Evaluation of Antioxidant activity of *Camellia sinensis* and *Vachellia nilotica* formulation

Gajapriya M[1] , Anju Cecil[2] *, Rajeshkumar S[3]

Gajapriya M
Saveetha Dental College and hospitals,
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University,
Chennai-600077,
Tamil Nadu, India.
Email.id:151801099.sdc@saveetha.com

Anju Cecil,
Senior lecturer,
Department of Periodontics,
Saveetha Dental College and hospitals
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University,
Chennai-600077,
Tamil Nadu, India.
Email id: anjuc.sdc@saveetha.com

Rajeshkumar S
Professor,
Department of Pharmacology,
Saveetha Dental College and hospitals,
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University,
Chennai-600077,
Tamil Nadu, India.
Email: rajeshkumars.sdc@saveetha.com

Corresponding Author:
Anju Cecil,
Senior Lecturer,
Department of Periodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences [SIMATS],
Saveetha University,
Chennai - 600077. Tamil Nadu, India.
E-mail Id: anjuc.sdc@saveetha.com

ABSTRACT :

Aim: To evaluate the antioxidant property of *Camellia sinensis* (Green Tea) and *Vachellia nilotica* (Babul) herbal formulation.

Background: In ancient times, green tea and barks of Babul tree were considered by traditional Chinese medicine. In th recent year studies for human suggest that babul the plant which is used in our study might contribute to a reduction in the risk of cardiovascular disease, cancer, hypertension , for controlling body weight, also has antibacterial and antiviral activity, and also has bone mineral density increasing property and anti-fibrotic properties.

Materials and Methods: Green tea and babul plants extract was produced with 1 gram dried green tea leaves and 1 gram Babul leaf powder dissolved in 100ml distilled water. For 10 mins the prepared extract was boiled , at a temperature of 60-80°C on a heating mantle. Whatman No.1 filter paper was used for filtering the prepared extract. Followed by the green tea and babul herbal formulation was tested to DPPH assay and H2O2 assay to identify the antioxidant activity.

Results: The Green tea and Babul herbal formulation has moderate antioxidant activity at high concentrations (50 µL). However, two standards of both assays have comparatively higher antioxidant activity compared to Green tea and Babul herbal formulation.

Conclusion: This study was concluded that the Green tea and Babul herbal formulations have a moderately efficient antioxidant activity at high concentrations.

Keywords : Antioxidants, Babul, Herbal formulation, Green tea.

INTRODUCTION:

In ancient times, camellia sinensis (green tea) was considered by traditional Chinese medicine (1). In th recent year studies for human suggest that babul the plant which is used in our study might contribute to a reduction in the risk of cardiovascular disease, cancer, hypertension(2), for controlling body weight (3)also has antibacterial and antiviral activity, and also has bone mineral density increasing property (4) and anti-fibrotic properties. Babul is an important multipurpose plant, which is widely distributed throughout specific places of India(5). The Treating potential and the whole plant part of Babul used as a medication (6). In the Indian medicine practices and in other traditional medicinal practices like siddha, Unami, both of these plants play an immense role in curing and treating different types of infections and diseases(7). The Babul plant is widely recommended as medication for the treatment of various diseases of human being and disorders like in skin, stomach and tooth problems etc in human beings as well as in animals(8).

The antioxidants play a major role in food preservation in packed food available commercially by avoiding oxidation processes occurring in all foods and having a main role in health promotion by many nutraceuticals, food supplements and functional food ingredients available in markets(9–11). The extract of Green tea has too much of polyphenolic components which is known for its antioxidant properties and the

flavanol monomers is the most active components in green tea (12)which is called as epigallocatechin-3-gallate. The epicatechin-3-gallate are more effective antioxidant components of green tea(13). Catechins are chemical antioxidants present in the green tea which can eradicates the free radical species and chelate transition metals in the body(14). There are so many articles which provide evidence that induction of oxidative stress caused by the effects of catechins in green tea(15).

Babul plant is known for its medicinal values which is known for its phenolics, which contains of tannin and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, catechin, epi- gallocatechin-7-gallate and epigallocatechin-5, 7-digallate(16). Babul plant has a huge value of medicinal values with high antioxidant properties(17). In a study the extract of Babul has proven the antioxidant activity of green tea and babul tested in the assay of lipid peroxidation which has the peroxyl radical scavenging capacity(18). Hydroxyl groups which are in the Babul plant present in the phenolic compounds which possess increased scavenging properties that can scavenge the free radicals too(19). The need to evaluate the Babul plant extract functional components for antioxidant activity(20). However the efficacy of gallotannins which is present in the Babul plant extract has antiplasmodial agents so more investigation is required in future studies(21). Aim of our study is to determine the antioxidant properties of the formulation prepared with camellia sinensis(green tea) and Vachellia nilotica (Babul). Till date no data is available exploring the combined antioxidant property of 2 well known established plants. Our college research team has extensive knowledge and research experience that has translate into high quality publications (22–37)

MATERIAL AND METHODS :

HERBAL FORMULATION PREPARATION:

We commercially obtained Green tea and Babul extract. In a 100 mL of distilled water 1 milligrams of dried Green tea tea and 1 milligrams of Babul leaf powder was dispersed and boiled for 10 minutes with a temperature range between 60-80°C. The Whatman No.1 filter paper was used to filter the prepared extract. Then again reheating of the filtered solution was done in a heating mantle for exactly 20 to 30 minutes to condense the 100ml extract into a 5ml extract of Green tea and Babul herbal solution.

Figure 1: Herbal formulation preparation.

Figure 2: Herbal formulation preparation.

DPPH RADICAL ASSAY:

The free radical scavenging activity of Green tea and Babul herbal formulation was evaluated by DPPH Assay and 5 Different concentrations (from 10 to 50 µg/ml) of the extract was taken and are mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris- HCl buffer (pH 7.4) and then it was incubated for 30 minutes exactly. After incubation, the lowering in the number of DPPH free radicals was determined based on the absorbance at 517 nm and here the BHT was used as control. The % of inhibition was evaluated from the following equation:

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

H2O2 SCAVENGING CAPACITY ASSAY:

Under physiological conditions as invivo in the lab Hydrogen peroxide (H2O2) is obtained by peroxisomes and also by several oxidative enzymes and by dismutation of superoxide radical and catalyzed by superoxide dismutase. The reduction product OH• results from the increased evidence that H2O2, in which OH• acts as a messenger molecule in the development and induction of inflammatory mediators in human body. One of the commonest methods for evaluating the antioxidant activity against this molecule is based on the intrinsic absorption of H2O2 in the UV region . Antioxidant activity is directly correlated to hydroxyl radical scavenging capacity of our extract. The method involves in-vitro Fenton reaction was used for production of hydroxyl radicals using Fe3+/ascorbate/EDTA/H2O2 system. Scavenging of this hydroxyl radical in presence of antioxidants is measured. In one of the methods the formaldehyde was yielded by hydroxyl radicals which is synthesized by the oxidation which are allowed to react with dimethyl sulphoxide. And the formaldehyde which is formed develops intense yellow color with Nash reagent (2 M ammonium acetate with 0.05 M acetic acid and 0.02 M acetyl acetone in distilled water). The intensity of yellow color formed which is measured at 412 nm spectrophotometrically acted against reagent blank. The activity is expressed as % of hydroxyl radical scavenging. The % of inhibition was evaluated from the following same as H2O2 Assay equation:

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

RESULT:

The antioxidant activity of Green tea and Babul herbal formulation was evaluated using DPPH assay. Free radicals scavenging property is the most important role of antioxidants. Descriptive statistics was used. The [Fig.3] the graph denotes Green tea and Babul herbal formulation **antioxidant activity**. The results show that the Green tea and Babul herbal formulation has moderately efficient activity at its high concentrations of (50 µL). Using an alternative Assay called H2O2 Assay also demonstrated the same antioxidants activity. Here the descriptive statistics was used. The [Fig.4] the graph denotes Green tea and Babul herbal formulation antioxidant activity. The results show that the moderately efficient activity at its high concentrations of (50 µL) and which is clearly the same as in DPPH Assay.

Table 1 denotes the Green tea and Babul herbal formulation the antioxidant activity in DPPH Assay. At 10µL Standard value is 76.56 and GT-Bab value is 65.85, at 20µL standard value is 78.52 and GT-Bab value is 66.58, at 30µL standard value is 85.63 and GT-Bab value is 73.25, at 40µL standard value is 88.68 and GT-Bab value is 80.65, at 50µL standard value is 93.15 and AgNP value is 81.45.

Table 2 denotes the Green tea and Babul herbal formulation the antioxidant activity in H2O2 Assay. At 10µL Standard value is 66.25 and GT-Bab value is 45.65, at 20µL standard value is 73.52 and GT-Bab value is 55.62, at 30µL standard value is 80.65 and GT-Bab value is 73.25, at 40µL standard value is 87.95 and GT-Bab value is 78.65, at 50µL standard value is 90.52 and AgNP value is 83.95.

Table 1. Shows the Green tea and Babul herbal formulation antioxidant activity in DPPH Assay

Concentration(µL)	Standard	GT-Bab
10µL	72.56	65.85
20µL	78.52	66.58
30µL	85.63	73.25
40µL	88.68	80.65
50µL	93.15	81.45

Fig.3. Shows the green tea and Babul herbal formulation antioxidant activity in DPPH assay. The x axis shows the concentration (µL) while the Y axis is shows of the percentage of inhibition. Blue colour depicts the standard and green colour depicts the green tea and Babul

herbal formulation. The green tea and Babul herbal formulation has moderately efficient antioxidant activity at its high concentrations (50 µL) in DPPH Assay.

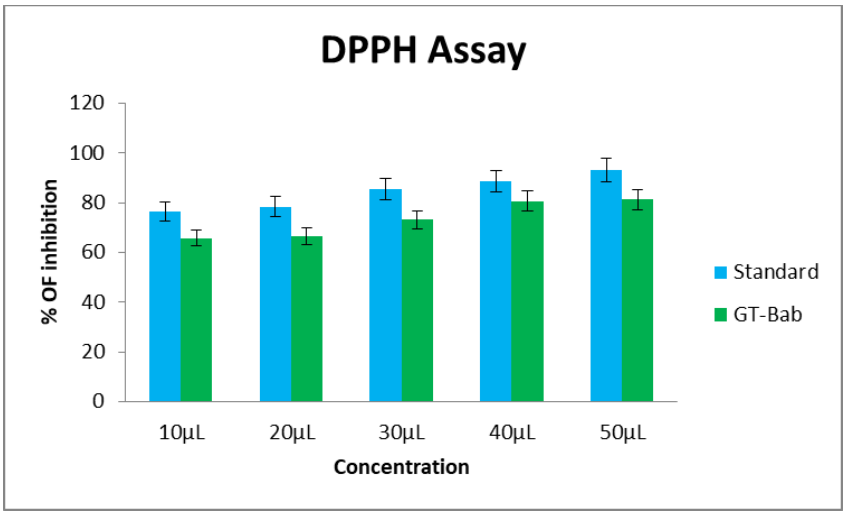
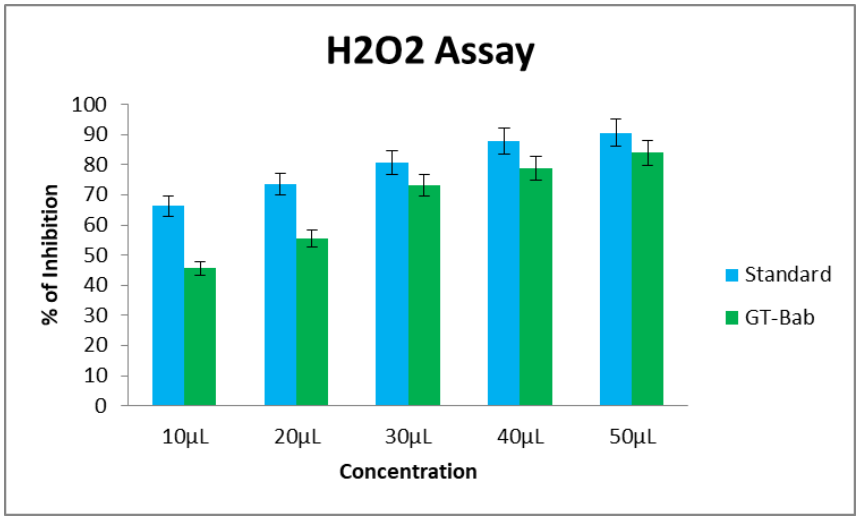


Table 2. Shows the Green tea and Babul herbal formulation antioxidant activity in H2O2 Assay

Concentration(µL)	Standard	GT-Bab
10µL	66.25	45.65
20µL	73.52	55.62
30µL	80.65	73.25
40µL	87.95	78.65
50µL	90.52	83.95

Fig.4 . Shows the green tea and Babul herbal formulation antioxidant activity in H2O2 assay. The x axis shows the concentration (µL) while the Y axis shows the percentage of inhibition. Blue color depicts the standard and green color depicts the green tea and Babul herbal formulation. The green tea and Babul herbal formulation has considerably efficient antioxidant activity at high concentrations (50 µL) in H2O2 Assay.



DISCUSSION:

In this study the green tea and Babul extract shows effective antioxidant activity in DPPH assay at 50µL is 81.45% when compared to standard is 93.15 and in H2O2 Assay at 50µL is 83.95% when compared to standard is 90.52. In another study(38) they evaluated the green tea antioxidant activity which has been possiblely increased in both DPPH and ABTS studies, although its amount of phenolic compounds has been less. So the study indicates that the quality of phenolic compounds does not always correlate directly with its antioxidant activity.

In another study(39), the protective properties of green tea leaf extract develops the primary and secondary oxidation products which act against the fats in biscuits and it is concluded that the source of active antioxidants is green tea leaf extract .

In another study(40) , the antioxidant activity of extract of Babul bark was evaluated and the results of the study showed that the Babul has considerable antioxidant activity. The study was carried out mainly based on the chemical composition of the plant extracts in *invivo* assays are essential in assessing the plants as biological antioxidants.

CONCLUSION:

From the present study, we concluded that a considerably moderate antioxidant activity of the Green tea and Babul herbal formulations is at higher concentrations. Our herbal formulation can be used in the future for investigating and employing them as less biotoxic alternatives medication and biomaterials in dentistry to all other already existing chemical biomaterials which is been used in the field causing side effects.

ACKNOWLEDGEMENT:

The authors are thankful to saveetha dental college for providing a platform to conduct the study.

CONFLICT OF INTEREST :

The authors declared that there is no conflict of interest.

FUNDING:

The present project is supported by

- Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai
- Little Holy Angels Hr Sec School, Chennai.

Reference:

1. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—a review. J Am Coll Nutr [Internet]. 2006; Available from: https://www.tandfonline.com/doi/abs/10.1080/07315724.2006.10719518

2. Bogdanski P, Suliburska J, Szulinska M, Stepien M. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese …. Nutr Res [Internet]. 2012; Available from: https://www.sciencedirect.com/science/article/pii/S0271531712001066

3. Kumar A, Kumar A, Thakur P, Patil S. Antibacterial activity of green tea (Camellia sinensis) extracts against various bacteria isolated from environmental sources. Recent Research in [Internet]. 2012; Available from: https://www.researchgate.net/profile/Sandip-Patil-13/publication/282151785_Antibacterial_activity_of_green_tea_Camellia_sinensis_extracts_against_various_bacteria_isolated_from_environmental_sources/links/5cbd6052299bf120977668bf/Antibacterial-activity-of-green-tea-Camellia-sinensis-extracts-against-various-bacteria-isolated-from-environmental-sources.pdf

4. Xu J, Xu Z, Zheng W. A Review of the Antiviral Role of Green Tea Catechins. Molecules [Internet]. 2017 Aug 12;22(8). Available from: http://dx.doi.org/10.3390/molecules22081337

5. Shen CL, Wang P, Guerrieri J, Yeh JK, Wang JS. Protective effect of green tea polyphenols on bone loss in middle-aged female rats. Osteoporos Int. 2008 Jul;19(7):979–90.

6. Tripathi S, Varma S, Goldey P. Using plants for health: Indigenous knowledge in health care in a tribal region of Bihar, India. Int J Sustainable Dev World Ecol. 2000 Dec 1;7(4):321–32.

7. Kumari M, Jain S. Development of tannin containing nutraceutical additive from Babul (Acacia nilotica) and its acceptability in food products for management of Diabetes. Med plants. 2013;5(3):150.

8. Janani M, Ahamed MIN. A review on traditional uses, phytochemistry and pharmacological activities of Acacia nilotica (Indian babul). International Journal of Biomedical Nanoscience and Nanotechnology. 2020 Jan 1;4(3-4):148–74.

9. Gadilohar, Girase, Suryawanshi. A Descriptive Study of Acacia nilotica

Linn. of Pharmaceutical … [Internet]. 2018; Available from: https://asianjpr.com/HTML_Papers/Asian%20Journal%20of%20Pharmaceutical%20Research__PID__2018-8-3-10.html

10. Nagalingam M, Rajeshkumar S, Balu SK, Tharani M, Arunachalam K. Anticancer and Antioxidant Activity of Morinda Citrifolia Leaf Mediated Selenium Nanoparticles. J Nanomater [Internet]. 2022;2022. Available from: https://www.hindawi.com/journals/jnm/2022/2155772/

11. Chaithanya MV, Maheswari TNU, Rajeshkumar S. Anti-inflammatory and antioxidant activity of lycopene, raspberry, green tea herbal formulation mediated silver nanoparticle. Journal of Indian Academy of Oral Medicine and Radiology. 2021 Oct 1;33(4):397.

12. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods. 2015 Oct 1;18:757–81.

13. Senanayake. Green tea extract: Chemistry, antioxidant properties and food applications–A review. J Funct Foods [Internet]. 2013; Available from: https://www.sciencedirect.com/science/article/pii/S1756464613001953

14. Sung H, Nah J, Chun S, Park H, Yang SE, Min WK. In vivo antioxidant effect of green tea. Eur J Clin Nutr. 2000 Jul;54(7):527–9.

15. Lambert JD, Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys. 2010 Sep 1;501(1):65–72.

16. Lee, Lee, Lee. Antioxidant Activity of Black Tea vs. Green Tea. 2001;

17. Atif, Naveed, Barkat. Acacia nilotica: A plant of multipurpose medicinal uses. J Med Arom Plant Sci [Internet]. 2012; Available from: https://academicjournals.org/journal/JMPR/article-full-text-pdf/4EC523629622

18. Farzana, Al Tharique. A review of ethnomedicine, phytochemical and pharmacological activities of Acacia nilotica (Linn) willd. J Pharmacogn Nat Prod [Internet]. 2014; Available from: https://www.phytojournal.com/archives/2014.v3.i1.315/a-review-of-ethnomedicine-phytochemical-and-pharmacological-activities-of-acacia-nilotica-linn-willd

19. Kalaivani T, Mathew L. Free radical scavenging activity from leaves of Acacia nilotica (L.) Wild. ex Delile, an Indian medicinal tree. Food Chem Toxicol. 2010 Jan;48(1):298–305.

20. Kaur K, Michael H, Arora S, Härkönen P, Kumar S. In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from Acacia nilotica (L.) Willd. ex Del. J Ethnopharmacol. 2005 Jul 14;99(3):353–60.

21. Rajvaidhya S, Nagori BP, Singh GK, Dubey BK. A review on Acacia Arabica-an Indian medicinal plant. International

Journal of [Internet]. 2012; Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.437.4093&rep=rep1&type=pdf

22. Ganapathy D, Shivalingam C, Shanmugam R, Sundramoorthy AK, Murthykumar K, Pitchiah S, et al. Recent Breakthrough of Bismuth-Based Nanostructured Materials for Multimodal Theranostic Applications. J Nanomater [Internet]. 2022 Jul 4 [cited 2022 Oct 13];2022. Available from: https://www.hindawi.com/journals/jnm/2022/4944320/

23. Viswanathan S, Palaniyandi T, Kannaki P, Shanmugam R, Baskar G, Rahaman AM, et al. Biogenic synthesis of gold nanoparticles using red seaweed Champia parvula and its anti-oxidant and anticarcinogenic activity on lung cancer. Part Sci Technol. 2022 May 19;1–9.

24. Perumalsamy, Shanmugam, Kim. Nanoemulsion and Encapsulation Strategy of Hydrophobic Oregano Essential Oil Increased Human Prostate Cancer Cell Death via Apoptosis by Attenuating …. Bioinorg Chem [Internet]. 2022; Available from: https://www.hindawi.com/journals/bca/2022/9569226/

25. Ganapathy D, Shanmugam R, Pitchiah S, Murugan P, Chinnathambi A, Alharbi SA, et al. Potential Applications of Halloysite Nanotubes as Drug Carriers: A Review. J Nanomater [Internet]. 2022 Apr 20 [cited 2022 Oct 13];2022. Available from: https://www.hindawi.com/journals/jnm/2022/1068536/

26. Jayakodi S, Shanmugam R, Almutairi BO, Almutairi MH, Mahboob S, Kavipriya MR, et al. Azadirachta indica-wrapped copper oxide nanoparticles as a novel functional material in cardiomyocyte cells: An ecotoxicity assessment on the embryonic development of Danio rerio. Environ Res. 2022 Sep 1;212:113153.

27. Mi XJ, Choi HS, Perumalsamy H, Shanmugam R, Thangavelu L, Balusamy SR, et al. Biosynthesis and cytotoxic effect of silymarin-functionalized selenium nanoparticles induced autophagy mediated cellular apoptosis via downregulation of PI3K/Akt/mTOR pathway in gastric cancer. Phytomedicine. 2022 Feb 26;99:154014.

28. Nivedha VM, Priyadarshini R. Anti-diabetic and Antioxidant Activity of Pterocarpus santalinus and Stevia Herbal Formulation. Journal of [Internet]. 2021; Available from: http://apacademicpress.com/id/eprint/382/

29. Rajeshkumar S, Santhoshkumar J, Vanaja M, Sivaperumal P, Ponnanikajamideen M, Ali D, et al. Evaluation of Zebrafish Toxicology and Biomedical Potential of Aeromonas hydrophila Mediated Copper Sulfide Nanoparticles. Oxid Med Cell Longev. 2022 Jan 28;2022:7969825.

30. Rajeshkumar S, Subramanian AK. In vitro anti-inflammatory activity of silymarin/hydroxyapatite/chitosan nanocomposites and its cytotoxic effect using brine shrimp lethality assay: nanocomposite for …. J Popul [Internet]. 2021; Available from: https://www.jptcp.com/index.php/jptcp/article/view/874

31. Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. J Popul Ther Clin Pharmacol. 2022 Jan 6;28(2):e29–40.

32. Rajeshkumar, Vanaja, Kalirajan. Degradation of Toxic Dye Using Phytomediated Copper Nanoparticles and Its Free-Radical Scavenging Potential and Antimicrobial Activity against …. Bioinorg Chem Appl [Internet]. 2021; Available from: https://www.hindawi.com/journals/bca/2021/1222908/

33. Viswanathan S, Palaniyandi T. Biomedical potential of silver nanoparticles capped with active ingredients of Hypnea valentiae, red algae species. Part Sci Technol [Internet]. 2022; Available from: https://www.tandfonline.com/doi/abs/10.1080/02726351.2021.1992059

34. Rajeshkumar S, Santhoshkumar J, Jule LT, Ramaswamy K. Phytosynthesis of Titanium Dioxide Nanoparticles Using King of Bitter Andrographis paniculata and Its Embryonic Toxicology Evaluation and Biomedical Potential. Bioinorg Chem Appl. 2021 Oct 7;2021:6267634.

35. Selvaraj A, George AM, Rajeshkumar S. Efficacy of zirconium oxide nanoparticles coated on stainless steel and nickel titanium wires in orthodontic treatment. Bioinformation. 2021 Aug 31;17(8):760–6.

36. Pandiyan I, Prabakar J, Indiran MA, Rathinavelu PK, Doraikannan S, Leelavathi L, et al. Comparing the Antimicrobial efficacy of Dentifrice containing Rosmarinus officinalis and Fluoride containing Dentifrice-An In vitro study. Research Journal of Pharmacy and Technology. 2021;14(7):3651–6.

37. Dhanraj G, Rajeshkumar S. Anticariogenic effect of selenium nanoparticles synthesized using brassica oleracea. J Nanomater [Internet]. 2021; Available from: https://www.hindawi.com/journals/jnm/2021/8115585/

38. Vilma, Kristina, Audrius. The analysis of quality and antioxidant activity of green tea extracts. J Med Arom Plant Sci [Internet]. 2011; Available from: https://academicjournals.org/journal/JMPR/article-full-text-pdf/392DCD719019

39. Mildner-Szkudlarz S. Evaluation of antioxidant activity of green tea extract and its effect on the biscuits lipid fraction oxidative stability. J Foodservice [Internet]. 2009; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1750-3841.2009.01313.x

40. Sini, Sinha, Karpagavalli. Determining the antioxidant activity of certain medicinal plants of Attapady,(Palakkad), India using DPPH assay. Curr bot [Internet]. 2011; Available from: https://www.researchgate.net/profile/Sini-Radhakrishnan/publication/281850076_Determining_the_antioxidant_activity_of_certain_medicinal_plants_of_Attapady_Palakkad_India_using_DPPH_assay/links/55fb3e1108aeafc8ac41b880/Determining-the-antioxidant-activity-of-certain-medicinal-plants-of-Attapady-Palakkad-India-using-DPPH-assay.pdf