Structure activity modeling of essential oils compounds and plant secondary metabolites: a Mini review of Antimicrobial Activity

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Abstract: The increasing incidence of microorganisms becoming resistant to antibiotics has continuously become a scientific community concern. Many essential oil components and plant secondary metabolites are known to possess broad spectrum antimicrobial activity, including against antibiotic resistant bacteria. These compounds may be a useful source of new and novel antimicrobials. However, there is limited research on the structure-activity relationship (SAR) of essential oil compounds, which is important for a target identification and lead optimization. Quantitative Structure Activity Relationships (QSAR or SAR) have helped scientists to establish mathematical relationships between molecular structures and their biological activities. This study aimed to elucidate SARs of essential oil components from experimental and literature sources. Minimum Inhibitory Concentrations (MICs) of essential oil components were collected against *Escherichia coli* and *Staphylococcus aureus* and then compared to those published in literature for identify or predict the best model for describing the antibacterial activity of new biologically active agents in the plant.

Keywords: Antimicrobial, Essential oil (EO); Escherichia coli, Structure-activity relationship, Staphylococcus aureus.

1-Introduction

Resistance and multi-drug resistance to antibiotics pose serious challenges for modern medicine. Infections caused by multi-resistant bacteria are especially dangerous for patients in intensive care units. Antimicrobial resistance has greatly increased in recent years and is now considered a global public health threat (Laxminarayan et al. 2016). Novel antimicrobials are needed to continue

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treating antibiotic resistant infections, yet the production of new antibiotics has stalled (Laxminarayan et al. 2013).

Natural products are a reservoir of structurally diverse compounds, so may be a source for the development of novel antimicrobial agents. Essential Oils (EOs) have been the subject of scientific interest over recent decades, with extensive screening indicating that many of these plant extracts and their isolated components possess antimicrobial activity (Pandey et al. 2016). Moreover, EOs also possesses antifungal or insecticide and deterrent activities. All parts of aromatic plants may contain essential oils as follows:

•Flowers, of course, including: orange, pink, lavender, and the (clove) flower bud or (ylang-ylang) bracts, Leaves, most often, including: eucalyptus, mint, thyme, bay leaf, savory, sage, pine needles, and tree underground organs, e.g., roots (vetiver),

• Rhizomes (ginger, sweet flag),

•Seeds (carvi, coriander), Fruits, including: fennel, anise, Citrus epicarps,

• Wood and bark, including: cinnamon, sandalwood, rosewood (Dhifi et al. 2016).

Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils (Abdelouaheb and Dicko 2012).

Natural products may influence a plethora of biological processes in human body. In many cases, the mechanism of action of these compounds is directly connected with their structures. The following compounds can be considered as examples of metabolites characterized with proved biological activity: polyphenols, alkaloids, or terpenoids (Załuski, Cieśla and Janeczko 2015b). To this effect, several techniques can be used to extract EOs from different parts of the aromatic plant, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions (Edris 2007) .

This article provides an overview about the growth inhibitory effects of substances from essential oils and second metabolites of plants as documented in scientific literature sources to predict future antimicrobial drugs.

Essential oils

EOs are complex mixture of volatile, low molecular weight organic compounds (Raut and Karuppayil 2014). EOs are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colorless at room temperature, they are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colorless at room temperature. They have a refractive index and a very high optical activity. These volatile oils contained in herbs are responsible for different scents that plants emit. They are widely used in the cosmetics industry, and perfumery (Dhifi et al. 2016). The EOs and their volatile constituents are also used widely to prevent and treat human disease. The possible role and mode of action of these natural products is diverse with regard to the prevention and treatment of cancer, cardiovascular diseases including atherosclerosis and thrombosis, as well as their bioactivity as antibacterial, antiviral, antioxidants and antidiabetic agents. They are also an application as natural skin penetration enhancers for transdermal drug delivery and the therapeutic properties of EOs in aroma and massage therapy (Edris 2007).

Terpenes and their oxygenated derivatives, terpenoids, are the most common EO compounds (Rehman et al. 2016), while phenylpropanoids and benzenoid compounds are less abundant (Dudareva et al. 2013).

Numerous EO components inhibit an array of clinically relevant pathogenic bacteria, including antibiotic resistant isolates, suggesting they may be candidates for the development of new antimicrobials. For example, carvacrol, thymol and menthol inhibited 11 important foodborne pathogens at Minimum Inhibitory Concentration (MIC) ranging 0.02–4.0µg/mL (Dudareva et al. 2013).

The EO compounds 1,8-cineole, carvacrol, terpinen-4-ol, eugenol and cinnamaldehyde inhibited *S. aureus* and Methicillin Resistant *S. aureus* (MRSA) at MICs ranging 0.006–1.6% and *Enterococcus faecalis* at MICs ranging 0.012 to>3.2% (Hammer, Carson and Riley 2012a).

Wang et al. (Wang et al. 2016) reported that hinokitiol inhibited MRSA and *Escherichia coli* at 60 and 40µg/ml, respectively. Orhan et al.(Orhan et al. 2011) showed that thirty-five EO components inhibited 11 isolates of *Klebsiella pneumoniae*, including extended spectrum beta-lactamase producing strains, at MICs ranging 8–64µg/mL. The mechanism by which EO components exert their antimicrobial effect is incompletely understood. But Much of the published research has concluded that EO components change the structure and function of bacterial cell membranes; it has been proposed that the hydrophobic nature of these compounds allows them to partition in the membrane (Nazzaro et al. 2013). This non-specific mechanism of action is assumed to bypass many

antibiotic resistance mechanisms and inhibit antibiotic resistant isolates. Moreover, it has been hypothesised that the risk of antibiotic resistance developing is lower than other antibiotics (Langeveld, Veldhuizen and Burt 2014), as per other membrane-targeting antimicrobials such as cationic antimicrobial peptides (Hurdle et al. 2011).

The hydrophobicity of EO components, measured by octanal/water partitioning coefficient (log P) has been correlated with their antimicrobial activity [(Andrade-Ochoa et al. 2015),(Ben Arfa et al. 2006). For example, Ben Arfa et al. reported that carvacrol (log P=3.52) was more antimicrobial than eugenol (logP=2.73) (Ben Arfa et al. 2006). The positive correlation of hydrophobicity and antimicrobial activity relates to a greater affinity for partitioning in the bacterial cell membrane, this correlation does not hold true at logP greater than 4, indicating that there are other important structural characteristics (Ben Arfa et al. 2006),(Ultee, Bennik and Moezelaar 2002).

Indeed, Several published papers have emphasised the role of a phenolic group in activity , for example, Andrade-Ochoa et al. reported that thymol, which possesses a phenolic group had a significantly lower MIC than menthol, which has the equivalent structure but is alicyclic (Andrade-Ochoa et al. 2015). The importance of a hydroxyl moiety alongside a phenolic group was demonstrated by comparing with aromatic compounds with alkyl substituents [(Andrade-Ochoa et al. 2015), (Ben Arfa et al. 2006),(Veldhuizen et al. 2006). It is hypothesised that the delocalised electron system of the phenolic moiety facilitates protons exchange through the hydroxyl group, which dissipates proton motive force (Ben Arfa, Combes et al. 2006).

Secondary metabolites

Secondary metabolites are being the subject of many research studies because these compounds exhibits many biological activities. These include antimicrobial, antifungal, anticancer, anticholinesterase and anti-inflammatory activities.

For example anticholinesterase inhibitors constitute the biggest group of drugs used in the treatment of Alzheimer's disease symptoms. Galanthamine, an alkaloid isolated from plants of the Amaryllidaceae family is one of the most popular medicines from the aforementioned group. (Załuski et al. 2015b)

For this review, only the antimicrobial activity of different secondary metabolite compounds from different plants will be presented. Table2 summarizes these different secondary metabolites and their antibacterial activities.

Table1: Different secondary metabolites and their antibacterial activities

Secondary metabolite and plant families	Antibacterial activity	References
Alkaloids		
Fabaceae	*Escherichia coli*	Carson and Hammer (2010)
Arnaryllidaceae	*Staphylococcus aureus*	(Savoia 2012)
Rutaceae	Methicillin-resistant *Staphylococcus aureus* (MRSA	(Sibi et al. 2012)
Flavonoids		
Rubiaceae	*Staphylococcus aureus*	(Sibi et al. 2012)
Rutaceae	*Staphylococcus epidermulis*	
	Methicillin-resistant *Staphylococcus aureus* (MRSA)	
Tannins		
Myrtaceae	*Staphylococcus aureus*	(Abdulhamid et al. 2014)
Rubiaceae	*Escherichia coli*	(Oboh 2006)
Terpenes		
Labiatae	Methicillin-resistant	(Korir et al. 2012)
Rutaceae	*Staphylococcus aureus* (MRSA)	
Quinones		
Boraginaceae	*Staphylococcus aureus*	(Papageorgiou, Assimopoulou and Ballis 2008)

The terpenoids, alkaloids, and phenolics may act as inhibitors of bacterial growth. MIC values<10 mg/L were obtained for plumbagin against *E. coli.* The phenolic compounds with antibacterial activity are pinocembrin (5,7-dihydroxyflavanone) and pinocembrin chalcone (2,4,6,-trihydroxychalcone) whose antibacterial activity was studied of *N. gonorrhoeae* with MIC of 64µg/mL, while, pinocembrin chalcone inhibited the growth with MIC of 128 µg/mL. (Table3)(Załuski, Cieśla and Janeczko 2015a).

Table 2: Data of principal secondary metabolites and their antibacterial activities

Secondary metabolites	Structure	MIC	Reference
Plumbagin	*E. coli*	<10 mg/L	(Passos et al. 2013)
Pinocembrin	*Neisseria Gonorrhoeae*	64 µg/mL 128 µg/mL	(Kuete et al. 2011)
t-Cinnamaldehyde Cinnamon oil Oregano oil Carvacrol 2,5-Dihydroxybenzaldehyde 2-Hydroxy-5-methoxybenzaldehyde	*Mycobacterium avium subsp paratuberculosis*	25.9 µg/mL 26.2 µg/mL 68.2 µg/mL 72.2 µg/mL 74 µg/mL 90.4 µg/mL	(Friedman et al. 2009)

The exact mechanism of action and a Structure activity relationships (SAR) have been known for a little number of natural compounds (mainly polyphenols, alkaloids, and terpenes). It might be concluded that if a secondary plant metabolite contains, in its structure, the following moieties: hydroxyl groups attached to phenyl ring (optimally two hydroxyl groups in ortho position) as well as conjugated πbonds, it is highly probable that it will be characterized by potent free radical scavenging and anti-enzymatic activity. As already stated such a significant activity is due to the fact that the resulting antioxidant radical, formed in the reaction with ROS, is stabilized by charge delocalization over the molecule or through intramolecular hydrogen bonding. Resonance-stabilized structures quickly terminate radical chain reactions. Plant metabolites possessing both phenolic ring and conjugated double bonds are the most potent direct antioxidants, for example, eugenol, cinammic acid derivatives, and flavonols. However it should be also remembered that the activity can be modified by the presence of other moieties. Further studies are needed to explain antioxidant activity of compounds without the aforementioned moieties, as featured: bicyclic monoterpenes (e.g., α-pinene) or ursane-type triterpenes (Załuski et al. 2015a).

Approach SAR and QSAR

SAR and quantitative analogs (QSAR) have long been employed to understand the links between molecular descriptors and biological activity.

EO components could be an attractive class of compounds for the development of new antimicrobial therapies. Understanding the SAR of antimicrobial agents is important in antimicrobial development to identify the most potent compounds and allow optimization of lead compounds; however there are limited published studies on the SAR of EO components as antimicrobial agents.

This antibacterial activity is evaluated in vitro by broth microdilution and agar dilution techniques as a minimum inhibitory concentration (MIC).

Biological activities in terms of MICs against *E. coli* and *S. aureus* have been collated and used as activity model predictors for the QSAR framework. Data from both strains were cumulated in order to obtain more generalised, unified relationships between EO components and their effect on both Gram-positive and Gram-negative bacterial strains and their growth. This enables us to consider drug targets with the broadest possible spectrum for biological activity and bacterial growth inhibition. Once molecules were aligned to the carvacrol reference (lowest MIC), a conformational hunt was initiated in order to consider the training and test set molecules based on collective torsions and rotational degrees of freedom. The sets were aligned by substructure to carvacrol before being subject to field point analysis.

Descriptors are therefore rescaled before running the partial least squares analysis. Once scaling has taken place, the standard deviation of each block of descriptors becomes equal. The simple algorithm as a regression model allows correlated descriptors to be considered. The latent variables are extracted as orthogonal components, or linear combinations of the descriptors considered in the first instance. In order to amplify the covariance between descriptors and biological activity, the latent variables are chosen selectively, and an equation is formulated predicting activity as a linear combination of the latent variables. Implementing cross-validation through leave one out and leave many out techniques defines the best possible number of latent variables. This will by nature also correspond to the highest value of q2 and predictive ability of the model. From -27 molecules with known biological activity (MIC), the dataset was partitioned to 20% test and 80% training set through a combined activity/random selection algorithm in order to robustly test the QSAR model. Carvacrol as the most potent in the training set with MIC=1.98mM, was used as the reference and to align the training set to the compound.

Table 3: Data of Essential oils and their antibacterial activities

	MIC (mM)		Authors
	E. coli	*S. aureus*	
1,8-cineole	18.95	18.15	(Rosato et al. 2007)
Allyl isothiocyanat	1.58	1.58	(Laxminarayan et al. 2013)
α-pinene	0.06	0.04	(Sokovic et al. 2008)
α-Terpineol	36.18	42.3	(Zengin and Baysal 2014)
α-thujone	0.39	0.39	(Jirovetz et al. 2006)
β-Caryophyllene	0.29	0.29	(Jirovetz et al. 2006)
β-pinene	0.06	0.04	Sokovic, Marin et al. 2008)
β-Thujone	0.04	0.39	Jirovetz, Buchbauer et al. 2006)
Camphor	0.05	0.39	Sokovic, Marin et al. 2008, Jirovetz, Buchbauer et al. 2006)
Carvone	6.38	50.19	(Knight and McKellar 2007)
Citronellol	8.95	4.48	(Rosato et al. 2007)
Eugenol		6.5	(Hammer, Carson and Riley 2012b)
Geraniol	9.08	4.54	(Rosato et al. 2007)
Hinokitiol	0.24		Jirovetz, Buchbauer et al. 2006)
Limonene	0.004	0.44	Sokovic, Marin et al. 2008)
Linalyl acetate	0.05	0.31	Sokovic, Marin et al. 2008)
Menthol	0.01	0.01	Sokovic, Marin et al. 2008)
Terpinen-4-ol	0.04	12.10	(Hammer et al. 2012a)
Thymol	9.32	4.6	(Rosato et al. 2007)

Carvacrol was the most antimicrobial agent tested, with an MIC of 1.98mM against both *E. coli* and *S. aureus* (Table 1). Cumin aldehyde was also strongly antimicrobial against *E. coli* but weakly antimicrobial against *S. aureus*, with MICs of 2.10 and 33.60mM, respectively. Linalool, p-cymene and γ-terpinene were weakly antimicrobial with MICs ranging 7.98–250.46 mM. MICs of 12 of 21 EO components obtained from literature sources were considerably lower than that of carvacrol, ranging 0.004–0.59mM, where limonene possessed the greatest reported antimicrobial activity (Table 1). Differences in MICs observed may be attributed to susceptibility of bacterial isolates

used between studies, which can vary significantly, or methodological differences as inoculum size, inoculum preparation method, growth medium and incubation time, which are known influence MIC values (ISO 2006).

Much of the published research has concluded that EO components change the structure and function of bacterial cell membranes; it has been proposed that the hydrophobic nature of these compounds allows them to partition in the membrane (Nazzaro et al. 2013). This non-specific mechanism of action is assumed to bypass many antibiotic resistance mechanisms and inhibit antibiotic resistant isolates. Moreover, The pharmacological and toxicological properties of a compound of interest have to be taken into consideration (Pauli 2001)

Conclusion

The QSAR study on antimicrobial effects of EO components provides some promising targets for identification and future bacterial screening. Indeed, the steric effects of some EO components have been shown as a structural commonality between inhibitory molecules, with the electrostatic properties and topography correlating well with the lowest MICs. Carvacrol and menthol were found to have the lowest MICs against E. coli and S. aureus, with highly correlated electrostatic topography. Most notably, the ligand efficiency, defined as a function of the MIC and the number of heavy (non-hydrogen atoms), has been noted to correlate well with EO components exhibiting high antimicrobial activity. This is a promising finding which will help lead target identification for future studies of essential oils, their components, and the use of natural products in antimicrobial treatments.

These antibacterial properties are important for future pharmacological uses. Future research will be able to identify and learn about the mechanisms these secondary metabolites. In addition, future researchers should isolate all compounds from plants, to be able to determine exactly which compound is contributing to the antibacterial activity. Lastly, the future of ethnopharmacology seems very fascinating, there is hope to eliminate these antibiotic-resistant microorganisms with compounds of plant using mathematical models of prediction antimicrobial activity.

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