

A current review on disrupting the sessile microbial army

Apoorva Udayashankara¹, K Manjunath^{2*}

¹ Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka, India

² Professor Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bangalore, Karnataka, India

Abstract

The sessile army of microbes enclosed in an extracellular polymeric substance (EPS) is known as the Biofilm. Biofilm formation is commonly seen in bacteria and some fungi. The high tolerance of these sessile army to exogenous stress and other cidal agents poses as a major problem in many fields ranging from industrial biofouling to chronic infections in humans hence researchers all around the globe are testing effective ways to prevent and control the biofilm formation. Chemical intervention of these are found to be unsatisfactory due to various reasons like resistance and toxicity hence in this current review the use of natural products like medicinal plants and its bioactive compounds to combat the formation resistant microbial communities with least toxicity and utmost efficacy is studied. This can be a useful data and guidance for future antibiofilm studies.

Keywords: anti-bio film, plant extracts, essential oil, bioactive compound, secondary metabolites

Introduction

The sessile microbial army enclosed within a extracellular polymeric substance is known as the Biofilm. It's a complex habitat for not just one kind of cells but is a diverse range of community irreversibly attached to a biotic or a abiotic surface [22]. It is hostile environment composed of matrix of polysaccharides, proteins, lipids, nucleic acids and other chemical materials. Biofilm resistance can be attributed to various factors like the reduced permeability of chemicals into the thick matrix, expression of the multidrug efflux pumps, environmental stress, differences in physiological property, bacteriophages and phage particles helps in releasing eDNA helping in the development of antibiotic tolerant colonies, quorum sensing defense, horizontal gene transfer, secretion of antibiotic modifying enzymes and the type IV secretion systems [4]. Hence the go to treatment for Biofilms has been combination of antibacterial agents at high doses which has eventually resulted in the development of multi drug resistant strains [6].

This concern has forced many to investigate alternative anti bio film agent with least side effects. The development of novel therapeutic measures which rely on inhibiting the biofilm formation rather than killing the pathogen using plant based products seems to be a promising approach. The aim of this review is to summarize the effectiveness of various plant based products like solvent extracts, secondary metabolites, bioactive compounds, and essential oils which have proven to have potent anti-bio film activity.

Bio film Formation

The planktonic cells enter the sessile phase of life to form a resistant army by firstly Pre- conditioning the biotic or abiotic substratum followed by the attachment. The cells then rapidly grow and divide, producing extracellular polysaccharides through quorum sensing resulting in the formation of a mature biofilm army composed of various different organisms. This is a reversibly association where the planktonic cells can get detached to form a new attachment [3, 19, 5].

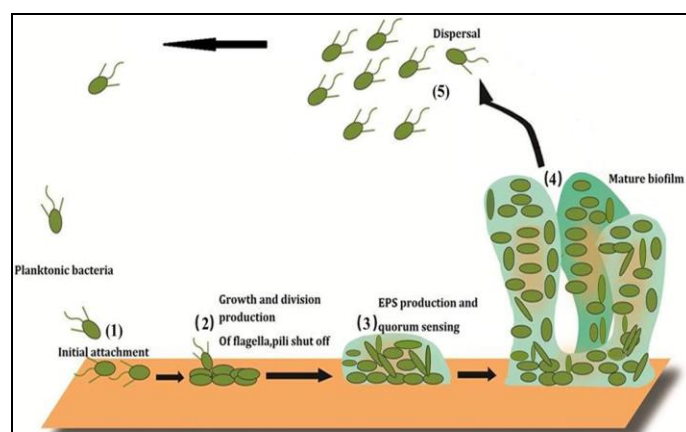


Fig 1

Bio film threat

Biofilms are ubiquitous, they are not only a threat at medical and industrial level but can be a serious problem at our very own houses colonizing a variety of surfaces like the kitchen slabs, toilet seats, sinks, chopping boards, bathrooms, Ac vents and even the toys. The sessile army is a daily challenge in food industry as they can be the potent source of contamination and spoilage of food products [32, 37]. In medical they are responsible for dental plaque to serious chronic illness, nosocomial infections and even the life threatening infections like bacterial endocarditis, Otitis media, cystic fibrosis, lung infections, urinary tract infections etc. Their major concern lies in the implantable devices and prosthetics. Key factors such as surface charge, surface texture, surface hydrophobicity, energy and biomaterial composition plays a critical role in colonizing implants during or any time post-surgery [1]. Marine fouling is also caused by the aggregation of microbial biofilm on ship hulls, metal corrosion, blockage of pipes contamination of water and reduced efficacy of purification are some of the numerous problems associated with biofilms in the water and sewage treatment plants [2].

Plants as Anti bio film agents

Nature has been a source of medicinal agents for thousands of years with a number of modern drugs isolated from natural sources on the basis of their use in traditional medicine. A number of plants derived products or drugs have been used for the treatment of various infections for

centuries [13]. Plants produce a diverse range of bioactive molecules, making them a rich source of therapeutic agents and contribute towards human health and well-being. Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, etc. that may contain active components. The antimicrobial property of the plants is attributed to the secondary metabolites like tannins, terpenoids, alkaloids and flavonoids produced by the plants as a means of defense against pathogens or due to environmental stress [7, 20, 36]. Due to the relatively lower side effects in comparison with modern synthetic drugs, coupled with reduced cost, plants play an important role in the development of novel drugs. Most of the phytochemicals are not highly effective as antimicrobial agents but they possess anti-pathogenic or anti-virulence properties, which are neither bactericidal nor bacteriostatic as these phytochemical compounds attenuate the expression of genes responsible for pathogenesis or virulence. This property of herbal constituents eliminates the development of resistance among the microorganisms [15].

Plant extracts as Bio film inhibitors

A number of research findings show the effectiveness of plants and its products in controlling and preventing the formation of the biofilm but most of these studies are *in-vitro*. The review is tabularized for the ease of understanding. The section bellow from Table 1 lists out the potent natural biofilm inhibitors of plant kingdom.

Table 1: List of plant based potent Antibiofilm inhibitors

Sl. No.	Plant species/ bioactive compound	Material used	Solvent	Effective against	Reference
1.	<i>Salvadora persica</i>	Chewing sticks	Methanol	<i>Streptococcus mutans</i>	[10]
2.	<i>Piper betle</i>	Leaf	Ethanol	<i>Streptococcus mutans</i> <i>A actinomycetemcomitans</i>	[3]
3.	<i>C robusta</i>	Beans	Aqueous	<i>Streptococcus mutans</i>	[11]
4.	<i>Peganum harmala</i>	Whole plant	Ethanol	<i>C albicans</i>	[12]
5.	<i>P nigrum</i> <i>A indica</i> <i>P betle</i> <i>M indica</i>	Leaf	Aqueous	<i>Enterococcus faecalis</i> <i>Staphylococcus aureus</i>	[13]
6.	<i>Trachyspermum ammi</i>	Seed	Aqueous Petroleum ether	<i>Streptococcus mutans</i>	[14]
7.	<i>Dioscorea altissima</i> <i>Annona hypogauca</i>	Aerial organs Flowers	Aqueous	<i>Streptococcus mutans</i>	[15]
8.	<i>Citrus limonum</i> <i>Zingiber officinale</i>	Essential oil	-	<i>Klebsiella ornithinolytica</i> <i>K. oxytoca</i> <i>K. terrigena</i>	[16]
9.	<i>Semecarpus anacardium</i> , <i>Glochidion lanceolarium</i> , and <i>Bridelia retusa</i>	Silver nanoparticles from leaf	-	<i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> <i>Staphylococcus aureus</i> .	[17]
10.	Myrtle Pomegranate	Leaf Fruit peel	Polyphenolic	<i>Streptococcus mutans</i> <i>Streptococcus oralis</i> <i>Streptococcus mitis</i> <i>Rothia dentoca</i>	[18]
11.	Umbelliferone	-	Methanol	MRSE	[19]
12.	Curcumin	-	Methanol	<i>Acinetobacter baumannii</i>	[20]

Table 2

Sl. No.	Plant species/ bioactive compound	Material used	Solvent	Effective against	Reference
13.	<i>Eugenia natalitia</i> <i>Eugenia woodii</i> <i>Evan Wyk</i> <i>Eugenia zeyheri</i> <i>Syzygium legatii</i>	Leaf	Acetone	<i>Bacillus cereus</i> <i>Enterococcus faecalis</i> <i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i>	[21]

	<i>Syzygium masukuense</i>			<i>Salmonella Typhimurium</i>	
14.	<i>Adiantum philippense</i>	Whole plant	Methanol	<i>S. flexneri</i> <i>S. aureus</i> <i>P. aeruginosa</i> <i>Escherichia coli</i>	[22]
15.	<i>Plectranthus barbatus</i>	Essential oil	-	<i>Pseudomonas aeruginosa</i>	[23]
16.	Grape fruit	Seed	Aqueous	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	[24]
17.	Grape fruit	Seed	Aqueous	<i>Candida albicans</i>	[25]
18.	N-cerotoyltryptamine Asimicin ent-19-carbomethoxykauran -17-oic acid	<i>Annona senegalensis</i> seed	Methanol	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Candida albicans</i>	[26]
19.	<i>Opuntia ficus-indica</i>	Cladodes	Polyphenolic	<i>Staphylococcus aureus</i>	[27]
20.	<i>Coriandrum sativum</i> L. <i>Mentha × piperita</i> L. <i>Pimpinella anisum</i> L	Plant extracts and essential oil	Methanol	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	[28]
21.	<i>Malva parviflora</i>	Leaf	Ethanol	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Klebsiella pneumonia</i>	[29]
22.	Cinnamon Eucalyptus Garlic Pomegranate, Tea Tree	Essential oil	-	<i>Proteus</i> <i>Aeromonas</i> <i>Pseudomonas</i>	[30]
23.	Chelerythrine Sanguinarine DiHydroxyBenzoFuran-proAnthocyanidin A2-phosphatidylCholine	Bioactive compound	-	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>	[31]
24.	Phenylpropanoids	Bioactive compound	-	<i>Candida albicans</i>	[32]
25.	<i>C. pumila</i> ε-Viniferin	Whole plant Bioactive compound	Ethanol	<i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i>	[33]

Table 3

Sl. No.	Plant species/ bioactive compound	Material used	Solvent	Effective against	Reference
26.	Terpenoids	Bioactive compound	Dimethyl sulphoxide	<i>Candida albicans</i>	[34]
27.	NiO nanoparticles	<i>Eucalyptus globulus</i> leaf	-	<i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i> MRSA	[35]
28.	Thyme	Essential oil	-	<i>Acinetobacter</i> <i>Sphingomonas</i> <i>Stenotrophomonas</i>	[37]
29.	<i>Coriandrum sativum</i> L.	Essential oil	-	<i>Candida albicans</i>	[38]
30.	Thyme	Essential oil	-	<i>Bacillus cereus</i>	[39]
31.	Pure oregano Thymol Cavacrol	Essential oil	-	<i>Salmonella</i> <i>Escherichia coli</i>	[40]
32.	Tea tree oil Bergamot oil Basil oil	Essential oil sophorolipids	-	<i>Vibrio cholerae</i>	[41]
33.	<i>Trigonella foenum</i>	Seed	Methanol	<i>Pseudomonas aeruginosa</i> <i>Aeromonas hydrophila</i>	[42]
34.	<i>Achyranthes aspera</i> L.	Stem	Methanol Benzene Petroleum Ether Aqueous	<i>Streptococcus mutans</i>	[43]
35.	<i>Capparis spinosa</i>	Fruit	Methanol	<i>Escherichia coli</i> <i>Proteus mirabilis</i> <i>Serratia marcescens</i> <i>Pseudomonas aeruginosa</i>	[44]
36.	<i>Salvadora persica</i> L	Fruit	Methanol	<i>Staphylococcus sp</i>	[45]
37.	Naringenin Quercetin Sinensetin Apigenin	Citrus flavonoids	Dimethyl sulphoxide	<i>Escherichia coli</i> <i>Vibrio harveyi</i>	[46]
38.	Embelin Piperine	Bioactive compounds	Dimethyl sulphoxide	<i>Streptococcus mutans</i>	[47]

39.	Casbane Diterpene from <i>Croton nepetaefolius</i>	Bark	Ethanol	Oral <i>Streptococcus</i>	[48]
40.	Reserpine	Bioactive compound	Dimethyl sulphoxide	<i>Klebsiella pneumoniae</i>	[49]
41.	Thyme peppermint	Essential oil	-	<i>Klebsiella pneumoniae</i>	[50]

Conclusion

An attempt has been made to review the potential of plant and its products as a promising agent to control and prevent the microbial biofilm formation and as a source for the development of therapeutic agents to combat biofilm induced infections.

Reference

- Tran PA, Hocking DM, O'Connor AJ. "In situ formation of antimicrobial silver nanoparticles and the impregnation of hydrophobic polycaprolactone matrix for antimicrobial medical device applications". *Mater Sci Eng C Mater Biol Appl*,2015;47:63-9. doi: 10.1016/j.msec.2014.11.016. Epub 2014 Nov 7. PMID: 25492173.
- Coetser SE, Cloete TE. "Biofouling and Biocorrosion in Industrial Water Systems", Taylor and Francis,2005;31(4):213-232.
- Khatoun, Zohr, Christopher D, Mc Tiernam, ErikJ. Suuronen, Thien- Fah Mah, Emilio I. Alarcon, "Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention." *Heliyon*,2018;4:12.
- Floyd KA, Eberly AR, Hadjifrangiskou M. "Adhesion of bacteria to surfaces and biofilm formation on medical devices" *Book on biofilms and implantable medical devices*, 2017, 47-95.
- Mizan MFR, Jahid IK, Ha SD. "Microbial biofilms in seafood: a food-hygiene challenge". *Food Microbiol*,2015;49:41-5
- T. Bjarnsholt, "The role of bacterial biofilms in chronic infections" *APMIS*,2013;121:1-58.
- Hafizah Y, Chenia "Anti-Quorum Sensing Potential of Crude *Kigelia africana* Fruit Extracts" *Sensors*,2013;13:2802-2817.
- Nantiya Joycharat, Surasak Limsuwan, Sanan Subhadhirasakul, Supayang Piyawan, Voravuthikunchai, Saranyabhong Pratumwan, Idris Madahin, Wanlapa Nuankaew, and Atchariya Promsawat "Anti-*Streptococcus mutans* efficacy of Thai herbal formula used as a remedy for dental caries" *Pharmaceutical Biology*,2012;50(8):941-947.
- Rawee Teanpaisan, Pajaree Kawsud, Nuntiya Pahumunto, and Jindaporn Puripattanavong "Screening for antibacterial and antibiofilm activity in Thai medicinal plant extracts against oral microorganisms". *Elsevier Journal of Traditional and Complementary Medicine*, 2016, 1-6.
- Sales Al-Shohaibani, Kasi Murugan. "Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of *Streptococcus mutans*: in vitro and molecular docking studies". *Biofouling, The Journal of bioadhesion and biofilm research*,2012;28(1):29-38.
- Monica Stauder, Adele Papetti, Dora Mascherpa, Anna Maria Schito, Gabriella Gazzani, Carla Pruzzo, and Maria Daglia "Antiadhesion and Antibiofilm Activities of High Molecular Weight Coffee Components against *Streptococcus mutans*" *Journal of Agricultural and Food Chemistry*, 2010, Vol. XXX, No. XX, XXXX
- Elham Aboualigalehdari, Nourkhoda Sadeghifard, Morovat Taherikalani, Zaynab Zargoush, Zahra Tahmasebi, Behzad Badakhsh, Arman Rostamzad, Sobhan Ghafourian, and Iraj Pakzad "Anti-biofilm properties of *Peganum harmala* against *Candida albicans*". *Osong Public Health Research Perspect*,2016;7(2):116-118.
- Geethashri R, Manikandan B, Ravishankar A. Veena Shetty "Comparative evaluation of biofilm suppression by plant extracts on oral pathogenic bacteria". *Applied Pharmaceutical Science*,2014;4(03):020-023.
- Rosina Khan, Mohd Adil, Mohd Danishuddin, Praveen K Verma, Asad U. Khan "In- vitro and in-vivo inhibition of *Streptococcus mutans* biofilm by *Trachyspermum ammi* seeds: An approach of alternative medicine" *Elsevier Phytomedicine*, 2012, 747-755.
- Barnabé, Michele *et al.* "The influence of Brazilian plant extracts on *Streptococcus mutans* biofilm." *Journal of applied oral science: revista FOB*,2014;22(5):366-72.doi:10.1590/1678-775720140085
- Avcioğlu, Nermin Hande "Antibio film Effects of *Citrus limonum* and *Zingiber officinale* Oils on Biofilm Formation of *Klebsiella ornithinolytica*, *Klebsiella oxytoca* and *Klebsiella terrigena* Species." *African journal of traditional, complementary, and alternative medicines: AJTCAM* 2016,2016;13(6):61-67. 29 Sep., doi:10.21010/ajtcam.v13i6.10
- Yugal Kishore Mohanta, Kunal Biswas, Santosh Kumar Jena, Abeer Hashem, Elsayed Fathi Abd_Allah and Tapan Kumar Mohanta, "Anti-biofilm and Antibacterial Activities of Silver Nanoparticles Synthesized by the Reducing Activity of Phytoconstituents Present in the Indian Medicinal Plants" *Front. Microbiol*, 2020.
- Daniela Sateriale, Roberta Imperatore, Roberta Colicchio, Chiara Pagliuca, Ettore Varricchio, Maria Grazia Volpe, Paola Salvatore, Marina Paolucci and Caterina Pagliarulo, "Phyto compounds vs. Dental Plaque Bacteria: In vitro Effects of Myrtle and Pomegranate Polyphenolic Extracts Against Single-Species and Multispecies Oral Biofilms" *Front. Microbiol*, 2020.
- Thirukannamangai Krishnan Swetha, Murugesan Pooranachithra, Ganapathy Ashwinkumar Subramenium, Velayutham Divya, Krishnaswamy Balamurugan and Shunmugiah Karutha Pandian, "Umbelliferone Impedes Biofilm Formation and Virulence of Methicillin-Resistant *Staphylococcus epidermidis* via Impairment of Initial Attachment and Inter-cellular Adhesion" *Front. Microbiol*, 2019.
- Chaitany Jayprakash Raorane, Yong-Guy Kim, Satish Kumar Rajasekharan, Rodolfo García-Contreras and Jintae Lee 1, "Antibiofilm and Antivirulence Efficacies of Flavonoids and Curcumin

- against *Acinetobacter baumannii*” *Front. Microbiol*, 2019.
21. Famuyide, Ibukun M. “Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African *Eugenia* and *Syzygium* (Myrtaceae) species and their selectivity indices.” *BMC complementary and alternative medicine*, 2019;19(1):141.
 22. Adnan, Mohd *et al.* “Effect of *Adiantum philippense* Extract on Biofilm Formation, Adhesion With Its Antibacterial Activities Against Foodborne Pathogens, and Characterization of Bioactive Metabolites: An in vitro-in silico Approach.” *Frontiers in microbiology*, 2020;11(823):13.
 23. Chatterjee B, Vittal RR. “Quorum sensing modulatory and biofilm inhibitory activity of *Plectranthus barbatus* essential oil: a novel intervention strategy”. *Arch Microbiol*, 2021;203(4):1767-1778. doi: 10.1007/s00203-020-02171-9. Epub 2021
 24. Song YJ, Yu HH, Kim YJ, Lee NK, Paik HD. “Anti-Biofilm Activity of Grapefruit Seed Extract against *Staphylococcus aureus* and *Escherichia coli*”. *J Microbiol Biotechnol*, 2019.
 25. Tsutsumi-Arai C, Takakusaki K, Arai Y, Terada-Ito C, Takebe Y, Imamura T *et al.* “Grapefruit seed extract effectively inhibits the *Candida albicans* biofilms development on poly methyl methacrylate denture-base resin”. *PLoS One*, 2019.
 26. Tamfu AN, Ceylan O, Fru GC, Ozturk M, Duru ME, Shaheen F. “Antibiofilm, antiquorum sensing and antioxidant activity of secondary metabolites from seeds of *Annona senegalensis*”, *Persoon. Microb Pathog*, 2020;144:104191.
 27. Raut JS, Karuppaiyl SM. “Phytochemicals as Inhibitors of *Candida* Biofilm”. *Curr Pharm Des*, 2016;22(27):4111-34
 28. Mitra Mohammadi Bazargani, Jens Rohl off. “Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms”, *Food Control*, 2016;61:156-164.
 29. Marwa Fady Abozed, Nadia Hashish, Magda Gazer “Efficacy of Ethanol Extract from Leaves of *Malva parviflora* to Inhibit Bacterial Biofilm Formation”, *Journal of Molecular Biology Research*, 2018;8:1
 30. Yusra Y. Agha “Effect of some plant oils on swarming motility and Biofilm formation in *Proteus*, *Aeromonas*, and *Pseudomonas*”, *J Adv Pharm Edu Res*, 2020;10(4):64-71.
 31. Artini M, Papa R, Barbato G, Scoarughi GL, Cellini A, Morazzoni P *et al.* Selan, “Bacterial biofilm formation inhibitory activity revealed for plant derived natural compounds”, *Bioorganic & Medicinal Chemistry*, 2012;20(2):920-926.
 32. Jayant Shankar Raut, Ravi kumar Bapurao Shinde, Nitin Mahendra Chauhan, and Sankunny Mohan Karuppaiyl, “Phenylpropanoids of Plant Origin as Inhibitors of Biofilm Formation by *Candida albicans*”, *J. Microbiol. Biotechnol*, 2014;24(9):1216-1225 <http://dx.doi.org/10.4014/jmb.1402.02056>
 33. Hyun Seob Cho, Jin - Hyung Lee, Shi Yong Ryu, Sang Woo Joo, Moo Hwan Cho, Jintae Lee. “Inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* O157:H7 Biofilm Formation by Plant Metabolite ϵ -Viniferin, *Journal of Agricultural and Food Chemistry*, 2013;61(29):7120-7126. DOI: 10.1021/jf4009313
 34. Jayant S Raut, Ravikumar B Shinde, Nitin M Chauhan, Mohan Karuppaiyl S. “Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by *Candida albicans*,” *Journal of Bioadhesion and Biofilm Research*, 2013;29:1.
 35. Samia Saleem, Bilal Ahmed, Mohammad Saghir Khan, Majed Al-Shaeri, Javed Musarrat. “Inhibition of growth and biofilm formation of clinical bacterial isolates by NiO nanoparticles synthesized from *Eucalyptus globulus* plants”, *Microbial Pathogenesis*, 2017;111:375-387. ISSN 0882-4010, <https://doi.org/10.1016/j.micpath.2017.09.019>.
 36. Reen FJ, Gutiérrez-Barranquero JA, Parages ML *et al.* “Coumarin: a novel player in microbial quorum sensing and biofilm formation inhibition”. *Appl Microbiol Biotechnol*, 2018;102:2063-2073. <https://doi.org/10.1007/s00253-018-8787-x>
 37. Sandra Szczepanski, André Lipski. “Essential oils show specific inhibiting effects on bacterial biofilm formation”, *Food Control*, 2013, 36(1). 2014, Pages 224-229, ISSN 0956-7135, <https://doi.org/10.1016/j.foodcont.2013.08.023>.
 38. Furletti VF, Teixeira IP, Obando-Pereda G, Mardegan RC, Sartoratto A, Figueira GM *et al.* Höfling, “Action of *Coriandrum sativum* L. Essential Oil upon Oral *Candida albicans* Biofilm Formation”, *Evidence-Based Complementary and Alternative Medicine*, 2011, Article ID 985832, 9 pages, 2011. <https://doi.org/10.1155/2011/985832>
 39. Kang J, Liu L, Wu X. *et al.* “Effect of thyme essential oil against *Bacillus cereus* planktonic growth and biofilm formation”. *Appl Microbiol Biotechnol*, 2018;102:10209–10218 <https://doi.org/10.1007/s00253-018-9401-y>
 40. Oh SY, Yun W, Lee JH. *et al.* “Effects of essential oil (blended and single essential oils) on anti-biofilm formation of *Salmonella* and *Escherichia coli*”. *J Anim Sci Technol*, 2017;59:4. <https://doi.org/10.1186/s40781-017-0127-7>
 41. Ruchira Mukherji, Asmita Prabhune, “Novel Glycolipids Synthesized Using Plant Essential Oils and Their Application in Quorum Sensing Inhibition and as Anti bio film Agents”, *The Scientific World Journal*, 2014, Article ID 890709, 7 pages, 2014. <https://doi.org/10.1155/2014/890709>
 42. Husain FM, Ahmad I, Khan MS, Al-Shabib NA. “*Trigonella foenum-graceum* (Seed) Extract Interferes with Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of *Pseudomonas aeruginosa* and *Aeromonas hydrophila*”. *Evid Based Complement Alternat Med*, 2015:879540. doi: 10.1155/2015/879540.
 43. Murugan K, Sekar K, Sangeetha S, Ranjitha S, Sohaibani SA. “Antibiofilm and quorum sensing inhibitory activity of *Achyranthes aspera* on cariogenic *Streptococcus mutans*: an in vitro and in silico study”. *Pharm Biol*, 2013;51(6):728-36. doi: 10.3109/13880209.2013.764330.
 44. Issac Abraham SV, Palani A, Ramaswamy BR, Shunmugiah KP, Arumugam VR, “Antiquorum sensing and antibiofilm potential of *Capparis spinosa*” *Arch Med Res*, 2011;42(8):658-68. doi:

- 10.1016/j.arcmed.2011.12.002. Epub Jan 2. PMID: 22222491.
45. Noumi E, Snoussi M, Merghni A, Nazzaro F, Quindós G, Akdamar G, Mastouri M, Al-Sieni A, Ceylan O. “Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of *Salvadora persica* L. methanolic extracts. *Microb Pathog*,2017:109:169-176. doi: 10.1016/j.micpath.2017.05.036. Epub 2017 May 26. PMID: 28552808.
 46. Vikram A, Jayaprakasha GK, Jesudhasan PR, Pillai SD, Patil BS. “Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids”. *J Appl Microbiol*,2010:109(2):515-527. doi: 10.1111/j.1365-2672.2010.04677.x. Epub 2010 Jan 19. PMID: 20163489.
 47. Dwivedi D, Singh V. “Effects of the natural compounds embelin and piperine on the biofilm-producing property of *Streptococcus mutans*”. *Journal of traditional and complementary medicine*,2015:6(1):57-61. <https://doi.org/10.1016/j.jtcme.2014.11.025>
 48. Cardoso SáN, Cavalcante TT, Araújo AX, dos Santos HS, Albuquerque MR, Bandeira PN *et al.* “Antimicrobial and antibiofilm action of Casbane Diterpene from *Croton nepetaefolius* against oral bacteria”. *Arch Oral Biol*,2012:2011:57(5):550-5. doi: 10.1016/j.archoralbio.2011.10.016. Epub 2011 Nov 25. PMID: 22119044.
 49. Magesh H, Kumar A, Alam A, Priyam, Sekar U, Sumantran VN *et al.* Vaidyanathan R, “Identification of natural compounds which inhibit biofilm formation in clinical isolates of *Klebsiella pneumonia*”. *Indian J Exp Biol*,2013:51(9):764-72. PMID:24377137.
 50. Mohamed SH, Mohamed MSM, Khalil MS, Azmy M, Mabrouk MI. “Combination of essential oil and ciprofloxacin to inhibit/eradicate biofilms in multidrug-resistant *Klebsiella pneumonia*”. *J Appl Microbiol*,2018:125(1):84-95. doi: 10.1111/jam.13755. Epub 2018 Apr 19. PMID: 29517825.