Baig MF, Siddareddi A. Open versus closed treatment of unilateral subcondylar and condylar neck fractures: a prospective, randomized clinical study. J Oral Maxillofac Surg [Internet]. 2010 Jun [cited 2021 Sep 15];68(6). Available from: https://pubmed.ncbi.nlm.nih.gov/20303209/

64. Molecular structure and vibrational spectra of 2,6-bis(benzylidene)cyclohexanone: A density functional theoretical study. Spectrochim Acta A Mol Biomol Spectrosc. 2011 Jan 1;78(1):113–21.

65. Putchala MC, Ramani P, Herald J. Sherlin, Premkumar P, Natesan A. Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity – A systematic review [Internet]. Vol. 58, Archives of Oral Biology. 2013. p. 563–74. Available from: http://dx.doi.org/10.1016/j.archoralbio.2013.01.016

66. Neelakantan P, Grotra D, Sharma S. Retreatability of 2 mineral trioxide aggregate-based root canal sealers: a cone-beam computed tomography analysis. J Endod. 2013 Jul;39(7):893–6.

67. Suresh P, Marimuthu K, Ranganathan S, Rajmohan T. Optimization of machining parameters in turning of Al-SiC-Gr hybrid metal matrix composites using grey-fuzzy algorithm [Internet]. Vol. 24, Transactions of Nonferrous Metals Society of China. 2014. p. 2805–14. Available from: http://dx.doi.org/10.1016/s1003-6326(14)63412-9