JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH
Journal of Complementary Medicine Research, ISSN: 2146-8397 Vol. 13, No. 2, 2022 (pp. 87-91)
ORIGINAL STUDY
WWW.JOCMR.COM

Preparation of Herbal Formulation of Stevia and Piper Longum and its Antiinflammatory and Cytotoxic Effect

M. Vignesh[1], S. Rajeshkumar[2], Anitha Roy[3], T.Lakshmi[4*]

[1]*Saveetha Dental college and Hospital, Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai-77, Tamilnadu, India.*

[2]*Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute Of medical and Technical Sciences, Saveetha University, Chennai-77, Tamilnadu, India.*

[3]*Professor, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India*

[4]*Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamilnadu, India.*

ABSTRACT

AIM: The aim of the study was to prepare the herbal formulation of stevia and the piper longum nanoparticle and to evaluate its anti-inflammatory and cytotoxic effects.

Materials and Methods: For the evaluation of antiinflammatory activity we used albumin denaturation assay and for the cytotoxic activity we used a brine shrimp lethality assay and evaluated the result according to the test results and the graph was made with the results

Result : The nanoparticle of stevia and piper longum showed a marked anti inflammatory and cytotoxic activity with increased in their concentrations

Conclusion: Stevia and piper longum nanoparticle markedly increase the anti inflammatory and cytotoxic activity compared to the standard and should be employed as a formulations

Corresponding Author: lakshmi@saveetha.com

How to cite this article: Vignesh M, Rajeshkumar S, Roy A, Lakshmi T. Preparation of Herbal Formulation of Stevia and Piper Longum and its Antiinflammatory and Cytotoxic Effect. Journal of Complementary Medicine Research, Vol. 13, No. 2, 2022 (pp. 87-91).

INTRODUCTION

Spices are the plant substance which is derived from exotic or indigenous origin aromatic with strong taste and which is used to enhance the taste of food.[(1)].Spices provide protection against some diseases and promote healing as they are rich in phytonutrients and other active ingredients.[(2)]One commonly used spices are *piper longum*. *L* which comes under the family piperaceae commonly known as a long pepper distributed nearly all over the world except some areas and It is an important component of Indian traditional medicine reported to be used as a remedy for treating respiratory tract infection, chronic gut related pain, gonorrhea, menstrual pain, tuberculosis, and arthritic conditions[(3-4)] and stevia known as a sweet leaf belongs to the family asteraceae and its herbaceous perennial shrub and it has good antioxidant properties and stevia also possesses antiinflammatory properties through its polyphenol derivatives and through their monomeric precursors that controls chronic inflammation and even several extracts of stevia like ethanol,methanol,chloroform extract exhibit the marked significant anti inflammatory effect[(5-6)] and the stevia plant polyphenol derivative also used against phytophagous insects, fungi, or bacteria and thus it also exhibit the antimicrobial activity and cytotoxic effect.[(7)] *Stevia pilosa* methanolic root extract (*SPME*) and *Stevia eupatoria* methanolic root extract *(SEME)* have an inhibitory effect on the viability and migration of prostate cancer cells and do not interfere with the enzalutamide anticancer effect[(8)] and compound Stevioside, a natural noncaloric sweetener isolated from *Stevia rebaudiana* Bertoni, possesses anti-inflammatory and antitumor promoting properties which is a major breakthrough.[(9-10)] In our study we made nanoparticle of both the sample of stevia and *piper longum* to achieve both anti inflammatory and cytotoxic effect and our team has extensive knowledge and experience that has translated into high quality publication.[(11-20)]

KEYWORDS:
Anti inflammatory, Brine shrimp lethality assay, Cytotoxic activity, Piperlongum Stevia, .

ARTICLE HISTORY:
Received: Jan 18, 2022
Accepted: Mar 27, 2022
Published: May 21, 2022

DOI:
10.5455/jcmr.2022.13.02.17

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT

Piper longum and stevia were dried and made into a powder. 1g of each plant powders were collected and dissolved in distilled water. And were boiled for 5-10 min at 60-70 Celsius. The solution was filtered by using Whatman no. 1 filter paper. The filtered extract was collected and stored in 4 degree Celsius.

CYTOTOXIC EFFECT

BRINE SHRIMP LETHALITY ASSAY

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL.80 µL,100 µL). Then the Stevia and piper longum nanoparticles were added according to the concentration level. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100

Anti-inflammatory activity:

ALBUMIN DENATURATION ASSAY:

The anti-inflammatory activity for stevia and piper longum nanoparticle was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations from the article(21). 0.05 mL of stevia and piper longum nanoparticle of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55°C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION

From the figure 1 it shows the significant increase in the graph in antiinflammatory activity of piper longum and stevia nanoparticle there is an increase in the inhibition of protein albumin by the test albumin denaturation assay when the concentration of the nanoparticle increases as 10¤l,20¤l,30¤,40¤l,50¤l and compared with the standard diclofenac sodium and DMSO is used as a control and showed a marked antiinflammatory activity.

In previous study stevia and piper longum sample show increased activity of anti inflammatory and cytotoxic activity

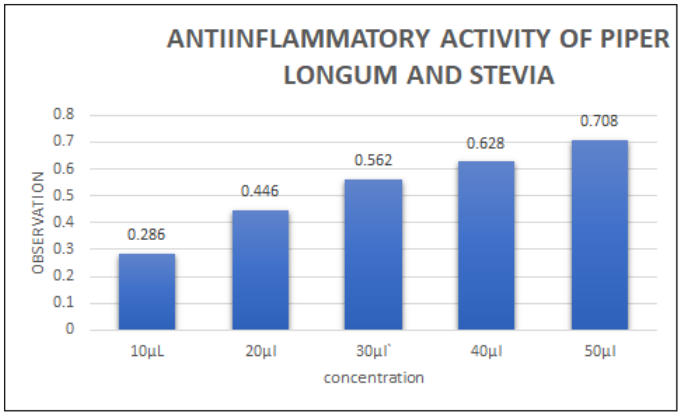

Fig. 1: Bar Graph showing the antiinflammatory activity of piper longum and stevia

Where X-axis represents the concentration in ¤l and Y-axis represents the observation

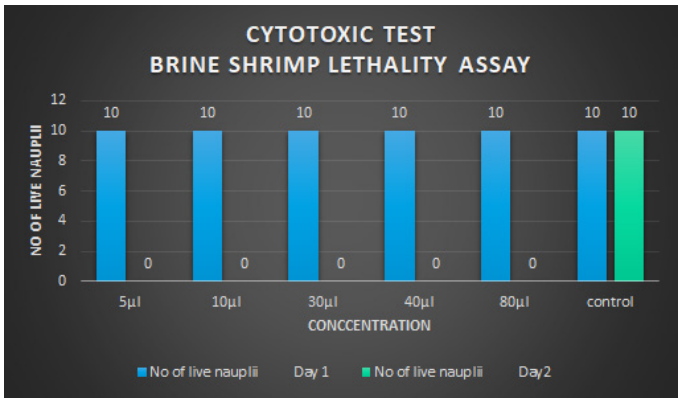

Fig 2: Showing the bar graph where X-axis represent the concentration of stevia and piper longum nanoparticle and Y-axis represent the No of the live nauplii

with supercritical fluid extraction from the plant[10] [22] [23] and previous study ginger is used as antiinflammatory agent[24] and previous study done gold nanoparticles to evaluate cytotoxic activity and antimicrobial activity.[25-26]

Further there previous studies stevioside the compound from the plant stevia shows the Increased cytotoxic activity against breast cancer cell line[27] and on further study there is a cinnamon oil mediated gold nanoparticle were used to test cytotoxic activity in brine shrimp[28-30] and on further study silver and graphene is used to achieve cytotoxic activity.[31]

From the figure 2 there is cytotoxic activity using brine shrimp lethality assay On day1 the sample of nauplii in elisa plates were kept without any nanoparticle there is no death of nauplii occurs and on the second day several concentrations of nanoparticle of stevia and piper longum(5µL,10µL,30µL,40µL,80µL) were added and last well is kept as control where there is no death of the nauplii before and after adding the nanoparticle of stevia and piper longum. In our study it is proved that both of stevia and piper longum nanoparticle showed the increased anti-inflammatory and cytotoxic activity

CONCLUSION

Stevia and piper longum nanoparticle extract showed very good dose dependant anti inflammatory and cytotoxic activity

very effectively and thus proven that the nanoparticle of the stevia and piper longum showed a marked increase on cytotoxic and antiinflammatory activity than synthetic chemicals or drugs thus we can employ the stevia and piper longum as a formulation in future due to its marked anti inflammatory and cytotoxic effects and even several properties are there in this plant extract and they are yet to be identified later

Acknowledgement

The authors are thankful to Saveetha Dental college for providing a platform to carry out this study.

REFERENCES

1. Buyukcombak SE, Esra Buyukcombak S, INTERNATIONAL BURCH UNIVERSITY. Effect of Spices on Biofilm Forming Capacity of Bacteria [Internet]. Vol. V7, International Journal of Engineering Research and. 2018. Available from: http://dx.doi.org/10.17577/ijertv7is100012
2. Premkumar LS. Fascinating Facts about Phytonutrients in Spices and Healthy Food. Xlibris Corporation; 2014. 246 p.
3. Prasad AK, Kumar V, Arya P, Kumar S, Dabur R, Singh N, et al. Investigations toward new lead compounds from medicinally important plants [Internet]. Vol. 77, Pure and Applied Chemistry. 2005. p. 25–40. Available from: http://dx.doi.org/10.1351/pac200577010025
4. Barma MD. Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study [Internet]. Vol. 13, Bioscience Biotechnology Research Communications. 2020. p. 74–8. Available from: http://dx.doi.org/10.21786/bbrc/13.7/14
5. Ibrahim NA, El-Gengaihi S, Motawe H, Riad SA. Phytochemical and biological investigation of Stevia rebaudiana Bertoni; 1-labdane-type diterpene [Internet]. Vol. 224, European Food Research and Technology. 2007. p. 483–8. Available from: http://dx.doi.org/10.1007/s00217-006-0400-3
6. Nandhini JT, Ezhilarasan D, Rajeshkumar S. An ecofriendly synthesized gold nanoparticles induces cytotoxicity via apoptosis in HepG2 cells [Internet]. Vol. 36, Environmental Toxicology. 2021. p. 24–32. Available from: http://dx.doi.org/10.1002/tox.23007
7. Myint KZ, Wu K, Xia Y, Fan Y, Shen J, Zhang P, et al. Polyphenols from Stevia rebaudiana (Bertoni) leaves and their functional properties. J Food Sci. 2020 Feb;85(2):240–8.
8. Koka P, Mundre RS, Rangarajan R, Chandramohan Y, Subramanian RK, Dhanasekaran A. Uncoupling Warburg effect and stemness in CD133 cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia. Mol Biol Rep. 2018 Dec;45(6):1653–62.
9. Asokkumar S, Naveenkumar C, Raghunandhakumar S, Kamaraj S, Anandakumar P, Jagan S, et al. Antiproliferative and antioxidant potential of beta-ionone against benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. Mol Cell Biochem. 2012 Apr;363(1-2):335–45.
10. Mohapatra S, Leelavathi L, Rajeshkumar S, D. SS, P. J. Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation - An In-Vitro Study [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 1859–64. Available from: http://dx.doi.org/10.14260/jemds/2020/405
11. 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 [Internet]. Vol. 277, Fuel. 2020. p. 118166. Available from: http://dx.doi.org/10.1016/j.fuel.2020.118166
12. 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
13. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of Passiflora caerulea [Internet]. Vol. 29, South African Journal of Chemical Engineering. 2019. p. 17–23. Available from: http://dx.doi.org/10.1016/j.sajce.2019.04.001
14. R KR, Kathiswar RR, Ezhilarasan D, Rajeshkumar S. Β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line [Internet]. Vol. 108, Journal of Biomedical Materials Research Part A. 2020. p. 1899–908. Available from: http://dx.doi.org/10.1002/jbm.a.36953
15. 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 [Internet]. Vol. 117, Microbial Pathogenesis. 2018. p. 68–72. Available from: http://dx.doi.org/10.1016/j.micpath.2018.02.008
16. 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.
17. 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.
18. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Sivaprakasam AN. Compatibility of Nonoriginal Abutments With Implants [Internet]. Vol. 28, Implant Dentistry. 2019. p. 289–95. Available from: http://dx.doi.org/10.1097/id.0000000000000885
19. 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.
20. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products [Internet]. Vol. 507, Aquaculture. 2019. p. 1–6. Available from: http://dx.doi.org/10.1016/j.aquaculture.2019.04.004
21. Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. J Adv Pharm Technol Res. 2012 Apr;3(2):136–8.
22. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells [Internet]. Vol. 27, Environmental Science and Pollution Research. 2020. p. 8166–75. Available from: http://dx.doi.org/10.1007/s11356-019-07511-x
23. Francis T, Rajeshkumar S, Roy A, Lakshmi T. Anti-inflammatory and Cytotoxic Effect of Arrow Root Mediated Selenium Nanoparticles [Internet]. Vol. 12, Pharmacognosy Journal. 2020. p. 1363–7. Available from: http://dx.doi.org/10.5530/pj.2020.12.188
24. Ganta SSL, Jeevitha M, Preetha S, Rajeshkumar S. Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles [Internet]. Journal of Pharmaceutical Research International. 2020. p. 14–9. Available from: http://dx.doi.org/10.9734/jpri/2020/v32i2830866
25. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Employing sulphated polysaccharide (fucoidan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines [Internet]. Vol. 26, Materials Today Communications. 2021. p. 101975. Available from: http://dx.doi.org/10.1016/j.mtcomm.2020.101975
26. Nasim I, Kamath K, Rajeshkumar S. Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study [Internet]. Vol. 23, Journal of Conservative Dentistry. 2020. p. 390. Available from: http://dx.doi.org/10.4103/jcd.jcd_315_20
27. Sreenivasagan S, Subramanian AK, Rajeshkumar SRS. Assessment of antimicrobial activity and cytotoxic effect of green mediated

silver nanoparticles and its coating onto mini-implants [Internet]. Vol. 9, Annals of Phytomedicine: An International Journal. 2020. Available from: http://dx.doi.org/10.21276/ap.2020.9.1.27

28. S. RJ, Roy A, Shanmugam R, E. DW. Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp LethalityAssay [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 2894–7. Available from: http://dx.doi.org/10.14260/jemds/2020/633
29. Shunmugam R, Balusamy SR, Kumar V, Menon S, Lakshmi T, Perumalsamy H. Biosynthesis of gold nanoparticles using marine microbe (Vibrio alginolyticus) and its anticancer and antioxidant analysis [Internet]. Vol. 33, Journal of King Saud University - Science. 2021. p. 101260. Available from: http://dx.doi.org/10.1016/j.jksus.2020.101260
30. Rajeshkumar S, Sherif MH, Malarkodi C, Ponnanikajamideen M, Arasu MV, Al-Dhabi NA, et al. Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica [Internet]. Vol. 1228, Journal of Molecular Structure. 2021. p. 129440. Available from: http://dx.doi.org/10.1016/j.molstruc.2020.129440
31. Nasim I, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles [Internet]. Vol. 16, Bioinformation. 2020. p. 831–6. Available from: http://dx.doi.org/10.6026/97320630016831
32. Buyukcombak SE, Esra Buyukcombak S, INTERNATIONAL BURCH UNIVERSITY. Effect of Spices on Biofilm Forming Capacity of Bacteria [Internet]. Vol. V7, International Journal of Engineering Research and. 2018. Available from: http://dx.doi.org/10.17577/ijertv7is100012
33. Premkumar LS. Fascinating Facts about Phytonutrients in Spices and Healthy Food. Xlibris Corporation; 2014. 246 p.
34. Prasad AK, Kumar V, Arya P, Kumar S, Dabur R, Singh N, et al. Investigations toward new lead compounds from medicinally important plants [Internet]. Vol. 77, Pure and Applied Chemistry. 2005. p. 25–40. Available from: http://dx.doi.org/10.1351/pac200577010025
35. Barma MD. Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study [Internet]. Vol. 13, Bioscience Biotechnology Research Communications. 2020. p. 74–8. Available from: http://dx.doi.org/10.21786/bbrc/13.7/14
36. Ibrahim NA, El-Gengaihi S, Motawe H, Riad SA. Phytochemical and biological investigation of Stevia rebaudiana Bertoni; 1-labdane-type diterpene [Internet]. Vol. 224, European Food Research and Technology. 2007. p. 483–8. Available from: http://dx.doi.org/10.1007/s00217-006-0400-3
37. Nandhini JT, Ezhilarasan D, Rajeshkumar S. An ecofriendly synthesized gold nanoparticles induces cytotoxicity via apoptosis in HepG2 cells [Internet]. Vol. 36, Environmental Toxicology. 2021. p. 24–32. Available from: http://dx.doi.org/10.1002/tox.23007
38. Myint KZ, Wu K, Xia Y, Fan Y, Shen J, Zhang P, et al. Polyphenols from Stevia rebaudiana (Bertoni) leaves and their functional properties. J Food Sci. 2020 Feb;85(2):240–8.
39. Koka P, Mundre RS, Rangarajan R, Chandramohan Y, Subramanian RK, Dhanasekaran A. Uncoupling Warburg effect and stemness in CD133 cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia. Mol Biol Rep. 2018 Dec;45(6):1653–62.
40. Asokkumar S, Naveenkumar C, Raghunandhakumar S, Kamaraj S, Anandakumar P, Jagan S, et al. Antiproliferative and antioxidant potential of beta-ionone against benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. Mol Cell Biochem. 2012 Apr;363(1-2):335–45.
41. Mohapatra S, Leelavathi L, Rajeshkumar S, D. SS, P. J. Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation - An In-Vitro Study [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 1859–64. Available from: http://dx.doi.org/10.14260/jemds/2020/405
42. 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.
43. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation [Internet]. Vol. 19, Biocatalysis and Agricultural Biotechnology. 2019. p. 101138. Available from: http://dx.doi.org/10.1016/j.bcab.2019.101138
44. 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
45. Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. J Adv Pharm Technol Res. 2012 Apr;3(2):136–8.
46. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells [Internet]. Vol. 27, Environmental Science and Pollution Research. 2020. p. 8166–75. Available from: http://dx.doi.org/10.1007/s11356-019-07511-x
47. Francis T, Rajeshkumar S, Roy A, Lakshmi T. Anti-inflammatory and Cytotoxic Effect of Arrow Root Mediated Selenium Nanoparticles [Internet]. Vol. 12, Pharmacognosy Journal. 2020. p. 1363–7. Available from: http://dx.doi.org/10.5530/pj.2020.12.188
48. Ganta SSL, Jeevitha M, Preetha S, Rajeshkumar S. Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles [Internet]. Journal of Pharmaceutical Research International. 2020. p. 14–9. Available from: http://dx.doi.org/10.9734/jpri/2020/v32i2830866
49. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Employing sulphated polysaccharide (fucoidan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines [Internet]. Vol. 26, Materials Today Communications. 2021. p. 101975. Available from: http://dx.doi.org/10.1016/j.mtcomm.2020.101975
50. Nasim I, Kamath K, Rajeshkumar S. Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study [Internet]. Vol. 23, Journal of Conservative Dentistry. 2020. p. 390. Available from: http://dx.doi.org/10.4103/jcd.jcd_315_20
51. Sreenivasagan S, Subramanian AK, Rajeshkumar SRS. Assessment of antimicrobial activity and cytotoxic effect of green mediated silver nanoparticles and its coating onto mini-implants [Internet]. Vol. 9, Annals of Phytomedicine: An International Journal. 2020. Available from: http://dx.doi.org/10.21276/ap.2020.9.1.27
52. S. RJ, Roy A, Shanmugam R, E. DW. Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp LethalityAssay [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 2894–7. Available from: http://dx.doi.org/10.14260/jemds/2020/633
53. Shunmugam R, Balusamy SR, Kumar V, Menon S, Lakshmi T, Perumalsamy H. Biosynthesis of gold nanoparticles using marine microbe (Vibrio alginolyticus) and its anticancer and antioxidant analysis [Internet]. Vol. 33, Journal of King Saud University - Science. 2021. p. 101260. Available from: http://dx.doi.org/10.1016/j.jksus.2020.101260
54. Rajeshkumar S, Sherif MH, Malarkodi C, Ponnanikajamideen M, Arasu MV, Al-Dhabi NA, et al. Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica [Internet]. Vol. 1228, Journal of Molecular Structure. 2021. p. 129440. Available from: http://dx.doi.org/10.1016/j.molstruc.2020.129440
55. Nasim I, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India. Cytotoxicity and anti-microbial analysis of silver and graphene

oxide bio nanoparticles [Internet]. Vol. 16, Bioinformation. 2020. p. 831–6. Available from: http://dx.doi.org/10.6026/97320630016831

56. Lakshmi, T., Geetha, R.V., Roy, A., Aravind Kumar, S. Yarrow (Achillea millefolium Linn.) a herbal medicinal plant with broad therapeutic use - A review, International Journal of Pharmaceutical Sciences Review and Research,2011,9(2), 136-141.
57. Dua, K., Wadhwa, R., Singhvi, G., Rapalli, V., Shukla, S.D., Shastri, M.D., Gupta, G., Satija, S., Mehta, M., Khurana, N., Awasthi, R., Maurya, P.K., Thangavelu, L., Rajeshkumar, S., Tambuwala, M.M., Collet, T., Hansbro, P.M., Chellappan, D.K., The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress, Drug Development Research(2019).,80(6), 714-730
58. Lakshmi, T.L., Aravind kumar, S., Preliminary phytochemical analysis & invitro antibacterial activity of Acacia catechu willd bark against streptococcus mitis, streptococcus sanguis & Lactobacillus acidophilus, International Journal of Phytomedicine,2011,3(4), 579-584.
59. Lakshmi, T., Geetha, R.V., Glycyrrhiza glabra linn commonly known as licorice: A therapeutic review, International Journal of Pharmacy and Pharmaceutical Sciences/2011,3(4),20-25.
60. Mehta, M., Dhanjal, D.S., Paudel, K.R., Singh, B., Gupta, G., Rajeshkumar, S., Thangavelu, L., Tambuwala, M.M., Bakshi, H.A., Chellappan, D.K., Pandey, P., Dureja, H., Charbe, N.B., Singh, S.K., Shukla, S.D., Nammi, S., Aljabali, A.A., Wich, P.R., Hansbro, P.M., Satija, S., Dua, K., Cellular signalling pathways mediating the pathogenesis of chronic inflammatory respiratory diseases: an update, Inflammopharmacology,2020,28(4), 795- 817.
61. Kandhan, T.S., Roy, A., Lakshmi, T., Rajeshkumar, S., Green synthesis of rosemary oleoresin mediated silver nanoparticles and its effect on oral pathogens, Research Journal of Pharmacy and Technology,2019,12(11), 5579-5582.
62. Vignesh, S., Anitha, R., Rajesh Kumar, S., Lakshmi, T., Evaluation of the antimicrobial activity of cumin oil mediated silver nanoparticles on oral microbes, Research Journal of Pharmacy and Technology(2019),12(8), 3709-3712.
63. Pranati, T., Anitha, R., Rajeshkumar, S., Lakshmi, T., Preparation of silver nanoparticles using nutmeg oleoresin and its antimicrobial activity against oral pathogens, Research Journal of Pharmacy and Technology,2019,12(6), 2799-2803.
64. Keerthiga, N., Anitha, R., Rajeshkumar, S., Lakshmi, T., Antioxidant activity of cumin oil mediated silver nanoparticles, Pharmacognosy Journal,2019,11(4),787-789.
65. Madhusudhanan, N., Lakshmi, T., Gowtham, K.S., Ramakrishanan, N., Venu Gopala Rao, K., Roy, A., Geetha, R.V., Invitro antioxidant and free radical scavenging activity of aqueous and ethanolic flower extract of Nymphaea alba, International Journal of Drug Development and Research,2011,3(3),252-258.
66. Gayathri, K., Roy, A., Lakshmi, T., Rajeshkumar, S., Controlling of oral pathogens using ginger oleoresin mediated silver nanoparticles, International Journal of Research in Pharmaceutical Sciences, 2019,10(4), 2988-2991.
67. Roy, A., Geetha, R.V., Lakshmi, T., Averrhoa bilimbi Linn-Nature's Drug store-A pharmacological review, International Journal of Drug Development and Research,2011,3(3), 101-106.
68. Preety, R., Anitha, R., Rajeshkumar, S., Lakshmi, T., Anti-diabetic activity of silver nanoparticles prepared from cumin oil using alpha amylase inhibitory assay, International Journal of Research in Pharmaceutical Sciences,2020,11(12), 1267-1269.