EXPLORATION OF ANTIOXIDANT EFFECTS OF CRUDE EXTRACT OF MANGROVE PLANT - *Avicennia marina*

Running Title: Antioxidant activity of ***Avicennia marina***

ABSTRACT:

Introduction: Antioxidants are efficient in the prevention of human diseases. Mangroves are high bioactive compounds with good holistic bioactivities including insecticides. *Avicennia marina,* a mangrove plant which has its origin in South Africa included in the family *Acanthaceae.*

Aim : To explore the antioxidant potential of ethanolic extract of mangrove plants, *Avicennia marina.*

Materials and Method: The fresh leaves of *Avicennia marina* were collected from Pichavaram mangrove forest area. The leaves were washed and then shade dried for 2-3weeks and turned into a fine powder. Crude methanolic extract of *Avicenna marina* was prepared. Total antioxidant activity, DPPH Assay and scavenging activity of hydrogen peroxide (H_2O_2) were performed and antioxidant potential was assessed using ascorbic acid as standard.

Result: The scavenging activity increased with increase in concentration of the extract and thus antioxidant activity was dose dependent. The total antioxidant activity was more close to the ascorbic acid equivalence.

Conclusion: This study concludes that the methanolic extract of *Avicenna marina* is capable of scavenging a wide range of free radicals for which it can be exploited for the treatment of various free radical mediated diseases. It is evident that mangrove species as a collective are rich sources of antioxidants, phenolics and antimicrobial compounds.

Keywords: Antioxidant, mangrove plant, innovative technique, ascorbic acid, free radicals.

INTRODUCTION:

The unique and dynamic environment in mangroves ecosystems owes to its geochemical characteristics and nutrient concentration that is modified by tidal flooding regularly (1). Mangrove is the second most productive marine ecosystem after coral reefs in marine (2). Mangroves are bounded with high bioactive compounds such as steroids, alkaloids, terpenoids, saponins and tannins with good holistic bioactivities including insecticides. Marine natural

products possess rich sources of bioactive secondary metabolites with promising potential for biomedical applications (3). Secondary metabolites primarily perform defense mechanisms on parasites and in extreme environmental conditions. Mangroves were able to survive in environments with high salinity, extreme humidity and pH levels as they make secondary metabolites (4).

Mangroves like *Avicennia marina* may possess some endophytic microbes in their tissues which are capable of producing biological or secondary metabolites. It was known that endophytic microbes isolated from a plant are able to produce secondary metabolites similar to those of the original plants (5). *Avicennia marina* belongs to the family Acanthaceae (6). The plant is commonly known as the gray or white mangrove (Fig. 1). *Avicennia marina* is the most widely distributed mangrove species in the Indo-Western Pacific area (7). *A. marina* is extremely resistant to environmental stress and can grow under environmental conditions of extreme tides, high salinity, high temperature and anaerobic soil (8). The unique adaptability and history of the plant make us engrossed to investigate the opportunities which can be afforded by the plant for the evolution of new medicines and adequate preparation to combat prevailing health problems and drug resistance diseases. *A. marina* plant has been used traditionally in folk medicine. The leaves of the plant have been potentially used as medical treatment for ulcers, abscess and burns (9). It has been traditionally used to reduce arthritic pain (10) and used worldwide for the treatment of smallpox, snake- bites. Fruits of *A. marina* have been used for digestive disorders such as constipation. The leaves and roots are used to treat wounds (11). The plant is a valuable source of many active constituents that have shown important pharmacological activities and has been reported that it is a rich source of various phytochemicals (12). Generally, many studies exploring anti-inflammatory, antidiabetic, antioxidant, anticancer and cytotoxicity, were reported using plant extract as such or after making nanoparticles (13) (14–22). However antioxidants are essential in the prevention of human diseases. Antioxidants possess the ability to reduce the oxidative damage associated with many diseases and disorders leading to cancer, cataracts, atherosclerosis, diabetes, arthritis and aging (23). Antioxidants work to protect lipids from peroxidation by free radicals (24). Oxidants can damage cells by inducing chain reactions such as lipid peroxidation or by oxidizing DNA or proteins (25). Bioactive compounds derived from the plant have been successfully used to reduce lipid oxidation. It is necessary to understand the

ability of natural extracts and preparations to inflect the metabolizing enzymes that can help the health system for proper diagnosis and treatment of patients, thereby can avoid adverse effects associated with it. Our team has extensive knowledge and research experience that has translated into high quality publications (26–30) (31–36).Thus the aim of the study was to explore its antioxidant potential using the ethanolic extract of *Avicennia marina.*

MATERIALS AND METHODS:

Ethical approval: Prior to the initiation of the study, clearance was obtained by the Scientific review board with Ethical approval number [IHEC/SDC/UG-1948/21/92]

Collection of plant material and preparation:

The fresh leaves of *Avicennia marina* were collected from Pichavaram mangrove forest area, Tamil Nadu. The leaves were washed thoroughly with tap water and then shade dried for 2-3weeks and turned into a fine powder. The following process has been carried out in the Marine biomedical and environmental research section of Blue lab at Saveetha Dental College, Chennai, under the guidance of faculties.

Preparation of extraction:

20g of dried powdered mangrove leaf samples were mixed with 100ml of methanol and allowed to stand at ambient temperature for 24 hours. Then the mixture was passed through whatman filter paper and the filtrate was centrifuged at 3000 rpm for 10 minutes and further filtered by a 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis.

Total antioxidant activity:

The 0.3 ml sample was prepared in different concentrations (0.5– 3mg/ml) with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95ºc for 90 minutes in a water bath. Absorbance of all sample

mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

DPPH Assay:

The antioxidant potential of mangrove crude extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml DPPH solution (120µM) in methanol and incubated in darkness at 37°c for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

Percentage of Inhibition (I %) = $(A_{control} - A_{sample}) / A_{control} \times 100$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample. Ascorbic acid was used as positive control.

Scavenging of hydrogen peroxide (H_2O_2):

40mM H_2O_2 was prepared and the concentration was determined spectrophotometrically by measuring the absorption with the extraction coefficient for H_2O_2 of 81 M^{-1} cm^{-1}. Mangrove extract and the standard ascorbic acid (0.5-3 mg/ml) were added to 0.6 ml of 40mM H_2O_2 solution and the absorbance of H_2O_2 was determined at 230 nm after 10 min incubation against the control, containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of H_2O_2 was calculated as follows:

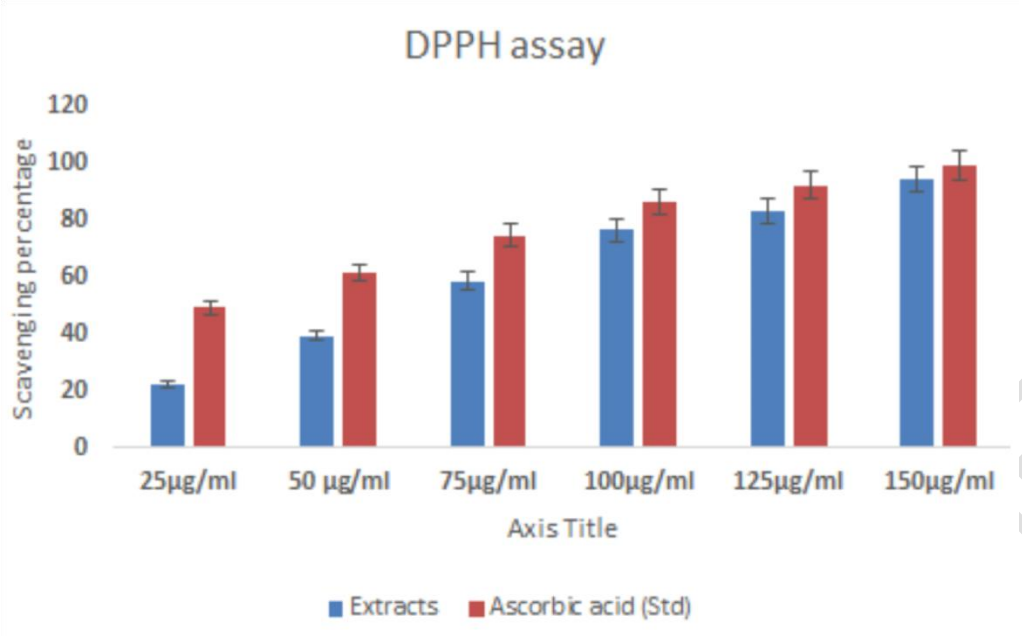
Percentage of Inhibition (I %) = $(A_{control} - A_{sample}) / A_{control} \times 100$

Figure 1: a) ***Avicennia marina*** **b) methanolic extract of** ***Avicennia marina***

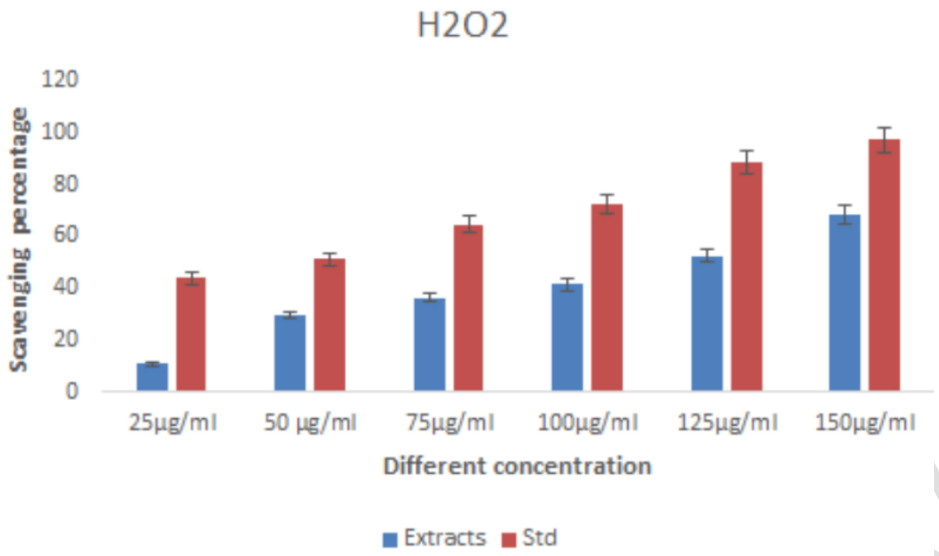
RESULTS:

Table 1: The shows total antioxidant assay where n = 3 and the values were given as mean± SE

Concentration of extract (□g/ml)	**Ascorbic acid equivalence**
25	27.81 ±1.21
50	49.73 ±1.30
75	68.29 ±0.81
100	92.57 ±1.22
125	112.64 ±1.31
150	138.49 ±1.28



Graph 1: Bar graph shows the free radical scavenging activity in DPPH assay of the extract in different concentrations against the standard. X axis represents different concentrations of methanolic extract and ascorbic acid, Y axis represents scavenging percentage, while blue colour represents the extracts and red represents the standard, ascorbic acid. Values were done in triplicate n=3 with mean ±SE . From this, it can be inferred that as concentration of extract increases the scavenging activity increases. at highest concentration, the scavenging percentage was almost near to that of the standard.



Graph 2: Graph shows the free radical scavenging activity in H_2O_2 of the extract in different concentrations against the standard. X axis represents different concentrations of methanolic extract and ascorbic acid, Y axis represents scavenging percentage while blue colour represents the extracts and red represents the standard, ascorbic acid. Values were done in triplicate n=3 with mean ±SE. From this, it can be inferred that as concentration of extract increases, the scavenging activity increases.

The total antioxidant assay involved 25□g/ml to 150 µg/ml of the extract in which the values observed were more close to ascorbic acid equivalent. At the highest concentration (150 µg/ml) the ascorbic acid equivalent was 138.49 ±1.28. However, the lowest concentration (25µg/ml) of crude extract showed the ascorbic acid equivalence was 27.81 ±1.21 (Table 1). From the DPPH assay, it can be observed that the scavenging activity of the extract increases with increase in concentration and thus it is a dose dependent activity (graph 1). In the lowest concentration (25□g/ml) the percentage of scavenging was 22.58 % while the standard, ascorbic acid showed 49.27% of scavenging. But with increase in concentration, the scavenging activity also increased as in the concentration of 100□g/ml, scavenging percentage was 76.42 and that of standard was 86.37% which is slightly a higher value. At the highest concentration (150□g/ml), scavenging percentage of the extract was 94.3 and that of standard was 99.2% which is a closer value to the extract. Thus this plant has better potential to act as antioxidant as its scavenging activity remains more similar to that to ascorbic acid.

On assessing the scavenging activity of extract against the standard in hydroxyl scavenging assay, it was found that with increase in concentration of the extract the scavenging percentage also increases (graph 2). In the lowest concentration (25□g/ml) the percentage of scavenging was only 10.85% while the standard, ascorbic acid showed 43.82% of scavenging. But with increase in concentration, the scavenging activity also increases as at 100□g/ml of concentration, scavenging percentage was 41.38% and that of standard was 72.61%. At the highest concentration (150□g/ml), scavenging percentage of the extract was 68.51% and that of standard was 97.09% and thus the scavenging potential of the extract was comparatively lesser than the standard.

DISCUSSION:

A prior study suggests that in the presence of an antioxidant, DPPH radical acquires one more electron and the absorbance decreases (37). There are certain methods available to assess antioxidant activity of compounds. DPPH free radical scavenging assay is a rapid and sensitive method for the antioxidant screening of plant extracts. In presence of an antioxidant, DPPH radicals gain one more electron and thus absorbance decreases. Another similar study with concentration ranging from 50-800□g/ml with ascorbic acid as standard, 96.25% and 74.55 % were the percentage of scavenging activity in ethyl acetate and methanolic extract on DPPH respectively (38). This study remains in contrast to the present study. Yet another study established that all the concentrations of *A. marina* extracts showed higher activity than the standard ascorbic acid except petroleum ether and methanolic extracts (39). Though this study was in accordance with the present study, the difference in scavenging activity between the extract and the standard remains here.

Previous study shows that the highest scavenging was seen in 100□g/ml in a DPPH radical scavenging assay (40). In recent years much attention has been devoted to natural antioxidants and their association with health benefits. In a similar study, at 800µg/mL concentration of methanolic extract of *A. marina* pneumatophore showed 69.12% which is a supportive finding of the present study (41). Similar studies on antioxidant, anti-inflammatory potential are in accordance with our study (15), (42)-(43). This study proved the limitation that the antioxidant property has been assessed through *in vitro* method whereas its effect and potential in living cells has to be explored. Thus further study will be progressed as an in vivo model to assessment of

antioxidant activity of *Avicennia marina.*

CONCLUSION:

From the results obtained, it can be concluded that methanolic extract of *Avicennia marina* is capable of scavenging a wide range of free radicals(44-53). In vitro assay study confirms *Avicennia marina* is a natural antioxidant and can be eminently used for pharmaceutical needs.

REFERENCES:

1. Arfi Y, Buée M, Marchand C, Levasseur A, Record E. Multiple markers pyrosequencing reveals highly diverse and host-specific fungal communities on the mangrove trees Avicennia marina and Rhizophora stylosa [Internet]. Vol. 79, FEMS Microbiology Ecology. 2012. p. 433–44. Available from: http://dx.doi.org/10.1111/j.1574-6941.2011.01236.x

2. Feller IC, Lovelock CE, Berger U, McKee KL, Joye SB, Ball MC. Biocomplexity in mangrove ecosystems. Ann Rev Mar Sci. 2010;2:395–417.

3. Sivaperumal P, Kamala K, Rajaram R. Adsorption of cesium ion by marine actinobacterium Nocardiopsis sp. 13H and their extracellular polymeric substances (EPS) role in bioremediation [Internet]. Vol. 25, Environmental Science and Pollution Research. 2018. p. 4254–67. Available from: http://dx.doi.org/10.1007/s11356-017-0818-0

4. Silalahi M. Pemanfaatan Citrus aurantifolia (Christm. et Panz.) sebagai Bahan Pangan dan Obat serta Bioaktivitas [Internet]. Vol. 17, Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam. 2020. p. 80. Available from: http://dx.doi.org/10.31851/sainmatika.v17i1.3637

5. Radji M. PERANAN BIOTEKNOLOGI DAN MIKROBA ENDOFIT DALAM PENGEMBANGAN OBAT HERBAL [Internet]. Vol. 2, Majalah Ilmu Kefarmasian. 2005. p. 113–26. Available from: http://dx.doi.org/10.7454/psr.v2i3.3388

6. Karami L, Majd A, Mehrabian S, Nabiuni M, Salehi M, Irian S. Antimutagenic and anticancer effects of Avicennia marina leaf extract on Salmonella typhimurium TA100 bacterium and human promyelocytic leukaemia HL-60 cells [Internet]. Vol. 38, ScienceAsia. 2012. p. 349. Available from: http://dx.doi.org/10.2306/scienceasia1513-1874.2012.38.349

7. Thatoi H, Samantaray D, Das SK. The genusAvicennia, a pioneer group of dominant mangrove plant species with potential medicinal values: a review [Internet]. Vol. 9, Frontiers in Life Science. 2016. p. 267–91. Available from: http://dx.doi.org/10.1080/21553769.2016.1235619

8. Das SK, Patra JK, Thatoi H. Antioxidative response to abiotic and biotic stresses in

mangrove plants: A review [Internet]. Vol. 101, International Review of Hydrobiology. 2016. p. 3–19. Available from: http://dx.doi.org/10.1002/iroh.201401744

9. Bibi N, Fawzi, Gokhan, Rajesh, Nadeem, Kannan RR, et al. Ethnopharmacology, Phytochemistry, and Global Distribution of Mangroves—A Comprehensive Review [Internet]. Vol. 17, Marine Drugs. 2019. p. 231. Available from: http://dx.doi.org/10.3390/md17040231

10. Lolok N, Ikawati N, Siharis F. The Potency of Antifertility Effect of Stem Bark Extract of Mangrove (Avicennia Marina) on Male White Rats (Rattus Novergicus) [Internet]. Proceedings of the 6th International Conference on Advanced Molecular Bioscience and Biomedical Engineering. 2019. Available from: http://dx.doi.org/10.5220/0009587701410146

11. Khasim SM, Long C, Thammasiri K, Lutken H. Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation. Springer Nature; 2020. 824 p.

12. Osman NAR, Faculty of Marine Science and Fisheries, Red Sea University, Sudan P, Box PO, Sudan, et al. The content of some selected metabolites of Hypnea valentiae (Turner) Montagne from the Red Sea coast of Sudan [Internet]. Vol. 4, Journal of Coastal Life Medicine. 2016. p. 458–64. Available from: http://dx.doi.org/10.12980/jclm.4.2016j6-49

13. Devaraj E, Roy A, Veeraragavan GR, Magesh A, Sleeba AV, Arivarasu L, et al. β-Sitosterol attenuates carbon tetrachloride–induced oxidative stress and chronic liver injury in rats [Internet]. Vol. 393, Naunyn-Schmiedeberg's Archives of Pharmacology. 2020. p. 1067–75. Available from: http://dx.doi.org/10.1007/s00210-020-01810-8

14. Meenapriya M, Anitha R, Lakshmi T. Effect of Lutein on Cytochrome P450 (Isoform CYP3A4) - An in vitro Study [Internet]. Vol. 10, Pharmacognosy Journal. 2018. p. 1093–5. Available from: http://dx.doi.org/10.5530/pj.2018.6.185

15. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Vol. 10, Pharmacognosy Research. 2018. p. 343. Available from: http://dx.doi.org/10.4103/pr.pr_46_18

16. Cinthura C, Thangavelu L, Rajeshkumar S, Gurunadhan D, Pradeep Kumar R, Roy A. COX2 Inhibitory activity of Abutilon indicum-An Invitro Study [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3523. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04131.7

17. Karthikeyan G, Lakshmi T, Rajeshkumar S, Roy A, Gurunadhan D, Geetha RV, et al. Glucose Uptake Potential in L6 Myotubes by Ficus racemosa [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3527. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04132.9

18. Roy A, Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Nadu T, et al. Molecular docking

analysis of compounds from Lycopersicon esculentum with the insulin receptor to combat type 2 diabetes [Internet]. Vol. 16, Bioinformation. 2020. p. 748–52. Available from: http://dx.doi.org/10.6026/97320630016748

19. Anitha R, Aneesa N, Varghese S. Antidiabetic activity of ajwain oil in different in vitro models [Internet]. Vol. 11, Journal of Pharmacy And Bioallied Sciences. 2019. p. 142. Available from: http://dx.doi.org/10.4103/jpbs.jpbs_128_18

20. S B, Balamithra S, Rajeshkumar S, Roy A, Lakshmi T. Antibacterial activity of selenium nanoparticles synthesized using Maranta arundinaecea root extract [Internet]. Vol. 11, International Journal of Research in Pharmaceutical Sciences. 2020. p. 2695–700. Available from: http://dx.doi.org/10.26452/ijrps.v11i2.2289

21. Roy A, Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Nadu- T, et al. Molecular docking analysis of compounds from Andrographis paniculata with EGFR [Internet]. Vol. 17, Bioinformation. 2021. p. 23–8. Available from: http://dx.doi.org/10.6026/97320630017023

22. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic Effect of Caralluma fimbriata Against Human Colon Cancer Cells [Internet]. Vol. 9, Pharmacognosy Journal. 2017. p. 204–7. Available from: http://dx.doi.org/10.5530/pj.2017.2.34

23. Website [Internet]. [cited 2021 Feb 23]. Available from: http://jsrr.in 113 ISSN: 2249-7846 (Online) M Packia Lincy et al., Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D, 2009. In vitro antioxidant activity of hot aqueous extract of Helieteres isora Linn.fruits. Nat Prod Radi., 8: 483-487.

24. Keerthiga N, Anitha R, Rajeshkumar RS, Lakshmi T. Antioxidant Activity of Cumin Oil Mediated Silver Nanoparticles [Internet]. Vol. 11, Pharmacognosy Journal. 2019. p. 787–9. Available from: http://dx.doi.org/10.5530/pj.2019.11.125

25. Ozturk M, Hakeem KR. Plant and Human Health, Volume 2: Phytochemistry and Molecular Aspects. Springer; 2019. 697 p.

26. 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.

27. 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.

28. 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.

29. 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

30. 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.

31. 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.

32. 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.

33. 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.

34. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115–21.

35. 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.

36. Veerasamy R, Roy A, Karunakaran R, Rajak H. Structure-Activity Relationship Analysis of Benzimidazoles as Emerging Anti-Inflammatory Agents: An Overview. Pharmaceuticals [Internet]. 2021 Jul 11;14(7). Available from: http://dx.doi.org/10.3390/ph14070663

37. C J, Jayachitra C, Karthika K, Paulsamy S. PHYTOCHEMICAL SCREENING AND IN VITRO ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF CISSUS SETOSA ROXB [Internet]. Vol. 2, INTERNATIONAL RESEARCH JOURNAL OF PHARMACY. 2013. p. 166–8. Available from: http://dx.doi.org/10.7897/2230-8407.04935

38. Al-Khayat JA, Alatalo JM. Relationship Between Tree Size, Sediment Mud Content, Oxygen Levels, and Pneumatophore Abundance in the Mangrove Tree Species Avicennia Marina (Forssk.) Vierh [Internet]. Vol. 9, Journal of Marine Science and Engineering. 2021. p. 100. Available from: http://dx.doi.org/10.3390/jmse9010100

39. Chitra V, Tamilanban T, Manasa K, Narayanan J. Evaluation of In-vitro Antioxidant Activity of Ethanolic Extract of Ugli Fruit [Internet]. Vol. 10, Research Journal of Pharmacy and Technology. 2017. p. 3826. Available from: http://dx.doi.org/10.5958/0974-360x.2017.00694.1

40. Kamala K, Sivaperumal P, Gobalakrishnan R, Swarnakumar NS, Rajaram R. Isolation and

characterization of biologically active alkaloids from marine actinobacteria Nocardiopsis sp. NCS1 [Internet]. Vol. 4, Biocatalysis and Agricultural Biotechnology. 2015. p. 63–9. Available from: http://dx.doi.org/10.1016/j.bcab.2014.10.005

41. H DRM, Manjunatha H DR. Evaluation of Antimicrobial Activity and Antioxidant Activity by Electrochemical Method of Ethanolic Extract of Pterocarpus marsupium Roxb Bark [Internet]. Vol. 04, Journal of Biotechnology & Biomaterials. 2014. Available from: http://dx.doi.org/10.4172/2155-952x.1000166

42. Dhayanithi J, Rajeshkumar S, Roy A, Lakshmi T. Preparation and Evaluation of Antifungal Activity of Arrow Root Mediated Selenium Nanoparticles Against Candida Albicans -. Journal of Complementary Medicine Research. 2020;11(5):83–8.

43. Role of Nanomedicine in Novel Corona Virus Pandemic: A perspective [Internet]. 2020 [cited 2021 Aug 31]. Available from: http://bbrc.in/bbrc/role-of-nanomedicine-in-novel-corona-virus-pandemic-a-perspective/

44. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. Pesqui Bras Odontopediatria Clin Integr. 2019 Mar 12;19(0):4668.

45. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol [Internet]. 2017 Mar [cited 2021 Sep 15];44(3). Available from: https://pubmed.ncbi.nlm.nih.gov/28004401/

46. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. Case Rep Dent [Internet]. 2019 Jan 22 [cited 2021 Sep 15];2019. Available from: https://pubmed.ncbi.nlm.nih.gov/30805222/

47. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev [Internet]. 2014 Jun 30 [cited 2021 Sep 15];8(1). Available from: https://pubmed.ncbi.nlm.nih.gov/25992232/

48. Menon S, Ks SD, R S, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces [Internet]. 2018 Oct 1 [cited 2021 Sep 15];170. Available from: https://pubmed.ncbi.nlm.nih.gov/29936381/

49. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent [Internet]. 2020 Jan [cited 2021 Sep 15];80(1). Available from: https://pubmed.ncbi.nlm.nih.gov/31710096/

50. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available from: https://pubmed.ncbi.nlm.nih.gov/11286449/

51. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J [Internet]. 2011 Jun [cited 2021 Sep 15];44(6). Available from: https://pubmed.ncbi.nlm.nih.gov/21255047/

52. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206–15.

53. Kumar MS, Vamsi G, Sripriya R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J Periodontol. 2006 Nov;77(11):1803–8.