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„Quorum Sensing and Essential Oils“

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Abstract

Quorum Sensing (QS) describes the ability of bacteria and other organisms to sense the quantity of individuals in their surrounding by means of signal molecules. As soon as a certain concentration is reached, the population can react with different responses, such as production of exotoxins or biofilms. This way of communication is of pharmaceutical interest, as for example biofilm-formation complicates the treatment of infections and therefore increases their pathogenicity. Consequently, inhibition of QS is a possible new target of antibiotics.

Essential oils (EOs) represent a group of natural QS-inhibitors. This thesis gives an overview of QS-systems in different organisms and discusses research results as well as the used methods to identify inhibitory potential of EOs.

Abstract

Quorum Sensing (QS) beschreibt die Fähigkeit von Bakterien und anderen Organismen, mithilfe von Signalmolekülen die Zahl der sie umgebenden Individuen zu registrieren. Beim Erreichen einer bestimmten Konzentration dieser Moleküle reagiert die Population mit zB. der Produktion von Exotoxinen oder mit der Bildung von Biofilmen. Diese Fähigkeit der Kommunikation ist unter anderem von pharmazeutischem Interesse, da die Konsequenzen von Biofilmbildung zum Beispiel die Behandlung von Infektionen erschwert bzw. die Pathogenität von Bakterienpopulationen steigert. Die Inhibition von QS ist daher ein alternativer Angriffspunkt für Antibiotika.

Ätherische Öle stellen hierbei eine natürliche Gruppe von QS-Inhibitoren dar. Diese Arbeit gibt einen Überblick über QS –Systeme verschiedener Organismen und erörtert Forschungsergebnisse sowie die angewandten Methoden zur Identifizierung der inhibitorischen Aktivität von ätherischen Ölen.

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1. Introduction

a.Cell-to-cell communication

Bacteria have the ability to communicate with each other by producing signal molecules, which they release into their environment. The fundaments of cell-to-cell communication can be subdivided in three stages: detection, signal transduction and response. Signal molecules are being detected when binding to extracellular receptors or intracellular receptor proteins. Ligand-binding leads to a change of confirmation on the receptor - which then causes an intracellular signalling cascade. This cascade then leads to a specific reaction- for example the activation of genes. [1]

b. Quorum Sensing – Definiton

QS describes die ability of bacteria to sense the number of bacteria in their environment by using signal molecules, also called autoinducers. This way of communication is important to coordinate group behaviour depending on the number of bacteria in the environment of a bacterial population. As soon as a critical external concentration of signal molecules ("Quorum") is reached, the population reacts by modifying its gene expression. This means the production of these signals is only effective when being secreted from a large number of cells.8[2]

Various species of bacteria have developed different signalling molecules for QS, which means that a direct communication between different classes is not necessarily possible. If there is an interspecies communication it is called "cross talk". It is also possible for one species of bacteria to have several different QS-signals which lead to different reactions – whereby the main reason for this kind of cell-to-cell communication appears to be the adaption to changing environmental conditions or the defence of toxic substances or other organisms.[3] Examples of cell-reactions caused by QS are bioluminescence, the expression of virulence factors, proliferation, sporulation, mating and biofilm-formation. [4] Most research on Quorum sensing signalling-circuits refer to systems based on either acyl-homoserine-lactones (AHLs) or peptides as signal molecules.

2. QS in bacteria

a. Gram-negative bacteria

1) The LUXRI -System

The research on QS began in the early 1970s, when Nealson, Platt and Hastings found out that *Vibrio fisheri* and *Vibrio harveyi* produce signal molecules when growing. As soon as a critical concentration of these molecules is reached, luminescence of the bacteria is activated. This team was also the first to assign the term "autoinducer"- for Quorum Sensing molecules [5]. The term "Quorum Sensing" was first mentioned 1995 by Fuqua, Winans and Greenberg in a journal of bacteriology – their research on QS was based on the question why the Gram-negative marine bacterium *V. fischeri* contains high levels of luciferase only when a large number of individuals is present. [6]

In nature, *V.fisheri* populations live- inter alia - in the light organs of the squid *Euprymna scolopes*. Visik et al. found out that this symbiosis is essential for the bacteria to reach the necessary cell-density for the synthesis of luciferase. [7] Eberhard et al. isolated an identified the QS-signal of *V.fisheri* in 1981 – the signal **3-O-C6-homoserine-lactone** (N-(3-oxohexanoyl)-L-homoserinlacton) (HSL or AHL) is an N-acyl-lactone [see page 10, Fig.1] [8]

Engelbrecht and Silverman found out that the luciferase operon is controlled by the expression of the proteins LUXI and LUXR, whereas LUXI is necessary for the synthesis of AHL and LUXR is a specific cytoplasmic receptor for the autoinducer and therefore regulates the response to the autoinducer.[9] Enzymes such as luciferase and being part of the luminescence system in bacteria are encoded by lux (lux lat. for light) genes. Hence, proteins with names starting with LUX indicate their correspondence to luminescence genes (lux). [10] Stevens et al. proved that as soon as a critical concentration is reached, LUXR specifically binds the QS-molecule and activates an operon which then leads to the transcription of the luciferase.[11]

AHL molecules differ in their ability to permeate cell membranes. Whitehead et. al suggested that this is caused by their structural variability (acyl side chain). [12] For example: Kaplan & Greenberg compared the cellular and external concentrations of autoinducer and found out that *V.fisheri* cells are freely permeable for the molecules – the autoinducer can diffuse into and out of the cell, [13] whereas Pearson et al. showed that there is an active efflux pump for HSL in *P. aeruginosa* – which produces – amongst others- a 3OC12-HSL as AHL-QS-molecule [see Fig. 1 / Lasl] [14]

In summary, this signalling circuit consisting of a synthase-regulator complex which is dependant to an AHL and when being activated leading to the expression of specific genes and is typically for Gram-negative bacteria. There are over 50 species using AHL molecules as autoinducers. These systems are referred to as LUXRI-homologous systems, whereas the nomenclature consists out of the genes / corresponding proteins controlling the QS-circuit (e.g. las/LAS) and other letters indicating the respective function (e.g. I = AHL synthesizing protein, R= AHL-receptor) resulting in QS-system designated e.g. lasRI in *P. aeruginosa.* [4] Other bacteria using the LUXRI –homologous system for QS are for instance:*, Aeromonas hydrophila, A. salmonicida,* and *Chromobacterium violaceum*. [6] To give an example of QS-Systems being partly homologous to LUXRI, the QS-systems of *V.fisheri*, *P. aeruginosa* are being presented. As *C. violaceum* is a typical strain used for QS-detection its QS-system is also being introduced.

Vibrio Harveyi

Besides the LUXRI System, Gram-negative bacteria also use other signalling pathways; one example is *V.harveyi*, a marine Gram-negative bacterium that also uses QS for bioluminescence. *V.harveyi* produces three different autoinducers:

1) Cao & Meighen identified an N-acyl-homoserin-lacton, as autoinducer (**3-hydroxy-C4-HSL** [Fig.1.], named **harveyi autoinducer 1** (HAI-1) [15] However it was observed that the genes involved in the QS-circuit are not homologous to the ones in the LUXRI-System described earlier. [16]

2) The second autoinducer discovers was named AI-2 (autoinducer 2) , a furanosyl borate diester.[17,18] Surette et al. identified the genes for luxS which is responsible for the production of AI-2.[19] LUXS binds periplasmatic to LUXP- this complex then interacts with a membrane-bound histidine kinase – LuxQ. [Lux Q, just like LUXN has the same response regulator domains like those in two-component signal transduction systems in grampositive bacteria.[17]
3) Henke & Bassler discovered a third QS-System in *V. harveyi* – being named after the bacterial strain in which the system was primary discovered: *Vibrio cholerae*, a bacterium causing the disease Cholera. The autoinducer (AI) is called *cholerae* autoinducer 1 (CAI-1) which is produced by CqsA (Cqs= *cholerae* quorum-sensing autoinducer) and binds to CqsS (=sensor). [20]

Pseudomonas aeruginosa

P. aeruginosa can cause severe and persistent nosocomial and wound infections as they are often resistant to several antibiotics. Affected patient groups of those nosocomial infections are for example cystic fibrosis patients, immune-compromised, elderly or long term intubated patients. It has been shown that QS plays a role in virulence gene expression- for instance by murine models with pneumonia, showing reduced virulence of the bacteria when deleting QS-genes. As *P.aeruginosa* is such an important human pathogen, it is often used as a model organism for QS-research.

P.aeruginosa has several virulence mechanisms, namely Iron scavenging (proteases, siderophores), cytotoxity (pyocyanin, exotoxin A), Antibiotic resistance (efflux pumps, modifying enzymes), immune evasion (elastase, alkaline protease), biofilm-development (alginate, rhamnolipids) and motility (flagella, type IV pili).

Some of the genes regulating those virulence mechanisms are known to be regulated by QS, examples are elastase, proteases, hydrogen cyanide, exotoxin A, secretion proteins, catalase, rhamnolipid, pyocyanin, lectins, acylated HSLs and pyoverdin. It has also been shown, that QSM of *P.aeruginosa* influence the production of several eucaroytic cytokines (IL-8, IL12, TNF-β) – which may also influence its pathogenicity. [21–23]

There are four main QS systems identified in *P.aeruginosa*, whereas 1) and 2) are LUXRI-homologous systems. The QS-systems are closely connected to each other

showing a complex communication network including various components mutually influencing each other.

1) The LasRI- System,named after its **elastas**e-regulating properties has the AI **N-(3-oxododecanoyl)-homoserine lactone (OdDHL)** [Fig.1.].
2) The RhlIR- System, designated after the RhlR. RhlR works as AI-receptor of this system and is a protein encoded by a **rhamnolipid** synthase gene cluster. The belonging AIs of this QS-system are **N-butyryl-L-homoserine-lactone**s=C4-HSL= **BHL**). [Fig.1.][23]

Both of the pathways where shown to be regulated by various mechanisms, an example are QscR (quorum-sensing-control repressor) [24] and VqsR (virulence and quorum-sensing regulator) [25], being LuxR homologues. QscR inhibits both pathways by forming LasR-QSM / RhIR-QSM – complexes, on the other hand it binds to OdDHL and activated its own regulon. VqsR positively activates the LasRI-system and is regulated by the LasR-QSM-complex. Just like QscR and VqsR , there are many other super-regulators which reflects the complexity of QS-regulation in *P.aeruginosa.* [23]

Virulence factors controlled by the las-system are for example elastase- causing degradation of different matrix proteins (collagen, elastin) and protease- causing disruption of epithelial barriers. A gene (RhlAB) controlled by the RhlIR-system codes for rhamnolipids and causes necrosis of host macrophages and polymorphonuclear lymphocytes.[23] Reimmann et al. also showed the correlation between OdDHL-presence, virulence-factors (pyocyanine, elastase, rhamnolipids, hydrogen cyanide,) and swarming motility- as both the virulence factors and motility decreased when OdDHL was reduced. [26]

3) Quinolone-based QS

The main QSM was found to be **2-heptyl- 3-hydroxy-4-quinolone** [Fig.1.], also known as PQS – the pseudomonas quinolone signal, the belonging receptor is PqsR (pseudomonas quinolone signal receptor). Research also determined a connection between the quinolone-system and LasR, as part of the PQS synthesis cluster is controlled by LasR and turn-controlled of PQS by the LasR-OdDHL. Quinolone-based QS was shown to influence biofilm-formation and virulence-factor-production

(pyocyanin, elastase, PA-IL lectin, rhamnolipids) as null mutation of the PQS-system leads to reduced biofilm-formation and virulence-factor-production respectively. [23]

4) Integrated Quorum Sensing Signal (IQS) based QS

The belonging AI is **2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde** [Fig.1.] belonging to a new class of QSM. Disruption of this system causes reduced production of the QSM PQS and BHL and also pyocyanin, rhamnolipids and elastase are decreased. [23]

Summarized, there is a clear connection between virulence and QS and therefore inhibition of QS-pathways could lead to reduced virulence in *P.aeruginosa* being a promising approach for new treatments of infections caused by those bacteria. It is worth a mention, that as explained above, QS plays a role in biofilm formation but is only one of several regulating factors such as swarming motility, rhamnolipids and siderophors. [27]

Structures of V.fishery, V.harveyi and P.aeruginosa QS-signals

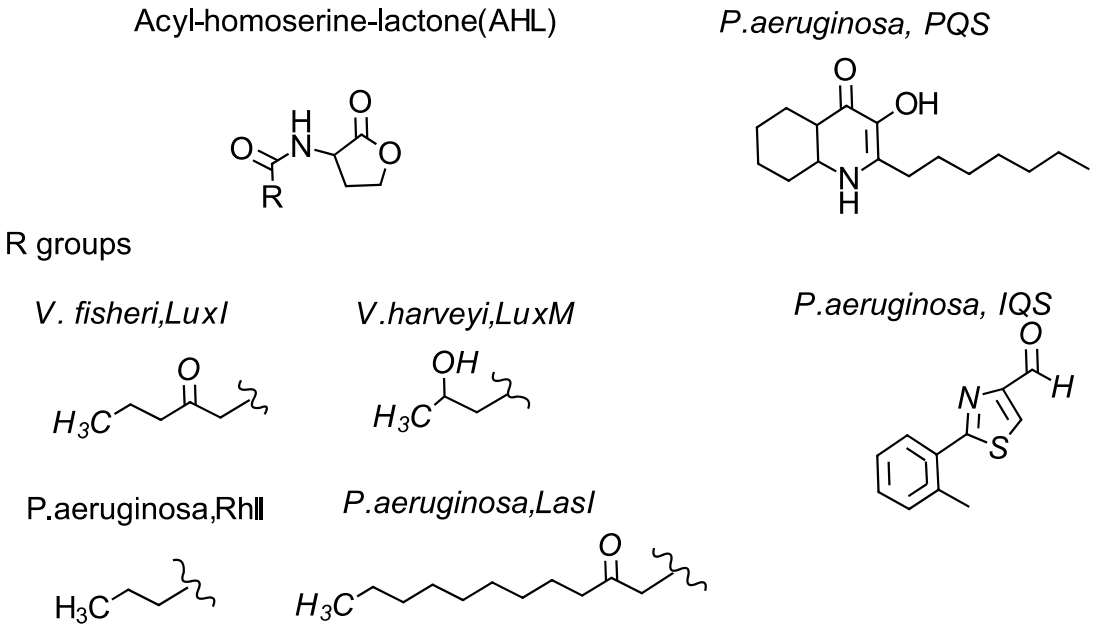

Fig.1. [adapted and newly drawn from Waters,Bassler][2]

Chromobacterium Violaceum

Given the fact that in most of the research done on EOs as QS-inhibitors, different strains of *C.violaceum* where being used as sensor, its QS-system is presented her. *C.violaceum* is an aquatic bacterium growing in soil and water which can cause abscesses and bacteraemia in human. The QS-system consists of LuxRI-homologues, namely **CviIR** (Cv =*C.violaceum*).

Research showed that antagonists of natural AHLs can reduce the pathogenicity by means of confirmation-modification of CviR of *C.violaceum* on the nematode *Caenorhabditis elegans*, allowing the presumption that QSI may also reduce virulence in of *C.violaceum* in human infections. One well studied gene product controlled by QS in *C.violaceum* is the purple pigment violacein. The violacein gene cluster consists out of four genes: vioABCD, whereas the vioA-promotor is directly controlled by CviR.

To detect QS-inhibition, different strains of *C.violaceum* are being used, they are listed in the chapter "Materials and methods". [28,29]

b. Gram-positive bacteria

Gram-positive bacteria use autoinducing peptides (AIPs) as signals. AIPs cannot freely diffuse through the membrane; they bind on extracellular histidine kinase receptors. This signalling way is classified as a "two-component" type, as it consists of a sensor and a response-regulator protein. As soon as the molecule binds, the kinase activity of the receptor is activated which leads to a phosphorylation cascade resulting in the binding of a responsive regulator, a DNA-binding regulator controlling the transcription of QS-genes. Bacteria using this kind of signalling path are *Bacillus subtilis, Streptococcus pneumonaiae* and *Staphylococcus aureus*. For the excretion of AIPs, bacteria often express ATP-binding cassette ABC-transporter[30]

3. QS in fungi

Although there has been done a lot of research on QS in bacteria in the last decades, QS in fungi was not investigated until 2001, when Hornby et al. discovered the extracellular QS-molecule farnesol in the dimorphic fungus *Candida albicans*. [31] *C.albicans* is one of the most frequent human fungal pathogens causing for example. catheter-related infections. [32]

In yeast-mycelium fungi, there is a direct correlation between cell morphology – either formation of budding yeasts (high cell density) or germ tubes (low cell density) - on initial cell density. This dependence is called the "inoculum size effect", firstly mentioned by Kulkarni and Nickerson in 1980. [33] The isoprenoid farnesol *in Candida albicans* causes a shift in morphology, resulting in inhibition of mycelial growth and formation of budding yeasts. Hornby et al. set up the hypothesis that Farnesol may be a QSM as its physical properties are suitable, in contrast to other extracellular molecules produced by *C.albicans* (geranylgeraniol, geraniol). These characteristics include the solubility in water, the lipophilicity and the fact that farnesol is produced continuously during cell growth in an amount proportionally to cell mass. [31]

Ramage et al.[32] showed a biofilm-inhibiting effect of farnesol, the inhibition was shown to be dependent on the time cells had to adhere to a surface before farnesol was added. They hypothesized that in presence of high farnesol-concentrations, mycelial development is inhibited and therefore biofilm formation is reduced. The cause of this may be the detachment of cells in order to colonize new substrate areas and to prevent overpopulation. [32,34]

In 2001, Oh et al. identified farnesoic acid as another autoregulatory substance also inhibiting filamentous growth in a strain of *C.albicans*.[35]Cho et al. showed that tyrosol is an autoregulatory molecule in *C.albicans*. It's effects are the shortening of the lag-phase of diluted cultures as well as the opposite effect of farnesol regarding germ tube formation– it accelerates the conversion from yeasts to mycelium. [36]

EOs as biofilm-inhibitors in C.albicans?

As mentioned above, farnesol, a sesquiterpene-alcohol, inhibits biofilm-formation due to morphological changes. As terpenoid structures are also constituents of essential oils, maybe EOs interfere with the fungal QS-system similarly and are therefore able to inhibit biofilm-formation. Agarwal et al. positively tested several plant oils on their biofilm-inhibiting activity, although it has not been clarified if this has any connection to QS. [37]

4. QS in *Saccharomyces cerevisiae*

Chen et al. showed that nitrogen starvation in *S. cerevisiae* results in the production of aromatic alcohols functioning as QSM. These molecules again lead to a morphogenetic switch from budding yeasts to the filamentous form. [38]

Besides *C.albicans and S.cerevisiae*, QS activities in other species (*Ceratocystis ulmi, Cryptococcus neoformans, Uromyces phaseoli, Neurospora crassa*,..) have been described, but research on which molecules are responsible and how the signalling pathways work is still at the beginning and processes are not yet understood. [34,39]

5. QS and Biofilm formation

A biofilm is "a thin layer of microorganisms adhering to the surface of a structure, which may be organic or inorganic, together with the polymers that they secrete". [40]

In several species, QS is involved in biofilm development, however the research on this topic is still at the beginning and there is only little knowledge about mutual influence between the biofilm (formation, pathogenic potential, structure,.) and the QS-system (onset of gene expression, interspecies signalling) [41].

Biofilms can not only be found attached on medical devices and implants including for instance catheters, orthopaedic implants or cardiac pacemakers but also adhere to endothelial or epithelial lining in lungs, intestinal or vaginal mucus layer or teeth. Biofilm-formation can result in significantly increased resistance against antibiotics and host defences and can therefore lead to chronic inflammations or impaired wound healing. [42]

Other fields, where biofilms cause worries are agriculture, fishery (fish - pathogenic bacteria like *Aeromonas hydrophila or A. salmonicida)* and water treatment plants (especially biofilms on reverse osmosis membranes built by the species *Legionella* in interaction with mainly γ-proteobacteria cause economic losses) . As biofilms occur ubiquitous, they are generally associated with deterioration of materials they populate.[43]Biofilm-formation can be divided in three stages: adhesion to a surface, maturation of the biofilm meaning cell-division and production an extracellular matrix in order to protect the bacteria from environmental stresses. The last stage is dispersal to allow bacteria to escape the biofilm community. [44]

Whereas Solano et al. believe that QS is most reasonable in the last step of biofilm disassembly as the signal density reaches sufficient concentrations only then [45], research has showed that QS influences all stages of biofilm formation [41]. In human pathogens *Staphylococcus aureus* [46] , *Heliobacter pylori* [47] and *Salmonella enterica* [48] QS plays a role in attachement-phase of biofilm -formation[49].
An example for an AHL-based QS System responsible for biofilm-maturation is *Aeromonas hydrophila* [50]. Lynch et al. found out that the plant pathogen *Xanthomonas campestris* uses a QS-system which is involved in biofilm dispersal. [50]

6. QS-inhibitors (QSIs)

As described earlier, QS is involved in biofilm formation and production of virulence factors and the inhibition of this way of communicating is therefore a promising target for new antibiotics.

Kalia summarized the possible targeting points of QS inhibitors (with regard to AHL QS-systems): Activity-reduction of the AHL receptor protein or synthase inhibition of the AI-production, degradation of the QSM, usage of QSM-analogues, decoy receptors or antibodies targeting the AHL receptors.

To effectively select QS molecules it has been proposed that QSI should be highly specific small molecules - preferably longer than the native AHL- to efficiently reduce QS-regulated gene expression. There should preferably be no adverse effects neither on bacteria nor host. QSM should be chemically stable and as far as possible resistant to the host's metabolic degradation. Meeting all this criteria, the chance of resistance-formation is reduced, the risk of harming beneficial bacteria in the present of the host is low and due to the low molecular the risk of antigenicity is low too. [43]

A term often used when discussing QSI is "Quorum Quenching" , meaning the quench or interference with Quorum sensing systems. [51]

There can be made rough division in natural (prokaryotic, animal-based, fungus based, plant based, ..) and synthetical QS-inhibitors (e.g. modified AHL-molecules, antagonists of receptor-ligand-interactions) [43]

7. Essential Oils as QS-inhibitors

An Essential oil (EO) was defined by the International Organization for Standardization (ISO 9235:2013) as "a product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after separation of the aqueous phase — if any — by physical processes". [52] EOs represent a great reservoir of possible new therapeutics as a single oil may contain more than 50 components. The research on EO as QSI in human medicine as well as in food industry, plant pathology and so on has been growing in the last years. [53,54] EOs already are widely used because of their antibacterial properties in for example antiseptics and preservatives (for example food preservation). [55] Main advantages of EOs used as antimicrobials are that their mammalian toxicity is low, methods of obtaining of EOs are relatively easy and they are environmentally friendly as they are being degraded quickly in water and soil. [53]

As mentioned before, Kalia et al. suggested, that QSI should preferably be small molecules with longer side chain than the natural AHLs. EOs are small molecules and there can be found constituents with longer acyl chains than those of some natural AHLs. [43]

When looking at examples of EO-components like the acyclic monoterpene citral compared to a typical AHL-AI, namely N-hexanoyl-homoserinelactone (C-6-HSL) produced by *C.violaceum* there is a clear resemblance, from which one might assume that EO-components could bind to AHL-receptors and therefore function as antagonists. Especially those acyclic monoterpens show resemblance due to their partly branched side chain as well as sesquiterpenes like farnesol, whereas on the first sight, cyclic components like menthol or the phenylporpanoide eugenol show less resemblance.

Several EOs have been tested on their anti-QS activity, most of them using strains of *C.violaceum* or *P.aeruginosa* as sensor organisms. This means, research on EO as QSI is currently concentrated on AHL-based quorum sensing. It is not without relevance to mention that especially biofilms are problematic in human infections or in industry. As current QSI- tests on *C.violaceum* concentrate only on the mechanism of signal inhibiting causing less pigment production, not including information on biofilm–formation. Those assays can therefore be considered as rough screening methods for inhibitors of luxRI-based QS but it is not possible to give a presumption on whether the EO would inhibit biofilm formation or inhibit QS in other organisms with similar QS-systems too.

Researches done on the QS-inhibitory effect of EOs on *P.aeruginosa* are more advanced, as they quantify virulence-factor-expression/ swarming motility and biofilm formation. As it is known that QS plays a major role in these areas and even some of the associated genes are known, this seems to be a more applicable model, as the tested factors are jointly responsible for the pathogenicity of *P.aeruginosa* infections.

Principle of QSI-Activity Testing of EOs:

EOs are chosen; usually different concentrations are being tested. As many of the EOs are known to inhibit bacterial growth, the research team often use sub-MIC-concentrations to detect QS independently from growth inhibition. Other methods to observe QS independently are concurrent cell viability tests.

In order to identify the components of the used essential oils, most of the research teams did GC-MS-analyses. As EOs differ in their composition depending on factors like collecting point, growth conditions, production method etc. this is an important step before the following testing's of the oils. An alternative method is to use EOs complying to a pharmacopoeia, as quality and percentages of components have to meet certain criteria.

In order to prove an inhibitory activity of the oils, different bacterial strains with known QS-systems can be used showing a certain reaction when QS is inhibited.

This can be e.g. less pigment production (*C.violaceum*), reduced virulence-factor-production (*P.aeruginosa*) or reduced fluorescence.

a. Materials and Methods

This chapter gives an overview on possible methods to detect QS-Inhibition of EOs and are based on those used in the discussed research results in the following chapter, however no warranty on completeness or accuracy of the given data is taken over. Primary, sensor strains and their belonging QS-inhibition detection method as well as their autoinducers (AI) are summarized; more detailed characterization of the strains is given subsequent to the table. Afterwards, common QS-inhibition assays are being presented.

Sensor strains used for QS-inhibition-detection

Sensor strain	**detection method**	**AI**
C.violaceum (CV) ATCC12472	violacein	3-OH-C10-HSL
CV VIR07	violacein	C6-C10 AHL
CV ATCC31532	violacein	C6-HSL
CV ATCC 31592	violacein	
CV026	violacein	C4 to C8-HSL
QSIS 1	green fluorescence	3-oxo-C6-HSL
Pseudomonas putida F117 +(pRK-C12)	green fluorescence	C12-HSL
E.coli MT102 + (pJBA132)	green fluorescence	C6-HSL
E.coli [pSB401]	luminescence	short-chain-AHLs
E. coli [pSB1075]	luminescence	long-chain-AHLs
E. coli DH5α [pJN105L + pSC11]	β-galactosidase	AHLs
E.coli + pKDT17	β-galactosidase	
P. aeruginosa PAO1	virulence factors, biofilm, swarming motility	several
P. aeruginosa qsc 119	β-galactosidase	AHLs *(P.aeruginosa)*
P.aeruginosa HT5	Virulence factors, biofilm	
Aeromonas hydrophila	virulence factors, biofilm	C-4-HSL , C-6-HSL

***C.Violaceum* strains:**

ATCC12472 : This wild type strain produces and responds to the AHLs by producing the pigment violacein. In presence of a QS-inhibitor, violacein production is inhibited or decreases which can be evaluated as QSI-activity. The major AHL is 3-hydroxy-C10-HSL.[56]

VIR07: VIR07 is a mutant strain of ATCC12472, the gene for AHL-Production (cviI, LUXi-homologue) was deleted. Violacein production is inhibited by C6-C8 HSLs and C4HSLs. Violacein-production is induced by long chain HSLs C10-C16 (mainly C10). [56]

ATCC31532: This wild type strain has N-hexanoyl-L-homoserine lactone (C6-HSL) as QSM and produces violacein in presence of this QSM. (production of antibiotics, protease, chitinase are controlled by QS too) [57]

CV026 : CV026 is a mutant of the wild-type strain ATCC31532 which is unable to produce AHL-molecules on its own, therefore exogenous C6-HSL molecules have to be added. Violacein-production is induced by C4-C8-HSLs, whereas C10-C14 HSLs inhibit it. [28,56]

CV ATCC31592: Alvarez et al [58]used this strain as a biosensor to test the EO of Hyptis Dilatata via Violacein production.

QSIS 1 : This strain is one of the constructed strains used for the QSI-Selector method A. [It was developed by Rassmussen et al. [59]Based on a luxI-gfp-reporter, the lux-I promotor region was fused to ohIA from *S.liquefaciens*. If a QSI is present (and 3-oxo-C6-HSL), cells can survive. [59]

Pseudomonas putida F117 (pRK-C12) + E.coli MT102 (pJBA132)

These strains are not able to produce AHL on their own. They sense AHLs with a sensor plasmid. This plasmid expresses a promotor controlled by AHL and is fused

to a gfp-gene. The LuxR-receptor protein is specific for long chain-AHLs (C12) in *P.putida* and for short chain AHLs (C6) in *E.coli.*

E.coli [pSB401] and [pSB1075]

These strains contain an N-acyl-HSL-sensor plasmids, constructed by Winson et al. [60]

PsB401: The plasmid contains a fusion of the genes luxRI' (*Photobacterium fisheri*) and luxCDABE (*Photorhabdus luminescence*). The strains shows bioluminescence in response to short-chain-AHLs.

PsB1075: The plasmid contains a fusion of the genes lasRI'(*P.aeruginosa PAO1*) and luxCDABE (*Photorhabdus luminescence*). The strain shows bioluminescence in response to long-chain-AHLs. [60,61]

E. coli DH5α harbouring pJN105L and pSC11

This strain contains lasR and PlasI::lacZ and therefore can be used as bioreporter strain, as their activity depends on the presence of AHL-molecules. Kalia et al used this strain to detect 3-oxo-C12-HSL produced by PAO1. [27]

E.coli harbouring pKDT17:

This is another sensor strain containing a plasmid with a lasB'-lacZ reporter showing lasB promoter activity and lasR being controlled by the lac promoter. [62] Again β-galactosidase-production is dependent on the presence of AHL-molecules.

P. aeruginosa – strains

PAO1: PAO1 is a wild type strain of *P. aeruginosa*, its QS systems were explained previously.

qsc 119

This strain is a mutant, which is not able to produce AHLs on its own. When external AHL is added, the strain reacts by producing β-galactosidase. [63]

HT5 ("Hospital Tucumán 5")

This strain was used by Luciardi et al.[63] to test the effect of mandarin EO on QS and virulence-factor-production. It's a strain isolated from a patient with food poisoning which is resistant to several antibiotics. As reference strain, they used *P. aeruginosa ATC 27853.*

Aeromonas hydrophila

This pathogen has an LUXRI-homologous QS-system and produces C-4 and C-6-HSL (mainly C4-HSL), as QS-molecules. *A.hydrophila* builds biofilms on different surfaces. Lynch et al.[50] showed the correlation between QS and biofilm-structure, showing less differentiated biofilm architecture in AHL deficient ahyI mutant strains, although other differences (height, surface coverage) were not observed. [50]

Pseudomonas flourescens KM121- and indirect sensor strain:

P. flourescens can be used in combination with CV026. CV026 produces Violacein if AHL is produced by *P.flourescens*. When examining the AHL profile, Myszka et al. found out that this strain produces C6-HSL, 3-oxo-C6 HSL and 3-OH-C8-HSL and 3-OH-C8-HSL. [64]

QS-inhibition detection assays

Disc-diffusion-assay

McLean et al.[57] developed a simple screening test for bacteria or plant material (leaf, stem, flower) for its QS-inhibitory potential (AHL-dependent signalling). For this method, they used either *Pseudomonas aurofaciens 30-84* , *CV ATCC 12472* or *CV026* as indicator strains. They basically incubated the test bacteria or placed the plant material on a petri plate (Lysogeny broth LB) and overlayed it with previously incubated indicator strains (LB broth / LB broth + antibiotics).

After incubating the colour was examined again. A lack of pigment production indicated QSI-activity of the used bacteria /plant material. Hence, QSI was detected by a colourless, opaque halo. [57]

Adonizio et al. [65]adjusted this method for testing different plant extracts. They used CV12472 and CV026 as sensor strains and referring to a standard disc diffusion test by Bauer et al. [66]The extracts (20µl) were loaded on sterile paper discs (6 mm diameter) and placed on the overnight cultures of indicator strains. They were incubated overnight. QSI was measured via measuring the colourless but viable cells around the disc in mm. This agar-diffusion method, slightly adjusted (incubation

time, diameter of the discs, volume of EO) became a common and fast way to test QSI-potential of EOs. [65,67]

Flask incubation assay

To determine the QSI-potential of vanilla extract, Choo et al.[67] used this assay in 2006. CV026 was incubated for 16-18h and then inoculated in Erlenmeyer flasks containing either LB/ LB with HHL (N-hexanoyl-HSL) or LB with HHL and the potential QSI. Afterwards, flasks were incubated in a shaken incubator for 24h (27°, 150rev/min). QSI was measured then via "Blosser and Gray method" to quantify violacein production by absorbance-measurement at 585 nm. They obtained violacein after flask incubation assay by taking 1 ml of each flask, vortexing it and dissolving the resulting insoluble pellet containing violacein in DMSO after discarding the supernatant. [67,68]

The QSI-selector (QSIS)

This assay, developed by Rassmussen et al.[59] in 2005 is another method making it possible to screen for QSI-activity of EOs. Method A, established in *E.coli* is based on a gene encoding a lethal protein being fused to a promotor controlling QS. This leads to cell death in presence of AHL signal molecules; therefore cell growth is only possible when QSI are present. Method B bases of a repressor-controlled antibiotic-resistance-gene. This repressor is controlled by a QS-regulated promotor. If AHL is present, the expression of the gene is inhibited; leading to an antibiotic effect and cell growth is inhibited. In presence of QSI, bacteria can grow. To detect QSI, they used for example a luxI-gfp-reporter in QSIS1 (see "sensor strains"). [59]

Fluorescence-based QSI-Detection

Jaramillo-Colorado et al.[69] used this method for the detection of QSI, which is based on the research of Wagner-Döbler et al. [70]The group screened several α-proteobacteria for AHL-production by using a sensor strain which carry plasmids consisting of gfp-reporter constructs (luxR-gfp). LuxR works as a reporter protein, initiating fluorescence by activating the transcription of gfp (green fluorescence protein). They used two sensor strains: *P.putida F117 (pRK-C12)* and *E.coli MT102*

(pJBA132) (see "sensor strains"). Fluorescence was measured at an excitation wavelength of 485 nm.

Bioluminescence-based QSI-Detection:

Winson et al. [60]developed this method to investigate AHL-based QS. They constructed reporter strains with plasmid vectors containing luxR, LasR or Rh1R in fusion with Lux-I homologues and luxCDABE from *Photorabdus luminescens ATCC29999*. In presence of specific AHLs, the strains show bioluminescence via ecpression of LuxCDABE.

β-Galactosidase-based QSI-Detection:

An example of using β-Galactosidase with the encoding gene LacZ was an assay carried out by Luciardi et al.[63], they used the reporter strain *P.aeruginosa qsc119*. to detect inhibition of QS by mandarin oil. External AHLs where obtained fom *P.aeruginosa* ATCC27853 or HT5, being wild type strains. After incubating with different concentrations of EO, β-galactosidase was measured spectrophotometrically.

Swarming motility of *P. aeruginosa.*

A method to test swarming motility was described by Vattem et al.[71], they used 40µl of the test-substances (dietary phytochemical extracts) on 0.3 % LB-agar plates. Then they inoculated the plates with PAO1. After incubating, they measured the swarming motility by determining the diameter of the motility swarms. Another assay, being the basis for Kalia et al. [27]who tested the effect of cinnamon oil on swarming motility of PAO1 was done by Krishnan et al.[72] They pured the test-extract on prewarmed swarm plates containing bacto agar, glucose and yeast extract. They point- inoculated the plates with PAO1. After incubation, swarming was measured by lengths of dendrites on the swarm plates.

Swimming motility of *P.flourescens/ PAO1*:

Myszka et al.[64] used this assay to determine flagella-based motility of *P.flourescens* by measuring the distance bacteria move from an inoculation point in a certain incubation period. Swimming motility is a bacterial movement from individual cells,

rotating their flagella to move in liquid environments. Swarming however describes a multicellular movement of bacteria in biofilms over solid surfaces through flagella. Bala et al. [73] described a similar method to detect swimming motility of PAO1, assessing the zone diameter of swim in mm.

Virulence factor production in *P. aeruginosa / A. hydrophila*

For the measurements of changing virulence-factor production based on QS-systems different assays where used, mainly based on an appropriate chemical processing followed by absorbance measurements.

Biofilm-visualization by Crystal Violet Assay

Crystal Violet Assay is an indirect quantification method of death cells. Crystal violet binds to protein and DNA on detached cells. Reduced cell attachment because of cell death leads to reduced violet staining in a culture. This simple assay can be used for the effect of EO on biofilms.[73]

Biofilm-assay by Le Thi et al.[74]

This assay was used by Myszka et al. [64]to detect adhesion of *P.flourescens* on stainless steel plates. After removing unattached cells with PBS (phosphate-buffered saline), bacteria are stained with acridine-orange and then observed in a fluorescence microscope to characterize the biofilm.

b. Research on Essential Oils as QSI

In this chapter, research done on EOs will be presented sorted by publishing year. (squared brackets indicate the publishing year) To achieve a better overview and comparability, contents and outcomes will be presented for each study. After that, information on the used EOs (components, extraction method and concentrations of EOs; if specified), sensor strains and methods are listed. Detailed data on QS-inhibition of the oils are then shown in charts. If additional research was done not being directly traced to QS, these results are explained after the charts. An overall table summarising all the discussed papers will be shown in the next chapter. The presented research results are concentrated on QS-related results; therefore, other results concerning for example the effect on bacterial growth are not discussed. Many investigations include the effect of EOs on biofilm formation. These results are mentioned but it is important to consider that biofilm formation is a complex mechanism where QS is only one of many signalling systems playing a role here. Hence, relating to the studies discussed in this work, one must not conclude a direct cause and effect relationship between QS-inhibition of the oils and decreased biofilm-formation; it is often more likely an inhibition caused by EOs, whereas the mechanism of inhibition is not known or not investigated in respective papers.

1) Clove, cinnamon, peppermint and lavender oil [2009]

Khan et al. [75]evaluated the anti-QS activity in 20 different EOs and showed QSI-activity in 4 of them: clove , cinnamon, peppermint and lavender.

EOs: subMICs

Sensor strains: CV12472, CVO26

AHL producing strain: CV31532 (C6-AHL)

Performed assay *:* Disc-diffusion assay: (CV 12472) / (CV026+CV12472)

Inhibition of QS regulated violacein production in C.violaceum strains (inhibition zone size in mm)

EO	**CV12472**	**CV026+ C-6-AHL**
Cinnamomum verum	12 mm	11 mm
Lavandula angustifolia	11 mm	10 mm
Mentha piperita	11 mm	10 mm
Syzigium aromaticum	19 mm	17 mm

Negatively tested EOs

Apium graveolens	*Foeniculum vulgare*	*Rosmarinus officinalis*
Citrus limon	*Cymbopogon martini*	*Santalum album*
Citrus paradisi	*Eucalyptus sp.*	*Thymus vulgaris*
Citrus sinensis	*Myristica fragrans*	*Trachyspermum ammi*
Cymbopogon citratus	*Olea europaea*	*Zea mays*
Foeniculum vulgare	*Petroselinum crispum*	*Zingiber officinale*

Further investigations on clove oil:

In addition to these analyses, Khan et al. [75]performed further research on clove oil (0,04-0,20 (v/v%). In order to prove, that the violacein production inhibition is not caused by the antibiotic effect of clove oil, quantitative Violacein measurements in clove-oil treated and non-treated cultures where done concurrently with cell viability tests in both of the cultures. The test showed, that clove oil works as QSI up to a concentration of 0.12%; higher concentrations lead to growth inhibition. Using GC/MS, the team revealed eugenol as the main component of clove oil but they discoverd, that pure eugenol has no effect on the inhibition of pigment production in *CV026.* Hence, other components like α/β-caryophyllene could be the effective substances.

Effect of clove oil on swarming motility in PAO1

Kahn et al. [75] analysed the effect of clove oil on swarming motility in a *P.aeruginosa strain (*Vattem et al.[71] method). The research revealed that sub-MICs of clove oil show a concentration dependant effect on the reduction of swarming motility in PAO1, whereas the main component of clove oil, eugenol, exerted no effect.

2) Rose, lavender, geranium and rosemary oil [2010]

Szabo et al. [76] investigated the effect of different essential oils on bacterial growth and QS in CV026 using either *E. coli* ATTC 31298 or Ezf 10-as AHL-producing strains. They following EO were used, most of them being already known as inhibitors of bacterial growth: rose, lavender, chamomile, orange, eucalyptus, geranium, citrus and rosemary oils. QS-inhibition was proven in rose, lavender, geranium and rosemary when using pure oil in both combinations, whereas eucalyptus oil only inhibited violacein production in the CV026 + E. coli ATTC 31298 combination and citrus oil only inhibited the CV026 + Ezf 10-17 combination. The most potent QS-inhibitor was rose oil. Interestingly, rose oil showed a comparable QSI-activity in the 10% dilution as the concentrated oil on CV026 + *E. coli* ATTC 31298 combination- whereas the other tested oils had a higher QSI-potential when being higher concentrated.

EOs: Quality of the Hungarian pharmacopoeia (Ph. Hg. VII.), concentrated or diluted (10%) in DMSO

Sensor strain: CV026

AHL-Producing strains: *E. coli* ATTC 31298 and Ezf 10-17 as, a strain isolated from a grapevine crown galltumor

Performed assay*:* Disc diffusion assay

Inhibition of QS regulated violacein production in C.violaceum strains (inhibition zone size in mm)[76]

	CV026+E. coli ATTC 31298	**CV026+ Ezf 10-17**
EO	10% / 100% EO	10% / 100% EO
Rosa damascena	15-20 / 18 mm	10 / 20 mm
Lavandula angustifolia L.	0 / 10	5 / 15
Eucalyptus globulus L	0 / 6	3 / 0
Geranium robertianum	0 / 13	3 / 15
Citrus sinensis L	0 / 0	2,5 / 16
Rosmarinus officinalis L	0 / 18	3 / 18

Negatively tested oils [76]

Juniperus communis L., *orange oil*

Matricaria recutica L.,

[76]

3) Oils of piper species [2010]

Pájaro et al.[77] investigated the anti-QS-activity of different Piper species EOs (*P. bogotense*, *P.brachypodom* and *P.bredemeyeri*). In this study, an IC50 for QS-inhibition was defined, representing the concentration of EOs leading to a 50% reduction of violacein production compared to the amount produced by a fully induced CV026 strain. The investigation revealed the highest anti-QS-activity in *P.bredemeyeri*, following *P.brachypodom* and *P.bogotens*e. The EO-concentrations for IC50-values also had a minor effect on cell growth inhibition but as these results did not differ significantly from the control cells, the research group concluded QSI being basically independent from cell growth inhibition.

Connection between molecular structure and QS-inhibition

The main components of the oils (<10%) are listed below:

- ➔ *P.bredemeyeri*: sabinene/β-pinene and α-pinene
- ➔ *P.brachypodom*: ; trans-β-caryophyllene and caryophyllene oxide
- ➔ *P.bogotense:* trans- sabinene hydrate and α-phellandrene

The major components of *P.bredemeyeri* oil – (with the exception of α-pinene which also can be found in *P.bogotense*) – cannot be found in the other *Piper* species and

may therefore be partly responsible for the high activity of this oil. Pajaro et al. also made the hypothesis that those components may be competitive inhibitors because of their similarity in size and polarity with HHLs. [77]

EOs: Plant material was collected in different Columbian states; oils were produced by microwave-assisted hydrodestillation. For the EO of *P. bogotense* and *P.bredemeyeri* herbs were used, for *P.brachypodom* oil the whole plant was processed. Different dilutions with DMSO where tested. To evaluate the connection between EOs and their constituents, the team refers to literature where components of the oils have been analysed. [77]

Sensor Strain: *CV026* + C6-HSL

Performed assay : Flask incubation assay

Inhibition of QS regulated violacein production in C.violaceum (IC50)

EO	**IC50 µg/ml**
P.bogotense	93.1 (77.0-112.6) [R^2=0.942]
P.brachypodom	513.8 (429.6-614.4) [R^2=0.925]
P.bredemeyeri	45.6 (41.2-50.5) µg/ml [R^2=0.711]

4) EOs from Columbian plants [2012]

Jaramillo-Colorado et al.[69] investigated the anti-QS-activity of different Columbian plants on a *P. putida* as well as on an *E.coli* strain. As the chemical composition of *Lippia alba* oils depends on the collection location of the plants, tests were carried out with oils from different collection sites. Additionally, the team differed between oils extracted by means of microwave-assisted hydrodestillation (MWHD) or hydrodestillation (HD). In concentrations of 1.2 mg / ml, anti-QS-activity was measured for some of the *L.alba* oils, *Ocotea sp*. and *Elettaria cardamomum* in *P.putida* (the highest activity inhibition was reached by a the geranial/neral chemotype of *L.alba*), whereas only two of the limonene/carvone chemotypes showed a low inhibitory activity of 9% and 1% respectively). All of the oils were active QSI-inhibitors in *E.coli*, the most active was an oil of *L.alba* chemotype 2 and *Ocotea sp*. [69]

Connection between molecular structure and QS-inhibition

When taking a look at the main components of the investigated oils, it is apparent that CT1 is a more active QS-inhibitor of C12-HSL regulated QS. The research group hypothesised that this is maybe because of the linear molecular structure of the components. Limonene and carvone are cyclic molecules and therefor show more similarity to C6-AHLs. The research group also investigated the QSI of different constituents of EOs separately. Carvone, geranyl-acetat, α – pinene and citral were investigated. Only citral was active in *P.putida* (long chain AHL), all of the other molecules only showed activity *in E.coli* (short chain AHL.). This result coincides with the tests on the EOs as again the linear citral inhibits long-chain-AHL-dependent QS and branched structures only inhibit QS in the short-chain-AHL-controlled *E.coli* strain.[69]

EOs: several *Lippia alba* oils 1-5 (MWHD/HD) belonging to
chemotype 1 with the main components geranial and neral or
chemotype 2 with limonene and carvone as main components.
Swinglea glutinosa (MWHD) *Myntotachys mollis* (HD) Ocotea sp. (MWHD) , *E. cardamomum* (HD)and *Zingiber officinale* (HD)
components of the oils were characterized via GC-MS.
Sensor strains: *E.coli* + pJBA132 (C6). *P. putida* +pRK12 (C12)
Performed assay:Fluorescence-based QSI – assay: *P.putida* + *E.coli*

Inhibition of QS regulated fluorescence in E.coli and P.putida strains (% of QS-decrease)[69]

EOs	***P.putida (C12-AHL)***	***E.coli (C6-AHL)***
Lippia alba (1) CT 1	65%	18%
L.alba (2) CT 2	-	15-84%
L.alba (3) CT 2	1%	72-78%
L.alba (4) CT2	-	61-62%
L.alba (5) CT2	9%	44-72%
Swinglea glutinosa	-	28-73%
Myntotachys mollis	-	56-65%
Ocotea sp.	25%	71-76%
Elettaria cardamomum	31%	21-22%
Zingiber officinale	-	34-69%

5) Clary sage, juniper, lemon and majoram oil [2013]

Kerekes et al. [53]investigated EOs of clary sage, juniper, lemon and marjoram on their QSI-potential. Their decision to use those oils was made because of their lack of phenolic compounds. Oils themselves as well as the main component of each oil where tested. All of the oils inhibited QS in the tested bacterial strains, except lemon and limonene showing only very little or no effect. Interestingly, the parent oils showed better inhibitory activity than their major components, in opposite to the biofilm-inhibitory-potential which was also tested in this study.

EOs: 1/2/3 µl, components were determined by the producer of the oils

Sensor strain: *CV6269*

Performed assay:Disc-diffusion assay:*CV6269*

Inhibition of QS regulated violacein production in C.violaceum by EOs and their main components (inhibition zone size in mm)[53]

	1/2/3 µl EO	1/2/3 µl main component
Salvia sclarea (linalool)	10/20/25mm	10/15/20 mm
Juniperus communis (α-pinene)	0/15/20 mm	0.1/0.1/0.1 mm
Origanum majorana (terpinen-4-ol)	10/15/20 mm	0/15/20 mm

Negatively tested EO: Citrus lemon (limonene) [53]

Effect on biofilm formation in different organisms

Biofilm-inhibition in *Bacillus cereus var. mycoides 0042, E. coli 0582 and P. putida 291* and in the yeast *Pichia anomaila 8061Mo* was detected via Crystal Violet Assay, additionally there has been done a structural analysis of the biofilm modifications with scanning electron microscopy (SEM). The team revealed that only majoram EO has an inhibitory effect on biofilms of *B.cereus*, wheres all of the tested oils inhibit biofilm formation in *E.coli*. Biofilms formed by *Pichia anomaila* are inhibited by all tested oils and also by α-pinene and terpninen-4-ol, whereas only majoram and its main component terpinen-4-ol inhibited biofilms in *P. putida*, the other oils had too high MICs to allow further investigations. When examining mixed biofilm (*E. coli* and *P. putida* 1:1), marjoram and cinnamon oils showed inhibitory effects. [53]

Connection between molecular structure and QS-inhibition

The oils investigated in this study mainly contain terpenoids. As mentioned above, their inhibitory potential is comparable, only lemon oil did not show any activity. When correlating these results to the main components of the oils, it seems that terpene alcohols (terpineol, linalool) possess a higher activity than monoterpenes (limonene and α-pinene). Then again it is apparent, that the multicomponent character of EOs should be included in such observations as α-pinene, being the main component of juniper oil has no significant QS-inhibiting effect by itself, whereas the oil was proven to be a potent QS-inhibitor, leading to the conclusion that other components have to be responsible for this effect. [53]

6) Clove, rose, chamomile and pine turpentine oil [2013]

Eris and Ulusoy [78]analysed the QSI-potential of different essential oils and determined the highest inhibitory potential in clove, rose, chamomile and pine turpentine EO. They started their investigations by screening the oils by QSIS-method and only the oils showing the highest activity in this assay where submitted to further tests. 42 essential and edible oils were screened by QSIS method 12 of them showed positive results. The activity was classified in weak (*) /medium (**) /strong positive (***) or negative, whereas the most active substances were underwent further tests. The team performed an agar-plate-assay, but results are not discussed in this paper as only pictures were published indicating the QSI activity of rose, clove, pine turpentine and chamomile EO on CV026, as quantification of the results is missing, those values are not mentioned in the table. Flask incubation method revealed the highest QS-inhibition for clove and rose oil. Concerning biofilm-formation, the study showed a significant reduction in PCI (*P.aeruginosa* clinical isolate) for rose, clove, pine turpentine and chamomile oil but interestingly, the oils did not show a significant change in PAO1. [78]

EO: ethanol-dilutions of filter-sterilized EOs, MICs were not evaluated but biomonitor strain cell counts treated with EOs were compared to those without treatment and no significant deviations in could be determined.

Sensor Strains: QSIS1, CV026, VIR07, ATCC12472, PAO1, PCI

Performed assays :

- QSIS: QSIS1
- Disc diffusion assay: CV026+C6AHL , VIR07 + C12AHL , ATCC1247
- Flask incubation assay : CV026,VIR07,ATCC12472
- Biofilm-inhibition via crystal violet assay : PAO1, PCI

Inhibition of QS regulated fluorescence in QSIS1 , violacein production in CV026, VIR07 and CV12472 (% of QS-decrease) and biofilm-inhibition in PCI (% of biofilm-decrease) by EOs [78]

EO	**QSIS1**	**CV026**	**VIR07**	**CV12472**	**PCI**
Rosa damascena	****	80%	70%	43%	53%
Eugenia caryophyllata	***	80%	72%	39%	52%
Matricaria recutita	**	75%	61%	32%	58%
Cinnamomum zeylanicum	**				
Cymbopogon nardus	*				
Juniperus communis	*				
Myrtus communis	*				
Pinus sylvestris	**	67%	65%	25%	59%
Salvia officinalis	*				
Thymus vulgaris	*				
[Vanilla fragrans	**				
Lavandula angustfolia	*				

Negatively tested oils (QSIS) [78]

Cedrus sp.	*Laurus nobilis*	*Pinus sp.*
Citrus aurantium	*Lilium candium*	*Punica granatum*
Citrus bergamia	*Melissa officinalis*	*Rosmarinus officinalis*
Citrus limonum	*Mentha piperita*	*Santalum album*
Citrus reticulata	*Myroxylon pereirae*	*Viola odorata*
Eucalyptus globulus	*Nigella sativa*	*Vitis vinifera*

Foeniculum vulgare Miller	*Ocimum basilicum*	*Zingiber officinale*
Jasminum officinale	*Olea Europa*	

Negatively tested oils (biofilm-formation in PAO1) [78]

Eugenia caryophyllata	*Pinus sylvestris*
Matricaria recutita	*Rosa damascena*

7) Clove oil [2013]

Husain et al.[79] investigated clove oil which also was already proven to be a potent QSI in *C.violaceum* strains and an inhibitor of swarming motility in PAO1 in 2009[75]. To understand the inhitbition of clove oil more precisely, the team used the *E.coli* strain MG4 pKDT17 to find out if the Anti-QS-effect of clove oil is due to inhibition of the LasR/RhlR- system. They proved this assumption as AHL-concentrations were decreased significantly (56%), being shown by the reduced β-galactosidase activity of the sensor strain. The team continued the research on the effects on PAO1, proving an inhibitory effect on QS-related virulence factors as well as on swimming motility.

SubMICs of clove oil additionally lead to enhanced survival of a PAO1 infected *Caenorhabditis elegans.* Clove-oil treatment lead to decreased biofilm-formation and virulence factor production (protease, exopolysaccharide [EPS]) in *A.hydrophila.*[79]

EO: subMICs of *Syzigium aromaticum* EO

Sensor strains: PAO1*, Aeromonas hydrophila WAF-79* (isolated from hospital wastewater) , *E. coli MG4 pKDT17*

Performed assays:[79]

- β-galactosidase – assay (*E. coli* MG4 pKDT17*)* to quantify AHLs produced by PAO1
- Virulence factor-production: : protease, chitinase , pyocyanin LasB, EPS
- Swimming motility of PAO1
- Biofilm-Assay via Crystal Violet staining : PAO1
- Survival of *Caenorhabditis elegans* infected with PAO1

Inhibition of QS regulated β-galactosidase production in PAO1 by clove oil (% of QS-decrease) [79]

EO	PAO1
Syzigium aromaticum	65%

8) Oregano and carvacrol [2014]

Alvarez et al. [80]investigated the QSI-activity of Mexican Oregano EO. QSI via Flask incubation assay was quantified as % of Violacein-reduction. As exact inhibition values were not published, the listed results are rough estimates. Concentrations used were 0.0156 mg/ml to 0.125 mg/ml showing a reduction of Violacein production of ~50% even in the lowest concentration and about 80% for 0.0625% .In the highest concentration cell viability was reduced too whereas the other concentrations did not affect viability significantly.

EOs: *Lippia graveolens Kunt*

Sensor strain: ATCC12472

Performed assays: Disc-diffusion assay, Flask incubation method with concurrent cell-viability testing [80]

Inhibition of QS regulated violacein production in C. violaceum ATCC12472 (inhibition zone size in mm) by oregano EO [80]

EO concentration	QSI
6.25 mg/ml	4.6±0,29 mm
12.5 mg/ml	5.6±0,29 mm
25 mg/ml	5.8+-1,04 mm

Inhibition of QS regulated violacein production in C. violaceum ATCC12472 (% of QS-decrease) [80]

ÊO concentration	QSI
0.0156%	>50%
0.0312%%	>60%
0.0625%	>75%
0.125%	>95%

Burt et al. found out that carvacrol, the main component of OEO inhibits Quorum sensing (Flask incubation method) in CV ATCC12472. [81]

9) Lemongrass and cinnamon oil [2014]

Mukherji and Prabhune [82]performed tests on various EOs and proved Lemongrass oil and Cinnamon oil to be active QS-inhibitors. . The group was the first one to adapt the EOs chemically in order to change their polarity by converting them into EO-sophorolipids (=glycolipids) produced by a *Candidia bombicola* strain to obtain oils that are more active in aquatic environments. They tested the EOs themselves , EOs with added oleic acid sophorolipid (OASL) and the EO-sophorolipids and compared the results. The test results revealed a higher QS-inhibition when adding oleic acid sophorolipid into the growth medium, caused by its function as an emulsifier, even to of the inactive oils showed QSI-activity then (Basil, Ylang Ylang). After converting the oils to their respective EO-sophorolipid, all of the tested oils showed inhibitory activity. [82] The outcome of the study indicates that QSI is not only on account of constituents of the EOs, but physical properties play an important role too.

EOs: main components are indicated but no reference on this data is mentioned

Sensor strain: CV026+C6HSL

Performed assay:Disc-diffusion assay

Inhibition of QS regulated violacein production in C.violaceum by EOs (inhibition zone size in mm) [82]

EO (concentration)	**CV026+C-6-HSL**
Cymbopogon citratus (20%)	15mm
Cinnamomum verum (10%)	15mm

Negatively tested oils[82]

Boswellia carteri	*Cymbopogon nardus*	*Ocimum basilicum*
Cananga odorata	*Eucalyptus sp*	*Rosmarinus off.*
Citrus bergamia	*Melaleuca alternifolia*	

Citrus sinensis *Mentha piperata*

10)EOs from South American Species [2014]

The research done by Pellegrini et al. [83]who tested several oils from south American plant species came to the result that *Salvia officinalis, Artemisia annua, Lepechinia floribunda, Schinus molle, Satureja odora* and *Minthostachys mollis* are QS-inhibitors. To quantify the results of the flask incubation assay, minimum QS inhibitory concentration (MQSIC) was defined as the concentration of EO that results in 50 % of QS (measured through violacein production). *S.molle, M.mollis* and *S.odora* showed the lowest MQSIS (=highest QS-inhibition).With the exception of *S.odora* when being tested via flask incubation assay and *L.floribunda* tested via disc diffusion assay, all oils showed QS-inhibition independently from cell growth inhibition. Hence, *S.odora* and *L.floribunda* QS-inhibition was just due to an antimicrobial effect and the results of the respective methods can therefore not be counted as positive.

EOs: extraction by steam distillation, components were analysed in previous reports via solid-phase microextraction (SPME) coupled to GC/MS, undiluted oils where used for disc diffusion test, flask incubation assay was performed with different concentrations.

Sensor Strain: ATCC1247

Performed assays: disc-diffusion assay, flask incubation assay with concurrent cell viability testing

Inhibition of QS regulated violacein production in C.violaceum by EOs (inhibition zone size in mm and MQSIC) [83]

EO	**Inhibition zone size**	**MQSIC**
Salvia officinalis	>90 ±0,1 mm	0.0259 %
Minthostachys mollis	>90 ±0,1 mm	0.0649 %
Satureja odora	90 ±1 mm	QSI=Growth Inhibition
Schinus molle	50±1.2 mm	0.005 %
Artemisia annua	70±0.2 mm	0.0138 %
Lepechinia floribunda	QSI=Growth inhibition	0.0137 %

11)Lavender oil [2014]

Lavender oil inhibits QS in *E.coli psB1075* and *E.coli pSB401*, these sensor strains react to the presence of either long chain or short chain AHLs by light production. Yap et al.[61] proved the oil to be an inhibitor of the long chain-sensitive strain *E.coli psB107.* The team did not determine MICs of the oil but they tested the bactericidal effect of the used concentrations and no changes of cell survival where detected.

EO: 0.01- 0.025%, components were analysed via GC-MS, main components are linalyl anthranilate (38.4%) and linalool (34.6%)

Sensor strains: *E. coli* [pSB401]+ 3-oxo-C6-HSL or and [pSB1075] +3-oxo-C12-HSL

Performed assay: luminescence based - QSI-assay (quantified as reduction of total light production)

Inhibition of QS regulated fluorescence in E.coli [psB1075] by lavender oil [61]

EO	**E.coli [pSB1075] +3-oxo-C12**
Lavandula angustifolia	✓

Lavender oil was negatively tested on *E Coli [pSB401]*+ 3-oxo-C6-HSL.

12)Lippia alba oils [2014]

Olivero-Verbel et al.[84] continued the research on *Lippia alba* EOs, which have already been investigated in 2012. [69] The authors also divided them in different groups depending on their extraction-method (MWDH/ HD) and their chemotype (geranial/neral =CT1, limonene/Carvone= CT2). In difference to the paper discussed before, this group also investigated the effect on cell growth of the tested oil concentrations and found out to that QSI is independent from the effect on cell growth. They found out, that the most active QS-inhibiting *L.alba* oils were those with high concentrations of geranial / neral (CT1) followed by the oils with limonene/Carvone (CT 2) as main constituents. In other words, CT1 is a more active QSI than CT2 in CV026 and ATCC31532, responding to C6HSL . As mentioned before, Jaramillo-Colorado et al.[69] came to the result, that CT2 is more active in the C6-AHL-sensible *E-coli* strain.

EOs: MWHD/ HD-oils from different Columbian locations, the components were characterized via GC-MS, Different DMSO-dilutions were used.
Sensor strains: CV026 + C6-HSL
Performed assay: Flask Incubation assay, determination of IC50-values

Inhibition of QS regulated violacein production in CV026 (% of maximal violacein inhibition and IC50) [84]

EO and chemotype	**%inhibition**	**IC50**
L.alba (A) CT2	>60%	66.91 (55.89-80.11) µg/mL R^2=0.95
L.alba(B) CT2	>70%	2.24 (1,98-2,54) µg/mL R^2=0.88
L.alba (C) CT1	>75%	0.62(0,53-0,72) µg/mL R^2=0.85
L.alba (D) CT1(Leaves)	>90%	15.79(14,3-17,42) µg/mL R^2=0.82
L.alba (E) CT2	>50%	30.32(27,27-33,71) µg/mL R^2=0.84

13) Ferula and dorema oil [2015]

Sepahi et al.[85] investigated the EOs of ferula and dorema, both belonging to the Apiaceae family; on their QSI-potential. Both of the oils where active, ferula oil even fully inhibited violacein production in CV026, although the exact diameters of the inhibitory halos weren't published. Regarding QS in *P.aeruginosa*, the team showed reduced production of different QS-controlled virulence-factors (elastase, pyoverdin, pyocyanin) as well as inhibition of biofilm-formation in PAO1.

EO: extraction by hydro-distillation

Sensor Strains: CV026, PAO1

Performed assays: Disc-diffusion assay : CV026, Virulence Factor Production: PAO1

Inhibition of virulence factors and biofilm-formation in PAO1 by EOs [85]

EO	**elastase**	**pyoverdin**	**pyocyanin**	**biofilm-formation**
Ferula asafoetida L.	✓	✓	✓	✓
Dorema aucheri Boiss.	✓	✓	X	X

✓= Inhibition X= no inhibition

14) *Hyptis dilatata* oil [2015]

Hyptis dilatata, being traditionally used for the treatment of wound infections of cattle in Columbia was proven to be a QS-inhibitor by Alvarez et al. [58]The main components of the oil are monoterpenes (2-ß-pinene, camphor, 1-ß-pinene), sesquiterpenes (mainly aromadendrene) and oxygenated derivates of these substance classes. As inhibition diameters and used oil-concentrations are not published, a comparison to other EOs is not possible.

EO: extraction through steam distillation, GC/MS-Analysis of the components, methanol solutions of the oils where used

Sensor Strain: CVATCC 31592

Performed assay: Disc-diffusion assay

Inhibition of QS regulated violacein production in C.violaceum ATCC31592[58]

EO	**CV ATCC 31592**
Hyptis dilatata	✓

15)Spice oil nanoemulsions [2015]

Venkadesaperumal et al. [86]investigated the QS-inhibitory potential of *Cuminum cyminum, Piper nigrum* and *Foeniculum vulgare*. In contrast to the other QSI-tests done by other groups who performed assays with pure or diluted oils, this team used nanoemulsions of the EOs, formulated by ultrasonic emulsification of Tween 80 and water. One reason the team mentioned to use this emulsions instead of pure EOs, is that EOs lose their bactericidal activity after certain dilutions because of changes in droplet structures. Other advantages are the high stability, high solubility and low turbidity and enlarged surface, allowing a rapid penetration of nanoemulsions into the cell. All of the tested oils showed inhibitory activity when using disc diffusion assay and flask incubation assay, results are shown below (rough estimates for FIA are given for results without exact data). Additionally to QS-inhibition-assays, the team investigated the effect of the nanoemulsions on biofilm formation and EPS production in *E.coli, Klebsiella peumoniae*, *Salmonella typhimurium* and *C.violaceum* strains, showing a concentration-dependent inhibitory potential of the oils.

EOs: subMICs (30 / 50µl/ml) of EOs in different emulsion ratios
Bacterial strains: CV026 + C6HSL
Performed assay: Disc diffusion assay, Flask incubation assay

Inhibition of QS regulated violacein production in CV026 by EOs (inhibition zone size in mm and %reduction of violacein) [86]

EO	**Disc diffusion assay**	**% reduction of violacein (50µl/ml)**
Cuminum cyminum	✓	>70%
Piper nigrum	✓	>55%
Foeniculum vulgare	✓	75,69%

✓= Inhibition of QS

16) Peppermint oil and menthol [2015]

Husain et al. [87] tested peppermint oil and its main component menthol on QSI-activity. Additionally, they investigated the effect of these substances on biofilm-formation in *P.aeruginosa* and *Aeromonas hydrophila,* as well as their inhibitory potential on QS-regulated virulence factors. This research group was the same team that revealed the QSI-activity of clove, cinnamon, peppermint and lavender in 2009. As peppermint oil was proven to be an active QS-inhibitor, they decided to continue research on this oil. Besides carrying out tests mentioned above, the team also performed molecular docking analysis of different constituents of the oil (menthone, limonene, menthol, menthylacetat) to an AHL – binding site of LASR having the natural autoinducer 3-oxo-C12-AHL.

Using different sub-MICs of the oil, a maximal violacein-reduction of 83,3 % of was recorded in CV026, showing no significant difference in cell viability in comparison to non-treated cultures. Peppermint oil lead to a decrease of LasB elastase, protease, chitinase and pyocyanin activity in PAO1. The oil also inhibited swarming motility of PAO1 and decreased production of EPS. Biofilm-reduction was shown too. The oil also showed reduction of protease, EPS production and biofilm-formation in *A. hydrophila*. Molecular docking of peppermint oil constituents revealed menthol as the best docked structure (based on binding energy scores) followed by isopulegol and 1-hydroxyaceton. Non-cyclic aliphatic constituents showed low affinities.

When investigating the effects of menthol on QSI, a maximum reduction of Violacein production of 85% was reached (400µg/ml). Just like the oil itself, biofilm reduction and reduced production of different virulence factors in the tested strains was shown too. [87]

EO: subMICs, GC/MS was carried out to analyse the components

Sensor Strains: CV026, PAO1*, Aeromonas hydrophila* (WAF-38)

Performed assays: [87]

- Flask Incubation Method + Cell Viability testing: CV026
- Biofilm inhibition via Crystal Violet Assay : PAO1*, A. hydrophila*
- Virulence-factor inhibition: LasB elastase, protease, chitinase and pyocyanin in PAO1, protease and exopolymeric substance (EPS) in *A.hydrophila*
- Swarming motility: PAO1

QS regulated violacein production in CV026 (% of QS-decrease) [87]

EO	**% reduction of violacein**
Mentha piperata	83.30%

Inhibited QS-associated processes in PAO1 by peppermint oil: Biofilm-formation, elastase/protease/pyoverdine/chitinase/EPS-production, swarming motility [87]

Inhibited QS-associated processes in A.hydrophila by peppermint oil:
Biofilm-formation, protease and EPS-production [87]

17)Cinnamon Oil [2015]

Cinnamon oil was tested regarding QS- inhibition (CV026, PAO1) and its effect on biofilm-formation, virulence-factor-production and swarming activity (PAO1). Whereas QS-inhibition is often quantified via flask incubation assay, Kalia et al. [27]additionally applied a β-galactosidase – assay in order to quantify AHLs produced by PAO1. Cinnamon oil was already tested positively on QSI in 2009 by Khan et al. [75]using the sensor strains CV026 and CV12472 (disc diffusion assay).

The oil revealed a concentration-dependant inhibition of violacein in CV026 with a maximum inhibition of 78% (0,6µl/ml). 3-oxo-C12HSL-production in PAO1 was reduced by 38% (0.2µl/ml), higher concentrations showed higher inhibition but that was most likely reducible to growth inhibition. All the other tested virulence-factors as well as swarming motility where inhibited. Biofilm-formation was reduced by 31%, further microscopy analysis showed changes in surface topology, integrity, structure, EPS, cell viability and DNA-content.

EO: subMICs, *Cinnamomum verum*

Sensor strains: CV026, PAO1 (GFP-tagged), E. coli DH5α pJN105L (containing lasR gene) and pSC11 (PlasI::lacZ)

Performed assays: [27]

- Flask incubation assay : CV026
- β-galactosidase – assay: To quantify 3-oxo-C12HSL produced by PAO1
- Virulence-factor production: pyocyanin, alginate, protease in PAO1
- Swarming motility of PAO1

- Biofilm-assay (Crystal violet staining) , further biofilm analysis by Scanning Electron Microscopy, Confocal Laser Scanning microscopy

Inhibition of QS regulated violacein production in CV026 and β-galactosidase-production in PAO1 (% of decrease) by cinnamon oil[27]

EO	**CV026**	**PAO1**
Cinnamomum verum	78%	38%

Inhibited QS-associated processes in PAO1 by cinnamon oil: biofilm-formation, pyocyanin/ alginate/ protease-production, swarming motility [27]

[27]

18)Coriander and (S)-(+)-linalool [2015]

A study carried out by Duarte et al.[88] proved that coriander EO as well as its main component (S)-(+)-linalool possesses a concentration-dependent QSI-potential. Disc diffusion assay revealed a higher inhibitory potential of (S)-(+)-linalool than the oil itself, proving that this component is mainly responsible for the inhibitory effect of the oil. Additionally, both of the substances inhibit biofilm formation in *Camylobacter jejuni* and *Camylobacter coli* strains, whereas the oil is the more active inhibitor.

EO:. pure or dilutions in DMSO,
Sensor Strain: ATCC12472
Performed assays: Disc diffusion assay. Flask incubation assay

Inhibition of QS regulated violacein production in ATCC12472 strains (inhibition zone size in mm and % of QS-decrease) by coriander oil and linalool[88]

EO	**mm QSI-radius (100% EO)**	**% inhibition of violacein (5µl/ml)**
Coriandrum sativum EO	63.50 +-2.12 * mm	>90 %
(S)-(+)-linalool	69.50-+0,71* mm	>90 %

*Mean +- standard variation (p<0,05), highest inhibition shown

19)Eucalyptus oils [2016]

Eucalyptus globulus and *Eucalyptus radiata* inhibit QS concentration-dependent in CV ATCC12472. [89]

EOs: extraction through hydrodestillation (leaves, small branches), GC-MS analysis of the components

Sensor Strain: ATCC12472

Performed assays: Disc Diffusion assay, Flask incubation assay

Inhibition of QS regulated violacein production in ATCC12472 strains (inhibition zone size in mm and % of QS-decrease) by eucalyptus oils

EO	**mm QSI-radius (100% EO)**	**% inhibition of violacein (5µl/ml)**
Eucalyptus globulus	10mm	>90%
Eucalyptus radiata	20mm	>90%

20)Mandarin oil [2016]

Luciardi et al.[63] investigated mandarin oil and the main component limonene on its effects on QS, virulence factors and biofilm- formation in *P.aeruginosa* strains. This research group differed between two oils depending on their production method which was either cold-pressing (=EOP) or cold pressing followed by steam distillation (=EOPD). EOP, EOPD and limonene reduced AHL concentration-dependent in both of the tested strains. AHL-reduction was shown to be higher than cell growth inhibition (CGI). An interesting fact the group mentioned in the paper was the increased AHL production in presence of the antibiotic azithromycin-showing a possible connection between bacterial stress and AHL production. Concerning biofilm-formation, the oils and limonene are concentration-dependent inhibitors. The team also showed a reduced elastase-B-production (>70%) in all of the tested strains and with all tested substances. The analysis of the components revealed monoterpenes as main components (97.66 %, EOP / 96.58 EOPD mainly represented by limonene, followed by γ-terpinene, myrcene and α-pinene). When comparing EOP and EOPD, EOPD contains more oxygenated monoterpens and sesequiterpene fractions. The research team believes that EOPD shows a more pronounced biofilm inhibition because of the higher amount of linalolol, α-terpineol, γ-terpinene and terpinolene respectively. [63]

EO: subMICs of *Citrus reticulata* oils [EOP/ EOPD], GC-MS-analysis of constituents was carried out

Sensor Strains : *P.aeruginosa ATCC27853* and *HT5* (Hospital Tucuman, strain resistant to several antibiotics) as AHL-producers, P. aeruginosa qsc 119 as sensor strain

Performed assays:

- β-galactosidase – assay (*P. aeruginosa* qsc 119) to quantify AHLs produced by *ATCC27853* and *HT5*
- Biofilm-assay (crystal violet staining): *ATCC27853* , *HT5*
  Further tests on the biofilm where done regarding metabolic activity, bacterial viability and the relation to growth inhibition:
- Elastase-B-activity-assay: *ATCC27853* , *HT5*

Inhibition of QS regulated β-galactosidase-production in ATCC 27853 and HT5 quantified by P.aeruginosa qsc119 (% of QS-decrease) by mandarin oil[63]

	ATCC27853	**HT5**
EOP 4mg/ml	49% (CGI: 17%)	38% (CGI: 33%)
EOPD 4mg/ml	50% (no cell CGI)	37% (CGI: 25%)
Limonene	34% (no CGI)	30% (CGI: 24%)

CGI=Cell Growth Inhibition

Inhibition of biofilm-formation in ATCC27853 and HT5 by mandarin oil (% of decrease) [63]

	ATCC27853	**HT5**
EOP 4mg/ml	82%	73%
EOPD 4mg/ml	88%	92%
Limonene 4mg/ml	49%	58%

* only highest inhibition showed

21)Thyme oil, thymol and carvacrol [2016]

The influence of *Thymus vulgare* EO (0.1 to 0.5 µl/ml) and its main components carvacrol and thymol (0.001 to 0.01 µl/ml), on QSI, flagella gene (fglA, responsible for motility)-expression (AHL-related) and biofilm formation in a *P.fluorescens* strain was investigated by Myszka et al. [64]The reason for the team to investigate the oil was the fact that *P.fluorescens* biofilms are a concern of food industry as mature biofilms are difficult to remove and available antimicrobial agents often show proper disinfection only staying in contact to the surface for a long time period. As the flagella may be the first part of the bacteria to have contact to surfaces and its production is controlled by autoinducers, the team hypothesised that this is a possible point of blocking to inhibit biofilm formation. Violacein production was reduced significantly for all tested substances. 90% (EO),80% (carvacrol) and 78% (thymol) – reduction was shown in the 72h cultures. When analysing the AHLs, treated cultures showed no AHL-molecules after treatment with the EO/thymol/carvacrol (72h) whereas reference samples contained C6-HSL, 3-oxo-C6 HSL, 3-OH-C8-HSL and 3-OH-C8-HSL.The investigation on the effect of subMICs of the oil and its main components on the gene expression of flgA in *P.flourescens* revealed inhibitory effects of all the tested substances, the EO had the highest inhibitory effect on both gene expression and motility. Regarding the biofilm, formation was inhibited by all the tested substances, the EO again again showed the highest inhibitory effect.[64]

EO: subMICs of *Thymus vulgare* EO (leaves) produced by hydro-destillation (0.1 to 0.5 µl/ml) , GC-MS-analysis was done to figure out the components of the oil - main EO- Components (<5%) where found to be thymol (55,42), carvacrol (6,84%) and p-cymene (5,33%).

Sensor Strain : *P. fluorescens* KM121, isolated from stainless steal surface in a food process plant as AHL-producing strain, CV026 as biosensor to measure AHL-production from KM121

Performed assays:

1) Flask Incubation assay for AHL quantification, AHL-profile was analysed
2) Swimming motility assay + flgA-gene expression in KM121
3) Biofilm assay (acridine-orange-staining, examination of the biofilm by fluorescence microscopy)

Inhibition of QS regulated violacein production in C.violaceum (% of QS-decrease)by thyme oil [64]

EO	**CV026**
Thymus vulgare	90%

Inhibited QS-associated processes in KM121 by thyme oil : biofilm-formation, swimming motility, flgA-gene expression[64]

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8. Overview of the results

Table 1) EOs positively tested on QS-inhibition

- *EO-concentrations:* If oils where tested in different concentrations below their minimal inhibitory concentrations (MIC) of the sensor strains, the label "**subMIC(s)"** is used. If no MIC was evaluated in the respective study or higher concentrations where used, concentrations or volumes are shown. In case of a study not clearly stating the concentration, the line is left empty.
- *M…Methods:*
- **DD**= Disc diffusion assay

  V= Flask Incubation assay, spectrophotometrical Violacein-quantification

  QSIS: QS-inhibitor-selector assay

  β: β-galactosidase assay

  F: Fluorescence-based assay

  L: Luminescence-based assay
- *I ….Quantification of QSI for the respective methods*

  DD: diameter of colourless (but viable) cells around EO-impregnated paper discs in mm

  V/F/L/QSIS/ β:percentages equal the reduction of violacein/ fluorescence/ luminescence/ β-galactosidase after treatment with the EO compared to control cells.

  Most of the oils where tested in different concentrations, this table only lists the maximal reductions at the respective concentrations of EOs. For cases not indicating exact values for maximal Violacein production, there is either given the IC50/MQSIC or a ✓ indicates a positive result.

Table 2) Overview of EOs negatively tested on QS-inhibition

Table 3) Overview of EOs tested on inhibition QS-related processes like virulence factor-production / biofilm formation/swimming motility and swarming motility in different organisms

Table 1) EOs positively tested on QS-inhibition

Plant	Concentration	M	sensor strain	I	Ref.
Artemisia annua	100%	DD	ATCC12472	70±0,2 mm	83
Artemisia annua	MQSIC = 0,0138 %	V	ATCC12472	50%	83
Cinnamomum verum	subMICs	DD	CV026	11 mm	75
Cinnamomum verum	subMICs	DD	ATCC12472	12 mm	75
Cinnamomum verum	subMICs	V	CV026	78%	27
Cinnamomum verum	subMICs	B	PAO1	38%	27
Cinnamomum verum	10%	DD	CV026	15 mm	82
Cinnamomum zeylanicum	50ml	QSIS	QSIS4	✓	78
Citrus reticulata	4mg/ml	ß	ATCC27853	49%	63
Citrus reticulata	4mg/ml	ß	ATCC27853	50%	63
Citrus reticulata	4mg/ml	ß	HT5	38%	63
Citrus reticulata	4mg/ml	ß	HT5	37%	63
Citrus sinensis L	100%	V	CV026+Ezf 10-21	15	76
Coriandrum sativum	5µl/ml	V	ATCC12472	>90 %	88
Coriandrum sativum	100%	DD	ATCC12472	63.50 ±2.12	88
Cuminum cyminum	subMICs	DD	CV026	✓	86
Cuminum cyminum	subMICs	V	CV026	>70%	86
Cymbopogon citratus	20%	DD	CV026	15 mm	82
Cymbopogon nardus	50ml	QSIS	QSIS5	✓	78
Dorema aucheri Boiss.	/	DD	CV026	✓	85
Elettaria cardamomum	1,2 mg / ml	F	*P. putida* +pRK12 (C12)	31%	69
Elettaria cardamomum	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	21-22%	69
Eucalyptus globulus	5µl/ml	V	ATCC12472	>90%	89
Eucalyptus globulus	100%	DD	ATCC12472	10 mm	89
Eucalyptus globulus L	100%	V	CV026+ATTC 31300	6 mm	76
Eucalyptus radiata	5µl/ml	V	ATCC12472	>90%	89
Eucalyptus radiata	100%	DD	ATCC12472	20 mm	89
Eugenia Caryophyllata	50ml	QSIS	QSIS2	✓	78
Eugenia caryophyllata	50ml (dil.)	V	CV026	80%	78
Eugenia caryophyllata	50ml (dil.)	V	VIR07	72%	78
Eugenia caryophyllata	50ml (dil.)	V	ATCC12472	39%	78
Ferula asafoetida L.	/	DD	CV026	✓	85
Foeniculum vulgare	subMICs	V	CV026+C6HSL	75,69%	85
Foeniculum vulgare	subMICs	DD	CV026	✓	86
Geranium robertianum	100%	V	CV026+ATTC 31301	13 mm	76
Geranium robertianum	100%	V	CV026+Ezf 10-20	15mm	76
Hyptis dilatata	30/60 µl (dil.)	DD	CV31592	✓	58
Juniperus communis	3 µl	DD	CV6269	20 mm	53

Juniperus communis	50ml	QSIS	QSIS6	✓	78
L.alba (3) CT 2	1,2 mg / ml	F	*P. putida* +pRK12 (C12)	1%	69
L.alba (3) CT 2	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	72-78%	69
L.alba (C) CT1	IC50= 0,62 µg/ml	V	CV026+C6HSL	50%	84
L.alba (2) CT 2	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	15-84%	69
L.alba (4) CT2	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	61-62%	69
L.alba (5) CT2	1,2 mg / ml	F	*P. putida* +pRK12 (C12)	9%	69
L.alba (5) CT2	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	44-72%	69
L.alba (A) CT2	I50= 66,91µg/ml	V	CV026+C6HSL	50%	84
L.alba (D) CT1(Leaves)	IC50=15,79 µg/ml	V	CV026+C6HSL	50%	84
L.alba (E) CT2	IC50=30,32 µg/ml	V	CV026+C6HSL	50%	84
L.alba(B) CT2	IC50= 2,24 µg/ml	V	CV026+C6HSL	50%	84
Lavandula angustfolia	50ml	QSIS	QSIS12	✓	78
Lavandula angustifolia	/	L	[pSB1075] +3-oxo-C12-HSL	✓	61
Lavandula angustifolia	subMICs	DD	CV026	10 mm	75
Lavandula angustifolia	subMICs	DD	ATCC12472	11 mm	75
Lavandula angustifolia L.	100%	V	CV026+ATTC 31299	10 mm	76
Lavandula angustifolia L.	100%	V	CV026+Ezf 10-18	15 mm	76
Lepechinia floribunda	MQSIC = 0,0137 %	V	ATCC12472	50%	83
Linalool	100%	DD	ATCC12472	69,50±0,71	84
Linalool	5µl/ml	V	ATCC12472	>90 %	88
Lippia alba (1) CT 1	1,2 mg / ml	F	*P. putida* +pRK12 (C12)	65%	69
Lippia alba (1) CT 1	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	18%	69
Lippia graveolens Kunt	25 mg/ml	DD	ATCC12472	5,8±1,04 mm	80
Lippia graveolens Kunt	0,13%	V	ATCC12472	>95%	80
Matricaria Recutita	50ml	QSIS	QSIS3	✓	78
Matricaria Recutita	50ml (dil.)	V	CV026	67%	78
Matricaria Recutita	50ml (dil.)	V	VIR07	65%	78
Matricaria Recutita	50ml (dil.)	V	ATCC12472	25%	78
Mentha piperita	subMICs	DD	CV026	10 mm	75
Mentha piperita	subMICs	DD	ATCC12472	11 mm	75
Mentha piperita	subMIC	V	CV026	83,30%	87
Minthostachys mollis	MQSIC = 0,0649 %	V	ATCC12472	50%	83
Minthostachys mollis	100%	DD	ATCC12472	>90 ±0,1	83
Myntotachys mollis	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	56-65%	69
Myrtus communis	50ml	QSIS	QSIS7	✓	78
Ocotea sp.	1,2 mg / ml	F	*P. putida* +pRK12 (C12)	25%	69
Ocotea sp.	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	71-76%	69
Origanum majorana	3 µl	DD	CV6269	20 mm	53
Pinus sylvestris	50ml	QSIS	QSIS8	✓	78

Pinus sylvestris	50ml (dil.)	V	CV026	75%	78
Pinus sylvestris	50ml (dil.)	V	VIR07	61%	78
Pinus sylvestris	50ml (dil.)	V	CV12472	32%	78
Piper bogotense	IC50 = 93,1 µl/ml	V	CV026+ C6AHL	50%	77
Piper brachypodom	IC50 = 513,8 µl/ml	V	CV026+ C6AHL	50%	77
Piper bredemeyeri	IC50 = 45,6 µl/ml	V	CV026+ C6AHL	50%	77
Piper nigrum	subMICs	V	CV026+C6HSL	>55%	86
Piper nigrum	subMICs	DD	CV026	✓	86
Rosa damascena	50ml	QSIS	QSIS1	✓	78
Rosa damascena	50ml (dil.)	V	CV026	80%	78
Rosa damascena	50ml (dil.)	V	VIR07	70%	78
Rosa damascena	50ml (dil.)	V	CV12472	43%	78
Rosa damascena L	100%	V	CV026+ATTC 31298	18 mm	76
Rosa damascena L	100%	V	CV026+Ezf 10-17	20 mm	76
Rosmarinus officinalis L	100%	V	CV026+ATTC 31303	18 mm	76
Rosmarinus officinalis L	100%	V	CV026+Ezf 10-22	18mm	76
Salvia off.	MQSIC = 0,0259%	V	ATCC1247	50%	83
Salvia off.	100%	DD	ATCC12472	>90 ±0,1 mm	83
Salvia officinalis	50ml	QSIS	QSIS9	✓	78
Salvia sclarea	3 µl	DD	CV6269	25 mm	53
Satureja odora	100%	DD	ATCC12472	90 ±1 mm	83
Schinus molle	MQSIC = 0,005 %	V	ATCC12472	50%	83
Schinus molle	100%	DD	ATCC12472	50±1,2 mm	83
Swinglea glutinosa	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	28-73%	69
Syzigium aromaticum	subMICs	DD	CV026	17 mm	75
Syzigium aromaticum	subMICs	DD	ATCC12472	19 mm	75
Syzigium aromaticum	subMICs	V	ATCC12472	78,40%	75
Syzigium aromaticum	subMICs	B	PAO1	56%	79
Thymus vulgare	subMICs	V	CV026+ KM121	90%	64
Thymus vulgaris	50ml	QSIS	QSIS10	✓	78
Vanilla fragrans	50ml	QSIS	QSIS11	✓	78
Zingiber officinale	1,2 mg / ml	F	*E.coli* + pJBA132 (C6)	34-69%	69

Table 2) EOs negatively tested on QS-inhibition

EO	method	sensor strain	ref.
Cymbopogon martini	DD	CV026+ C6AHL	75
Cymbopogon martini	DD	CV12472	75
Apium graveolens	DD	CV026+ C6AHL	75
Apium graveolens	DD	CV12472	75

Boswellia carteri	DD	CV026+ C6AHL	82
Cananga odorata	DD	CV026+ C6AHL	82
Cedrus sp.	QSIS	QSIS1	78
Citrus aurantium	QSIS	QSIS1	78
Citrus bergamia	QSIS	QSIS1	78
Citrus bergamia	DD	CV026+ C6AHL	82
Citrus limon	DD	CV026+ C6AHL	75
Citrus limon	DD	CV12472	75
Citrus limon	DD	CV6269	53
Citrus limonum	QSIS	QSIS1	78
Citrus paradisi	DD	CV026+ C6AHL	75
Citrus paradisi	DD	CV12472	75
Citrus reticulata	QSIS	QSIS1	78
Citrus sinensis	DD	CV026+ C6AHL	75
Citrus sinensis	DD	CV12472	75
Citrus sinensis	DD	CV026+ C6AHL	82
Citrus sinensis L	V	CV026+ATTC 31302	76
Cymbopogon citratus	DD	CV026+ C6AHL	75
Cymbopogon citratus	DD	CV12472	75
Cymbopogon nardus	DD	CV026+ C6AHL	82
Eucalyptus globulus	QSIS	QSIS1	78
Eucalyptus globulus L	V	CV026+Ezf 10-19	76
Eucalyptus sp	DD	CV026+ C6AHL	82
Eucalyptus sp.	DD	CV026+ C6AHL	75
Eucalyptus sp.	DD	CV12472	75
Foeniculum vulgare	DD	CV026+ C6AHL	75
Foeniculum vulgare	DD	CV026+ C6AHL	75
Foeniculum vulgare	DD	CV12472	75
Foeniculum vulgare	DD	CV12472	75
Foeniculum vulgare Miller	QSIS	QSIS1	78
Jasminum officinale	QSIS	QSIS1	78
Juniperus communis L.,	V	CV026+ATTC 31302/Ezf 10-20	76
L.alba (2) CT 2	F	*P. putida* +pRK12 (C12)	69
L.alba (4) CT2	F	*P. putida* +pRK12 (C12)	69
Laurus nobilis	QSIS	QSIS1	78
Lavandula angustifolia	F	*E.coli* [pSB401]+ 3-oxo-C6-	61
Lilium candium	QSIS	QSIS1	78
Matricaria recutica L.,	V	CV026+ATTC 31302/Ezf 10-21	76
Melaleuca alternifolia	DD	CV026+ C6AHL	82
Melissa officinalis	QSIS	QSIS1	78

Mentha piperata	DD	CV026+ C6AHL	82
Mentha piperita	QSIS	QSIS1	78
Myntotachys mollis	F	P. putida +pRK12 (C12)	69
Myristica fragrans	DD	CV026+ C6AHL	75
Myristica fragrans	DD	CV12472	75
Myroxylon pereirae	QSIS	QSIS1	78
Nigella sativa	QSIS	QSIS1	78
Ocimum basilicum	QSIS	QSIS1	78
Ocimum basilicum	DD	CV026+ C6AHL	82
Olea europa	QSIS	QSIS1	78
Olea europaea	DD	CV026+ C6AHL	75
Olea europaea	DD	CV12472	75
Orange	V	CV026+ATTC 31302/Ezf 10-19	76
Petroselinum crispum	DD	CV026+ C6AHL	75
Petroselinum crispum	DD	CV12472	75
Pinus sp.	QSIS	QSIS1	78
Punica granatum	QSIS	QSIS1	78
Rosmarinus off.	DD	CV026+ C6AHL	82
Rosmarinus officinalis	DD	CV026+ C6AHL	75
Rosmarinus officinalis	DD	CV12472	75
Rosmarinus officinalis	QSIS	QSIS1	78
Santalum album	DD	CV026+ C6AHL	75
Santalum album	DD	CV12472	75
Santalum album	QSIS	QSIS1	78
Swinglea glutinosa	F	P. putida +pRK12 (C12)	69
Thymus vulgaris	DD	CV026+ C6AHL	75
Thymus vulgaris	DD	CV12472	75
Trachyspermum ammi	DD	CV026+ C6AHL	75
Trachyspermum ammi	DD	CV12472	75
Viola odorata	QSIS	QSIS1	78
Vitis vinifera	QSIS	QSIS1	78
Zea mays	DD	CV026+ C6AHL	75
Zea mays	DD	CV12472	75
Zingiber officinale	F	*P.putida* +pRK12 (C12)	69
Zingiber officinale	DD	CV026+ C6AHL	75
Zingiber officinale	DD	CV12472	75
Zingiber officinale	QSIS	QSIS1	78

Table 3) EOs tested on inhibition QS-related processes

EO	sensor strain	positive	negative	ref
Cinnamonum verum	*PAO1*	*biofilm, protease, pyocyanin, alginate, swarmin g motility*		27
Citrus reticulata EOP	*ATCC27853*	*biofilm, elastase B*		63
Citrus reticulata EOP	*HT5*	*biofilm, elastase B*		63
Citrus reticulata EOPD	*ATCC27853*	*biofilm, elastase B*		63
Citrus reticulata EOPD	*HT5*	*biofilm, elastase B*		63
Dorema aucheri Boiss.	*PAO1*	*biofilm, elastase, pyoverdin*	*pyocyanin*	85
Eugenia caryophyllata	*PAO1*		*Biofilm*	78
Eugenia caryophyllata	*PCI*	*Biofilm*		78
Ferula asafoetida L.	*PAO1*	*biofilm, elastase, pyoverdin, pyocyanin*		85
Matricaria Recutita	*PAO1*		*Biofilm*	78
Matricaria Recutita	*PCI*	*Biofilm*		78
Mentha piperata	*PAO1*	*biofilm, elastase, protease, pyoverdin, pyocyanin, chitinase, EPS, swarming*		87
Mentha piperita	*A.hydrophila*	*biofilm, protease,EPS*		87
Pinus sylvestris	*PAO1*		*Biofilm*	78
Pinus sylvestris	*PCI*	*Biofilm*		78
Rosa damascena	*PAO1*		*Biofilm*	78
Rosa damascena	*PCI*	*Biofilm*		78
Syzigium aromaticum	*PAO1*	*biofilm, swarming*		75
Syzigium aromaticum	*A.hydrophila*	*biofilm, protease, EPS*		79
Syzigium aromaticum	*PAO1*	*biofilm, elastase, protease, pyocyanin, chitinase, EPS, swimming*		79
Thymus vulgare	*P.fluorescens*	*biofilm, swimming, fg1A-gene-expression*		64

9. Discussion

As presented before, several EOs where tested positively on QS-inhibition or inhibition of QS-related processes like biofilm-formation and virulence factor production in several different sensor strains. The large variety of assays used to detect QS-inhibition of EOs and deviations in their execution makes it difficult to compare the activity of the oils. To illustrate this point, the aspects of the studies complicating comparability are present here:

The QS-activity of EOs can only be valued individually for the respective assay

The most active QS-inhibitors in CVATCC12472, tested via disc diffusion assay, are EOs from *Minthostachys mollis, Salvia off.* and *Satureja odora* with QS-inhibitory halos over 90mm, followed by *Artemisia annua* and *Coriandrum sativum* EO. QS-inhibition tested via flask incubation assay showed different results, as coriander and eucalyptus oil seem to bear high inhibitory potential. Although, oils in the respective studies have not been tested on their growth-inhibitory potential and therefore these results may be due to growth-inhibition. An interesting result showing the difficulty of compare disc diffusion assay and flask incubation assay is the result of oregano oil (*Lippa graveolens Kunt*) as the inhibitory activity is very low compared to other oils in disc diffusion assay but shows a high inhibitory potential in flask incubation assay. In CV026, the most active oils inhibiting QS in CV026 (disc diffusion assay) are rose and rosemary oil, followed by clove, cinnamon and geranium oil.
When tested via flask incubation assay, thyme oil is the most active oil, followed by clove, rose and peppermint oil.

An EO being an active inhibitor of AHL-based QS in one strain is not necessarily active in another strain also producing AHLs

Due to the varying side chain of AHLs in different organisms, EOs show different activities depending on the QS-system of the tested organism which was demonstrated by Yap et al.[61]when investigating the effect of lavender oil on either short chain or long chain AHL sensitive sensor strains. For one thing, this aspect it important to consider when conducting studies on EOs as the respective AHLs have

to be defined. This ambiguity exist e.g. in the study done by Szabo et al.[76], as the research team did not specify the AHLs produced by *E. coli* ATTC 31298 and Ezf 10-17, they just referred to an unpublished work by Szegedi. This point should be considered before assuming that specific EOs already tested positively might inhibit QS in other bacteria with AHL-based QS.

EOs often not only inhibit QS but also they do inhibit growth

As mentioned before, disc diffusion assay makes a clear differentiation between QS-inhibition and growth-inhibition possible because a strain which is only QS-inhibited is colourless and turbid whereas a clear halo indicates cell death, other assays often include concurrent cell viability tests in order to individually determine QS-inhibition or EOs are used in subMIC concentrations. Studies not clearly declaring this aspects discussed in this paper are 1), 4), 18) and 19) therefore these results cannot be ambiguously assigned to QS-inhibition.

Extraction method and collecting site of the EOs influences test results

Constituents of EOs vary significantly depending on their production method resulting in different QS-activity, which was shown e.g. by Jaramillo-Colorado et al. when investigating clove oil either extracted by microwave-assisted hydrodestillation or hydrodestillation. The relevance of collecting site regarding constituents of the EOs was illustrated when comparing different EOs from *L.alba*. [69]

As mentioned in chapter 7), EOs are defined according to their extraction method, therefore it is quite relevant to include this information in publications, although this specification is often not mentioned in the discussed studies in this paper. This aspect should be considered when interpreting study results. An example is a study published by Ganesh et al.[90] in which they discussed the anti-QS-activity of "EOs". However, they used supercritical CO_2-extraction to obtain the oils which is an extraction method not being in accordance to the ISO-definition of EOs. [90] GC/MS-analysis can also give a clue on the extraction method, as oil ingredients may indicate an extraction method not being rule-consistent. This was demonstrated by a study conducted by Eris and Ulusoy as they analysed the components of the tested oils by GC/MS. The main clove oil components mentioned in the paper where the solvents propylenglycol (73.2%) and diethylenglycol (16.0%). They also found

proplylenglycol in chamomile oil. The team also mentioned “vanilla EO”, although vanilla does not contain an EO. [78]

10. Conclusion

QS-systems in different organisms varies widely, research on those pathways is especially concentrated on AHL-based QS which may be due to the fact that many pathogenic bacteria possess LUXRI-homologous QS-systems. As QS regulates a wide range of bacterial functions such as biofilm-formation, virulence-factor production or swarming motility, QS-inhibitors represent a group of active substances, possibly having multifaceted effects on bacterial populations differing markedly from classical antibiotic targets. Research on EOs on QS-inhibitors only started a few years ago with *in-vitro* studies on *C.violaceum.* Given the fact that EOs already are well studied, safe substances which are easy to gain, they meet important criteria for possible new drugs. *In vitro* studies on different sensor strains, all based on AHLs as autoinducers revealed a significant QS-inhibitory potential of many different EOs. Not only did several oils inhibit QS itself but also do they inhibit QS-related processes. However, methods of detecting QS-inhibition are widely differing from each other and therefor prevent a transparent comparison of the EOs. In summary, EOs are potent QS-inhibitors in the tested sensor strains but research on this topic is still at its beginning. Closer investigations are necessary to e.g. understand the exact inhibition mechanisms or to define the effective components of the oils.

11. Abbreviations

"I" in e.g. LuxI / LasI	AI synthesis protein
"R" in e.g. LuxR / LasR	AI receptor protein
AHL or HSL	Acyl-homoserine-lacton
AI	Autoinducer
AI-2	*V.harveyi* autoinducer 2, a furanosyl borate diester
ATCC12472	*C.violaceum* wild type strain
ATCC31532	*C.violaceum* wild type strain
BHL	N-butyryl-L-homoserine-lactones
CAI-1	*V.cholerae* autoinducer 1
CGI	cell growth inhibition
CqsA	*V.cholerae* quorum-sensing autoinducer
CqsS	*V. cholerae* quorum-sensing sensor
CT	Chemotype
CV ATCC31592	*C.violaceum* strain
CV026	*C.violaceum* mutant strain of ATCC31532
CviIR	QS system in *C.violaceum*
DD	Disc diffusion assay
EO(s)	Essential Oil(s)
EPS	Exopolysacharide
F	Flourescence based assay
Gfp	Green flourescence protein
HAI-1	*V.harveyi* autoinducer 1, an AHL
HD	Hydrodestillation
HHL	N-hexanoyl-HSL
HT5	"Hospital Tucuman 5", a *P.aeruginosa* strain isolated from a patient
IC50	Concentration of EO leading to a 50% reduction of violacein production
L	Luminescence based assay
LasRI	*P.aeruginosa* QS system , designated after ist elastase-regulating properties
LB agar	Luria Bertani agar/ lysogeny broth, a growth medium for bacteria
LUX	proteins coded by luminescence genes (lux)
lux	luminescence genes (lux = lat. light)
LUXRI homologous systems	QS systems homologous to LUXRI-QS in e.g. *V.fisheri* using AHL as Ais
MIC	Minimal inhibitory concentration
MQSIC	Minimum QS inhibitory concentration = IC50
MWHD	Microwave assisted hydrodestillation

OdDHL	N-(3-oxododecanoyl)-homoserine lactone
PAO1	Wild type strain of *P.aeruginosa*
PBS	Phosphate Buffered Saline, a buffer solution
PCI	*P.aeruginosa* Clinical Isolate
PQS	pseudomonas quinolone signal
PqsR	PQS receptor
QS	Quorum Sensing
QscR	QS-control repressor
QSI	QS-inhibition / QS-inhibitor(s)
QSIS	QSI-selector, a QS-detection method
QSIS 1	QS-strain constructed via QSI-Selector method A
QSM	QS molecule
RhlIR	*P.aeruginosa* QS system , designated after RhlR
RhlR	Protein encoded by a rhamnolipid synthase gene cluster, AI receptor of BHL
SEM	Scanning electron microscopy
SPME	Solid-phase microextraction
ß	ß-galactosidase assay
subMIC	Concentrations below MIC
V	Flask incubation assay
VIR07	*C.violaceum* mutant strain of ATCC12472
VqsR	virulence and QS regulator

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