



**UNIVERSITI PUTRA MALAYSIA**

**MEDICINAL HERBS AS GROWTH AND HEALTH PROMOTERS  
IN AFRICAN CATFISH (*Clarias gariepinus*, Burchell)**

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**FP 2012 10**

**MEDICINAL HERBS AS GROWTH AND HEALTH PROMOTERS IN AFRICAN  
CATFISH (*Clarias gariepinus*, Burchell)**

By

**ATEFEH SHEIKHLAR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**August 2012**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**MEDICINAL HERBS AS GROWTH AND HEALTH PROMOTERS IN AFRICAN CATFISH (*Clarias gariepinus*, Burchell)**

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**August 2012**

**Chairman: Professor Abdul Razak Alimon, PhD**

**Faculty: Agriculture**

In order to investigate the antimicrobial activity of aqueous and methanolic extracts of four plants, *Euphorbia hirta* (asthma herb), *Trigonella foenum-graecum* seed (fenugreek), *Citrus limon* peel (lemon) and *Morus alba* foliage (mulberry) on growth performance and prevention of *Aeromonas hydrophila* in African catfish (*Clarias gariepinus*), five experiments were carried out. In the first experiment, the effect of aqueous and methanolic extracts of aforementioned plants were investigated against two Gram-negative bacteria, *Aeromonas hydrophila* and *Escherichia coli* and two Gram-positive bacteria, *Streptococcus agalactiae*, and *Staphylococcus aureus* and the phytochemical compounds of the tested herbal extracts were determined. The 2<sup>nd</sup> study was designed to evaluate the effects of dietary incorporating of these extracts (at different levels) in African catfish (*Clarias gariepinus*). In this regard, assessing the effect of EHE and MFE supplemented diets on the growth, hematology and histology (kidney and liver)

was conducted. The effect of inclusion of EHE in the diet on growth and disease prevention in the catfish was evaluated in the 3<sup>rd</sup> study. In the 4<sup>th</sup> study, The effect of inclusion of EHE in the diet on growth and disease prevention in the catfish was investigated. In the 5<sup>th</sup> study, the effects of dietary mixed-herbal (*E. hirta* and *Morus alba*) methanolic extracts (E-ME) on growth, nutrient digestibility, hematological and intestinal indices, antioxidant activity and disease prevention in the catfish were investigated. In the first experiment, the aqueous and methanolic extracts were obtained using distilled water and methanol. To examine antimicrobial characteristics of each extracts against tested bacteria the paper disc diffusion method was used. The experiments were conducted at an Aquatic Animal Health Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Fingerling African catfish (*Clarias gariepinus*) used in this study, were obtained from a local farm. The fish were weighed and kept in 100-l aquaria with 30 fish per aquarium. The initial weight per fish was around 9.5. Each treatment had three replicates and fish were fed over a period of 60 days (feeding experiment) and 30 days (challenge test). Growth performance, hematological parameters, meat antioxidant activity and cumulative mortality were determined in these studies. The results of first study indicated that based on the inhibition zone, the aqueous extracts of *Trigonella foenum-graecum* seed (TS) and *Citrus limon* peel (CP) revealed weak antibacterial activity against the bacteria. However, *E. hirta* (EH) and *M. alba* foliage (MF) aqueous extract at a concentration of 100 mg/ml showed moderate and weak activities respectively. The methanolic extracts of all herbs exhibited stronger antimicrobial activities against the tested pathogens compared to the water extracts. Among the entire methanolic extracts, the EH and MF had the strongest activities,

while the others exhibited moderate or weak activities. Moreover, the results indicated that *A. hydrophila* was the most sensitive microorganism tested, with the highest inhibition zone in the presence of the methanolic extracts obtained from EH and MF. The phytochemical screening of the methanol extract of *E.hirta* (EHE) and methanol extract of *M. alba* foliage (MFE) showed the presence of secondary metabolites such as phenols, volatile oils, tannins, saponins, steroids, flavonoid, terpenoids and alkaloids. Results of 2<sup>nd</sup> revealed no negative effects of EHE and MFE (at 7 g/Kg DM of diet) in the experimental diets on the fish, while by increasing the level of EHE and MFE to 9 g/Kg, it showed some negative changes in the growth, hematological characteristics and histological assessment.

Results of 3<sup>rd</sup> study showed that growth performance was positively affected by dietary supplements. Mortality rate decreased in fish fed EHE-5 and EHE-7 (5 and 7g/Kg of DM) supplemented diets. Red blood cells, albumin and total protein increased in fish fed with EHE-7 diet compared to other groups. The meat from fish fed with the EHE supplemented diet (EHE-7) was higher for total phenols content and the free radical-scavenging effect (DPPH) than the other dietary groups. Dietary EHE did not change the lipid oxidation (TBARS) of meat. It was shown that storage time had significant effect on meat antioxidative potential. The results of hematological profile after artificial infection with *A. hydrophila* revealed that RBC, Hb, Ht, total protein, albumin and globulin were better for group offered with EHE-7 diet compared to untreated control, EHE-2 and EHE-5. All the measurements in the *A.hydrophila* infected fish which fed in the EHE at 7 g/kg DM (EHE-7) showed similar values compared with the control and the treatment groups which received antibiotic. The fish fed EHE-5 showed lower cumulative mortality than

fish fed with EHE-2 and untreated control, while the group fed with EHE-7 had the lowest cumulative mortality among others. The results of the 4<sup>th</sup> study showed that inclusion of *M.alba* foliage extract (MFE) did not improve the growth performance. The values of RBC, Hb and serum albumin and total protein were all higher for the treatment MFE-5 and MFE-7 than other treatments. The meat from fish fed MFE-5 and MFE-7 had significantly greater total phenols content than other dietary groups. The free radical-scavenging (DPPH) activity of meat from fish fed MFE was significantly improved. The DPPH-scavenging effect of the MFE -7 diet was higher than that of the control, MFE-2 and MFE-5 diets. Dietary MFE (at any level) did not affect the lipid oxidation (TBARS) of meat. It was shown that storage time had significant effect on meat antioxidative potential. In the challenge test, all the measurements (RBC, Hb, Ht, total protein, albumin and globulin) in the *A. hydrophila* infected fish fed with the MFE-5 and MFE-7 diets showed similar values compared with the control treatments (healthy control and antibiotic treated control). Cumulative mortality decreased by inclusion of the extract in the diet and the fish fed MFE-7 had the lowest cumulative mortality over the period of infection. Results of the 5<sup>th</sup> study showed that growth performance improved in fish fed EHE supplemented diet compared to other groups. The values of RBC counts, Hb and serum albumin and total protein were all higher for the treatments with EHE, MFE and E-ME than for the control treatment. The meat from fish fed with different extracts (EHE, MFE and E-ME diets) had significantly greater total phenols content as well as free radical-scavenging (DPPH) effect than the meat of fish fed with the control diet. The lipid oxidation (TBARS) values of fish fed EHE, MFE and E-ME diets did not increase during storage, while that of control group increased. In the present experiment,

determination of apparent digestibility showed that the digestibility for dry matter and crude protein were higher for the fish fed EHE diet than the MFE and control diets. Post infection hematological profile revealed that fish fed with EHE, MFE and E-ME diets had RBC, Hb, Ht, total protein, albumin and globulin values comparable with healthy and antibiotic treated controls. Furthermore, the cumulative mortality in the fish that received EHE, MFE and E-ME diets were much lower than untreated groups. Histological assessment of intestine in this experiment showed no abnormalities.

In conclusion, these studies suggested that the methanolic extract of *E. hirta* and *M. alba* foliage were found to be effective in growth improvement and bacterial disease prevention in African catfish. On the other hand, the benefits obtained from the inclusion of mixed-herbal extracts were not synergistic.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**HERBA UBATAN SEBAGAI PENGGALAK PERTUMBUHAN DAN KESIHATAN PADA IKAN KELI AFRIKA (*Clarias gariepinus*, Burchell)**

Oleh

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Dalam mengkaji potensi antimikrob ekstrak akuas dan metanol empat jenis tumbuhan, bahagian aerial *Euphorbia hirta*, biji halba, kulit lemon dan daun *Morus alba* pada kadar pertumbuhan dan rawatan *Aeromonas hydrophila* pada ikan keli Afrika, lima siri eksperimen dijalankan. Untuk eksperimen pertama, kesan ekstrak akuas dan metanol bahagian aerial *Euphorbia hirta*, biji halba, kulit lemon dan daun *Morus alba* pada dua jenis bakteria Gram negatif (*Aeromonas hydrophila*, *Escherichia coli*) dan dua jenis bakteria Gram-positif (*Streptococcus agalactiae*, *Staphylococcus aureus*) dikaji dan sebatian fitokimia ekstrak herba ditentukan. Pada eksperimen pertama, ekstrak akua dan metanolik diperolehi dengan menggunakan air dan metanol. Teknik resapan cakerakertas digunakan untuk memeriksa ciri-ciri antimicrobial pada setiap ekstrak terhadap bakteria



yang diuji. Eksperimen telah dijalankan di Unit Kesihatan Haiwan Akuatik, Fakulti Perubatan Veterinar, Universiti Putra Malaysia. Benih ikan keli Afrika (*Clarias gariepinus*) yang digunakan untuk kajian ini diperolehi daripada ladang tempatan. Ikan ditimbang dan ditempatkan di 100-l akuaria dengan kapasiti 30 ikan per akuarium. Berat awal setiap ikan ialah sekitar 9.5. Setiap rawatan mempunyai tiga replikat dan ikan diberi makan selama 60 hari (eksperimen pemberian makanan) dan 30 hari (ujian cabaran). Prestasi pertumbuhan, parameter hematologi, aktiviti antioksidan daging dan kematian kumulatif telah ditentukan di dalam kajian ini. Hasil kajian berdasarkan zon perencatan, menunjukkan bahawa ekstrak akuas herba dan kulit lemon mempunyai aktiviti antibakteria yang lemah. Walaubagaimanapun, ekstrak akuas *E. hirta* dan daun *Morus alba* (EH dan MF) pada kepekatan 100 mg/ml menunjukkan aktiviti yang sederhana. Ekstrak methanolic kesemua herba mempamerkan aktiviti antimikrob yang kuat terhadap patogen yang dikaji jika dibandingkan dengan ekstrak akuas. Antara keseluruhan ekstrak methanolic, EH dan MF (pada 100 mg/ml) mempunyai aktiviti yang paling kuat, sementara yang lain menunjukkan aktiviti sederhana atau lemah. Tambahan lagi, hasil menunjukkan *Aeromonas hydrophila* adalah mikroorganisma yang dikaji paling sensitif, dengan zon perencatan terbesar dengan kehadiran ekstrak methanolic yang didapati daripada EH dan MF. Kajian diteruskan dengan pengesanan kandungan aktif ekstrak herba yang tersebut sebelumnya. Pengesanan fitokimia ekstrak methanolic *E. hirta* dan daun *Morus alba* (EHE dan MFE) didapati mengandungi metabolit sekunder (fitokimia) seperti fenol, minyak mudah meruap, tanin, saponin, steroid, flavonoid, terpenoid dan alkaloids. Kajian seterusnya direka bentuk untuk menilai penambahan diet ekstrak tersebut (pada tahap yang berbeza) pada ikan keli Afrika. Berknaan perkara ini,

penilaian kesan EHE dan MFE sebagai diet tambahan untuk pertumbuhan hematologi dan histologi (ginjal dan hati) dilakukan dan keputusan menunjukkan tiada kesan negatif EHE dan MFE (7g/kg DM) pada ikan yang diberi makan diet secara ujikaji, sementara penambahan tahap EHE dan MFE kepada 9 g/kg terdapat sebahagian perubahan negatif pada pertumbuhan, ciri-ciri hematologi dan penilaian histologi diperhatikan.

Kesan penambahan EHE dalam diet untuk pertumbuhan dan rawatan penyakit pada ikan dinilai pada kajian seterusnya. Keputusan menunjukkan prestasi pertumbuhan dipengaruhi oleh rawatan pemakanan, tambahan pula kadar kematian menurun pada ikan yang diberi makan EHE-5 dan EHE-7 (5 dan 7 g/kg DM) diet tambahan. Sel darah merah, Hb dan albumin meningkat dalam ikan yang diberi makan diet EHE-7 berbanding kumpulan lain. Isi daging daripada ikan yang diberi diet tambahan EHE (EHE-7) mempunyai kandungan jumlah fenol dan kesan hapus sisa radikal (DPPH) yang tinggi berbanding kumpulan diet yang lain. Diet EHE-7 memberi kesan pengoksidaan lipid (TBARS) pada isi daging juga. Hasil profil hematologi selepas infeksi *A. hydrophila* menunjukkan RBC, Hb, Ht, jumlah protin, albumin dan globulin adalah lebih baik untuk kumpulan yang diberikan diet EHE-7 berbanding kumpulan kawalan, EHE-2 dan EHE-5. Semua pengukuran ikan yang diinfeksi *A. hydrophila* dimana diberi makan EHE 7g/kg DM (EHE-7) menunjukkan kepekatan sama berbanding dengan kawalan dan rawatan yang menerima antibiotik Ikan yang diberi diet EHE-5 menunjukkan kematian kumulatif yang rendah jika dibandingkan dengan EHE-2 dan kawalan yang tidak dirawat sementara kumpulan yang diberi makan EHE-7 mempunyai kematian kumulatif yang paling rendah berbanding kumpulan lain yang diberi diet EHE-2 dan EHE-5.

Keputusan kajian yang seterusnya menunjukkan penambahan ekstrak daun mulberi (MFE) tidak memperbaiki pertumbuhan. Kepekatan RBC, Hb, Ht dan albumin serum adalah tinggi untuk rawatan MFE-5 dan MFE-7 berbanding rawatan lain. Dalam kajian kebolehhidupan, semua pengukuran (RBC, Hb, Ht, jumlah protein, albumin dan globulin) dalam ikan terjangkit *A. hydrophila* yang diberi makan diet MFE-5 dan MFE-7 menunjukkan nilai yang sama jika dibandingkan dengan rawatan kawalan (kawalan ikan yang sihat dan diberi rawatan antibiotik). Kematian kumulatif berkurangan dengan penambahan ekstrak dalam diet dan ikan yang diberi makan MFE-7 mempunyai kematian kumulatif terendah sepanjang tempoh selepas jangkitan. Dalam kajian kelima, kesan hematologi dan indeks usus, aktiviti antioksidan dan rawatan jangkitan bakteria (*A. hydrophila*) dikaji. Keputusan menunjukkan prestasi pertumbuhan lebih baik pada ikan yang diberi diet tambahan EHE berbanding kumpulan lain. Kepekatan RBC, Hb dan albumin serum adalah lebih tinggi untuk rawatan EHE, MFE dan E-ME berbanding kawalan. Isi ikan yang diberi diet ekstrak yang berbeza (EHE, MFE dan E-ME) mempunyai kandungan jumlah fenol dan kesan hapus sisa radikal bebas (DPPH) lebih signifikan berbanding isi ikan yang diberi diet kawalan.

Nilai pengoksidaan lipid (TBARS) ikan yang diberi diet EHE, MFE dan E-ME tidak bertambah sepanjang proses penyimpanan, sementara kumpulan kawalan adalah meningkat. Dalam eksperimen ini, penentuan kebolehcernaan ketara menunjukkan kebolehcernaan bahan kering dan protin mentah adalah tinggi pada ikan yang diberi diet EHE berbanding diet MFE dan kawalan. Profil hematologi selepas jangkitan menunjukkan ikan yang diberi diet EHE, MFE dan E-ME mempunyai nilai RBC,

Hb,Ht, jumlah protin, albumin dan globulin berbanding dengan kumpulan yang sihat dan kumpulan kawalan yang dirawat dengan antibiotik. Tambahan lagi, kematian kumulatif pada ikan yang diberi diet EHE, MFE dan E-ME adalah rendah jika dibandingkan dengan kumpulan yang tidak menerima rawatan dimana menyokong pemerhatian eksperimen sebelum ini.

Penaksiran histologi usus dalam eksperimen ini menunjukkan tiada keabnormalan. Akhir sekali, kajian ini mencadangkan ekstrak methanol of *E. hirta* dan daun *M. alba* didapati efektif dalam memperbaiki pertumbuhan dan rawatan penyakit bakteria pada ikan keli Afrika dan kelebihan didapati daripada tindakan penambahan ekstrak.

## ACKNOWLEDGEMENTS

First of all, I express thanks to God. I hope to live each day better than the last so that I can please him. I would like to express my profound gratitude and very sincere thanks to my supervisor Professor Dr. Abdul Razak Alimon, for his invaluable guidance, concern, patience, encouragement and constructive instructions throughout my research. I am very grateful to my co-supervisors Associate Professor Dr. Hassan Mohd Daud and Associate Professor Dr. Che Roos Saad for their assistance, suggestion and advice. Most of all, I deeply appreciate the respect and confidence they have shown me, to allow me to flourish as a scientist and a person.

I wish to thank all the staff and graduate students of the Department of Animal Science and Faculty of Veterinary Medicine, especially Aquatic Animal Health Laboratory members for their helpfulness and friendship, with special thanks to Miss Ruhil Hayati Hamdan and other friends.

I would like to express many thanks to my beloved family for supporting me throughout my graduate studies. They have taught me that having good morals, working hard, and believing in yourself can take you far in life.

I certify that a Thesis Examination Committee has met on 8.8.2012 to conduct the final examination of Atefeh Sheikhlar on his Doctor of Philosophy thesis entitled “Medicinal herbs as growth and health promoters in African catfish (*Clarias gariepinus*, Burchell, 1822) ” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy (PhD).

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## **Declaration**

I declare that the thesis is my original work except for qualification and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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**ATEFEH SHEIKHLAR**

Date: 8.August.2012



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**8 THE EFFECTS OF MIXED-HERBAL (*Euphorbia hirta* AND *Morus alba* FOLIAGE) EXTRACT ON GROWTH PERFORMANCE, ANTIOXIDATIVE ACTIVITY, NUTRIENT DIGESTIBILITY, HEMATOLOGICAL PARAMETERS, INTESTINAL CHARACTERISTICS AND PREVENTION OF INFECTION WITH *Aeromonas hydrophila* IN AFRICAN CATFISH (*Clarias gariepinus*)**

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