Section: Research Paper

BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING TENDER COCONUT WATER AND EVALUATION OF ITS TOXICITY

Pradeep Veerappan , Sandhya Sundar, Rajeshkumar Shanmugam, Ramya Ramadoss, Suganya Paneerselvam, Pratibha Ramani

Authors:

Pradeep Veerappan

Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai - 600077

Sandhya Sundar,

Senior lecturer, Department of Oral pthology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077

Tamil Nadu, India

Rajesh kumar Shanmugam,

Professor Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077

Tamil Nadu, India

Ramya Ramadoss,

Professor, Department of Oral pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077

Tamil Nadu, India

Suganya Paneerselvem,

Senior lecturer, Department of Oral pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077

Tamil Nadu, India

Dr. Pratibha Ramani,

Professor and Head, Department of Oral pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077 Tamil Nadu, India

Corresponding Author:

Section: Research Paper

Dr. S.Sandhya,
Senior lecturer, Department of Oral pathology, Saveetha Dental College and Hospitals,
No. 162, Poonamallee High Road, Velappanchavadi, Chennai - 600077

ABSTRACT:

BACKGROUND : Selenium nanoparticles (SeNPs) have the capacity to be used for various purposes . Therefore, they have attracted more attention in recent years and several synthesis methods have been exploited. Green synthesis or biological synthesis using plant extracts or bacterial synthesis has gained popularity .

AIM: To prepare and assess the toxicity of the selenium nanoparticles prepared using Cocos nucifera (tender coconut).

MATERIALS AND METHODS: Water from Cocos nucifera was obtained and used in the green synthesis of selenium nanoparticles from sodium selenite and ascorbate. Brine shrimp lethality assay and Zebrafish toxicology were performed to evaluate the toxicity of the prepared material.

RESULTS: Selenium nanoparticles synthesised from Cocos nucifera said to possess cytotoxic effect against the cancerous cell . Cytotoxicity of selenium nanoparticles synthesised from Cocos nucifera depends on the level of concentration through that therapeutic effect can be studied .

CONCLUSION : Cytotoxicity of selenium nanoparticles synthesised from Cocos nucifera depends on the level of concentration through which therapeutic effect can be achieved .

KEYWORDS :

Cytotoxicity , biogenic compounds , therapeutic effect , selenium nanoparticles , cancerous cells .

INTRODUCTION:

Nanoparticle is an ultra fine particle which is normally defined as a matter which is between 1 to 100 nanometres in diameter . In recent years , these nanoparticles have become one of the emerging resources in modern day medicine . The nanoparticles can very accurately locate and transfer drugs to the target cells . Examples of certain nanoparticle drugs include anti cancer drugs like cisplatin , doxorubin , 5 - fluorouracil and dexamethasone . (1)

Selenium (Se) is more often used as a dietary supplement because of its effect on immunity and cancer . Se compounds are said to be known for its detoxification properties (2) . Selenium plays a vital role in several important metabolic pathways, such as thyroid hormone

metabolism and immune functions. It also prevents cellular damage caused by free radicals by incorporation into antioxidant enzymes. Se deficiency is concerned with a range of serious complications such as cancer, cardiovascular and inflammatory diseases . (3) However, long-term Se supplementation or higher concentrations of selenium could cause toxicity. When used as nanoparticles, the concentration of selenium can be greatly reduced. (4)

Selenium nanoparticles (SeNPs) have gained popularity recently. SeNPs are different from other nanoparticles in terms of their inorganic and organic characteristics. These characteristics, along with shape and size, depend on a number of factors, including as the synthesis method, the use of surfactants or additives, reaction temperature, and reaction duration. They can also have polymers and surfactants applied to their surface to coat or cover it. In compared to selenium's inorganic and organic forms, SeNPs' reported toxicity was reduced.(5)

Biological synthesis of selenium nanoparticles have gained a huge importance in recent years as they are a cost effective and natural method of synthesis. (6) The essential components of the SeNPs biosynthesis or green synthesis process are reducing and stabilising agents. Polysaccharides, phenolic chemicals, flavonoids, tannins, saponins, amino acids, enzymes, proteins, and sugars are just a few examples of the biomolecules found in plant extracts that are recognised to have medical value and be potential selenium lowering agents. Because nanoparticles have a tendency to aggregate, a stabiliser is frequently employed to prevent their excessive growth by coating the surface with a thin layer of a polymer or surfactant, which lessens interactions between the nanoparticles.(7)

While less rigorous toxicological assessment is possible for in vitro applications of nanoparticles, in vivo applications of nanoparticles necessitate a full understanding of the kinetics and toxicity of the particles. There are more and more published in vitro cytotoxicity studies of nanoparticles employing various cell lines, incubation durations, and colorimetric tests. However, given the wide range of nanoparticle concentrations and exposure times used in these experiments, it is difficult to determine if the observed cytotoxicity is physiologically meaningful. (8)

Cell-culture investigations are frequently the initial step in determining how an agent will respond when absorbed into the body. Comparatively speaking, cellular testing is less morally dubious, more manageable and reproducible, and less expensive than animal experimentation. Understanding that cell cultures are sensitive to changes in their environment, including variations in temperature, pH, and the amounts of nutrients and waste, in addition to the concentration of the potentially harmful chemical being studied, is crucial when discussing cytotoxicity. (9) Controlling the experimental setup is essential to verify that the measured cell mortality reflects the additional nanoparticles' toxicity rather than the unstable culturing circumstances. Furthermore, because nanoparticles may bind to dyes and are redox active.(10)

Coconut liquid (coconut liquid endosperm) has excellent nutritional content, and numerous possible medicinal properties, cocos nucifera is consumed worldwide. (11)The only remaining species of the genus Cocos is Cocos nucifera, which belongs to the family Arecaceae. It is more like a Drupe than a fruit. The endosperm of coconuts is suspended in coconut water. Numerous health issues, such as dehydration, digestive issues, exhaustion, diarrhoea, kidney stones, and constipation were thought to be helped by coconut water. Additionally, it is utilised as a growth-promoting supplement in the plant tissue culture sector. Phytohormones, especially cytokinins, are among the most intriguing substances found in coconut water. xic impact and selenium's toxicity in developing organisms.(12)

Previous research had revealed some notable anti-aging, anti-carcinogenic, and anti-thrombotic actions that aided in the numerous health advantages. Among the cytokinin substances found in coconut water are N6-isopentenyladenine, dihydrozeatin, trans-zeatin, kinetin, ortho-topolin, dihydrozeatin O-glucoside, trans-zeatin O-glucoside, trans-zeatin riboside, kinetin riboside, and trans-zeatin riboside-5'-monophosphate.(13)

Our team has extensive knowledge and research experience that has translate into high quality publications (Chellapa et al. 2020; Kumar et al. 2020; Ramesh Kumar et al. 2011; Ganapathy et al. 2022; Anita et al. 2020; PradeepKumar et al. 2021; Barabadi et al. 2021; Mathivadani et al. 2020; Subramaniam and Muthukrishnan 2019; Felicita 2017)

The current study aims to evaluate the cytotoxic effect and embryonic toxicology of selenium nanoparticles synthesised from *Cocos nucifera* . *(14)*

MATERIALS AND METHODS :

A. COCONUT WATER SAMPLE PREPARATION:

Two *Cocos nucifera* was bought from nearby local retailer . It was cut open and bifurcated , all of its water were collected and 200 ml of it is taken in cylindrical measuring tube , later it was filtered and collected in a conical flask . Filtration is done with the help of whatman filter paper .

B. SYNTHESIS OF SELENIUM NANOPARTICLES:

Sodium selenite is reduced with ascorbic acid to produce selenium nanoparticles. The reducing properties of ascorbate acid are used to produce the selenium nanoparticles. Ascorbic acid ($C_6H_8O_6$) and sodium selenite (Na_2SeO_3). Ascorbic acid was used to reduce sodium selenite, and polysorbate 20 was used to stabilise the reaction. 90 mL of Milli-Q water was mixed with 30 mg of $Na_2SeO_3.5H_2O$. To the sodium selenite solution, ascorbic acid (10 mL, 56.7 mM) was added dropwise while being vigorously stirred. Each 2 ml of ascorbic acid was followed by 10 L of polysorbate. Ascorbic acid was then added, resulting in the formation of selenium nanoparticles. The reactant solution changes from clear white to clear crimson to demonstrate this.All solutions were prepared in a sterile setting with double-distilled water in a sterile cabinet. The fluid was then centrifuged at 12000 rpm to separate out the selenium nanoparticles. Before being used in bacteria investigations, the pellet was resuspended in sterile double distilled water. Using inductively coupled plasma optical emission spectroscopy, the selenium content of nanoparticles was measured.

C. BRINE SHRIMP LETHALITY ASSAY:

The brine shrimp lethality assay (BSLA) is a straightforward and low-cost bioassay that is used to evaluate the potency of phytochemicals found in plant extracts. The results of the current investigation showed that the extract's concentration directly correlated with the degree of lethality. Brine shrimps were picked because they are readily available year-round and are simple to hatch from dry cysts. An essential technique for the preliminary cytotoxicity testing of plant extracts and other substances based on their capacity to kill lab-cultured larvae is the brine shrimp lethality assay (nauplii). For 24 hours, the nauplii were exposed to various amounts of

plant extract. The efficacy of the extract was determined by counting the number of motile nauplii. It is straightforward, economical, and just minimal testing is necessary**.**

The brine shrimp assay provides a number of benefits, including low prices, ease of handling, sensitivity, repeatability, and lack of ongoing culturing. A straightforward, high throughput cytotoxicity test for bioactive compounds is the brine shrimp lethality bioassay. It is based on test chemicals' ability to kill brine shrimp (Artemia salina), a straightforward zoological creature. Michael et al. made the initial suggestion for this assay, and numerous other groups later developed it.

Salt water preparation: 200 ml of distilled water was used to dissolve 2g of iodine-free salt. 10–12 ml of saline water were added to 6 well ELISA plates. That was followed by the progressive addition of 10 nauplii (5, 10, 20, 40, 80, and control) to each well. The nanoparticles were then put in the appropriate amounts based on concentration. The plates underwent a 24-hour incubation period. After 24 hours, the ELISA plates were examined, noted for the quantity of live nauplii present, and the formula was used to compute their presence.

FORMULA : number of dead nauplii/number of dead nauplii+number of live nauplii×100

RESULT & DISCUSSION :

Cytotoxic analysis of the samples in present work showed direct dose concentration response relationship in which cell viability decreased at higher concentration . It is known that pharmacological effect and toxicity are highly dependent on concentration (1).Through the graph attached above we are able to infer that the increase in concentration will decrease the viability and hatching rate. Examination of toxicity is a crucial concern for any kind of nanoparticle based drug . It may be anti cancer drugs, anti inflammatory drugs, antifungal drugs . So the development of selenium based nanoparticle anti cancer drug is dependent on toxicity examination . Generation of ROS (Radical Oxygen Species) was found to be associated with the toxicity of selenium . In general in vivo analysis , selenium nanoparticle showed much lesser toxicity in comparison to organic and inorganic compounds of selenium . Drug interaction is easily evaluated by microtitration methods, which allow multiple varied ratios of interacting

medicines to be investigated at once. The investigation of cytotoxicity frequently involves the study of drug interactions. (15) An isobologram can be used to evaluate the data and do drug interaction analysis.Selenium nanoparticles demonstrated decreased toxicity, increased bioavailability and stronger activity in comparison to organic and inorganic seleno compounds . (16)

Selenium nanoparticles at a dose of 0.7 mg selenium / kg suppressed cell proliferation by 99 percent in case of small sized nanoparticles without perceived toxicity . Nano selenium has potential chemopreventive ability with reduced toxicity . So nano selenium can function as a potential chemopreventive agent with reduced risk of selenium toxicity . (17) The selenium nanoparticle synthesis from *Cocos nucifera* at a concentration of 1 microgram / liter exhibits cytotoxicity to the cancerous cells present without affecting or causing any harm to the surrounding cells or tissues . The cytotoxic nature of selenium nanoparticles depends on the concentration. The majority of the in vitro investigations included in this publication evaluate dosimetry just by looking at the dose-response relationship following the external injection of various nanoparticle concentrations. However, Chang et al. found that the number of ingested nanoparticles related to cytotoxicity despite the fact that cells are observed to quickly internalise nanoparticles. (18)

Numerous studies have shown that selenium can be used as a protective agent against various types of cancers . Chemoprevention of prostate cancer can be achieved with nutritional doses of antioxidant vitamins and selenium nanoparticle based drugs . Methyl selenocysteine has been shown to be the most effective seleno compound identified in reduction of tumors . (19)(20–28)

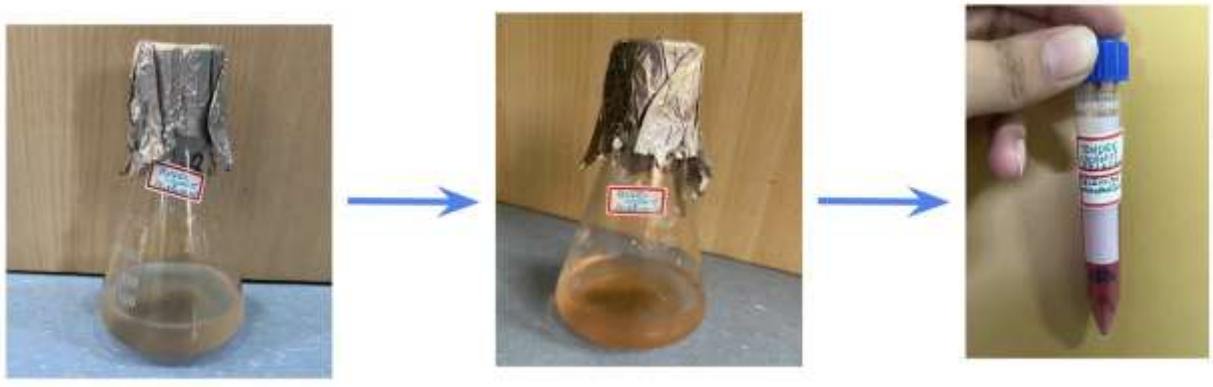

Figure 1 : Preparation of Selenium nanoparticle using *Cocos nucifera* water : 50ml of Cocos nucifera water and sodium selenite + ascorbate mixture were added together in a beaker (a). Following progressive stages of color changes (b), final meron coloured selenium nanoparticles were evidenced (c)which is taken as the final step of synthesis.

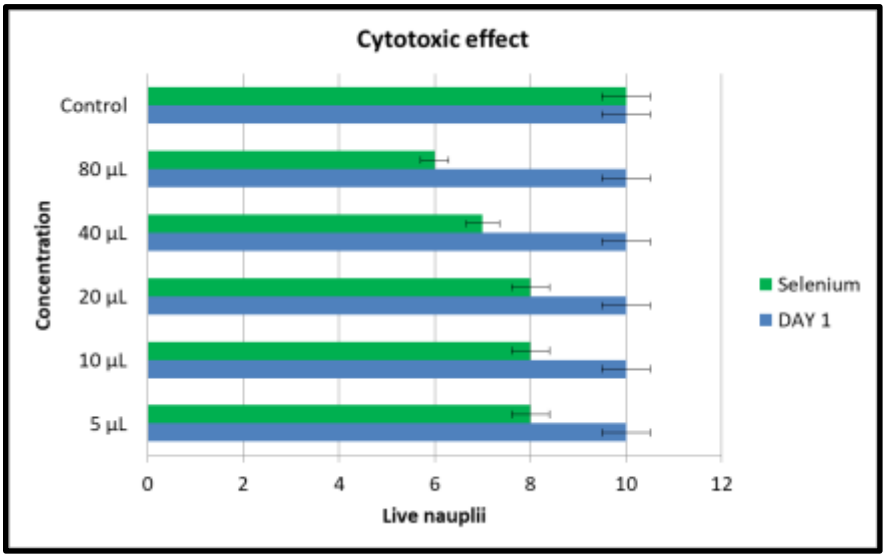

Figure 2 Brine Shrimp Lethality Assay depicts the concentration dependent toxicity of the prepared selenium nanoparticles to the live brine shrimps. The X axis denotes the number of live nauplii cells counted opposed to the different concentrations of the selenium nanoparticle and controls in the y-axis. The maximum cytotoxicity of 30% was shown by the selenium nanoparticles at a concentration of 80µL.

Embryonic toxicology

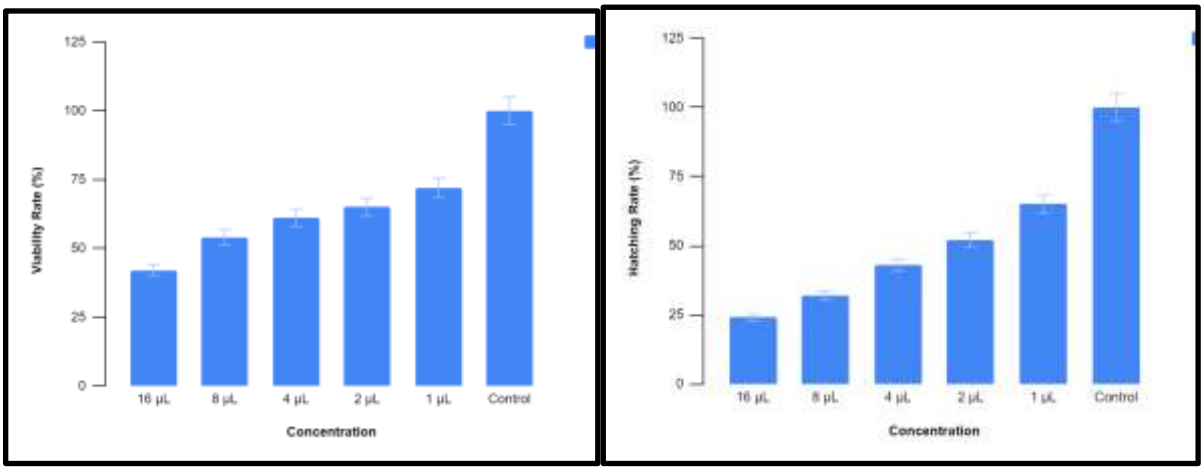

Figure 3 : The figure depicts the concentration dependent toxicity of the prepared selenium nanoparticles to the viability and hatching rate of zebrafish embryos. The X axis denotes the different concentrations of the selenium nanoparticle opposed to the number of live zebrafish embryos(a) and their hatching rate(b) in the y-axis. The maximum cytotoxicity of 30% was shown by the selenium nanoparticles at a concentration of 80µL.

FUTURE SCOPE OF RESEARCH:

Further evaluation of the cytotoxic effect of Se NP on the cell lines and experimental animal models have to be performed.

CONCLUSION:

The selenium nanoparticle synthesis from *Cocos nucifera* in appropriate concentration has a valuable effect. Cytotoxicity of selenium nanoparticles synthesized from Cocos nucifera is concentration dependent. The nanoparticle, at its lowest concentration, is absolutely toxic-free and can be employed for the antimicrobial as well as other biological applications.

AUTHOR CONTRIBUTIONS

Study design, Conceptualisation – Dr.S.Sandhya, Dr. R.Ramya, Dr.Rajeshkumar shanmugam

data verification, manuscript drafting – Pradeep V R, Dr.rajeshkumar shanmugam, Dr.Suganya paneerselvam, Dr.S.Sandhya

Literature search, experimental data collection, analysis, manuscript writing – Pradeep V R, Dr.rajeshkumar shanmugam, Dr. S.Sandhya, Dr. R.Ramya

Manuscript review – Dr.R.Ramya , Dr.S.Sandhya, Dr. Pratibha Ramani

FUNDING CONTRIBUTION:

Self-funded

ACKNOWLEDGMENT

We extend our sincere gratitude to Saveetha dental College and hospitals for their constant support and successful completion of this work.

ETHICAL APPROVAL:

Ethical approval was given by the institutional Standard Review Board.

CONFLICT OF INTEREST:

There is no conflict of interest.

REFERENCES:

1. Kesharwani P, Singh KK. Nanoparticle Therapeutics: Production Technologies, Types of Nanoparticles, and Regulatory Aspects. Academic Press; 2021. 672 p.
2. Vrček IV. Selenium Nanoparticles: Biomedical Applications [Internet]. Molecular and Integrative Toxicology. 2018. p. 393–412. Available from: http://dx.doi.org/10.1007/978-3-319-95390-8_21
3. Role of Cancer C ell M etabolism in Cancer Therapy and Cancer Prevention [Internet]. Cancer Cell Metabolism and Cancer Treatment. 2001. p. 219–58. Available from: http://dx.doi.org/10.1201/9780203091906-14
4. Chung, Chung. Investigating the use of selenium nanoparticles in protecting cells from external stress [Internet]. Available from: http://dx.doi.org/10.17760/d20262528
5. Cremonini E. Antimicrobial and anti-biofilm activities of biologically synthesized selenium nanoparticles [Internet]. Available from: http://dx.doi.org/10.26226/morressier.56d6be7ad462b80296c97d38
6. Jain R. Biogenic Nanoparticles of Elemental Selenium: Synthesis, Characterization and

Relevance in Wastewater Treatment. CRC Press; 2015. 272 p.

7. Rai M, Patel M, Patel R. Nanotechnology in Medicine: Toxicity and Safety. John Wiley & Sons; 2021. 448 p.

8. Soliman MKY, Salem SS, Abu-Elghait M, Azab MS. Biosynthesis of Silver and Gold Nanoparticles and Their Efficacy Towards Antibacterial, Antibiofilm, Cytotoxicity, and Antioxidant Activities. Appl Biochem Biotechnol [Internet]. 2022 Nov 7; Available from: http://dx.doi.org/10.1007/s12010-022-04199-7

9. Production from cell culture [Internet]. Animal Cell Culture and Technology. Available from: http://dx.doi.org/10.4324/9780203427835_chapter_11

10. Oancea F. Bioaccumulation Potential of Selenium Nanoparticles [Internet]. Priochem 2021. 2022. Available from: http://dx.doi.org/10.3390/chemproc2022007029

11. Rethinam P, Krishnakumar V. Tender Coconut Varieties [Internet]. Coconut Water. 2022. p. 37–76. Available from: http://dx.doi.org/10.1007/978-3-031-10713-9_3

12. Prathapan A, Rajamohan T. ANTIOXIDANT AND ANTITHROMBOTIC ACTIVITY OF TENDER COCONUT WATER IN EXPERIMENTAL MYOCARDIAL INFARCTION [Internet]. Vol. 35, Journal of Food Biochemistry. 2011. p. 1501–7. Available from: http://dx.doi.org/10.1111/j.1745-4514.2010.00471.x

13. Spiess LD. Comparative Activity of Isomers of Zeatin and Ribosyl-Zeatin on *Funaria hygrometrica* [Internet]. Vol. 55, Plant Physiology. 1975. p. 583–5. Available from: http://dx.doi.org/10.1104/pp.55.3.583

14. Alva S. Analysis of Potassium Ion (K), Sodium Ion (Na), and Proteins from Coconut Water Variety of Coconut and Hybrid Coconut [Internet]. Vol. 3, Journal of Chemical Natural Resources. 2022. p. 78–84. Available from: http://dx.doi.org/10.32734/jcnar.v3i1.9340

15. SITKOVSKY, HENKART. Cytotoxic Cells: Recognition, Effector Function, Generation, and Methods. Springer Science & Business Media; 2012. 528 p.

16. Angeli A, Velluzzi A, Selleri S, Capasso C, Spadini C, Iannarelli M, et al. Seleno Containing Compounds as Potent and Selective Antifungal Agents. ACS Infect Dis. 2022 Sep 9;8(9):1905–19.

17. Ecobichon DJ. The Basis of Toxicity Testing. CRC Press; 1997. 240 p.

18. Srivastava S, Bhargava A. Green Nanoparticles: The Future of Nanobiotechnology. Springer; 2021. 352 p.

19. Morikawa T. Chemopreventive Activities of Phytochemicals. MDPI; 2020. 210 p.

20. Felicita AS. Quantification of intrusive/retraction force and moment generated during en-masse retraction of maxillary anterior teeth using mini-implants: A conceptual approach. Dental Press J Orthod. 2017 Sep-Oct;22(5):47–55.

21. Barabadi H, Saravanan M, Mostafavi E, Vahidi H. Bioengineered Nanomaterials for Wound Healing and Infection Control. Woodhead Publishing; 2023.

22. Kumar BS, Krishnamoorthy S, Shanmugam S, PradeepKumar AR. The time taken for retrieval of separated instrument and the change in root canal volume after two different techniques using CBCT: An study. Indian J Dent Res. 2021 Oct-Dec;32(4):489–94.

23. Mihai IF, Anita D, Dorneanu OS, Luca CM, Manciuc CD, Budacu CC, et al. Seroprevalence of Anti-Hepatitis E Virus Antibodies among Patients from a Tertiary Hospital from Northeast Romania. Medicina [Internet]. 2022 Jul 29;58(8). Available from: http://dx.doi.org/10.3390/medicina58081020

24. Shenoy A, Ahmed N, Rajaraman V, Maiti S, Ganapathy DM. Comparative analysis of weld strength of nickel-chromium and cobalt-chromium base metal alloys when submitted to tungsten inert gas welding. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S442–6.

25. Ramesh Kumar KR, Shanta Sundari KK, Venkatesan A, Chandrasekar S. Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study. Am J Orthod Dentofacial Orthop. 2011 Oct;140(4):479–85.

26. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach [Internet]. Vol. 64, Acta virologica. 2020. p. 93–9. Available from: http://dx.doi.org/10.4149/av_2020_111

27. Muthukrishnan S, Palanisamy S, Subramanian S, Selvaraj S, Mari KR, Kuppulingam R. Phytochemical Profile of Erythrina variegata by Using High-Performance Liquid Chromatography and Gas Chromatography-Mass Spectroscopy Analyses. J Acupunct Meridian Stud. 2016 Aug;9(4):207–12.

28. Muthukrishnan K, Mohanraj E, Pankaj P, Subramanian J. A COMPREHENSIVE STUDY ON TRACHEOSTOMY IN A RURAL TERTIARY CARE CENTRE [Internet]. Vol. 5, Journal of Evidence Based Medicine and Healthcare. 2018. p. 2669–74. Available from: http://dx.doi.org/10.18410/jebmh/2018/548