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Application of rosemary *Rosmarinus officinalis*  
extract as an anthelmintic agent against  
Monogenean parasite in common carp *Cyprinus*  
*carpio*

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博士学位論文内容要旨  
Abstract

専攻 Major	Applied Marine Biosciences	氏名 Name	Zoral Mehmet Arif
論文題目 Title	Application of rosemary <i>Rosmarinus officinalis</i> extract as an anthelmintic agent against Monogenean parasite in common carp <i>Cyprinus carpio</i> (ローズマリー抽出物のコイ単生類に対する抗寄生虫薬への応用)		

The common carp (*Cyprinus carpio*) is an economically important species in the aquaculture industry. It is third in overall production world-wide and cultured in approximately 100 countries. The common carp production has increased steadily and will likely continue to increase in the future. However, intensive aquaculture has resulted in some serious parasitic diseases, affecting the health and productivity of carp and other species. Ectoparasites of freshwater and marine farmed fish cause significant economic losses. Chemotherapy by immersion has been the standard treatment against these parasitic diseases. However, these aquaculture drugs also leave harmful residues and adversely affect the environment and fish health. Recently, aquaculturists have focused on alternative approaches including the use of herbs as effective and environmentally friendly treatments. Medicinal plants have been shown to have antipathogenic, anti-inflammatory and immunostimulant activities. In addition, medicinal plants were reported to have low toxicity to fish and have fewer adverse environmental impacts. The objective of this research is to determine to find new effective candidate herb against parasitic diseases. Also, effective doses are determined to treat infected fish by herb extract in immersion and oral treatment methods. In addition, this study is investigated the side effects of plant diet used in high doses against fish in oral treatment. In last part, effective component profile in blood and mucus are investigated in pharmacokinetic study. First of all, I examined the effects of some natural herbs, such as rosemary, clary sage and thyme leaf extracts against *Ichthyophthirius multifiliis* (protozoa), *Trichodina* spp. (protozoa), *Dactylogyrus minutus* (monogenea), *D. extensus* (monogenea) and *Argulus* spp. (arthropoda) *in vitro*. In this screening, I found that the rosemary extract have strong anthelmintic activity against *D. minutus*. Based on the result of screening, I focused on the effects of rosemary extract against monogenean parasites, and conducted various experiments for applying herb to control parasitic diseases. In chapter 3, I evaluated the efficacy of rosemary extract by bath treatment and oral administration to prevent and control monogenean infestation. In the chapter 4, I examined the safety of rosemary extract against fish. In last chapter, I investigated 1,8-Cineole component in fish blood and mucus.

*In vitro* parasite survival experiments revealed that both of ethanol and aqueous rosemary extracts reduce parasite survival time in dose dependent manner and ethanol extracts of rosemary had higher anti-parasitic activity against the *Ichthyophthirius multifiliis*, *Trichodina* spp., *D. minutus* and *D. extensus* than that of aqueous extracts. The pure component of rosemary extract obtained commercially used in *in vitro* experiments showed that 1,8-Cineole was the most toxic component against *D. minutus*. The parasite intensity and prevalence in fish exposed to 50 and 100 g aqueous rosemary solution/L water for 30 min were significantly lower than those in controls ( $p < 0.05$ ). In oral treatment experiments, diets of *C. carpio* were supplemented with eight different concentrations of aqueous rosemary extract. The intensity of *D. minutus* was significantly less in fish fed for 30 days with feed containing 60, 80 and 100 ml aqueous extract/100 g feed than in control ( $p < 0.05$ ). No abnormal behavior was observed in any experimental fish group in the 30 day feeding period. Together these results indicate that rosemary is a promising candidate for prevention and control of monogenean infection. Aqueous rosemary extract demonstrated lower toxicity against host, and although its anthelmintic activity was slightly inferior to that of the ethanolic extract, the aqueous extract is a relatively safe and beneficial method for practical control of monogenean parasites.

In order to examine the safety of rosemary aqueous extract, toxicological study were conducted in fish were fed experimental diets supplemented with 10, 20, 40, 80, 100 ml extract/100 g feed for 20 days. According to the histopathological examination, the notable histological changes were detected in liver and kidney. Atrophy and nuclear pyknosis of hepatocytes were caused in the groups of  $\geq 20$  ml aqueous extract/100 g feed administration. In kidney, granular degeneration lead to necrosis and vascular irregularity were observed in groups of  $\geq 40$  ml aqueous extract/100 g feed. It was estimated that the LOAEL of rosemary extract against fish was 20 ml aqueous extract/100 g feed by the hepatotoxic effect. These findings indicate that high doses of rosemary extract in diet for 20 days can be damage to liver and kidney.

In plasma chemistry analysis, fish were fed three experimental diets: control (without extract), 10 ml and 80 ml rosemary extract/100 g of feed for 20 days. Serum samples were taken at the end of 10 and 20 days feeding. AST (aspartate aminotransferase) activity of rosemary diet groups was tended to higher than of control group although the difference was not significant. The number of specimens that showed higher AST activity was increased with dose dependent manner. The AST is indicator of hepatocytes damage in vertebrates. It is suggested that the results of AST activity reflected hepatotoxicity of rosemary extract obtained

by histopathological examination. In other clinical biochemical parameters, there were no significant differences among the experimental groups. These results suggest that the liver function and kidney damage were not serious.

1,8-Cineole was detected in blood 80 ml aqueous extract/100 g feed for 1 day. Following oral administration, 1,8-Cineole level in blood was reached to peak ( $117.89 \pm 3.47$  ng/ml) at 60 min after administration. After that, the amount of 1,8-Cineole in blood decreased exponentially from 2 to 72 h. The elimination half-lives ( $T_{1/2}$ ) of 1,8-Cineole was calculated as 248 min in blood. 1,8-Cineole were detected  $15.08 \pm 7.6$  ng,  $49.8 \pm 41.1$  ng and  $60.9 \pm 37.7$  ng in 10 mg crude dried mucus 5, 10 and 20 days administration, respectively. These results indicate that components of rosemary extract, such as 1,8-Cineole, absorbed by oral administration and travel through the blood, and can be secreted to mucus.

In summary, rosemary extract and its active compound of 1,8-Cineole showed anthelmintic activity against monogenean parasite. Both of oral administration and immersion of rosemary extract were effective to cure monogenean parasitic infection. In addition, component of rosemary extract was absorbed by oral administration and travel through the blood and secreted into mucus. Based on these results, I concluded that rosemary extract would be candidate anthelmintic agent that apply to control of parasitic infection of farmed fish. On the other hand, it was revealed that rosemary extract had hepatotoxicity and nephrotoxicity in histopathological study and its LOAEL might be lower than that of effective dose. Further studies are recommended to establish suitable treatment regime for practical use of rosemary extract to cure parasitic infection in farmed fish.

