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Exploration of Antioxidant Effects of Crude Extract of Mangrove Plant - *Avicennia marina*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 methanolic extract of mangrove plants, *Avicennia marina.*
Materials and Methods: 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 were performed and antioxidant potential was assessed using ascorbic acid as standard.

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Results: 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.

1. 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 – 36]. Thus the aim of the study was to explore its antioxidant potential using the methanolic extract of *Avicennia marina.*

2. MATERIALS AND METHODS

2.1 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 study was carried out in the Marine biomedical and environmental research section of Blue lab at Saveetha Dental College, Chennai.

2.2 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 whatsman 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.

2.3 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 95°c 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.

2.4 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.

2.5 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 %) = $(A_{control} - A_{sample}) / A_{control} \times 100$

Fig. 1. a] *Avicennia marina* b] methanolic extract of *Avicennia marina*

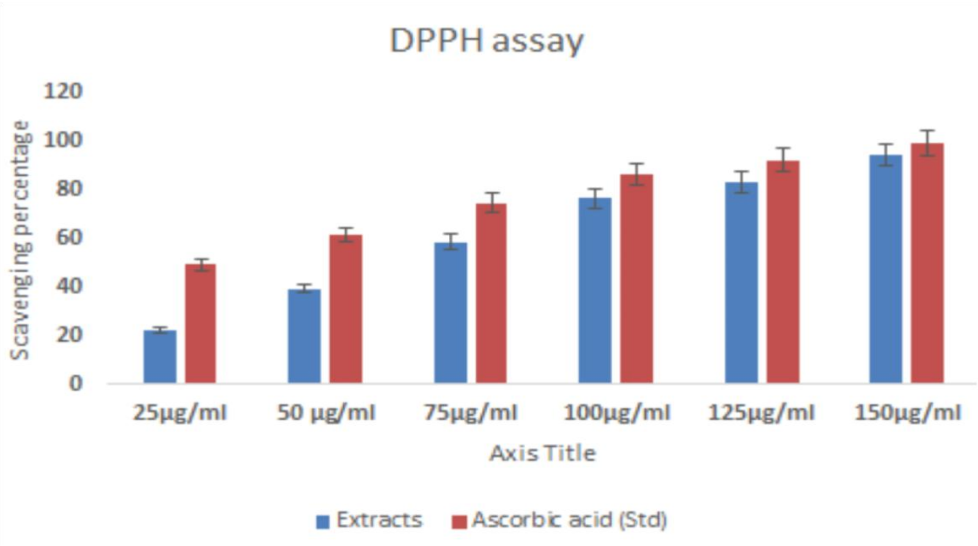
3. RESULTS

The total antioxidant assay involved 25µg/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 (25µg/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 100µg/ml, scavenging percentage was 76.42 and that of standard was 86.37% which is slightly a higher value. At the highest concentration (150µg/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.

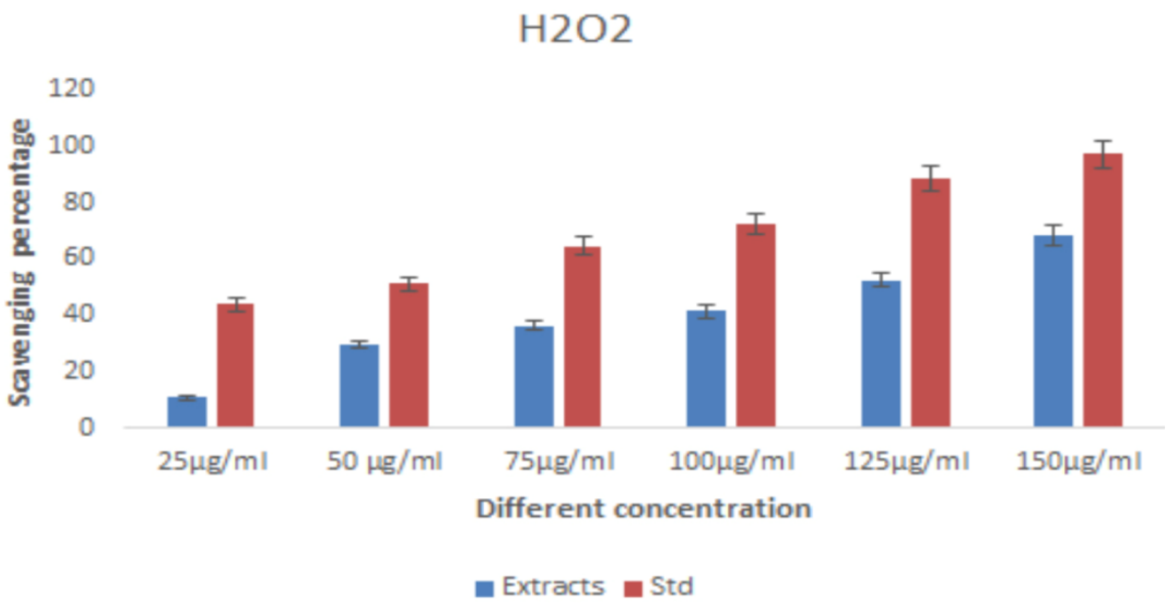
On assessing the scavenging activity of extract against the standard in hydroxyl scavenging assay, it was found that with increase in concentration of the extract the scavenging percentage also increases (Graph 2). In the lowest concentration (25µg/ml) the percentage of scavenging was only 10.85% while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

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

Concentration of extract [µg/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 H_2O_2 free radical scavenging activity 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

4. DISCUSSION

One previous 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 50-80 µ 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].

Previous study shows that the highest scavenging was seen in 100µg/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 [15,42-52]. 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.*

5. CONCLUSION

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

ETHICAL APPROVAL

Prior to the initiation of the study, Ethical clearance was obtained by the scientific review board with approval number (IHEC/SDC/UG-1948/21/92)

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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). FEMS Microbiology Ecology. 2012;79:433–44. Available: 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). Environmental Science and Pollution Research. 2018;25:4254–67. Available: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). Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam. 2020;17:80. Available:http://dx.doi.org/10.31851/sainmatika.v17i1.3637
5. Radji M. Peranan Bioteknologi Dan Mikroba Endofit Dalam Pengembangan Obat Herbal (Internet). Majalah Ilmu Kefarmasian. 2005;2:113–26. Available: 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). ScienceAsia. 2012;38:349. Available: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). Frontiers in Life Science. 2016; 9:267–91. Available: 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). International Review of Hydrobiology. 2016;101:3–19. Available: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). Marine Drugs. 2019;17: 231. Available: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: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). Journal of Coastal Life Medicine. 2016;4:458–64. Available: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). Naunyn-Schmiedeberg’s Archives of Pharmacology. 2020;393:1067–75.

Available: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). Pharmacognosy Journal. 2018;10: 1093–5. Available: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). Pharmacognosy Research. 2018; 10:343. Available: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). Indian Journal of Public Health Research & Development. 2019;10:3523. Available: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). Indian Journal of Public Health Research & Development. 2019;10:3527. Available: http://dx.doi.org/10.5958/0976-5506.2019.04132.9
18. Roy A, Rajagopal P, Thangavelu L. Molecular docking analysis of compounds from Lycopersicon esculentum with the insulin receptor to combat type 2 diabetes (Internet). Bioinformation. 2020;16(10),: 748–52. Available: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). Journal of Pharmacy And Bioallied Sciences. 2019;11:142. Available: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). International Journal of Research in Pharmaceutical Sciences. 2020;11:2695–700. Available:http://dx.doi.org/10.26452/ijrps.v11i2.2289
21. Roy A. Molecular docking analysis of compounds from *Andrographis paniculata* with EGFR (Internet). Bioinformation. 2021;17:23–8. Available: 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). Pharmacognosy Journal. 2017; 9:204–7. Available:http://dx.doi.org/10.5530/pj.2017.2.34
23. M Packia Lincy , Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D. In vitro antioxidant activity of hot aqueous extract of Helieteres isora Linn.fruits. Nat Prod Radi. 2009;8:483-487.
24. Keerthiga N, Anitha R, Rajeshkumar RS, Lakshmi T. Antioxidant activity of cumin oil mediated silver nanoparticles (Internet). Pharmacognosy Journal. 2019;11:787–9. Available:http://dx.doi.org/10.5530/pj.2019.11.125
25. Ozturk M, Hakeem KR. Plant and Human Health, Phytochemistry and Molecular Aspects. Springer. 2019;2:697.
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;32:1–4. Available: 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: 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)., International Research Journal Of Pharmacy. 2013;2:166–8. Available: 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). Journal of Marine Science and Engineering. 2021;9: 100. Available: 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;10:3826. Available: 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;4:63–9. Available: 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: 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. Thangavelu L .Role of Nanomedicine in Novel Corona Virus Pandemic: A perspective (Internet). 2020 (cited 2021 Aug 31). Available: 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: 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: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: 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: 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: 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: 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: https://pubmed.ncbi.nlm.nih.gov/21255047/
52. 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;77(11):1803–8.

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