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In vitro bactericidal effect of various commercial essential oils and their major chemical constituents on *Aeromonas salmonicida* subsp. *Salmonicida*

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INTRODUCTION

- ❖ Antimicrobial resistance crises lead to restriction of antibiotic treatments. Several studies have evaluated the efficacy of plant essential oils (EOs) against fish diseases to improve fish health while reducing the potential for the spread of antimicrobial resistance [1].
- ❖ However, little evidence is available for antibacterial activity of EO major compounds (EOCs) against *Aeromonas salmonicida subsp. salmonicida*, causal agent of furunculosis in salmonid fish. Furthermore, the synergic effect of various associations of EOs/EOCs have not been studied against *Aeromonas spp.*

AIM

- To determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of several EOs/EOCs single or in combination against *A. salmonicida subsp. salmonicida (Ass)* strains.
- To suggest efficient EOs to overcome the problem of antimicrobial resistant micro-organisms.

METHODS

- ❖ Four *A. salmonicida subsp. salmonicida* bacterial strains (AERO31, CAE235, CAE452 and CAE258) were used for evaluating 13 EOs and 16 EOCs anti-microbial sensitivity.
- ❖ To determine the antibacterial activity of EOs/EOCs, MIC (24 and 48h) and MBC (48h) of each product or their binary combinations (10-90%) were determined by performing broth dilution methods (CLSI, 2006).
- ❖ MBC/MIC ratio against tested strains were calculated to determine the tolerance to bactericidal activity of each EO/EOC.
- ❖ Checkerboard synergy test through the lowest FIC (fractional inhibitory concentration) index method was performed [2] to determine the synergistic, antagonistic or additive effects between the most efficient EOs/EOCs.

RESULTS

Minimum inhibitory concentration (MIC) of various EOs for *A. salmonicida subsp. salmonicida* strains

Essential oils	MIC 24h AERO31	MIC 48h AERO31	MIC 24h CAE235	MIC 48h CAE235	MIC 24h CAE452	MIC 48h CAE452	MIC 24h CAE258	MIC 48h CAE258
Ceylon cinnamon bark (<i>Cinnamomum zeylanicum / verum</i>)	245	245	245	490	61	123	490	490
oregano vulgaris (<i>Origanum vulgare</i>)	226	226	226	226	113	226	453	453
compact oregano (<i>Origanum compactum</i>)	458	458	458	458	229	229	458	458
Provence green oregano (<i>Origanum heracleoticum</i>)	458	458	458	458	458	458	458	458
clove (<i>Eugenia caryophyllata</i>)	520	520	520	520	520	520	520	520
geraniol thyme vulgaris (<i>thymus vulgaris</i>)	880	880	880	880	440	440	440	1760
thymol thyme vulgaris (<i>thymus vulgaris</i>)	907	907	907	907	907	907	907	907
thyme saturoides (<i>Thymus saturoides</i>)	1840	1840	1840	1840	1840	1840	1840	3680
thujanol thyme thymus (<i>Thymus vulgaris</i>)	1784	1784	3568	3568	892	892	≥3568	≥3568
tea tree (<i>Melaleuca alternifolia</i>)	3624	3624	3624	3624	3624	≥3624	≥3624	≥3624
ravintsara (<i>Cinnamomum camphora</i>)	≥3592	≥3592	3592	3592	≥3592	≥3592	≥3592	≥3592
Provence linalool thyme vulgaris (<i>Thymus vulgaris</i>)	3360	3360	≥3360	≥3360	≥3360	≥3360	≥3360	≥3360
Cineole rosemary (<i>rosemary officinalis</i>)	≥3628	≥3628	≥3628	≥3628	≥3628	≥3628	≥3628	≥3628

- MIC and MBC values of each EO for Ass varied slightly from one strain to another, but the difference between strains was not remarkable.
- Mostly EOs showed MBC/MIC ratio of 1 or 2.
- Ceylon cinnamon bark (*Cinnamomum zeylanicum / verum*), oregano (*Origanum vulgare*, *Origanum compactum*, *Origanum heracleoticum*) and clove (*Eugenia caryophyllata*) were the most effective EOs for Ass strains.

DISCUSSION / CONCLUSION

- The mechanisms of action of EOs depend on their chemical composition which are able to damage bacteria [3].
- MBC/MIC ratio of tested EOs/EOCs resulted to their bactericidal activity for Ass.
- A single preparation of cinnamon, oregano, clove and thyme EOs showed the highest antibacterial activity against Ass strains.
- The major compounds of cinnamon, oregano, clove and thyme EOs (cinnamaldehyde, carvacrol, eugenol and thymol, *resp.*) were responsible for their antibacterial activity against Ass strains.
- Most notably, this is the first report on the antimicrobial effects of various EOs and their EOCs, combinedly against furunculosis. However, further study is needed to determine their antibacterial effect *in vivo* essay.

REFERENCES

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Minimum inhibitory concentration (MIC) of various EOCs for *A. salmonicida subsp. salmonicida* strains

Essential oil compounds	MIC 24h AERO31	MIC 48h AERO31	MIC 24h CAE235	MIC 48h CAE235	MIC 24h CAE452	MIC 48h CAE452	MIC 24h CAE258	MIC 48h CAE258
cinnamaldehyde	125	249	125	125	62	62	125	125
eugenol	250	500	250	500	125	250	250	250
thymol	500	500	500	500	500	500	500	500
carvacrol	344	688	688	1375	344	344	344	688
geraniol	870	870	870	870	870	870	870	870
(-)-terpinen-4-ol	500	1000	1000	2000	500	1000	1000	2000
α-terpineol	2000	2000	500	1000	500	1000	1000	2000
sabinene hydrate: 4-thujanol	1000	2000	1000	2000	500	1000	1000	2000
(-)-borneol	2000	2000	1000	2000	1000	2000	2000	2000
(-)-menthol	2000	2000	1000	2000	1000	2000	2000	2000
citral	1740	3480	1740	3480	1740	1740	1740	1740
(R)-(+)-limonene	3334	3334	3334	3334	1667	3334	3334	3334
linalool	≥3445	≥3445	≥3445	≥3445	≥3445	≥3445	3445	3445
citronellal	≥3256	≥3256	≥3256	≥3256	≥3256	≥3256	≥3256	≥3256
(S)-(-)-limonene	≥3342	≥3342	≥3342	≥3342	3342	≥3342	3342	≥3342
eucalyptol	≥3647	≥3647	≥3647	≥3647	≥3647	≥3647	≥3647	≥3647

- MIC and MBC values of each EOC for Ass varied slightly from one strain to another, but the difference between strains was not remarkable.
- Mostly EOCs showed MBC/MIC ratio of 1 or 2.
- cinnamaldehyde, eugenol, thymol and carvacrol showed the highest antibacterial activity.

Synergistic test of different EOs/EOCs associations for *A. salmonicida subsp. salmonicida* strains

Associations	Bacterial strains							
	AERO31		CAE235		CAE452		CAE258	
	FIC	FICI	FIC	FICI	FIC	FICI	FIC	FICI
cinnamaldehyde + eugenol								
cinnamaldehyde 90%	3.61	3.66	1.79	1.84	0.89	0.91	1.79	1.84
eugenol 10%	0.05		0.05		0.02		0.05	
cinnamaldehyde 70%	1.40	1.47	0.69	0.76	1.40	1.55	0.69	0.76
eugenol 30%	0.07		0.07		0.15		0.07	
cinnamaldehyde 50%	2.01	2.26	1	1.25	1	1.25	1	1.25
eugenol 50%	0.25		0.25		0.25		0.25	
cinnamaldehyde 30%	0.59	0.76	0.29	*0.47	0.29	*0.47	0.29	*0.47
eugenol 70%	0.17		0.17		0.17		0.17	
cinnamaldehyde 10%	0.80	1.7	0.40	1.3	0.40	1.3	0.40	1.3
eugenol 90%	0.90		0.90		0.90		0.90	

- The different association of EOs/EOCs did not show a synergistic activity against all strains tested except the combination of cinnamaldehyde (30%) and eugenol (70%) against CAE235, CAE452 and CAE258 (FICI <0.5).
- There was a neutral or an additive effect for all associations tested (0,5 < FICI < 4).
- None of the combinations tested had an antagonist effect (FICI >4).