



EFFICACY OF THE ESSENTIAL OILS OF *Hedychium coronarium* J. KOENIG, AN ENDANGERED MEDICINAL PLANT TO WORK IN SYNERGY WITH ANTIBIOTICS AND ITS BIOFILM-INHIBITING PROPERTIES

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ABSTRACT Nature has been the source of medicines to humankind since time immemorial. Of the various natural sources, medicinal / aromatic plants remain the most sought after for maintenance of health, longevity, prevention and cure. The pharmacological properties of plants are attributed to the various phytochemicals present in them. Of all the biologically active phytochemicals, essential oils are gaining special significance. *Hedychium coronarium* J. Koenig, belonging to the Zingiberaceae family, is an endangered, medicinal and aromatic plant. The essential oils of this plant are known to have antimicrobial (antibacterial, antifungal) properties. As there is a significant rise in the emergence of multi-drug resistant bacteria due to reasons like rampant use of antibiotics, casual use in the dosages, plants are being looked upon as a source of novel compounds that will combat them. The present study attempts to find the synergistic effects of the essential oils of *Hedychium coronarium* with antibiotics and also its biofilm-inhibiting properties. Amikacin, Gentamicin and Vancomycin showed synergistic effect against *Escherichia coli* NCIM 2065 but an additive effect was seen in case of tetracycline. In case of *Staphylococcus aureus* NCIM 2127, though synergistic effect was not seen, the outcome of the combined treatment was more effective than the individual treatment. A synergistic effect was observed against *Pseudomonas aeruginosa* NCIM 5210. The percentage reduction in biofilm formation was 33.47%, 15.85% and 13.67% against *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 5210 and *Staphylococcus aureus* NCIM 2127 respectively.

KEYWORDS : antibiotics, biofilms, essential oils, multi-drug resistance, synergy

INTRODUCTION

Medicinal and Aromatic Plants are nature's gift to mankind and form the backbone of traditional medicine. Aromatic plants also referred to as 'aromatic herbs' produce and exude aromatic substances. Included in the same group are the essential oil plants that accumulate oils in certain specific organs that are used to produce essential (ethereal) oils (Sharifi-Rad et al., 2017). The chemical ingredients or phytochemicals present in plants can serve as starting and /or model materials for pharmaceutical research and medicine production. The phytochemicals include alkaloids, flavonoids, glycosides, gums, oils, resins, saponins, tannins and waxes. Medicinal plants are an integral part of research and development in the pharmaceutical and drug development industry (Larayetan et al., 2019). The twentieth century witnessed a shift from extraction of medicinal compounds from plants to making these compounds or their analogues synthetically. The chemical structures of natural products were used as templates for designing perfect new drugs referred to by industry as "new chemical, entities". Thus, medicinal, aromatic and cosmetic plants are economically important plants.

The microbial world with its immense diversity has been known to exist even before the advent of the human world. The microbes have developed different kinds of relationships with humans in order to survive; some beneficial and some harmful. Antibiotic resistance, forming large bacterial communities called biofilms are some of them. Antibiotics are chemical compounds of biological origin. Antibiotics are one of the most important antibacterial compounds used against infectious diseases and have to a large extent enhanced human health since their introduction. Though antibiotic therapy has advanced well, we still live in an era where there is a rapid rise in incidents of antibiotic resistant infections. Amongst the most common are the MRSA also known as Methicillin Resistant *Staphylococcus aureus* which kills around 50,000 individuals every year in the United States and Europe with many more mortalities in other countries. Similar observation in the developing countries has been made in the recent years for the occurrence of Antibiotic-resistant tuberculosis (TB). Data collected since 2013, mentions approx. 480,000 cases of MDR TB. It is predicted that infections will reach to nearly 10 million deaths per year by 2050, if appropriate actions against the rampant use of antibiotics are not taken (Chokshi et al., 2019). Antibiotic resistance has become a global concern in line with the pollution and environment challenges that we face (Parekh et al., 2007). There are many mechanisms of gaining drug resistance: chromosomal changes,

the exchange of genetic material via plasmids and transposons and change in the active sites via mutations etc. to list a few (Neu, 1992). Today, bacteria have developed resistance to not only one drug but to multiple drugs simultaneously, leading to emergence of MDR (Multiple Drug Resistant) bacteria also called the superbugs. The resistance to multiple antibiotics is increasing rapidly and the medical community has to face multiple challenges (Zhao et al., 2020). Antibiotic resistance may lead to an increase in treatment failure, treatment costs and fatality rate. The emergence in MDR bacteria has forced scientists worldwide to search for new antimicrobial substances from various natural sources, plants being the most important one, leading to the resurgence in the use of herbal medicines.

Apart from the reasons stated above, the underlying cause of resistance is the selective pressure enforced on the pathogen. This can be considered a life and death situation for the bacteria, a situation enough to evolve drug resistance strategies (Zhao et al., 2020). One of the ways that the organisms have adopted is the extensive formation of biofilms. These structures are effective drug barriers providing a special microenvironment to the bacteria. A newer approach to combat drug resistance due to non-permeability to antibiotics could be to use strategies to control biofilms as a preliminary step. The best way would be to screen for molecules which would inhibit the biofilm production in the bacteria (Wei et al., 2020). It has been perceived and proven that bacteria are self-sufficient organisms and maintain a strictly 'unicellular' lifestyle. However, it has been observed in nature that bacteria rarely exist as planktonic growth of pure cultures. In fact, they exist as a complex, dynamic, surface-associated community called a 'biofilm' (O'Toole, 2011). Thus, a biofilm is an association of microorganisms that is irreversibly attached to a surface and cannot be removed by gentle rinsing. It is enclosed within a self-produced matrix of polymeric substance, primarily a polysaccharide. Biofilms are formed on a wide variety of surfaces. When biofilms are formed on medical devices, they often are difficult to treat. In a biofilm, bacteria are well-protected against antibiotics or a hostile environment or against the host's defence mechanisms. This is due to the 'persister' cells present in the biofilm (Lewis, 2005). The 'persister' cells are genetically identical to the rest of the population but they are non-dividing. They also have toxin-antitoxin systems present in them. The target of the antibiotics is blocked because of the toxin molecules thus helping the biofilm to survive in a hostile environment (Lewis, 2005). In addition to 'persister' cells, the presence of an extracellular polysaccharide or matrix also protects the individual cells. Bacteria in

biofilms communicate by secreting low molecular weight, extracellular molecules also called autoinducers thus maintaining a high degree of co-ordinated, multicellular behaviour (Huang et al., 2011). This complex coordination occurs due to a mechanism called "quorum sensing". In 2018, Feng *et al.*, have reported on biofilms of *Borrelia burgdorferi* explaining formation of various dormant, non-growing "persisters" in stationary phase cultures that impart antibiotic-resistance to biofilms (Feng et al., 2019). The stationary phase is the initial phase in biofilm formation. This indicates that even stationary phase cultures can be used as a target for antibiofilm activities. In 2005, some authors have reported that alteration of adherence factors like adhesin in the initial phase of biofilm formation can also be used for inhibiting it (Al-Shuneigat et al., 2005)

Essential oils have been reported by several workers to have an effect on biofilms. Essential oils of tea tree, rosemary, ginger, rose, chamomile, eucalyptus, marjoram, clarysage, juniper have moderate to intense quorum quenching effect (Kerekes et al., 2015). Studies also reveal that different essential oils have been observed to be effective against biofilms formed by pathogens like *Salmonella*, *Listeria*, *Pseudomonas* and *Staphylococcus* (Hyldgaard et al., 2012). In 2019, Das *et al.*, have shown antibiofilm activity of essential oil extracted from the rhizomes of *Zingiber officinale* Rosc., a member of family Zingiberaceae against biofilms of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis* (Das et al., 2019).

Hedychium coronarium J. Koenig, belonging to the family Zingiberaceae is also known as Gulbawali or Dolan Champa or butterfly ginger lily (Tailor et al., 2015). It is found at altitudes from sea level (Verma et al., 2020). It is cultivated in many parts of the world and widely distributed in tropical and subtropical regions among China, India and South-East countries. It has tremendous medicinal and economic value (Verma et al., 2010). Every part of the plant is useful and serves to be a source of income for the grower. The dried stems with 43%-48% cellulose is used as raw material for making paper (Bisht et al., 2006). Flowers are widely cultivated for perfume, essence and ethnomedicine (Pachurekar et al., 2017). *Hedychium coronarium* contains essential oils whose major contents are α -pinene, β -pinene, linalool, 1,8-cineole, α -terpineol, α -humulene and caryophyllene. The rhizome has anti-cancerous, antioxidant, anti-hypertensive, diuretic, leishmanicidal, antimalarial activities and is used in irregular menstruation, piles bleeding and stone in urinary tract (Pachurekar et al., 2017). The biological activities like antimicrobial (antibacterial, antifungal), cytotoxic, chemopreventive, antiallergic, larvicidal, antioxidant, anti-inflammatory, anti-helminthic, antiangiogenic, fibrinolytic, coagulant and hepatoprotective activities of this plant is due to the presence of essential oils.

Considering the limitations of synthetic drugs such as prolonged use inducing multi-drug resistance in bacteria over a period of time, the essential oils from the type species of the genus *Hedychium*, *Hedychium coronarium* J. Koenig has been studied for its potential to work in synergy with commonly used antibiotics in addition to its biofilm-inhibiting property.

MATERIALS AND METHODS

Collection of plant material and sample preparation

Fresh rhizomes of *Hedychium coronarium* were obtained from the greenhouse of Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai. They were washed in continuous running water for 1 hour. The rhizomes were again washed with Tween 20 and a soft brush was used to remove all adhering soil particles. The cleaned rhizomes were deskinning and were grated to be used for further extraction. The grated rhizomes were weighed and kept in a flask covered with aluminium foil. To 6 grams of grated rhizomes, 60 ml of distilled water was added. Hydro distillation of grated rhizomes was carried out for one hour in a water bath at 69 °C. To collect the oil, the distillate was poured in a separating funnel and hexane was added in the ratio 10:30 (hexane: water). The lower aqueous layer was drained from the hexane layer after the partitioning. This process was repeated several times. The system was vented continuously and vigorous shaking was avoided. All the hexane layers were pooled together and kept for drying overnight in a pre-weighed vial (Akinyele et al., 2011). The percentage of essential oils were calculated. The dried residue was dissolved in 10% dimethyl sulfoxide (DMSO) to get a final concentration of 2.5 mg/mL.

Microorganisms and antibiotics

Antibacterial and synergistic effects between the essential oil extracted from the rhizomes of *Hedychium coronarium* and conventional antibiotics were examined. Microorganisms were obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India. They were maintained at 4°C on nutrient agar slants. They were sub cultured weekly. The organisms used in the present study were *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 5210, *Staphylococcus aureus* NCIM 2127. Pre-impregnated antibiotic discs were used for the assay (Himedia Laboratories Ltd., Mumbai).

Study of antimicrobial activity of the essential oils

The antimicrobial activity of crude essential oil from *Hedychium coronarium* J. Koenig was performed by agar cup method on sterile Muller and Hinton agar using the standard Kirby Bauer method of antibiotic sensitivity test. The dried essential oil was reconstituted in 10% Dimethyl sulphoxide (DMSO) in 1:1 proportion. This was used as an undiluted or stock solution of essential oil. Control was maintained using 10% DMSO (Balouiri et al., 2016)

Synergistic studies of essential oils and antibiotics

The synergistic activity of crude essential oil from *Hedychium coronarium* and antibiotics was performed by disc diffusion method (Beargie et al., 1965). Mueller Hinton Agar (HiMedia Ltd.) was seed inoculated with 1ml of the inoculum (1×10^8 cfu/ml) and poured into the sterile petri plates and the plates were incubated overnight at 37°C. The synergistic activity was determined by measuring the diameter of the zone of inhibition. For sample preparation, sterile pre-impregnated antibiotic discs, sterile discs with 50 μ l of reconstituted essential oil prepared in 10% DMSO and sterile impregnated antibiotic discs coated with 50 μ l of reconstituted essential oil prepared in 10% DMSO were used. For each bacterial strain, controls used were sterile discs coated with 50 μ l of 10% DMSO. The experiment was done in triplicates and mean values were presented.

Biofilm-inhibition studies of the essential oils.

The antibiofilm activity was carried out using the microtiter dish Biofilm Formation Assay which uses the dye, crystal violet (CV) (O'Toole et al., 2011). The above-mentioned bacterial strains were allowed to enrich in a sterile Luria Bertanii medium overnight. The overnight culture was diluted 1:100 in the same medium. 100 μ l of the dilution was added per well in a sterile 96-well micro-titre dish. Nine wells/microorganism were inoculated for biofilm formation. The essential oil sample was added to the microtiter wells and the plates were incubated for 24 hours at 37 °C. In control no essential oil was added. After incubation, the plate was turned over and shaken to remove any unbound cells. The plate was gently washed with sterile water to remove unattached cells and media components. The washing step was repeated three times. 125 μ l of 1% solution of crystal violet was added to each well and the plate was incubated at room temperature for 15 minutes. The plate was rinsed 3-4 times in distilled water as mentioned above to remove excess stain and unbound cells. 200 μ l of ethanol was added to release and dissolve the stain in each well and allowed to stand for 15 minutes (Pourkhorasravi et al., 2021). The plate was shaken and blotted vigorously on a stack of tissue paper. The absorbance was measured at 630 nm using an Elisa plate reader (Meril diagnostics, Ltd.). The percentage biomass removal was calculated as:

$$\% \text{ biomass removal} = \frac{\text{O.D control at 630 nm} - \text{O.D. essential oil at 630 nm}}{\text{O.D. control at 630 nm}} \times 100$$

Red blood cell hemolytic assay for qualitative cytotoxicity of essential oils.

Hemolytic activity of crude essential oil of *Hedychium coronarium* J. Koenig was performed by modifying the method given by Ribeiro (Ribeiro et al., 2020). The essential oil was spot inoculated on sterile superimposed blood agar plate (SIBA). It was allowed to be absorbed for 15 minutes. The plate was incubated at 37°C for 24 hours and the zone of haemolysis was checked. The experiment was done in triplicates (Ribeiro et al., 2020).

RESULTS AND DISCUSSION

Hedychium coronarium J. Koenig essential oils extract was tested for its synergistic effect with four standard antibiotics impregnated on discs i.e. amikacin, gentamicin, tetracycline and vancomycin and the results are presented in Figure 1

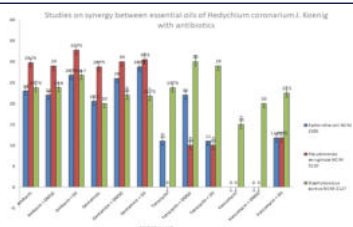
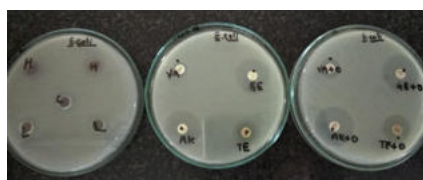


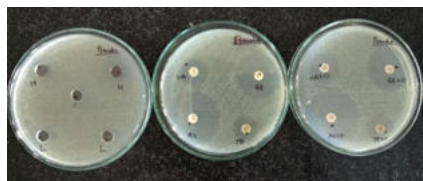
Figure 1: Studies on synergy between essential oils of Hedychium coronarium

J. Koenig with antibiotics

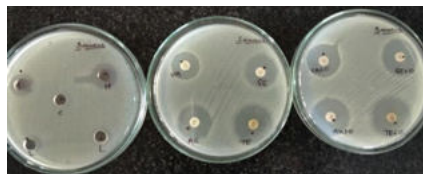
A significant synergistic effect was clearly seen between the essential oil and all four antibiotics against *Pseudomonas aeruginosa* NCIM 5210. Against *Escherichia coli* NCIM 2065, antibiotics amikacin, gentamicin and vancomycin showed synergistic effect but an additive effect was seen in case of tetracycline. In case of *Staphylococcus aureus* NCIM 2127, though a synergistic effect is not seen, the outcome of the combined treatment was more effective than the individual treatment. *Escherichia coli* NCIM 2065 showed sensitivity against vancomycin as well as the essential oil. *Pseudomonas aeruginosa* NCIM 5210 showed resistance against tetracycline and vancomycin. Both organisms became susceptible or a reversal of resistance was observed when the essential oil was used in combination with tetracycline and vancomycin (Figure 2).



A



B



C

Figure 2. Synergistic effects of antibiotics with essential oils of Hedychium coronarium

AK=Amikacin GE=Gentamycin, TE=Tetracycline and V=Vancomycin, O=oil, H= high concentration, L= low concentration, C= control

A. With *Escherichia coli* NCIM 2065 B. *Pseudomonas aeruginosa* NCIM 5210 C. *Staphylococcus aureus* NCIM 2127.

Explicit research has been reported on the antibacterial activity of the essential oils of this plant (Joshi et al., 2008). In the present study, the extracted oil had no effect against *Escherichia coli* NCIM 2065 when used individually. It has been cited in literature that various essential oils are synergy enhancers when used in combination with the standard drugs. This may be because natural compounds like terpenes present in essential oils disrupt the bacterial cell membrane in two ways; either by inducing changes in membrane structure or by inducing changes in membrane function. (Yap et al., 2014). Bacterial cell membrane is designed in such a way that it maintains the cell shape, allows cell growth, division and protection. Essential oils of oregano, tea-tree, some flavonoids from galangal, thyme, clove, gallic acids and essential oils from garlic have shown to have altered cell shape, cell structure, cell wall permeability, alteration in cell charge, integrity and

permeability of cytoplasmic membrane (Horne et al., 2001). There are very few reports of bacteria developing spontaneous resistance against essential oils. This may be because essential oils target numerous target sites. Also, if permeabilising effects are one of the modes of action of essential oils, then resistance is unlikely to develop. Gibbons et al. reported resistance modifying diterpenes, against strains of *Staphylococcus aureus* possessing the Tet (K), Msr (A), and Nor (A) multidrug resistance efflux mechanisms (Gibbons, 2005). In their study, none of the compounds displayed any antibacterial activity individually but in combination with tetracycline and erythromycin, a two-fold increase in the activities of these antibiotics was observed against two strains of *Staphylococcus aureus*. Many reported assays show additivity or moderate synergism, indicating that essential oils may offer possibilities for reducing antibiotic use (Langeveld et al., 2014).

Biofilm-inhibiting activity of extracted essential oil of Hedychium coronarium J. Koenig was tested against biofilms of *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 5210 and *Staphylococcus aureus* NCIM 2127 by the standard Microtiter Dish Biofilm Formation Assay (Figure 3).

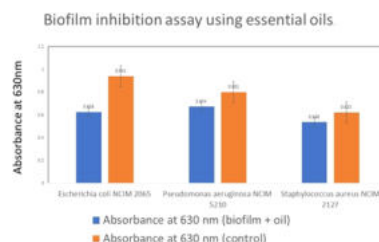


Figure 3. Biofilm reduction assay of the essential oil against *Escherichia coli* NCIM 2065

Biofilm-inhibiting activity was seen against all three microbes. The reduction against *Escherichia coli* NCIM 2065 was 33.47 %, against *Pseudomonas aeruginosa* NCIM 5210 15.85% and against *Staphylococcus aureus* NCIM 2127 13.67% (Figure 4).

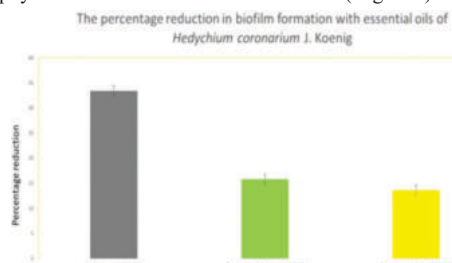


Figure 4: Percentage reduction in biofilm formation of *Escherichia coli* NCIM 2065,

Pseudomonas aeruginosa NCIM 5210, *Staphylococcus aureus* NCIM 2127 Biofilm-inhibiting activity of this medicinal plant has not been reported. Among the Zingiberaceae family members, the ethanolic extract of *Zingiber officinale* showed moderate antibiofilm activity against *Pseudomonas aeruginosa* ATCC 27853, the percentage reduction being 10.5 (Nikolić et al., 2014). Das et al., 2019 has found the percentage of biofilm inhibition by ginger essential oil to be highest against *Staphylococcus aureus* (94 %) followed by *Klebsiella pneumoniae* (91 %), *Escherichia coli* (89 %) and *Enterococcus faecalis* (83 %). Essential oils of oregano, carvacrol, thymol inhibit biofilm formation of *Staphylococcus aureus*, *Staphylococcus epidermidis* (Kerekes et al., 2015). The essential oil of *Satureja thymra* as well as its hydrosol fraction exhibited a strong antimicrobial action against both mono species and mixed-culture biofilms (Chorianopoulos et al., 2008). Szczepanski et al. and Kim et al. have reported various essential oils to inhibit biofilm formation and virulence of *Escherichia coli* 0157:H7 (Kim et al., 2016; Szczepanski & Lipski, 2013). Selected natural products that originate in plants can influence microbial biofilm formation through different mechanisms. The components of essential oils act as membrane permeabilizing agents which leads to proton, phosphate and potassium leakage. This further affects pH, homeostasis and equilibrium of inorganic ions (Lambert et al., 2001). The cytoplasmic membrane is damaged and the nucleic acids are lost. In 2012, Tian et al. reported that essential oils inhibit mitochondrial

ATPase activity (Tian et al., 2012) Carvacrol and thymol, the constituents of oregano essential oils diffuse through the polysaccharide matrix of the biofilm and destabilise it (Nostro et al., 2007). In 2014, Kumar et al., verified that phenolic compounds isolated from Zingiber officinale are quorum-sensing inhibitors of *Pseudomonas aeruginosa* MTCC 2297 biofilm (Kumar et al., 2014). Several studies have shown that the presence of terpenes in essential oils might be responsible for a wide spectrum of antibacterial activity.

Any product irrespective of origin has to comply with certain pharmacological and cytotoxicity analysis. Cytotoxicity studies have been carried out on the extracted oil. The method used here is a preliminary, qualitative test. The extracted essential oil showed no zone of haemolysis as seen in Figure 5, indicating the safety of its use. A similar test but a tube method and quantitative assay has been indicated by authors (Ribeiro et al., 2020).



Figure 5 : Hemolysis assay for effect of oil on red blood cells (No zone of inhibition)

Blood cells are the first to come across during the application of the topical preparations and also considering the fact that their cell membranes are extremely fragile. Prior to going for cell lines and animal testing this method could prove to be a fast and inexpensive tool in assessing the cytotoxicity of all preparations.

CONCLUSION

Medicinal and Aromatic Plants have been a source of medicines since ancient times. The medicinal properties of plants are due to various phytochemicals present in them; essential oils being one of them. It is well-known that the family Zingiberaceae consists of a variety of aromatic plants with high medicinal value. *Hedychium coronarium* J. Koenig belonging to this family is found to be rich in terpenes. Essential oils present in the rhizomes of this plant are known to be antimicrobial. When used in combination with antibiotics, a good synergistic effect was observed against bacteria as seen in the present study. The essential oils also showed good biofilm-inhibiting properties.

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REFERENCES

- Akinyele, T. A., Akinpelu, D. A., & Okoh, A. I. (2011). In vitro antilisterial properties of crude aqueous and n-hexane extracts of the husk of *Cocos nucifera*. *African Journal of Biotechnology*, 10(41), 8117–8121. <https://doi.org/10.5897/AJB11.330>
- Al-Shuneigat, J., Cox, S. D., & Markham, J. L. (2005). Effects of a topical essential oil-containing formulation on biofilm-forming coagulase-negative staphylococci. *Letters in Applied Microbiology*, 41(1), 52–55. <https://doi.org/10.1111/j.1472-765X.2005.01699.x>
- Balouiri, M., Sadiki, M., & Ibensouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpba.2015.11.005>
- Beargie, R. A., Bracken, E. C., Riley, H. D. Jr. (1965). Micromethod (Spot-Plate) Determination of In Vitro Antibiotic Susceptibility. *Applied Microbiology*, 13, (2), 279–280. <https://doi.org/10.1128/am.13.2.279-280.1965>
- Bisht, G. S., Awasthi, A. K., & Dhole, T. N. (2006). Antimicrobial activity of *Hedychium spicatum*. *Fitoterapia*, 77(3), 240–242. <https://doi.org/10.1016/j.fitote.2006.02.004>
- Chokshi, A., Sifri, Z., Cennimo, D., & Homg, H. (2019). Global contributors to antibiotic resistance. *Journal of Global Infectious Diseases*, 11(1), 36–42. <https://doi.org/10.4103/jgid.jgid.110.18>
- Chorianopoulos, N. G., Gaiouris, E. D., Skandamis, P. N., Haroutounian, S. A., & Nychas, G.-J. E. (2008). Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid-base sanitizers. *The Society for Applied Microbiology*, 104, 1586–1596. <https://doi.org/10.1111/j.1365-2672.2007.03694.x>
- Das, A., Dey, S., Sahoo, R. K., Sahoo, S., & Subudhi, E. (2019). Antibiofilm and Antibacterial Activity of Essential Oil Bearing Zingiber officinale Rosc. (Ginger) Rhizome Against Multi-drug Resistant Isolates. *Journal of Essential Oil-Bearing Plants*,

- 22(4), 1163–1171. <https://doi.org/10.1080/0972060X.2019.1683080>
- Feng, J., Tingting, L., Yee, R., Yuan Y., & Bai C. (2019). Stationary phase persister/biofilm microcolony of *Borrelia burgdorferi* causes more severe disease in a mouse model of Lyme arthritis: implications for understanding persistence. *Post-treatment Lyme Disease Syndrome (PTLDS)*, and treatment failure. *Discovery Medicine*, 27, 125–138.
- Gibbons, S. (2005). Plants as a source of bacterial resistance modulators and anti-infective agents. *Phytochemistry Reviews*, 4(1), 63–78. <https://doi.org/10.1007/s11101-005-2494-9>
- Horne, D., Holm, M., Ober, C., Chao, S., & Young, D. G. (2001). Antimicrobial effects of essential oils on *Streptococcus pneumoniae*. *Journal of Essential Oil Research*, 13(5), 387–392. <https://doi.org/10.1080/10412905.2001.9712241>
- Huang, R., Li, M., & Gregory, R. L. (2011). Bacterial interactions in dental biofilm. *Virulence*, 2(5), 435–444. <https://doi.org/10.4161/viru.2.5.16140>
- Hyldgaard, M., Mygind, T., Meyer, R. L., Hayashi, M. A. F., & Knapp, C. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, 1(3), 1–24. <https://doi.org/10.3389/fmicb.2012.00012>
- Joshi, S., Chanotiya, C. S., Agarwal, G., Prakesh, O., Pant, A. K., & Methela, C. S. (2008). Terpenoid compositions and antioxidant and antimicrobial properties of the rhizome essential oil of different *Hedychium* species. *Chemistry and Biodiversity*, 5, 299–309.
- Kerekes, E.-B., Deák, É., Takó, M., Tserenadmird, R., Petkovits, T., Vágvolgyi, C., & Krisch, J. (2013). Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. *Journal of Applied Microbiology*, 115, 933–942.
- Kim, Y. G., Lee, J. H., Gwon, G., Kim, S. I., Park, J. G., & Lee, J. (2016). Essential Oils and Eugenols Inhibit Biofilm Formation and the Virulence of *Escherichia coli* O157:H7. *Scientific Reports*, 6, 36377. <https://doi.org/10.1038/srep36377>
- Kumar, V. N., Murthy, P. S., Manjunatha, J. R., & Bettadaiah, B. K. (2014). Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. *Food Chemistry*, 159, 451–457. <https://doi.org/10.1016/j.foodchem.2014.03.039>
- Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G.-J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, 91, 453–462.
- Langeveld, W. T., Veldhuizen, E. J. A., & Burt, S. A. (2014). Synergy between essential oil components and antibiotics: A review. *Critical Reviews in Microbiology*, 40(1), 76–94. <https://doi.org/10.3109/1040841X.2013.763219>
- Larayetan, R., Ololade, Z. S., Ogunmola, O. O., & Ladokun, A. (2019). Antimicrobial, Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of *Callistemon citrinus*. *Evidence-Based Complementary and Alternative Medicine*, 1–14. <https://doi.org/10.1155/2019/5410923>
- Lewis K. (2005). *Persister Cells and the Riddle of Biofilm Survival*. *Biochemistry (Moscow)*, 70(2), 267–274.
- Neu Harold C. (1992). The crisis in antibiotic resistance.
- Nikolić, M., Glamočlija, J., Ferreira, I. C. F. R., Calhella, R. C., Fernandes, Á., Marković, T., Marković, D., Giweli, A., & Soković, M. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and *Reut and Thymus vulgaris* L. essential oils. *Industrial Crops and Products*, 52, 183–190. <https://doi.org/10.1016/j.indcrop.2013.10.006>
- Nostro, A., Roccaro, A. S., Bisignano, G., Marino, A., Cannatelli, M. A., Pizzimenti, F. C., Cioni, P. L., Procopio, F., & Blanco, A. R. (2007). Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Journal of Medical Microbiology*, 56(4), 519–523. <https://doi.org/10.1099/jmm.0.46804-0>
- O'Toole G. A. (2011). Microtiter dish biofilm formation assay. *Journal of visualized experiments: JoVE*, (47), 2437. <https://doi.org/10.3791/2437>
- Pachurekar, P., & Dixit, A. K. (2017b). A Review on Pharmacognostical Phytochemical and Ethnomedicinal Properties of *Hedychium coronarium* J. Koenig an Endangered Medicine. *International Journal of Chinese Medicine*, 1(2), 49–61. <https://doi.org/10.11648/j.ijcm.20170102.13>
- Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 10. <http://www.ajbrui.com/http://www.bioline.br/mdhttp://www.ajol.com>
- Pourkhoravani, E., Delghani Nayeri, F., & Mohammadi Bazargani, M. (2021). Decoding antibacterial and antibiofilm properties of cinnamon and cardamom essential oils: a combined molecular docking and experimental study. *AMB Express*, 11(1). <https://doi.org/10.1186/s13568-021-01305-6>
- Ribeiro, N. M., Reboças De Araújo, I. D., Carlos, A., Júnior, V., Araújo, G. M., Araújo, R. M., Cavalcanti De Albuquerque, C., Fernandes, J. V., & Andrade, V. S. (2020). Red Blood Cell Hemolytic Assay: An Alternative To Assess Cytotoxicity Of Essential Oils. <http://www.journalijdr.com>
- Sharifi-Rad, J., Sureda, A., Tenore, G. C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M. R., Ademiulyi, A. O., Sharifi-Rad, R., Ayatollahi, S. A., Iriti, M., & Mephee, D. J. (2017). molecules Biological Activities of Essential Oils: From Plant Chemecology to Traditional Healing Systems. *Molecules*, 22, 70. <https://doi.org/10.3390/molecules22010070>
- Szczepanski, S., & Lipski, A. (2013). Essential oils show specific inhibiting effects on bacterial biofilm formation. *Food Control*, 36(1), 224–229. <https://doi.org/10.1016/j.foodcont.2013.08.023>
- Taylor, C. S., & Goyal, A. (2015). A Comprehensive Review On *Hedychium coronarium* J. Koenig. (Dolanchampa/Kapurkachri). *International Journal of Research in Ayurveda & Pharmacology*, 6(1), 98–100. <https://doi.org/10.7897/2277-4343.06121>
- Tian, J., Ban, X., Zeng, H., He, J., & Chen, Y. (2012). The Mechanism of Antifungal Action of Essential Oil from *Dill* (*Anethum graveolens* L.) on *Aspergillus flavus*. *PLoS ONE*, 7(1). <https://doi.org/10.1371/journal.pone.0030147>
- Verma M., & Bansal Y. K. (2010). Butterfly Lilly (*Hedychium coronarium* J.Koenig): An Endangered Medicinal Plant. *Plant Archives*, 10(2), 841–843. <https://doi.org/10.13140/2.1.4350.0168>
- Verma, P. & Kundu, R. (2020). The Ginger Prophecy: A Review of the unexplored genus *Hedychium* for Cancer. *Indian Journal of Pharmaceutical Sciences*, 82(1), 11–20. <https://doi.org/10.36468/pharmaceutical-sciences.618>
- Wei, Q., Bhasme, P., Wang, Z., Wang, L., Wang, S., Zeng, Y., Wang, Y., Ma, L. Z., & Li, Y. (2020). Chinese medicinal herb extract inhibits PQS-mediated quorum sensing system in *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology*, 248. <https://doi.org/10.1016/j.jep.2019.112272>
- Yap, P. S., Yiap, B. C., Ping, H. C., & Lim, S. H. (2014). Essential oils, A new horizon in combating bacterial antibiotic resistance. *The Open Microbiology Journal*, 8, 6–14. <https://doi.org/10.2174/1874285801408010006>
- Zhao, X., Yu, Z., & Ding, T. (2020). Quorum-sensing regulation of antimicrobial resistance in bacteria. *Microorganisms*, 8, (3). MDPI AG. <https://doi.org/10.3390/microorganisms8030425>